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Подано експериментальні дані про особливості будови, розвитку та функціонування рослинних і тваринних організмів, флору та фауну України, одержані на основі досліджень, що проводяться науковцями ННЦ "Інститут біології" в галузях фізіології рослин і тварин, генетики, ботаніки, зоології, мікробіології, вірусології. Викладено також нові дані стосовно біохімічних і біофізичних основ регуляції у клітинах і органах у нормі й після впливу різноманітних фізико-хімічних факторів, наведено результати нових методичних розробок.

Для викладачів, наукових співробітників, аспірантів та студентів.

Подано экспериментальные данные об особенностях строения, развития и функционирования растительных и животных организмов, флору и фауну Украина, полученные на основе исследований, проводимых учеными ОНЦ "Институт биологии" в области физиологии растений и животных, генетики, ботаники, зоологии, микробиологии, вирусологии. Изложены также новые данные обиохимических и биофизических основ регуляции в клетках и органах в норме и после воздействия различных физико-химических факторов, приведены результаты новых методических разработок.

Для преподавателей, научных сотрудников, аспирантов и студентов.

Collection of articles written by the scientists of ESC "Institute of Biology" contains data on research in molecular biology, physiology, genetics, microbiology, virology, botanics, zoology concerning the structure, development and function of the plant and animal organisms, flora and fauna of Ukraine. Results of newly developed biophysical methods of biological research, biochemical data regarding metabolic regulation under the influence of different factors are presented.

For scientists, professors, aspirants and students.

ЗМІСТ

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EXPRESSION OF *CHGA* GENE IN RAT DUODENAL EPITHELIAL CELLS UPON LONG-TERM GASTRIC HYPOACIDITY AND WITH ADMINISTRATION OF MULTIPROBIOTIC SYMBITER

The increasing of Chga gene's expression in rat's duodenal villus and crypt epithelial cells upon gastric hypoacidic conditions were shown. The level of Chga mRNA was similar to the control value both in villus and crypt epitheliocytes upon treatment of hypoacidic rats with multiprobiotic Symbiter.

Key words: gastric hypoacidity, duodenal, rats, Chga gene expression, multiprobiotic.

Introduction. In recent decades, proton-pump inhibitors (PPI) of gastric parietal cells, such as omeprazole, remain the most effective therapeutic agents against acid-related disorders [18, 19]. Development of dysbiosis is one of the key consequences of long-term hypoacidity. Colonization of gastrointestinal tract (GIT) by opportunistic microbiota forms stable sources of endogenous infection and besides the effect of hypergastrinemia additionally promotes gastric carcinogenesis and tumorigenesis both in other parts of GIT and associated organs [1, 20].

Chromogranin A (encoded by *Chga* gene) – is the protein of granin's family, it expresses in neuroendocrine cells of APUD–system (for example, endocrine cells if GIT), which is the integrated control diffuse neuroendocrine system of human organism [8, 11, 14, 22, 23]. Expression of this gene is regulated exactly by gastrin and chromogranin A is necessary for processing of histamine propeptide in the gastric epithelial cells [5]. In small intestines, in particular in duodenum, chromogranin are produced by different types of enteroendocrine cells: P/D1, EC (enterochromaffin cell), D, G, S, N and so on [1, 11].

Analysis of scientific literature showed, that chromogranin A is a potentially useful approach for identification of inflammatory intestinal disorders: unspecific ulcerative colitis and Crohn's disease [14, 22, 23].

It was found more than 20 types of neuroendocrine cells (pancreatic islets, cells of APUD-system and so on) which can develop into respective malignancies under progression of cancers (nesidioblastoma, glucagonoma, gastrinoma, somatostatinoma, carcinoids of GIT and so on) [6, 8, 11, 14, 22, 23]. Thus, it was shown overexpression of *Chga* gene, focal hyperplasia of ECL-cells (enterochromaffin-like cells) with future development of malignant carcinoid from these cells upon long-term uninterrupted administration of PPIs from omeprazole, lansoprazole and other groups [5, 10, 13].

Overexpression of *Chga* gene mRNA is demonstrated in carcinomas with metastatic tissue and in GI tumors, in particular in duodenal carcinoid (extremely rare tumor which biological behavior has not been fully elucidated) [22]. Chromogranin A is found in neuroendocrine tumors, which whether secrete or do not extract hormones and amines, what is especially important for early diagnosing of their concealed functional activity, when characteristic clinic manifestations of disorder are absent as overexpression of appropriate hormones, but the tumor is located. So, the determination of *Chga* mRNA level is a useful tumor marker for the monitoring of gastrointestinal neuroendocrine carcinoids [14].

It was proved in clinical trials that probiotics were able not only to cure dysbiotic states, but also to instantly reduce damage ratio of GIT [3, 7]. Multiprobiotics of "Symbiter® acidophilic" concentrated group (hereinafter referred to as Symbiter) are characterized by complexity, wide array of bioactivity, high level of safety for organism and composition that is maximally close to nature microbial populations of human and animals [7, 12].

Analysis of scientific literature showed lack of data on the pattern of above mentioned gene expression in duodenal upon experimental or natural hypoacidity. Data about effect of probiotics on *Chga* gene expression in duodenal upon these conditions are also absent.

Consequently, the aim of current investigation was to determine the expression of *Chga* gene in rat duodenal epithelial cells upon long-term gastric hypoacidity by means of injection of omeprazole (and thereafter upon the excess of gastrin in blood) and with administration of Symbiter.

Materials and methods. The International recommendations on performance of medical and biological investigations with the use of animals according to European Convention for the Protection of Vertebrate Animals Used for Experimental and other Scientific Purposes were followed. Experiments were carried out on white non-strain male rats with initial weight around 180-200 g.

All animals were divided into four groups. The rats injected abdominally with 0,2 ml of physiological solution and 0,5 ml of water for injections orally were used as a control (first group). Animals of second group were treated with Symbiter (manufactured by LLC "O.D. Prolisok") orally (0,14 ml/kg) during 28 days. Hypoacidity (third group) was modeled by everyday intraperitoneal injection of omeprazole (14 mg/kg) during 28 days [21]. Fourth experimental group simultaneously with omeprazole obtained Symbiter in the same dose. Number of animals in each experimental group was 6. Crypts and villi of duodenal epithelial cells were extracted by means of low-temperature method [9]. RNA was isolated following Chomczynski and Sacchi [2]; cDNA was synthesized in 20 μl of reaction mix containing 2 μg of RNA, 1 mM dNTP, 50 U of reverse transcriptase "MultiScribeTM Reverse Transcriptase", corresponding buffer, 20 U of ribonuclease inhibitor "RNase Inhibitor" ("Applied Biosystems", США), 20 pmol (1,0 μM) of reverse primer. Synthesis was carried out in the following conditions: 37° C – 2 hour. Polymerase chain reaction was performed in 30 μl of reaction mix containing 10 μl of cDNA, PCR buffer, 200 μM of each dNTP, 30 pmol (1,0 μM) of each primer, 2,5 mM of MgCl2 and 1,5 U of Taq DNA polymerase ("iTaqTM", "Bio-Rad", США).

PCR amplifications consisted of an initial denaturing step of 95ºC for 3 min, followed by 35 (28 for Actb – gene used as internal control of reaction due to its constitutive expression) cycles of 95ºC for 45 s, the annealing step (with optimal annealing temperature): *Chga* (620 b.p., 59ºC – 45 s) and *Actb* (521 b. p., 49º C – 40 s); the extending step at 72º C for 1 min 15 s (for *Chga*) or 1 min (for *Actb*). Final extension step was performed upon 72º C for 5 min.

Such primer sequences were used in reactions: for *Chga* – forward – GGCCCAGCAGCCGCTGAAGCAGCA and reverse – CTCTGCGGTTGGCGCTGCCCTCCT; for Actb – forward – TGGGACGATATGGAGAAGAT and reverse – ATTGCCGATAGTGATGACCT. Reproducibility of the amplification results was evaluated in parallel experiments by the repetition of the PCR reactions with all ani-

mals and each primer at least three times. Separation of PCR products was performed electrophoretically in 1,6 % agarose gel with 0,5 x TBE buffer following Sambrook et al. [17]. For semi-quantitative analysis of amplicons expression based on densitometry the ImageJ 1.45s program was used. Indices of mRNA expression were calculated for each sample following Konturek et al. [15].

Mathematical and statistical processing of experimental data was performed using GraphPad Prism 4.03 ("GraphPad Software Inc.", USA). The normal Gaussian distribution of the data was verified by the Shapiro-Wilk normality test. Two-way analysis of variance (two-way ANOVA) and Bonferroni post tests were performed on obtained data. Statistical significance was set at $p \leq 0,05$. The data are expressed as means and standard deviations.

Results and discussion. PCR analysis of cDNA samples generated in the rat's duodenal villous and crypt epithelial cells indicated the presence of a specific signal with the expected length (620 b. p.) for *Chga* gene both in the control and second (animals treated only with Symbiter) groups of investigated animals (Fig. 1.).

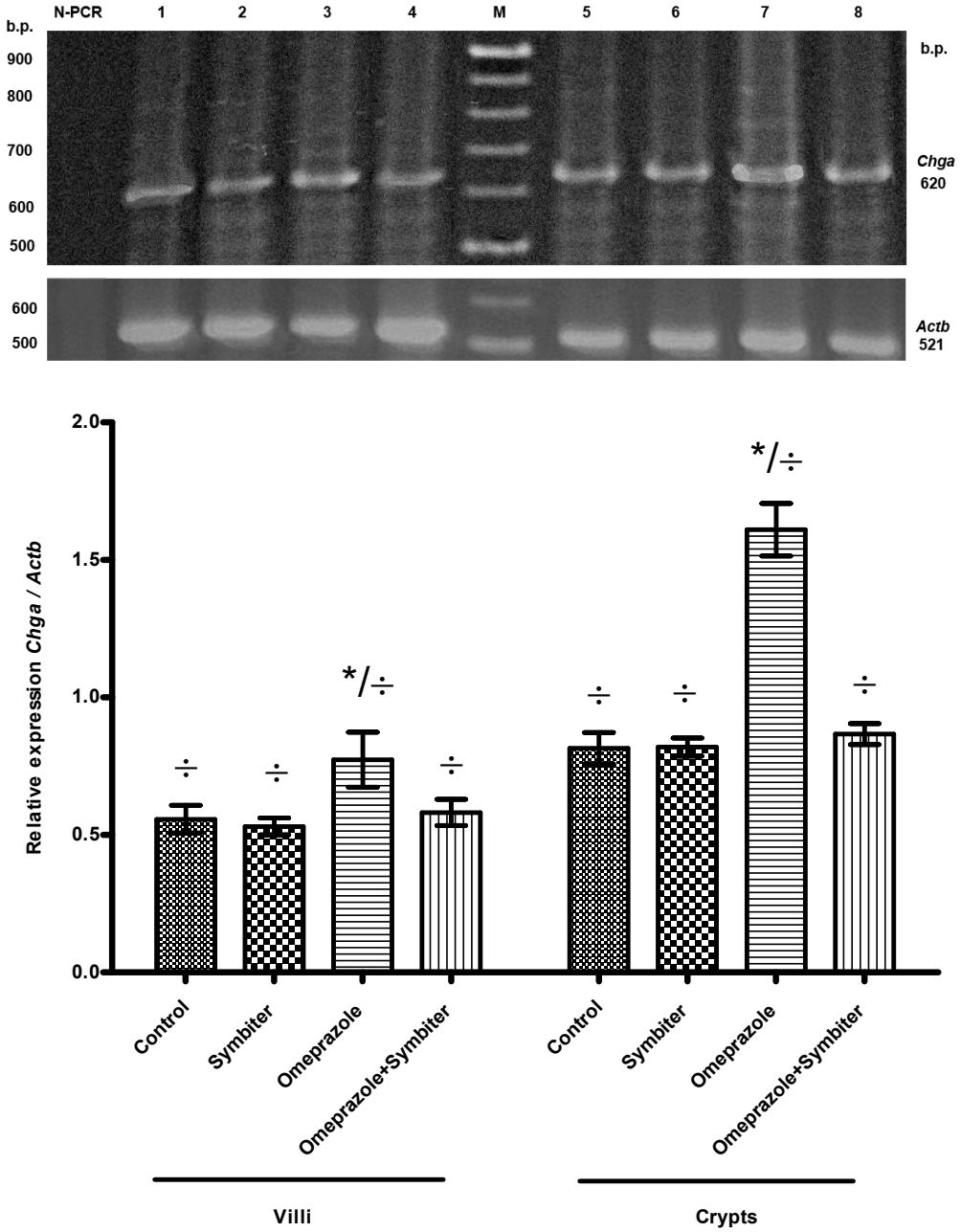

Fig. 1. Level of *Chga* gene mRNA in rat duodenal upon long-term hypoacidity and with administration of multiprobiotic Symbiter.
M – molecular mass marker; villus epithelial cells: 1 – control; 2 – Symbiter; 3 – omeprazole;
4 – omeprazole + Symbiter; crypts epithelial cells: 5 – control; 6 – Symbiter; 7 – omeprazole;
8 – omeprazole + Symbiter; N-PCR – negative PCR control; * – $p \leq 0,0001$ in relation to control; ÷ – $p \leq 0,0001$ villi in comparison with crypts

It was established that the levels of *Chga* gene's expression did not significantly differ in the control and second groups from one another both in villus epitheliocytes and crypts comparing with the types of epithelial cells. While, the levels of this gene mRNA in duodenal samples of animals injected with omeprazole during 28 days and rats of fours group (Omeprazole + Symbiter) as significantly differentiate both in villous epithelium and crypt cells as between analyzed types of epitheliocytes ($p \leq 0,0001$) (Fig. 1., Table. 1.).

Table 1. **Level of *Chga* gene mRNA in rat duodenal upon long-term hypoacidity and with administration of multiprobiotic Symbiter (m ± SD, n = 6)**

Groups of animals	Typo of epithelial cells	Relative expression *Chga / Actb*
Control	villi	0,557 ± 0,0481 ÷
	crypts	0,815 ± 0,0543 ÷
Symbiter	villi	0,530 ± 0,0292 ÷
	crypts	0,819 ± 0,0312 ÷
Omeprazole	villi	0,773 ± 0,0951* ÷
	crypts	1,61 ± 0,0912* ÷
Omeprazole + Symbiter	villi	0,581 ± 0,0452 ÷
	crypts	0,866 ± 0,0365 ÷

Notes: SD – standard deviation;
** – p ≤ 0,0001 in relation to control;*
÷ – p ≤ 0,0001 villi in comparison with crypts.

As we see from the *Table 1*., the level of *Chga* gene's expression was higher than control values in 1,4 times in villi and approximately in 2 times in crypts of animals upon long-term gastric hypoacidity (p≤0,0001). At the same time upon simultaneous administration of multiprobiotic Symbiter this parameter was in 1,3 and about 1,9 times lower than in animals of third group (p≤0,0001).

It is well known, *Chga* gene is expressed by different duodenal enteroendocrine cells. Message of *Chga* are expressed at ≈ 1000 × the level in malignant gastro-intestinal carcinoids compared with normal mucosa. Detectable levels of *Chga* in normal mucosa (small intestine/gastric) reflects the presence of *Chga*-expressing endocrine cells in these tissues and further emphasizes the sensitivity of the technique since it detects neuroendocrine cells, which represent approximately 1 per 2000 epithelial cells in duodenum (there are more enteroendocrine cells in crypts then in villi, although the functional activity of these cells are approximately the same). Since endocrine cells constitute ≈ 1 % by volume of the gastro-intestinal mucosa, the detection in normal mucosa further confirms the sensitivity of PCR as an identification tool and emphasizes its ability to detect disease at a cellular level (1, 11, 14). In our experiment we demonstrated the elevation of *Chga* mRNA level in villi and crypts upon hypoacidic conditions (Fig. 1., Table. 1.). It can be assumed not only about intensification of inflammatory processes in duodenal [4], but about possible neoplasia in epithelial cells on later stages of pathology process development [4]. Thus, determination of *Chga* gene's level of expression could be used as one of the most sensitive marker of neuroendocrine tumors [8, 14, 22, 23].

Thus, the obtained changes in expression of *Chga* gene in rat duodenal villus and crypts epithelial cells upon hypoacidic conditions should point out the development of teratoid displacements in duodenal tissue. Different rates in alterations of above mentioned gene's expression in villus and crypts epithelial cells is determined by their structural and functional characteristics (1, 11, 14). Besides this, according to a literature, it is well known, that an early feature of some inflammatory diseases, including pathological disorders in intestines, is the formation of crypt abscesses, which are composed of neutrophils that have migrated across the epithelium and into the crypt lumen [1].

Among probable mechanisms of Symbiter's action on gene expression in rat duodenal, firstly, it should be pointed out its ability to liquidate dysbiosis and bacterial colonization of GIT. As a result, the burden of pathogenic microbiota is removed from GIT and associated organs [3, 7]. Furthermore, multicomponent probiotic Symbiter is able to increase de novo synthesis of the main low-molecular cellular antioxidant – reduced glutathione and, thus, to raise its content both in GIT and duodenal. Besides this, the products of Symbiter's bacterials vital functions (vitamins, exopolysaccharides, short chain fat acids, immune-response modulating agents and so on) are characterized by antioxidant properties, on the basis of what they can delay the development of oxidative stress and decrease the intensity of inflammation and pathological actions in duodenal [4, 6, 12, 16]. The reduction of gastrin level in the blood upon prolonged administration of Symbiter has recently been observed [7]. On the basis of binding studies, it may be suggested that observed effects of Symbiter are linked not only with normalization of GIT microbiota, but also with restriction of hypergastrinemia effects [7, 16]. But final acceptance or rejection of this suggestion requires further investigations, which will allow us to distinctly distinguish the consequences of hypergastrinemia and bacterial colonization of GIT by use of selective antagonists of gastrin receptor.

Conclusion. In summary, we have shown, that long-term experimental hypoacidity is accompanied by changes in expression of *Chga* gene in rat duodenal epithelial cells. While upon simultaneous administration of multiprobiotic Symbiter the expression pattern of this gene is similar to control both in villous and crypt ephiteliel cells. Based on the obtained data, it can be assumed, that analyzed gene is involved in the development of pathological processes in duodenal and there is some potential risk of duodenal carcinogenesis upon long-term use of omeprazole (and probably other PPIs).

References

1. K., Ghishan F., Merchant J. etc. Physiology of the gastrointestinal tract. 4th edition. – New York: Academic Press, 2006. – 2080 p.

2. Chomczynski P., Sacchi N. Single-step method of RNA isolation by acid guanidiniumthiocyanate-phenol-chloroform extraction // Anal. Biochem. – 1987. – Vol. 162. – P. 156-159.

3. Culligan E., Hill C., Sleator R. Probiotics and gastrointestinal disease: successes, problems and future prospects // Gut Pathogens. – 2009. – 1, N 19. – P. 38-49.

4. Dranitsina A., Dvorchshenko K., Savko U., Ostapchenko L. Expression of Reg1a gene in rat duodenal upon long-term hypoacidity and with administration of multiprobiotic "Symbiter®" acidophilic concentrated // Bulletin of Taras Shevchenko National University of Kiev. Series Biology. – 2013. – Vol. 2 (64). – P. 5-8.

5. Dufresne M., Seva C., Fourmy D. Cholecystokinin and gastrin receptors// Physiol Rev – 2006. – Vol. 86. – P. 805-847.

6. Dvorshchenko K. O., Vakal S. E., Dranitsina A. S., Senin S. A., Ostapchenko L. I. Stress-responsive systems in rat pancreas upon long-term gastric hypochlorhydria and administration of multiprobiotic "Symbiter®" // Ukrain'skyi Biokhimichnyi Zhurnal-2013. – 85, N 2. – P. 68-77.

7. Falaleeva T.M., Virchenko O.V., Beregova T.V., Iankovsky D.S. Correction of stress-induced changes in hypothalamic-pituitary-adrenal system by multiprobiotic "Symbiter" // World of medicine and biology. – 2012. – N 1. – P. 173-178.

8. Fukui H., Takada M., Chiba T. et al. Concurrent occurrence of gastric adenocarcinoma and duodenal neuroendocrine cell carcinoma: a composite tumour or collision tumours ? // Gut. – 2001. – Vol. 48. – P. 853–856.

9. Flint N., Cove F.L., Evans G.S.H. A low-temperature method for the isolation of small-intestinal epithelium along the crypt-vinlus axis // Biochem J. – 1991. – Vol. 280 (Pt 2). – P. 331–334.

10. Glaser S.S., Rodgers R.E., Phinizy J.L. et al. Gastrin inhibits secretin-induced ductal secretion by interaction with specific receptors on rat

cholangiocytes // Am J Physiol Gastrointest Liver Physiol. – 1997. – Vol. 273 – P. G1061–G1070.

11. Habib A. M., Richards P., Cairns L. S. et al. Overlap of endocrine hormone expression in the mouse intestine revealed by transcriptional profiling and flow cytometry // Endocrinology. – 2012. – Vol. 153 (7). – P. 3054 – 3065.

12. Iankovsky D., Dyment G. Microbiota and human health. – Kyiv: LLC Chervona Ruta-Turs, 2008. – 552 p.

13. Ivashkin V.T., Sheptulin A.A. The perspectives of usage of proton-pump inhibitors in gastroenterology // The diseases of gastrointestinal tract. – 2001. – Vol. 3 (1). – P. 3-6.

14. Kidd M., Modlin I., Mane S.M. et al. Q RT-PCR detection of chromogranin A. A new standard in the identification of neuroendocrine tumor // Annals of Surgery – 2006. – Vol. 243 (2). – P. 273-280.

15. Konturek P., Brzozowski T., Pierzchalski P. et al. Activation of genes for spasmolytic peptide, transforming growth factor alpha and for cyclooxygenase COX-1 and COX-2 during gastric adaptation to aspirin damage in rats // Aliment. Pharmacol. Ther. – 1998. – Vol. 12 (8). – P. 767-777.

16. Lutgendorff F., Trulsson L. M., van Minnen L. P., Rijkers G. T., Timmerman H. M., Franzén L. E., Gooszen H. G., Akkermans L. M., Söderholm J. D., Sandström P. A. Probiotics enhance pancreatic glutathione biosynthesis and reduce oxidative stress in experimental acute pancreatitis // Am. J. Physiol. Gastrointest. Liver Physiol. – 2008. – 295, N 5. – P. 1111-1121.

17. Sambrook J., Russell D. Molecular cloning: a laboratory manual.– Cold Spring Harbor Laboratory Pr.; 3rd edition, 2000. – 2344 p.

18. Shin J., Vagin O., Munson K., Munson K., Kidd M., Modlin I. M., Sachs G. Molecular mechanisms in therapy of acid-related diseases // Cell. Mol. Life Sci. – 2008. – 65, N 2. – P. 264-281.

19. Sundstrom A., Blomgren K., Alfredson L., Wiholm B. Acid-suppressing drugs and gastroesophageal reflux disease as risk factors for acute pancreatitis – results from a Swedish Case-Control Study // Pharmacoepidemiology and drug safety. – 2006. – 15, N 3. – P. 141-149.

20. Thomson A., Sauve M., Kassam N., Kamitakahara H. Safety of long-term use of proton pump inhibitors // World J. Gastroenterol. – 2010. – 16, N 19. – P. 2323-2330.

21. Tsyriuk O.I., Beregova T.V. Effect of omeprazole-induced hypergastrinemia on the basal gastric secretion in rats // Bulletin of biological and medical issues. – 2007. – № 3. – P. 38-43.

22. Waisberg J., Joppert-Netto G., Vasconcellos C. et al. Carcinoid tumor of the duodenum: a rare tumor at an unusual site. Case series from a single institution // Arq. Gastroenterol. – 2013. – Vol. 50 (1). – P. 3–9.

23. Zatelli M.C., Torta M., Leon A. et al. Chromogranin A as a marker of neuroendocrine neoplasia: an Italian multicenter study // Endocrine-Related Cancer. – 2007. – Vol. 14. – P. 473–482.

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ЕКСПРЕСІЯ ГЕНА CHGA В ЕПІТЕЛІОЦИТАХ ДВАНАДЦЯТИПАЛОЇ КИШКИ ЗА УМОВ ТРИВАЛОЇ ГІПОАЦИДНОСТІ ШЛУНКА ТА ПРИ ВВЕДЕННІ МУЛЬТИПРОБІОТИКА СИМБІТЕР

Показано зростання рівня експресії гена Chga в епітеліоцитах ворсинок та крипт дванадцятипалої кишки щурів за гіпоацидних умов. При введенні мультипробіотика Симбітер за тих самих умов патерн експресії вищезазначеного гена в епітеліоцитах як ворсинок, так і крипт був подібний до контролю.

Ключові слова: шлункова гіпоацидність, дванадцятипала кишка, щури, експресія гена Chga, мультипробіотик.

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ЭКСПРЕССИЯ ГЕНА *CHGA* В ЭПИТЕЛИОЦИТАХ ДВЕНАДЦАТИПЕРСТНОЙ КИШКИ КРЫС ПРИ ДЛИТЕЛЬНОЙ ЖЕЛУДОЧНОЙ ГИПОАЦИДНОСТИ И ПРИ ВВЕДЕНИИ МУЛЬТИПРОБИОТИКА СИМБИТЕР

Показано увеличение уровня экспрессии гена Chga в эпителиоцитах ворсинок и крипт двенадцатиперстной кишки крыс в гипоацидных условиях. При введении мультипробиотика Симбитер в тех же условиях содержание мРНК Chga в эпителиоцитах как ворсинок, так и крипт было на уровне контрольных значений.

Ключевые слова: желудочная гипоацидность, двенадцатиперстная кишка, крысы, экспрессия гена Chga, мультипробиотик.

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THE CHANGES IN FUNCTIONING OF MUCUS BARRIER OF STOMACH IN CONDITIONS OF LONG-TERM HYPOACIDITY AND THEIR CORRECTION

Increase in the duration of hypoacidity of gastric juice evoked by daily injection of blocker of gastric acid secretion omeprazole from 7 to 28 days accompanied by substantial rise of the level of oxiproline, fucose, N-acetylneuraminic acid and hexuronic acids in gastric mucus in rats. It is witness of intensification of degradation collagenic and noncollagenic proteins in gastric mucus. Injection of multiprobiotic Symbiter against the background of hypoacidity evoked by omeprazole led to decrease the level of studied parameters to control values in all terms of investigations.

Key words: gastric mucus, omeprazole, multiprobiotic.

Introduction. The basis mucous layer of the stomach are polymerized structural glycoproteins of mucus. Due to its polymer structure and hydrophobic properties gel mucus protects gastric mucosa from direct contact with xenobiotics, endogenous nitroso compounds, free radicals, bacterial toxins. Long-term hypoacidity of gastric juice and anacidity are risk factors for carcinogenesis in stomach. In conditions of hypoacidity in stomach dysbiosis develops [5], this can lead to structural changes of mucus. Dysbiosis [9] and disturbance of structure of gastric mucus [4] in turn accelerate the development of neoplastic changes in stomach.

In connection with this the aim of our work was to investigate effect of multiprobiotic "Symbiter® acidophilic" as drug for prophylaxis of dysbiosis, on state of mucus barrier in stomach in conditions of hypoacidity of different duration evoked by omeprazole.

Materials and methods. The study was done on white rats which were divided into 12 group. To the rats of 4 groups during 7, 14, 21, 28 days consequently were injected blocker of gastric acid secretion omeprazole ("Sigma", USA) (14 mg/kg intraperitonealy once a day). To the rats of others 4 groups during the same terms simultaneously with omeprazole we injected multiprobiotic "Symbiter® acidophilic" (Symbiter) (limited company "O.D. Prolisok") (0.14 ml/kg per os once a day). To the rats of 4 control groups were injected during 7, 14, 21 and 28 days 0.2 ml H_2O intraperitonealy and 0.5 ml H_2O per os. Symbiter is concentrated fluid biomass of bioplasts of symbiosis of 14 microorganisms strains. The composition of one dose (10 ml) of Symbiter is concentrated biomass of bioplasts of bacterium's symbiosis CFU/cm^3, no less: Lactobacillus and Lactococcus – $6.0x10^{10}$, Propionic bacterium – $3.0x10^{10}$, Bifidobacterium – $1.0x10^{10}$, Acetic bacterium – $1.0x10^{6}$. For assessment of mucus barrier state in stomach in a day of last injection of drugs in parietal mucus we determined the levels of oxiproline using method as described earlier [8] ,

fucose using method as described earlier [6], N-acetylneuraminic acid using method as described previously [1] and hexuronic acids using method as described earlier [7]. All results are performed as Mean±SD by using Students t test.

Results and discussion. In 7, 14, 21 and 28 days of omeprasole injection the levels of oxiproline were increased respectively by 7.9% (p<0.001), 125.3% (p<0.001), 146.3% (p<0.001) and 163.2% (p<0.001) (Fig.1).

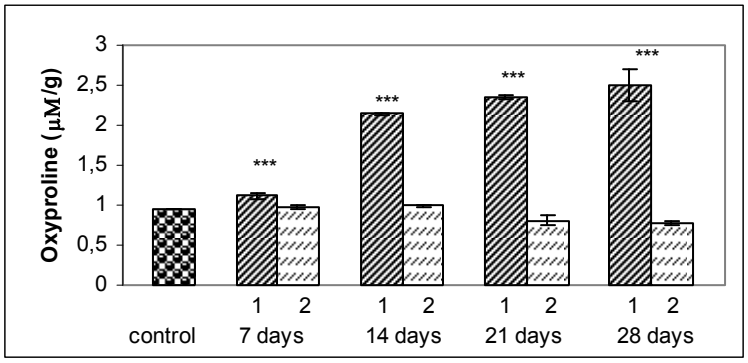

Fig.1. Changes of oxiproline concentration in gastric mucus in rats after long-term injection of omeprazole (1) and simultaneous injection of omeprazole and multiprobiotic "Symbiter" (2). Changes that are statistically different from the control group (p<0.001 by Student t test) are indicated with stars

This indicates that with enhance of duration of hypoacidity of gastric juice degradation of collagenic proteins in gastric mucus intensifies.

In 7, 14, 21 and 28 days of omeprasole injection the levels of fucose were increased respectively by 23.6% (p<0.001), 49.7% (p<0.001), 52.3% (p<0.001) and 107.0% (p<0.001) (Fig.2).

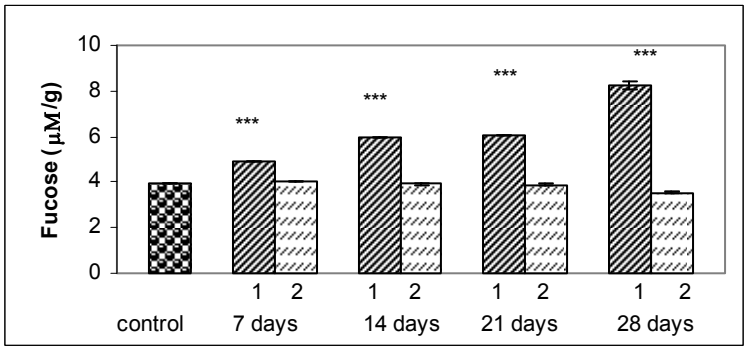

Fig. 2. Changes of fucose concentration in gastric mucus in rats after long-term injection of omeprazole (1) and simultaneous injection of omeprazole and multiprobiotic "Symbiter" (2). Changes that are statistically different from the control group (p<0.001 by Student t test) are indicated with stars

In 7, 14, 21 and 28 days of omeprazole injection the levels of N-acetylneuraminic acid were increased by 7.6% (p>0.05), 65.2% (p<0.001), 79.3% (p<0.001) and 111.0% (p<0.001) (Fig.3).

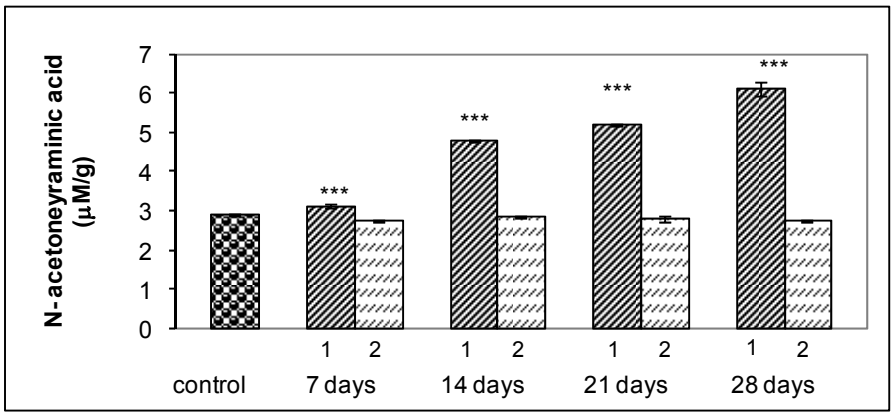

Fig.3. Changes of N-acetylneuraminic acid concentration in gastric mucus in rats after long-term injection of omeprazole (1) and simultaneous injection of omeprazole and multiprobiotic "Symbiter" (2). Changes that are statistically different from the control group (p<0.001 by Student t test) are indicated with stars

In 7, 14, 21 and 28 days of omeprazole injection the levels of hexuronic acids were increased by 17.2% ($p<0.001$), 33.9% ($p<0.001$), 78.5% ($p<0.001$) and 74% ($p<0.001$) compared with the control (Fig.4).

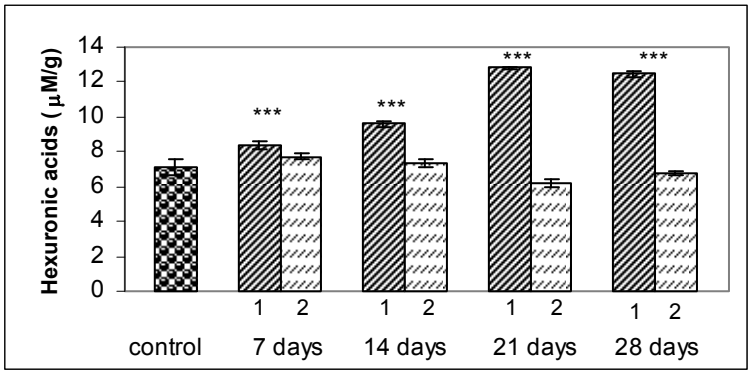

Fig.4. Changes of hexuronic acids concentration in gastric mucus in rats after long-term injection of omeprazole (1) and simultaneous injection of omeprazole and multiprobiotic "Symbiter" (2). Changes that are statistically different from the control group ($p<0.001$ by Student t test) are indicated with stars

The increase the levels in gastric mucus such glycoproteins as fucose, N-acetylneuraminic acid and hexuronic acids is evidence of degradation of non-collagenic proteins in gastric mucus.

Injection of multiprobiotic Symbiter against the background of hypoacidity evoked by omeprazole led to decrease the level of studied parameters to control values in all terms of investigations.

Long-term suppression of gastric acid secretion increases depolymerization of protective as collagenic and non-collagenic proteins in gastric mucus. As result the levels of oxiproline, fucose, N-acetylneuraminic acid and hexuronic acid in gastric mucus were substantially increased. Our results are in agreement with the studies of Ekambaram et al. [2] which shows that the levels of glycoproteins (hexose, hexosamine, sialic acid and fucose) were increased in rats with gastric cancer induced by N-methyl-N'-nitro-N-nitrosoguanidine. To take into account our previous date that injection of omeprazole to the rats during 28 days evoked in one group of rats hyperplasia and in other group metaplasia in gastric mucosa we supported the idea [3] that mucins are diagnostic markers in cancer. Multiprobiotic Symbiter prevented enhanced catabolism of protective proteins in gastric mucus. We hypothesize that regulation of glycoprotein levels by multiprobiotic could be associated with the regression of omeprazole-induced gastric hyperplasia and metaplasia.

Conclusions. Long-term inhibition of gastric acid secretion leads to degradation of protective collagenic and noncollagenic proteins in gastric mucus. Injection of multiprobiotic Symbiter against the background of hypoacidity evoked by omeprazole decreased the level of studied parameters to control values in all terms of investigations. These results shows the gastroprotective effect of multiprobiotic Symbiter and make probiotic perspective means of prophylaxis of negative consequences of hypoacidity.

References

1. Colb V.G. Clinic Biochemistry: Belarus, 1976. – p. 303.
2. Ekambaram G. Impact of naringenin on glycoprotein levels in N-methyl-N'-nitro-N-nitrosoguanidine-induced gastric carcinogenesis in rats / Rajendran P., Devaraja R., Muthuvel R., Sakthisekaran D. // Anti-cancer Drugs. – 2008. – Vol.19. – P. 885-890.
3. Hollingsworth M.A. Swanson BJ. Musins in cancer: protection and control of the cell surface// Nature Reviews Cancer. – 2004. – Vol.4. – P 45-60.
4. Karakeshysheva M.B. Morfo-funkcionalnye osobennosti slizistoi obolochki zheludka i biochimicheskiy sostav slizi pri predrakovych izmeneniyah i rake zheludka. Avtoref.dis...k-ta. biol. nauk, – K, 2008.
5. Martinsen T.C. Spontaneous ECL cell carcinomas in cotton rats: natural course and prevention by a gastrin receptor antagonist// Carcinogenesis. – 2003. – Vol.24 (12). – P. 1887.
6. Sharaev P.N. Method for determining of fucosa not attached to proteins. //Klin. lab. diagnostika. – 1997. – № 4. – P. 17-18.
7. Sharaev P.N. Method for determining of glykozaminoglycans in biological fluids.// Lab. delo. – 1987. – № 5. – P. 530-532.
8. Tetyanets S. S. Method for determining freely oxyproline in blood serum.// Lab. delo. – 1985. – № 1. – P. 61-62.
9. Waldum H.L., Brenna E., Sandvik A.K. Long-term safety of proton pump inhibitors: risks of gastric neoplasia and infections. // Expert Opin Drug Saf. – 2002. – Vol.1(1). – P.29-38.

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ЗМІНИ У ФУНКЦІОНУВАННІ СЛИЗОВОГО БАР'ЄРУ ШЛУНКА В УМОВАХ ГІПОАЦИДНОСТІ ТА ЇХ КОРЕКЦІЇ

Збільшення тривалості гіпоацидності шлункового соку, викликаної щоденним введенням блокатора секреції соляної кислоти в шлунку омепразолу, з 7 до 28 днів супроводжувалось суттєвим зростанням рівня оксипроліну, фукози, N-ацетилнейрамінової кислоти та гексуронових кислот у шлунковому слизу щурів. Це є свідченням інтенсифікації процесу деградації колагенових та неколагенових білків шлункового слизу. Введення мультипробіотика "Симбітер" на фоні гіпоацидності, викликаної омепразолом, призводило до зменшення рівня досліджуваних показників до контрольних в усі терміни експерименту.

Ключові слова:шлунковий слиз, омепразол, мультипробіотик.

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ИЗМЕНЕНИЯ В ФУНКЦИОНИРОВАНИИ СЛИЗИСТОГО БАРЬЕРА ЖЕЛУДКА В УСЛОВИЯХ ГИПОАЦИДНОСТИ И ИХ КОРЕКЦИЯ

Увеличение продолжительности гипоацидности желудочного сока, вызванного ежедневным введением блокатора секреции соляной кислоты в желудке омепразола, с 7 до 28 дней сопровождалось существенным ростом уровня оксипролина, фукозы, N- ацетилнейраминовой кислоты и гексуроновых кислот в желудочной слизи крыс. Это является свидетельством интенсификации процесса деградации коллагеновых и неколлагеновых белков желудочной слизи. Введение мультипробиотика "Симбитер" на фоне гипоацидности, вызванной омепразолом, приводило к уменьшению уровня исследуемых показателей до контрольных во все сроки эксперимента.

Ключевые слова: желудочная слизь, омепразол, мультипробіотик.

UDK 578.76

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THE PREVALENCE OF HIV-1 STRAINS RESISTANT TO ANTIRETROVIRAL DRUGS AMONG CHILDREN RECEIVING INEFFICIENT HIGHLY ACTIVE ANTIRETROVIRAL THERAPY

Here we present the results of analysis of prevalence of HIV strains with drug resistance mutations among HIV-infected children receiving inefficient HAART. Blood samples from 60 HIV-infected children aged <15 years were taken to perform the viral resistance genotyping. The prevalence of HIV-1 strains characterized with high resistance to any drug constituted 65.0%. In total, 51.67% of children required a correction of HAART scheme. The majority of isolated HIV strains (96.67%) belonged to subtype A of HIV-1.

Key words: HIV-1, HAART.

Introduction. The drugs currently used to treat HIV-infection belong to six distinct classes: nucleoside-analog reverse transcriptase inhibitors (NRTIs), nonnucleoside reverse-transcriptase inhibitors (NNRTIs), protease inhibitors (PIs), integrase inhibitors, fusion inhibitors and receptor antagonists [1]. Combinations of antiretroviral drugs are used now for the treatment of HIV infection – so-called highly active antiretroviral therapy (HAART). HAART regimens generally comprise three antiretroviral drugs, usually two nucleoside analogues and either a protease inhibitor or a nonnucleoside reverse-transcriptase inhibitor.

One of the major causes of treatment failure is the development of drug resistance to antiretroviral drugs (ARVs). Resistance testing is recommended for optimization antiretroviral therapy after treatment failure, for effective changing regimens of HAART. In this connection the research to identify resistant to ARVs HIV strains is necessary to achieve the efficiency of treatment. Given the fact that the vast majority of children in Ukraine and in the world at large infected by vertical HIV transmission from HIV-infected mothers, HIV-infected infants may acquire resistant HIV strains from the mother in utero or during the intrapartum period. Resistance may also emerge from exposure to antiretroviral drugs given to the infant for prophylaxis against HIV transmission. Resistant HIV strains may also emerge from exposure to antiretroviral drugs given to the infant for prophylaxis against HIV transmission [2]. On the other hand, receiving suboptimal doses of drugs during HAART in turn leads to the formation of resistant strains of HIV too.

Antiretroviral therapy is considered like virologic ineffective if the level of HIV viral load is not reduced to a level less than 1000 RNA-copies/ml blood after 24 weeks of HAART.

The aim of this work was to establish the prevalence of resistant strains of HIV in HIV-infected children with virologic failure of HAART.

Materials and methods. Samples of plasma HIV- infected children under 15 years of age receiving HAART were used for investigation. Whole blood samples were obtained by venipuncture into EDTA-containing tubes. After centrifugation, plasma was separated and stored at –70 °C for RNA viral extraction. Samples of plasma were pretested by PCR to determine the level of HIV-1 RNA using the Abbott RealTime HIV-1 (Abbott RT HIV-1) to assess the effectiveness of HAART. Samples in which the viral load of HIV-1 RNA exceeded 2000 copies VIL-1/ml plasma were selected for further sequencing. HIV genome sequencing was performed on the genetic analyzer ABI PRISM 3100 (Applied Biosystem) using the test system ViroSeqTM HIV-1 Genotyping System (Abbott, USA), which detects mutations in the reverse transcriptase (RT) and protease regions of the pol gene and provides the physician with a report indicating genetic evidence of viral resistance. The entire protease gene and two-thirds of the RT gene are amplified to generate a 1.8 kb amplicon. The amplicon is used as a sequencing template for seven primers that generate an approximately 1.3 kb consensus sequence. The Viroseq HIV-1 Genotyping System software assembles, edits, and identifies mutations within this 1.3kb sequence. The software compares the consensus sequence with a known reference, HXB-2, to determine mutations present in the sample. Analysis of primary nucleotide sequences was performed using BioEdit (v.7.0.0). Evaluation of nucleotide substitutions were performed using the database at Stanford University, USA (hivdb.stanford.edu). All HIV-1 pol sequences for genotyping were analyzed using the REGA HIV-1 Subtyping Tool, version 2.0.

Results and discussion. The presence of mutations of HIV-1 resistance to antiretroviral drugs was determined in HIV-1 RNAs isolated from blood samples of 60 HIV-infected children under 15 years of age. All children received HAART for at least one year. All children included in the study have virological failure of HAART: indexes of viral load of HIV-1 in samples of their blood were higher than 2000 RNA-copies/ml after 6 months after initiation of HAART or after the last modification scheme of HAART and ranged from 2681 to 10 million RNA-copies/ml plasma. Among the children included in the study 30 (50.0 %) received the first scheme of therapy, in 30 (50%) children the scheme of therapy was changed: in 18 children – twice, in 7 – three times, and in 5 – four times.

HIV RNA was isolated from all 60 specimens of blood. Determination of subtypes of HIV-1 was based on the analysis of polymerase gene sequences (a protease and RT regions). According to an analysis of the database of Stanford University, two of the selected strains of viruses can be classified as subtype B HIV -1 (3.33 %), 29 – subtype A HIV -1 (48.33 %), 28 samples were CRF01-AE (46.67%), one – CRF02 – AG (1.67%). But all CRFs were classified as subtype A in additional analysis with REGA HIV-1 Automated Subtyping Tool (Version 2.0). Thus, most of the sequences belonged to subtype A (96.67%), and only two sequences belonged to subtype B (3.33%) (fig. 1).

The resistant strains of HIV-1 were detected in 40 (66.67 %) of 60 samples of plasma HIV-infected children, in 39 (65.0%) samples HIV strains with high resistance to at least one antiretroviral drug were found. In total group 31 (31/60, 51.67%) children needed for the correction of the scheme of HAART. Ten from 39 children (25.64 %) had levels of HIV viral load higher than 100 000 RNA copies/ml plasma. It is known that mutations of HIV resistance to antiretroviral drugs contribute to reducing its replicative capacity compared to the "wild" sensitive virus [3]. At the same time, the HIV genome can form spontaneously polymorphic compensatory mutations that contribute to the restoration of replicative capacity of the virus. Therefore, high HIV viral load (more than 100 000 RNA-copies/ml plasma) in the presence of resistance mutations may be explained by manifestation of polymorphism mutation.

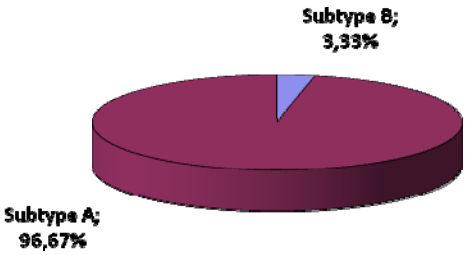

Fig.1. Distribution of HIV-1 subtypes among HIV-infected children

The prevalence of HIV drug resistance mutations was analyzed according to the drug class family. The most commonly detected mutations were mutations of resistance to NRTIs – in 33 from 60 patients were identified strains of HIV with at least one mutation from this group (Table 1).

***Table* 1.The prevalence of HIV strains with resistance mutations among children with virological failure of HAART**

Indicator	Quantity of samples/total group	Rate,%
High-level resistance to at least one antiretroviral drug	39/60	65.0
Resistance to the drug classes::		
NRTIs	33/60	55.0
NNRTIs	29/60	48.33
PIs	8/60	13.33
Resistance to two classes of drugs	24/60	40.0
Resistance to two classes of drugs	4/60	6.67

There are two main mechanisms of resistance to NRTIs. Both of these mechanisms are realized through the formation of mutations in the region of the gene pol, which encodes reverse transcriptase (RT) of HIV. The first – increasing the RT's phosphorolytic activity, which in the presence of a pyrophosphate donor (usually ATP) allows to remove chain-terminating inhibitors from the 3′ end of the primer. This mechanism is associated with mutations M41L, D67N, K70R, L210W, T215Y, T215F, K219Q, K219E – family of mutations known as thymidine analogue mutations (TAMs) because they are selected by thymidine analogs zidovudine (AZT) and stavudine (d4T) [4]. The development of resistance to thymidine analogues is a result of the gradual accumulation of specific mutations in 41, 67, 70, 210, 215 and 219 positions of HIV reverse transcriptase. Viruses acquire phenotypic resistance to thymidine analogues resulting combined mutations at positions 41 and 215, or storage of at least four of the six mutations. Another mechanism – the formation of conformational changes in the molecule reverse transcriptase of HIV that result in loss of the ability of the enzyme to bind to NRTIs, which makes it impossible to include them in the chain of provirus DNA of HIV- 1[5]. Among mutations that result in the inability to include NRTIs in chain of provirus DNA in HIV-1 there are M148V, L74V, M184I, K65R. Among them the most common is the M184V mutation. The RT mutation M184V confers high-level phenotypic resistance to the cytidine analogs lamivudine (3TC) and emtricitabine (FTC) and low-level cross-resistance to abacavir (ABC) and didanosine(DDI). Despite the high level of phenotypic 3TC and FTC resistance caused by M184V, there is often some benefit in including 3TC or FTC in a salvage therapy regimen because M184V increases susceptibility to AZT, d4T, and tenofovir (TDF) and causes a decrease in HIV-1 replication capacity [6].

In the spectrum of detected mutations of HIV resistance to NRTIs in the investigated samples at the mutation M184V (M184MV) was dominant by the frequency of detection – it was found in 32 samples from 60 (53.33 %). Other mutations of HIV resistance to NRTIs, were found with less frequently, but their range was wide. TAMs were found in 20 samples (20/60, 33.33%): in 4 samples M41L mutation were found, in 11- strains with substitution at position D67 (D67N, D67DN), in 7 – at position K70 (K70R, K70KR), in 7 – at position T215 (T215Y, T215 F),in 8 – at position K219 (K219E, K219Q, K219EQO). In samples of blood of 3 children HIV strains with four TAMs were found, in one sample – HIV strain with five TAMs. Also other mutations were found: in 6 samples – mutations at position L74 (L74V, L74LV), in two samples – L210W (L210 LW), V75M – in one.

The second group by the frequency of detection was the group of mutations that provide the resistance to the non-nucleoside reverse transcriptase inhibitors of HIV-1. These mutations were found in HIV strains isolated from 29 samples of blood (29/60, 48.33%). Drugs of this class trigger conformational changes in the HIV reverse transcriptase molecule with the formation of the so-called hydrophobic "pocket" – this region is NNRTI-binding site. The binding of NNRTIs, in turn, causes changes in the conformation of the active center of the enzyme and leads to loss its inability to synthesis of provirus DNA chain of HIV-1. Mutations that cause changes in the amino acid sequence in hydrophobic "pocket", lead to the formation of the stability of the virus to several or all drugs from this class [7]. These mutations include nucleotide substitutions in positions 100-110, 180-190 and 225-235. We found some mutation of that class, most frequently were following: K101E – in 8 samples, G190S – in 16 samples, K103N – in 10 samples, P225H (met in combination with K103N) – in 4 samples, Y181C – in 2 samples. Mutations G190S and K103N are causing high level of HIV resistance to nevirapine (NVP) and efavirenz (EFV). K103 reduces NVP and EFV susceptibility by about 50 and 20-fold, respectively [8]. G190S is mutation that accumulates during prolonged ineffective therapy with most NNRTIs. The nucleotide substitution K101E causes the average level of resistance of HIV to these drugs. P225H is a nonpolymorphic accessory mutation which in combination with K103N causes >50-fold reduced susceptibility to NVP and EFV [9]. Y181C causes >25-fold reduced susceptibility to NVP [10].

The mechanism of action of protease inhibitors is to block the activity of the enzyme, causing it to lose the ability to cleavage of precursor viral proteins (gag and gag–pol) permitting the final assembly of the inner core of viral particles [11]. Resistance to this class of drugs is caused by a complex of mutations that are divided into major (that cause reduced sensitivity of the virus to specific drugs of this class) and minor (that do not affect the stability of the virus to the PIs, but in the presence of major mutations can

enhance the level of HIV resistance to these drugs). Mutations of resistance to PIs were detected in 11 samples, but mutations associated with high levels of resistance to PIs were detected in 8 of them (8/60, 13.33%). Among the major mutations of resistance to PIs nucleotide substitutions at position 46 (M46L, M46LI) were detected – in 7 of the investigated sequences, at position 82 (V82A, V82F) – in 5 samples. M46I/L contribute reduced susceptibility to few PIs (indinavir (IDV), nelfinavir (NFV), fosamprenavir (FPV), atazanavir (ATV), and lopinavir (LPV)) [10]. V82A decreases susceptibility to IDV and LPV and confers cross-resistance to ATV and NFV [12]. The mutation I54V and L76V were detected in 3 and 2 samples, respectively. Among the minor mutations substitutions at position L10I dominated, which were found in 13 of the samples.

Conclusions. Thus, HIV strains with mutations of high level resistance to ARVs were found in the majority (in 39 from 60) of blood samples obtained from children with virological inefficiency HAART. The frequency of detection of resistance mutations to NRTIs was 55.0 %, to NNRTIs – 48.33%, to PIs – 13.33 %. In total group 21 children (35.0 %) had treatment failure, probably related to their low adherence to therapy, abnormalities in the mode of taking the drugs. These results indicate the high relevance of problem of the formation and spread of resistant strains of HIV among HIV- positive children in Ukraine and the necessity for further study of that problem.

References

1. E.J. Arts, D.J. Hazuda 2012.HIV-1 Antiretroviral Drug Therapy. Cold Spring Harb Perspect Med. April; 2(4).

2. Weidle P.J., Nesheim S. 2010. HIV drug resistance and mother-to-child transmission of HIV. Clin Perinatol. Dec;37(4):825-42.

3. Martinez-Picado J., Savara A. V., Sutton L. et al. 1999. Replicative Fitness of Protease Inhibitor-Resistant Mutants of Human Immunodeficiency Virus Type 1. J Virol.,73(5): 3744–3752.

4. F. Clavel, A. J. Hance. 2004. HIV Drug Resistance.N Engl J Med; 350:1023-35.

5. L. Menéndez-Arias. Retroviral Reverse Transcription Mechanisms of resistance to nucleoside analogue inhibitors of HIV-1 reverse transcriptase.2008. Virus Research, 134 (1–2):124–146.

6. Shafer RW, Schapiro JM. 2008.HIV-1 drug resistance mutations: an updated framework for the second decade of HAART. AIDS Rev.;10:67-84.

7. Shiro Ibe, Wataru Sugiura. 2011. Clinical Significance of HIV Reverse-transcriptase Inhibitor-resistance Mutations. Disclosures Future Microbiol. ;6 (3):295-315.

8. Zhang, Z., Xu, W., Koh, Y.H. et al. 2007. A novel nonnucleoside analogue that inhibits human immunodeficiency virus type 1 isolates resistant to current nonnucleoside reverse transcriptase inhibitors. Antimicrobial agents and chemotherapy, 51;429-437.

9. Alcaro, S., Alteri, C., Artese, A. et al. 2011. Docking analysis and resistance evaluation of clinically relevant mutations associated with the HIV-1 non-nucleoside reverse transcriptase inhibitors nevirapine, efavirenz and etravirine.ChemMedChem, 6;2203-2213.

10. Vingerhoets J., Tambuyzer L., Azijn H. 2010.Resistance profile of etravirine: combined analysis of baseline genotypic and phenotypic data from the randomized, controlled Phase III clinical studies.AIDS 24;503-514.

11. Barber T.J., Harrison L., Asboe D. 2012. Frequency and patterns of protease gene resistance mutations in HIV-infected patients treated with lopinavir/ritonavir as their first protease inhibitor. The Journal of antimicrobial chemotherapy, 67;995-1000.

12. Rhee S.Y., Taylor J., Fessel W.J. 2010. HIV-1 protease mutations and protease inhibitor cross-resistance. Antimicrobial agents and chemotherapy, 54; 4253-4261.

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ПОШИРЕННІСТЬ ШТАМІВ ВІЛ-1, СТІЙКИХ ДО АНТИРЕТРОВІРУСНИХ ПРЕПАРАТІВ, СЕРЕД ДІТЕЙ, ЯКІ ОТРИМУЮТЬ НЕЕФЕКТИВНУ ВИСОКОАКТИВНУ АНТИРЕТРОВІРУСНУ ТЕРАПІЮ

Представлені результати аналізу поширеності резистентних до АРВ – препаратів штамів ВІЛ- 1 серед дітей з неефективною ВААРТ. Для проведення досліджень з виявлення резистентних до АРВ – препаратів штамів ВІЛ були відібрані зразки крові 60 ВІЛ-інфікованих дітей у віці до 15 років. Частота виявлення штамів ВІЛ з мутаціями, що забезпечують стійкість високого рівня хоча б до одного препарату, включеному в схему лікування, склала 65,0 %, 51,67 % дітей потребували корекції схеми терапії. Більшість проаналізованих послідовностей РНК ВІЛ (96,67%) належали до субтипу А ВІЛ-1.

Ключові слова: АРВ прапарати штамів ВІЛ-1, ВААРТ.

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РАСПРЕДЕЛЕНИЕ ШТАММОВ ВИЧ-1, УСТОЙЧИВЫХ К АНТИРЕТРОВИРУСНЫМ ПРЕПАРАТАМ, СРЕДИ ДЕТЕЙ, КОТОРЫЕ ПОЛУЧАЮТ НЕЭФФЕКТИВНУЮ ВЫСОКОАКТИВНУЮ АНТИРЕТРОВИРУСНУЮ ТЕРАПИЮ

Представлены результаты анализа распространенности резистентных к АРВ-препаратам штаммов ВИЧ-1 среди детей с неэффективной ВААРТ. Для проведения исследований по выявлению резистентных к АРВ-препаратам штаммов ВИЧ были отобраны образцы крови 60 ВИЧ-инфицированных детей в возрасте до 15 лет. Частота выявления штаммов ВИЧ с мутациями, обеспечивающими устойчивость высокого уровня хотя бы к одному препарату, включенному в схему лечения, составила 65,0%, 51,67% детей нуждались в коррекции схемы терапии. Большинство проанализированных последовательностей РНК ВИЧ (96,67%) принадлежали к субтипу А ВИЧ-1.

Ключевіе слова: АРВ препараты штаммов ВИЧ-1, ВААРТ.

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ADVANCED APPROACHES TO IDENTIFICATION OF VIRUSES INFECTING OF WILD HERBACEOUS PLANTS

Recently, interest in studying viruses in wild flora was gradually increasing. This is connected with necessity of better understanding plant virus evolution, ecology, virulence, and even to avoid economic losses due to crop-wild hybridization, followed by introgression of pathogen-resistant transgenes to wild populations. In this review brief information about last contributions in development of wild plant virology is given. Different approaches to the researches are present here.

Key words: viruses in wild flora, transgenes to wild populations.

Introduction. Viruses commonly infect wild plants. However, virus infection is easily overlooked in wild plant populations. Although infections can be visually unapparent, it is frequently assumed that absence of visual symptoms (such as leaf mottling or malformation) indicates lack of virus infection. Moreover, symptoms of virus infection are sometimes difficult to distinguish from environmental stresses. For these reasons, in part, virus ecology in natu-

ral plant populations is poorly studied [1]. Little is known about the prevalence or effects of virus infection in wild plant populations, much of our understanding of plant-virus interactions comes from economically important plants (e.g., crops, horticultural varieties, and pasture plants). In crops, virus infection can reduce plant growth by depressing photosynthesis, changing metabolism, and altering resource allocation. Virus infections can drastically reduce crop yield, resulting in economic losses. Moreover, many virus vectors are difficult to control, and for this reason, genetic resistance to virus infection is often the most practical means of controlling crop losses [2]. The use of transgenic crops with virus resistance offers promise for control of problematic viruses. The commercial release of virus-resistant transgenic crops has motivated research on plant-virus ecology in natural populations [1]. Knowledge from studies of viruses of cultivated plants may not be adequate for explaining the ecology and evolution of plant viruses in nature. Anthropogenic activities such as domestication of crops, travel and exploitation of natural habitats seem to influence plant virus spread and evolution in cultivated conditions.

Little is known about plant virus diversity, host specificity and evolution under natural conditions where human influence is limited. In wild plants, virus infection can affect plant growth, mortality, and seed production, but these effects vary among populations, species, and environments. Although these data indicate that viruses can affect community dynamics and have fitness impact on many wild plants, remarkably little is known about virus prevalence in wild populations [3]. Assuming recent information, which is connected with investigation of viruses in wild plants, several main reasons for further research on the given theme can be established. Regarding evolution, the phylogenetic relationships between the viruses in wild and domestic plants should be clearly assessed. In respect of virulence, several studies of the plant viruses in natural systems indicate no harmful effects or a mild influence on their host plant's fitness [4]. Moreover, new species of plant viruses can probably be found (e.g. Asclepias asymptomatic virus [5]). High rates of using transgenic crops may cause crop-wild hybridization, followed by introgression of pathogen-resistant transgenes to wild plant, which is an ecological risk of increasing wild population in size or becoming weedier [6]. Viruses are known to be presented in wild fungi and algae, woody perennials, and herbaceous plants. This review is focused on the recent investigations of the viruses found in wild herbaceous species.

Discovery of novel viruses from wild plants. During 21st century, the level of concern with viruses, which must exist in plants under wild conditions, was rising. Several researches directed to find new virus species in wild plants in Alaska have been accomplished. A new virus named Nootka lupine vein-clearing virus (NLVCV) was isolated from *Lupinus nootkatensis* plants that were confined to a relatively small area in the Talkeetna mountains of south-central Alaska. There were apparent leaf symptoms of pronounced vein clearing and mosaic on 3- to 4-week-old plants. The nucleotide sequence of RNA-dependent RNA polymerase (RDRP) did not match any known virus, but contained short regions of identity to several carmoviruses. Only species belonging to the *Fabaceae* were susceptible to NLVCV by mechanical inoculation. Based on precise studies of this virus and similarity of the RDRP gene to that of other carmoviruses, it is suggested that NLVCV is a member of the family *Tombusviridae*, and tentatively of the genus *Carmovirus* [12].

A novel potyvirus was discovered in wild celery and kneeling Angelica (family *Apiaceae*) in the Matanuska-Susitna Valley, Alaska, named after its natural plant hosts, angelica virus Y (AnVY) (Table 1) [13].

Recent studies describe a novel mastrevirus found in grass *Digitaria didactyla* in Africa. Analysis of the sequence shows the virus to be a typical mastrevirus, with four open reading frames, two in each orientation, separated by two noncoding intergenic regions. Although it showed the highest levels of sequence identity to CSMV (68.7%), their sequences are sufficiently diverse for the virus to be considered a member of a new species in the genus *Mastrevirus*, based on the present species demarcation criteria. It was proposed that the name first used during the 1980s be used for this species, Digitaria didactyla striate mosaic virus (DDSMV) [14].

Yellow oat-grass (*Trisetum flavescens* L. Beauv) is a perennial grass native to Europe, West Asia and North Africa. Yellow oat-grass plants with mild mosaic and pronounced dwarfing symptoms were observed at different locations in the Czech Republic. Serological assays of infected plant extracts using antiserum specific to the closest species in the family *Potyviridae* were negative. Based on phylogenetic analyses of the coat protein cistron and flanking genomic regions, it was proposed to be a distinct viral species of the genus *Tritimovirus*, tentatively named Yellow oat-grass mosaic virus (YOgMV) [15].

Research in Australia revealed that a range of viruses, both indigenous and exotic, infect native orchids. A novel potyvirus was identified from a wild plant of *Diuris laxiflora* that did not exhibit symptoms. The name Donkey orchid virus A (DOVA), isolate SW3.1 was applied. Its predicted genome organization was typical of those of other potyviruses [16].

***Table* 1. Examples of viruses identified in wild herbaceous plants, presented in chronological order**

Name of virus	Family and/or Genus of virus	Name of wild host plant	Family of wild host plant	Symptoms	Author(s) and year of indication
Glycine mosaic virus (GMV)	*Comovirus*	*Glycine clandestina* and *G. tabacina*		mosaic symptoms and marginal deformation in leaves	Bowyer et al. 1980
Turnip yellow mosaic virus	*Tymoviridae Tymovirus*	*Cardamine lilacina*	*Brassicaceae*	mild	Guy and Gibbs 1985
Tobacco mild green mosaic virus (TMGMV)	*Virgaviridae, Tobamovirus*	*Nicotiana glauca*	*Solanaceae*	mild or unapparent	Rodríguez-Cerezo et al. 1991
Kennedya yellow mosaic virus	*Tymoviridae Tymovirus*	*Desmodium scorpiurus; Kennedya rubicunda*	*Fabaceae*	unapparent	Skotnicki et al. 1996
Beet western yellow virus (BWYV)	*Luteoviridae*	*Brassica oleracea*	*Brassicaceae*	yellowing of tissue	Raybould et al. 1999
Barley yellow dwarf virus (BYDV)	*Luteoviridae*	1) *Bromus hordeaceus* 2) *Setaria viridis* 1) *Setaria lutescens*	*Poaceae*	1) unupparent 2) reduction in panicle length 3) increase in panicle length	Remold 2002
Hardenbergia mosaic virus	*Potyviridae, Potivirus*	*Hardenbergia comptoniana*	*Fabaceae*	unapparent	Webster et al. 2007

Closing table 1

Name of virus	Family and/or Genus of virus	Name of wild host plant	Family of wild host plant	Symptoms	Author(s) and year of indication
Passion fruit woodiness virus	*Potyviridae, Potivirus*	*Passiflora aurantia*	*Passifloraceae*	unapparent	Webster et al. 2007
Angelica virus Y (AnVY)	*Potyviridae, Potivirus*	*Angelica lucida* L. and *A. genuflexa* Nutt.	*Apiaceae*	leaf mottling	Robertson 2007
Polygonum ringspot virus (PolRSV)	*Buniaviridae, Tospovirus*	*Polygonum convolvulus*	*Polygonaceae*	chlorotic or necrotic ringspots, mosaic and deformation	Ciuffo et al. 2008
Eragrostis curvula streak virus (ECSV)	*Geminivirigae*	*Eragrostis curvula*	*Poaceae*	mild streak	Varsani et al. 2009
Yellow oat-grass mosaic virus (YOgMV)	*Potyviridae Tritimovirus*	*Trisetum flavescens*	*Poaceae*	mild mosaic and dwarfing	Hassan 2009
Sweet potato feathery mottle virus (SPFMV)	*Potyviridae, Potivirus*	*Ipomoea* sp., *Hewittia* sp., and *Lepistemon* sp.	*Convolvulaceae*	unapparent	Arthur et al. 2010
Narcissus late season yellows virus (NLSYV)	*Potyviridae, Potivirus*	*Narcissus* sp.	*Amaryllidaceae*	leaf streaking and yellowing, leaf distortion and plant stunting	Wylie et al. 2010
Digitaria didactyla striate mosaic virus (DDSMV).	*Geminivirigae, Mastrevirus*	*Digitaria didactyla*	*Poaceae*	streak	Briddon et al. 2010
Asclepias asymptomatic virus (AsAV)	*Tymoviridae Tymovirus*	Asclepias viridis	*Apocynaceae*	unapparent	Melcher 2013
1)Bean yellow mosaic virus; Ornithogalum mosaic virus; Blue squill virus A 2) Turnip yellows virus	*1) Potyviridae, Potivirus 2)Luteoviridae, Polerovirus*	*Diuris* sp.	*Orchidaceae*	unapparent	Wylie et al. 2013

Transgenic crops and viruses. Because not so much is known about the prevalence or effects of virus infection in wild plant populations, most of our understanding of plant-virus interactions comes from economically important plants. In crops, virus infection can reduce plant growth by depressing photosynthesis, changing metabolism, and altering resource allocation. Controlling the virus vectors is mostly difficult; hence using transgenic crops is one of the most novel and popular methods to avoid losses of yield. For example, in the United States, 27 crop species with virus-resistance transgenes have been issued permits for field trials, and a handful of crops have been deregulated for commercial production (i.e., squash, papaya, and potato; Information Systems for Biotechnology, 2012). Dealing with transgenic plants we must be aware of crop–wild hybridization followed by the introgression of transgenes into wild relatives, which located in proximity to crops. This process may have certain negative consequence: the size or dynamics of the wild plant population, which is limited by pathogen attack, may increase, or wild population may become weedier due to ecological advantages obtained from virus-resistant transgenes. A research has been conducted to contribute in our awareness of such very problem. It surveyed wild *Cucurbita pepo* populations in the south-central United States over 4 years for five virus species. These populations were examined for the presence of virus-resistance transgenes. Although results of the survey constitute that the virus-resistance transgene was not present in any of the 1256 leaf samples of wild *C. pepo* collected from 21 sites over 4 years in south-central United States [2], we cannot still certainly exclude the possibility of the transgene introgression, for instance, into other wild plant in the other countries. In addition, prevalence of viruses in wild *C. pepo* was examined. Cucumber mosaic virus (CMV), Squash mosaic virus (SqMV), Watermelon mosaic virus (WMV), and Zucchini yellow mosaic virus (ZYMV) were detected. It was also confirmed that RT-PCR is more sensitive than ELISA, and results suggest that neither method is 100% accurate. At least one of these viruses surveyed was present in 17 of 21 wild *C. pepo* populations and prevalence ranged from 4–74%, and the average prevalence for all viruses was 23%. Another interesting data is that, in field survey, 80% of infections were visually unapparent [2].

Ecogenomics: advantages and disadvantages. Several recent metagenomic studies have analyzed prokaryotic viruses in a variety of unexpected environments [7]. Metagenomics has been very valuable in directing the rethinking of the global 'virome', i.e. there are orders of magnitude more viruses in nature than previously anticipated, but it has not been able to link any viruses found in environmental samples to their hosts. Ecogenomics can fill this gap in our understanding. In addition, almost all metagenomics studies of viruses have characterized bacterial viruses, while the methods described here give us a way to analyze eukaryotic hosts and their viruses. However, the sample processing for this type of study is much more labour intensive than what is used in metagenomics, and hence ecogenomics can simply give a different perspective on the global virome [8] and, particularly, on investigation of viruses of wild plants. Clear example of this kind of method should be noticed. That was remarkable survey of viruses in wild flora of the Tall Grass Prairie Preserve in northeastern Oklahoma, and the Area de Conservacio´n Guanacaste in northwestern Costa Rica. These areas have low and high level of biodiversity respectively. dsRNA were used, as a form of nucleic acids that is generally unique to viruses, to assess RNA virus infection in plants, by converting it to cDNA through a process specific for dsRNA. The resulting cDNA then was amplified with tagged primers that could cross reference each sample to the sequences obtained by pyrosequencing in pools of 24 to 96 uniquely tagged samples [8]. As a result, there were discovering thousands of plant viruses that are generally unique, and only distantly related to known viruses. The term 'Ecogenomics' to distinguish this study from the metagenomic studies from environmental samples since given sequences are directly linked to the original plant hosts. Another essential survey on the same territory (Tall Grass Prairie Preserve) was accomplished. One considerable result was discovering novel *Tymovirus* – Asclepias as-

ymptomatic virus (Table 1) in *Asclepias viridis* [5]. Plant samples were screened for virus-like sequences in double-stranded RNA and in nucleic acids associated with particulate fractions of plant homogenates. Furthermore, among the plant specimens analyzed for amplifiable VLP-VNA, only 2.3% were noted at collection as having any symptoms of disease. In each year of harvest the proportion of samples that were PCR positive was the same among plants with and without symptoms. Thus, presence or absence of amplification was not an indicator of disease, manifested as symptoms [9]. Therefore this massive sequencing cannot allow evaluating of virulence, and gives information to understand evolutionary and ecological relationships among plant viruses in wild flora. Similar approach to identification plant viruses in wild plants is described by another research. A remarkable recent advance in plant virus discovery has been the utilization of massively parallel pyrosequencing (next-generation sequencing, 'deep' sequencing), which is capable of yielding megabases to gigabases of sequence information, coupled with bioinformatics [10]. It was described the use of a massively parallel sequencing approach whereby polyadenylated plant RNA from multiple plants was pooled and sequenced together before the output was analyzed for the presence of viral genomes. This research represents part of a project to describe the ecological roles viruses play in the indigenous flora of the south-west Australian floristic region. After analysis, complete or partial genome sequences representing 20 virus isolates of 16 polyadenylated RNA species were identified. In three cases, 2-3 distinct isolates of a virus species co-infected the same plant. Twelve of the viruses identified were described previously and belonged to the genera *Potyvirus*, *Nepovirus*, *Allexivirus*, and *Carlavirus*. Four were unknown and are proposed as members of the genera *Potyvirus*, *Sadwavirus*, and *Trichovirus* [11].

Nowadays, studying viruses persisting in non-cultivated plants become more popular. The knowledge allows us to understand general plant virus ecology better, because, previously, only viruses of economically essential plants were discovered, and an ability to make whole picture of ecological processes of plant viruses was restricted by lack of information about viruses in wild plants. Obviously, wild plants are natural reservoir of plant viruses. Thus, the plenty of surveys in order to indicate viruses of crops in wild populations have been done. As a result, it became clear that visual symptoms in wild plants are mostly unapparent. It could be suggested that milder symptoms in wild plants are connected with long-term co-evolution with certain virus, which, obviously, is not present in the population of cultivated plants. Moreover, studying viruses in wild populations seems to be useful for biotechnology: to assess a risk of virus-resistant transgene introgression into wild plant. Development of new methods of sequencing, coupled with bioinformatics, caused metagenomics, while metagenomics was followed by ecogenomics – beneficial to massive simultaneous discovery of viruses in wild flora, linked to their hosts.

References

1. Cooper J. I., Jones R. A. C. Wild plants and viruses: Under-investigated ecosystems // Advances in Virus Research. – 2006. – Vol. 67. – P. 1 – 47.

2. Prendeville H. R, Ye X., Morris T. J., Pilson D. Virus infections in wild plant populations are both frequent and often unapparent // American Journal of Botany. – 2012. – Vol. 99. – №6. – P. 1033–1042.

3. Thapa V. Investigations on the ecology and evolution of wild plant viruses // PhD. dissertation Ecology of Plant Viruses. – Oklahoma State University. – December 2010 – 86 p.

4. Fargette, D., Konate, G., Fauquet, C., Muller E., Peterscmitt, M. and Thresh J. M. Molecular ecology and emergence of tropical plant viruses// Annual Review of Phytopathology. – 2006. – Vol. 44. – P. 235 – 260.

5. Melcher U. Ecological and evolutionary implications of sequence variation of Asclepias asymptomatic virus in non-cultivated plant // 12th International Symposium on Plant Virus Epidemiology. – Arusha, Tanzania, 2013. – P. – 69.

6. Tepfer M. Risk assessment of virus-resistant transgenic plants // Annual Review of Phytopathology. – 2002. – Vol. 40. – P. 467 – 491.

7. Robertson N. L. Biology of a new virus isolated from Lupinus nootkatensis plants in Alaska // Plant Pathology. – 2004. – Vol. – 53. – P. 569–576.

8. Robertson N. L. Identification and characterization of a new virus in the genus Potyvirus from wild populations of Angelica lucida L. and A. genuflexa Nutt., family Apiaceae // Arch Virol. – 2007. – Vol. – 152. – P. 1603–1611.

9. Briddon R. W., Martin D. P., Owor B. E., Donaldson L., Markham P. G., Greber R. S., Varsani A. A novel species of mastrevirus (family Geminiviridae) isolated from Digitaria didactyla grass from Australia // Arch Virol. – 2010. – Vol. – 155. – P. 1529–1534.

10. Hassan M., Sirlova L., Jokes M., Vacke J. Identification and characterization of a novel Tritimovirus species isolated from wild Trisetum flavescens L., family Poaceae // Virus Genes. – 2009. – Vol. – 39. – P. 146 – 152.

11. Wyliea S. J., Lia H., Dixonb K. W., Richardsc H., Jonesa M. G. K. Exotic and indigenous viruses infect wild populations and captive collections of temperate terrestrial orchids (Diuris species) in Australia // Virus Research. – 2013. – Vol. 171. – P. 22 – 32.

12. Azam F., Worden AZ. Microbes, molecules, and marine ecosystems // Science. – 2004. – Vol. – 303. – P. 1622–1624.

13. Roossinck M. J., Saha P., Wiley G.B., Quan J., White J.D., Lai H., Chavarria F., Shen G., Roe B.A. Ecogenomics: using massively parallel pyrosequencing to understand virus ecology // Molecular Ecology. – 2010. – Vol. – 19. – P. 81–88.

14. Muthukumar V., Melcher U., Pierce M., Wiley G. B., Roe B. A., Palmer M. W., Thapa V., Ali A., Ding T. Non cultivated plants of the Tallgrass Prairie Preserve of northeastern Oklahoma frequently contain virus-like sequences in particulate fractions // Virus Research. – 2009. – Vol. 141. – P. 169–173.

15. Adams I.P., Glover R.H., Monger W.A., Mumford R., Jackeviciene E., Navalinskiene M., Samuitiene M., Boonham N. Next-generation sequencing and metagenomic analysis: a universal diagnostic tool in plant virology// Mol Plant Pathol. – 2009. – Vol. – 10. – P. 537 – 545.

16. Wylie S.J., Luo H., Lia H., Jones M. G. K. Multiple polyadenylated RNA viruses detected in pooled cultivated and wild plant samples // Arch Virol – 2012. – Vol. – 157. – P. 271–284.

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НОВІ ПІДХОДИ ДО ІДЕНТИФІКАЦІЇ ВІРУСІВ, ЯКІ УРАЖАЮТЬ ДИКОРОСЛІ ТРАВ'ЯНИСТІ РОСЛИНИ

Останнім часом зацікавленість у вивченні вірусів дикорослої флори поступово зростає. Це пов'язано з необхідністю кращого розуміння еволюції, екології, вірулентності вірусів рослин, і також задля уникнення економічних втрат через взаємну гібридизацію трансгенних технічних культур та диких рослин та наступної інтрогресії вірус-стійких трансгенів в дикі популяції. В даному огляді надана коротка інформація про останні внески в розвиток вивчення вірусів диких рослин. Представлені різні підходи до досліджень.

Ключові слова: віруси дикорослої флори, транс гени в дикій популяції.

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НОВЫЕ ПОДХОДЫ К ИДЕНТИФИКАЦИИ ВИРУСОВ, КОТОРЫЕ ПОРАЖАЮТ ДИКОРАСТУЩИЕ ТРАВЯНИСТЫЕ РАСТЕНИЯ

За последнее время интерес к изучению вирусов дикорастущей флоры постепенно возрастает. Это связано с необходимостью лучшего понимания эволюции, экологии, вирулентности вирусов растений, и также во избежание экономических потерь из-за взаимной гибридизации трансгенных технических культур с дикими растениями, и, впоследствии, интрогрессии вирус-устойчивых трансгенов в дикие популяции. В данном обзоре дана короткая информация о последних вкладах в изучение вирусов диких растений. Здесь представлены разные подходы к исследованиям.

Ключевые слова: вирусы дикорастущей флоры, трансгены в дикой популяции.

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IDENTIFICATION OF MAREK'S DISEASE IN POULTRY FARMS OF UKRAINE AND ESTABLISHMENT THE FORMS OF DISEASE

Marek's disease virus is one of a pathogen who remains to cause a significant losses in poultry veterinary medicine in all over the world. In this paper, Marek's disease was identified in 234 of 304 observed samples by histological diagnosis and established the dominant manifestation of the disease. The main form that was detected in positive samples was classic manifestation of MD. The identification of Marek's disease and different forms in poultry flocks pointed on circulation of herpes virus and the existence of virulent and highly virulent variants of the virus in poultry farms of Ukraine.

Key words: Marek's disease virus, histological diagnosis.

Introduction. Marek's disease (MD) is the most widespread lymph proliferative disease of chickens and turkeys. The disease is characterized by the development of malignant lymphomas in visceral organs (acute form) and nerve damage (classical form). Thus, the annual loss of MD in global poultry industry is estimated at 1-2 billion dollars [1]. World Society of Agriculture and Food (FAO) has about 2002 the largest poultry farms, containing 45 billion broilers and laying hens and in addition 57 million tons of eggs. Thus, the poultry industry adds global budget for 100 – $ 200 billion, Marek's disease virus in turn reduces it to 1%. However, the trend of Marek's disease virus and industrial rearing of poultry is not always observed in such way. By 1960, when the industrial poultry breeding was intensified, existing variety of Marek's disease virus showed pathogenicity in a very mild form. Thus, since the first case of diagnostic Marek's disease was noticeable change in the typical clinical manifestations [5]. The list of chronic polyneuritis (multiple peripheral nerve hypertrophy) gradually joined by visceral lymphoma (formation of tumours in organs such as the heart, liver, kidneys, lungs, etc.) more acute transient paralysis, immunosuppression, brain swelling and dermatitis. However, common symptoms of the disease with lesions of different genetic variants of the virus remains paralysis and chronic inflammation that occurs directly in response cytolytic infection of B- cell proliferation, manifested as lymphoma and degenerative changes in the form of arterial atherosclerosis [6].

Another factor that directly influenced on the evolution of Marek's disease pathogenicity was the creation and using vaccines. Thus, the first vaccine was created on the base of attenuated strains (HPRS- 16) of Marek's disease virus [1]. Soon, the prevention was perfected with the development of new vaccines based on the strain that genetically related to the herpes virus of turkeys (HVT) [2]. Mass vaccination of poultry decreased the percentage of losses up to 99% and created the major barrier of protection.

It should be noted that birds losing from Marek's virus was quickly stopped after vaccine using based on strain of HVT in the early of 70's. But success maintained only to the end of 70's and loss was recovered by the emergence of more virulent variants of the virus. But, the new bivalent vaccine consisting of HVT and strain from the second serotype of MDV (MDV -2) have become a response to the virus aggression. A new approach to vaccination of poultry allowed to keep the pressure from the virus a short period of time. After a relative lull in the mid of 90s, the losses in the poultry industry gained momentum in the answer of the emergence of new highly virulent strains of Marek's disease. Thus, the industry has been forced to take an empirical approach by introducing more effective vaccine, namely CVI988. Displayed vaccine was based on a first serotype of the virus (MDV-1) [3]. Used immunization strategy proved very successful way and allowed to control losses in the industrial poultry breeding. Nevertheless, the experience of previous years, tells us that as more stable control strategy we have as sooner we get the following changes "shift" in virulence by the emergence of new, more virulent variants of the virus. The vivid proof of this theory is the recent emergence of virus isolates that show a very high level of pathogenicity in chickens that were vaccinated with CVI988 [4].

Currently, the disease is controlled by the highly efficient mono-, bi -and polyvalent vaccines. However, Marek's disease virus remains exemplary model of the evolution of virus virulence during the past 60 years and continues to move towards its increase. Pathogenesis and disease manifestation is very different and depends on various factors, namely the serotype and patotype of virus, from genetic conditions of poultry, age of infection, the immune system, and welfare of flocks.

Currently, distinguish 3 main forms of Marek's disease. Thus, an acute form manifests as malignant tumours in visceral organs (liver, spleen, kidneys, lungs, stomach), the classical form – lymphocytes proliferation in pereferiynyh nerves and mixed, in which tumours are localized in both nerves and visceral organs. The development of mixed forms cause by Marek's disease virus pointed on the circulation of the virulent and highly virulent genetic variants of the virus. Of course, the classical method determination of the pathogenicity is a selection of agent, inoculate susceptible host by this agent and take the same disease, which is characterized as the control samples. However, takes in mind the manifestation of the disease, the time of infection and the presence or absence of a vaccine can talk about the presence of virulent strains of MD.

Thus, the aim of our study was to identify the Marek's disease on poultry farms Ukraine with current manifestations.

Materials and methods. The analyse of the pathological condition of samples from 117 poultry farms and sampling was carried out in the laboratory of pathological anatomy in the laboratory of diagnostic centre of "BioTestLaboratory". The study was beginning in 2009 up to 2012 and took place in different regions of Ukraine. For studies was selected bird with such external clinical signs of disease as: lameness, paralysis of the limbs and wings, change of iris colour and it's shape and size, blindness poultry and with the sudden death. Bird with such pathological changes as diffuse focal thickening of the nerve roots of the lumbar and brachial plexus, which become dull gray swelling in the lungs, kidneys, liver, heart, spleen, gonads, glandular stomach, muscle, liver congestion and spleen, and thickening of the walls of the glandular stomach were taken for histological examination. The size of selected samples was 2-4 cm selected materials immediately transferred to a 10% solution of formalin. Container with sampled was labelled in such follows: date of sampling, age of birds, a

household name, input the number in the LTD. Sectional material for histological examination left in tanks of formaldehyde at room temperature.

Fixation of tissue samples was performed by immersion in 80 ml of 10% formalin solution and holding in the microwave for 30 seconds at a power of 400 W with a water load of 400 ml. After initial fixation was performed by immersing the dehydrated tissue samples in 80 ml of 96% ethanol and exposure in the microwave for 10 min at 100 W power [10]. Then cut tissue samples (width of cut was less than 1.5 cm), taking into account the structural features of each body and the presence of visible lesions and placed in plastic cassettes Turboflowe [11].

After fixation was performed pre-processing studied tissues using automated station MicromSTP- 120. To prepare paraffin blocks, cooling the samples and filling was used the "MicromEC 350" station [81]. Preparation of thin sections of pathological material flooded in paraffin was performed using a microtome "Microm HM 340 E," and knives for histological studies "Sec- 130". Cutting was done by setting "step" microtome 50 microns, and for the manufacture of tissue samples – 5 microns. Slices of tissue samples were transferred from the water bath (water temperature 39 °C) directly into glass slides [11].

Made histological sections were kept for 1-2 hours at temperature of + 37° C for drying. Painting histological sections was performed hematoxylin and eosin solutions [81].

The sample's microscoping was carried out by an increase in the 100 and 200 times using appropriate lenses and an increase in 1000 using immersion lens microscope Axioskop 2 plus (CarlZeiss).

Results and Discussion. In 68% of the clinical cases were reported substantial polymorphic lymphoid irregular infiltrates that located in the perivascular and parenchyma of the body (Fig.1). It should be emphasized that the degree of substitution of the liver parenchyma intractable tumours ranged from 60 to 80% of body tissue.

Compared to the frequency of changes in the incidence of intractable liver tumours caused by Marek's disease virus was significantly lower. Thus, polymorphic lymphocytic infiltrates in the lungs detected only in 41 % of the cases whereas intractable tumours in the kidneys revealed only 30 % (Fig.2).

Often in studies recorded changes in the glandular part of the stomach (80 % of all samples). Taking into account poly-etiological cause of this disease (infectious bronchitis virus effect, Gumboro disease virus, poisoning, etc..), we took into account only those cases of proventrykulus in which changes were found not only in the glands and mucosa, but also in muscular glandular mucosa of the stomach. It should be emphasized that the localization of infiltrates in the muscular membrane as the stomach (15% of cases) and intestinal (detected in 32% of cases) is characterized by the classic signs of Marek's disease virus as polymorphic lymphocytic infiltrates in the peripheral nerves.

It should be added that tumours caused by Marek's disease virus in the tissues of the nervous system were found in 32% of the sample studied.

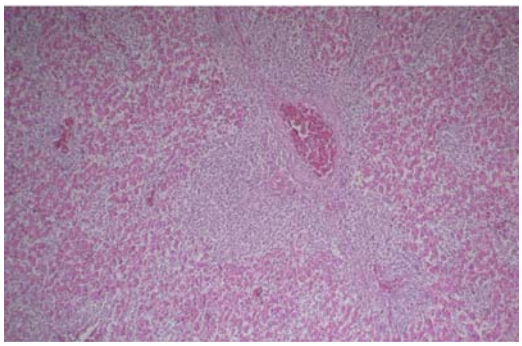


Fig. 1. Liver of damaged broiler in age of 45 days by MDV.
Heavy infiltration of proliferating mixed lymphoid cells in a liver. H&E. x100

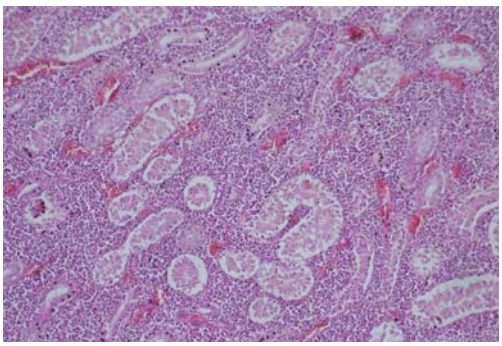
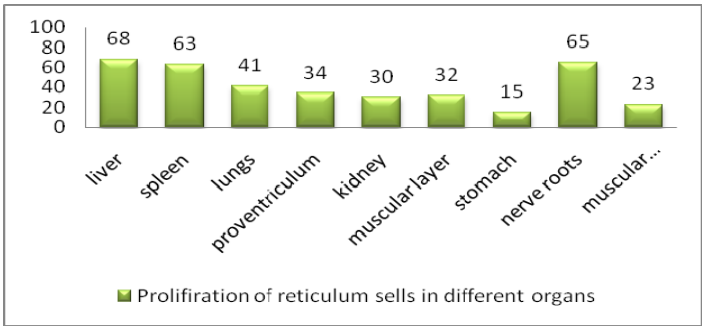


Fig 2. Heavy infiltration of proliferating mixed lymphoid cells in a kidney. H&E. x200

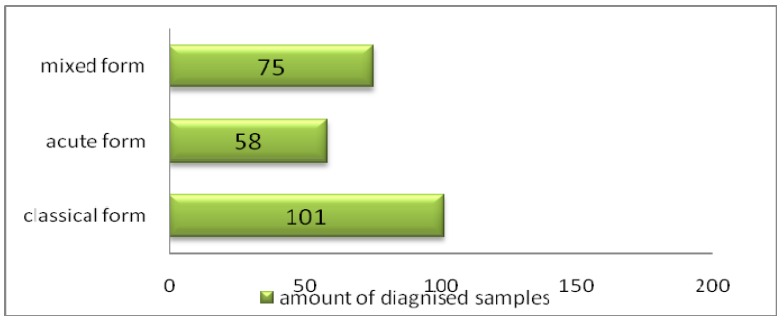
Thus, the most often tumors was met in the liver – 68%, peripheral nerves – 65%, spleen – 63%, in a somewhat lesser extent in the lung – 41% and glandular stomach – 34% (Table 1).

***Table*.1. Damaged visceral organs and peripheral nerves by Marek's disease virus**



I want to emphasize that the Marek's disease was identified in 234 samples from 117 farms of Ukraine. With the help of histological tools of diagnostic, it was determined that the classic form of disease was identify in 101 sample, while mixed form in 75 studied cases. It should be noted that the mixed form of Marek's disease was detected in 58 of observed samples.(Table 2).

***Table*. 2 Forms of Marek's disease manifestation in observed samples**



Thus, by pathomorphological and histological tools of diagnostic was analyzed 304 samples from 117 poultry farms Ukraine from 2009 up to 2012. Marek's disease was identified in 234 samples, that pointed on the circulation of this pathogen in Ukraine. Given the age of the birds, livestock vaccination, cross and nature of lesions in various organs was established that the dominant form of expression is the classic form of the disease, in which all visceral organs of birds are affected. Number of acute and mixed form in observed cases concerning about existence of virulent and highly virulent strains of Marek's disease within our state.

References

1. Morrow C. Fehler F. Marek's disease a worldwide problem. In Marek's disease, An Evolving Problem / Davison F., Nair V. // Elsevier Academic Press. – 2004. – P.49-61.

2. Witter R.L. Increased virulence of Marek's disease virus field isolate. // Avian Dis. – 1997. – Vol. 41. – P. 149–163.

3.Nair, V. Evolution of Marek's disease–a paradigm for incessant race between the pathogen and the host. // Vet. Journey. – 2005. – Vol. 170. – P. 175–183.

4. Osterrieder N., Kamil J.P., Schumacher D., Tischer B.K., and Trapp S. Marek's disease virus: from miasma to model. // Nat. Rev. Microbiology. – 2006. – Vol. 4. – P. 753–761.

5. Atkins, K. E., A. F. Read, N. J. Savill, K. G. Renz, S. W. Walken-Brown, and M. E.Woolhouse. Modelling Marek's disease virus (MDV) infection parameter estimates formortality rate and infectiousness. // BMC Veterenary Research. – 2011. – Vol. 7.

6. Nair, V., and H. J. Kung. Marek's disease virus oncogenicity: molecular mechanisms, and V. Nair (ed.), Marek's disease, an evolving problem. // Elsevier, Oxford, United Kingdom. – 2004. – Academic Press – P. 32-48.

7. Witter, R.L. Classification of Marek's disease virus according to pathotype-philosophy and methodology.// Avian Pathology. – 2005. – Vol. 31. – P. 525-538.

8. Witter, R.L. Very virulent Marek's disease viruses: Importance and control / R.L. Witter // In Proc 18th World's Poultry Congress // World's Poultry Congress, Nagoya, Japan. – 1988. – P. 92-97.

9. Norman L J Ross, R. Wadekar, A. Lopes, A. Dagnall. Methylation of two Homo sapiens-specific X-Y homologous genes in Klinefelter's syndrome (XXY). // American Journal of Medical Genetics Part B: Neuropsychiatric Genetics. – 2006. – Vol. 5. – P. 544 – 548.

10. Bacon L.D, Hunt H.D, Cheng HH: A review of the development of chicken lines to resolve genes determining resistance to diseases. // Poult Scientist. – 2000. – Vol. 79(8). – P. 1082-1093.

11. Fredericksen T.L, Longenecker B.M, Pazderka F, Gilmour DG, Ruth R.F. T-cell antigen system of chickens: Ly-4 and Marek's disease. // Immunogenetics. – 1977. – Vol.5. – P. 535-552.

12. Kaiser P, Underwood G, Davison F. Differential cytokine responses following Marek's disease virus infection of chickens differing in resistance to Marek's disease. // J Virol. – 2003 – Vol. 77(1). – P. 762-768.

13. Churchill, A.E. Immunization against Marek's disease using a live attenuated virus / A.E Churchill, L.N. Payne, and R.C. // Chubb // Nature – 1969. – Vol. 221 – P. 744-747.

14. Witter, R.L. Protection by attenuated and polyvalent vaccines against highly virulent strains of Marek's Disease virus / R.L. Witter // Avian Pathol. – 1982. – Vol. 11.-P. 49-62.

15. Rispens, B.H. Control of Marek's disease in the Netherlands. I. Isolation of an avirulent Marek's disease virus (strain CVI988) and its use in laboratory vaccination trials / B.H. Rispens [et al] // Avian Dis. – 1972. – Vol. 16. – P. 108-125.

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ІДЕНТИФІКАЦІЯ ХВОРОБИ МАРЕКА НА ПТАХОФЕРМАХ УКРАЇНИ ТА ВИЗНАЧЕННЯ ФОРМИ ЗАХВОРЮВАННЯ

Вірус хвороби Марека один із патогенів, який викликає значних економічних втрат у ветеринарії птахівництва в усьому світі. В роботі, хвороба Марека була ідентифікована в 234 зразках з 304 досліджуваних. За допомогою гістологічного методу діагностики встановили, що домінантною формою прояву була класична форма. Ідентифікація хвороби Марека в різних господарствах України з різною маніфестацією, вказує на циркуляцію збудника і на різну міру патогенності самого збудника.

Ключові слова: Вірус хвороби Марека, гістологічна діагностика.

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ИДЕНТИФИКАЦИЯ БОЛЕЗНИ МАРЕКА НА ПТИЦЕФЕРМАХ УКРАИНЫ И ОПРЕДЕЛЕНИЕ ФОРМЫ БОЛЕЗНИ

Вирус болезни Марека – один из патогенов, который вызывает значительные экономические потери в ветеринарии птицеводства во всем мире. В работе, болезнь Марека была идентифицирована в 234 образцах из 304 исследуемых образцов. С помошью гистологического метода диагностики устновлено, что доминантной формой оказалась классическая форма заболевания. Болезнь Марека идентифицированная в разных хозяйствах Украины, что указывает на циркуляцию возбудителя и на разную сепень патогенности самого возбудителя.

Ключевые слова: вирус болезни Марека, гистологическая диагностика.

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RETROSPECTIVE ANALYSIS OF INFLUENZA-INDUCED MORBIDITY IN POPULATION OF ZHYTOMYR REGION DURING 1999-2011

The epidemiological analysis of morbidity of influenza population Zhytomyr region for 13 calendar years was carried out. Dynamic of morbidity was characterized by periodic decrease and increase. The biggest data of morbidity of influenza of population from 1999-2011, were in the 1st quarter in February. The epidemic increasing of incidence in the epidemic season 2009-2010 was observed in October-December. Indicators of influenza of Zhytomyr region population coincide with the course of disease in Ukraine in general, and in some years over and above the Republican approximately 1,5 – 2. Morbidity of influenza Zhytomyr region populations has winter-spring seasonality and cyclic recurrence.

Key wods: flu, illness, seasonal, cyclical disease.

Introduction: Influenza viruses are the most prevalent pathogens of human respiratory infections and one of the most significant because they cause to high morbidity and mortality [1, 2].

The rapid pace of evolution of influenza viruses by various selection pressures, the production of novel genotypes through reassortment following mixed infections and their ability to constantly adapt to new avian and mammalian species, which makes monitoring and predictions influenza outbreaks is particularly difficult [3, 4, 5].

In the United States approximately 36,000 deaths occur annually following influenza infection. There is concern about the continuation of zoonotic infections of highly pathogenic avian influenza H5N1 [6, 7]. The worst influenza outbreak in recorded history, the so-called 'Spanish' influenza pandemic of 1918–1919 [8].

Pandemics of influenza virus appeared sporadically and were more than 1,000 years ago. In the past century there were four pandemics: 1918–1919 'Spanish' H1N1 influenza; 1957–1958 'Asian' H2N2 influenza; 1968–1969 'Hong Kong' H3N2 influenza; and 2009–2010 'Swine-origin' H1N1 influenza [6].

The emergence of pandemic (H1N1) 2009 in Mexico confirmed the need to understand the epidemiology of past pandemics in the world, which should be investigated in the future to further prevent pandemic [10].

Thus, influenza viruses continue to remain relevant pathogens, and therefore the aim of study – a comparison of the morbidity of influenza Zhytomyr region populations and Ukraine as a whole. The primary goal of this study – to analyse seasonality, cyclical recurrence of morbidity Zhytomyr region populations during from 1999 till 2011.

Materials and Methods: The information and statistical data Zhytomyr regional sanitary-epidemiological station from 1999 till 2011 (reporting forms on the annual morbidity – f.2 and monthly morbidity – f.1) were used.

Results and Discussion

Flu is inherent seasonality. In the Zhytomyr region, as in Ukraine as a whole an annual seasonal epidemic rises of influenza are recorded. Analyzing monthly dynamics of influenza population of Zhytomyr region of each calendar year from 1999 to 2011 may be noted that it has been registered in the region during all calendar years of our observation of a distinct seasonality. Increase in incidence occurs in the winter-spring period (January – March).The biggest data of influenza morbility of populations from 1999-2011, were in the 1st quarter of February (1288,86 per 100 000 population – 2008; 549,81 per 100 000 population – 2007; 395,35 per 100 000 population – 2004; 2302, 65 per 100 000 population – 2003; 637,52 per 100 000 population – 2001; 2046,18 per 100 000 population – 2000; 1946, 51 per 100 000 population – 1999). Increased morbidity associated with the output pupils and students from holidays (large crowd of people promote the spread of infection), after which increases morbidity not only in influenza, but also from ARVI.

During the summer, there is a significant decrease in morbidity of influenza. These cases are sporadic. Primarily due to the direct dependence of influenza on the temperature of the environment – the flu virus is well preserved at low temperatures, and the second – with an increase in nonspecific immunity in the population. Continuity epidemiological process also explains that in the northern hemisphere of the globe disease occurs in autumn and winter (November – March) and in the southern hemisphere – April – October. Thus there is a transfer of influenza viruses from one hemisphere to the second. There is a hypothesis about the persistence of influenza virus in the body recover from the person, and if low immunity can cause disease. People who have no immune protection against these viruses often suffer. Analyzing the dynamics of long-term (13 calendar years) influenza morbidity of Zhytomyr region populations and comparing its indicators with Ukraine as a whole can conclude that during surveillance in epidemic process of this infection proved respect to some cyclical recurrence (years of increase : 1999 (3785,6 per 100 000 population); 2003 (3556,39 per 100 000 population); 2005 (2034,57 per 100 000 population); 2009 (1595,75 per 100 000 population) and years of decrease : 2002 (438,62 per 100 000 population); 2004 (1186,41 per

100 000 population); 2006 (689,4 per 100 000 population); 2011 (223,36 per 100 000 population).

Indicators of morbidity of influenza Zhytomyr region populations coincide with the course of disease in Ukraine in general, and in some years over and above the Republican approximately 1,5 – 2 (Fig. 1).

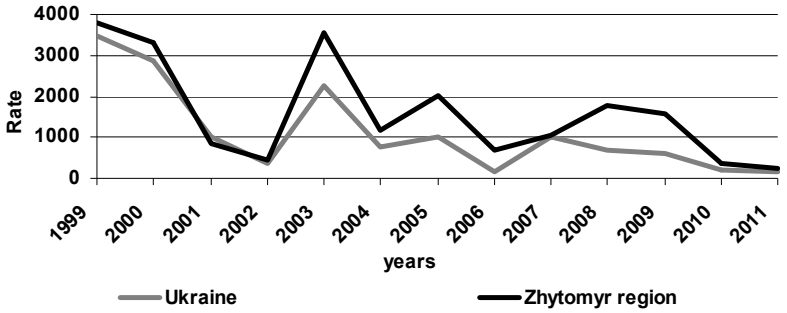

Fig. 1. Dynamic of influenza-induced morbidity in population of Zhytomyr region during 1999-2011 (rate of morbidity per 100000 population)

Morbidity is also dependent on the coincidence of epidemic strains of influenza virus vaccine. If they match, the vaccine is effective and allows you to prevent the flu in the population, thereby reducing these figures.

Currently, after a slight rise of morbidity Zhytomyr region of influenza in 2009 has tended to fall, well represented in the graph. The same pattern region and in other regions and in Ukraine in general. Decrease primarily due to the increase in population immunity due to large numbers recover and vaccination among the population of the respective vaccines, both in the Zhytomyr region and in other regions of Ukraine. There has been a systematic health education outreach on measures of personal and social prevention, the need for timely seeking medical help and self-harm, rules "cough etiquette" and so on.

It is confirmed that the flu for the period 1999-2011 he keeps the main characteristics of the epidemic process, namely affects all age groups, a winter-spring seasonality and long-term cycles.

References

1. Wright PF, Neumann G, Kawaoka Y. Orthomyxoviruses. // In Fields Virology, ed. DM Knipe, PM Howley. – 2007. – P. 1691 – 40.

2. Taubenberger K. J., Morens M. D. The Pathology of Influenza Virus Infections // Annu. Rev. Pathol. Mech. Dis. – 2008. – №3. – P. 499 – 522.

3. Palese P., Shaw M.L. Orthomyxoviridae: the viruses and their replication // In: Knipe, D.M., Howley, P.M. (Eds.), Fields Virology. – 2007. – № 2. – P. 1647 – 1689.

4. Taubenberger, J.K., Kash, J.C. Influenza virus evolution, host adaptation, and pandemic formation // Cell Host Microbe. – 2010. – 7 (6). – P. 440 – 451.

5. Morens D.M., Taubenberger J.K. 1918 Influenza, a Puzzle with Missing Pieces. // Emerging Infectious Diseases. – 2012. – Vol. 18, No. 2. – P. 332 – 335.

6. Morens D.M., Taubenberger J.K., Fauci A.S. The persistent legacy of the 1918 influenza virus. // N. Engl. J. Med. – 2009. – 361 (3). – P. 225 – 229.

7. Peiris J.S., de Jong M.D., Guan Y. Avian influenza virus (H5N1): a threat to human health. // Clin. Microbiol. Rev. – 2007. – 20 (2). – P. 243 – 267.

8. Taubenberger J.K., Morens D.M. 1918 influenza: the mother of all pandemics. // Emerg. Infect. Dis. – 2006. – 12 (1). – P. 15 – 22.

9. Morens D.M., Taubenberger J.K. Pandemic influenza: certain uncertainties. // Rev. Med. Virol. – 2011. – 21 (5). – P. 262 – 284.

10. Johnson N.P., Mueller J. Updating the accounts: global mortality of the 1918–1920 Spanish influenza pandemic. // Bull. Hist. Med. – 2002. – 76 (1). – P. 105 – 115.

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РЕТРОСПЕКТИВНИЙ АНАЛІЗ ЗАХВОРЮВАНОСТІ НА ГРИП НАСЕЛЕННЯ ЖИТОМИРСЬКОЇ ОБЛАСТІ ЗА ПЕРІОД 1999-2011 РР.

Проведено аналіз захворюваності на грип населення Житомирської області за 13 календарних років. Динаміка захворюваності характеризується періодичними підйомами та спадами. Епідемічний підйом захворюваності в епідемічному сезоні 2009–2010 рр. спостерігався у жовтні – грудні. Показники захворюваності на грип населення Житомирської області практично співпадають з перебігом захворюваності по Україні в цілому, а в деяких роках із перевищенням республіканських приблизно у 1,5 – 2 рази. Захворюваність на грип населення Житомирської області має зимово-весняну сезонність та багаторічну циклічність.

Ключові слова: грип, захворюваність, сезонність, циклічність, захворювання.

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РЕТРОСПЕКТИВНЫЙ АНАЛИЗ ЗАБОЛЕВАЕМОСТИ ГРИППОМ НАСЕЛЕНИЯ ЖИТОМИРСКОЙ ОБЛАСТИ ЗА ПЕРИОД 1999-2011 ГГ.

Проведен анализ заболеваемости гриппом населения Житомирской области за 13 календарных лет. Динамика заболеваемости характеризуется периодическими подъёмами и снижениями. Епидемический подъём заболеваемости в епидемическом сезоне 2009–2010 гг. наблюдался в октябре – декабре. Показатели заболеваемости гриппом населения Житомирской области практически совпадают с течением заболеваемости по Украине в целом, а в некоторых годах даже с превышением республиканских приблизительно в 1,5 – 2 раза. Заболеваемость гриппом населения Житомирской области имеет зимне-весеннюю сезонность и многолетнюю цикличность.

Ключевые слова: грипп, заболеваемость, сезонность, цикличность, заболевание.

UDK 578.4+633.88

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HEAVY METALS CONTENT IN VIRUS INFECTED PURPLE CONEFLOWER PLANTS

It was revealed that purple coneflower plants infected Tomato spotted wilt virus. It was investigated that concentration of nine microelements (As, V, Sb, Cr, Fe, Ba, Sr, B, Mn) in purple coneflower plants under viral infection is higher than in healthy plants. Content of seven metals (Cd, Ni, Mo, Ti, Al, Zn i Al) was less than in the control samples. Differences for other elements (Pb, Hg, Cu, Co, Li) are not educed. It is necessary to notice that content of high-toxic elements (As, V, Sb, Cr, Fe) in the infected plants exceeded maximum allowable concentrations (MAC) in 1.2, 7, 2.3, 2.5 and 3.4 times respectively, unlike control samples in which concentration of these metals was within the limits of norm.

Key words: plant viruses, Tomato spotted wilt virus, purple coneflower, heavy metals.

Introduction: It is known that medical plants contain many important microelements which are necessary for the proper functioning of human organism. On the other side, except the positive effect of microelements, is also negative: there is a list of elements the concentration of that in foods is subject to hard control. Ability of medical plants to accumulate heavy metals from an environment is studied in the last ten years widely enough and does not cause doubts [4, 5, 6, 11, 14, 15, 18, 25, 26], that stipulates actuality of researches in this direction.

For today following classification of microelements is developed in relation to their operating on the human organism: microelements which matter in the human feed (Co, Cr, Ce, F, Fe, I, Mo, Mn, Ni, Se, Si, V, Zn); microelements which have a toxicological value (As, Be, Cd, Co, Cr, F, Hg, Mn, Mo, Ni, Pb, Pd, Se, Sn, Ti, V, Zn) [20]. It is needed to notice, that 10 from the transferred elements is taken in both groups. Thus, it is not always possible to set a difference between vitally necessary and toxic metals. All metals can show toxicity, if they enter organism in a surplus amount.

However, there are metals which show the expressed toxicological properties at the lowest concentrations and do not execute which that was not by a useful function – mercury, cadmium, lead, arsenic. In this connection, the common commission of FAO and WHO in obedience to a food code (*Codex Alimentarius Europaeus*) included mercury, cadmium, lead, arsenic, copper, strontium, zinc, iron in the number of components, content of which is controlled at the international trading in the products of feed. In Russia and CIS 7 elements (furnace, nickel, chrome, aluminium, fluorine, iodine, tin) are controlled. Some other metals can be controlled at presence of the certificates [16]. Content of these metals must be regulated and in medical plants because for today there are a few data about the degree of metals transition in to medicamental forms that is made from raw material of these plants [13, 17, 22]. In addition, information about element composition of medical plants is needed both for estimation of pharmacological properties of preparations from them and for standardization, development of analytically-normative documentation on a medical digester, from that get the plant-based preparations [12, 19, 26].

Mechanisms of viruses influence on heavy metals content in the plants are not found out. Data about determination of their concentration in virus infected herbs that are grown on territory of Ukraine are not present.

Aim of the research was to investigate heavy metals concentration in purple coneflower plants infected with viruses.

Materials and methods. For diagnostics of viruses in the plants applied the methods of visual diagnostics, ELISA and transmission electronic microscopy (EM). Contrasting has been made with 2% solution of phosphorus – tungstic acid. Virions are investigated using electron microscope JEM 1230 (JEOL, Japan) [23].

Detection and identification of viruses has been carried out with enzyme-linked immunosorbent assay (DAS-modification) using commercial test-systems of firm LOEWE (Germany). The results of reaction registered on the rider Termo Labsystems Opsis MR(THE USA) with Dynex Revelation Quicklink software at lengths of waves of 405/630 нм. For reliable took on values that exceeded negative control in three times [1].

Studying of the concentration microelements in plants taken in the flowering stage (both with symptoms of the disease and symptomless samples) has been carried out by mass spectrometry method using ISP-MC X-Series 2 (Termo Fisher Scientific) [8].

As raw material from echinacea is used in an untilled kind – in the plant-based preparations, infusions and biologically active additives (BAA), the analysis of data on determination of heavy metals concentration was carried out by comparison with: 1) level of maximum allowable concentrations (MAC) that is regulated by medical and biological requirements and sanitary norms of food quality № 5061-89 [21]; 2) possible norms that is regulated by State Pharmacopoeia of Ukraine (SPU) – not more than 0,1% [12]; 3) 'Hygienic Requirements for Foodstuff Safety and Nutritional Value' (SanPiN 2.3.2.1078-01) that establish hygienic requirements for substances and materials that come into contact with foodstuffs [24]; 4). WHO norms [10].

Results and discussion. Under monitoring of purple coneflower plantations (*Echinacea purpurea* (L.) Moench.) we revealed plants with yellow spotted symptoms on the leaves (Fig. 1).

Figure. 1. Purple coneflower plants with symptoms of yellow spotted on the leaves (control – at the left)

Spherical viral particles 100±20 nm in diameter were detected in the sap of the sick coneflower plants (Fig.2).

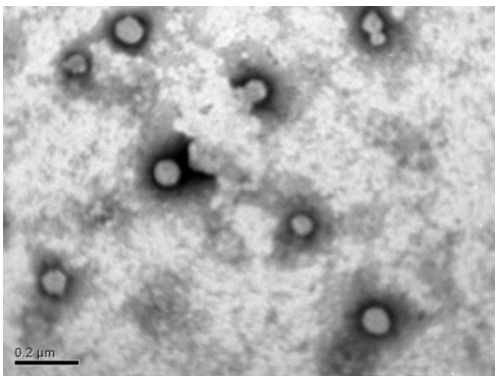

Figure. 2. Electron microscopy picture of the virions detected in the purple coneflower leaves

Viral particles presented on the Fig. 2 are similar to the viruses of the genus *Tospovirus* family *Bunyaviridae* in their morphology and size. It is known that tospoviruses are spherical virions with diameter 80-120 nm and have a wide circle of sensitive plants [9]. The typical member of the genus is *Tomato spotted wilt virus* (TSWV). It is necessary to mark that TSWV and also *Impatiens necrotic spot virus* (INSV) were already detected in the purple coneflower plants in Bulgaria and Lithuania [2,3,7].

Having regard to said early, the plants of Echinacea were tested by us in the presence of TSWV and INSV. ELISA results showed a presence of TSWV antigens in the purple coneflower plants (Fig. 3).

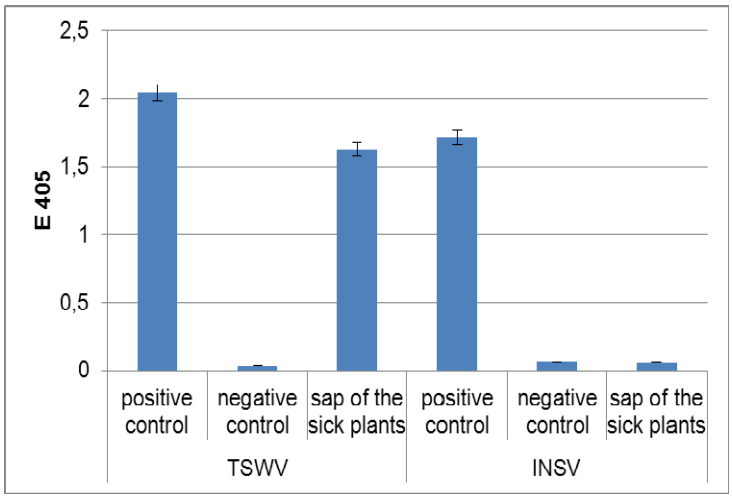

Figure 3. Content of the TSWV and INSV antigens in purple coneflower plants

Some debate exists as to exactly what constitutes a "heavy metal" and which elements should properly be classified as such. Some authors have based the definition on atomic weight, others point to those metals with a specific gravity of greater than 4.0, or greater than 5.0. The actinides may or may not be included. Most recently, the term "heavy metal" has been used as a general term for those metals and semimetals with potential human or environmental toxicity. That's why analysis of the results on research of heavy metals concentration was carried out in

relation to their toxicity for the human organism. So, all investigated metal were divided into three groups: high-toxic, "mildly toxic" and low toxic metals.

The results of our research have not revealed differences in the content of such heavy metals as Pb, Hg, Cu between healthy and virus infected samples. Norms regulated by medical and biological requirements 5061-89 (MBR), SPU and WHO are not is not exceeded (table 1).

***Table* 1. Concentration of heavy metals in the purple coneflower plants, mg/kg** p≤0,01

Metal	Virus infected plants	Healthy plants	MBR 5061-89	SanPiN 2. 3.2.107801	WHO
Pb	0,48	0,52	0,5	5,0	10,0
Cd	0	0,09	0,03	1,0	3,0
As	0,25	0,06	0,2	3,0	-
Hg	0	0	0,02	1,0	-
Cu	4,50	4,81	5,0	-	-
Zn	11,12	27,68	10,0	-	-

However, it is necessary to notice that concentration of such high-toxic element as As in the Echinacea infected with viruses was higher in 4,5 times in comparison with healthy plants (table 1). Content of Cd in healthy plants was 0,085, that exceeds MAC in 2,8 times, unlike sick plants in which this element is not educed. A tendency to the reduce of concentration at a viral infection is marked for Zn (in 2,5 times), thus in the healthy plants MAC is exceeded in 2,7 times. On the SanPiN requirements, that estimate biologically active additives, content of all four metals (Pb, Cd, As, Hg) and in healthy, and in the virus infected samples of coneflower was within the limits of norm.

Except described 6 elements, Tl, V, Be, Ni and Bi are high-toxic metals too. Our research educed that the concentration of vanadium in all samples had exceeded MAC (0,5 mg/kg) and in TSWV-infected plants been in 1,3 times higher, than in healthy (Fig. 4).

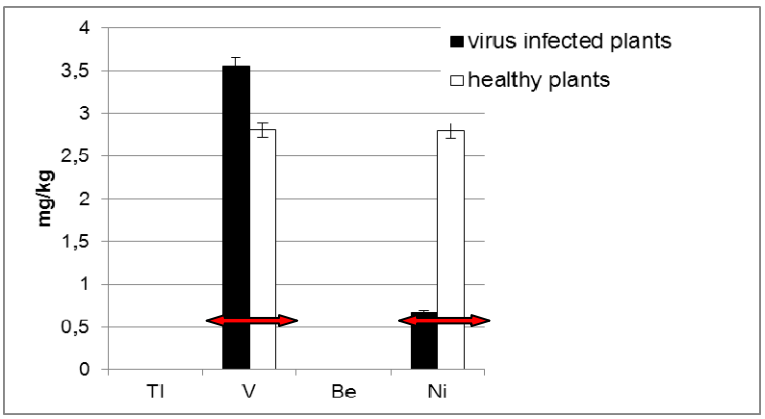

Figure 4. Concentration of high-toxic metals in purple coneflower plants (MAC is marked a pointer)

Thallium and beryllium are educed neither in sick plants nor in control (fig. 4). Concentration of nickel in infected plants was on verge of MAC (0,5 mg/kg), and here in healthy exceeded it in 5,6 (!) times. The same conformity revealed by us and for Bi. Its to law is a concentration was in 3 times higher in healthy plants than in sick plants and presented 0,65 mg/kg. The accumulation of such potentially dangerous metals even in healthy plants testifies to the necessity of an increase control of their content in plant raw material.

Concentration of four so-called "mildly toxic" for a man metals (Sb, Cr, Mn, Ba) in the coneflower plants under viral infection was higher, than in control (fig. 5, 6). Thus MAC for Sb and Cr is exceeded in 2,5 times (fig. 5).

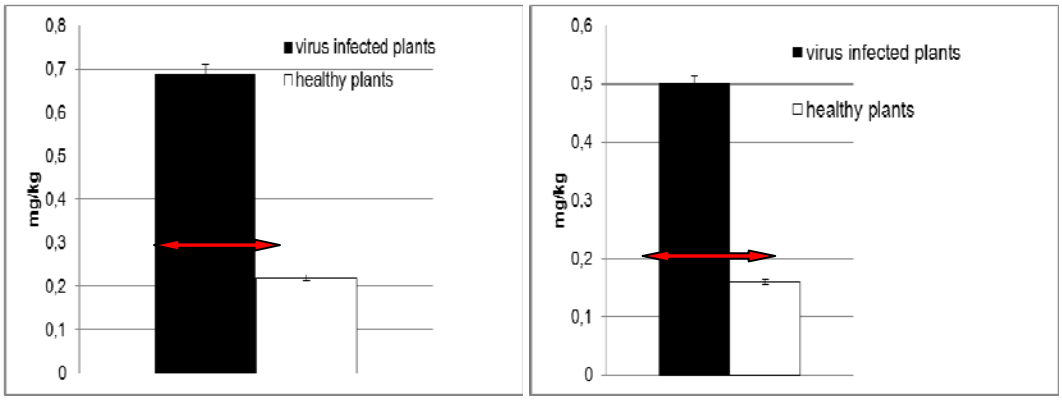

Figure 5. Concentration of Sb (at the left) and Cr (at the right) in purple coneflower plants (MAC is marked a pointer)

Increase of concentration under viral infection is marked by us and for such metals as a barium (Ba) and manganese (Mn) – in 1,3 and 1,2 times accordingly (fig. 6).

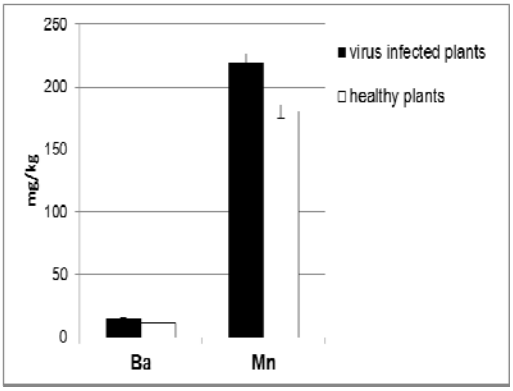

Figure 6. Concentration of Ba and Mn in purple coneflower plants

Content of iron that is subject to hard control in food, in healthy coneflower plants exceeded MAC in 2 times, in virus infected – in 3,4 times. In addition, this index exceeded in 1,7 times possible norms regulated SPU (Fig. 7).

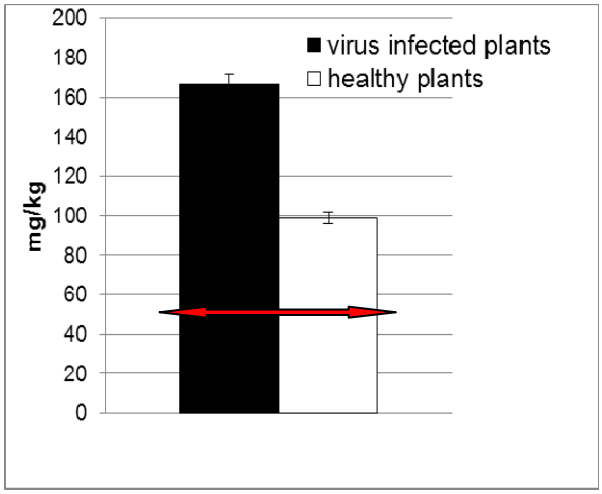

Figure. 7. Content of iron in the purple coneflower plants (MAC is marked a pointer)

Investigation of concentration of "low toxic metals" is shown that content of Sr and B was higher in TSWV-infected coneflower in 1,3 and 1,6 times in comparison with healthy plants (table 2).

***Table* 2. Concentration of "low toxic metals" in purple coneflower plants, mg/kg p≤0,01**

Metal	Virus infected plants	Healthy plants
B	253,4	156,6
Sr	150,5	116,7
Li	2,9	2,9
Ti	11,0	16,1
Al	35,7	62,1

Content of lithium in the coneflower plants infected with TSWV did not differ from such in the control. And the concentration of Ti and Al was lower in sick plants in 1,5 and 1,7 times accordingly (table 2).

Conclusions. Thus, it was investigated that viral infection substantially effects on the microelements content in the purple coneflower plants. It is necessary to notice that the conducted research found out the tendency of accumulation of some heavy metals in these plants under TSWV infection in amounts which exceed MAC in food products. Although these indexes were within the limits of norms, regulated State Pharmacopeia of Ukraine and SanPiN. But presently and until now there is not clear information about passing of heavy metals to the medical forms. Thus, our studies demonstrated the obvious negative role of viruses in the production of high-quality medical raw material and to the necessity of the viral of the monitoring of medical plants with further development of protective methods from the detected viruses.

References

1. Clark M. F., Adams A. N. Characteristics of the microplate method of enzyme-linked immunosorbent assay for the detection of plant viruses // J. Gen. Virology. – 1977. – Vol. 34. – P. 574– 586.

2. Dikova B. Tomato spotted wilt virus on some medicinal and essential oil-bearing plants in Bulgaria // Bulgarian Journal of Agricultural Science. – 2011. – Vol. 17 (3). – P. 306-313.

3. Dikova B., Djourmanski A., Lambev H. Establishment of economically important viruses on Echinacea purpurea and their influence on the yield // Материалы междунар. науч. конф. "Инновационные подходы к изучению эхинацеи". – Полтава, 25-25 июня 2013 г. – Полтава: Дивосвіт, 2013. – С. 36-45.

4. Heavy Metal Accumulation in Some Herbal Drugs / V. Rai, P. Kakkar, S. Khatoon et al. // Pharmaceutical biology. – 2001, Vol. 39, No. 5. – P. 384-387.

5. Heavy metals and nutritional composition of some selected herbal plants of Soon Valley, Khushab, Punjab, Pakistan / A. Ghani, S. Saeed, Z. Ali et al.M. // African Journal of Biotechnology. – 2012. – Vol. 11(76). – P. 14064-14068.

6. Heavy metals contamination in herbal plants from some Ghanaian markets / C. K. Bempah, J. Boateng, J. Asomaning et al. // Journal of Microbiology, Biotechnology and Food Sciences. – 2012/13. – Vol. 2 (3). – P. 886-896.

7. Samuitienė M., Navalinskienė M., Jackevičienė E. Detection of Tospovirus Infection in Ornamental Plants by DAS-ELISA // Lua Research paper (ISSN 1648 – 116X). – 2003. – Nr. 57 (10).

8. Uptake of various trace elements during germination of wheat, buckwheatand quinoa / J. Lintschinger, N. Fuchs, H. Moser et al. // Plant Foods for Human Nutrition. – 1997. – Vol. 50. – P. 223-237.

9. Virus taxonomy. Ninth report of the International Committee on Taxonomy of Viruses / eds. A.M.Q. King, M. J. Adams, E. B. Carstens, E. J. Lefkowitz. –Elsevier. – 2012. – 1327 p.

10. Who monographs on selected medicinal plants. – Vol. 1, Geneva, 1999. – 293 p.

11. Великанова Н.А., Гапонов С.П., Сливкин А.И. Изучение динамики накопления тяжелых металлов травой горца птичьего и листьями подорожника большого в процессе вегетации в городе Воронеже и его окрестностях // Современные проблемы науки и образования. – 2012. – № 5.

12. Державна фармакопея України. Випуск 1. Доповнення 2.: Державне підприємство "Науково-експертний фармакопейний центр", Харків. – 2008. – С. 120.

13. Динаміка вмісту важких металів (Cd, Cu, Pb, Fe) у препаратах на основі кореня валеріани / Т.Я. Врублевська, Н.П. Тамчук, О.І. Соловей та ін. // Вісник фармації. – 2001. – № 4. – С. 26-29.

14. Журавель Т.О. Інтродукція видів роду Echinacea Moench. на південний схід України // Автореф. дис... канд. біол. наук: 03.00.05. – К., 2005. – 22 с.

15. Єренко О.К., Мазулін О.В., Смойловська Г.П. Нітратне забруднення екстемпоральних лікарських засобів і рослинної сировини видів роду Inula L. флори України // Запорожский медицинский журнал. – 2012. – № 4 (73). – С. 65-67.

16. Копнов В. Принципы качества жизни \\ Стандарты и качество. – 2003. – № 2. – С. 37-41.

17. Листов С.А., Непесов Г.А., Сахатов Э.С. Содержание тяжелых металлов в настоях из лекарственного растительного сырья // Фармация. – 1992. – № 4. – С. 37-41.

18. Ловкова М.Я., Бузук Г.Н. Лекарственные растения – концентраторы микроэлементов. Новые аспекты применения этих видов в медицине // Вопросы биологической, медицинской и фармацевтической химии. – 2013. – №4. – С.43-49.

19. Мазулин А.В., Калошина Н.А., Доля В.С. Экологическая оценка лекарственного сырья – важный фактор совершенствования его стандартизации // Человек и его здоровье: сб. научн. работ. – Курск, 1999. – Вып. 2. – С. 243–244.

20. Макро- та мікроелементи (обмін, патологія та методи визначення): монографія / М.В. Погорєлов, В.І. Бумейстер, Г.Ф. Ткач, С.Д. Бончев, В.З. Сікора, Л.Ф. Суходуб, С.М. Данильченко. – Суми: Вид-во СумДУ, 2010. – 147 с.

21. Медико-биологические требования и санитарные нормы качества продовольственного сырья и пищевых продуктов № 5061-89. Утверждены Минздравом СССР 1 августа 1989 г.

22. Проблемы нормирования тяжелых металлов в лекарственном растительном сырье / О.И. Терёшкина, И.П. Рудакова, И.В. Гравель и др. // Фармация. – 2010. – №2. – С. 7-11.

23. Салига Ю. Т., Снітинський В. В. Електронна мікроскопія біологічних об'єктів. – Львів, 1999. – 152 с.

24. СанПіН 2. 3.2.1078 01 "Гігієнічні вимоги безпеки і харчової цінності харчових продуктів".

25. Сметанюк О.І., Черновська Н.В. Просторово-часова мінливість вмісту свинцю в лікарських рослинах // Клінічна та експериментальна патологія. – 2009. – № 3 (29). – С. 101-102.

26. Экологические аспекты заготовки и использования лекарственного растительного сырья / Н.Э. Коломиец, Г.И. Калинкина, А.А. Марьин и др.// Известия Самарского научного центра Российской академии наук. – 2010. – Т. 12, № 1 (8). – С. 2051-2054.

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ВМІСТ ВАЖКИХ МЕТАЛІВ У ВІРУСІНФІКОВАНИХ РОСЛИНАХ ЕХІНАЦЕЇ ПУРПУРОВОЇ

Виявлено, що рослини ехінацеї пурпурової уражені вірусом плямистого в'янення томатів. Встановлено, що із 21-го проаналізованого мікроелемента концентрація дев'яти (As, V, Sb, Cr, Fe, Ba, Sr, B, Mn) у вірусінфікованих рослинах ехінацеї пурпурової є вищою, ніж у контролі, семи (Cd, Ni, Mo, Ti, Al, Zn і Al) – нижчою, по інших (Pb, Hg, Cu, Co, Li) – різниці не виявлено. Показано, що вміст високотоксичних елементів (As, V, Sb, Cr, Fe) у рослинах, уражених вірусом, перевищував гранично допустимі концентрації (ГДК) у 1.2, 7, 2.3, 2.5 та 3.4 рази відповідно, на відміну від контрольних зразків, у яких концентрація вказаних мікроелементів була у межах норми.

Ключові слова: фітовіруси, вірус плямистого в'янення томатів, ехінацея пурпурова, важкі метали

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СОДРЖАНИЕ ТЯЖЕЛЫХ МЕТАЛЛОВ И ВИРУСИНФИЦИРОВАННЫХ РАСТЕНИЯХ ЭХИНАЦЕИ ПУРПУРНОЙ

Обнаружено, что растения эхинацеи пурпурной поражены вирусом пятнистого увядания томата. Установлено, что из 21-го проанализированного микроэлемента концентрация девяти (As, V, Sb, Cr, Fe, Ba, Sr, B, Mn) в вирусинфицированной эхинацее пурпурной выше, чем в здоровых растениях, семи – (Cd, Ni, Mo, Ti, Al, Zn і Al) – ниже, по другим – (Pb, Hg, Cu, Co, Li) – разницы не выявлено. Показано, что количественное содержание высокотоксических элементов (As, V, Sb, Cr, Fe) в пораженных вирусом растениях превышало гранично-допустимые концентрации в 1.2, 7, 2.3, 2.5 и 3.4 раза соответственно, в отличие от контрольных образцов, в которых концентрация указанных металлов была в пределах нормы.

Ключевые слова: фитовирусы, вирус пятнистого увядания томата, эхинацея пурпурная, тяжелые металлы.

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MANTAINING THE STATUS OF UKRAINE AS A POLIO-FREE TERRITORY

The article presents data on polio immunization coverage in Ukraine by age group during 2009-2012 and the results of investigating immunity to polioviruses in various population groups during 2009-2011.Considering the current poliomyelitis situation in the world and the possibility of wild poliovirus importation from endemic countries, continued monitoring of herd immunity is necessary for Ukraine to maintain its status as a polio-free territory.

Key words: poliomyelitis, Ukraine, immunity.

Introduction. Poliomyelitis is an acute infectious disease affecting the nervous system, primarily the grey matter of the spinal cord [1, 2]. Poliovirus, the causative agent of poliomyelitis, is a human enterovirus and member of the family of *Picornaviridae*.

Poliomyelitis has appeared in epidemic form, become endemic on a global scale, and been reduced to near-elimination, all within the span of documented medical history [1]. Global expansion of polio immunization resulted in a reduction of paralytic disease from an estimated annual prevaccine level of at least 600,000 cases to fewer than 1,000 cases in 2000 [3]. Indigenous wild poliovirus type 2 was eradicated in 1999, but unbroken localized circulation of poliovirus types 1 continues in 3 countries in Asia and Africa

[4, 5]. Current challenges to the final eradication of paralytic poliomyelitis include the continued transmission of wild polioviruses in endemic reservoirs, reinfection of polio-free areas, outbreaks due to circulating vaccine-derived polioviruses, and persistent excretion of vaccine-derived poliovirus by a few vaccines with B-cell immunodeficiency [6, 7].

In 2002, the WHO European region which includes Ukraine was certified free of poliomyelitis. Maintaining the status of Ukraine as a polio-free territory depends primarily on immunization, the effectiveness of which can be assessed by the level of herd immunity in the country. The data obtained during an investigation of the level of specific antibodies and the data on polio immunization in regions allowed us to assess the polio epidemic situation in Ukraine.

Methods. The official Ukraine Ministry of Health data on scheduled polio immunization of children in 2009-2012 have been analyzed [8]. The results of a serological monitoring of collective immunity of the population of Ukraine in 2009-2012 were analyzed, too. Virological departments of sanitary epidemiological stations selectively investigate specific immunity to assess the effectiveness of polio immunization and summit official statistical reporting forms to the Kiev Institute of Epidemiology and Infectious Diseases on an annual basis [9]. The results of our own serological investigations of collective immunity in the adult population have been used, too.

Results and Discussion. According to the Ministry of Health of Ukraine decree entitled "The action plan for maintaining the status of Ukraine as a polio-free country for 2011-2013", polio immunization coverage of children less than 1 year of age should be at least 95% in all administrative regions.

A comparative analysis of polio immunization of children in Ukraine by age in 2009-2012 showed declining levels of immunization coverage in all age groups (Fig. 1.).

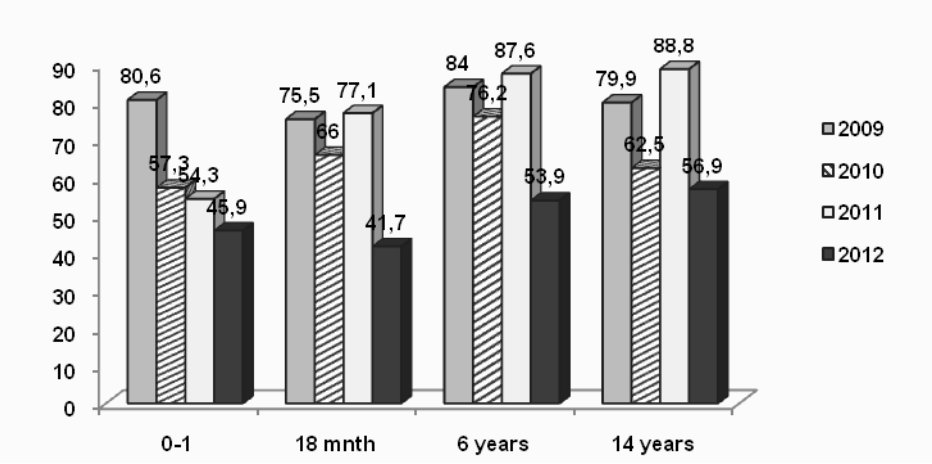

Figure 1. Polio immunization coverage of children by age group, Ukraine, 2009-2012

In 2009, polio immunization coverage of children under one was 80.6%, the coverage with the first booster at 18 months was 75.5%, and the coverage with boosters at 6 and 14 years was 84% and 79.9% respectively. In 2010, polio immunization coverage significantly decreased. It amounted to 57.3% among children under one year of age, 66% in children eligible for the first revaccination, 76.2% in children aged 6 years, and 62.5% at 14 years of age. In 2011, 88.8% of older children aged 14 years received a polio booster; the coverage among 6 year olds was 87.6%. However, only 54.3% of children less than one year of age got vaccinated. During the first 9 months of 2012, polio immunization coverage in Ukraine amounted to 45.9% in children less than one year old, 41.7% among children aged 18 months, 53.9% at 6 years of age, and 56.9% at 14 years of age.

The above results are indicative of an increasing number of people susceptible to poliovirus, which can present a threat to the epidemiological wellbeing of Ukraine in case of wild poliovirus importation.

A serological study using sera from healthy individuals of various age groups was conducted at the Laboratory of Respiratory and other Viral Infections to assess population immunity to serotypes 1, 2 and 3 of vaccine strains of poliovirus.

The data analysis indicated that the majority of the population aged 20-65 years had low-to-medium levels of protective antibodies to the three polioviruses (Fig. 2.).

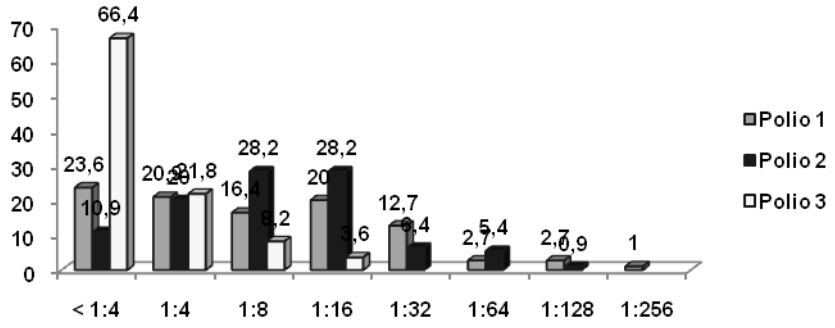

Figure 2. Antibody levels to poliovirus types 1, 2 and 3 among the adult population of Ukraine in 2012

Considering that the last polio booster is given to children at the age of 14 years, a decrease of immunity was observed in older individuals not exposed to the virus.

For example, a significant proportion (23.6%) of the tested individuals was not protected against poliovirus type 1; their antibody titers were lower than 1:4. Twenty percent of the population had protective antibody titers of 1:16. High antibody titers to poliovirus type 1 were observed in 2.7% of the population, and only 1% of the population had antibody titers of 1:256.

10.9% of the population had no immunity to type 2 of poliovirus; 28.2% of the individuals had protective antibody titers ranging from 1:8 to 1:16. Only 0.9% of the population had high antibody titers.

With respect to poliovirus type 3, a significant decrease of the share of the population with medium and high immunity was observed. For example, the majority (66.4%) of the tested individuals had antibody levels less than 1:4; 21.8% of the population had low antibody levels. Not a single person was shown to have antibody levels higher than 1:16.

We also analyzed herd immunity in various regions of Ukraine in 2009-2011 (Table 1.).81.3% of the tested individuals were children less than 14 years of age. The data indicated that the share of the tested individuals with no or low antibody titers to type 3 poliovirus was 3 times higher than the share of people with similar antibody titers to poliovirus types 1 and 2. We established that the majority of the tested population had antibody titers of 1:64 to types 1 and 2 of poliovirus and 1:32 to poliovirus type 3.

Conclusions

The following conclusions can be drawn from the investigation. During 2009-2012, immunization coverage of the population of Ukraine decreased which led to an increase of the proportion of the population susceptible to poliovirus. A significant proportion of the adult population of Ukraine has low antibody titers to poliovirus types 1 and 2; 66.4% of the population had no protective antibodies against type 3 of poliovirus. Children under 14 years of age had protective antibody titers to poliovirus types 1, 2 and 3 of 1:32 to 1:64. The adequate antibody level among the individuals below the age of 14 years can be explained by recent vaccination.

***Table* 1. Immunity to poliovirus types 1, 2 and 3 among the population of Ukraine, 2009-2011**

Year	Poliovirus Type 1									
	Antibody titers									
	0 M±m	1:4 M±m	1:8 M±m	1:16 M±m	1:32 M±m	1:64 M±m	1:128 M±m	1:256 M±m	1:512 M±m	1:1024 M±m
2009	2,2±0,1	5,5±0,4	10,8±0,5	15,8±0,4	18,9±0,7	21,5±0,7	16,2±0,6	6,7±0,4	1,3±0,2	0,3±0,1
2010	2,7±0,2	3,8±0,3	11,5±0,5	16,1±0,6	20,5±0,7	20,2±0,7	17,2±0,6	6,9±0,4	1,0±0,1	0,1±0,05
2011	2,2±0,2	3,3±0,3	10,3±0,5	15,7±0,6	22,0±0,7	21,9±0,6	15,1±0,6	5,9±0,4	3,3±0,3	0,3±0,09
	Poliovirus Type 2									
2009	1,5±0,2	5,3±0,4	11,4±0,5	17,8±0,6	21,3±0,7	22,1±0,7	14,1±0,6	5,0±0,4	1,0±0,2	0,05±0,04
2010	2,4±0,2	3,9±0,3	11,8±0,5	16,6±0,6	23,5±0,7	20,0±0,4	14,9±0,6	5,8±0,4	1,1±0,2	0,1±0,05
2011	2,1±0,2	3,3±0,3	9,7±0,5	17,7±0,6	23,9±0,7	20,5±0,6	14,2±0,6	5,6±0,4	2,8±0,3	0,2±0,07
	Poliovirus Type 3									
2009	4,5±0,3	8,1±0,5	18,7±0,7	19,4±0,7	21,6±0,7	16,1±0,6	8,0±0,5	2,6±0,3	0,4±0,1	0,02±0,02
2010	5,8±0,4	7,8±0,4	17,6±0,6	20,3±0,6	21,6±0,6	15,7±0,6	8,7±0,4	2,3±0,2	0,2±0,1	
2011	5,6±0,2	6,4±0,4	16,6±0,6	22,1±0,7	23,0±0,7	15,8±0,6	7,2±0,4	2,4±0,2	0,8±0,1	

References

1. Nathanson N., Olen M. FromEmergence to Eradication: The Epidemiology of Poliomyelitis Deconstructed. Am. J. Epidemiol. – 2010. – Vol. 17, №1 – P. 1-17.
2. M. Tardy-Panit, B. Blonder, A. Martin et al. A mutation in the RNA polymerase of polio type 1 contribute to attention in mice. Journal of Virology. 1993 August; №67:4630-4638.
3. World Health Organization. Global eradication of poliomyelitis by the year 2000. Geneva: WHO; 1988. World Health Assembly Resolution WHA41.28.
4. R.B. Aylward, J. Linking Policy and Practice. Polio eradication: mobilizing and managing the human resources. Bulletin of WHO 2005;83: 268-273.
5. World Health Organization Media Centre. Fact sheet №114, October 2012. Available at: http://www.who.int/mediacentre.
6. Bill & Melinda Gates Foundation. Polio strategy. November 13, 2009. Available at: http://www.gatesfoundation.org/polio/Documents/polio-white-paper.pdf.
7. Update: risk assessments and risk mitigation activities in polio-free areas. Working Group on Risk Assessments for Polio-free Regions. October 2012. Available at: http://www.polioeradication.org.
8. Decree of the Ministry of Health of Ukraine #276 "On procedures for conducting prophylactic vaccinations in Ukraine" dated 31 October, 2010.
9. Decree of the Ministry of Health of Ukraine #196 "On strengthening measures for preventing poliomyelitis in Ukraine" dated 14 July, 1998.

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ПІДТРИМАННЯ СТАТУСУ УКРАЇНИ, ЯК КРАЇНИ, ВІЛЬНОЇ ВІД ПОЛІМІЄЛІТУ

У статті представлені дані за охопленням вакцинацією проти поліомієліту вікових груп населення України в 2009-2012 роках. Представлені результати дослідження імунітету до вірусів поліомієліту в різних групах населення в 2009-2011 рр.. Враховуючи поточну ситуацію з поліомієліту у світі, можливість завезення диких поліовірусів з ендемічних країн на територію України, необхідним є контроль стану колективного імунітету, для підтримки статусу України як території, вільної від поліомієліту.

Ключові слова: поліомієліт, Україна, імунітет.

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ПОДДЕРЖКА СТАТУСА УКРАИНЫ, КАК СТРАНЫ СВОБОДНОЙ ОТ ПОЛИОМИЕЛИТА

В статье представлены данные по охвату вакцинацией против полиомиелита возрастных групп населения Украины в 2009-2012 годах. Представлены результаты исследования иммунитета к вирусам полиомиелита в различных группах населения в 2009-2011 гг. Учитывая текущую ситуацию по полиомиелиту в мире, возможность завоза диких полиовирусов из эндемичных стран на территорию Украины, необходимым является контроль состояния коллективного иммунитета, для поддержания статуса Украины как территории, свободной от полиомиелита.

Ключевые слова: полиомиелит, Украина, иммунитет.

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TOMATO SPOTTED WILT VIRUS ON PEPPER *(CAPSCIUM ANNUUM* L.) PLANTS IN HUNGARY

In Hungary resurgence of Tomato spotted wilt virus (TSWV) frequently causes heavy crop losses in pepper production since the mid nineties. Management of TSWV control was first directed against the thrips (using different insecticides or plastic traps), and against weeds as host plants of the virus and the thrips. Later on Tsw resistance gene was introduced into different types of pepper. In 2010 and 2011 sporadically, but in 2012 more frequently a resistance breaking strain of TSWV on resistant pepper cultivars was observed in the Szentes region (Hungary). It is supposed that outbreaks of TSWV infection was due to the fact that protection against Frankliniella occidentalis was neglected and some effective pesticides (like Unifos 50 EC) were withdrawn.

Key wods: Tomato spotted wilt virus, Frankliniella occidentalis.

Introduction. Tomato spotted wilt virus (TSWV) is the type member of the genus Tospovirus (family Bunyaviridae), causes an important disease of horticultural and agronomic crops. The virus distributed worldwide is having extremly broad host range and is now considered as one of the ten most economically destructive plant viruses [1, 6, 15]. TSWV is transmitted by thrips in a persistant manner [4]. The virion varies in size from 80 to 120 nm and has spherical enveloped character [5]. The genome of TSWV consists of three ssRNA segments: small (S) and medium (M) RNAs have ambisense coding strategies, whereas the large (L) RNA is of negative polarity.

In Hungary TSWV was described in 1972, but the virus was not considered as an important pathogen. In 1995 very severe damage of TSWV infection was observed in tomato and pepper production in the Szentes vegetable growing region (Hungary) (Fig.1). The introduction and spread of western flower thrips (*Frankliniella occidentalis*), an efficient TSWV vector, in that time certainly played an important role in TSWV emergence [5].

Fig.1. Symptoms of TSWV infection on susceptible pepper cultivar: chlorotic spots and rings on leaves (A) and fruits (B)

Management of TSWV control was first directed against the thrips using different insecticides or plastic traps, and against weeds as host plants of the virus and the thrips. Later on *Tsw* resistance gene [3] was introduced into different types of pepper (conical white, long pale green hot and sweet, tomato shape, spice pepper and blocky types) (Csilléry unpublished). Pepper cultivars carrying *Tsw* resistance gene upon TSWV inoculation show necrotic local lesions on the leaves or other parts of the plant without systemic infection (Fig. 2).

In 2010 and 2011 sporadically, but in 2012 more frequently systemic virus symptoms were observed on resistant pepper cultivars in Szentes region [2, 4, 12] (Fig. 3). The presence of new resistance breaking strain of TSWV was proved by virological (test-plant, serological and RT-PCR) methods. It was demonstated that TSWV can adapt very rapidly to plant resistance, and the *Tsw* resistance gene was broken down only a few years after its deployment in pepper crops [9, 11, 13, 14].

Fig. 2. Symptoms of TSWV infection on resistant pepper varieties: necrotic spots on stem (A) and fruit (B, C)

Fig. 3. Systemic symptoms of TSWV infection on resistant pepper cultivars

Materials and methods. *Virus isolates.* TSWV isolates originated from pepper cultivars susceptible and resistant against TSWV from Szentes region (South-east Hungary). Fruit samples were collected from plants exhibiting typical symptoms of virus infection such as stunting, mosaic, chlorotic and/or necrotic spots, rings and distortion on the leaves and fruits (Figure 3). The isolates were used for ELISA serological tests, RT-PCR and maintained by mechanical inoculation on *Nicotiana tabacum* cv. Xanthi-nc test plants.

*RNA extraction, RT-PCR.*Total RNA was extracted from leaves of *N. tabacum* cv. Xanthi-nc plants systemically infected by TSWV or from infected pepper fruits using the Spectrum Plant Total RNA Kit (Sigma) following the manufacturer's instructions. RT-PCR reactions for synthesis of first-strand cDNA were performed with Revert Aid H Minus First Strand cDNA Synthesis Kit (Thermo Science). Specific primers TSWV-185CPforward (5'-AAT TTC TCC GCA ATC TAT TTC AGT TG-3') and TSWV-183CP reverse (5'-GGG GAT CCA GAG CAA TTG TGT CAA TTTT-3') amplified 1720 bp fragment of N and the non-coding genomregions. PCR reaction was performed in 25µl – 50 µl final volume. Amplification consisted of one cycle at 94°C for 5 min, followed by 35 cycles of 30 sec of denaturation at 94°C, 1 min of annealing at 50°C; and 2 min of extension at 72°C; and then one cycle of final extension for 10 min at 72°C. PCR products were electrophoresed in 1% agarose and stained with ethidium bromide.

Results. The collected samples showed typical symptoms of Tomato spotted wilt virus infection. The virus was transmitted by mechanical inoculation onto test plants. On *Nicotiana tabacum* cv *Xanthi*-nc plants chlorotic and necrotic spots and rings on inoculated leaves and systemic mosaic or necrotic rings or necrosis were observed (Fig. 4). Slight differences on symptoms were observed among different isolates independently whether originated from TSWV susceptible or resistant pepper cultivars. Samples for ELISA serological test were taken from the original fruits and from test plants. In all samples TSWV were detected.

Fig. 4. TSWV symptoms on *Nicotiana tabacum* cv *Xanthi*-nc plants

TSWV specific PCR-product was amplifyed by RT-PCR method (Fig. 5). Our results confirmed the presence of Tomato spotted wilt virus both in TSWV susceptible and resistance cultivars in Hungary. Our results confirmed the presence of the resistance breaking isolate of Tomato spotted wilt virus in Hungary. Further investigations needed to characterize the resistance breaking TSWV isolates from Hungary.

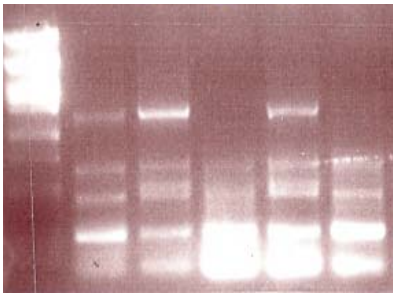

Fig. 5. Separation of amplified RT-PCR products of TSWV infected pepper plants on 1 % agarose gel stained with ethidium bromide. 1 – DNA lenght marker Pst I digested λDNA, Lane 2,3 and 5 TSWV infected pepper plants, Lane 4 – uninfected pepper plant, Lane 6 – healthy pepper plant

Earlier experiments predicted the determinant for breakdown *Tsw* resistance locating on RNA S of TSWV [7, 8]. For this reason our aim is in the future to characterize the S RNA of Hungarian resistance breaking isolates of TSWV and to compare them to other TSWV isolates.

It is supposed that outbreaks of TSWV infection in Szentes vegetable growing region is due to the fact that protection against *Frankliniella occidentalis* was neglected and some effective pesticide (like Unifos 50 EC) were withdrawn.

References

1. Adkins S. Tomato spotted wilt virus – positive steps towards negative success // Molecular Plant Pathology. – 2000. – V. 1. – P. 151-157.

2. Bese G., Krizbai L., Takács A.P. A paradicsom foltos hervadás vírus (Tomato spotted wilt virus, TSWV) rezisztencia áttörő törzs első megjelenése Magyarországon / Növényvédelmi Tudományos Napok. – Budapest, 2012.

3. Black L.L., Hobbs H.A., Kammerlohr D.S. Resistance of Capsicum chinense lines to tomato spotted wilt virus from Louisiana, USA, and inheritance of resistance // Acta Horticulturae. – 1996. – V. 431. – P. 393-401.

4. Csilléry G., Almási A., Tóbiás I. Occurence of resistance breaking strain of Tomato spotted wilt virus on resistant pepper cultivars in Hungary: The 21st Internatioanl Pepper Conference, November 4-6. – Florida, 2012.

5. Gáborjányi R., Csilléry G., Tóbiás I., Jenser G. Tomato spotted wilt virus: A new threat for pepper production in Hungary // IXth Eucapia Meeting, Budapest, – 1995.

6. Goldbach R., Peters D. Molecular and biological aspects of tospoviruses / Ed. by R. Elliott. – New York, 1996.

7. Jahn M., Paran I., Hoffmann K., Radwanski E.R., Livingstone K.D., Grube R.C., Aftergoot E., Lapidot M., Moyer J. Genetic mapping of the TSW locus for resistance to the Tospovirus Tomato spotted wilt virus in Capsicum spp. And its relationship to Sw-5 gene for resistance to the same pathogen in tomato // Mol. Plant Microbe Interact. – 2000. – V. 13. P. – 673-682.

8. Margaria P., Ciuffo M., Pacifico D., Turina M. Evidence that the nonstructural protein of Tomato spotted wilt virus is the avirulence determinant in the interaction with resistant pepper carrying the Tsw gene // Mol. Plant Microbe Interact. – 2007. – V. 20. – P. 547-558.

9. Margaria P., Ciuffo M., Turina M. Reistance breaking strain of Tomato spotted wilt virus (Tospovirus, Bunyaviridae) on resistant pepper cultivars in Almeria, Spain // Plant Pathol. – 2004. – V. 53. P. – 795.

10. Prins M., Goldbach R. The emerging problem of tospovirus infection and nonconventional methods of control // Trends Microbiol. – 1998. – V. 6. P. – 31-35.

11. Roggero P., Masenga V., Tavella L. Field isolates of Tomato spotted wilt virus overcoming resistance in pepper and their spread to other hosts in Italy // Plant Disease. – 2002. – 86. P. – 950-954.

12. Salamon P., Nemes K., Salánki K. A paradicsom foltos hervadás vírus (Tomato spotted wilt virus, TSWV) rezisztenciatörő törzsének első izolálása paprikáról (Capsicum annuum L) Magyarországon / Növényvédelmi Tudományos Napok. – Budapest, 2010.

13. Sharman M., Persley D.M. Field isolates of Tomato spotted wilt virus overcoming resistance in Capsicum in Australia // Australasian Plant Pathology. – 2006. – V. 35. P. – 123-128.

14. Thomas-Caroll M.L., Jones R.A.C. Selection, biological properties and fitness of resistance-breaking strains of Tomato spotted wilt virus // Ann Appl. Biol. – 2003. – V. 142. P. – 235-243.

15. Tomlinson J.A. Epidemiology and control of virus diseases of vegetables // Annals of Applied Biology. – 1987. – V. 110. – P. 661-681.

16. Whitefield A.E., Ullman D.E., German T.L. Tospovirus-thrips interactions // Ann. Rev. Phytopath. – 2005. – V. 43. – P. 459-489.

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ВІРУС ПЛЯМИСТОГО В'ЯНЕННЯ ТОМАТУ НА РОСЛИНАХ ПЕРЦЮ СОЛОДКОГО ІРУС (*CAPSICUM ANNUM* L.) В УГОРЩИНІ

З середини 1990-х років спалахи захворювання, спричиненого вірусом плямистого в'янення (бронзовості) томату (Tomato spotted wilt virus (TSWV)) в Угорщині, часто призводять до значних втрат врожаю комерційних посівів перцю солодкого. Початково зусилля при боротьбі з TSWV спрямовувалися на контролювання векторів вірусу – трипсів (шляхом використання різних інсектицидів чи пластикових пасток) та бур'янів, які виступають хазяями для вірусу та трипсів. Пізніше були створені різноманітні трансгенні сорти перцю з інтродукованим геном стійкості до даного вірусу – Tsw. Починаючи з 2010-2011 рр. у регіоні Сентеш в Угорщині траплялися поодинокі, а з 2012 р. – все частіші випадки появи нової форми TSWV, яка була здатна до подолання стійкості трансгенних рослин перцю. Вважається, що спалахи інфекції, викликаної TSWV, спричинені невиконанням рекомендацій щодо контролю трипса Frankliniella occidentalis та припиненням використання деяких ефективних пестицидів (наприклад, Unifos 50 EC). Дана робота присвячена вивченню цієї проблеми.

Ключові слова: вірус плямистого в'янення томату, трипс Frankliniella occidentalis.

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ВИРУС ПЯТНИСТОГО УВЯДАНИЯ ТОМАТА НА РАСТЕНИЯХ ПЕРЦА СЛАДКОГО (*CAPSICUM ANNUM L.*) В ВЕНГРИИ

С середины 1990-х годов вспышки заболевания, вызванного вирусом пятнистого увядания (бронзовости) томата (Tomato spotted wilt virus (TSWV)) в Венгрии, часто приводит к значительным потерям урожая коммерческих посевов перца сладкого. Изначально усилия при борьбе с TSWV направлялись на контроль векторов вируса – трипсов (путем использования различных инсектицидов или пластиковых ловушек) и сорняков, которые выступают хозяевами для вируса и трипсов. Позже были созданы разнообразные трансгенные сорта перца с интродуцированным геном устойчивости к данному вирусу – Tsw. Начиная с 2010-2011 гг в регионе Сентеш в Венгрии случались единичные, а с 2012 г. – все чаще случаи появления новой формы TSWV, которая была способна к преодолению устойчивости трансгенных растений перца. Считается, что вспышки инфекции, вызванной TSWV, вызванные невыполнением рекомендаций по контролю трипса Frankliniella occidentalis и прекращением использования некоторых эффективных пестицидов (например, Unifos 50 EC). Данная работа посвящена изучению этой проблемы.

Ключевые слова: вирус пятнистого увядания томата, трипс Frankliniella occidentalis.

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EFFICIENCY OF SEROLOGICAL KITS OF DIFFERENT MANUFACTURERS IN DEFECTION OF ANTIBODIES AGAINST PRRVS CIRCULATION IN UKRAINE

Stadejek et al. have studied the efficiency of five serological test kits, which are in wide use across the world, showing that their sensitivity differs in relation to the geography of PRRSV isolation. Karniychuk et al. confirmed an essential genetic and antigenic distinctions between East European PRRSV isolates and West European and North American strains of the virus. Our results on differences in the sensitivity of serological kits to detect anti-PRRSV antibodies comply with the data published earlier. When using a BIONOTE kit false positive results were received whereas the application of a CIVTESTsuisPRRS kit might lead to the significant number of false negatives questioning the expediency to use these test kits. Our findings lead us to conclude that a serological IDEXX HerdCheck PRRS 3XR ELISA kit is the most sensitive to Ukrainian PRRSV strains while an Ingezim PRRS Universal test kit may also be used to detect antibodies against this virus in Ukraine.

Key words: PRRVS, strains, serological kits, antibodies.

Introduction. A porcine reproductive and respiratory syndrome virus (PRRSV) is a member of Arterevirus genus, Artereviridae family and Nidovirales order [1]. Virus isolates are usually attributed either to the North American and European strains that have some distinctions in genome. The fact that these genetically different types of the virus appeared practically simultaneously presents one of the today's mysteries [2]. The analysis of their nucleotide sequences and antigen properties demonstrated that the North American (type 2) and European (type 1) PRRSV strains are only 63% identical at genome level [1]. Evolutional variability of this virus is suggested to be the highest among RNA viruses [3].

Until 2010 it was thought that the ORF7 is the most conservative gene of PRRSV. That is why it was widely used in diagnostic in test kits based on RT-PCR and real time PCR techniques [4]. However, Stadejek et al. analyzing European isolates of the virus found out the significant genetic variability among the ORF7 sequences which was especially high in East Europe where four main virus subgroups were revealed [4, 5]. Such high variability makes significantly harder to diagnose the related disease correctly, to develop efficient and safe vaccines as well as to control the disease.

Clinical signs of PRRSV infection vary depending on the virus virulence, immune status of the herd and age of infected animals. Viremia leads to clinical manifestation of the disease. PRRSV capable to cross transplacentary barrier and infect fetus causing abortions, stillbirths and births of weakened piglets [6, 7].

The International Epidemiological Bureau marks out, as the most efficient, several techniques of PRRSV diagnostics: virus isolation, serological tests, PCR and real time PCR. Serological tests are a potent and sensitive approach used in schemes of PRRSV control [3, 8]. Seroconversion can be identified 7-11 days after animals been infected using proven, highly sensitive and specific serological kits [9]. An analysis is performed in serum sampled from animals of an infected herd belonging to different age groups.

Blood serum specimens are tested with time intervals (for example, at the time of clinical signs development and then in 2–3 weeks) providing the basis for serological diagnostics. Such approach is also applied to control the results of vaccination. It is important to take into account the presence of maternal anti-PRRSV antibodies in serum specimens. The level of these antibodies gradually decreases up to 9th week of animal life [10]. According to literature the number of blood serum specimens sampled from a single

farm has to be no less than 12 in order to obtain statistically valid results [11, 12].

At the moment, an immuno-enzyme analysis (IEA) is regarded as an easily available, sufficiently reliable technique the advantages of which include fast results and high specificity. Taking into account the widespread occurrence of PRRSV in Ukraine [13, 14] as well as its antigen variability [15] the aim of our study was to evaluate the efficiency of four basic serological test kits being used in Ukraine for detection of antibodies against Ukrainian isolates of the virus.

Material and methods

Blood serum samples were collected from animals of the main herd in different farms to assess titers of anti-PRRSV antibodies. The biological material was transported in thermo containers filled with ice. The volume of a sample was equal to 3 ml. When sampling specimens the following data were recorded: clinical signs, farm's technology details, animal age, farm name, province. In total, 93 samples of blood serum from eight farms in seven provinces of Ukraine were taken for analysis.

The titer of anti-PRRSV antibodies in blood serum samples was assessed with IEA using a diagnostic HerdChek*PRRS X3 kit manufactured by IDEEX (USA). Tests were performed in accordance with the instruction of a kit manufacturer.

11 serum samples originated from five Ukrainian farms were selected. Tests were performed simultaneously in order to minimize the influence of a sample quality, test conditions and kit series. Farms providing serum samples use different breeding companies to rebuild their herds, are territorially separated and do not trade with one another. Herds in these farms were serologically PRRSV positive during six months preceding the tests. This allowed us to exclude management as a factor influencing the similarity/difference of properties for pathogens circulating in these farms.

Further, blood serum samples having different titers of anti-PRRSV antibodies were selected (low titer, high titer and titer near the cutoff). These samples were analyzed on the presence of anti-PRRSV antibodies using the following kits: Ingezim PRRS Universal 11.PRU.K1 (Spain), BIONOTE (Korea), CIVTESTsuisPRRS (HIPRA, Spain) and repeatedly IDEXX HerdCheck PRRS 3XR ELISA (USA). Tests were performed in accordance with the instruction of a kit manufacturer.

Results and Discussion

A PRRSV variability presents the greatest challenge to its efficient diagnostics. Stadejek et al. have studied the efficiency of five serological test kits which are in wide use across the world demonstrating that their sensitivity differs in relation to the geography of PRRSV isolation. In addition, Stadejek stressed that a strict separation in genetic characteristics of PRRSV exists between East and West Europe explaining it by the absence of active trade between countries of the former Soviet Union and Europe. For example, according to their genetic characteristics PRRSV samples isolated on the territory of Poland were attributed to a single PRRSV subgroup while Belorussian isolates were thought to belong to three genetic subgroups [4].

Data received in two Ukrainian farms show that all four test kits produced similar results in detection of anti-PRRSV antibodies (Table 1).

***Table* 1. Comparison of serological kits in tests of pig blood serum samples on the presence of anti-PRRSV antibodies**

Farm	№ Sample	"IDEXX HerdCheck PRRS 3XR ELISA", USA *			"CIVTESTsuisPRRS", HIPRA, Spain **		"BIONOTE", Korea ***		"Ingezim PRRS Universal 11.PRU.K1", Spain ****	
		OD	Titre	Result	OD	Result	OD	Result	OD	Result
1	1	2,984	7554	pos	150	pos	1,53	pos	2,504	pos
2	2	3,012	7612	pos	80	pos	1,53	pos	2,845	pos
	3	0,699	1551	pos	19	sub	0,91	pos	0, 53	pos
3	4	2,932	7480	pos	125	pos	1,53	pos	1,606	pos
	5	0,396	837	sub	9	neg	0,77	pos	0,412	sub
	6	0,059	105	neg	0	neg	0,46	pos	0,119	neg
	7	0,456	976	pos	2	neg	0,74	pos	0,432	sub
4	8	0,556	1208	pos	0	neg	0,18	neg		neg
	9	2,601	6494	pos	0	neg	0,27	neg		neg
	10	0,042	74	neg	0	neg	0,24	neg	0,141	neg
5	11	0,384	805	sub	24	pos	0,84	pos	0,609	pos

* "IDEXX HerdCheck PRRS 3XR ELISA", USA – pos >0,4; titer> 844.
** "CIVTESTsuisPRRS", HIPRA, Spain – pos >20.
***"BIONOTE", Korea – pos >0,4.
****"Ingezim PRRS Universal 11.PRU.K1", Spain – pos >0,450.

Only an IDEXX HerdCheck PRRS 3XR ELISA kit identified anti-PRRSV antibodies in farm #4 samples while the results of tests in these samples performed with other test kits were negative ones. It is worth to note that false negative results enable the spread of the disease, which is highly contagious, among animals of the herd causing significant economic losses due to abortions, stillbirths, births of weakened piglets and development of respiratory symptoms in adult animals. From the other hand, false positives are also impermissible drawbacks in PRRSV diagnostics. In order to exclude the possibility that test results for farm #4 samples were false positives, blood serum was sampled again from the same animals. Titers of anti-PRRSV antibodies and their dynamics pointed to the presence of PRRSV infection.

False positives were received in two specimens when using a BIONOTE kit. A CIVTESTsuisPRRS kit appeared to be less sensitive to detect anti-PRRSV antibodies in samples with levels near the cutoff. It is also necessary to add that sample #11 was shown to be PRRSV positive by tests performed with a Ingezim PRRS Universal, BIONOTE and CIVTESTsuisPRRS kits whereas IDEXX HerdCheck PRRS 3XR ELISA kit showed a negative result the value of which was, however, near the cutoff.

Thus, data received indicate the difference in the sensitivity between all commercial serological kits being currently used in Ukraine that comply with results Karniychuk et al. published earlier. These authors confirmed significant genetic and antigenic distinctions between PRRSV isolated in East Europe and the virus isolated in West Europe and USA [15].

The study of a reproductive and respiratory swine syndrome is greatly important because this disease may cause enormous economic losses for pig farms. Contagiousness,

high levels of abortions and mortality related to aforementioned disease stipulates the necessity to implement efficient diagnostic strategy, as one of the measures allowing to combat the spread of PRRSV in pig farms of Ukraine. The use of serological tests is one of approaches to solve this problem. These tests help to ascertain a farm's epizootic situation, to identify infection times and intensity that is necessary for comprehensive estimate of PRRSV involvement in the development of respiratory and reproduction-related symptoms. In order to design an optimal preventive vaccination scheme it would be the most practical to obtain serological profiles at farm level.

Taking the aforementioned into account one may conclude that the sensitivity of serological test kits is a critical factor assuring that test results comply with real data. False positives are possible when using a BIONOTE kit whereas CIVTESTsuisPRRS kit may show significant number of false negatives questioning the expediency to apply these test kits. Data obtained allow to conclude that a serological IDEXX HerdCheck PRRS 3XR ELISA kit is the most sensitive with respect to Ukrainian PRRSV isolates. However, it should be stressed that the use of an Ingezim PRRS Universal test kit is also possible in Ukraine.

References:

1.Van Breedam W., Costers S., Vanhee M. et. al. Porcine reproductive and respiratory syndrome virus (PRRSV) – specific mAbs: supporting diagnostics and providing new insights into the antigenic properties of the virus // Veterinary Immunology and Immunopathology. – 2011. – P.1-12.

2. Kedkovid R. et. al. NSP2 genevariation of the North American genotype of the ThaiPRRSV incentral Thailand // VirologyJournal. – 2010. – Vol.7. – P.340-352.

3. Duinhof T.F., Schaik G. van, Esch E.J.B. van, Wellenberg G.J. Detection of PRRSV circulation in herds without clinical signs of PRRSV: Comparison of five age groups to assess the preferred age group and sample siza // Veterinary Microbiology. – 2001. – Vol.150. – P. 180-184.

4. Stadejek T., Balka G., Chabros K., Lipej Z., Novosel D., Stankevicius A. Genetic diversity of European genotype PRRS in Central an Eastern Europe – an update // 21st international pig veterinary society congress. – Vancuver, 2010.

5. Lipej Z., Novosel D. An overview about PRRSS in Croatia // The Balkan meeting on PRRS diagnostic. – Hrvatska, 2011.

6. Kim H.K., Yang J.S., Moon H.J. et al. Genetic analysis of ORF5 of recent Korean porcine reproductive and respiratory syndrome virus (PRRSVs) in viremic sera collected from MLV-vaccinating or non-vaccinating farms // Journal of Veterinary Science. – 2009. – Vol.10. – P.121-133.

7. Forsberg R. Divergense time of porcine reproductive and respiratory syndrome virus subtypes// Mol. Boil. Evol. – 2005. – Vol 2. – P. 2131-2134.

8. World organization for modern health. PRRS: the disease, its diagnosis, prevention and control // Report of the OIE AD HOC group on reproductiveandrespiratorysyndrome. – Paris. – 2004.

9. Rownald R.R. The interaction between PRRSV and the late gestation pig fetus // Virus Research – 2010. – Vol. 154 – P.114-122.

10. Virus taxonomy. Classification and nomenclature of viruses : eight repot of the International Comettee of Viruses // C.M.Fauquet, M.A. Mayo, J. Maniloff, U. Desselberger.

11. Kim S.H., Roh I.S., Choi E.J., Lee C., Lee C.H., Lee K.H., Lee K.K., Song Y.K., Lee O.S., Park C.K. A molecular analysis of European porcine reproductive and respiratory syndrome virus isolated in south Korea // Vet Microbiol. – 2010. – Vol. 143. – P. 394-400.

12. Nodelijk G., Nielen M., De Jong M.C.M., Verheijden J.H.M. A review of porcine reproductive and respiratory syndrome virus in Dutch breeding: population dynamics and clinical relevance // Prev.Vet. Met. – 2003. – Vol. 60. – P. 37-52.

13. Ivashchenko O. The spread of porcine reproductive and respiratory syndrome virus in ukraine from 2005 to July 2012 year // EuroPRRS2012 (Budapest, Hungary, 10-12 October 2012). – P. 74-78.

14. Dudar L., Ivashchenko O. Prevalance of PRRSV and PCV-2 associated abortions in Ukraine in 2007-2012 period // 5th European symposium of porcine health management (Edinburgh, United Kindom, 22nd-24th May 2013). – P. 132.

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ЕФЕКТИВНІСТЬ ВИКОРИСТАННЯ СЕРОЛОГІЧНИХ ТЕСТ-СИСТЕМ РІЗНИХ ВИРОБНИЦТВ ДЛЯ ДЕТЕКЦІЇ АНТИТІЛ ВРРСС, ЩО ЦИРКУЛЮЮТЬ В УКРАЇНІ

Tomasz Stadejek та його колеги дослідили ефективність 5-ти найпоширеніших серологічних тест-систем у світі та показали їх різну чутливість в залежності від географії виділення ізолятів ВРРСС. Karniychuk та співав. підтвердили значну генетичну та антигенну відмінність ВРРСС, ізольованого зі східної Європи, у порівнянні із західноєвропейським та американським штамами ВРРСС. Наші результати різності чутливості серологічних тест-систем для детекції антитіл до ВРРСС корелюють з раніше опублікованими результатами. При використанні тест-системи виробництва "BIONOTE" детектовані хибно позитивні результати, тоді як тест-система "CIVTESTsuisPRRS" може показувати значну кількість хибно негативних результатів, що в свою чергу вказує на недоцільність використання даних тест-систем. За отриманими даними можна зробити висновок, що найчутливішою до українських штамів ВРРСС є серологічна тест-система "IDEXX HerdCheck PRRS 3XR ELISA", проте використання в Україні тест-системи "Ingezim PRRS Universal" також можливе.

Ключові слова: PRRVS, серологічні комплекти, антитіла.

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ЭФФЕКТИВНОСТЬ ИСПОЛЬЗОВАНИЯ СЕРОЛОГИЧЕСЧКИХ ТЕСТ-СИСТЕМ РАЗНЫХ ПРОИЗВОДИТЕЛЕЙ ДЛЯ ДЕТЕКЦИИ АНТИТЕЛ ВРРСС, КОТОРЫЕ ЦИРКУЛИРУЮТ НА ТЕРРИТОРИИ УКРАИНЫ

Tomasz Stadejek и его коллеги исследовали эффективность 5-ти распространенных серологических тест систем в мире и показали их различную чувствительность в зависимости от географии выделения изолятов ВРРСС. Karniychuk и соавт. подтвердили значительное генетическое и антигенное различие ВРРСС, изолированных из восточной Европы по сравнению с западноевропейским и американским штаммами ВРРСС. Наши результаты о разной чувствительности серологических тест- систем для детекции антител к ВРРСС коррелируют с ранее опубликованными результатами. При использовании тест-системы производства "BIONOTE" детектировались ложноположительные результаты, тогда как тест-система "CIVTESTsuisPRRS" может показывать значительное количество ложноотрицательных результатов, что в свою очередь указывает на нецелесообразность использования данных тест-систем. По полученным данным можно сделать вывод, что наиболее чувствительной к украинским штаммам ВРРСС является серологическая тест-система "IDEXX HerdCheck PRRS 3XR ELISA", однако использование в Украине тест-системы "Ingezim PRRS Universal" также возможно.

Ключевые слова: PRRVS, серологические комплекты, антитела.

UDC 578.85/.86

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IDENTIFICATION OF VIRUSES AFFECTING GLADIOLUS IN KYIV REGION

The plants of different cultivars of Gladiolus from Kyiv region were analyzed for viruses. The viruses were identified by the methods of bioassay, ELISA and electron microscopy. Tomato aspermy virus was identified in the samples of Gladiolus plants.

Key words: ELISA, viruses, gladiolus.

Introduction. The *Gladiolus* genus belongs to the *Iridaceae Juss* family. It includes more then 150 species. Most of them are native to Africa and few originated from the Mediterranean area, Asia and South Europe. Cultivars of gladiolus exhibit a great diversity of colour, size, shape, flowering time, and bulbing and dormancy behaviour [2]. Gladiolus is an important ornamental plant grown for commercial purposes, bouquets, floral baskets and cut flowers in Ukraine. Viruses represent a major problem for gladiolus and other bulb crops because the plants are propagated each year by bulbs that may harbor a virus. These viruses can cause extensive losses in the quality and quantity of flowers and corms. They cause significant economic losses to floriculture. The most widespread symptoms of virus infections in gladiolus plantings are overall stunting, color-breaking, flower distortion, reduced flower and cormel production, severe mosaic in leaves, stunted plants, and color -breaking in flowers, notched leaf blade margin symptoms.

Gladiolus plants may be infected by a number of different viruses or by strains of a particular virus [1]. The main viruses reported from gladioli in different countries are: *Tomato ringspot virus* (ToRSV), *Tomato black ring virus* (ToBRV), *Tomato spotted wilt virus* (TSWV), *Tobacco mosaic virus* (TMV), *Tobacco ringspot virus* (TRSV), *Arabis mosaic virus* (AMV), *Bean yellow mosaic virus* (BYMV), *Cucumber mosaic virus* (CMV), *Tobacco rattle virus* (TRV), *Tobacco streak virus* (TSV) [3, 4].

None of the viruses found on gladioli was specific to the crop species or to the *Iridaceae* family. All of these viruses have a wide host range as well as geographic distribution and some of them can be transmitted by vectors. Unfortunately the early diagnosis of virus diseases is not essential for establishing management measures. In addition to the aboveground parts of plants the corms could be a source of virus infection. To improve the quality of planting material and minimizing viral affection of gladiolus the timely diagnosis is of essential importance. Thus, identification of the source of infection, including the presence of the pathogen in planting material, is effective in controlling spread of viruses of gladioli and termination of epiphytoties.

The aim of the work was to investigate and identify the viruses affecting gladiolus.

Marerials and methods. For detection and identification of viruses affecting gladiolus we used 22 samples of 3 different cultivars ('Nizhnist', 'Nichnyj bluz', 'Asol') from private gardens of flower growers collected in Baryshivka town in Kyiv region. The samples were collected from gladioli expressing viral symptoms on leaves and flovers.

Virus identification was carried out using standard DAS-ELISA and indirect ELISA [9]. The samples were prepared by homogenizing plant tissue with 0.1 M phosphate buffer (pH 7.4) in the ratio 1:3 (m/v), followed by centrifugation at 5000 rpm for 20 min. For the diagnostics we used polyclonal antiserums to TMV (antiserum obtained at the virology department, the sensitivity and specificity confirmed experimentally), *Turnip mosaic virus* (TuMV) (antiserum kindly provided by Lesemann D.E., Julius Kühn Institute, Federal Research Center for Cultivated Plants, Institute for Epidemiology and Pathogen Diagnostics, Germany), *Tomato aspermy virus* (TAV), ToRSV, TRSV, CMV, *Arabis mosaic virus* (ArMV), *Pepper mild mottle virus* (PMMoV), *Tomato spotted wilt virus* (TSWV), *Tobaco rattle virus* (TRV) (Loewe, Germany).

Futher, viruses were identified by bioassay and study of virus particle morphology. Biological properties of viruses were studied using the range of test plants: *Nicotiana tabacum, Cucumis sativus, Lycopersicon esculentum, Phaseolus vulgaris, Capsicum annum, Tetragonia expansa, Zinnia elegans*. The test plants were inoculated on early growth stages by mechanical sap transmission, applying carborundum as an abrasive.

The morphology of virions was studied in leaf dip preparations negatively stained with 2% uranyl acetate. Electron microscopy (EM) was carried out using a JEOL-1400 electron microscope at the magnification of 40 000 and 60 000.

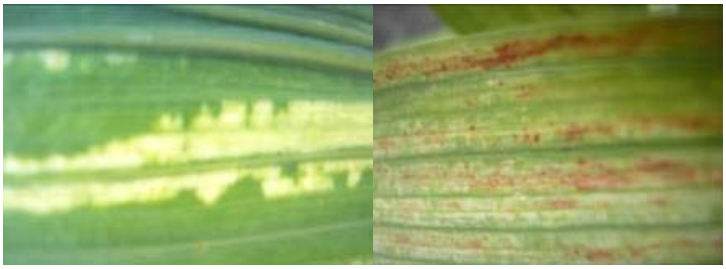
Results and discussion. Viral symptoms on gladiolus plants are not specific and common for wide range of viruses. Moreover, imbalance of mineral elements, their lack, high intensity lighting, damage by insects and mites, bacterial and fungal infections or genetic disorders can cause symptoms similar to those of viral infection. Although viral infection could be diagnosed in advance by specific symptoms such as ring spot, mosaic and the necrosis.

The samples were collected from gladioli expressing viral symptoms on leaves and flovers. Leaves of naturally infected gladiolus plants exposed chlorotic spots and streaks of irregular shape. Some samples demonstrated coarse breaking patterns, expressed as disappearance of pigment in flowers (Fig.1). Severe color breaking and deformation of flowers are the most common symptoms associated with CMV infection in gladiolus [9].

A B C

Fig.1. White and light gray blotches in flowers of *Gladiolus* 'Nichnyj bluz' (A), 'Asol' (B) and 'Nizhnist' (C)

Such symptoms as green mosaic, local necrotic lesions and striped mosaic had been detected on leaves of gladiolus 'Nizhnist' (Fig.2A). According to the literature data, the symptoms of mosaics could be caused by BYMV and TRV [5, 6]. As the mixed viral infection is common in gladiolus, the symptoms may be very variable. The different cultivars of gladiolus also contribute to variability of symptoms [7]. As the symptoms can be not only of viral origin, identification of the virus, based on symptoms alone, is difficult because the gladiolus can react differently even to the same virus.



A **B**

Fig.2. Leaf of infected gladiolus showing green mosaic (A), local necrotic lesions and striped mosaic (B)

To confirm the infectivity of the plant sap and to detect the biological properties of pathogens, we used biological testing on the range of test plants typical for gladioli viruses [7]. They produced different virus-like symptoms (Table 1).

***Table* 1. Symptoms on leaves of test plants inoculated with the sap of infected gladiolus**

Test plants	Symptoms					
	Nicotiana tabacum	*Cucumis sativus*	*Lycopersicon esculentum*	*Tetraggonia expansa*	*Capsicum annum*	*Phaseolis vilgaris*
Gladiolus 'Nichnyj bluz'	-	M	-	-	-	N
Gladiolus 'Nizhnist'	M	Cl.sp.	M, D	-	-	N
Gladiolus 'Asol'	-	M	-	-	-	-

M – mosaic; N – necrotic lesions; D – deformation of leaves; Cl.sp. – chlorotic spots; – symptomless.

Mosaic symptoms were observed on leaves of *Nicotiana tabacum* after inoculation with sap from plant *Gladiolus* 'Nizhnist'. These symptoms are typical for wide range of gladiolus viruses, such as TAV, CMV. However, symptoms of small local chlorotic spots on cotyledons of *Cucumis sativus* are typical for TAV infection. Besides, this virus causes necrotic reaction on leaves of *Phaseolus vulgaris* [8]. Symptoms registered on leaves of *Nicotiana tabacum* and *Phaseolus vulgaris* are characteristic for BYMV [6]. Other inoculated test plants showed no reaction on inoculation with gladiolus sap.

Hence, the biotesting confirmed the infectivity of the sap samples of gladiolus plants. Absence of symptoms on other testing plants after sap inoculation, in our opinion, cannot provide evidence of non-infectious nature of the disease because the pathogens may not be readily transmitted by mechanical inoculation and also may have different diagnostically susceptible host species.

For the virus identification we used DAS-ELISA and indirect ELISA with antisera to viruses, which are reported from gladioli: TMV, CMV, PMMoV, ToRSV, TRSV, TuMV, TAV, ArMV, TSWV and TRV. According to ELISA results, the antigens of TAV were detected in the samples of gladiolus 'Nizhnist', while the results of the testing of gladiolus 'Nichnyj blus" and 'Asol' with this serum were negative. Antigens of TMV, CMV, PMMoV, ToRSV, TRSV, TuMV, ArMV, TSWV and TRV have not been detected in tested samples.

The electron microscopy of the plant sap was carried out simultaneously with biotesting. One type of spherical virus-like particles was revealed in infected plant tissues. The particles of about 30 nm in diameter were detected in plants of Gladiolus 'Nizhnist' with stripe mosaic symptoms (Fig.3).

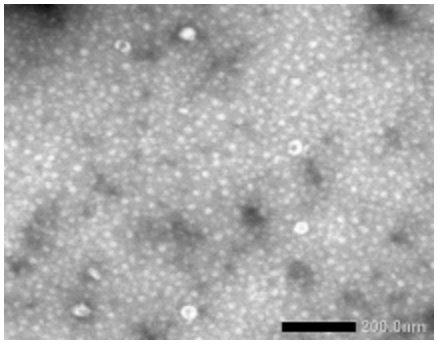


Fig.3. Electron micrograph of virus particles in the sap of gladiolus plants cultivar 'Nizhnist', bar 200 nm

On the basis or test plants' reaction data, ELISA, particle morphology and literature data [4, 8] the virus was identified as TAV.

Conclusion. The plants of different cultivars of gladiolus from Kyiv region were analyzed for presence of viruses. The pathogen detected in gladiolus plant was identified as *Tomato aspermy virus*. Methods of controlling viral diseases assume growing and propagating healthy planting material tested as virus-free, inspection of plants during vegetation for occurrence of symptoms and elimination of affected plants.

References

1. Arneodo J.D., S. de Breuil, Lenardon S.L., Conci V.C, Conci L.R. Detection of Bean yellow mosaic virus and Cucumber mosaic virus infecting gladiolus in Argentina // Agriscentia. – 2005. – Vol. XXII (2). – P. 87-89.

2. Bozarth R.F., Corbett M.K. Virus diseases of gladiolus in Florida // Florida state agricultural experiment stations journal series. – 1957. – N. 661. – P. 363-370.

3. Dubey V. K., Aminuddin, Singh V. P. Molecular characterization of Cucumber mosaic virus infecting Gladiolus, revealing its phylogeny distinct from the Indian isolate and alike the Fny strain of CMV // Virus Genes. – 2010. – N. 41. – P. 126–134.

4. Katoch M., Abdin M.Z., Ram R., Zaidi A.A. An overview of diagnostics for viruses infecting gladiolus // Crop Protection. – 2003. – N. 22. – P. 153–156.

5. Katoch M., Abdin M.Z., Zaidi A.A. First report of Tobacco rattle virus occurring in gladiolus in India // Plant Pathology. – 2004. – N. 53. – P. 236.

6. Katoch M., Ra R., Zaid A.A., Garg I.D. Status of Bean yellow mosaic virus on Gladiolus // Crop Protection. – 2002. – N. 21. – P. 861–865.

7. Navalinskienė M., Samuitienė M. Identification of agents of viral and phytoplasmal diseases affecting gladioli (Gladiolus L.). Acta Biol. Univ. Daugavp. – 2010. – Suppl. 2. – P.: 137 – 144.

8. Raj S.K., Kumar S., Verma D.K., Snehi S.K. First report on molecular detection and identification of Tomato aspermy virus naturally occurring on gladiolus in India // Journal of Phytopathology. – 2011. – Vol. 39, № 3. – P. 303-307.

9. Raj S. K., Srivastava A., Chandra G., Singh B.P. Characterization of Cucumber mosaic virus isolate infecting Gladiolus cultivars and comparative evaluation of serological and molecular methods for sensitive diagnosis// Current Science. – 2002. – N. 83. – P.1132–1137.

10. Серология и иммунохимия вирусов растений/ Р.В. Гнутова. – М.: Наука, 1993. – 301 с.

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ІДЕНТИФІКАЦІЯ ВІРУСІВ, ЯКІ УРАЖУЮТЬ ГЛАДІОЛУС У КИЇВСЬКІЙ ОБЛАСТІ

Проведено обстеження рослин різних сортів роду Gladiolus, відібраних на території Київської області, на наявність вірусних патогенів. Віруси детектували за допомогою методів біотестування, імоунофрментого аналізу та електронної мікроскопії. У зразках рослин було ідентифіковано вірус аспермії томатів.

Ключові слова: імуноферментний аналіз, віруси, гладіолус

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ИДЕНТИФИКАЦИЯ ВИРУСОВ, КОТОРЫЕ ПОРАЖАЮТ ГЛАДИОЛУС В КИЕВСКОЙ ОБЛАСТИ

Проведено обследование растений разных сортов рода Gladiolus, отобранных на территории Киевской области, на наличие вирусных патогенов. Вирусы детектировали с помощью методов биотестирования, имоунофрментого анализа и электронной микроскопии. В образцах растений был идентифицирован вирус аспермии томатов.

Ключевые слова: иммуноферментный анализ, вирусы, гладиолус

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PHYLOGENETIC ANALYSIS OF B/VICTORIA-LIKE INFLUENZA VIRUSES ISOLATED IN 2008-2012 IN UKRAINE

The article presents results of phylogenetic analysis of B/Victoria-like influenza viruses isolated in 2008-2012 epidemic seasons in Ukraine. Key mutations in amino acid sequences in hemagglutinin and neuraminidase proteins of Ukrainian isolates were analyzed. All Ukrainian B/Victoria-like influenza viruses type B located in Brisbane/60 cluster as the most isolates from all over the world according our data.

Key words: B/Victoria-like, phylogenetic analysis, Ukraine, Brisbane/60.

Introductoin. Family *Ortomyxoviridae* contains five different genes: *Influenza virus* A, *Influenza virus* B, *Influenza virus* C, *Isavirus* and *Togotovirus* [1]. Influenza B viruses belonged to the two genetic lineages: B/Victoria/2/87 and B/Yamagata/16/88 since the end of the XX century. Influenza viruses type B are less discovered than A type. Type B haven't animal host reservoir and pandemic potential. However, influenza B viruses could cause severe diseases and become the main infectious agent of influenza epidemic every three years [2,3].

The phylogenetic analysis applied to new influenza isolates allows monitor the rate and intensity of virus variations practically in real time. Moreover, the comparative analysis of their protein sequences allows reveal point amino acid replacements providing the mechanism of virus adaptation to human immune system [4]. Sequences of surface antigens – hemagglutinin (HA) and neuraminidase (NA) – are usually used for genetic analyses [5].

The **aim** of our work was to analyze the genetic diversity of B/Victoria-like influenza viruses isolated in Ukraine in 2008-2012.

Materials and methods. Nasal-throat swabs taken from patients with influenza-like illnesses (ILI) or severe acute respiratory infection (SARI) from different regions of Ukraine during 2008-2012 epidemic seasons in the study. They were analyzed using real-time polymerase chain reaction (RT-PCR). Influenza viruses type B were isolated in MDCK cell culture from samples positive in PCR. Hemagglutinin (HA) and neuraminidase (NA) gene sequences of Ukrainian isolates were selected to perform phylogenetic comparisons. Phylogenetic analysis was performed using MEGA 5 software [6].

Sequences of influenza viruses from other countries were received from GISAID resource (http://platform.gisaid.org/), using BLAST (Basic Local Alignment Search Tool) analysis (http://www.ncbi.nlm.nih.gov/ Blast.cgi). Sequences were aligned using ClustalW algorithm. Phylogenetic trees were built by the neighbor joining method [7] applying Kimura 2-parameter model [8]. Evolutional distances were calculated in terms of the number of base substitutions per site. A bootstrap technique with 1000 replications was used to test statistical validity of received data [9]. Nucleotide sequences were translated into amino acid sequences using MEGA 5 software.

Results and discussion. The B/Victoria-like influenza viruses circulated in 2008-2009, 2010-2011 and 2011-2012 epidemic seasons.

Season 2008-2009

*Comparison of hemagglutinin (HA) genes***.** Genetic comparison of the influenza virus type B HA genes shown that all investigated isolates were genetically related to vaccines strain B/Brisbane/60/2008 (Fig. 1). The Brisbane/60 cluster consists mainly of viruses among the different countries, but a small virus population from Middle East genetically related to the strain B/Malaysia/2506/2004.

Amino acid analyses of explored influenza HA genes identified four substitutions (K48E, K80R, K129N, A199T) in viruses of 2008-2009 influenza season compared with B/Brisbane/32/2002. That indicates the lower mutation rate of influenza viruses type B than type A, approved by the theoretical date [158]. The Brisbane/60 cluster viruses selected additional substitutions: N75K, N165K and S172P, also majority of viruses contain also mutation V146I, including explored isolates B/Kiev/69/2009 and B/Kiev/222/2009. The substitutions L58P (together with isolate from Madagascar), K136Q and S140T were occurred in the B/Kiev/58/2009 virus (Fig. 1).

*Comparison of neuraminidase (NA) genes***.** Genetic comparison of the 2008-2009 influenza season virus type B NA genes shown that all investigated isolates were closed to vaccines strain B/Brisbane/60/2008, estimated rate 98% (Fig. 2). As in the comparison of HA genes (Fig. 1), most of viruses from that season were presented in the Brisbane/60 cluster, but a short virus population from Middle East preserved genetical similarity to the strain B/Malaysia/2506/2004.

For all isolates from 2008-2009 influenza season were distinguished substitutions I248V and L396F, compared with the B/Brisbane/32/2002 strain. For B/Malaysia-like viruses group were noted by presence of mutations S41P and D463N (Fig. 2).

The Brisbane/60 cluster was indicated by existence of substitutions I204V and A358E, significant part of viruses also contain – D329N, including investigated isolates B/Kiev/69/2009 and B/Kiev/222/2009. Either Kiev isolates № 69 and 222, together with viruses from Norway, Russia, USA, Germany etc. received additional amino acid substitutions G378E and D463N (Fig. 2). The B/Kiev/58/2009 isolate, as in case with HA gene, became certain differences in neiraminidase that distanced it from other Kiev isolates on the phylogenetic tree. Along with Uganda virus Kiev isolate B/Kiev/58/2009 gain the I249V substitution.

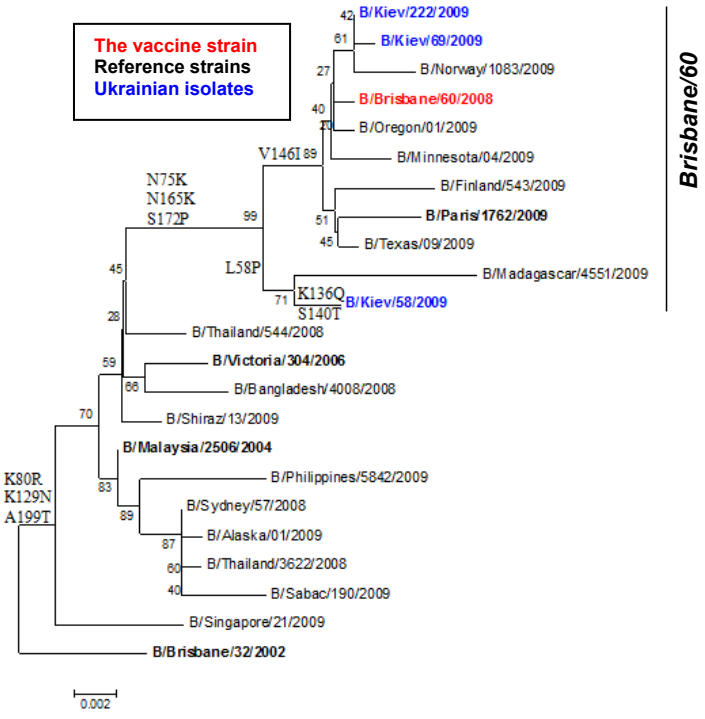

Figure 1. Molecular phylogenetic analysis of HA nucleotide sequences B/Victoria-like influenza viruses isolated in 2008-2009

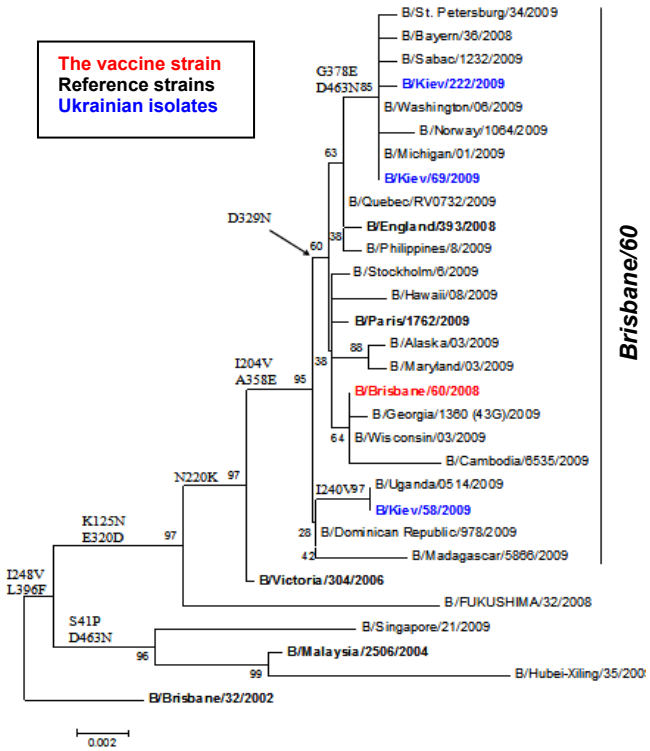

Figure 2. Molecular phylogenetic analysis of NA nucleotide sequences B/Victoria-like influenza viruses isolated in 2008-2009

Season 2010-2011. B/Victoria lineage viruses were prevalence in the world during that epidemic season (approximately 85%).

Comparison of hemagglutinin (HA) genes. Results of phylogenetic analyses are shown in the Figure 3. As in the previous 2008-2009 influenza season (Fig.1), that season viruses were similar to the vaccine strain B/Brisbane/60/2008. Low mutation rate with single amino substitution was observed in the investigated samples.

The B/Brisbane cluster viruses proceed selection of the mutations N75K, N165K, S172P i I199T. Isolates with L58P substitution developed new subcluster 2, which contains majority of the Ukrainian isolates from current season (Fig. 3). Urkainian isolate B/Odessa/3886/2010 was elected as reference strain by the Center for disease control and prevention (CDC) in London (Fig. 3).

In the investigated isolates were revealed single amino acid substitutions (Fig. 3): H122N – in the B/Zaporizzya/210/10; N197S – in the B/Kharkov/4260/10; T222A – in the B/Odessa/145/10 i B/Odessa/3886/10.

Comparison of neuraminidase (NA) genes. By genetic analyses results of neiraminidase genes (Fig. 4.), mostly explored isolates, including all Ukrainian viruses, were related to the vaccine strain Brisbane/60/2008 and belonged to the equal cluster. The isolates from Brisbane/60 cluster received the mutations I204V, A358E and most viruses had additional substitution P51S, L73F, N199D, but part of virus population also contained S27L.

Our isolated influenza viruses type B of the discovered influenza season selected such additional substitution: I45T and I455T – in the B/Ukraine/7/11 and B/Zaporizzya/87/11 together with isolates from Myrmansk and Minsk; L75F – in the B/Ukraine/7/11; E44K and D329N – in the B/Zaporizzya/87/11; V248I – in the B/Zaporizzya/210/11 and isolate from England; S51L – in the B/Ukraine/142/11.

Partly isolates from 2010-2011 influenza season (viruses from Bolivia, Indianan, Irkytsk and England) by NA sequence were related to the reference strain B/Malaysia/2506/2004. In the all idem isolates revealed substitution K220N, however viruses from Irkytsk and England also contained mutations P42S, N127K and D463N (Fig. 4).

The phylogenetic analyses of the surface influenza B/Victoria virus antigens revealed that all investigated Ukrainian isolates were related to vaccine strain B/Brisbane/60/2008 in the 2010-2011 influenza season with estimated rate 97%.

Season 2011-2012. Influenza B viruses has been isolated in the world much less compared with A type during the 2011-2012 epidemic season. They formed 9.5% of the total number of isolated influenza viruses, but 31.5% in the previous 2010-2011 season. We isolated only 2 B/Victori-like influenza viruses in Ukraine.

Comparison of hemagglutinin (HA) genes. The results of hemagglutinin genes phylogenetic comparison are presented in theFigure 5. All influenza viruses have taken for the analysis continued to carry the amino acid substitution N75K, N165K and S172P, compared with strain B/Malaysia/2506/2004. They located within the dominant in Victorian branch Brisbane/60 cluster (Fig. 5). There three groups of viruses are isolated during the 2011-2012 season have been formed in this cluster. The amino acid sequences of viruses from different groups were allmost identical with the exception of single substitutions.

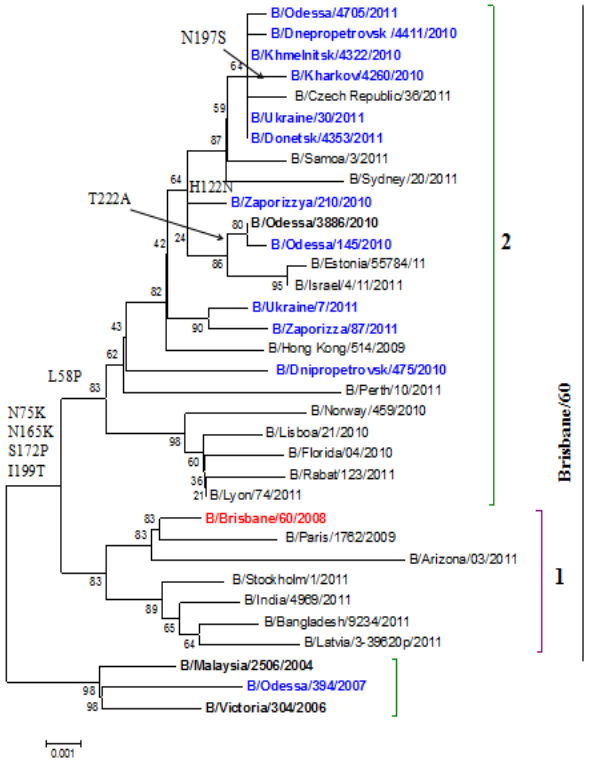

Figure 3. Molecular phylogenetic analysis of HA nucleotide sequences B/Victoria-like influenza viruses isolated in 2010-2011

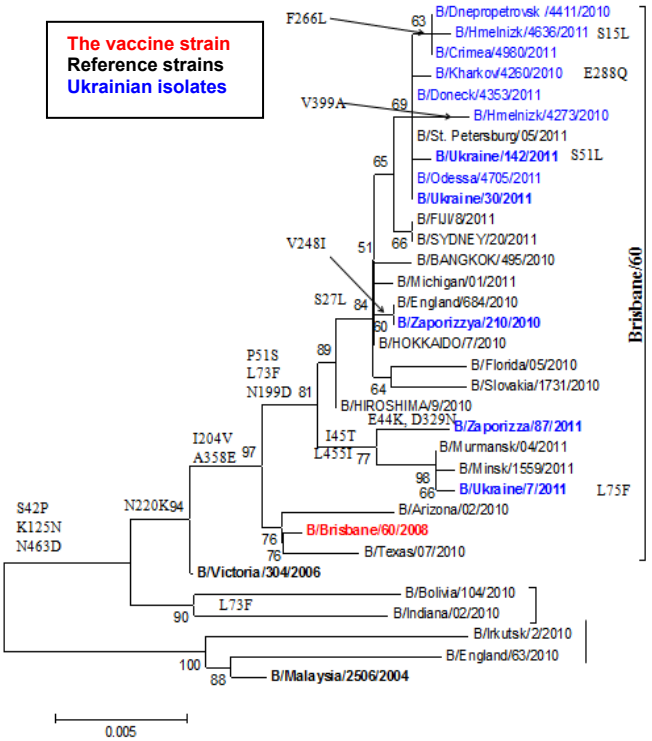

Figure 4. Molecular phylogenetic analysis of NA nucleotide sequences B/Victoria-like influenza viruses isolated in 2010-2011

B/Ukraine/104/2012, the only one sequenced in Ukraine influenza B isolate, was similar to viruses from group 2. Its sequence of hemagglutinin gene was the 100% similar to the virus from Italy – B/Milano/03/2012. PCR positive results for influenza B in Italy showed from 2012 week 6, while the first positive in PCR in Ukraine was in 2012 week 10 according EuroFlu data. Therefore, the higher likelihood was the entry of influenza B from Italy to Ukraine than vice versa. Reference strain for the group 2 was B/Malta/Mv636714/2011 virus.

Comparison of neuraminidase (NA) genes. The isolate B/Ukraine/104/2012 located within the dominant

B/Brisbane cluster according to the analysis. Comparison the neuraminidase genes of all viruses isolated in 2011-2012 in the world showed that they arranged within three clusters. Viruses of clusters 1 and 2 gained P41S, S42P, K125N and E320D substitutions, compared with B/Malaysia/2506/2004 strain (Fig. 6).

The most circulating influenza viruses type B as the B/Ukraine/104/2012 strain has located in cluster B/Brisbane in this epidemic season. This cluster reflects the main direction of evolutionary changes among influenza B viruses circulating since 2006. Strain B/Ukraine/104/2012 has located in the "top" of cluster – from B/Florida/07/2012 to B/Ukraine/5374/2012 (Fig. 6) that gained S295R and E358K substitution.

The second cluster included isolates located in the United States, Japan and Iceland in 2011. They gained a number of additional amino acid substitutions in the neuraminidase (T8M, Q61H, L73F, M375K, A389T and S397R).

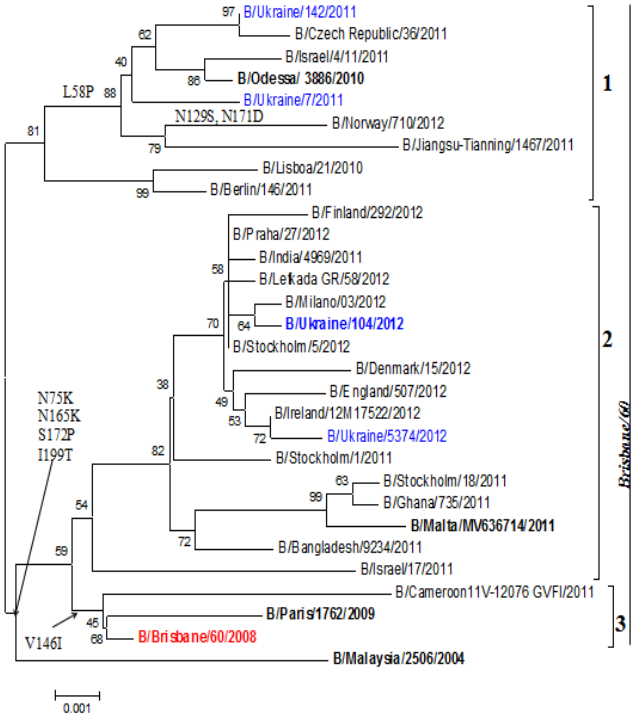

Figure 5. Molecular phylogenetic analysis of HA nucleotide sequences B/Victoria-like influenza viruses isolated in 2011-2012

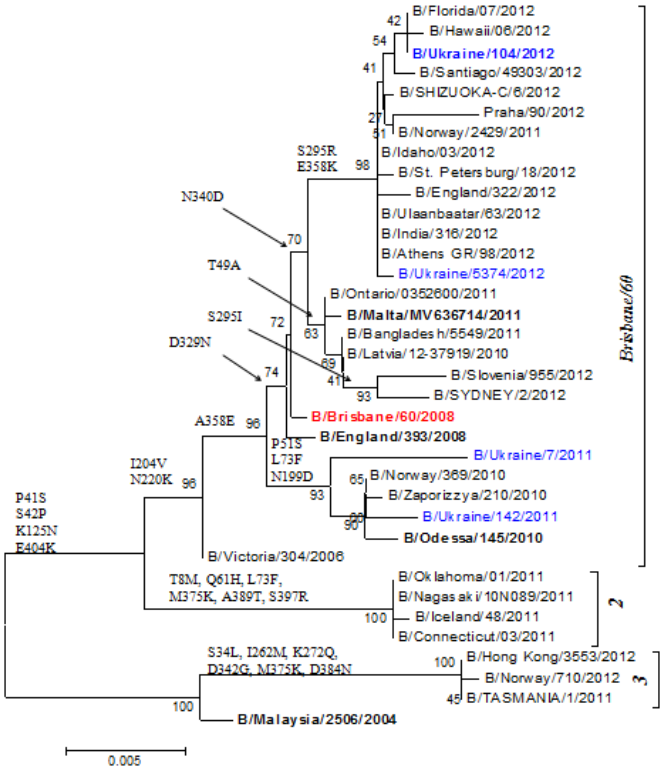

Figure 6. Molecular phylogenetic analysis of NA nucleotide sequences B/Victoria-like influenza viruses isolated in 2011-2012

A small group of viruses has been separated in third cluster – the branch genetically closer to B/Malaysia/2506/2004 strain than to viruses isolated from 2008. These viruses gained a number of amino acid substitutions: S34L, I267M, K272Q, D342G, M375K and D384N. The hemagglutinin genes of these isolates were similar to viruses from B/Brisbane cluster (Fig. 5), but their neuraminidases were separated into third cluster (Fig. 6).

Conclusions.

Influenza viruses type B isolated in Ukraine during 2008-2012 has located in common cluster Brisbane/60 and their hemagglutinin and neuraminidase genes almost hasn't changed. Most of B/Victoria-like influenza viruses allocated in the world also located in Brisbane/60 cluster. The evolutionary "towing" of Ukrainian isolates of the three seasons was shown from our analysis. The level of influenza B virus evolution is significantly lower than influenza A according to research of foreign authors [10]. Our studies confirmed this regularity.

References

1. Virus Taxonomy. Ninth Report of the International Committe on Taxonomy of Viruses / King M.Q., Adams M.J., Carstens E.B. [et al.] – London: Academic Press. – 2011. – P. 749–758.
2. Lin P. Recent changes among human influenza viruses / Lin P., Gregory V., Bennett M., Hay A. // Virus Res. – 2004. – V.103. – P. 47–52.
3. Chen R. The Evolutionary Dynamics of Human Influenza B Virus / Chen R., Holmes C. // J Mol Evol. – 2008. – V. 66(6). – P. 655–663.
4. Лукашов В.В. Молекулярная эволюция и филогенетический анализ Учебное пособие. – М. : БИНОМ. Лабаратория знаний. – 2009. – 256 с.
5. Gamblin S.J., Skehel J.J. Influenza Hemagglutinin and Neuraminidase Membrane Glycoproteins // J Biol Chem. – 2010. – 285, N 37. – P. 28403 – 28409.
6. Tamura K., Peterson D., Peterson N., Stecher G., Nei M., and Kumar S. MEGA5: Molecular Evolutionary Genetics Analysis using Maximum Likelihood, Evolutionary Distance, and Maximum Parsimony Methods // Mol Biol Evol. – 2011. – 28, N 10. – P. 2731–2739.
7. Saitou N. and Nei M. The neighbor-joining method: A new method for reconstructing phylogenetic trees // Molecular Biology and Evolution. – 1987. – 4, N 4. – P. 406–425.
8. Kimura M. A simple method for estimating evolutionary rate of base substitutions through comparative studies of nucleotide sequences // Journal of Molecular Evolution. – 1980. – 16. – P. 111-120.
9. Felsenstein J. Confidence limits on phylogenies: An approach using the bootstrap // Evolution. – 1985. – 39, N 4. – P. 783-791.
10. Jackson D. Molecular studies of influenza B virus in the reverse genetics era / Jackson D., Elderfield A., Barclay S. // J Gen Virol. – 2011. – V. 92(1). – P. 1-17.

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ФІЛОГЕНЕТИЧНИЙ АНАЛІЗ ВІРУСІВ ГРИПУ ТИПУ B/VICTORIA, ВИДІЛЕНИХ В УКРАЇНІ В 2008-2012 РОКАХ

В статті представлені результати філогенетичного аналіу вірусів шрипу типу В генетичної лінії B/Victoria, виділених в Україні в 2008-2012 епідемічних сезонах. В роботі проаналізовано основні заміни в амінокислотних послідовностях гемаглютиніту та нейрамінідази українських ізолятів. За нашими даними всі укріїнські B/Victoria-подібні віруси гриппу В розмістились в кластері Brisbane/60, як і більшість виділених вірусів в світі.

Ключові слова: B/Victoria, філогенетичний аналіз, Україна, Brisbane/60.

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ФИЛОГЕНЕТИЧЕСКИЙ АНАЛИЗ ВИРУСОВ ГРИППА ТИПА B/VICTORIA, ВЫДЕЛЕННЫХ В УКРАИНЕ В 2008-2012 ГОДАХ

В статье представлены результаты филогенетического анализа вирусов гриппа В генетичаской линии B/Victoria, выделенных в Украине в 2008-2012 эпидемических сезонах. В работе были проанализированы основные замещения в аминокислотных последовательностях гемагглютинина и нейраминидазы украинских изолятов. По нашим данным все украинские B/Victoria-подобные вирусы гриппа В расположились в кластере Brisbane/60 как и большинство выделенных вирусов в мире.

Ключевые слова: B/Victoria, филогенетический анализ, Украина, Brisbane/60.

UDK 578.81

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SYSTEMIC APPROACH TO STUDYING VIRUSES OF BACTERIA FROM ANTARCTIC SOIL BIOTOPES

Sub-Antarctic climate and specific geological/biological characteristics created unique environment for development of bacterial and viral populations in soil. This paper reviews environmental factors which influence the maintenance of virus infectivity and must be taken into account when treating the samples in the lab.

Key words: Sub-Antarctic climate, bacteriophages, soil biotopes.

Introduction. Exogenic viral pathogens traditionally remained the main aspect in research focused on soil ecology of viruses. As regarding bacterial viruses and their populational dynamics, most of the work was done using indicator species of bacteria. Classical approach for evaluation of virus numbers in soil samples typically came to counting negative colonies (plaques) on lawn of indicator bacterium. Such type of research allows obtaining rather limited data on a specific group of viruses and not the general knowledge on viral community. This is not enough, however, for comprehensive understanding of the ecological role the bacterial viruses play in soil biotopes. Moreover, complex insight in phage-bacteria-soil environment interactions requires paying attention to a number of factors affecting each element of this system, both separately and in combinative effect. In addition, influence of such factors must be considered in laboratory research.

It was demonstrated that relative bacteria number and diversity in soil may be fairly considerable and depend on sampling location and soil properties [1]. Research on density and species diversity of bacterial populations in biotopes of Dry Valleys confirmed that such locations might be characterized with 10^6-10^8 prokaryotic cells per gram of substrate [2]. For cold climates it was shown that gram-negative bacteria, α, β and γ-proteobacteria (*Pseudomonas spp*. and *Vibrio spp*.), as well as phylum *Cytophaga–Flavobacterium–Bacteroides* were detected most often. In case of gram-positive microorganisms, coryneforms (*Ar-*

throbacter and *Micrococcus sp*.) were most common. *Oscillatoria*, *Phormidium* and *Nostoc* genera typically were widespread *Cyanobacteria* representatives in Antarctica. Psychrophilic yeasts *Cryptococcus spp.* have also been isolated from soil samples.

Ukrainian Antarctic station 'Academician Vernadsky' is based on Galindez Island (65.245678° SLat, 64.257825° WLong). This region is characterized by relatively mild sub-Antarctic climate. Mean summer temperature is about 0°C but decreases to -20…-25°C in winter. Cyclones are typical for these latitudes bringing nebulosity, often rains and mists. At winter time, flaws of wind of 30-35 m/s and above, as well as drift-ice in the ocean, are common [3].

It is logical to assume that such atypical geoclimatic conditions would limit massive spread and development of biota. In part this hypothesis is confirmed by nearly total absence of vascular plants in the region. Only two species of higher plants are registered for the continent of Antarctica: *Deschampsia antarctica* and *Colobanthus quitensis*. However, many moss, lichen and algae species have been found.

In total, 114 species and intraspecific producer taxons have been described. Various groups of invertebrates (nematodes, mites) are found in moss sod, lichens, on plants and in soil substrate. These feed of plants or predate [4]. Substrate for biota development is usually represented by rocks and detritus eluvium. Initial soil is formed due to accumulation of organic matter, guano, dead vegetation and by wind run-ups. Accumulation of organic matter and guano is common for bird colonies [4].

These conditions are unique for evolution and development of microbial communities and respective bacteriophages. Environmental factors and substrate characteristics (i.e. temperature, humidity, pH, ionic strength, etc.) have strong effect on the development, preservation and spread of both bacteria and their viruses.

Total quantity of aerobic microorganisms in this region were just 2-3 times smaller comparing to regions with moderate climate and constituted 10^5-10^8 cells/g of sample. Quantity of microorganisms in different biotopes decreased in the following order (cells/g of sample): soil ($1x10^6$-$5x10^7$) → aboveground part of *Deschampsia antarctica* plants (10^6-10^8) → underground part of mosses ($1x10^6$-$5x10^8$) → sludge of freshwater reservoir (10^5-10^7) → aboveground part of mosses (10^3-10^6) → lichens (10^3-10^6). Identified microorganisms belonged to 'classic' taxons typical for various regions with moderate climate including *Bacillus, Actinomyces, Streptomyces, Pseudomonas, Methylobacterium, Enterobacter, Staphylococcus*, and *Brevibacterium* genera, etc. [5]. Isolated microorganisms showed tolerance to high ('over-bactericidal') concentrations of toxic metals – mercury, copper, chrome, cadmium, nickel, and cobalt. This tolerance could be determined by plasmids and/or transposons which typically correlated with bacteria resistance toward antibiotics. This may exemplify adaptive reaction of microorganisms on existence in conditions of severe competition for substrate [5-8].

Phages were detected in samples of superficial soil layers neighboring moss rhizoids. This research resulted in isolation and morphological descriptive classification of phages belonging to the families *Podoviridae* (C1 morphotype), *Siphoviridae* (B1 morphotype), and *Myoviridae* (A1 morphotype). However, the authors have not managed to select any sensitive bacterial host capable of maintaining virus replication in laboratory conditions [9].

Comprehensive understanding of mechanisms and means of spread and preservation of phages in such systems, as well as viruses' interactions with their respective hosts, requires systematic knowledge about factors which potentially influence the research objects directly. It is important to consider environmental factors and characteristics of soil substrates affecting viruses in soil. In addition, selection of proper laboratory techniques for obtaining most reliable results is crucial.

Influence of ecological factors. Different characteristics of soil and environment, as well as virus own properties, effect the adsorption characteristics of viral particles in soil. In particular it is known that viruses adsorb on soil particles and degree of adsorption commonly exceeds 90% for various viruses. Such factors as type of clay materials, cation-exchange capacity, ionic strength, soil-bound and unbound organic matter, and pH influence the efficiency of virus adsorption on soil particles [10]. Morphology and biochemical characteristics of viral particles (for instance, isoelectric point, etc.) also make significant contribution in this phenomenon. Soil is a heterogeneous substrate consisting of particles with different charge(s) and varying hydrophilic/ hydrophobic properties which may have a dramatic effect of the adsorption of virus particles.

In addition, a number of environmental factors may also lead to virus inactivation and their inert state in soil. Such factors include temperature, pH, clay minerals, organic matter (nutrients), heavy metals, acidic contaminants, aerobicity, ionic strength (saline soil), and humidity.

Temperature is one the major factors of the environment influencing preservation/inactivation of phages. Temperature dictates optimums for bacterial growth and hence microorganisms are divided on psychrophilic, mesophilic and termophilic. At this point we should say that optimal temperature for a phage does not always correlate with such for a bacterium. In general, low temperatures are more preferred for virus survival/preservation. This may be related to higher microbial and enzymatic activities seen at higher temperatures which favor phage inactivation and degradation [10]. Substrate acidity not only affects bacterial hosts but also may influence stages of adsorption, penetration, as well as latent stage duration in 'phage-host' system. In turn, pH value is a component of electrostatic properties of a substrate which play key role in virus adsorption on solid particles. At critical pH values (2.5-3,5 pH units higher than isoelectric point of a virus) a virion irreversibly adsorbs to a surface. At lower pH values this process is deemed reversible. It has been established, for instance, that the majority of bacteriophages demonstrated tolerance towards pH fluctuations in the range of pH 5-8. At low temperatures, however, this range of virus tolerance for pH might be somewhat wider, pH 4-10 [11].

Ionic strength represents another powerful ecological factor mediating virus binding with clay particles and favoring virus 'survival'. At higher values of ionic strength virion adsorption to clay particles is typically more efficient. Clay materials may 'screen' virions and hence contribute to virus protection from inactivation and loss on infectivity caused by UV irradiation. This allows more time for virus persistence outside a host cell. It was demonstrated that phages maintain their infectivity for longer when in loam or clay soil as compared to sandy substrates [10].

Organic matter content in a substrate also influences both virus infectivity and bacteria susceptibility. Antarctic soil is a poor substrate for growth of microorganisms and therefore host cells may show insusceptibility to viral infection in soil environment. One of the reasons of hosts' tolerance is resulting from alterations of superficial properties of a bacterial cell. For instance, phosphates' deficiency may lead to loss of teichoic acids in the cell wall of *B. subtilis W23* and subsequent loss of ability to adsorb SP50 phage at the initial stage of infection.

Metal oxides also affect virus adsorption and inactivation. Soils rich in metals (*in situ* represented in forms of

oxides) favor much faster virus inactivation preventing their further passive transportation in soil water [12]. At the same time, organic compounds in a substrate alleviate 'toxic' influence of heavy metals on virus particles by forming metalorganic complexes.

Epifluorescent microscopy analysis of virus-like particles (VLPs) in soils from Delaware, USA, showed significant correlation between VLPs abundance and water content. It was explained that there was a certain critical value of humidity needed to maintain virus infectivity. Otherwise, humidity decrease leads to elevation of virucidal effect of water evaporation. Evaporation *per se*, as well as humidity decrease, leads to soil drying which results in virus inactivation [10, 13].

Overcoming methodological problems. In the view of abovementioned factors and in order to prevent skewing of future results it's important to pay proper attention to every step of working with the samples, including sample collection, storage, transportation, and further processing in a laboratory. For instance, significant losses of virus at the stage of isolation/filtration, isolation of inactivated virus particles (partially degraded or without nucleic acid), and artifacts during epifluorescent or electron microscopy may lead to false conception about virus numbers, diversity and spread.

Based on such reasoning, it is of importance to select optimal method for elution of virus particles from soil/substrate samples. There are many buffer/media compositions known for overcoming bound/adsorbed virus state when isolating viruses from soil and bottom sediments (sludge) [10]. For instance, nutrient broth with or without ovalbumin, meat extract with or without NaCl or borate(s), cation-complete medium, glycine buffer, sodium pyrophosphate, potassium citrate, mineral buffers, etc. Meat extract (6-10%) is the most common medium used for soil samples.

For further virus separation from bacterial communities and other matter it's recommended to use membrane filters with pores of 0,22-0,45 mcm in diameter. In turn, this stage may result in significant losses of virus (up to 2/3). Careful choice of filter type is needed to account for virus ability to bind to some type of membranes.

When analyzing samples of six types of soil (two samples of agricultural soils and four samples of forest soils) [13], researchers concluded that potassium citrate was the optimal choice for elution. Having the same efficiency as sodium pyrophosphate buffer and glycine buffer, it does not lead to significant background fluorescence (during epifluorescent microscopy) and allows taking more detailed photographs.

When working with soil or substrate, it is advisable to repeatedly wash the samples at the initial isolation (elution) of viral particles. It was demonstrated that 1^{st} stage of elution yields up to 70% of VLPs, 2^{nd} stage – 20%, 3^{rd} stage – 10%, and 4^{th} stage – 1 % of total VLPs yield from soil matter. This trend was shown to be similar for different soil types providing means for more accurate determination/evaluation of viruses in various types of substrates [13].

Morphological analysis of bacteria viruses using electron microscopy has one significant disadvantage – wrong classification due to physical breakdown of tailed virus particles loosing a tail. Aggressive soil extraction typically leads to separation of phage tails. This may provide grounds for erroneous morphological description of viruses found in a sample [10].

In addition it should be remembered that influence of environmental factors, conditions of samples' transportation and non-optimal methods for working with samples and virus isolation may have great effects on the resulting visual diagnostics of viruses in substrates. It has been established, for instance, that the majority of bacteriophages demonstrated tolerance towards pH fluctuations in the range of pH 5-8. At low temperatures, however, this range of virus tolerance for pH might be somewhat wider, pH 4-10. Strong acidulation or alkalinization induced progressive degradation of viral particles with the release of nucleic acid, tail separation, tail sheath contraction, and particle breakdown into structural elements – protein head capsid, tail sheath, tail tube, and tail fibers. Similar effects were also shown for temperature stress. For instance, momentary or repeated freezing-thawing often led to viral particle degradation, DNA release, contraction of tail sheath, sheath separation from the tail, tail separation from the head, etc. Surprisingly, basal plate and fibers often preserved [11].

Conclusions. Taking into account great variety of soil types by components' content and physic-chemical indexes, region of sampling may impose certain additional requirements. Proper transportation of soil samples is not a bit less important as may result in transformation of mineral mass of soil/substrate and skew future experimental results. Such considerations dictate certain requirements for processing different types of samples. One of the ways out is to use several different techniques in parallel when every stage of processing a sample with further comparison and amendment of obtained results. This provides means for determining a complex of optimal methods for each group of samples (i.e. region) and preventing inaccuracies and artifacts.

Virus species composition for terrestrial and freshwater ecosystems still remains rather understudied. Phage research of surface layer of soil in Antarctica resulted in isolation and morphological descriptive classification of phages belonging to the families *Podoviridae* (C1 morphotype), *Siphoviridae* (B1 morphotype), and *Myoviridae* (A1 morphotype). However, sensitive bacterial hosts capable of maintaining these viruses' replication in laboratory conditions have not been identified. Wide application of novel approaches, such as metagenomics, would allow widening our understanding of viral communities inhabiting natural soil ecosystems in Antarctica, as well as of their genetic and population structure.

References

1. Abedon S.(ed.) Bacteriophage ecology. Cambridge University Press. – 2011. – P. 37–65.

2. Barrientos-Díaz L., Gidekel M., Gutiérrez-Moraga A. Characterization of rhizospheric bacteria isolated from Deschampsia antarctica Desv. // World Journal of Microbiology and Biotechnology. – 2008. – Volume: 24. – Issue: 10. – P. 2289-2296.

3. Мартазинова В.Ф., Тимофеев В.Е., Иванова Е.К. Погодные условия на станции Академик Вернадский в 2011 г. и ожидаемое состояние температурного режима в летний период 2011-2012 гг. по инструментальным наблюдениям 2011 г. // УАЖ. – 2011/2012. – № 10-11. –С. 320-332.

4. Поліщук В.П., Костіков І.Ю., Таран Н.Ю. та ін. Комплексне вивчення антарктичної біоти // Український Антарктичний Журнал. – 2009. – №8. – С. 284-292.

5. Tashyrev O.B. Complex researches of structure and function of Antarctic terrestrial microbial communities // Ukrainian Antarctic Journal. – 2009. –№ 8. –P. 228–242.

6. Tashyrev O.B., N.A. Matvieieva, V.A. Romanovskaya et al. Polyresistance and superresistance of Antarctic microorganisms to heavy metals, Reports of Nat. Acad. Sci. of Ukraine. – 2007, pp. 170–175.

7. Tashyrev O.B., V.A. Romanovskaya, I.B. Sioma et al. Antarctic microorganisms resistant to high concentration of Hg2+, Cu2+, Cd2+ and CrO 2- // Reports of Nat. Acad. Sci. of Ukraine. – 2008. – № 4 –1. – P. 169–176.

8. Tashyrev A., Romanovskaya V., Beregova T. et al. Trends and prospects for developing of new industrial biotechnologies on the base of Antarctic extremophilic microorganisms // Ukrainian Antarctic Journal. – 2010. – № 9, 158-186.

9. Жиленков Е.Л., Жиленков М.Е., Завальский Л.Ю. и др. Фаги в почвенных образцах Антарктиды // V Міжнародна конференція "Біоресурси та віруси". – Київ, 2007. – С. 154.

10. Kimura M., Zhong-Jun Jia, Nakayama N. et al. Ecology of viruses in soils: Past, present and future perspectives // Soil Science and Plant Nutrition. –2008. –№ 54:1. –P. 1-32.

11. Тихоненко А.С. Ультраструктура вирусов бактерий. – М., "Наука". – 1968. – Стр. 170.

12. Шевченко О.В., Будзанівська І.Г., Патика В.П., Бойка А.Л., Поліщук В.П. Вплив важких металів на перебіг вірусних інфекцій рослин. – К.: Фітоцентр, 2003. – 224 с.

13. Williamson K. E., Radosevich M., Wommack K. E. Abundance and diversity of viruses in soil // Applied and environmental microbiology. 2005. – Vol. 71. – N 6. – p. 3119–3125.

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СИСТЕМНИЙ ПІДХІД ПРИ ДОСЛІДЖЕННІ ВІРУСІВ БАКТЕРІЙ АНТАРКТИЧНИХ ҐРУНТОВИХ ЦЕНОЗІВ

Субантарктичний клімат та специфічні геолого-біологічні характеристики створюють унікальні умови для розвитку ґрунтових бактеріальних та вірусних популяцій. В статті розглядаються фактори оточуючого середовища які мають вплив на збереження інфекційності вірусів та які необхідно враховувати при роботі в лабораторії.

Ключові слова: Субантарктичний клімат, бактеріофаги, ґрунтові ценози.

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СИСТЕМНЫЙ ПОДХОД ПРИ ИССЛЕДОВАНИИ ВИРУСОВ БАКТЕРИЙ АНТИРКТИЧЕСКИХ ПОЧВЕННЫХ ЦЕНОЗОВ

Субантарктический климат и специфические геолого-биологические характеристики создают уникальные условия для развития почвенных бактериальных и вирусных популяций. В статье рассматриваются факторы окружающей среды, которые влияют на сохранение инфекцинности вирусов и которые необходимо учитывать при работе в лаборатории.

Ключевые слова: Субантарктический климат, бактериофаги, почвенные ценозы.

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AGE RELATED FEATURES OF WATER AND ELECTROLYTES TRANSPORT ACROSS THE EPITHELIUM OF RATS COLON AND THEIR CORRECTION WITH MULTIPROBIOTIC "SIMBITER ® ACIDOPHILUS" CONCENTRATED

The transport of water and electrolytes across the epithelium of the colon in rats of different ages was studied. It was found that at the 21 and 24 month old rats absorption of water and Na^+ and Cl^- ions significantly increased, which is one of the causes of constipation occurancens in aged and elderly rats. Periodic administration of multi-probiotic "Symbiter" (0.14 mg / kg) prevents age-related changes in the transport of water and electrolytes across the epithelium of the colon.

Keywords: colon, the total flow of water and Na^+ and Cl^- ions, multiprobiotic.

More than 60% of people over 60 years old have the problem with constipations. Such problem not only reduce the social activities of people, but also cause pressure increasing in the cavity of intestine, diverticulums, overgrowth and accumulation of endotoxin [3]. It was shown that the main reason of constipation in healthy individuals who are older than 80 years is slowed chyme transport through intestine, which occurs due to decreased of motor activity of colon [10]. Age related changes in colon are associated with degeneration of nerve fibers that innervate its smooth muscles [14]. With age neurotransmitter acetylcholine (main stimulator of smooth muscle motor activity) release decreases, that is the reason of constipations occurrence. Robert et al [11] established another mechanism of acetylcholine release reduction in colon during aging. They showed that aging is accompanied by decrease of calcium appearance in the colon neurons of rats, resulting in acetylcholine release reduction. Ayzman R. I. showed that aging causes changes in intestinal mucosa, which leads to hydrolysis and absorption decrease. There are several reason: surface of membrane digestion decrease because of atrophy, number of operating carriers in transport systems also reduce, Cl^-secretion decrease [1].

Thus, the most of literature sources associated the development of age related constipation with changes in colon motility and much less attention is paid to the study of age-related aspects of water and electrolytes transport across the epithelium of the colon.

Previously, we showed that water, Na + and Cl- absorption increases with age and that is the reason of age-related constipation [6]. These changes begin at the end of mature age. Despite the large number of laxatives drugs, the search of safe preparation and drugs without addiction is still actual. One of the most perspective preparations are probiotics. Especially taking into account that with age colon contamination with opportunistic flora increases [7].

The aim of our work was to study dynamics of water and electrolytes transport across the epithelium of the colon in rats of different ages with standart diet and with periodic consumption of multiprobiotic "Symbiter ® acidophilic" concentrated (Symbiter).

Methods: In chronic experiments the transport of water and electrolytes across the epithelium of the colon were studied on white nonlinear female rats in ontogenesis. Rats were born with different females in one day period and were randomly divided into 2 groups: control, rats were fed a standard maintenance diet, and experimental, with periodically multiprobiotic supplement to diet. Rats of control group at the age of 3, 6, 9, 12, 15 and 18 month old were administered 0.5 ml dechlorinated tap water of room temperature. Rats of experimental group in the first 10 days after birth got 1 drop of Symbiter daily. Hereafter these rats at the age of 3, 6, 9, 12, 15 and 18 month were injected 0.14 ml/kg multiprobiotic Symbiter diluted in 0.5 ml of water. One dose of Symbiter (10 ml) consists of biomass of 14 living probiotic stains in symbiosis: Lactobacillus and Lactococcus – non less than $6.0x10^{10}$ CFU/cm^3, propionic-acid bacteria – $3.0x10^{10}$ CFU/cm^3, bifidobacteria – $1.0x10^{10}$ CFU/cm^3, acetic-acid bacteria – $1.0x10^6$ CFU/cm^3. From each group at the age of 3 month (juvenile age), 6, 9 month (young age), 18 month (mature age), 21 month (eldery) and 24 month (senile) animals were taken for experimental tests. Rats were anaesthetized by urethane at the dose 1.15 g/kg intraperitoneally**.** The transport of water and electrolytes across the epithelium of the colon were measured in acute experiments by method of perfusion of isolated colon. After equilibration period (60 min.), samples were taken each 20 minutes. Total perfusion duration was 180 min. Changes in concentration of unabsorbed phenol red marker determined with photocolorimetric methods (λ=520 nm, λ =560 nm та λ=600 nm) represented the level of water absorption. Concentration of CL^- in perfused solution was measured with ion meter. Measure-

ments of the concentrations of Na^+ and K^+ were carried out using a flame photometric analyzer of liquids [13].

All obtained data were subjected to statistical analysis using software package "Statistics, 8.0". Shapiro-Wilks criterion was used for the analysis of data distribution type. As the data were abnormal distributed independent samples were compared by Mann-Whitney U test. We calculated median (Me) and lower and upper quartiles (Me [L.q... U.q]) [4].

Results. It was found that in 3 months old rats the level of water absorption was 31.01 [5.05... 68.93] ml/g*20 min, Na^+ – 24.81 [2.86... 46.85], K^+ – 0.74 [-3.01... -0.05] and Cl^- – 13.92 [4.46... 26.99] µmol/g*20 min. This results matched with other researchers data obtained in vivo and showed that the absorption of water is accompanied by absorption of Na^+ and Cl^-, K^+ secretion [1, 8, 10] (Fig. 1).

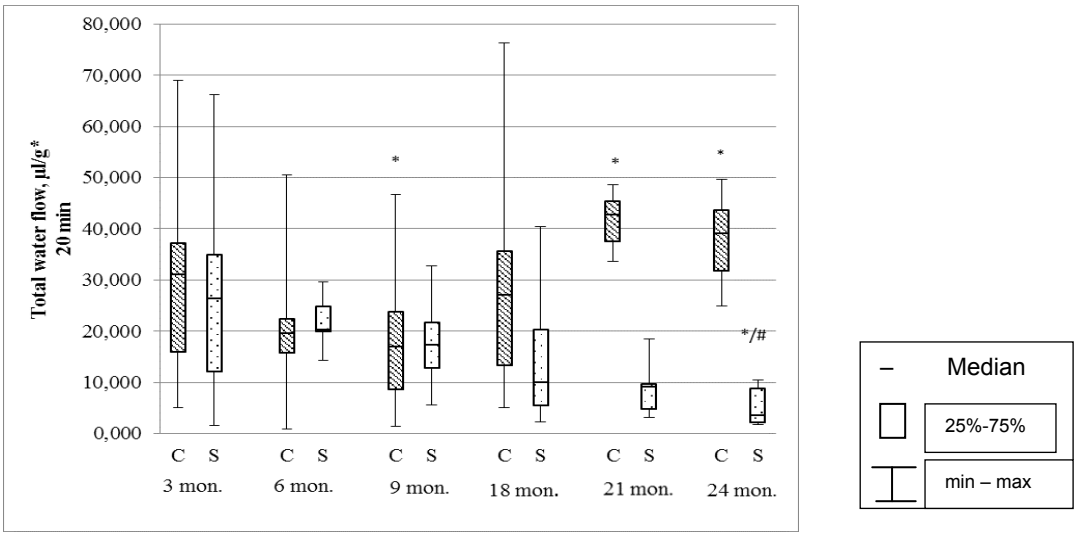

Figure 1. Total flow of water across the epithelium of the colon of different age groups rats (Me [L.q... U.q], n = 8)
K – rats of control group;
C – rats of experimental group that were periodically injected with multyprobiotic Symbiter;
* – p <0,05 compare to 3 months old control group; # – p <0,05 compare to same age control group

The level of water absorption in 6 and 18 months old rats of control group had no significant difference from rats of juvenile age and was equal to19.82 [3.5... 50.57] and 27.16 [12.72... 35.95] ml/g*20 min respectively. In the same time in 21 and 24 month old rats absorption of water and Na^+ and Cl^- ions was increased. Absorbtion of water in eldery rats increased by 33.7% ($p<0.05$), in senile – 26.0% ($p<0.05$) compare to juvenile. Periodic introduction of multiprobiotic decreased water absorption in colon of 18, 21 and 24 months old rats correspondently by 63.0% ($p<0.05$), 79.1% ($p<0.05$) and 90.8% ($p<0.05$) compare to control group of same age.

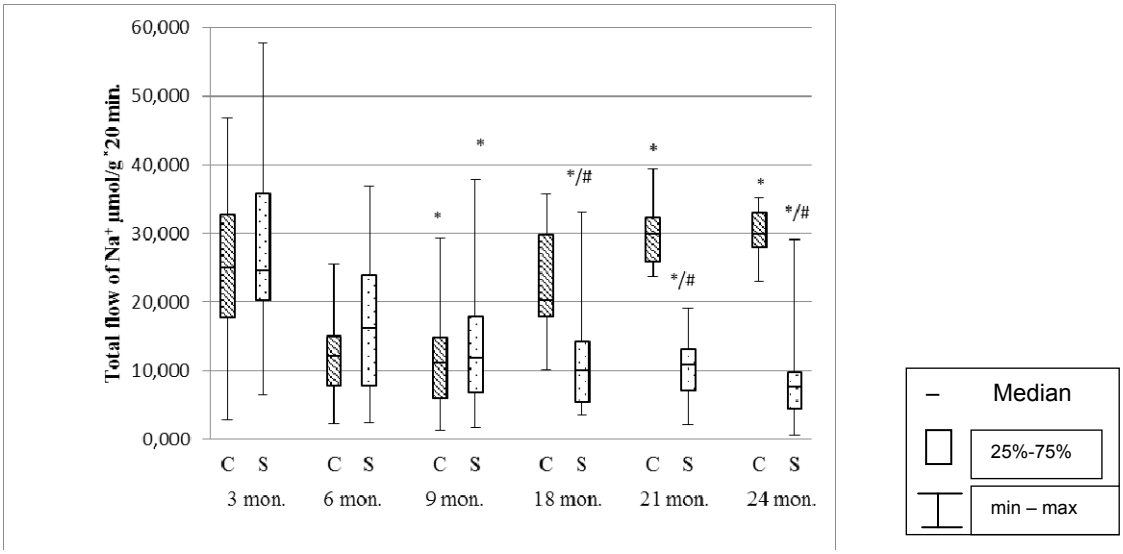

Figure 2. Total flow of Na^+ across the epithelium of the colon of different age groups rats (Me [L.q... U.q], n = 8)
K – rats of control group;
C – rats of experimental group that were periodically injected with multyprobiotic "Symbiter";
* – p <0,05 compare to 3 months old control group; # – p <0,05 compare to same age control group

The total flow of Na^+ in 6 months old rats was lower than in 3 months old, but there was no significant differences (Fig. 2). In 9 month old rats Na^+ absorption decreased by 57.7% ($p<0.05$) compare to rats of juvenile age. Thus, the total flow of electrolytes in 9 month old rats was the smallest. In experimental group same as in control, in 9

months old rats the total flow of ions decreased by 51.99% ($p<0.05$) in comparison with 3 months old control group.

In the eldery (21 months) and senile rats (24 months) total flow of Na^+ was increased by 19.7% ($p<0.05$) and 20.5% ($p<0.05$) compare to 3 month old rats of control group. Thus, the rate of Na^+ total flow, as well as water, was the biggest in the 24 month old rats and was 29.90 [23.04... 32.25] μmol/g*20 min. The total flow in mature, elderly and senile rats of experimental group of ions decreased by 50% ($p<0.05$) and 64.07% ($p<0.05$) and 74.05% ($p<0.05$) compare to control group of same age and was also lower than in 3 month old rats of control group.

The total flow of Cl^- ions in 6 and 18 months old rats did not differ from 3 months old. The lowest level of Cl^- ions absorption was in 9 months and was 9.03 [0.37... 23.43] μmol/g*20 min. In eldery and senile rats significant increase of ions total flow was observed. In 21, 24 month old rats data was equal to 22.73 [17.49... 30.93], 20.38 [10.61…26.92] μmol/g*20 min correspondently, which was higher by 63.3% ($p<0.05$) and 46.4% ($p<0.05$) compare to juvenile rats. As in the case with the level of water and Na^+ total flow periodic Symbiter introduction reduced the total flow of Cl^- ions across the epithelium of colon (Fig. 3).

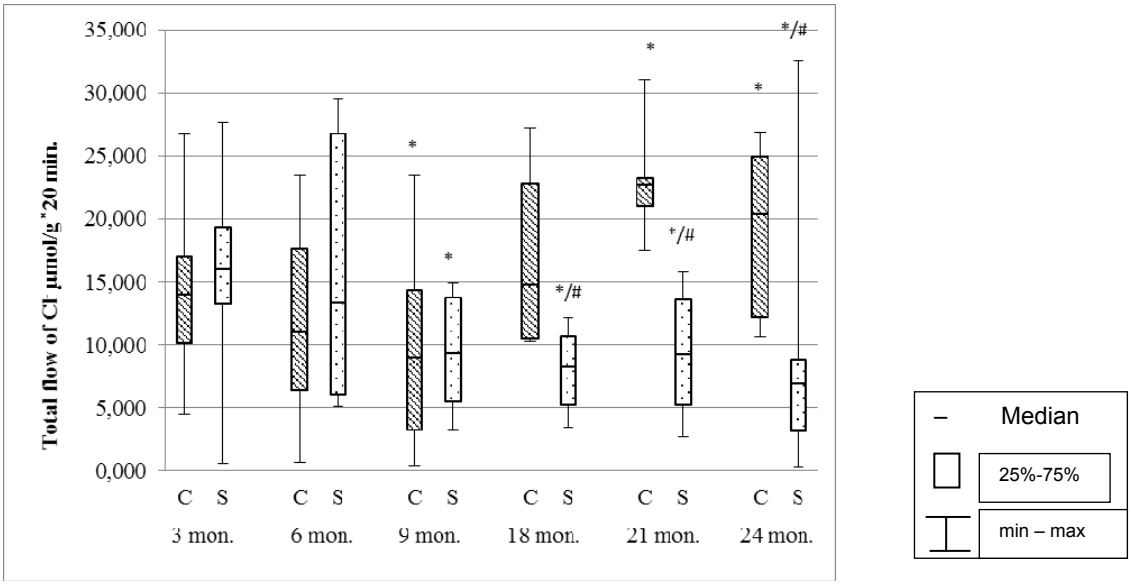

Figure 3. Total flow of Cl^- across the epithelium of the colon of different age groups rats (Me [L.q... U.q], n = 8)
K – rats of control group;
C – rats of experimental group that were periodically injected with multyprobiotic Symbiter;
* – p <0,05 compare to 3 months old control group; # – p <0,05 compare to same age control group

It was found that Na^+ and Cl^- total flow was accompanied with changes in the total flow of water across the epithelium of the colon in different age groups of rats. However, during the ageing the total flow of K^+ ions had no significant changes either in control or in experimental groups. It was clear that at the rest transcellular is the predominant type of water transport across the epithelium of the colon. That occurs passive by diffusion over osmotic gradient of NaCl concentration between intra-and intercellular fluids of intestinal epithelial cells. Our experiments confirm such theory.

Periodic addition of multyprobiotic Symbiter in the diet of rats prevented the reduction of water, Na^+ and Cl^- total flow in elderly and senile rats. We concluded that multiprobiotic consumption had prevented age-related changes in intestinal microflora.

The significance of microflora to the human body is still not full revealed and therefore is often underestimated. But today it is clear that bacteria play an important and a multidimensional role in the various functions of the body. For example they generate significant quantities of various physiologically active compounds. Among them short-chain fatty acids (SCFA) that assure water electrolyte balance and mineral metabolism (the content of carbonates in intestine lumen and pH of the intestinal contents), are the main source of colonocyte nutrition, regulate colon motility [2]. Thus, we consider that SCFA preservation might be the reason of decreased amount of constipations in eldery and senile rats of experimental group.

Conclusions:

1. In elderly and senile rats absorption of water, Na^+ and Cl^- increase.
2. Transport of K^+ ions does not change during ontogenesis.
3. Periodic administration of multiprobiotic "Symbiter ® acidophilic" concentrated prevents the reduction of water, Na^+ and Cl^- total flow in elderly and senile rats.
4. Multiprobiotic "Symbiter® acidophilic" concentrate may be consider an effective preparation for constipations prevention.

References

1. Айзман Р.И. Возрастные особенности транспорта калия в дистальном отделе толстого кишечника крыс / Айзман Р.И., Боровец Е.Н., Герасев А.Д. // Российский физиологический журнал. – 2000. – № 86. – С. 86-94.

2. Белобородова Н.В. Метаболиты анаэробных бактерий (летучие жирные кислоты) и реактивность макроорганизма / Н.В. Белобородова, С.М. Белобородов // Антибиотики и химиотерапия. – 2000. – № 2. – С. 28-36.

3. Белоусова Е.А. Лечение запора у пожилых пациентов / Е.А. Белоусова // Клиническая геронтология. – 2008. – № 1. – С. 58–65.

4. Гланц С. Медико-биологическая статистика / С. Гланц (Пер. с англ.). – М.: Практика, 1998. – 459 с.

5. Приходько В.Ю. Проблема хронічних закрепів у терапевтичній і геріатричній практиці / В.Ю. Приходько, О.В. Давидович, Н.Я. Давидович // Ліки України. – 2008. – № 5. – С. 43-46.

6. Черпак М.Ф. Вікові особливості транспорту води і електролітів через епітелій товстої кишки / М.Ф. Черпак, Т.В. Берегова // Шевченківська весна 2012: 10-а Міжнародна міждисциплінарна науково-практична конференції молодих вчених, 19-23 березня 2012 р., Київ: матеріали конференції. – Київ, 2012. – С. 326-327.

7. Enck P. The effects of ageing on the colonic bacterial microflora in adults / P. Enck, K. Zimmermann, K. Rusch [et al.] // Z Gastroenterol. – 2009. – Vol. 47, № 7. – P. 653-658.

8. Greger R. Regulation of ion transport in colonic crypts / R. Greger, M. Bleich, J. Leipziger [et al] // News Physiol Sci. – 1997. – Vol. 12. – P. 62-66.
9. Huang Z.C. Diagnosis and treatment of slow transit constipation complicated with adult megacolon / ZC. Huang, Q. Liu, SG. Li // Zhonghua Wei Chang Wai Ke Za Zhi. – 2011. –Vol. 14, № 12. – P. 41-44.
10. Madsen JL. Effects of aging on gastrointestinal motor function / JL. Madsen J.Graff // Age, Ageing. – 2004. – Vol. 33. – P. 154-159.
11. Roberts D. Evidence for age-associated reduction in acetylcholine release and smooth muscle response in the rat colon / D. Roberts, D. Gelperin, JW. Wilwey // Am J Physiol. – 1994. – Vol. 267. – P. 515-522.
12. Spring K.R. Routes and mechanism of fluid transport by epithelia / K.R. Spring // Annu Rev Physiol. – 1998. – Vol. 60. – P. 105–119.
13. Sladen G.E. Studies on the effects of unconjugated dyhidroxy bile salts on rat small intestinal function in vivo / G.E. Sladen, J.T. Harries // Biochem Biophys Acta. – 1972. – Vol. 288. – P. 443-456.
14. Voutilainen S. The gastrointestinal tract: safety pharmacology aspects relating to agening, diet and natural/herbal remedies / S. Voutilainen // Journal of Pre-Clinical Research. – 2009. – Vol. 3, № 2. – P. 076-083.
15. Winter D.C. Rapid effects of aldosterone on sodium-hydrogen exchange in isolated colonic crypts / D.C. Winter, M.F. Schneider, G.C. O'Sullivan // J Membr Biol. – 1999. – Vol. 170. – P. 17–26.

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ВІКОВІ ОСОБЛИВОСТІ ТРАНСПОРТУ ВОДИ ТА ЕЛЕКТРОЛІТІВ ЧЕРЕЗ ЕПІТЕЛІЙ ТОВСТОЇ КИШКИ ЩУРІВ ТА ЇХ КОРЕКЦІЯ МУЛЬТИПРОБІОТИКОМ СИМБІТЕР АЦИДОФІЛЬНИЙ КОНЦЕНТРОВАНИЙ

Досліджено транспорт води і електролітів через епітелій товстої кишки у щурів різного віку. Встановлено, що у віці 21-го та 24-х місяців всмоктування води та іонів Na^+ і Cl^- значуще збільшується, що є однією з причин виникнення закрепів. Періодичне додавання до стандартного корму мультипробіотика "Симбітер® ацидофільний" концентрований (0,14 мл/кг) запобігає віковим змінам у транспорті води і електролітів через епітелій товстої кишки.

Ключові слова: товстий кишечник, сумарний потік води та іонів Na^+ і Cl^-, мультипробіотик.

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ВОЗРАСТНЫЕ ИЗМЕНЕНИЯ ТРАНСПОРТА ВОДЫ И ЭЛЕКТРОЛИТОВ ЧЕРЕЗ ЭПИТЕЛИЙ ТОЛСТОГО КИШЕЧНИКА КРЫС И ИХ КОРЕКЦИЯ МУЛЬТИПРОБИОТИКОМ СИМБИТЕР АЦИДОФИЛЬНЫЙ КОНЦЕНТРИРОВАНЫЙ

Исследованы транспорт воды и электролитов через эпителий толстой кишки у крыс разного возраста. Установлено, что в возрасте 21-го и 24-х месяцев всасывания воды и ионов Na^+ и Cl^- значимое увеличивается, что является одной из причин возникновения запоров у крыс пожилого и старческого возраста. Периодическое добавление к стандартному корму мультипробиотика "Симбитер ® ацидофильный" концентрированный (0,14 мл/кг) предотвращает возрастные изменения в транспорте воды и электролитов через эпителий толстой кишки.

Ключевые слова: толстый кишечник, суммарный поток воды и ионов Na^+ и Cl^-, мультипробиотик.

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DETECTION OF SOME VIRUS PATHOGENS OF CACTUS IN UKRANIAN BOTANICAL GARDENS

Cactus collections of some Ukrainian botanical gardens were analyzed for virus contamination. Different virus-like symptoms including mosaics, chlorosis and local necroses have been detected on cactus plants in these collections. Biological properties of isolated viruses were defined by the methods of bioassay, transmission electron microscopy and indirect ELISA.

Key words: ELISA, isolated viruses, cactus.

Introduction: The *Cactaceae* are mostly spiny succulents with photosynthetic stems comprising 200 genera and more than 2,000 species [1]. Cactuses have been the object of amateur and professional botanical collectors because of unusual structures and exceptionally colorful and beautiful blossoms. Huge variety of cactus species coupled with their distinctive ecological and biological characteristics cause a number of difficulties associated primarily with cultivation of these plants in the greenhouses. Due to the ordinary vegetative propagation and long-term cultivation of cactus plants in the same collections, these plants may serve as reservoirs of different viruses.

In accordance with literary data about 11 viruses are able to affect the members of *Cactaceae* family: *Cactus virus X*(CVX)*, Schlumbergera virus X* (SVX), *Opuntia virus X* (OVX), *Zygocactus virus X (*ZVX), *Saguaro cactus virus* (SCV), *Sammons' Opuntia virus* (SOV), *Cactus virus 2* (CV2), *Cactus mild mottle virus* (CMMoV), *Rattail cactus necrosis-associated virus* (RCNAV), *Impatiens necrotic spot virus* (INSV) and *Tomato spot wilt virus* (TSWV) [3, 4, 5, 6, 7, 8].

That's why the aim of our work was to **analyze collection of cactus in Ukrainian botanical gardens for virus contamination.**

Materials and methods: The material for investigation was collected in different Ukrainian botanical gardens: Donetsk botanical garden of the National Academy of Sciences of Ukraine, Botanical garden of Ivan Franko National university of Lviv, Botanical garden of Odessa I.I. Mechnikov National university, Karazin' Botanic Garden of Kharkiv National University, Nikitsky Botanical Garden – National Scientific Centre. For detection and identification of viruses 67 samples of different cactus cultivars were selected. For the biological characteristics of the pathogen we used the method of bioassay. Infectivity of plant sap was confirmed proved using indicators plants such as *Chenopodium murale, Celosia cristata, Datura stramonium, Gomphrena globosa, Chenopodium murale, Nicotiana tabacum, Nicotiana rustica.* These plants are typical test-plants for majority cactus viruses.

Virus identification was carried out using indirect ELISA [2]. Samples were tested by ELISA with serums to *Tobaco mosaic virus (*TMV) (antiserum obtained at the virology department, the sensitivity and specificity confirmed experimentally), *Potato virus S* (PVS), *Potato virus M (PVM), Potato virus X* (PVX) (Institute of Agricultural Microbiology, Ukrainian Academy of Agrarian Sciences, Chernigiv), *Tomato spotted wilt virus* (TSWV*), Impatiens necrotic spot virus* (INSV) (Loewe, Germany).

The morphology of virions was studied in leaf dip preparations negatively stained with 2% uranyl acetate.

electron microscopy was carried out using a JEOL-1400 electron microscope at the magnification 30 000 [7].

Results and discussion: Cactus plants demonstrated different symptoms of virus infection. On plants *Mammilaria zeilmanniana, Opuntia* sp. (from collection of the Donetsk botanical garden of the National Academy of Sciences of Ukraine), *Ferocactus echidne, Gymnocalycium* sp., *Opuntia* sp. (from collection of Botanical garden of Ivan Franko National university of Lviv), *Mammillaria nivosa, Opuntia* sp. (from collection of Botanical garden of Odessa I.I. Mechnikov National university), *Mamillaria microhelia, Ferocactus sp., Thelocactus chrenbergii, Trichocereus bridgesii, Mamillaria magnimamma, Opuntia sp., Cereus sp., Astrophytum capricorne, Cereus sp., Astrophytum myriostigma (*from greenhouse collection of Nikitsky Botanical Garden) and *Ritterocereus pruinosus (*from the collection of Karazin' Botanic Garden of Kharkiv National University) we observed mosaic symptoms. On plants *Ausrocylindropuntia tunicata, Monvillea sp., Sulcorebutia sp., Bolivicereus samupatanus, Opuntia sp., Astrophytum myriostigma v. nudum* from greenhouse collection of Nikitsky Botanical Garden – National Scientific Centre the symptoms of necrosis had been detected (Fig.1. A., B.).

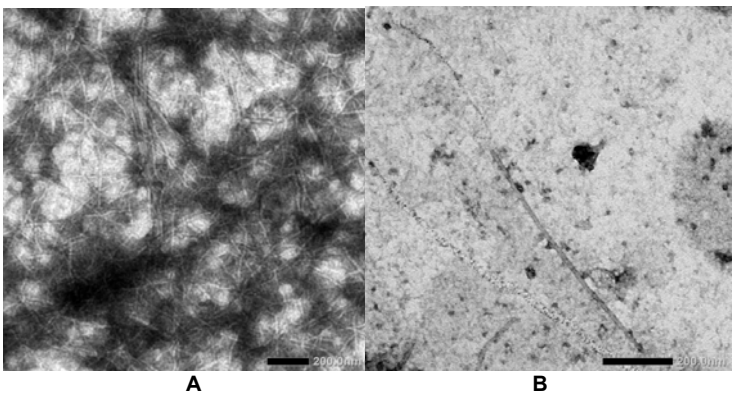
A B

Fig.1. Symptoms of cactus plants:
A. Mosaic on stem of *Opuntia* sp. from the collection of Botanical garden of Odessa I.I. Mechnikov National University
B. Chlorosis and necrotic spots on stem of *Opuntia microdasys* from the collection of Fomin Botanical Garden of Taras Shevchenko National university of Kyiv

To investigate the biological characteristics of viruses and their diversity samples of cactus with visual symptoms of disease were selected. All samples were tested for indicator plants typical for cactus virus (*Celosia argentea, Celosia cristata, Gomphrena globosa, Chenopodium amaranticolor, Chenopodium murale, Nicotiana tabacum, Nicotiana rustica)*. Local necrosis on the leaves of plants *Chenopodium murale* and *Gomphrena globosa* are typical evidence of a cactus X virus [3, 5, 6]. In *Nicotiana tabacum* and *Nicotiana rustica* the symptoms observed included small localized chlorotic lesions and in some plants systemic damage.

According to ELISA datas antigens of CVX were detected in plants matherial of *Mammilaria zeilmanniana, Opuntia* sp., *Ferocactus echidne, Gymnocalycium* sp., *Opuntia* sp., *Mammillaria nivosa, Opuntia* sp., *Consolea rubescens, Caralluma* sp., *Echinocereus* sp., *Opuntia brasiliensis, Opuntia microdasys* v. *rufida, Opuntia* sp. and *Pereskia aculeata* v. *godseffiana*, *Monvillea* sp., *Sulcorebutia* sp., *Bolivicereus samupatanus, Opuntia* sp., *Ferocactus* sp. *Chamaecereus silvestrii* f. cristata, *Echinopsis* sp. f. cristata, *Echinocereus pectinatus* f. cristata, and *Mammillaria elongata* f. cristata, *Ritterocereus pruinosus*, *Opuntia* sp., *Cereus* sp., *Astrophytum capricorne, Astrophytum myriostigma, Astrophytum myriostigma* v. *nudum*. According to ELISA datas antigens of CV2 were detected in plants *Mammilaria zeilmanniana, Opuntia* sp., *Ferocactus echidne, Gymnocalycium* sp., *Opuntia* sp., *Mammillaria nivosa, Opuntia* sp., *Consolea rubescens, Caralluma* sp., *Echinocereus* sp., *Opuntia brasiliensis, Opuntia microdasys* v. *rufida, Opuntia* sp. and *Pereskia aculeata* v. *godseffiana* and *Monvillea* sp., *Sulcorebutia* sp., *Bolivicereus samupatanus, Opuntia* sp., *Ferocactus* sp.

To study the morphology of the pathogen we carried out transmission electron microscopy. In the plant sap of *Mammilaria zeilmanniana, Opuntia* sp. (collection of the Donetsk botanical garden of the National Academy of Sciences of Ukraine), *Ferocactus echidne, Gymnocalycium* sp., *Opuntia* sp. (collection of Botanical garden of Ivan Franko National university of Lviv), *Mammillaria nivosa, Opuntia* sp. (collection of Botanical garden of Odessa I.I. Mechnikov National university), *Consolea rubescens, Caralluma* sp., *Echinocereus* sp., *Opuntia brasiliensis, Opuntia microdasys* v. *rufida, Opuntia* sp. and *Pereskia aculeata* v. *godseffiana* (collection of Fomin Botanical Garden of Taras Shevchenko National university of Kyiv) and *Monvillea* sp., *Sulcorebutia* sp., *Bolivicereus samupatanus, Opuntia* sp., *Ferocactus* sp. (collection of Nikitsky Botanical Garden – National Scientific Centre) we registered filamentous virions with size 580 × 13 nm which is typical *Cactus virus X*. In the plant sap of *Opuntia* sp. (collection of Botanical garden of Odessa I.I. Mechnikov National university), *Opuntia* sp. (collection of Botanical garden of Ivan Franko National university of Lviv), *Gymnocalycium mihanovichii* v. *friedrichii, Mammillaria magnimamma, Mammillaria microhelia, Opuntia* sp. and fasciated plants of *Chamaecereus silvestrii* f. cristata, *Echinopsis* sp. f. cristata, *Echinocereus pectinatus* f. cristata, and *Mammillaria elongata* f. cristata (collection of Fomin Botanical Garden of Taras Shevchenko National university of Kyiv), *Ritterocereus pruinosus* from the collection of Karazin' Botanic Garden of Kharkiv National University, *Opuntia* sp., *Cereus* sp., *Astrophytum capricorne, Astrophytum myriostigma, Astrophytum myriostigma* v. *nudum* (collection of Nikitsky Botanical Garden – National Scientific Centre) we registered filamentous virions with 650 × 12 nm (Fig.2), which is typical for *Cactus virus 2*. It was also found rod-shaped particle with size of 317 × 18 nm, which is typical for tobamovirus in fasciated plants of *Echinopsis* sp. f. cristata and *Mammillaria elongata* f. cristata.



A B

Fig.2. Electron micrograph of filamentous virus-like particles from plant material from:
A- *Mammillaria elongata* f. cristata
B- *Opuntia brasiliensis*

Comparing the results of bioassay, EM and ELISA tests CV2 had been detected in such plants as: *Mammilaria zeilmanniana, Opuntia* sp. (collection of the Donetsk botanical garden of the National Academy of Sciences of Ukraine), *Ferocactus echidne, Gymnocalycium* sp., *Opuntia* sp. (collection of Botanical garden of Ivan Franko National university of Lviv), *Mammillaria nivosa, Opuntia* sp. (collection of Botanical garden of Odessa I.I. Mechnikov National university), *Consolea rubescens, Caralluma* sp., *Echinocereus* sp., *Opuntia brasiliensis, Opuntia microdasys* v. *rufida, Opuntia* sp. and *Pereskia aculeata* v. *godseffiana* (collection of Fomin Botanical Garden of Taras Shevchenko National university of Kyiv) and *Monvillea* sp., *Sulcorebutia* sp., *Bolivicereus samupatanus, Opuntia* sp., *Ferocactus* sp. (collection of Nikitsky Botanical Garden – National Scientific Centre).

CVX has been identified in plants of *Opuntia* sp. from collection of Botanical garden of Odessa I.I. Mechnikov National university, *Opuntia* sp. from collection of Botanical garden of Ivan Franko National university of Lviv, *Gymnocalycium mihanovichii* v. *friedrichii, Mammillaria magnimamma, Mammillaria microhelia, Opuntia* sp. and fasciated plants of *Chamaecereus silvestrii* f. cristata, *Echinopsis* sp. f. cristata, *Echinocereus pectinatus* f. cristata, and *Mammillaria elongata* f. cristata from the collection of Fomin Botanical Garden of Taras Shevchenko National university of Kyiv, in plants of *Ritterocereus pruinosus* from the collection of Karazin' Botanic Garden of Kharkiv National University, *Opuntia* sp., *Cereus* sp., *Astrophytum capricorne, Astrophytum myriostigma, Astrophytum myriostigma* v. *nudum* from the collection of Nikitsky Botanical Garden – National Scientific Centre. Attracts attention the fact that CVX was in the all botanical gardens, but the species composition of affected plants was different. It should be noted that in all the studied collections plants of the genus *Opuntia* were the most infected

Moreover, virus related to tobamoviruses has been detected in plants of *Echinopsis* sp. f. cristata and *Mammillaria elongata* f. cristata from the collection of Fomin Botanical Garden of Taras Shevchenko National university of Kyiv. Definitive identification of this virus requires additional research because there are three different tobamoviruses which are able to infect the cactus plants.

Conclusions: Summarizing the obtained results it is possible to assert that collections of Donetsk botanical garden of the National Academy of Sciences of Ukraine, Botanical garden of Ivan Franko National university of Lviv, Botanical garden of Odessa I.I. Mechnikov National university, Karazin' Botanic Garden of Kharkiv National University, Nikitsky Botanical Garden – National Scientific Centre were contaminated by *Cactus virus X* and *Cactus virus 2*. It should be noted that before being placed in a greenhouse cactuses had not been tested for presence of viral pathogens. Besides the pests (nematodes) and mites had been registrated in some plants with the viral symptoms. Although ability of vector transmission not proven for majority of cactus viruses, it is possible that the presence of pests and non-viral disease causing deterioration of the physiological state of the plants. In addition, cactuses could support reproduction of viruses other types of plants and, thus, be the reservoirs of plant virus infections. A timely detection and continuous monitoring of cactus viruses is a main part of the system of plant virus protection.

References

1 Anderson, Edward F. The Cactus Family // Pentland, Oregon: Timber Press 2001. – P. 766.

2. M. Chessin, D. Lesemann. Distribution of Cactus Viruses in Wild Plants // Phytopatology. – 1972. –Vol.62. – P.97-99.

3. M.L. Duarte, M.A.V. Alexandre, E.B. Rivas, R. Harakava, S.R. Galleti and M.M. Barradas Potexvirus diversity in Cactaceae from Sao Paulo state in Brazil // Journal of Plant Pathology (2008), 90 (3), 545-551.

4. Virus Taxonomy Classification and Nomenclature of Viruses Ninth Report of the International Committee on Taxonomy of Viruses Editors Andrew M.Q. King, Michael J. Adams, Eric B. Carstens, and Elliot J. Lefkowitz International Union of Microbiological Societies Virology Division Elsevier academic press.

5. R. Koenig, C.W.A. Pleij, S. Loss, W. Burgermeister, H. Aust, J. Schiemann. Molecular characterization of potexviruses isolated from three different genera in the family Cactceae // Arch. Virol. – 2004. – №149. – P.903-914.

6. J.R. Lastra, Daisy Gaskin, y R.C. de Uzkątegui. Virus x del cactus en Venezuela // Agronomia Tropical.1976. – №26. – P.303-310.

7. Liou MR, Hung CL, Liou RF (2001) First report of Cactus virus X on Hylocereus undatus (Cactaceae) in Taiwan. Plant Dis 85: 229.

8. G.M. Milbrath, M.R. Nelson and R.E. Wheeler. The Distribution and Electron Microscopy of Viruses of Cacti in Southern Arizona // Phytopatology. – 1973. – №63. – P.1133-1139.

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ДЕТЕКЦІЯ ДЕЯКИХ ВІРУСНИХ ПАТОГЕНІВ КАКТУСІВ В БОТАНІЧНИХ САДАХ УКРАЇНИ

Колекції кактусових провідних українських ботанічних садів були проаналізовані на наявність вірусної інфекції. На кактусах були виявлені різні вірусоподібні симптоми, включаючи мозаїки, хлорози та локальні некрози. Біологічні властивості детектованих вірусів визначали методами біологічного тестування, електронної мікроскопії та непрямого імуноферментного аналізу.

Ключові слова:імуноферментний аналіз, віруси рослин, кактуси.

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ДЕТЕКЦИЯ НЕКОТОРЫХ ВИРУСНЫХ ПАТОГЕНОВ КАКТУСОВ В БОТАНИЧЕСКИХ САДАХ УКРАИНЫ

Коллекции кактусовых ведущих украинских ботанических садов были проанализированы на наличие вирусной инфекции. На кактусах были выявлены различные вирусоподобные симптомы, включая мозаики, хлороз и локальные некрозы. Биологические свойства детектированных вирусов определяли методами биологического тестирования, электронной микроскопии и косвенного иммуноферментного анализа.

Ключевые слова: иммуноферментный анализ, вирусы растений, кактусы.

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ETHANOL EFFECT ON BIOPRODUCTIVITY, PHOTOSYNTHESIS AND RESPIRATION OF MICROALGA *CHLAMYDOMONAS REINHARDTII*

Some organic compounds may significantly stimulate the growth of unicellular green alga Chlamydomonas reinhardtii. Among them the most effective growth enhancers are acetate and monohydric alcohol methanol. The aim of the present work was studying the effect on the productivity of C. reinhardtii another alcohol – ethanol, which transforms into acetic acid in the process of intracellular oxidation. The results showed that in the presence of ethanol respiration was stimulated, photosynthesis inhibited and the growth of the culture stopped. We concluded that the cause of growth inhibition of C. reinhardtii was pH decline of the cultural medium due to oxidation of ethanol to acetic acid.

Keywords: Chlamydomonas reinhardtii, photosynthesis, respiration, ethanol, mixotrophy.

Introduction. The green alga *Chlamydomonas reinhardtii* has importance as model for many biotechnological processes and algal biofuels [17]. Availability of a sequenced genome [10], a proteomic database [9], and metabolomics protocols [8] benefits the use of *Chlamydomonas* to establish many fundamental aspects of metabolic control in photoautotrophic organisms [8]. It can grow either photosynthetically in the light with atmospheric CO_2 as the sole carbon source, or under heterotrophic conditions in the dark using various exogenic carbon sources added to the growth medium or else mixotrophic conditions (light and carbon sources). Under all conditions, *C. reinhardtii* remains green and retains a normally developed chloroplast, which can thus metabolize a variety of carbon sources as located in the chloroplast (starch) or assimilated through the cytosol of the cultural medium [12].

Significant stimulation of microalgae growth by exogenic methanol at mixotrophic cultivation was shown for unicellular green algae *Chlorella minutissima, Scenedesmus obliquus* [16]*, Botryococcus braunii* [12] as well as *C. reinhardtii* [2]. Another alcohol – 2-carbon ethanol, following methanol in the homologous series of monohydric alcohols, is able to enhance the growth of microalga *Euglena gracilis,* being one of the most efficient carbon sources for this microalga [19]. Acetate, the product of ethanol oxidation, strongly stimulates C*. reinhardtii* growth [6]. *C. reinhardtii* is capable of heterotrophic and mixotrophic growth utilizing acetate as a source of carbon and energy.

In microanaerobiosis, which is naturally formed in habitat of microalgae when the respiration rate exceeds the rate of photosynthesis, the cells of *C. reinhardtii* excrete ethanol, formate and acetate [7]. Acetate can be metabolised to triose by an ATP-dependent entry into the glyoxylate or Krebs cycle to produce reducing equivalents, which can be used to reduce the plastoquinone pool [11]. It is incorporated into acetyl coenzyme A (acetyl-CoA) following two possible pathways: a direct conversion with acetyl-CoA synthetase or a two-step reaction involving acetate kinase and phosphate acetyltransferase. Acetyl-CoA enters into the glyoxylate cycle, where it is converted to succinate. Succinate is further utilized in the Krebs cycle. The carbon of ethanol, like in methanol, is oxidised to CO_2 at the final stage and may supplied as a substrate for photosynthesis.

From other hand, it was established that the addition of 0.3% v/v ethanol in the culture medium of *Dunaliella viridis* accompanied by cessation of culture growth and increased intracellular concentrations of DNA, RNA and total protein [1]. Ethanol increases ploidy of the cells and inhibits their metabolism. Microalgae pass from dormancy to intense growth after removal of ethanol from the cultural medium. It was found toxic effects of ethanol on the growth of *Chlorella vulgaris* and *Selenastrum capricornutum*, ethanol inhibited the growth of these algae at a concentration of 0.05% [4].

The ability of exogenic ethanol to regulate productivity of *C. reinhartdii* under aerobic conditions in the light and in the dark has not been investigated. The aim of our study was to determine the effect of exogenic ethanol on productivity of batch culture of *C. reinhartdii* and its effect on photosynthesis and respiration.

Materials and methods. Unicellular green alga *Chlamydomonas reinhartdii* was obtained from the microalgae collection of Kholodny Botany Institute of NAS of Ukraine (IBASU-B – 163). Batch autotrophic cultures were grown on liquid Kessler's medium [3] in 0.5 l flasks with magnetic stirrer agitation at room temperature. 24 h white fluorescent light with 100 μmol photons•m^{-2}•s^{-1} on the surface of flasks was used. The ethanol effects were studied at the stage of exponential growth phase of batch culture. The packed cell volume (PCV) was determined as a measure for the biomass accumulation. The PCV, the volume of the cell pellet in μl, was measured by the centrifugation of a defined volume of the cell suspensions at 1400×g for 5 min in haematocrite tubes [13]. The chlorophylls (Chl) were determined spectrophotometerically in ethanolic extracts by the method of Wintermans and De-Mots [18]. The concentration of chlorophylls was calculated using the formulas: Chl *a* =13,70(A_{665}-A_{750})-5,76(A_{649}-A_{750}); Chl *b* =25,80(A_{649}-A_{750})-7,60(A_{665}-A_{750}); Chl*a*+*b* = 6,10(A_{665}-A_{750})+20,04(A_{649}-A_{750}).

Intensity of visible photosynthesis (A) and dark respiration (R) was determined in the gas phase above the suspension of algae by IRGA method with QUBIT Systems S151 Carbon Dioxide Analyzer (Canada). Gas flow rate was 0.4 l/min and the concentration of carbon dioxide – 700-800 μM. Gas exchange measurements were carried out in a thermostated glass cell filled by 2 ml of concentrated suspension of microalgae (30-40 mg/l of chlorophyll). The rate of carbon dioxide uptake was determined under illumination with light intensity of 350 mol photons•m^{-2}•s^{-1}. Dark respiration measured with a low content CO_2 in the gas space above the suspension of microalgae after turn-

ing off the light. Before being introduced into the analyzer air was passed through the column with ascarite for CO_2 removing from the gas phase.

Each experiment was repeated several times (n) and the mean values (M) and standard deviation (m) calculated for each treatment. Mathematical-statistical data processing was performed using the software package Microsoft Excel 2010. Statistical difference between groups was determined by t-test, for statistically significant were taking changes with $P<0.05$.

Results and discussion. As an indicator of the growth intensity of *C. reinhartdii* culture, we have chosen the changing concentration of total chlorophyll in exponential growth phase. The rate of cell division is directly proportional to chlorophyll concentration in the culture [5]. By analogy with methanol [2], we examined the effects of 20 and 40 mM ethanol on the growth of *C. reinhardtii*. In the presence of this solvent, the accumulation of chlorophyll is reduced and accompanied by cell death both in the light and in the dark (Fig 1). Effects of 20 and 40 mM ethanol were the same.

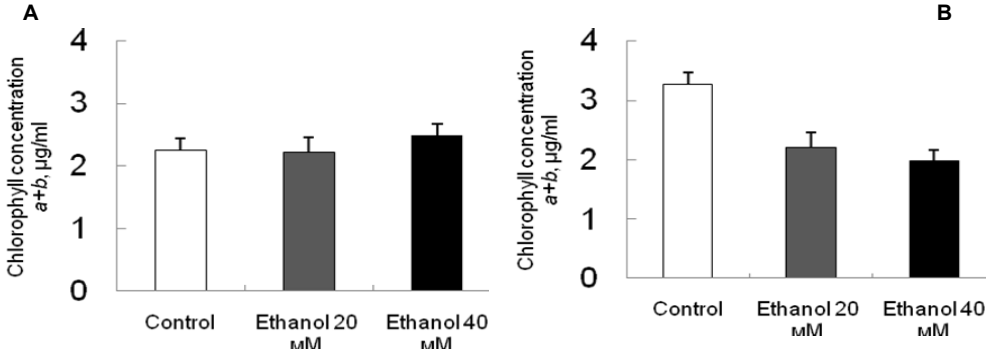

Fig. 1. The concentration of total chlorophyll in suspension *C. reinhardtii* on the fourth day of cultivation, µg/ml.
A – cultivation in the light, B – cultivation in the dark. (M+m, n=3)

Regardless of added ethanol concentration, the pH declines to 3.5 on the fourth day after ethanol application. The pH changes induced by ethanol are not depending on illumination. A gradual acidification of the medium takes place both in the light and in the dark. Apparently, acidification of the cultural medium in the presence of ethanol is due to its incomplete metabolization inside microalgal cells. Oxidation of ethanol occurs in several stages: 1. ethanol → acetaldehyde → acetic acid → CO_2. The result of the first stage of the oxidation is acetaldehyde, a toxic compound that can negatively affect the *C. reinhardtii* cells. Metabolization of ethanol by alcoholdehydrogenase occurs in both forward and backwards direction and depends on the ratio of substrates concentration – ethanol, acetaldehyde and nicotinamide coenzymes. The equilibrium concentration of acetaldehyde at the level of 1 mM may be established at addition of 40 mM exogenic ethanol [14]. However, under conditions of our experiments the formation of acetic acid proves that the enzymatic system involved in the detoxification of acetaldehyde to acetic acid are active. Since acetate is easily assimilated by the *C. reinhardtii* cells, its accumulation in the presence of exogenic ethanol may indicate a competition between acetate and ethanol at the level of the mitochondrial respiratory chain.

Microalgae begin secrete excess acetic acid in the culture medium when the rate of acetic acid formation exceeds the rate of complete intracellular ethanol utilization. As result of ethanol oxidation, acetic acid is accumulated in the liquid medium and induces its acidification. Toxic effect of ethanol can be explained by the formation of the equilibrium concentration of acetaldehyde in the culture medium, and its acidification due to secretion of excessive acetic acid from the cells.

In addition to the research culture growth by analyzing the content of chlorophyll, we determined the growth rate of the culture also by packed cell volume (PCV). PCV was determined on the third day after application of ethanol (10 µM, 100 µM, 1 mM, 10 mM) in the culture medium of *C. reinhardtii* and in control. Addition of ethanol at a concentration greater than 10 µM reduces the growth of *C. reinhardtii* biomass in in terms of PCV (Fig 2). Ethanol at a concentration of 100 µM inhibits the accumulation of biomass by 23% at a concentration of 1 mM and 10mM of ethanol inhibits the cellular growth by 33%. The presence of ethanol in the culture medium had a negative effect on the cells, resulting in a decrease in cell motility and increasing the proportion of fixed cells under a light microscope.

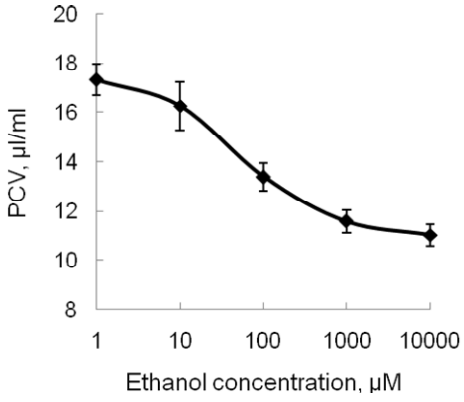

Fig. 2. PCV in autotrophic culture of *C. reinhardtii* on the third day after application of different amounts of ethanol. (M+m, n=3)

We analyzed the effect of ethanol (50 mM) on the rate of dark CO_2 release by *C. reinhardtii*, as a measure of dark mitochondrial respiration R. As seen in Fig 3, A, the rate of respiration in batch culture of *C. reinhardtii* increased twice after six hours of microalgae cultivation with ethanol compared with control. Increasing R can be explained by activation of turnover of the Krebs cycle as a result of utilization of excess acetyl-CoA.

The parameter of V_{max} can indirectly characterize the intracellular pool of pyruvate and malate. Ethanol at a concentration of 50 mM increases V_{max} by 29 % compared with control (Fig 3, B). Therefore, we can assume that ethanol increases the intracellular concentration of tricarboxylic acids. Ethanol oxidation lead to increasing of NADH content, which may lead to increasing intracellular concentrations of malate or pyruvate. Also R in culture with ethanol after six hours is equal to V_{max}, ie the rate of CO_2 release reaching V_{max} after switching off the light remains at this level for 20 minutes. This can be explained by the fact that the addition of ethanol saturates Krebs cycle in the dark and in the light tricarboxylic acids are accumulated.

For further establishing the toxic effects of ethanol at concentration of 50 mM on the metabolism of *C. reinhardtii*, we investigated the change in apparent photosynthetic CO_2 uptake (A). Adding ethanol depressed photosynthesis by 25% compared with the control (Fig 3, C). Inhibition of apparent photosynthesis can occur as a result of activation of the light mitochondrial respiration, or due to toxic effects of acetaldehyde on pigment-protein complexes of photosynthetic membranes. Change A, R and V_{max} with addition of ethanol in culture medium *C. reinhardtii* are similar to change these parameters during mixotrofic cultivation of *C. reinhardtii* in the medium with acetate [15]. However, unlike the cultivation with ethanol, acetate stimulates the growth of the alga. Therefore, we can assume that the toxic effects of ethanol on *C. reinhardtii* is not due to oxidation of acetate in Krebs cycle but rather due to oxidation of ethanol to acetaldehyde with participation of alcoholdehydrogenase.

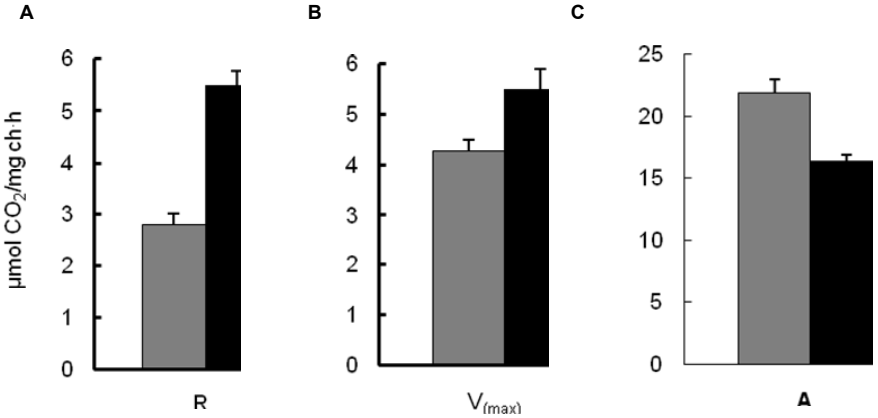

Fig. 3. Indicators of CO_2 gas exchange *C. reinhardtii* (□ – control, ■ – in the presence of 50 mM methanol).
A – dark respiration rate (R). B – the maximum rate of respiration (V_{max}). C – apparent photosynthetic rate (A).
(M+m, n=5)

Conclusions. Exogenic ethanol inhibits the growth of batch culture of *C. reinhardtii* in a concentration greater than 10 μM. Toxic effect of ethanol on the metabolism accompanied by a decrease in pH of cultivation medum both in the light and in the dark. The intensity of visible photosynthesis also decreases, and significantly increases the intensity of dark mitochondrial respiration of mixotrophic *C. reinhardtii* cultivated with ethanol.

References

1. Божков А.И., Мензянова Н.Г. Влияние этилового спирта на метаболизм водорослей. Метаболизм нуклеиновых кислот и белка в клетках Dunaliella viridis Teod // Альгология. – 2002. – 12, 3. – С. 300–308.

2. Степанов С. С., Золотарева Е. К. Метаболічний шлях метанолу у рослин // Альгология. – 2011. – т.21, № 2. – С. 178–190.

3. Bishop N.I., Senger H. Preparation and photosynthetic properties of synchronous cultures of Scenedesmus // Meth. Enzymol. – 1971. – Vol. 23, part A. –P. 53– 66.

4. El J. A Effects of organic solvents and solvent-atrazine interactions on two algae, Chlorella vulgaris and Selenastrum capricornutum // Arch Environ Contam Toxicol. – 1996. – Vol.31, No.1. – P.84-90.

5. Gitelson A., Grits Y., Etzion D. Optimal properties of Nannochloropsis sp and application to remote estimation of cell mass // Biotechnology Bioengineering. – 2000. – Vol. 69, No.5. – P. 516-525.

6. Hemschemeier A., Jacobs J., Happe T. Biochemical and physiological characterization of the pyruvate formate-lyase Pfl1 of Chlamydomonas reinhardtii, a typically bacterial enzyme in a eukaryotic alga // Eukaryot. Cell. – 2008. – Vol. 7, No. 3. – P. 518–526.

7. Johnson X., Alric J. Interaction between Starch Breakdown, Acetate Assimilation, and Photosynthetic Cyclic Electron Flow in Chlamydomonas reinhardtii // J. Biol. Chem. – 2012. – Vol. 287, No. 31. – P. 26445–26452.

8. Lee D., Fiehn O. High quality metabolomic data for Chlamydomonas reinhardtii // Plant Meth. – 2008. – Vol. 4, 7.

9. May P., Wienkoop S., Kempa S., et al. Metabolomics- and Proteomics-Assisted Genome Annotation and Analysis of the Draft Metabolic Network of Chlamydomonas reinhardtii // Genetics. – 2008. – Vol. 179. – P. 157–166.

10. Merchant S.S., Prochnik S.E., Vallon O., et al. The Chlamydomonas genome reveals the evolution of key animal and plant functions // Science.- 2007. – Vol. 318. – P. 245–250.

11. Mi H., Klughammer C., Schreiber U. Light-induced dynamic changes of NADPH fluorescence in Synechocystis PCC 6803 and its ndhB-defective mutant M55 // Plant and Cell Physiology. – 2000. – Vol. 41, № 10. – P. 1129–1135.

12. Nonomura A., Benson A. The path of carbon in photosynthesis: Improved crop yields with methanol // Proc. Natl. Acad.Sci. USA. – 1992. – Vol. 89, No. 20. – P. 9794–9798.

13. Navakoudis E., Ioannidis N.E., Dörnemann D., Kotzabasis K. Changes in the LHCII-mediated energy utilization and dissipation adjust the methanol-induced biomass increase // Biochim. Biophys. Acta. – 2007. – Vol. 1767, No. 7. – P. 948–955.

14. Perata P., Alpi A., LoSchiavo F. Influence of ethanol on plant cells and tissues // J. Plant Physiol. – 1986. – Vol. 126. – P. 181–188.

15. Roach T., Sedoud A. Krieger-Liszkay A. Acetate in mixotrophic growth medium affects photosystem II in Chlamydomonas reinhardtii and protects against photoinhibition // Biochim. Biophys.Acta. – 2013. – Vol. 1827, No.10. – P. 1183-1190.

16. Theodoridou A., Dörnemann D., Kotzabasis K. Light dependent induction of strongly increased microalgal growth by methanol // Biochim. Biophys. Acta – 2002. – Vol. 1573, No. 2. – P. 189–198.

17. Wang Z.T., Ullrich N., Joo S., et al. Algal Lipid Bodies: Stress Induction, Purification, and Biochemical Characterization in Wild-Type and Starchless Chlamydomonas reinhardtii // Eukar. Cell. – 2009. – Vol. 8. – P. 1856–1868.

18. Wintermans F.G., De Mots A. Chlorophyll determination A suitable method for Chlamydomonas. // Biochim. Biophys. Acta. – 1965. – Vol. 109. – P. 448–453.

19. Yoval-Sánchez B., Jasso-Chávez R., Lira-Silva E., et al. Novel mitochondrial alcohol metabolizing enzymes of Euglena gracilis // J. Bioenerg. Biomembr. – 2011. – Vol. 43, No.5. – P. 519-530.

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ВПЛИВ ЕТАНОЛУ НА БІОПРОДУКТИВНІСТЬ, ФОТОСИНТЕЗ ТА ДИХАННЯ МІКРОВОДОРОСТІ *CHLAMYDOMONAS REINHARDTII*

Деякі органічні сполуки здатні значно стимулювати ріст одноклітинної зеленої водорості Chlamydomonas reinhardtii. Серед них найбільш активними стимуляторами росту є ацетат і одноатомний спирт метанол. Метою даної роботи було вивчення впливу на продуктивність C. reinhartdii іншого спирту – етанолу, який при внутрішньоклітинному окиснені трансформується у ацетат. Отримані результати показали, що етанол стимулює дихання, пригнічує фотосинтез і зупиняє ріст культури. Зроблений висновок, що причиною гальмування росту C. reinhartdii було зниження pH середовища культивування внаслідок окиснення етанолу до оцтової кислоти.

Ключові слова : Chlamydomonas reinhardtii, фотосинтез, дихання, етанол, міксотрофія.

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ВЛИЯНИЕ ЭТАНОЛА НА БИОПРОДУКТИВНОСТЬ, ФОТОСИНТЕЗ И ДЫХАНИЕ МИКРОВОДОРОСЛИ *CHLAMYDOMONAS REINHARDTII*

Некоторые органические соединения способны значительно стимулировать рост одноклеточной зеленой водоросли Chlamydomonas reinhardtii. Среди них наиболее активными стимуляторами роста являются ацетат и одноатомный спирт метанол. Целью данной работы было изучение влияния на продуктивность C. reinhartdii другого спирта – этанола, окисляющегося внутри клеток в ацетат. Результаты показали, что этанол стимулирует дыхание, подавляет фотосинтез и останавливает рост культуры. Сделан вывод, что причиной торможения роста C. reinhartdii было снижение pH среды культивирования в результате окисления этанола до уксусной кислоты.

Ключевые слова : Chlamydomonas reinhardtii, фотосинтез, дыхание, этанол, миксотрофия.

UDK: 616.33-002.44

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THE INFLUENCE OF LOW MOLECULAR WEIGHT ORGANIC COMPOUNDS ON ANTIOXIDANT DEFENSE SYSTEMOF THE GASTRIC MUCOSA UNDER ETHANOL-INDUCED GASTRIC LESIONS IN RATS

It was investigated the preventive effect of low molecular weight organic compound (LMOC) on erosive and ulcerative lesions in the gastric mucosa of rats caused by ethanol. It was found that prophylactic injection of this substance at a dose of 1 mg/kg effectively protects the stomach from ethanol injuries. LMOC effectively restored the pro-/ antioxidant equilibrium by reducing the intensity of lipid peroxidation in the gastric mucosa of rats after ethanol injection and increase ofsuperoxidedismutase, catalase activityandactivity of glutathionesystem.

Keywords: ethanol-induced injuries, lipid peroxidation,low molecular weight organic compound.

Introduction. Gastric ulcer is a common disease affecting many people worldwide [1]. The peptic ulcer, characterize by mucosal damage, is predominantly caused by *Helicobacter pylori*, antiplatelet agents such as acetylsalicylic acid [2], nonsteroidal anti-inflammatory drugs (NSAIDs) such as oral bisphosphonates, potassium chloride, immunosuppressive medications [3], serotonin reuptake inhibitors [4], alcohol consumption, and cigarette smoking [5]. The ulcer disease may lead to upper gastrointestinal haemorrhage and perforation [6], which have high morbidity and mortality rates [7]. Thus,the search of the newnontoxic medications is very importanttoday. So the aim of the work was to investigate the preventive effect of low molecular weight organic compound (LMOC) on erosive and ulcerative lesions in the gastric mucosa undere-thanol-induced gastric lesions in rats.

Methods. The animals used in the study were bred and kepton a standard diet in terms of accredited vivarium of Educational and Scientific Center "Institute of Biology" Taras Shevchenko National University of Kyiv in accordance with the "standard rules on arrangement, furnishing and maintenance of experimental biological clinics (vivarium)".

The studywas carried out on 30 white laboratory Wistar rats. The research was conducted in accordancewith the Law of Ukraine dated 21.02.2006 № 3447-IV "On protection of animals from abuse" and the ethical standards and rules of working with laboratory animals (Guide for the Care and Use of Laboratory Animals, National Academy Press, Washington DC, 1996) [8]. All animals selected for the experiment were subjected to veterinary examination, were acclimated for five days, and then randomly divided into groups, numbered and marked appropriately.

For examine of the preventive actionof LMOC (sodium 2-(2-hydroxyphenoxy) acetyl)-L-prolinate)rats were divided into 3 groups of 10 animal seach: 1stgroup was intact rats, 2nd and 3rd – rats, which had ethanol-induced ulcer at ion of the gastric mucosa (GM). Rats of 2nd group were injected with normal saline at a volume 2 ml/kg 30 minutes before ulcerogenic factor action, they were the control for the 3rd group. Rats of the 3rd group were treated with LMOC which was injected at thedoseof 1 mg/kg(2 ml/kg of saline solution) 30 minutes before ethanol action (compound was syn the size dat Lomonosov Moscow State University).

Erosive and ulcerative lesions of GM of rats in the 2nd and 3rd groups were caused by intra gastric ethanol infusion. After 1 hour from ethanol exposure the rats were sacrificed.To assess the state of GM of rats after ulcerogenic factor action stomach was removed, cutalong the lesser curvature, turn mucous out and thoroughly was hed with saline. The area and number of ulcers was measure dusing experimental gastroscope.

In the homogenateof GM of rats the content of lipid peroxidation(LP) products(the concentration of hydrogen peroxide, dien conjugates, thiobarbituric acid (TBA)- active products and Shiff's bases) wasmeasured by standard biochemical methods [9-12]. Antioxidant protection of the GM under condition of ethanol administration was assessed by the superoxide dismutase, catalase and glutathione system activity [13-15]. To study the influence of NMOS on glutathione system we examined the content of reduced (GSH) and oxidized glutathione (GSSG), glu-

tathione peroxidase (GP), glutathione reductase (GR) and glutathione transferase (GT) activity [14, 16-19]. All obtained datawere subjected to statistical analysis using software package "Statistics, 8.0". Shapiro-Wilks criterion was used for the analysis of data distribution type. As the data were normally distributed, we used Levan criterion forevaluation the equality of variance and Student's t-test for independent samples. We calculated mean values (M) and standard error of mean (m). Significant difference was considered at $p \leq 0,05$ [20].

Results. After an hourfrom ethanol exposure in the group of rats which were injected with water simultaneously with ethanol ulcerative lesions of gastric mucosa were reported in 100% of animals. The number of ulcers per one stomach was 13.1±1.8, and the total area of the ulcerative lesionswas equal to256.1±68.0 mm^2. Prophylactic administration of LMOC had no effect on the number of ulcerative lesions in animals. However, the average size of lesions was 83±18.61 mm^2, so it was established the significant reduction in lesion area by 67.6 % ($p<0.05$) compare tothe control group (Fig.1).

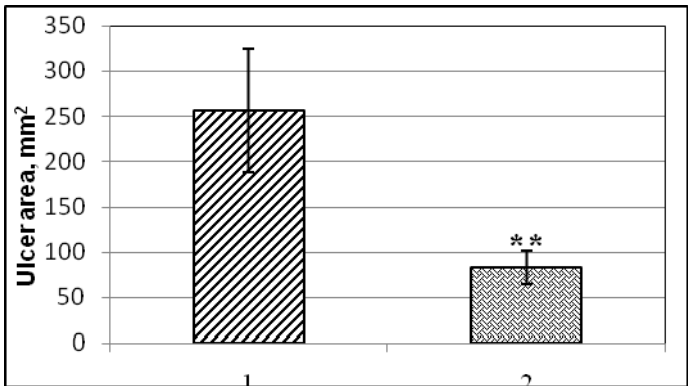

Figure 1. The ulcer area in the gastric mucosa of rats caused by the introduction of ethanol under treatment with low molecular weight organic compound (10 rats in each group, M±m):
1 – saline + ethanol;
2 – low molecular weight organic compound + ethanol

After the ethanol introduction it was established a significant increase of the intensity of LP in the GM of rats. The content of diene conjugates, TBA-active products and Shiff's bases was increased by 5.2 times, 2.3 times and 4.93 times accordingly compared with intact control (Table 1). This indicates the active accumulation of reactive oxygen species(ROS) after administration of ethanol. Indeed, hydrogen peroxide content inGMwas exaggeratedby 4.87 times. As a result of intensification of LPin the GM of rats superoxide dismutase and catalase activity was raised by 4.28 times ($p<0.001$) and 2.33 times ($p<0.01$) accordinglycompared with intact controls (Table 2).

***Table1*. Lipid peroxidation in the gastric mucosa of rats with erosive and ulcerate velesions caused by ethanol and prophylactic injection of low molecular weight organic compound (M+m)**

	Intact control	Saline group	Low molecular weight organic compound group
H_2O_2, μmol × mgof $protein^{-1}$	2.1±0.2	10.23±0.92***	10.51±0.94***
Dieneconjugates, nmol × mgof $protein^{-1}$	71.3±6.4	371.3±35.3***	239.6±21.9***/##
TBA-active products, nmol × mgof $protein^{-1}$	44.2±4.4	101.7±9.94***	63.6±5.95*/#
Shiff's bases, c.u. ×mgof $protein^{-1}$	1.2±0.1	5.91±0.58***	4.02±0.35*/#

*, *** – $p<0.05$, $p<0.001$ compared with intact control, #, ## – $p<0.05$, $p<0.01$ compared with saline group

LMOC decreased the content of diene conjugates by1.55 times ($p<0.01$), TBA-active products – by 1.6 times ($p<0.05$), Shiff's bases–by 1.47 times ($p<0.05$) in the GMof rats compared with the control group. The superoxide dismutase activity was increased by 1.65 times ($p<0.01$) under influence of LMOC compared with intact animals. However, comparing the group of rats injected with saline and LMOC, it should be noted that LMOCdiminished the enzyme activity compared with control group. Given the lower superoxide dismutase and higher catalase activity in rats treated with LMOC, the concentration of hydrogen peroxide in both groups was similar, suggesting acceleration of ROS scavenging by LMOC.

Examining of glutathione system activity it was established that GSH significantly decreased by 29 % ($p<0.05$) in the GM of the group of animals exposed to ethanol compared with intact control (Table 3). In contrast, oxidized glutathione content increased, indicating the use of its reduced form for the scavenging of ROS. This effect may be due to both increased use of glutathione by GP and its use for inactivation of ROS. Indeed, in our study found that the GP activity exceeded by 38 % ($p<0.05$) values of intact animals. One of the likely causes of a GSH decrease may also be a reduction in the GR activity, which restores GSSG to GSH, by 27 % ($p<0.05$) compared with intact rats. This enzyme uses NADPH as a reducing equivalent, and NADPH content is significantly reduced under conditions of ethanol-induced oxidative stress [21]. As a result of ethanol effect the GR activity decreased by 54 % ($p<0.001$) versusintact control. After the introduction of ethanol the GT activity was reduced by 43 % ($p<0.05$). This effect may be explained by depletion of glutathione systemunder the accumulation of excessive amounts of ROS.

***Table* 2. The antioxidant enzymes activity in the gastric mucosa of rats with erosive andulcerative lesions caused by ethanol and prophylactic injection of low molecular weight organic compound(M ± m)**

	Intact control	Saline group	Low molecular weight organic compound group
Superoxidedismutaseactivity, c.u. × min^{-1} × mgof $protein^{-1}$	0.16±0.01	0.686±0.050***	0.264±0.024*/###
Catalase activity, nmol × min^{-1} × mgof$protein^{-1}$	314.7±29.8	733.4±63.8**	1110.4±109.7***/##

, * – p<0.01,p<0.001 comparedwithintactcontrol, ##, ### – p<0.01, p<0.001 compared with saline group

Under conditions of LMOC treatment GSH content decreased by 52 % (p<0.01) compared to the intact control, and by 46.2 % (p<0.05) compared to the saline group. The content of the oxidized form of glutathione under conditions of prophylactic administration of LMOC was higher by 35 % (p <0.05) versus the intact rats, but decreased by 26.5% (p <0.05) compared with the control. Given increased activity of GT by 16 % (p <0.05) compared to the control in rats treated with LMOC, this suggested a more intensive use of glutathione in the inactivation ROS under LMOC treatment. This finding was confirmed by a decrease in the content of LP products under conditions of administration of the investigated compound. The GR activity was reduced by 54,3% (p<0.001) versus the intact rats, and was less by 37,3% (p<0.05) compared with the saline group. The high content of oxidized glutathione form gave the evidence of glutathione catabolism decrease under the treatment with LMOC.

***Table* 3. Glutathione content and enzymes activity of glutathione antioxidant defense system in the gastric mucosa of rats under condition of erosive and ulcerative lesions caused by ethanol and prophylactic injection of low molecular weight organic compound(M±m)**

	Intact control	Saline group	Low molecular weight organic compound group
Reducedglutathione, nmol of GSH ×mgof$protein^{-1}$	33.26±0.82	23.31±0.12*	15.94±1.04**/#
Oxidized glutathione,nmol of GSSG × mgof $protein^{-1}$	39.49±1.71	72.44±0.79**	53.36±2.83*/#
Glutathione transferaseactivity,nmolof conjugate glutathione with 1-chloro-2,4-dinitrobenzene × min ×mgof$protein^{-1}$	135.88±13.06	78.29±7.26**	90.93±8.78*/#
Glutathione reductase activity, nmolNADPH × min ×mgof$protein^{-1}$	1156.7±49.3	844.05±16.1*	528.7±48.5***/#
Glutathione peroxidaseactivity,nmol of GSH × min × mgof $protein^{-1}$	0.78±0.05	1.08±0.06*	0.8±0.03#

The GP activity did not differ from the intact control and significantly decreased by 26% (p<0.05) compared with the control group. These data indicate a resumption of the normal GP activity under conditions of ethanol administration and prophylactic administration of LMOC.

Thus, the results of the study showed that the LMOC reduces the intensity of LP in the GM of rats after administration of ethanol and possessesthe antioxidant effect by increasing superoxide dismutase, catalase and glutathione systemactivity.

Conclusions: 1.LMOC significantly reduces the intensity of LP in GM of rats under conditions of ethanol-induced injury. 2.LMOC increased the superoxide dismutase, catalase activity and activity of glutathione system.

References

1. Antiulcer activity of Trigonellafoenum-graecum leaves in cold restraint stress-induced ulcer model / S.Anand[et al.] // MolClinPharmacol. – 2012. – Vol.13, № 1. – P.90-99.

2. Gastroduodenal toxicity of low-dose acetylsalicylic acid: a comparison with non-steroidal anti-inflammatory drugs / N. D. Yeomans [et al.] // Curr Med Res Opin. – 2009. – Vol.25, № 11. – P.2785-2793.

3. Yuan YPeptic ulcer disease today / Y. Yuan, I. T. Padol, R. H. Hunt // Nat ClinPractGastroenterolHepatol. – 2006. – Vol.3, № 2. – P.80-89.

4. Use of selective serotonin reuptake inhibitors and upper gastrointestinal disease / T. Itatsu[et al.] // Intern Med. – 2011. – Vol.50, № 7. – P.713-717.

5. Risk factors for peptic ulcer bleeding / T. Soberg [et al.] // TidsskrNorLaegeforen. – 2010. – Vol.130, № 11. – P.1135-1139.

6. Acute upper GI bleeding: did anything change? Time trend analysis of incidence and outcome of acute upper GI bleeding between 1993/1994 and 2000 / M. E. van Leerdam [et al.] // Am J Gastroenterol. – 2003. – Vol.98, № 7. – P.1494-1499.

7. Systematic review of the epidemiology of complicated peptic ulcer disease: incidence, recurrence, risk factors and mortality / J. Y. Lau [et al.] // Digestion. – 2011. – Vol.84, № 2. – P.102-113.

8. Guide for the Care and Use of Laboratory Animals. – Washington DC: National Academy Press, 1996.

9. Nourooz-Zadeh J. Measurement of plasma hydroperoxide concentrations by the ferrous oxidation-xylenol orange assay in conjunction with triphenylphosphine / J. Nourooz-Zadeh, J. Tajaddini-Sarmadi, S. P. Wolff // Anal Biochem. – 1994. – Vol.220, № 2. – P.403-409.

10. Гаврилов В. БИзмерение диеновых конъюгатов в плазме крови по УФ-поглощениюгептановых и изопропанольных экстрактов / В. Б. Гаврилов, А. Р. Гаврилова, Н. Ф. Хмара // Лабораторное дело. – 1988. – № 2. – С.60-63.

11. Колесова О. Е. Перекисное окисление липидов и методы определения продуктов липопероксидации в биологических средах / О. Е. Колесова, А. А. Маркин, Т. Н. Федорова // Лабораторное дело. – 1984. – № 9. – С.540-546.

12. Jiang Z. Y. Hydrogen peroxide production during experimental protein glycation / Z. Y. Jiang, A. C. Woollard, S. P. Wolff // FEBS Lett. – 1990. – Vol.268, № 1. – P.69-71.

13. Королюк М. А.Метод определения активности каталазы / М. А. Королюк, Л. И. Иванова, И. Г. Майорова // Лабораторное дело. – 1988. – № 1. – С.16-18.

14. Орехович В. Н. Современные методы в биохимии / В. Н. Орехович. – М.: Медицина, 1977. – С.66-68.

15. Чевари С. Роль супероксиддисмутазы в окислительных процессах клетки и метод определения ее в биологических материалах / С. Чевари, И. Чаба, Й. Секей // Лабораторное дело. – 1985. – № 11. – С.678-681.

16. Habig W. H.Glutathione S-transferases. The first enzymatic step in mercapturic acid formation / W. H. Habig, M. J. Pabst, W. B. Jakoby // J Biol Chem. – 1974. – Vol.249, № 22. – P.7130-7139.

17. Mokrasch L. C. Glutathione content of cultured cells and rodent brain regions: a specific fluorometric assay / L. C. Mokrasch, E. J. Teschke // Anal Biochem. – 1984. – Vol.140, № 2. – P.506-509.

18. Власова С. Н.Активность глутатионзависимых ферментов эритроцитов при хронических заболеваниях печени у детей / С. Н. Власова, Е. И. Шабунина, И. А. Перслегина // Лабораторное дело. – 1990. – № 8. – С.19-22.

19. Hissin P. J.A fluorometric method for determination of oxidized and reduced glutathione in tissues / P. J. Hissin, R. Hilf // Anal Biochem. – 1976. – Vol.74, № 1. – P.214-226.

20. Гланц С. Медико-биологическая статистика / С. Гланц – М.: Практика. – 1998. –.459 с.

21. MinicisDe S. Oxidative stress in alcoholic liver disease: role of NADPH oxidase complex / De S.Minicis, D. A. Brenner // J GastroenterolHepatol. – 2008. – Vol.23 Suppl 1 – P.S98-103.

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ВПЛИВ НИЗЬКОМОЛЕКУЛЯРНОЇ ОРГАНІЧНОЇ СПОЛУКИ НА СИСТЕМУ АНТИОКСИДАНТНОГО ЗАХИСТУ СЛИЗОВОЇ ОБОЛОНКИ ШЛУНКА В УМОВАХ ЕТАНОЛ-ІНДУКУВАНОГО УРАЖЕННЯ ШЛУНКА У ЩУРІВ

Було досліджено профілактичний ефект низькомолекулярної органічної сполуки на ерозивно-виразкові ураження в слизовій оболонці шлунка щурів, викликані етанолом. Встановлено, що профілактичні ін'єкції цієї речовини в дозі 1 мг/кг ефективно захищали шлункові дуражень, викликани хетанолом. Сполука ефективно відновила про-/ антиоксидантну рівновагу шляхом зменшення інтенсивності перекисного окислення ліпідів у слизовій оболонці шлунка щурів після введення етанолу та підвищення супероксиддисмутазної, каталазної активності та активност іглутатіонової системи.

Ключові слова:етанол-індуковані ураження, перекисне окиснення ліпідів, низкомолекулярна органічна сполука.

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ВЛИЯНИЕ НИЗКОМОЛЕКУЛЯРНОГО ОРГАНИЧЕСКОГО СОЕДИНЕНИЯ НА СИСТЕМУ АНТИОКСИДАНТНОЙ ЗАЩИТЫ СЛИЗИСТОЙ ОБОЛОЧКИ ЖЕЛУДКА В УСЛОВИЯХ ЭТАНОЛ-ИНДУЦИРОВАННЫХПОРАЖЕНИЙ ЖЕЛУДКА У КРЫС

Было исследовано профилактический эффект низкомолекулярного органического соединения на эрозивно-язвенные поражения в слизистой оболочке желудка крыс, вызванные этанолом. Установлено, что профилактические инъекции этого вещества в дозе 1 мг/кг эффективно защищали желудок от поражений, вызванных этанолом. Соединение эффективно восстановило про-/антиоксидантное равновесие путем уменьшения интенсивности перекисного окисления липидов в слизистой оболочке желудка крыс после введения этанола и повышение супероксиддисмутазной, каталазнойактивности и активности глутатионовой системы.

Ключевые слова: этанол-индуцированные поражения, перекисное окисление липидов, низкомолекулярное органическое соединение.

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VERTEBRATES FROM THE METHOLITHIC SITE LASPI VII (CRIMEA, UKRAINE)

The article deals with the results of studying the remains of vertebrates from the Mesolithic site Laspi. It is established that a relatively small taxonomic diversity is inherent to this locality; it is mainly represented by four basic species: Great Bustard, Wels catfish (recently became extinct in the Crimea), Zander and the European hare, and four other species that have seemingly been hunted occasionally, as their bones are present, but few: Grey Partridge, Spotted Crake, European hamster and European hedgehog. In those days the basis of the local people's ration was bustard, and the successful hunting for this bird was probably the reason for the Mesolithic people to stop on this territory. Analysis of species diversity suggests that the animals were hunted in the winter. It is established that the rivers of the region, destroyed by an earthquake in 1790, were deep enough for catfish to live there.

Key words: mesolit, Crimea,vertebrates.

Introduction: In the territory of Ukraine, as well as in many other regions of the Palearctic, formation of the modern climatic zones with species richness close to the modern one began during the Mesolithic. Research into the history and trends of wildlife is extremely important for understanding the anthropogenic transformation of ecosystems. It is not possible without studying fossils. The historical aspect of description of the animal world is a basis for understanding the real ties that bind the living world into a single functioning system [10]. Information about findings of the fossil vertebrates, with their careful studying and interpretation, is rather important.

Taxonomic richness of the Crimean peninsula is much higher than that of the neighboring areas due to the great diversity of terrain and climate. The main game animals in the mountainous part of the peninsula were wild boar (25% of bones from archaeological sites belonging to this species), roe deer (20%), red deer (14%), and also rabbit (10 %). Saiga and carnivorous mammals were hunted much less frequently (5% and 10%, respectively). Bones of marine mammals (seals and dolphins) were found in some Crimean localities, which are remote from the sea (Zamil-Koba and Fatma-Koba) [2].

At the end of the Mesolithic basis of the population on the Southern coast of the Crimea were Tauri tribes associated with the Kizil-Koba culture [7]. Locations of most Mesolithic sites in the Crimea and in the rest of Ukraine were determined by their proximity to water and suitability for hunting, fishing and gathering [3].

Location. Laspi VII site was discovered in 1973, and excavated in 1974-76 under the guidance of Dmitri Telegin (60 m^2 were dug out). The site is located in a rock canopy, with several large stones in front of it serving as a wind barrier [Буров]. Radiocarbon dating indicates the age of 5670-7135 BC [Телегін]. The sea level was lower then and the site was further from the sea by 0.5-1.5 m [2].

Skeletal remains in Laspi VII are located in five layers that were signified by the letters of the Cyrillic alphabet during the field work: А (top layer), Б, В, Г and Д (bottom layer). Here we use the Latin alphabet, the letters of which correspond to Cyrillic. Top layer – A ("А" in Cyrillic) beneath layers: B ("Б"), C ("В"), D ("Г"), E ("Д"). Thin layers of clay, ash and the shellfish remains are deposited between bonyferous horizons. The type of sedimentation indicates that they are naturally transferred from the higher area (eastern part of the cliff).

Laspi VII is repeatedly mentioned in the papers (mainly by archaeologists). In addition to information about the instruments, we know about the discovery of a large number of mollusk shells (*Helix*, *Dreissena*) [2]. Most of the tools are different flint cores. But there are also bone harpoons (except for Laspi in the Crimea they were found only at Murzak-Koba and Kara-Koba). Spindly darts present at the Laspi site are also known from Shang-Koba and Fatma-Koba) [7]. Birds from this site are described by Tsvelykh and Taykova [8]. In our work we used other, previously non-published, material from the Laspi VII. Tsvelykh & Taykova did not mention all the birds in their paper, because those bones

were deposited in the back up funds of NMNH NASU during over 35 years and were found only in 2013.

Material: The total amount of skeletal remains is near 700, though it is possible to attribute only 201 of them to species or at least to a genus level. Recent fish and bird bones from the collection of department of vertebrate palaeozoology in the National Museum of Natural History at the National Academy of Sciences of Ukraine (NMNH NASU) were used for comparison. The bones are deposited in NMNH NASU (No. AZ 117-318).

Results of determination of the osteological material are presented in the table 1.

***Table* 1**

Layers	Bony fishes		Birds			Mammals		
	Silurus glanis	*Sander lucioperca*	*Otis tarda*	*Coturnix coturnix*	cf. *Porzana porzana*	*Erinaceus europaeus*	*Lepus europaeus*	*Cricetus cricetus*
A	v (2)	–	cor (5) hum (2) fem (1) tbt (1) tmt (2) ph (3)	–	–	–	–	–
A, B	v (19)	dn (1) v (3)	–	–	–	–	–	–
A, B, C	v (1)	dn (3) qua (1) art (1)	–	–	–	tb (1)	rad (2) cal (1)	–
B	–	–	tbt (1) ph (1)	–	–	–	–	–
C	v (8)	–	qua (1) cor (1) fem (1) tbt (1) ph (3)	–	–	–	–	–
B, C	–	–	–	–	–	–	rad (1) mt (1)	–
D	–	–	qua (1) sc (1) cor (1) ph (1)	–	–	–		–
D, E	–	–	–	–	–	–	hum (1) ul (1) cal (1) mt (1)	ul (1) tb (1)
E	v (1)	–	qua (5) cor (12) sc (16) ste (6) hum (5) ul (4) cmc (3) fem (1) tbt (15) tmt (11) ph (24)	tbt (1)	tbt (1)	–	ul (2) rad (2) tb (1) cal (1) mt (1) ph (1)	ul (1)

Abbreviations: *dn – dentale; qua – quadratum; art – articulare; v – vertebta; cor – coracoideum; sc – scapula; ste – sternum; hum – humerus; ul – ulna; rad – radius; cmc – carpometacarpus; fem – femur; tbt – tibiotarsus; tb – tibia; tmt – tarsometatarsus; cal – calcaneus; mt – metatarsale; ph – phalanx.*

Among the bone fragments, which could not be identified, 2 belong to the fish, 2 – to small mammals and more than 530 – to the birds. Regarding the bird bones, they do not have the epiphysis; it can be assumed that they probably belonged to the Great Bustard according to their sizes.

Discussion: Taxonomic diversity of vertebrates from the Laspi VII is peculiar. At first, it is low – only 8 species, 4 of which (*Coturnix coturnix*, cf. *Porzana porzana*, *Erinaceus europaeus*, *Cricetus cricetus*) can be considered an accidental prey, since they are represented by isolated findings, and these animals are lightweight (from 80 to 600 g). It can be assumed on the basis of the low taxonomic diversity and relatively small mass of prey that they are formed over a short period of time. The obtained results are slightly different from those for birds from Laspi VII, which were published by Tsvelykh & Taykova in 2011. They noted that the Great Bustard is the most abundant (common) species (the minimum possible number of individuals – 35), but also established the presence of 10 other bird species: *Gavia stellata* (1 individual), *Gavia arctica* (2), *Podiceps grisegena* (1), *Phalacrocorax carbo* (2), *Anas platyrhynchos* (2), *Aythya marila* (1), *Bucephala clangula* (1), *Mergus merganser* (1), *Coturnix coturnix* (2), *Columba livia* (1) [8]. This difference in taxonomic diversity of birds exists due to the fact that our research and studies of Tsvelykh & Taykova were based on bone material from the different wells with "cooking remains". So they could be formed at different times and perhaps even by different generations. Besides the difference in taxonomic diversity, there are other facts for such an assumption. At first, the ratio balance of bustard bones in different layers. In the paper of Tsvelykh & Taykova, 32.3% bones of this species were in the layer A and quite more – in the layer E. In our collection the most was in the layer E (74.2% from the total number of determined bones, and more than 90% based on bone fragments without epiphyses). Consequently, people who left the remains processed by Tsvelykh & Taykova belong to ate bustard meat during their stay in the Laspi VII more

or less regularly, adding other bird species to the ration. The remains processed by us belonged to people who had an opportunity to eat bustard just at the beginning of their stay. Probably a successful hunt for this species has been the main reason for the ancient people to stay in the Laspi. The amount of bustard they consumed was significantly smaller in subsequent time.

The nearly complete absence of bustard femurs is rather interesting. Only 3 small proximal femur fragments from the 136 that belonged to this species and from the 530 that supposedly belonged to the Great Bustard were found (2.2% or 0.45%, respectively) [8]. We assume that ancient people did not throw this bone away with others and it could be used for certain purposes. At the same time, femur contains more than 4% of bustard bones in materials of Tsvelykh & Taykova. It also strengthens us in the opinion that these sets of remains were left after different generations of people.

Bustard inhabits open plain areas and is absent in the mountains. These birds appear on the southern coast of Crimea only in some winters, when some of the specimens are unable to migrate through the Kerch peninsula due to harsh weather conditions [1]. The area near Mesolithic Laspi is also mountainous; therefore bustard was hunted in the winter.

The majority of identifiable fish bones belongs to the European catfish (*Silurus glanis*), slightly less – to Zander (*Sander lucioperca*). Body length of catfish from the layer A, recovered using the method of V.D. Lebedev [4] is about 70 cm, pike-perch – near 56 cm. Thus, ancient people preferred to catch relatively small-sized fish. Part of the zander bones (dentale, vertebrae) from the layers A and B likely belongs to young specimens. Catfish remains are rather interesting in many aspects. Firstly, this species does not exist in the Crimea anymore, having become extinct recently [5]. Secondly, there are practically no rivers in the region of Laspi. The nearest large water body – Chornaya River – is more than 9 km away (given the mountainous terrain, it is actually more than 15 km from the site). Sukhaya River, which is located near the Laspi, is a temporal watercourse [9]. It is known that before the earthquake in 1790 there were many rivers in that area, as shown in its name (from the Greek "*Λάσπη*" – "dirt", because this territory was nurtured by numerous watercourses). But the natural disaster destroyed existing water network [6]. Those now non-existent rivers were probably deep enough for catfish. A significant amount of catfish remains also suggests the existence of creeks and whirpools. Aquatic vegetation in this part of the river was absent or poorly developed, because it is known that Zander avoids overgrown areas [11]. With the ancient methods of fishing it was more convenient to catch the catfish in the winter, so it confirms our assumption that the bones from Laspi VII belong to animals caught during the winter.

Conclusions: The prevalent species in Laspi location are the Great Bustard (*Otis tarda*), Wels catfish (*Siluris glanis*), Zander (*Sander lucioperca*) and European hare (*Lepus europeus*). Grey Partridge (*Perdix perdix*), Spotted Crake (cf. *Porzana porzana*), European hedgehog (*Erinaceus europaeus*) and European hamster (*Cricetus cricetus*) are presented by single bones and are probably an accidental prey.

Animals that were found in the Laspi VII site have probably been hunted during the winter. It can explain the specific species composition of the locality, which slightly differs from those of the other Mesolithic sites in the Crimea. Rivers in Laspi, destroyed by an earthquake in 1790, were deep enough for catfish to live there (this species is now extinct in the faunal composition of the Crimea).

References

1. Антипина Е.Е. Антропогенное изменение ландшафтов Крымского полуострова и его авифауны на протяжении последних ста лет // Орнитология. – № 28. – 1998. – С. 60-65.
2. Буров Г.М., Колтухов С.Г., Зайцев Ю.П., Петрова Э.Б., Даниленко В.Н., Ефетов К.А. История Крыма с древнейших времён до наших дней. – Симферополь, 2009. – 420 с.
3. Долуханов П.М., Хотинский Н.А. Палеогеографические рубежи голоцена и мезо-неолитическая история Европы // Первобытный человек, его материальная культура и природная среда в плейстоцене и голоцене. – Ч. 1. – М., 1974. – С. 211-217.
4. Лебедев В.Д. Пресноводная четвертичная ихтиофауна Европейской части СССР. – М., 1960. – 404 с.
5. Мовчан Ю.В. Риби України. – К., 2011. – 420 с.
6. Неяченко И.И. Симеиз-Форос. – Симферополь: Таврия, 1986. – 94 с.
7. Телегін Д.Я. Мезолітичні пам'ятки України. – К., 1982. – 252 с.
8. Цвелых А.Н., Тайкова С.Ю. Птицы раннего голоцена из мезолитической стоянки Ласпи VII в Крыму // Тр. Мензбировского орнитол. обва. Т. 1: мат-лы XIII Международн. орнитол. конф. Евразии. – Махачкала: АЛЕФ (ИП Овчинников), 2011. – С. 186-194.
9. Швидкий А.В. Геологическое строение окрестностей бухты Мегало-Яло (юго-западный Крым) // Вестник СПб ГУ, 1999. – №1. – С.22-26.
10. Kovalchuk O.M. History of the fossil carp fishes (Teleostei, Cyprinidae) in Ukraine // Acta zoologica cracoviensia. – 2013. – 56 (1). – P. 41-51.
11. Sterba G. Süßwasserfische aus aller Welt. – Leipzig, 1971. – 350 s.

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ХРЕБЕТНІ З МЕЗОЛІТИЧНОЇ СТОЯНКИ ЛАСПІ VII (АР Крим, Україна)

У статті представлено результати дослідження решток хребетних із мезолітичної стоянки Ласпі VII. Встановлено, що даному місцезнаходженню притаманне відносно невелике видове різноманіття, представлене чотирма основними видами: дрохвою, сомом (вид, на сьогодні відсутній у Криму), судаком та зайцем і чотирма видами, які потрапили до складу здобичі випадково: перепілкою, погоничем звичайним, хом'яком та їжаком. Основу раціону становила дрохва, успішне полювання на яку, ймовірно, було причиною зупинки мезолітичних людей у Ласпі. Аналіз видового складу дає підстави припускати, що здобич було впольовано взимку. Встановлено, що річки регіону, знищені землетрусом 1790 р., були достатньо глибокі, оскільки в них мешкав сом.

Ключові слова: мезоліт, Крим, хребетні.

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ПОЗВОНОЧНЫЕ ИЗ МЕЗОЛИТИЧЕСКОЙ СТОЯНКИ ЛАСПИ VII (АР Крым, Украина)

В статье представлены результаты исследования остатков позвоночных из мезолитической стоянки Ласпи. Установлено, что этому местонахождению присуще относительно небольшое видовое разнообразие, представленное четырьмя основными видами: дрофой, сомом (вид, на сегодня отсутствующий в Крыму), судаком и зайцем, а также четырьмя видами, которые попали в добычу случайно: перепёлкой, обыкновенным погонышем, хомяком и ежом. Основу рациона составляла дрофа, успешная охота на которую, вероятно, была причиной остановки мезолитических людей в Ласпи. Анализ видового разнообразия даёт основания полагать, что животных добыли зимой. Установлено, что реки региона, уничтоженные землетрясением 1790 г., были достаточно глубоки, поскольку в них обитал сом.

Ключевые слова: мезолит, Крым, позвоночные.

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DETECTION OF *ARABIS MOSAIC* AND *TOMATO ASPERMY VIRUSES* INFECTING ORCHIDS OF NATURAL UKRAINIAN FLORA

The orchids collected from natural biocenosis and from collection of M. M. Gryshko' National Botanical Garden were studied. Two types of viral pathogens were identified in the samples of orchids: Arabis mosaic virus and Tomato aspermy virus.

Key words: orchids, Arabis mosaic virus, Tomato aspermy virus.

Introduction. *Orchidaceae* Juss. is one of the biggest families of flowering plants including about 35 000 species. They can be found all over the world excluding deserts and Polar Region [5]. Viral diseases of orchids are known from the middle of XX century [7]. As for today, more over 30 viruses of orchids have been described [9, 11]. The majority of these viruses were detected in tropical orchids cultivated *in situ*. On the other hand the viruses of terrestrial orchids of temperate zone are studied not enough and require immediate attention as they endanger rare plant species.

All orchids of Ukrainian natural flora are listed in the Red Book of Ukraine [2]. These plants are characterized by complicated developmental biology and require specific symbiotic fungi and distinct entomophily. Therefore, orchids have low degree of recovery, high sensitivity to environmental changes and anthropogenic factors. Viruses pose a biotic stress factor and can directly affect the general condition of the gene pool in plants' populations. Viruses transferred to natural biocenosis from agriecosystems are the most dangerous because they are often highly pathogenic for the new hosts.

The antigens of A*rabis mosaic virus* (ArMV)*, Bean yellow mosaic virus* (BYMV)*, Tomato aspermy virus* (TAV), *Tobacco rattle virus* (TRV) and *Turnip mosaic virus* (TuMV) were detected in orchids of natural Ukrainian flora (from Carpathians and Crimea regions) in previous investigations [2]. Also, the infection of *Cypripedium* sp., *Orchis sp.*, *Ophrys* sp. by TRV and TuMV has been described [7].

Materials and methods. The samples of 20 different species of orchids were surveyed during 2011-2013 yy. The plants were collected in Cherkasy (Kaniv Natural Reserve), Crimean, Kherson, Kyiv, Lviv, Zakarpattia regions and in collection of M. M. Gryshko' National Botanical Garden.

Virus identification was carried out using standard DAS-ELISA [1]. The samples were prepared by homogenizing plant tissue with 0.1 M phosphate buffer (pH 7.4) in the ratio 1:3 (m/v), and followed by centrifugation at 5000 rpm for 20 min. For the diagnostics were used polyclonal antiserums to TMV (antiserum obtained at the virology department, the sensitivity and specificity confirmed experimentally), TuMV (antiserum kindly provided by Lesemann D.E., Julius Kühn Institute, Federal Research Center for Cultivated Plants, Institute for Epidemiology and Pathogen Diagnostics, Germany), TAV, ArMV, *Tomato spotted wilt virus* (TSWV), *Impatiens necrotic spot virus* (INSV), *Iris yellow spot virus* (IYSV), (Loewe, Germany) and *Potato virus Y* (PVY) (Prime Diagnostics, The Netherlands).

Biological properties of viruses were studied using the range of test plants. The test plants were inoculated on early growth stages by mechanical sap transmission, applying carborundum as an abrasive.

The morphology of virions was studied in leaf dip preparations negatively stained with 2% uranyl acetate. EM was carried out using a JEOL-1400 electron microscope at the magnification 40 000 and 60 000.

Results and discussion. For detection and identification of viruses affecting terrestrial orchids were used 47 samples of plants collected in Ukraine.

To confirm the infectivity of the plant sap, we used biological testing on the range of test plants: *Amaranthus caudatus, Brassica oleracea, Capsicum annuum, Celosia argentea, Chenopodium amaranticolor, Cucumis sativus, Datura stramonium, Gomphrena globosa, Lycopersicon esculentum, Nicotiana alata, N. benthamiana, N. rustica, N. tabacum, Petunia hybrida, Phaseolus vulgaris, Tetragonia expansa, Zinnia elegans.* Such range of test plants is typical for many viruses of ornamental plants [5, 8].

According to biotesting the most typical symptoms were necrotic lesions, yellowing and deformation of leaves on *Datura stramonium*; chlorotic spots and deformation of leaves on *Petunia hybrida*; necrotic lesions and mosaic on leaves of *N. benthamiana*; necrotic lesions and vein chlorosis on leaves of *Chenopodium amaranticolor*; necrotic lesions, deformation of leaves and wilting on *Tetragonia expansa* (fig. 1).

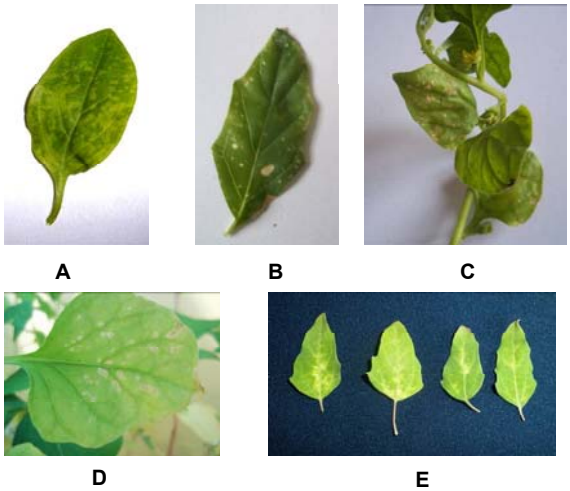

Figure 1. Symptoms on test plants inoculated with the sap of orchids: A – chlorotic spots on the leaves of *Petunia hybrida*; B – necrotic spots on the leaves of *Datura stramonium*; C – necrotic lesions and deformation of the leaves of *Tetragonia expansa*; D – necrotic spots on the leaves of *Nicotiana benthamiana*; E – vein chlorosis symptoms on the leaves of *Chenopodium amaranticolor*

The electron microscopy of the orchids sap was carried out simultaneously with biotesting. Three types of spherical virus-like particles was revealed in infected plant tissues. The particles with diameter of about 30 nm were detected in plants of *Anacamptis laxifolia, Dactylorhiza majalis, Platanthera bifolia* (Zakarpattia region), *A. palustris, Epipactis helleborine* (Kaniv Natural Reserve), *E.palustris, D. incarnata* (Kyiv region), *Gymnodenia conopsea* and *Listera ovata* (Lviv region) (fig.2, A, B).

The virus-like particles with diameter of 50 nm were found in plants of *A. picta* (Kherson region), *E.palustris, D. incarnata* (Kyiv region) and *Platanthera bifolia* (Kaniv Natural Reserve) (fig.2, C, D). According to literature data, spherical virions with diameter of about 50 nm are typical for representatives of *Caulimoviridae*: *Caulimovirus, Cavemovirus, Petuvirus, Soymovirus* [10]. The majority of viruses belonging to the listed genera can be transmitted by mechanical inoculation. The reaction of test plants eliminates such viruses as *Blueberry red ringspot virus*, *Cestrum leaf curling virus*, *Petunia vein clearing virus* and *Tobacco vein clearing virus* which are not transmitted in mechanical manner.

The particles with diameter of about 80-100 nm, typical for representatives of *Tospovirus*, were revealed in the samples of *A. picta* (Kherson region) (fig. 2, E, F).

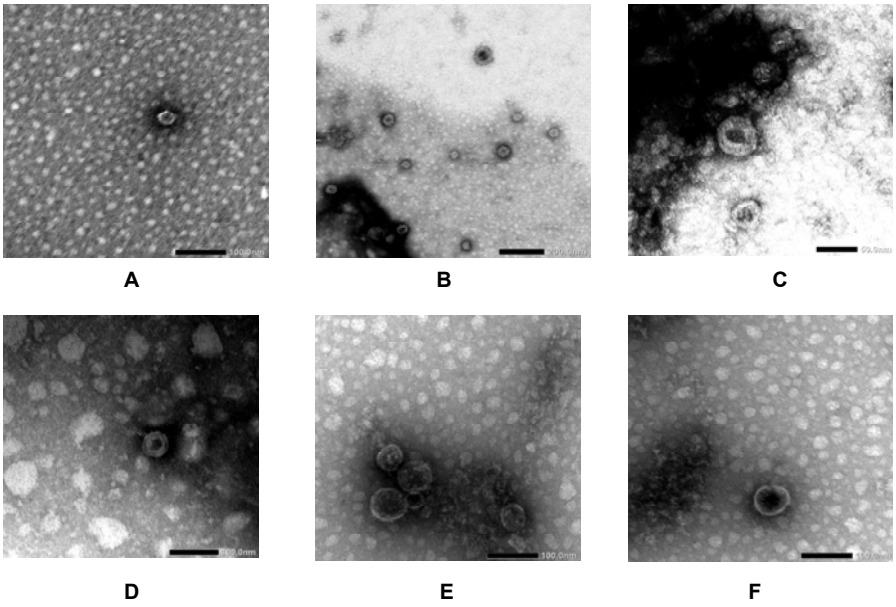

Figure 2. Electron micrograph of virus particles in the sap of infected plants. Virus-like particles with diameter of about 30 nm: A – *A. palustris* (bar 100 nm)*;* B – *E. palustris* (bar 200 nm). Virus-like particles with diameter of about 50 nm: C – *A. picta* (bar 50 nm)*;* D – *P. bifolia* (bar 100 nm). Virus-like particles with diameter of about 80-100 nm: E, F – *A. picta* (bar 100 nm)

For the virus identification in samples of orchids we used DAS-ELISA with antiserums to viruses, which are widespread in agriecosystems of Ukraine and to those which have been previously detected in orchids of temperate climatic zone [2, 7]. According to ELISA testing the antigens of *Tomato aspermy virus* were detected in the samples of orchids *Anacamptis laxifolia, Dactylorhiza majalis, Platanthera bifolia* (Zakarpattia region), *A. palustris, Epipactis helleborine* (Kaniv Natural Reserve), *E.palustris, D. incarnata* (Kyiv region), *Gymnodenia conopsea, Listera ovata* (Lviv region), *Cephalanthera longifolia, Cypripedium calceolus* (M. M. Gryshko National Botanical Garden); and antigens of *Arabis mosaic virus* were revealed in plants of *D. romana*, *Cephalanthera longifolia* and *P.bifolia* (M. M. Gryshko National Botanical Garden).

Conclusion. The biotesting confirmed the infectivity of the sap samples of orchids. EM studies also confirmed the presence of virus-like particles in infected plant tissues with the virions with diameter of about 30 nm, 50 nm and 80-100 nm. Two types of pathogens detected in orchids were identified as *Arabis mosaic virus* and *Tomato aspermy virus*.

References:

1.Серология и иммунохимия вирусов растений/ Р.В. Гнутова. – М.: Наука, 1993. – 301 с.

2.Віруси зозулинцевих природної флори України / Г.В. Коротєєва, В.П. Поліщук // Мікробіол. Журн. – 2004. – Т. 66 – № 2. – С. 74-80.

3. Червона книга України. Рослинний світ/ За ред. Дідух Я.П. – К.: Глобалконсалтинг, 2009. – 900 с.

4. Тропические и субтропические орхидеи /Т.М. Черевченко/ К.: Наукова думка,1993. – 253с.

5. Brunt, A.A., Crabtree, K., Dallwitz, M.J., Gibbs, A.J., Watson, L. and Zurcher, E.J. (eds.) (1996 onwards). `Plant Viruses Online: Descriptions and Lists from the VIDE Database. Version: 20th August 1996.

6. Mosaic of Cymbidium orchid/ D.D. Jensen // Phytopathology. – 1950. – Vol. 40. – P. 966–967.

7. The occurrence of tobacco rattle and turnip mosaic virus in Orchis ssp., and of an unidentified potyvirus of Cypripedium calceolus / D.E. Lesemann, H.J. Vetten // Acta Hortic. – 1985. –Vol.64. – P. 45–54.

8. Dekoratyvinių augalų virusinės ligos ir jų sukėlėjai Lietuvoje. Monografia / M. Navalinskienė, M. Samuitienė / Kaunas:Lututе, 2006, – 256 p.

9. Orchid biotechnology II // eds. Chen H.-W. and Chen H.-H. – World Scientific Publishing Co Pte Ltd, Singapore. – 2011. – 380 p.

10. Virus Taxonomy: Ninth Report of the International Committee on Taxonomy of Viruses / [King A. M. Q., Adams M. J., Carstens E.B., Lefkowitzl E.J.] – NewYork: Elselver Academic Press., 2012. – 1338 p.

11. Viruses of orchids and their control / F.W. Zettler, G.C. Wistler, M.S. Elliot, N.-J. Ko // Plant. Disease. – 1990. – Vol. 74. – P. 621–626.

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ВИЯВЛЕННЯ ВІРУСІВ МОЗАЇКИ АРАБІСУ ТА АСПЕРМІЇ ТОМАТІВ СЕРЕД ОРХІДНИХ ПРИРОДНОЇ ФЛОРИ УКРАЇНИ

Проведено обстеження террестріальних орхідних природніх біоценозів України та колекції орхідних Національного ботанічного саду імені М.М. Гришка. Ідентифіковано два вірусних патогени серед досліджених рослин: вірус мозаїки арабісу та вірус аспермії томату.

Ключові слова: віруси орхідних, вірус мозаїки арабісу, вірус аспермії томатів

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ВЫЯВЛЕНИЕ ВИРУСОВ МОЗАИКИ АРАБИСА И АСПЕРМИИ ТОМАТА СРЕДИ ОРХИДНЫХ ПРИРОДНОЙ ФЛОРЫ УКРАИНЫ

Проведено обследование террестриальных орхидных природных биоценозов Украины и коллекции орхидных Национального ботанического сада имени Н.Н. Гришка. Идентифицированы два вирусных патогена среди исследованных растений: вирус мозаики арабиса и вирус аспермии томата.

Ключевые слова: вирусы орхидных, вирус мозаики арабиса, вирус аспермии томата

UDK 578.85/.86: [581.19:547.56]

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THE INFLUENCE OF ANTROPOGENIC LOAD AND OF THE PHYTOVIRAL INFECTION ON THE SYNTHESIS OF PHENOLIC COMPOUNDS

Have been shown a direct relationship between the degree of anthropogenic load and the degree of infestation by viruses, and the content in these plants phenolic compounds. Also, shown that the reproduction of TMV in sugar beet plants inducses the synthesis of phenolic substances that accumulating, probably, start to inhibit virus accumulation.

Key words: anthropogenic load, infestation by viruses, phenolic substances.

Introduction. One of the ecological and biochemical mechanisms of plant adaptation in the complex influence of anthropogenic factors and viral infections. are the changes in the composition and in the quantitative ratio connections of the compounds in the antioxidantal group, in particular-phenolic nature [1].

Phenolic compounds – one of the most important classes of secondary metabolites, widely represented in plants. Various functions of phenolic compounds in the plant cell, and at the same time a wide range of biological effects on humans and animals, justify study of its participation in the development of protective reactions of organisms in adverse conditions of existence [2]. The literature on the effect of stress factors on the content of phenolic compounds in plants is fragmentary and contradictory. However, it clearly indicate the variability of these parameters [3].

This fact confirms the assumption of importante role of phenolic compounds in the development of resistance of plants to changes in the intensity of natural and anthropogenic stress factors [4].

According to the all written above, the goal of the research was:

– to analyze the influence of the location of the place of grown about the factors of anthropogenic load on the fitovirusological state of plants of sugar beet (*Beta vulgaris* L) and content of phenolic compounds in them;

– to confirm the in vitro correlation between virus reproduction and synthesis of phenolic compounds.

Materials and methods. Method of random samples from agrocenosis with varying degrees of anthropogenic load on visual symptoms were selected sugar beet plants, which further were investigated by undirected enzyme-linked immunosorbent assay for the presence of viral antigens and achohol extraction method for determining the concentration of phenolic compounds [5, 6]. Leaf blades, which were selected for the ELISA, were dried and measured their content of phenolic compounds.

Analysis of results. ELISA was demonstrated the dependence of the distribution and antigen detection percent in plants from the place of sampling. The highest content of phenolic compounds in plants we recorded in the areas with a high degree of anthropogenic load and, accordingly, the greatest defeat by viral pathogens.

This interconnection has been saved in relation to other locations.

To confirm the interconnection between the accumulation of viral particles and the change in the level of concentration of phenolic compounds, we made a laboratory experiment on the model of "sugar beet plant – TMV."

The content of phenolic compounds in plants infected by a virus increased irregularly, compared with the control.

When we compared the dynamics of accumulation of viral antigens and phenolic compounds in sugar beet plants infected with TMV, this dependence can be traced as: in the period between 3 and 6 day after an infection we observed a slight increase of the concentration of viral antigens against the background of the concentration of phenolic compounds. From 6 to 10 day a number of fenolic compounds increases even faster than in the previous period. The concentration of the TMV antigens in this time wasn't changing.

Findings. This way, we have shown a direct relationship between the degree of anthropogenic load and the degree of infestation by viruses, and the content in these plants phenolic compounds.

It is shown that the reproduction of TMV in sugar beet plants inducses the synthesis of phenolic substances that accumulating, probably, start to inhibit virus accumulation.

Sugar beet – biennial plant and can't be used for laboratory model "virus – a plant" and is not suitable for laboratory researches we are doing the selection of the optimal model system for the study of interference of viruses and synthesis of phenolic compounds in the plant and the use of phenolic compounds by the plants when there are some stress factors, like abiotic and biotic nature.

References

1. Rice-Evans C.A. Parker L. (Eds.) Flavonoids in Health and Diseases. –Marcel Dekker, New York, 1997.

2. Halliwell B., Gutteridge (Eds) Free Radicals in Biology and Medicine. – Clarendon Press, Oxford (Uk), 1985. – P. 167.

3. Paladini A. C., Marder M. Viola H. et al. Flavonoids and the central nervous system: from forgotten factors to potent anxiolytic compounds // J. Pharm. Pharmacol. – 1999. – Vol. 51. – P. 519–526.

4. Залесский В.Н., Великая Н.В. Антиапоптические, проапоптические и антитоксические реакции молекул флавоноидов – растительных фенолов // Проблеми харчування. – 2003. – №1. – С. 38–43.

5. Зайцев В.М. Методи концентрування та визначення фенольних сполук (огляд) / В.М. Зайцев, В.А. Халаф, Г.М. Зайцева // Методы и объекты химического анализа. – 2008. – Т.3, №1. – С. 4-21.

6. Постоєнко В.О., Бондарчук Л.І., Патика В.П. Методи оцінки якості та стандартизації апіфітопрепаратів у формі мазей. – К.: 2004 – 27 с.

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ВПЛИВ АНТРОПОГЕНИХ ФАКТОРІВ НА СИНТЕЗ ФЕНОЛЬНИХ СПОЛУК ПРИ ФІТОВІРУСНІЙ ІНФЕКЦІЇ

Було показано прямий взаємозв'язок між рівнем антропогенного навантаження та рівнем інфікованості вірусом, а також вмістом фенольних сполук в цих рослинах. Також, показано, що відтворення ВТМ в рослинах цукрового буряку індукують синтез фенольних сполук які акумулюючись, можливо, починають пригнічувати накопичення вірусу.

Ключові слова: антрпогене навантаження, рівень інфікованості вірусом, фенольні сполуки.

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ВЛИЯНИЕ АНТРОПОГЕНЫХ ФАКТОРОВ НА СИНТЕЗ ФЕНОЛЬНЫХ СОЕДИНЕНИЙ ПРИ ФИТОВИРУСНОЙ ИНФЕКЦИИ

Была показана прямая взаимосвязь между уровнем антропогенной нагрузки и уровнем инфицированности вирусом, а также содержанием фенольных соединений в этих растениях. Также, показано, что воспроизведение ВТМ в растениях сахарной свеклы индуцируют синтез фенольных соединений которые аккумулируясь, возможно, начинают подавлять накопление вируса.

Ключевые слова: антрпогенная нагрузка, уровень инфицированности вирусом, фенольные соединения.

UDK 576.858

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ISOLATION OF *INFECTIOUS PANCREATIC NECROSIS VIRUS* FROM WILD-LIFE RAINBOW TROUT *ONCORHYNCHUS MYKISS* IN WESTERN UKRAINE

During routine sampling and testing of catched wild-life fish species from Siret river of Chernivtsi region, an aquatic birnavirus was isolated from diseased rainbow trout Onhorhynchus mykiss. Preliminary examination of diseased fish revealed a range of lesions particularly in pancreatic tissue. Virus isolate grew in fish cell line RTG-2, FHM and EPC. IPNV caused morphological changes, such as vacuole enlargements and cells rounding. Subsequently cells scaled from a surface and characteristic cytopathic effect (CPE) of virus on cells was visible. Examination by electron microscopy demonstrated that the isolated virus was ultrastructurally similar to IPNV. In addition after amplification of viral RNA incorporating three different primer pairs the IPNV specified PCR products were visible on agarose gel stained with ethidium bromide. The nucleotide sequences of amplified fragments were analysed and the prevalance of Ukrainian isolate, which was named "Karpaty", to Sp strain was revealed. The comparison of sequences of IPNVs VP2 and NS genes from NCBI and amplified fragments of IPNV strain "Karpaty" confirmed the high identity of 95-99% with Sp strain, firstly isolated in Denmark. Among the isolates of Sp strain the most related to IPNV "Karpaty" were viruses found in Great Britain, Norway, France, Turkey and Iran.

Key words: Infectious pancreatic necrosis virus, cell culture, RT-PCR, sequence analysis.

Introduction. Infectious pancreatic necrosis virus (IPNV) belongs to the family *Birnaviridae* and is an agent of an acute, contagious fish disease causing high mortality not only in juvenile salmonids but also in non-salmonid fishes. Members of the family *Birnaviridae* are icosahedral viruses of approximately 65 nm in diameter composed of five polypeptides and two strands of double-stranded RNA [1].

In young salmonid fish, IPNV can cause high mortalities followed by a life-long, chronic infection in the survivors. Persistently infected fish are asymptomatic that have virus in many visceral organs and can shed live virions [2]. The IPNV is widely distributed in Europe and there are severel new reports about virus isolation in neighboring countries of Ukraine such as Poland, Czech Republic, Slovakia and Russia [3,4]. Since salmonids breeding are mainly located in the west region of Ukraine the IPNV is an economically important fish pathogen for all Ukrainian trout farms.

The **aim** of present study was to isolate IPNV from salmonids in Ukraine. Therefore the goals of the present study were to provide preliminary characterization of isolated strain in cell culture and electron-microscopy, to select valid oligonucleotide primers and test it in PCR assay; to provide sequencing of amplified products in way of verification of target amplification; and to accomplish the phylogenetic analysis of Ukrainian IPNV strain.

Materials and methods. During June 2011, a total of 14 fish samples (body weight 0.4-0.7 kg) were continuously collected from a diseased wild-life rainbow trout catched in Siret river, west region of Ukraine. Each fish was dissected, and the samples of pancreas, kidney and spleen were removed from individual fish and placed into a 1.5 ml microcentrifuge tube. Samples were transported on ice to the laboratory and processed immediately.

RTG-2, FHM and EPC cell lines were maintained in DMEM medium (SIGMA) supplemented with 100 U ml^{-1} penicillin, 100 μg ml^{-1} streptomycin and 10% fetal bovine serum (FBS). The samples of kidney and spleen of tested fish were homogenized with DMEM and filtred through the 0,45 μm membrane (Millipore). Then the virus suspension was inoculated onto 24-hours cell monolayers growing in 25 cm^2 flasks. After adsorption for 60 min at 20°C, DMEM medium supplemented with 2% FBS was added to the monolayers. When a complete viral cytopathic effect (CPE) was evident, the tissue culture supernatant was harvested and centrifuged at 2500 × *g* for 10 min at 4°C to remove cell debris. The 50% tissue culture infective dose ($TCID_{50}$ ml^{-1}) of the resulting supernatant was determined [5].

The IPNV was purified both from collected organs and the tissue culture supernatant by the method of ultracentrifugation. Briefly, after cell debris was separated by centrifugation at 2500 × *g* for 10 min at 4°C the pellet was discarded and the supernatant was centrifuged in ultracentrifuge Beckman L5-50B in a rotor SW-40 for 60 min at 70500 × *g* at 4°C. The virus pellet was suspended in TNE (50 mM Tris-HCl, 150 mM NaCl, 1 mM disodium ethylene diaminetetracetic acid [EDTA], pH 7.5) and centrifuged at 2500 × *g* for 5 min at 4°C. Then the virus suspension was used for electron-microscopy investigation and viral RNA extraction [6].

For electron-microscopy investigation the viral suspension was stained with 2% uranyl acetate and studied in electron microscopy EM-125.

Genomic viral RNA was extracted from collected fish organs, viruses-infected cells culture supernatant and purified virus suspension using GeneJET™ RNA Purification Kit (Fermentas) as described in manufacturer's protocol. After elution of the RNA in DEPC-treated water, it was kept at -20°C until required. The cDNA synthesis was conducted using RevertAid™ Premium First Strand cDNA Synthesis Kit (Fermentas) following the manufacturer's instructions and subjected to PCR amplification.

Three sets of virus specific-primers (one-step PCR) were used for amplification of fragments of viral dsRNA targeting the IPNV NS and VP-2 proteins [7-9]. PCR amplification was conducted with a pre-dwel cycle 50°C for 15 min and an initial cycle 95°C for 2 min and followed by 35 cycles of 95°C for 30 sec, 50°C for 30 sec and 72°C for 60 sec. A final extension step was conducted at 72°C for 7 min. The PCR products were analyzed by 2.0% agarose gel electrophoresis.

For extraction of DNA from agarose gel the Silica Bead DNA Extraction Kit was used. Sequencing was performed on a 3130 Genetic Analyzer and analyzed using BLASTN, Vector NTI 10 and MEGA version 5.2 software.

Results and Discussion. This study reports the isolation of IPNV from wild-life rainbow trout in Siret river Chernivtsi region. During June 2011, a total of 14 fish samples were continuously collected from a diseased wild-life rainbow trout catched in Siret river, west region of Ukraine. External signs of disease in catched trouts included uncoordinated spiral swimming, violent flexing of the body and developing of anaemia. Diseased fish were notably darker in colour and appeared weak and lethargic. Internally, the alimentary tract was empty of food but moderately distended and filled with greyish mucus. The liver was dark and inflamed and the kidney and spleen were pale and swollen.

Between 4 and 7 days post infection (d.p.i.), viral CPE was evident in the RTG-2, FHM and EPC cells. All three cell lines were sensitive to virus. IPNV caused morphological changes, such as vacuole enlargements and cells rounding. Infected cells became filamentous in the early stage of infection. Subsequently cells scaled from a surface and characteristic cytopathic effect (CPE) of virus on cells was visible. For cell lines of RTG-2 and FHM the complete destruction of monolayer was noted on 7-8 day after infection (d.a.i.). For culture of EPC characteristic CPE and complete destruction of cell monolayer were marked on 10-12 d.a.i. Infectious titer of IPNV "Carpathians" in studied cell lines was following for EPC $10^{5,5-5,8}$ $TCID_{50}$/ml, and for the lines of FHM and RTG-2 $10^{6,2-6,5}$ and $10^{6,9-7,4}$ $TCID_{50}$/ml respectively. The low infectious titer of virus in cells of EPC can be related to its slow reproduction in this culture. The greatest infectious titer was observed for the culture of RTG-2, that is fully appropriately, as this cell line was derived from a rainbow trout – natural IPNV reservoir. That is why the RTG-2 is the most appropriate cell lines for accumulation of the Ukrainian isolate IPNV "Carpathians". But for diagnostics of the Ukrainian isolate IPNV "Carpathians" all three cell cultures of RTG-2, FHM and EPC can be used.

Results of our electronic-microscopy researches of purified viral particles revealed basic for birnaviruses morphology and ultrastructure characteristics. Virions of the Ukrainian isolate of IPNV "Carpathians" had a hexagonal form, their diameter was 70 ± 5 nm. Viral particles were non-enveloped (Fig. 1).

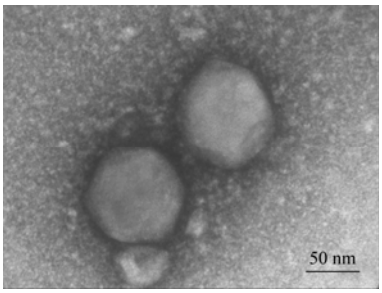

Fig. 1. Electron-microscopy of IPNV particles after purification (×80000)

For rapid diagnostic of Ukrainian IPNV strain the method of PCR was developed. Three sets of primers targeting NS and VP2 genes were used for virus identification and the parameters of PCR cycling were optimized. It was shown that WB primers are the most efficient for virus diagnostic, however the IPN and PrD primers also can be used. Amplified fragments were in size of 200 base pairs (bp) for WB primers, 620 and 175 bp for IPN and PrD primers respectively. In case of low concentration of target RNA only WB primers were enabled to identify the virus. It was noted that annealing temperature of 60^0C was the most suitable for all primer sets.

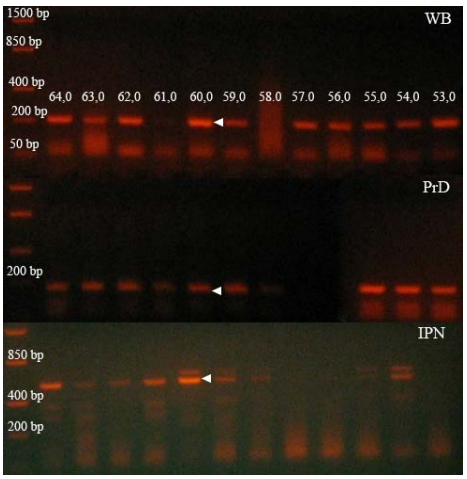

Fig. 2. Amplification in PCR of VP2 (IPN and WB primers) and (PrD primers) NS gene fragments of IPNV strain "Karpaty". The best result is shown ◄; left side DNA ladder FastRuler™ (Thermo Scientific); range of annealing temperature 53-64 ^{0}C

The nucleotide sequences of amplified fragments were analysed and the prevalance of Ukrainian isolate of IPNV "Karpaty" to Sp strain was revealed. The comparison of sequences of IPNVs VP2 and NS genes from NCBI and amplified fragments of IPNV strain "Karpaty" confirmed the high identity of 95-99% with Sp strain, firstly isolated in Denmark. Among the isolates of Sp strain the most related to IPNV "Karpaty" were viruses found in Great Britain, Norway, France, Turkey and Iran.

Thus the selected primers and developed PCR assay can be used for IPNV diagnostic in salmonids cultivated in fish-farming or native ponds of Ukraine. For rapid virus identification in PCR method the WB primers should be used. The complete monitoring of IPNV in Ukraine has to result in total data of virus distribution in Ukraine and also to identify another strains which are widespread in Europe. It will be the subject of our future research.

Infectious pancreatic necrosis can cause a significant economic impact on salmonids with a worldwide distribution. IPNV occurs in most major salmonid-farming countries and it is believed to be spread through the importation of salmonid fishes and their eggs. The virus is vertically transmitted; therefore, the detection of virus in broodstock, even in the absence of the disease, often means the destruction of these valuable fish species [10]. There will be a need to screen the farmed populations of trout near the river Siret in order to confirm of IPNV presence.

Rapid and accurate diagnosis of IPNV infection is critical to the control of the virus because trout surviving infections as juveniles may become life-long carriers and shed live virus. Therefore the screening of samples using RT-PCR would potentially be more sensitive than tissue culture, however suitable cell lines also can be used for surveillance of wild fish for IPNV. Additionally for Ukrainian strains of IPNV the moleqular technics are required and always must be used in purpose of serotype and genotype determination.

Conclusion. The water birnavirus was isolated from wild-life trout in Siret river of Chernivtsi region, Western Ukraine. Preliminary characterizaton of isolated virus revealed its relatedness to IPNV, which was nemed "Karpaty" strain. The nucleotide sequences of amplified fragments were analysed and the prevalance of "Karpaty" isolateto Sp strain was revealed. Among the isolates of Sp strain the most related to IPNV "Karpaty" were viruses found in Great Britain, Norway, France, Turkey and Iran.

References

1. Dixon P.F., Ngoh G.-H., Stone D.M. et al. Proposal for a fourth aquabirnavirus serogroup // Archeves of Virology. – 2008. – Vol. 153. – P. 1937–1941.

2. Rodriguez S.J, Borrego J.J., Perez-Prieto S.I. Infectious pancreatic necrosis virus: biology, pathogenesis, and diagnostic methods // Adv. Virus Res, 2003, pp. 113–165.

3. Antychowicz J., Wejman M., Grawinski E. The isolation of viral haemorrhagic septicaemia and infectious pancreatic necrosis viruses in trout in Poland // Med. Weter. – 2000. – Vol. 56 (4). – P. 255-258.

4. Reschova S., Pokorova D., Hulova J., et al. Surveillance of viral fish diseases in the Czech Republic over the period January 1999 – December 2006 // Veterinarni Medicina. – 2008. – Vol. 53, (2). – P. 86–92.

5. Reed L., Muench H. A simple method of estimating fifty percent endpoints // The American journal of clinical hypnosis. – 1938. – Vol. 27. – P. 493-497.

6. Orpetveit I., Küntziger T., Sindre H., et al. Infectious pancreatic necrosis virus (IPNV) from salmonid fish enters, but do not replicate in, mammalian cells // Virology Journal. – 2012. – Vol. 9:228. doi:10.1186/1743-422X-9-228.

7. Pryde A., Melvin W.T., Munro A.L.S. Nucleotide sequence analysis of the serotype-specific epitope of infections pancreatic necrosis virus // Arch Virol. – 1993. – Vol. 129. – P. 287-293.

8. Blake S.L., Schill W.B., McAllister P.E. et al. Detection and identification of aquatic birnaviruses by PCR assay // J. Clin. Microbiol. – 1995. – Vol. 33 (4). – P. 835.

9. Williams K., Blake S., Sweeney A. et al. Multiplex Reverse Transcriptase PCR Assay for Simultaneous Detection of Three Fish Viruses // J. Clin. Microbiol. – 1999. – Vol. 37(12). – P. 4139.

10. Espinoza J.C., Cort´es-Gutierrezb M., Kuznara J. Necrosis of infectious pancreatic necrosis virus (IPNV) infected cells rarely is preceded by apoptosis // Virus Research. – 2005. – Vol. 109. – P. 133–138.

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ВИДІЛЕННЯ ВІРУСУ ІНФЕКЦІЙНОГО ПАНКРЕАТИЧНОГО НЕКРОЗУ З ДИКИХ ПРЕДСТАВНИКІВ РАЙДУЖНОЇ ФОРЕЛІ *ONCORHYNCHUS MYKISS В ЗАХІДНІЙ УКРАЇНІ*

В роботі представлено результати дослідження вірусу інфекційного панкреатичного некрозу, виділеного від форелі з річки Сапет, Чернівецької області, Західна Україна. Досліджено репродукцію українського ізоляту IPNV "Карпати" в перевивних культурах клітин риб RTG-2, FHM та EPC. Всі три клітинні лінії виявились чутливими до вірусу. IPNV призводив до морфологічних змін, таких як вакуолізація цитоплазми та округлення клітин. Згодом клітини відшаровуватись від поверхні і проявлялась характерна цитопатична дія (ЦПД) вірусу на клітини. Результати електронно-мікроскопічних досліджень очищеної вірусної суспензії показали характерну для бірнавірусів морфологію та ультраструктуру. Віріони українського ізоляту IPNV "Карпати" мали гексагональну форму, їхній діаметр складав 70 ± 5 нм. Підібрано олігонуклеотидні праймери, специфічні до фрагментів генів VP2 та NS, та проведено оптимізацію постановки ПЛР. Аналіз нуклеотидних послідовностей ампліфікованих фрагментів IPNV "Карпати" свідчить, що український ізолят належить до штаму Sp. Ампліфіковані фрагменти кДНК на 95-99% ідентичні з послідовностями генів NS та VP2 інших ізолятів штаму Sp. Серед ізолятів штаму Sp найбільш спорідненими до українського ізоляту IPNV виявились віруси, виділені у Великобританії, Норвегії, Франції, Турції та Ірані.

Ключові слова: вірус інферкійного панкреатичного некрозу, культури клітин риб, бірнавіруси.

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ВЫДЕЛЕНИЕ ВИРУСА ИНФЕКЦИОННОГО ПАНКРЕАТИЧЕСКОГО НЕКРОЗА У ДИКИХ ПРЕДСТАВИТЕЛЕЙ РАДУЖНОЙ ФОРЕЛИ *ONCORHYNCHUS MYKISS* В ЗАПАДНОЙ УКРАИНЕ

В работе представлены результаты исследования вируса инфекционного панкреатического некроза, выделенного из форели реки Сапета, Черновицкой области, Западной Украины. Исследовали репродукцию украинского изолята IPNV "Карпаты" в культурах клеток рыб RTG-2, FHM и ЭДС. Все три клеточные линии оказались чувствительными к вирусу. IPNV приводил к морфологическим изменениям таким, как вакуолизация цитоплазмы и округление клеток. Впоследствии клетки отслаиваються от поверхности и проявляется характерное цитопатическое действие (ЦПД) вируса на клетки. Результаты электронно-микроскопических исследований очищенной вирусной суспензии показали характерную для бирнавирусив морфологию и ультраструктуру. Вирионы украинского изолята IPNV "Карпаты" имели гексагональную форму, их диаметр составлял 70 ± 5 нм. Подобраны олигонуклеотидные праймеры, специфичные к фрагментам генов VP2 и NS, и проведена оптимизация постановки ПЦР. Анализ нуклеотидных последовательностей амплифицированных фрагментов IPNV "Карпаты" свидетельствует, что украинский изолят относится к штамму Sp. Амплифицированные фрагменты кДНК на 95-99 % идентичны с последовательностями генов NS и VP2 других изолятов штамма Sp. Среди изолятов штамма Sp наиболее родственными к украинскому изоляту IPNV оказались вирусы, выделенные в Великобритании, Норвегии, Франции, Турции и Иране.

Ключевые слова: вирусинфекционного панкреатического некроза, бирнавирусы, культуры клеток рыб.

UDK 578.865

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NOVEL TOBAMOVIRUS ISOLATED FROM VEGETABLE CROPS IN UKRAINE

Here we report on the novel tobamovirus, Pepper mild mottle virus (PMMoV), endangering vegetable crops of Solanaceae family which has been isolated in Ukraine for the first time. Virus particles' size and morphology are studied. It has been established that Ukrainian PMMoV isolate belongs to a cluster with strains from Spain and Korea.

Keywords: Tobamovirus, polymerase chain reaction, phylogeny, PMMoV.

Introduction. During the recent years plant virologists (as well as other scientists) have witnessed an increased interest to the advance of knowledge at the level of population and similar ecologically-oriented research. This trend is common for many traditional molecular biology studies. The development of a new generation of diagnostic methods for plant viruses (such as ELISA, RIA, RIPA, PCR, RT-PCR, etc.) during the last decade enabled a new level of studying plant viruses' spread in the environment.

The relevance of this question lies in both its fundamental and practical significance. The determination of virus spread, mechanisms of virus transmission, natural range of host plants, research on virus response to environmental changes makes it possible not only to more fully characterize a given representative of the Vira kingdom, but also to predict the emergence and development of viral diseases for developing sound strategies of combating the viral infections. This includes search for resistant varieties, control of virus reservoirs and carriers, obtaining virus-free planting material, etc.

At present, the representatives of *Tobamovirus* genus are widespread in Ukraine and remain the point of interest for field virologist due to the harm they cause for crops, particularly for plants of *Solanaceae* family.

This work was aimed at establishing diversity and spread of tobamoviruses infecting plants of *Solanaceae* family, phylogenetic analysis of nucleotide sequences for coat protein of newly discovered pathogen (*Pepper Mild Mottle Virus*).

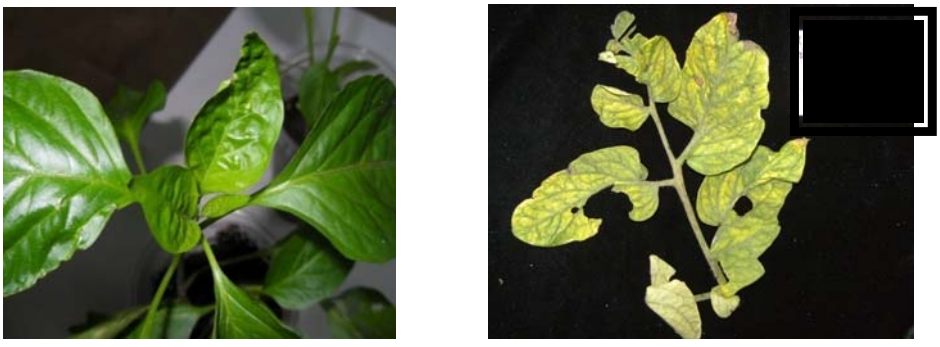
Materials and methods. We used samples of sweet pepper plants (*Capsicum annuum*) and tomato plants (*Lycopersicon esculentum*) collected in Crimea, Vinnytsia, Zhytomyr, Kyiv and Poltava regions – very differing parts of Ukraine in geographical terms. Sampled plants were characterized with virus-like symptoms.

These samples were then tested for the presence of antigens of *Tomato mosaic virus* (ToMV), *Tobacco mosaic virus* (TMV), and *Pepper mild mottle virus* (PMMoV) (typical tobamoviruses infecting these cultures) by DAS-ELISA using commercial antisera from Loewe (Germany) and Prime Diagnostics (The Netherlands).

We have also used the following metods: transmission electron microscopy, total RNA extraction, NA electrophoresis, RT-PCR, nucleic acid sequencing, phylogenetic analysis. For RT-PCR, we have used primers specific to a part of the coat protein gene of PMMoV: 5-TAC TTC GGC GTT AGG CAA TC-3 (forward), 5-GGA GTT GTA GCC CAG GTG AG-3 (reverse).

Results and discussion. DAS-ELISA analysis showed that 22% of symptomatic pepper samples collected in Poltava region contained PMMoV, and 22% of tomato samples were contaminated by ToMV. In Zhytomyr region, 11% of tomato samples contained PMMoV. In Kiev region, 33% of pepper samples were positive for ToMV, as well as 11% of tomato samples. In Vinnytsia region, we have shown that 22% of pepper samples and 22% of tomato plants were infected by ToMV. In Crimea (probably the biggest region in Ukraine for growing tomato and pepper in the open field conditions), 11% of pepper samples and 33% of tomatosamples were shown positive for ToMV.

Collected samples of *Capsicum annuum* and *Lycopersicum esculentum* plants had typical virus-like symptoms (Fig. 1A, 1B).



A B

Figure 1. Virus-like symptoms:
A – deformation of leaves of *Capsicum annuum*; B – light green mosaics on leaves of *Lycopersicum esculentum*

We have used transmission electron microscopy for confirmation of DAS-ELISA results and to study the morphology of virus particles (ToMV, PMMoV).

Microscopy studies demonstrated the presence of rod-shaped virus particles typical of *Tobamovirus* genus, namely, for the *Pepper mild mottle virus* and *Tomato mosaic virus* whose size constituted 310±3 x 15±3 nm and 300±3 x 19±3 nm, respectively (Fig. 2, 3).

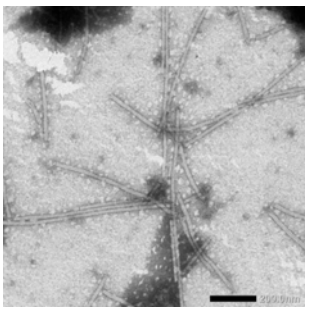


Figure 2. Electron micrography of PMMoV

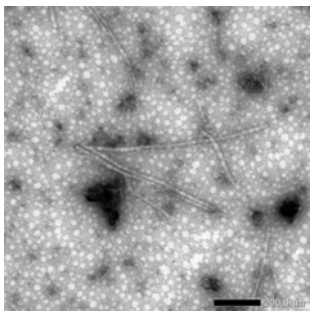


Figure 3. Electron micrography of ToMV

Further, we have extracted a total RNA preparation from PMMoV-infected tomato plants to use the resulting product in RT-PCR.

The total RNA extraction was carried out RNeasy Plant Mini kit (Qiagen, UK) following the manufacturer's recommendations. The results were checked by electrophoresis of nucleic acids in 1.5% agarose gel using TBE buffer.

The next step was the RT-PCR using primers specific to the coat protein gene of PMMoV (Fig. 4).

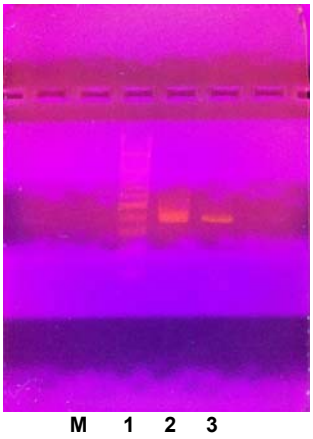


Figure 4. Results of RT-PCR for PMMoV:
M – marker (100bp, Fermentas);
1 – cDNA of capsid protein gene of PMMoV; 2 – positive control; 3 – negative control

We have obtained the cDNA of expected size of 387 bp which was further used for sequencing and construction of a phylogenetic tree (Fig. 5). Phylogenetic tree was constructed using the Neighbor-Joining method.

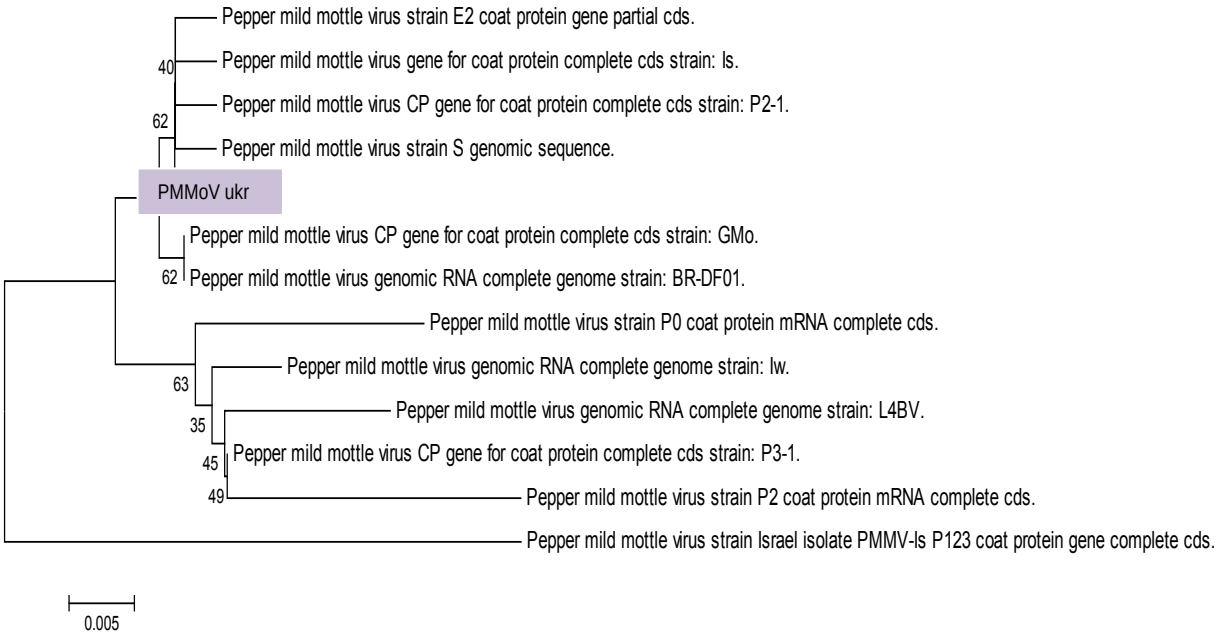


Figure 5. Phylogenetic tree (NJ) showing evolutionary relations of Ukrainian isolate of PMMoV with published virus sequences (based on the coat protein gene)

Thus, the Ukrainian PMMoV isolate belongs to a cluster containing virus strains found in Spain and Korea.

Conclusions. According to the DAS-ELISA, tobamoviruses PMMoV and ToMV are prevalent among vegetable crops in the studied regions of Ukraine. Electron microscopic studies confirmed the presence of rod-shaped virus particles typical of *Tobamovirus* genus, namely, for the *Pepper mild mottle virus* and *Tomato mosaic virus* whose size is approximately 310±3 x 15±3 nm and 300±3 x 19±3 nm, respectively. We have generated a cDNA of 387 bp corresponding to the coat protein gene of Ukrainian PMMoV isolate. Phylogenetic analysis of the coat protein gene of PMMoV showed that Ukrainian PMMoV isolate groups into a cluster with strains detected in Spain and Korea.

References

1. Andrew M.Q. King, Michael J. Adams, Eric B. Carstens. Virus taxonomy. Ninth report of the International Committee on Taxonomy of Viruses. – 2012. – 1139 p.
2. Jarret R.L., Gillaspie A.G., Pinnow D.L., Barkley N.A. The Occurrence and Control Pepper Mild Mottle Virus (PMMoV) in the USDA/ARS Capsicum Germplasm Collection // Seed Technology. – 2008. – Vol.30. – №1. – P. 27-36.
3. Jae-Gee Ryu, Sug-Ju Ko, Yong-Hwan Lee, Mi-Kyeong Kim, Kook-Hyung Kim, Heung-Tae Kim. Incidence and Distribution of Virus Diseases on Paprika (Capsicum annuum var. grossum) in Jeonnam Province of Korea // The plant path. J. – 2009. – Vol.25. – №1. – P. 95 – 98.
4. Thottappilly G. Virus and virus-like diseases of major crops in developing countries. – Gargening, 2003. – 800 p.

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НОВИЙ ПРЕДСТАВНИК РОДУ *TOBAMOVIRUS*, ВИДІЛЕНИЙ З ОВОЧЕВИХ КУЛЬТУР В УКРАЇНІ

Знайдено новий для України вірус овочевих культур, зокрема рослин родини Solanaceae, визначено розмір та морфологію його вірусних часток. Встановлено приналежність українського ізоляту PMMoV до кластерів штамів вірусів Іспанії та Кореї.

Ключові слова: Tobamovirus, полімеразна ланцюгова реакція, філогенія, PMMoV.

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НОВЫЙ ПРЕДСТАВИТЕЛЬ РОДА *TOBAMOVIRUS*, ВЫДЕЛЕНЫЙ ИЗ ОВОЩНЫХ КУЛЬТУР

Найден новый для Украины вирус овощных культур, в частности растений семейства Solanaceae, определен размер и морфология вирусных частиц. Установлено принадлежность украинского изолята PMMoV к кластерам штаммов вирусов Испании и Кореи.

Ключевые слова: Tobamovirus, полимеразная цепная реакция, филогения, PMMoV.

UDK 578

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PHYLOGENETIC ANALYSIS OF HOSTA VIRUS X ISOLATED IN UKRAINE

Hosta virus X (Potexvirus, HVX) was identified by ELISA in different cultivars of hosta selected from M.M. Hryshko National botanical garden of Ukraine (NAS of Ukraine). Coat protein gene sequences of Ukrainaian HVX isolate was determined and compared with all known strains and isolates from Gene Bank. Phylogenetic trees were constructed using neighbour-joining and maximum likelihood methods. The CP of Ukrainian isolate shared 99–100% nucleotide and amino acid sequence identity with American isolates.

Key words: Hosta virus X, ELISA, Gene Bank.

Introduction. Hosta spp. are popular herbaceous perennial plants with more than 7000 varieties, and widely cultivated due to their diversity in leaf shape and color patterns, shade tolerance and pest resistance. Hosta virus X *(Potexvirus*, HVX) is a very serious problem for hosta growers. This virus was first identified and described in Minnesota, USA in 1996. Since then, HVX has been reported from other US states, Canada, Europe as well as from other continents. HVX is generally considered to be the most economically important virus infecting hostas [1]. As HVX is an emerging disease that is causing problems for growers, garden centers, and gardeners, the EPPO Secretariat felt that HVX could usefully be added to the EPPO Alert List. As HVX is sap-transmissible, it is easily transmitted during vegetative plant propagation. Hostas can also be propagated by seeds, but the possible seed transmission of HVX needs to be clarified. As is the case for other potexviruses, HVX is also spread by mechanical contact. Therefore, it is easily transmitted from plant to plant on hands and tools (e.g. pruning tools when removing old leaves or flowers). Over long distances, trade of infected plants has probably been the most significant source of the disease. In addition, it is suggested that some cultivars which have been selected and commercialized because of their 'interesting foliage' were in fact infected by HVX, which has contributed to further spreading the virus. There is no evidence that HVX might be transmitted by insects or other vectors [2, 3].

As is the case for other viruses, the control of the disease is difficult and essentially based on the use of resistant cultivars and of prophylactic measures to minimize the possibility of mechanical transmission of HVX. The production of virus-free planting material through the implementation of certification schemes could also contribute to limiting the spread of HVX [4,5].

We have previously shown that HVX infected some varieties of Hosta plants from the M. M. Gryshko National Botanic Gardens. The aim of this study was to carry out a phylogenetic analysis of Ukrainian isolate of HVX.

Materials and methods:

The samples of various hosta cultivars were collected from M. M. Gryshko National Botanical Garden (NAS of Ukraine). The investigated hosta cultivars included: Hosta Sum and substance, Hosta Striptease, Hosta Lady Guinevere, Hosta spp, Hosta Venticosa, Hosta Udulata, Hosta. Halcyon, Hosta. Crispula Maek, Hosta Gold Standart, Hosta Great Expectation, Hosta Ultraviolet light, Hosta Mediovariegata, Hosta spp, Hosta, Hosta August Moon, Hosta Twilight, Hosta Paul Glory, Hosta Siboldiana, Hosta Whirlwind, Hosta Abigua, Hosta Wide Brim. The samples were selected from plants with the following symptoms: systemic chlorosis, veinal chlorosis interveinal chlo-

rosis, leaf discoloration, leaf rolling and curling, necrotic lesions. Indirect ELISA tests of hosta samples were carried out using antiserum obtained in our laboratory earlier. The results were measured automatically with ELISA reader Stat Fax 2100 (Awarennes Technology, USA) at 405 (for alkaline phosphatase conjugated antibodies). Reverse transcription polymerase chain reaction (RT–PCR) was accomplished using kit SuperScript II (Invitrogen, USA) and a primer pair that amplified a 706 bp fragment [6].

RT–PCR was carried out according to manufacturer recommendations. The amplified product from hosta cultivar *Sum and substance* was sequenced on Applied Biosystems 3730x1 DNA Analyzer using Big Dye terminators, version 3.1 (Applied Biosystems, USA). Obtained sequences were identified and compared using BLAST-analysis (http://www.ncbi.nlm.him.gov).

Phylogenetic analysis was realized through program packages of MEGA 5 [7]. Bootstrep test with 1000 bootstrep replications was applied to check the reliability of phylogenetic trees. Phylogenetic trees were constructed using neighbour-joining (NJ) and maximum likelihood (ML) methods. Statistical analyses of data obtained was carried out using MS Excel software with performing Student's *t* test.

Results and discussion:

HVX was identified by ELISA in the samples from the next cultivars of hosta: Sum and substance,Halcyon, Crispula Maek, Gold Standart, Great Expectation, Ultraviolet light. Further, the samples were used to optimize the conditions of RT-PSR for hosta virus X detection. The first phase of investigations included the extraction of the virus from plant material (non RNA extraction). After heating, the samples of isolated virus were subjected to RT-PCR (without prior RNA extraction). Agarose gel electrophoresis revealed the presence of amplified products with corresponding molecular weight (Fig. 1).

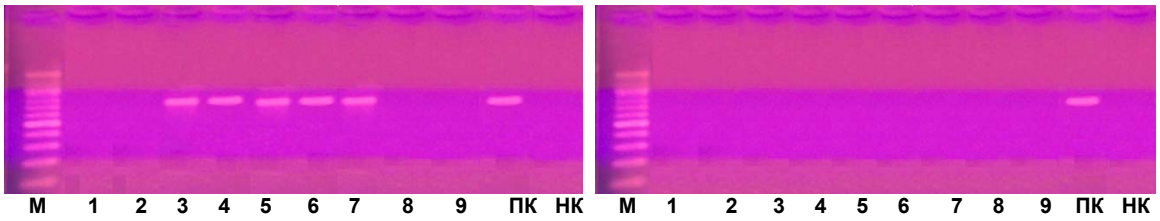

Fig. 1. Electrophoretic studies of RT-PCR products in agarose gel:
M – markers, Fermentas 100 bp, 1 – sample isolated from *Hosta Venticosa*, 2 – sample isolated from *H. Udulata, 3 – H. Halcyon, 4 – H. Crispula Maek, 5 – H. Gold Standart,*6 – *H. Great Expectation.,* 7 – *H. Ultraviolet light,* 8 – *H. Mediovariegata,* 9 – *Hosta spp.*, 10 – *Hosta spp*, 11 – *H. August Moon,* 12 – *H. Twilight,* 13 – *Hosta Paul Glory* 14 – *H. Paul Glory,* 15 – *H. Siboldiana,* 16 – *H. Whirlwind,* 17 – *H. Abigua,* 18 – *H. Wide Brim*, ПК- positive control (Sum and substance), НГ – negative control

As can be seen from Figure 1. five amplified products with expected size (706 b.p.) were obtained. Amplified product from hosta *Sum and substance* further was used for sequencing and construction of phylogenic trees.

Comparison of Ukrainian isolate nucleotide sequences with available in databases sequences of known HVX strains and isolates revealed high percent of their similarity (near 99% for nucleotide sequences and near 98% for amino acid sequences) to American isolates. Thus Ukrainian isolate and American isolates probably belong to the same strain (Table 1).

***Table* 1. Comparison of amino acid and nucleotide sequences of HVX isolated in Ukraine with different strains and isolates from GenBank**

Isolate	Nucleotide sequense, %	Amino acid, %
HVX_USA_Sum_and_Substance	99,1	97
HVX_Poland_Sum_it_Up	99,1	97
HVX_Korea	98,6	95,2
HVX-Kr	98,6	95,2
HVX_Poland_Vim_and_Vigor	98,9	96,4
HVX_Poland_Sum_and_Substance1	98,8	97
HVX_USA_Striptease	99,7	98,8
HVX_Poland_Sum_and_Substance	98,6	96,4
HVX_USA:_Tennessee	99,5	98,8
HVX_USA_Sum_and_Substance_2	99,2	97,6
HVX_USA_Sugar_and_Cream	99,2	98,2
HVX_USA_Hosta_fortunei_Antioch	99,2	98,2
HVX_USA_Gold_Standard	99,2	97,6
HVX_USA_Sum_and_Substance1	99,1	97
HVX-37_USA_Sum_and_Substance	99,1	97
HVX-36_USA_Sum_and_Substance	99,1	97
HVX_USA_HVX-U	99,1	97
HVX_China	98,9	97
HVX_Czech_Republic	98,9	97,6

Construction of phylogenetic tree by NJ method (Fig.2) demonstrated the similarity of Ukrainian isolates with other HVX isolates that is confirmed by date available in Table1.

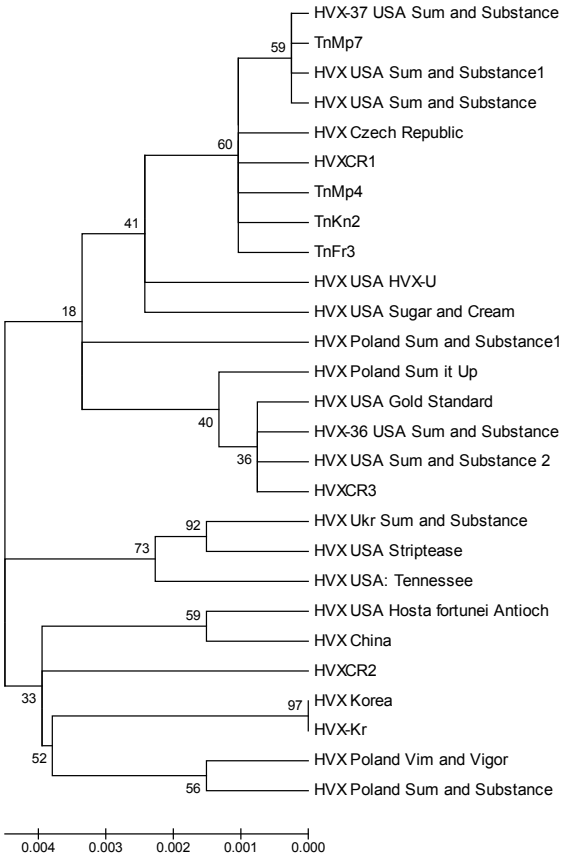

Fig.2. Evolution relationships of Ukrainian HVX isolate with known from Gene bank isolates (NJ is 1000 bootstrap replications)

To determine possible origin of Ukrainian isolate another approach to analysis of obtained nucleotide was applied. Maximum Likelihood method was chosen as discrete method and was performed using model JC+I. The chose of model for calculation was accomplished with program software MEGA5. As we can see from obtained phylogenetic tree (Fig.3), Ukrainian and American isolates are placed in the same cluster (have a joint origin) and possibly have a common ancestor.

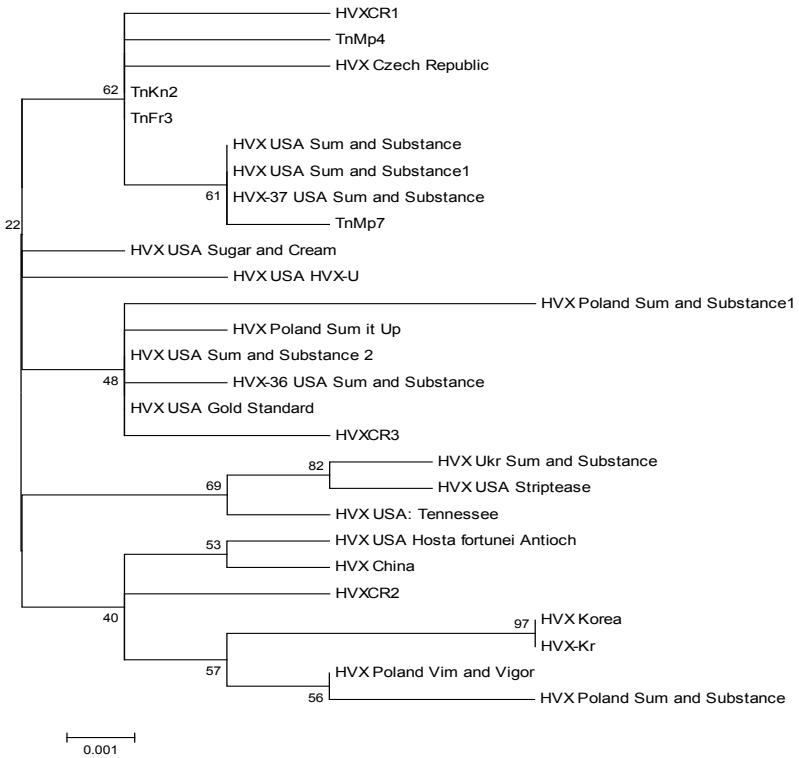

Fig.3. Molecular phylogenetic analysis of HVX capsid protein gene, constructed by Maximum likelihood method (ML is 1000 bootstrap replications)

We can see from phylogenetic tree that cluster separation depends neither on geography of virus isolation (Ukrainian isolate are similar to American) nor on plant cultivar as Ukraine isolate was extracted from *Sum and substance* hosta cultivar whereas similar American isolate was selected from hosta *Striptease*.

Our data is in agreement with literuture data according to with analisys of CP i TGB1 aminoacid sequenses of all known HVX strains confirmed their monophyletic origin. Never the less the relationships between different isolates of HVX are still unclear [8].

It was also shown that the HVX-CP gene is less variable than TGB1, which suggests that CP is possibly under more stringent selection pressure than TGB1. The substitutions observed among the isolates in their respective sequences of the two genes were irregularly distributed. The 3'-proximal part of CP was the least variable region. This is probably due to its critical role in mediating essential functions such as interaction with the genomic RNA, movement and encapsidation [9,10].

Observed HVX genetic variability possibly has biological value. There are many instances where it has been shown that a single amino acid changes in the CP of a plant virus has a significant impact on virus/host interactions. Hence, additional investigations are required to determine the biological significance of the observed amino acid sequence diversity in CP and TGB1 of HVX.

Reference

1. Currier S, Lockhart BEL. Characterization of a potexvirus infecting Hosta spp // Plant Dis. – 1996. – Vol. 80 – P. 1040–1043.
2. Koláčková A., Holková L. First report of Hosta virus Xinfecting Hostain the Czech Republic // New Disease Reports – 2011 – Vol. 24 – P:9.
3. Lockhart B., Currier S. Viruses occurring in Hosta spp. in the USA // Acta Hort – 1996 – Vol.19 – P. 62–67.
4. Park M.H., Ryu K.H. Molecular evidence supporting the classification of Hosta virus X as a distinct species of the genus Potexvirus // Arch Virol – 2003 – Vol.148 – P:2039–2045.
5. Adedire O.L., Wen R.H., Windham A., Windham M., Hajimorad M.R. Hosta virus X in Hostaidentified in Tennessee, USA // Plant Pathology – 2009 – Vol.58 – P:405.
6. Koenig R., Lesemann DE., Potexvirus group. Descriptions of Plant Viruses // CMI/AAB. – England – 1978 – No. 200].
7. Tamura K., Peterson D., Peterson N. et al. MEGA5: Molecular Evolutionary Genetics Analysis using Maximum Likelihood, Evolutionary Distance, and Maximum Parsimony Methods//Mol. Biol. Evol. – 2011. – V. 28, № 10. – P. 2731–2739.
8. Fajolu O., Wen R.-H., Windham S., Windham M. T., Moulton J. K., Hajimorad M.R. Genetic variability and phylogenetic analysis of hosta virus X // Arch Virol – 2009 – Vol.154 – P.1909–1916.
9. Lewandowski D. J. Hosta Virus X // Agriculture and Natural Resources – 2008 – HYG-3069-08.
10. Tang J., Hardy C., Lebas B.S.M., Ward L.I. Presence of Hosta virus X in New Zealand // Australasian Plant Dis. – 2012 – Vol.7 – P:39–40.

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ФІЛОГЕНЕТИЧНИЙ АНАЛІЗ Х ВІРУСУ ХОСТИ ДЕТЕКТОВАНОГО НА ТЕРИТОРІЇ УКРАЇНИ

Х-вірус хости (Potexvirus, HVX) був ідентифікований методом ІФА в різних сортах хоста з колекції Національного ботанічного садаНАН України ім. М.М. Гришка. Отримано сиквенс гена білка оболонки українського ізоляту HVX. Отримана послідовність порівнювалася з відомими штамами з Генбанка. Филогенетические дерева були побудовані за допомогою методу об'єднання сусідів і максимальної правдоподібності. Ген капсидного білка українського ізоляту на 99-100% подібні за нуклеотидним і амінокислотним послідовностей з американськими изолятами.

Ключові слова: х-вірус хости, імуноферментний аналіз, генбанк.

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ФИЛОГЕНЕТИЧЕСКИЙ АНАЛИЗ Х ВИРУСА ХОСТЫ ДЕТЕКТИРУЕМОГО НА ТЕРРИТОРИИ УКРАИНЫ

Х-вирус хосты (Potexvirus, HVX) был идентифицирован методом ИФА в различных сортах хосты из коллекции Национального ботанического сада НАН Украины им. Н.Н. Гришка. Получен сиквенс гена белка оболочки украинского изолята HVX. Полученная последовательность сравнивалась с известными штаммами из генбанка. Филогенетические деревья были построены с помощью метода объединения соседей и максимального правдоподобия. Ген капсидного белка украинского изолята на 99-100% подобен по нуклеотидным и аминокислотным последовательностям с американскими изолятами.

Ключовые слова: х-вирус хосты, иммуноферментный анализ, генбанк.

UDK 578.01

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COMPLEMENTARY RESULTS OF LUMINESCENT AND TRANSMISSION ELECTRON MICROSCOPY PROVIDE STRIKING EVIDENCE OF HEAVY METAL IONS' EFFECT ON THE FORMATION OF AGGREGATES OF *TOBACCO MOSAIC VIRUS* VIRIONS BOTH *IN VITRO* AND *IN VIVO*

In vitro electron microscopy studies showed that virus particles treated with heavy metals aggregate creating either 'typical' lateral (side-to-side) aggregates of virions or star-like ones not reported previously. Luminescent light microscopy of epidermal cells of virus-infected tobacco plants demonstrated that metal treatment has led to the appearance of mostly amorphous and noncompact inclusion bodies, which were not typical for cells of plants not stressed with a heavy metal. Finally, electron microscopy of thin sections of tissues of virus-infected tobacco plants showed that metal-affected cells contained higher numbers of larger crystalline multilayered inclusions consisting of virus particles.

Key words: virus-infected tobacco, metal-affected cells.

Introduction. Previously we have shown that heavy metal contamination of ecosystems favours plant virus spread [1, 2], more intense accumulation of viruses by systemically infected plants and delay in the onset of virus-specific symptoms [3]. We have also demonstrated positive correlation between the heavy metal content in soil and virus concentration in tissues of plants grown in such soil [4]. Long-term virus passaging in heavy metal-stressed plants has been shown to affect neither virus infectivity nor the appearance of local virus-specific symptoms [4, 5].

According to the proposed hypothesis (partially confirmed by the outcomes of laboratory and small-scale field

experiments), chronic effect of abiotic environmental stress factors may lead to intensification of plant virus infection development. Many plausible reasons for this may be suggested including: (i) more efficient intercellular and/or systemic virus transport; (ii) more efficient virus replication/accumulation at the cell level (for instance, due to the plant defenses' failure); (iii) formation of novel virus variants tolerant to the stress exerted by the metals.

This work has been focused on the second option, as we have studied the *in vitro* and *in vivo* effects of heavy metals on *Tobacco mosaic virus* (TMV) virions and formation of virus-induced inclusion bodies in infected cells.

Materials and methods. In this work we have used a well-studied model system "*Tobacco mosaic virus* – *Nicotiana tabacum* cv. Samsun plants".

Tobacco plants were virus-inoculated mechanically in two upper leaves at the stage of four true leaves using carborundum powder [6]. The concentration of inoculum was 150 μg/ml. The development of systemic viral infection was monitored visually by symptoms, and using indirect ELISA [7] to measure virus content in the plants (not shown here for the lack of space).

Heavy metals Zn and Pb in the form of water-soluble salts (ZnSO4x7H2O and Pb(NO3)2 (Alfarus, Ukraine) have been used to simulate a soil contamination. The compounds were dissolved in sterile distilled water and added to soil separately (monometal contamination) at the 5X maximum permissible concentrations (MPC). Values of 1X MPC for the metals under study were as follows: Zn – 300 mg/kg, and Pb – 100 mg/kg [8]. The heavy metals were applied to soil 5 days prior to plant inoculation with TMV.

For thin-sectioning studies, leaf tissue was processed, thin sectioned and analyzed with a transmission electron microscope according to generally-accepted protocols [9]. For microscopy, copper grids or blends (Sigma, USA) were coated with chloroform-dissolved 0.2% polyvinyl formaldehyde (Serva, Germany), dried overnight on filter paper at room temperature, and then strengthened with carbon coating. The samples deposited onto grids were stained with 2.5% uranyl acetate and 0.02 N lead citrate (Serva, Germany), and examined using JEM-1200 ex or JEM 1400 (JEOL, Japan) transmission electron microscopes. The sections were photographed at a magnification of 5,000-60,000x.

For luminescent microscopy studies we used fresh leaf tissue of tobacco plants, acridine orange dye and green light filter following generally-accepted protocol [10]. Photographs were made under UV-light at an instrumental magnification of 630x.

For *in vitro* studies, water-soluble salts of heavy metals (ZnSO4x7H2O and Pb(NO3)2 (Alfarus, Ukraine) have been used at the range of concentrations (5-22 mM, calculated for metal). TMV was used in the distilled water suspension of 0.165 mg/ml. Salt and virus suspensions were mixed 1:1 on a glass slide and incubated at room temperature for 30 min [11]. The resulting suspension was used for transmission electron microscopy studies following the generally-accepted procedure at a magnification of 5,000-30,000x.

Results and discussion. *In vitro* studies showed that TMV particles treated with heavy metals aggregate in two ways creating either 'typical' lateral (side-to-side) aggregates of virions known from literature or star-like ones not reported previously (Figure 1, 2). Together with available data [12], the importance of bivalent metal ions for the formation of TMV-specific inclusion bodies in infected plant cells has been suggested.

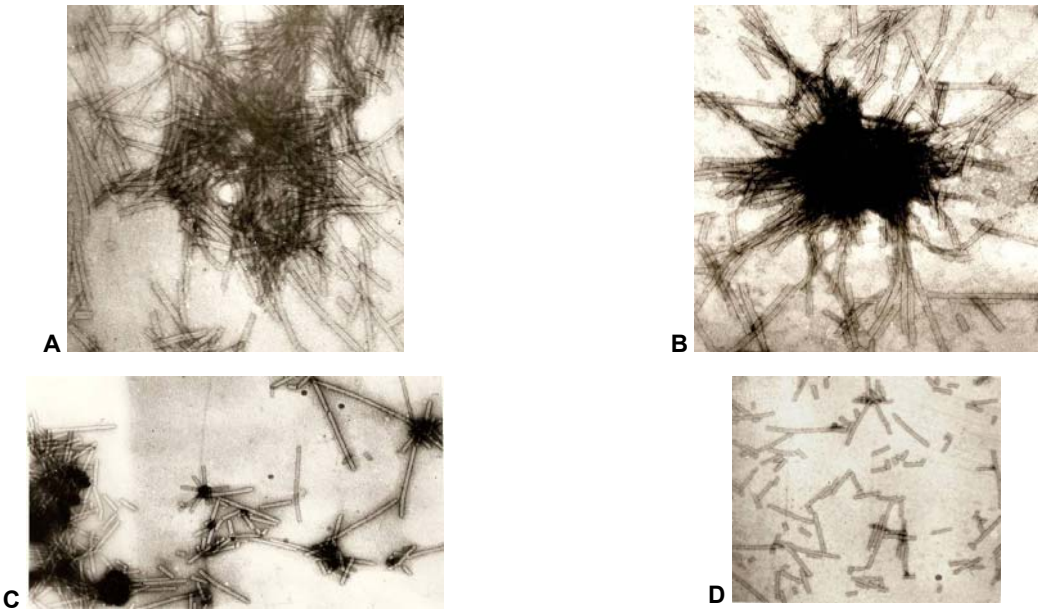

Figure 1. Electron micrograph of TMV suspension:
(A, B) incubated with Zn salt (instrumental magnification 40000x); (C) incubated with Pb salt (instrumental magnification 30000x); (D) not treated with metal (instrumental magnification 30000x)

Indeed, this has also been confirmed in part by *in vivo* luminescent analysis of epidermal cells of TMV-infected tobacco plants. Normally, TMV U1 strain induced the development of quasi-crystal intracellular inclusion bodies characterized by compactness, visual homogeneity and geometrically 'proper' shape. Zinc treatment has led to the appearance of the inclusion bodies, most of which being amorphous and noncompact, with visible 'fissures' (Figure 2).

Figure 2. Luminescent microscopy of TMV-induced inclusion bodies in epidermal hairs of leaves of infected tobacco plants (UV light, acridine orange dye, green light filter, instrumental magnification 630x):
(A) compact virus inclusion (orange) typical for plants not treated with heavy metals; (B) noncompact virus inclusion (orange) often found in metal-stressed plants

Further, we were primarily concerned with observing the progress of TMV infection in cells of systemically infected tobacco plants subjected to heavy metal stress. The point was to use electron microscopy as a proxy measure to see whether virus replicates more efficiently in a single cell, and to elucidate the consequences of dual stress at the cell level.

Intact palisade parenchyma cells of tobacco plants had a typical morphology and properly shaped nuclei, chloroplasts and mitochondria. The nucleus with nucleoli normally was located close to the centre of the cell, whereas large chloroplasts were oval in shape, had dense stroma, fully-formed thylacoids and lamellas, and typically resided at the cell periphery. Cells of tobacco plants systemically infected with TMV showed typical mild pathologies mainly involving a moderate vacuolization of the cytoplasm and deformation of chloroplasts. The nuclei of such cells were larger than those of intact cells, but the cell wall, mitochondria and cell membrane did not demonstrate significant alterations. Virus-specific crystalline inclusion bodies have been found in the cytoplasm close to the cell periphery (Figure 3).

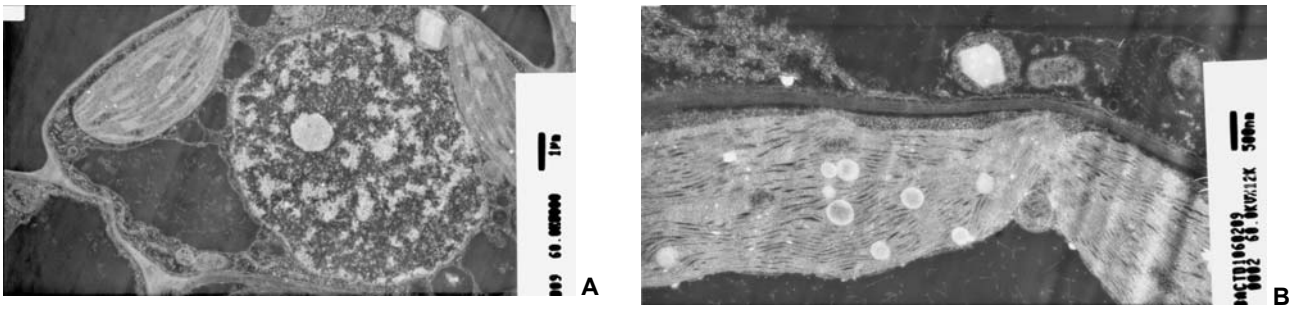

Figure 3. Electron micrographs of intact (A) and TMV-infected (B) cells of tobacco plants

Mesophyll cells of Zn-treated TMV-infected tobacco plants demonstrated higher degree of vacuolization. Their organelles (mainly, the nucleus and chloroplasts) were often significantly distorted and usually displaced close to the cell membrane. Virus particles formed numerous prominent crystalline inclusion bodies located close to the cell periphery forming layered structures composed of TMV virions. In Zn-treated cells TMV particles have been repeatedly observed in nuclei and chloroplasts.

Pb-treated plant cells also reacted on viral infection rather similarly (Fig.7). To our opinion, lead ions had more dramatic effect on the progress of TMV infection in tobacco parenchyma cells. As such, organelles (especially the nucleus and chloroplasts) were more damaged. Cells of virus-infected tobacco plants grown in Pb-enriched substrate also showed higher degree of vacuolization, distinct abnormalities of the cell wall structure (visual 'fragility') and expansion of the intercellular space. These virus-infected cells, as well as Zn-effected, also contained numerous abnormally large starch grains in their chloroplasts. It worth to note that according to microscopy data the cytoplasm of such cells contained more virions and fully-formed virus-specific crystalline inclusions as compared to the cells of Zn-treated virus-infected plants (Figure 4).

In this work we were primarily concerned with observing the progress of TMV infection in cells of systemically infected tobacco plants subjected to heavy metal stress. The point was to use electron microscopy as a proxy measure to see whether virus replicates more efficiently in a single cell, and to elucidate the consequences of dual stress at the cell level.

Figure 4. Electron micrographs of TMV-infected cells of tobacco plants grown in (A) Zn-amended or (B) Pb-amended soil

Visual estimates of the course of viral infection clearly show that heavy metals Zn or Pb potentiate virus accumulation in cells. This is in accordance with the quantitative ELISA results, when Zn and Pb induced respectively 2-times and 4,5-times increase in TMV content as compared to that in virus-infected plants not treated with heavy metals (i.e., representing virus load at the plant level). In addition, the microscopy provided evidence that metal-affected cells contained higher numbers of larger crystalline multilayered inclusions consisting of TMV particles.

In TMV-infected parenchyma cells of tobacco plants stressed by another stressor, heavy metal (Zn or Pb), similar pathological changes were observed: vacuolization, some distortion/damage of major organelles (nucleus and chloroplasts) more evident in Pb-treated cells, occurrence of large starch grains in the chloroplasts and trans-/malformation of the cell wall (Pb-specific effect). One can say that the overall cell degradation is much more severe when cells are affected by both TMV and a heavy metal.

Conclusions. In this work we have demonstrated plausible coherence of results obtained using different kinds of microscopy for TMV virions *in vitro* and viral infection *in vivo*. Presented data show that heavy metals may have direct effects on virions' aggregation and also can (indirectly) influence the formation of viral inclusions in the cell. Combined effect of viral infection and heavy metals has more serious consequences for cells of tobacco plants and may be indicative of more efficient virus replication in chronically stressed cells.

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References

1. Polischuk V.P. 2005. Effects of radioactive and chemical pollution on plant virus frequency / V.P. Polischuk, T.P. Shevchenko, I.G. Budzanivska [et al]. NATO Security through Science Series, Environmental Security: Vol.2., Equidosimetry (F. Brechignac and G. Desmet, eds.). – P.87-92.

2. Polischuk V.P., Budzanivska I.G., Shevchenko T.P. Abiotic environmental factors: effects on epidemiology of plant virus infections Cooper J.I. et al. (eds.). 2006. Virus Diseases and Crop Biosecurity. NATO Security through Science Series, Springer. – P.121-132.

3. Shevchenko O., Kamzel O., Budzanivska I., Shevchenko T., Spaar D. and Polischuk V. 2006. Heavy metal soil contamination delays the appearance of virus-induced symptoms on potato but favours virus accumulation. Archives of Phytopathology and Plant Protection, 40(6): 406-413.

4. Kamzel O.A., Petrenko S.M., Budzanivska I.G., Shevchenko O.V. 2007. Correlation between the zinc content and TMV replication efficiency in tobacco plants. The Bulletin of Taras Shevchenko' Kyiv National University. Biology Series, 49-50: 100-101.

5. Iutynska O.A., Bysov A.S., Shevchenko O.V. Reproduction and systemic transport of TMV in plants under modeled stress // Agroecology Journal. – Spec. issue. – 2011. – P.85-88.

6. Hill S.A. Methods in plant virology. Oxford: Alden Press, 1984. 167 p.

7. Crowther J.R. ELISA. Theory and practice. N.Y.: Humana Press, 1995. – 96 p.

8. Kabbata-Pendias, A. & Pendias. H. (1986) Trace Elements in Soils and Plants, Boca Raton, Florida: CRC press, Inc., 349 p.

9. Mendgen K. Electron Microscopy of Plant Pathogens. Edited by D.E. Lesemann. – Springer, 1991. – 336 p.

10. Konstantinova-Shlesinger, M.A., (Ed.) (1961) Luminescent analysis, Moscow: State Publisher of physical-mathematical literature, 405 p.

11. Nedoluzhko, A. & Douglas, T., (2001) Ordered association of tobacco mosaic virus in the presence of divalent metal ions. J. of Inorganic Chem. 84, pp.233-240.

12. Durham, A.C.H. & Hendry, D.A., (1977) Cation binding by tobacco mosaic virus. Virology 77, pp.510-519.

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ВЗАЄМОДОПОВНЮЮЧІ РЕЗУЛЬТАТИ ЛЮМІНЕСЦЕНТНОЇ ТА ТРАНСМІСІЙНОЇ ЕЛЕКТРОННОЇ МІКРОСКОПІЇ СВІДЧАТЬ ПРО ВПЛИВ ІОНІВ ВАЖКИХ МЕТАЛІВ НА УТВОРЕННЯ АГРЕГАТІВ ВІРІОНІВ ВІРУСУ ТЮТЮНОВОЇ МОЗАЇКИ ЯК В УМОВАХ *IN VITRO*, ТАК І *IN VIVO*

Електронномікроскопічні дослідження в умовах in vitro показали, що оброблені важкими металами вірусні частки агрегують у "типові" структури, в яких віріони з'єднані латерально, та утворюють зіркоподібні структури, неописані раніше. Результати світлової люмінесцентної мікроскопії продемонстрували, що під впливом важких металів в епідермальних клітинах вірус-інфікованих рослин тютюну утворюються головним чином аморфні некомпактні вірусні включення, нетипові для клітин рослин, які не підлягали впливу стресу, спричиненого важкими металами. Електронна мікроскопія ультратонких зрізів тканин вірус-інфікованих рослин тютюну показала, що важкі метали викликали появу більшої кількості великих багатошарових включень, які складалися з вірусних часток.

Ключові слова: люмінесцентна мікроскопія, мікроскопія ультратонких зрізів, важкі метали, вірус-інфіковані рослини тютюну, вірусні включення.

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ВЗАИМОДОПОЛНЯЮЩИЕ РЕЗУЛЬТАТЫ ЛЮМИНЕСЦЕНТНОЙ И ТРАНСМИССИВНОЙ ЭЛЕКТРОННОЙ МИКРОСКОПИИ СВИДЕТЕЛЬСТВУЮТ О ВЛИЯНИИ ИОНОВ ТЯЖЕЛЫХ МЕТАЛЛОВ НА ОБРАЗОВАНИЕ АГРЕГАТОВ ВИРИОНОВ ВИРУСА ТАБАЧНОЙ МОЗАКИ КАК В УСЛОВИЯХ *IN VITRO*, ТАК И *IN VIVO*

Электронномикроскопические исследования в условиях in vitro показали, что обработанные тяжелыми металлами вирусные частицы агрегируют в "типичные" структуры, в которых вирионы соединены латерально, и образуют звездоподобные структуры, описаные ранее. Результаты световой люминесцентной микроскопии показали, что под влиянием тяжелых металлов в эпидермальных клетках вирус-инфицированных растений табака образуются главным образом аморфные некомпактные вирусные включения, нетипичные для клеток растений, которые не подлежали воздействию стресса, вызванного тяжелыми металлами. Электронная микроскопия ультратонких срезов тканей вирус-инфицированных растений табака показала, что тяжелые металлы привели большему количеству многослойных включений, которые состояли из вирусных частиц.

Ключевые слова: люминесцентная микроскопия, микроскопия ультратонких срезов, тяжелые металлы, вирус-инфицированные растения табака, вирусные включения.

UDK 578.81

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BACTERIOPHAGES FROM SAMPLES OF *PULSATILLA PRATENSIS* PLANTS OF NATURAL FLORA OF UKRAINE

We segregated the bacteriophages with long tails, which have different litic activity, size and consist of proteins from Pulsatilla pratensis plants. These plants were selected in the Kaniv Nature Reserve.

Key words: bacteriophages, natural flora, Pulsatilla pratensis.

Introdaction. Bacteriophages are the most widespread in the environments with high density of active metabolically bacteria [1]. In phytocenosis the reprodaction of a set of bacteria which significantly influence a physiological condition of plants is supported and can show phytopathogenic properties. In this regard studying of the interconnected processes of ecology of bacteria and their phages is an actual task. Despite a large number of the works dedicated to segregation and identification of phages, the processes associated with their ecology and evolution in the nature are studied deficiency [2]. Evolutionary approach allows to study them under the influence of environment where the titre of phages in an ecosystem, density of bacterial population and a physiological condition of microorganisms are important factors. Research of interaction of system of populations of phages and bacteria in the conditions of natural environment gives the chance to research the dynamics of interaction and development of populations under the influence of environment factors. It's allows a better understanding about bacteriophages. The relation of quantity of virus particles to quantity of bacteria in averages 10:1. Moreover, the phages are the most numerical organisms on Earth according to some data. Population of phages exceeds 10^{30} virus particles [3]. Transduktion is an improbable event [4]. But, with such number of phages, it happens quite often. The number of virus particles consists not only in their huge number, but also in a specific variety [5].

Material and methods. Sampling for analyses of features of segregation made from the rhizomes of plants of *P. pratensis* and soil around the roots during the period from June, 2011 to June, 2013. In researches used isolates of bacteriophages which are susceptible to cultures of bacteria of *Pseudomonas fluorescens* and *Seratia marcienses L-2*. After series of passages, pure phage clones with different phage plaque morphology were obtained for every isolate. Viruses accumulated on the cultures in nutrient broth with aeration at 25°C. C.In researches used lysates with concentration10^{9}-10^{10} plaque forming units per ml (PFU/ml). Titers were determined by agar-layer technique by Gratia [6].

For research of a range of lytic activity used pathogenic for plants cultures of bacteria: *Erwinia carotovora 216, Pseudomonas syringae pv. atrofaciens 1025, Pseudomonas viridiflava 8868, Pseudomonas fluorescens 8573* and *Seratia marcienses L-2*, the cultures were provided by the museum of phytopathogenic bacteria of the Institute of Microbiology and Virology of the National Academy of Sciences of Ukraine.

The protein composition of the isolated viruses was researched by electrophoresis by Laemmli [7]. Morphology of virus particles researched by means of electronic microscope (JEOL JEM – 1400).

Results and discussion. Phages from samples formed small clear negative colonies on *Seratia marcienses L-2* and colonies with an aureole on *Pseudomonas fluorescens* with diameter about 0,5-1 mm (fig.1).

Research of features of segregation of phages from rhizomes of plants and soil around the roots showed existence of variety of isolates of phages on the basis of lytic ability to used indicator bacteria. The received isolates were characterized by high titers of lytic activity. In total, 4 phage isolates were isolated and described. For determine of a range of hosts was conducted the research of lytic activity of phages on 5 strains of phytopathogenic bacteria. It is revealed that from four checked samples two (the sample №1 and №6) showed lytic activity to strains of different genus of phytopathogenic bacteria (*Pseudomonas* and *Seratia marcienses L-2*) while other two isolates was monovalent (samples №2, №3) – showed lytic activity only to one bacterial strain (tabl.1).

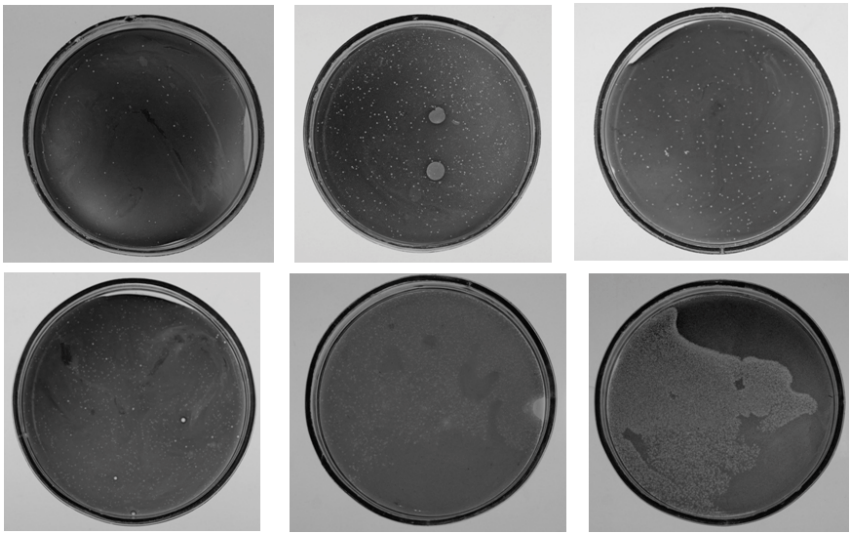

Fig.1. Negative colonies of phages on the culture:
Seratia marcienses L-2 – sample №1 (A), sample №2 (B), sample №3 (C), sample №6 (D)
and *Pseudomonas fluorescens* – sample №1 (E) and sample №6 (F)

Table 1. **Phage lytic activity to indicator bacteria**

Number of sample / Bacterial culture	№1	№2	№3	№6
Erwinia carotovora	-	-	-	-
Pseudomonas syringae pv. atrofaciens	-	-	-	-
Pseudomonas fluorescens	+	-	-	+
Pseudomonas viridiflava	-	-	-	-
Seratia marcienses L-2	+	+	+	+

Notes: "+" – negative colonias
"-" – colonias were absent

Virulent phages of samples №2 and №3 are narrowly specific. Phages of samples №1 and №6 show polyvalency reovercoming generic border, infecting 100 percent of representatives of *Pseudomonas fluorescens* and *Seratia marcienses L-2* respectively.

The nature of phage segregation allows to assume existence of the mechanisms providing preservation of populations of phages in biocenoses. The received results create a basis for studying of distribution of certain representatives of viruses of microorganisms in the nature, definition of their interrelations in biocenoses.

Analysis of the electron micrographs showed that the phages were different in structure and size of virions.

Among them the group of phages, with an icosahedral head and the long non-contractile tail, relates to family *Siphoviridae* and order *Caudovirales*. Phages have the sizes: diameter of a head – 97 ± 2 nm, length of a tail – 157 ± 3 nm, diameter of a head – 89 ± 2 nm, length of a tail – 154 ± 4 nm. Other isolate of a phage with an i icosahedral head without long tail relates to family *Podoviridae* and order *Caudovirales*. It had the size: diameter of a head – 37 ± 1 nm, length of a tail shoot – 137 ± 3 nm (fig.2).

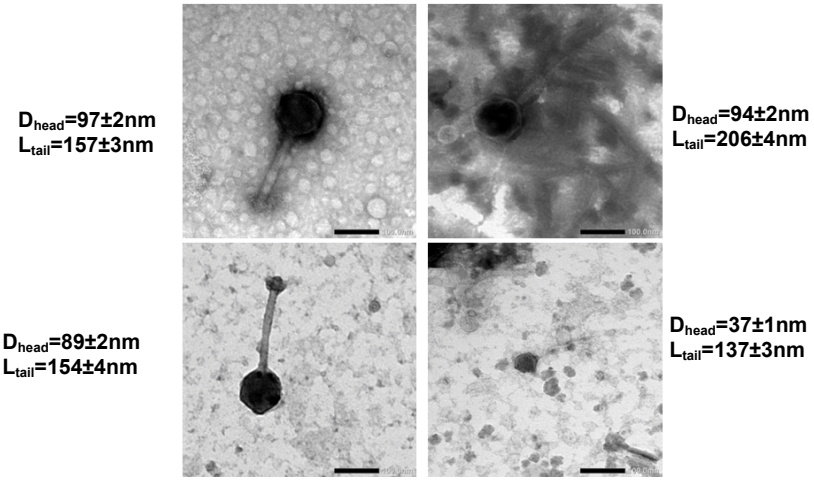

Fig.2. Electron micrographs of segregated phages. A – from sample №2, B – from sample №3, C-D – from sample №6

The protein composition of the isolated viruses was researched by electrophoresis by Laemmli. Research polypeptide structure of phages conducted for the purpose of detection of distinctive characteristics polypeptide structure. In work used preparations of the concentrated and cleared suspensions of phages. Analyzed allocation of samples on molecular masses. Compared on number and electrophoresis mobility the 4 isolates of phages: sample №1, №2, №3 and №6 – a susceptible bacteria of *Seratia marcienses L-2*, №21 and №26 – a susceptible bactera of *Pseudomonas fluorescens*. Them electron micrographes differed and had characteristic individual distinctions (tabl.2).

Table 2. **Molecular weight of phages polypeptides**

Ps. fluorescenns	Sample №6, kDa					66						31	27				16
	Sample №1, kDa						63			40	34						
S. marcienses L-2	Sample №6, kDa							60	47			31	26	24	19	18	
	Sample №3, kDa		167		104		63			39	33	31	28		19	18	
	Sample №2, kDa		167		104	66				40		31	27				
	Sample №1, kDa	190		123						40		31	28		19		

Thus, phages from samples №2 and №3 have high degree of similarity that allows to assume their general origin. Other samples of phages considerably differ on some polypeptides. Results of polypeptide structure can reflect a certain evolutionary process in population of phages. They had the general ancestor. Comparison of proteins of phages of different hosts shows a variety of molecular mass of their polypeptides. According to literary data a variety of structural proteins is characteristic for viruses of microorganisms into which structure can enter to several tens proteins.

Conclusions. Nature of allocation of phages allows to assume existence of the mechanisms providing preservation of populations of phages in biocenoses. The received results create a basis for studying of distribution of certain representatives of viruses of microorganisms in the nature, definition of their interrelations in biocenoses. Comparison of the allocated phages will allow to understand deeper an evolutionary way of viruses in natural ecosystems.

References

1. Vos M., Birkett P.J., Birch E., Griffiths R.I., Buckling A. Local adaptation of bacteriophages to their bacterial hosts in soil // Science. – 2009. – Vol. 325. – P. 833.

2. Ashelford K., Day M., Bailey M., Lilley A., and Fry J. In situ population dynamics of bacterial viruses in a terrestrial environment. Appl. Environ. Microbiol. 1999. 65, pp. 169-174.
3. Wommack K. and Colwell R. Virioplankton: viruses in aquatic ecosystems // Microbiol. Mol. Biol. Rev. – 2000. – p. 320.
4. Fineran P., Petty N., Salmond G., Schaechter M., Elsevier E. Transduction: Host DNA Transfer by Bacteriophages // In The Encyclopedia of Microbiology. – 2009. – P. 666 – 679.
5. Sandra Chibani-Chennoufi, Anne Bruttin, Marie-Lise Dillmann, and Harald Brüssow. Phage-Host Interaction // Ecological Perspective Journal of Bacteriology. Vol. 186, No. 12. – 2004. – p. 3677-3686.
6. Адамс М. // Бактериофаги. – М.: Мир, 1961. – 527 с.
7. Laemmli U. Cleavage of structural proteins during the assembly of the head of bacteriophage T4 // Nature. –1970. – № 5259. – p. 680-685.
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БАКТЕРИОФАГИ ЗІ ЗРАЗКІВ РОСЛИН *PULSATILLA PRATENSIS* ПРИРОДНОЇ ФЛОРИ УКРАЇНИ

При дослідженні зразків з рослин Pulsatilla pratensis, відібраних на території Канівського природного заповідника, було виділено 6 бактеріофагів з довгими хвостовими відростками, що відрізнялися за розмірами, біологічною (літичною) активністю та білковим складом.
Ключові слова:бактеріофаги, флора України, Pulsatilla pratensis.

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БАКТЕРИОФАГИ ИЗ ОБРАЗЦОВ РАСТЕНИЙ *PULSATILLA PRATENSIS* ПРИРОДНОЙ ФЛОРЫ УКРАИНЫ

При исследовании образцов из растений Pulsatilla pratensis, отобранных на территории Каневского природного заповедника, было выделено 6 бактериофагов с длинными хвостовыми отростками, которые отличались по размерам, биологической (литической) активностью и белковому составу.
Ключевые слова: бактериофаги, флора Украины, Pulsatilla pratensis.

UDK 578.85/86

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DIAGNOSTICS OF SEED-BORNE CEREAL VIRUSES IN AGRIECOSYSTEMS OF UKRAINE

Using various complementing diagnostics techniques, we have analyzed spread of cereal viruses capable of seed transmission for Ukraine. Testing different cultivars and lines of cereal plants massively cultivated in Ukraine showed that seed(s) of 10 cultivars (11,8% of their total quantity) contains Barley stripe mosaic virus (BSMV).

Key words: cereal viruses, seed transmission, Barley Stripe Mosaic Virus.

Introduction. Conservation of a virus in a seed for subsequent virus transmission is an ingenious strategy for virus survival because the seed virtually links sowing seasons. This approach is of special importance for viruses having narrow host range and for viruses which are not readily transmitted by vectors. For cereals, *Barley stripe mosaic virus* (BSMV) is a showcase of probably most specialized virus as the seed transmission is vital for its survival [1; 2; 3]. In addition to BSMV it is known that *Wheat streak mosaic virus* (WSMV) may also be transmitted with seeds with the rate of 0,1-0,2% for maize [4] and 0,5-1,5 % for different wheat genotypes [5]. In the early 2000ies, seed transmission has brought WSMV from the USA to Australia where this virus (having probably adopted to a new vector species, *Aceria tosichella*) induced heavy epidemics [6]. Different authors pointed that even such small seed transmission rate of the virus as 1% (i.e., when 1% of seeds contain virus) may lead to multiple virus infection of plant generation (105-107 infected plants per hectare). This is why annual yield losses of cereals attributed to WSMV in the North America make approximately 5%. However, local outbreaks favored by intensive virus spread by *Aceria tritici* at the early stage of plant growth may lead to total loss of the crop yield [1].

As regarding BSMV infection in the USA, natural virus infection typically leads to barley yield losses of 30-31%. Virus induced losses are normally due to flower sterility (BSMV is transmitted by both seeds and pollen) [2]. Efficiency of seed transmission of this virus depends on virus strain, stage of plant growth at which it became infected, and also on species and cultivar of the crop. Available data suggest that BSMV retains its infectivity even in the seeds stored for more than 19 years [2]. This fact is of importance when choosing material for plant selection aiming at breeding new virus-resistant cereal crops. In addition it's worth to say that BSMV-infected plants produce 20-50% less seed, mainly due to the decrease of the number productive stems and number of seeds in a spike. BSMV is spread worldwide where cereals are grown. The virus cannot be inactivated by chemical or temperature seed treatment (in spite of the fact that temperature point for virus inactivation is 70°C) [2; 3].

Starting from the 1960ies, many authors described diseases of cereal crops induced by WSMV and BSMV in Ukraine. Main foci of research were biological properties of these viruses, their spread, visual appearance of the diseases on various cultivars, harmfulness, etc. [7; 8; 9]. Today, however, these pathogens (and especially seed transmission) is totally neglected. BSMV is a good example of the virus which spread remains unknown.

This work was aimed at analyzing spread of WSMV and BSMV in Ukrainian agriecosystems using different diagnostic techniques, and also at testing plant selection material of major cereal crops (available at the Bank of plant genetic resources of Ukraine) for BSMV infection.

Materials and methods. For obtaining reliable data on detection and spread of seed-borne cereal viruses we have conducted 10-year monitoring of wheat and barley commercial sowings showing symptoms typical for these pathogens. The monitored areas were Vinnytsya, Dnipropetrivsk, Kyiv, Lviv, Mykolayiv, Odessa, Poltava, Kherson, Kmelnytskiy and Cherkassy regions. During the visual assessment of the fields attention was paid to the percentage and relative spread of diseased plants, to occurrence of insect vectors, and to the abundance of concurrent bacterial and fungi infections. WSMV and BSMV were detected using DAS-ELISA with commercial polyclonal test systems (Loewe Biochemica, Germany) following the manufacturer's recommendations. Samples with optical density of 0,2 and higher were considered positive in ELISA [10].

For direct virus detection, study of their morphological properties and dimensions transmission electron microscopy (TEM) was used. For microscopy, plant samples were homogenated in 0,1 M PBS, pH 7,4, and centrifuged at 4,000 rpm for 15 min. The supernatant was deposited on Formvare-coated copper grids further contrasted with 2% uranil acetate for 10 min [11].

For detection contamination of cereal seed bank selection material with viruses, seedlings and young plants (stage of 4 leaves) of 85 cultivars and lines were used. 10 plants of each genotype were tested for viruses using ELISA as described above.

Results and discussion. WSMV is demonstrated to be one of the most spread plant viruses in agriecosystems of Ukraine. By means of visual diagnostics and ELISA, we have detected WSMV in winter and spring wheat, winter and spring barley in sowings from Vinnytsya, Dnipropetrivsk, Kyiv, Odessa, Poltava, Kharkiv and Cherkassy regions. WSMV has been most widely spread in central, northern and eastern parts of Ukraine. Season of 2007/2008 yy has shown a peak in virus spread, as well as warm and humid autumn of 2012 (especially in northern and eastern regions). Different cultivars developed varying symptoms of WSMV infection ranging from small streak mosaics to light green stripe mosaics, even when grown on the same field. ELISA confirmed that plants with differing symptoms have been infected with the same virus, WSMV, in the form of monoinfection. Importantly, plants with stripe mosaic symptoms were grouped together forming a focus of infection in the field (Fig.1a), when separate plants with streak mosaic symptoms were more or less evenly distributed (Fig.1b).

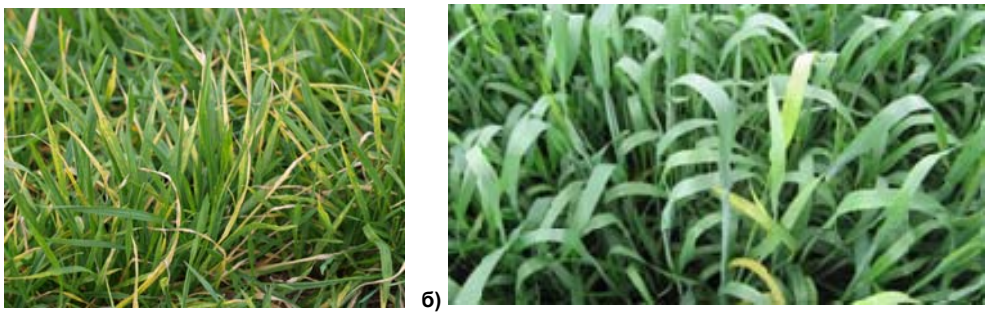

Fig.1. WSMV symptoms of winter wheat plants: (a) late autumn, Kharkiv region; (b) late spring, Kyiv region

In our opinion, these results may indicate different means of virus transmission in the field. Separate diseased plants were probably germinated from WSMV-contaminated seed, when in case of foci of infection the virus was rather vector-transmitted as *Aceria tritici* mites were found abundant in the leaves' sulci.

BSMV has been detected only sporadically on winter wheat in 2003 (Kyiv and Poltava regions), in 2006 (Vinnytsya region), and in 2008 (Kyiv region). This probably is connected to the unique mean of virus transmission by seed. In addition, BSMV-contaminated seed has low mass and normally is not used for sowing.

Microscopy analysis confirmed plants' infection with either WSMV or BSMV. We have detected typical particles of *Tritimovirus* genus, *Potyviridae*, 700x13-14 nm (Fig.2a), and rod-shaped particles of 120-150x20 nm typical for *Hordeivirus* genus (Fig.2b). ELISA confirmed the TEM results.

We have also tested cereal seed bank selection material for BSMV and demonstrated that 10 cultivars (11,8% of their total quantity) contained BSMV. These were spring wheat cultivars "Kharkivska 30", "Rannya 93", "Prohorovka", "Voronezhska 6", "Saratovska 60", and "Saratovska 68"; winter wheat cultivar "Skala"; winter barley cultivar "Avangard"; selection lines "D-253"and "D-257".

This information must be taken into account when breeding new varieties.

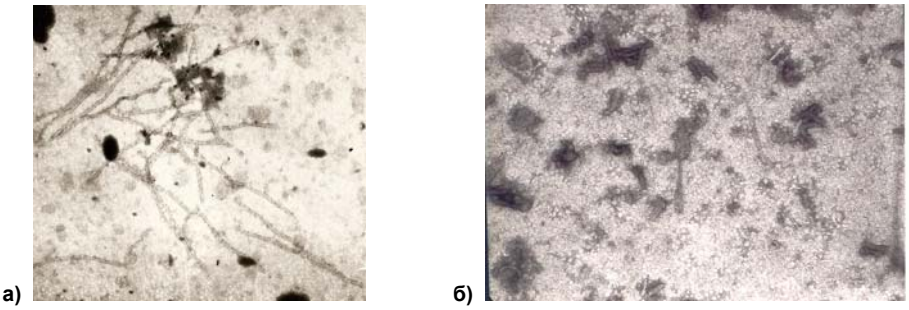

Fig.2. Electron micrography of WSMV (a) and BSMV (b) (x25000)

We need to say that various cultivars and lines of cereals demonstrated differing rate of BSMV infection. "Rannya 93", "Prohorovka", and "Saratovska 68" cultivars were most contaminated (45-65 %), when "Voronezhska 6" cultivar and lines "D-253"and "D-257" were characterized with only 10% of infection (Fig.3).

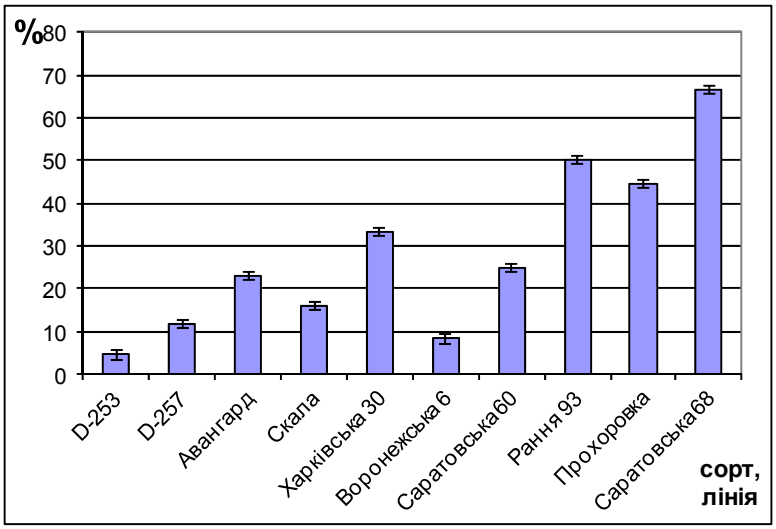

Fig.3. Percentage of seed contamination with BSMV for different cultivars and lines of cereals

The absolute number of initially infected seeds is vitally important for disease progression, especially in case of selection/breeding material. These results underline the need for careful testing of source genetic material.

Conclusions. We have analyzed agriecosystems in 11 cereal-growing regions of Ukraine for occurrence and spread of seed-borne viruses. Using different diagnostic approaches we have demonstrated significant spread of *Wheat streak mosaic virus* – the pathogen gathering its epidemic potential in many regions of the country. Moreover, we have also detected *Barley stripe mosaic virus i*n several agriecosystems and breeding material of major cereal cultures provided by the Bank of plant genetic resources of Ukraine. BSMV is highly specialized for seed transmission with unknown vectors. It's also spread mechanically by contact. BSMV is transmitted by seed of only susceptible/tolerant barley cultivars. The efficiency of its seed transmission by resistant cultivars is negligible [12; 13].

Hence, co-adaptation of the virus and the host favors seed transmission of BSMV. Mild strains of this virus are more readily seed-transmitted and do not induce severe visual symptoms. The symptoms depend on the growing conditions, underlying the need for careful monitoring of virus spread.

References

1. Jones R.A.C. Determining "threshold" levels for seed-borne virus infection in seed stocks // Virus Research, 2005, Vol. 71: 171-183.

2. Maule A.G., Wang D. Seed transmission of plant viruses: a lesson in biological complexity //Trends in Mikrobiology. 1996, Vol.4., № 4: 153-158.

3. Timian R.G. The range of symbiosis of barley and barley stripe mosaic virus // Phytopathology. 1974, Vol. 64: 342-345.

4. Hill J.H., Martinson C.A., Russell W.A. Seed transmission of maize dwarf mosaic and wheat streak mosaic viruses in maize and response of inbred lines. // Crop Science. 1974, Vol. 14, № 2: 232-235.

5. Jones R.A.C, Coulls B.A., Mackie A.E., Dwyer G.I. Seed transmission of Wheat streak mosaic virus shown unequivocally in wheat // Plant Dis. 2005, Vol. 89: 1048-1050.

6. Dwyer G.I., Gibbs M.J., Gibbs A.J., Jones R.A.C. Wheat streak mosaic virus in Australia: Relationship to isolates from the Pacific Northwest of the USA and its dispersion via seed transmission // Plant Dis. 2007, Vol. 91: 164-170.

7. Олейник А.Н. Полосатая мозаика пшеницы на Украине // Автореф. дис. канд.биол. наук. – К., 1968. – 15 с.

8. Шевченко Ж.П., Хельман Л.В., Недвига О.Є. та ін. Вірусні та мікоплазмові хвороби польових культур. К.: Урожай, 1995. 304 с.

9. Міщенко Л.Т. Вірусні хвороби озимої пшениці. К.: Фітосоціоцентр, 2009. – 352 с.

10. ELISA: theory and practice / edited by John R. Crowther. p. cm. (Methods in molecular biology, v. 42). 1995, 223 p.

11. Миронов А.А., Комиссарчик Я.Ю., Миронов В.А. Методы электронной микроскопии в биологии и медицине. С-П.: Наука, 1994. – 399 с.

12. Timian R.G. The range of symbiosis of barley and barley stripe mosaic virus // Phytopathology. 1974, Vol. 64: 342-345.

13. Jackson A.O., Petty I.T.D., Jones R.W., Edwards M.C., French R. Analysis of barley stripe mosaic virus pathogenicity // Sem. Virol, 1991, Vol. 2: 107-119.

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ДІАГНОСТИКА ВІРУСІВ ЗЕРНОВИХ КУЛЬТУР, ЩО ЗДАТНІ ДО НАСІННЄВОЇ ПЕРЕДАЧІ, В АГРОЕКОСИСТЕМАХ УКРАЇНИ

Використовуючи різні методи діагностики визначено поширення вірусів зернових культур, що здатні до насіннєвої передачі в агроекосистемах України. Тестування сортів та ліній зернових, які вирощуються в Україні, показало, що насіння лише 10 (11,8 %) сортів контаміноване вірусом штрихуватої мозаїки ячменю (ВШМЯ).

Ключові слова:віруси зернових культур, насіннєва передача, вірус штрихуватої мозаїки ячменю.

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ДИАГНОСТИКА ВИРУСОВ ЗЕРНОВЫХ КУЛЬТУР, КОТОРЫЕ ПЕРЕДАЮТСЯ СЕМЕНАМИ, В АГРОЭКОСИСТЕМАХ УКРАИНЫ

Используя различные методы диагностики определено распространение вирусов зерновых культур, способных к семенной передаче в агроэкосистемах Украины. Тестирование сортов и линий зерновых, которые произрастают в Украине, показало, что семена только 10 (11,8 %) сортов контаминированны вирусом штриховой мозаики ячменя (ВШМЯ).

Ключевые слова: вирусы зерновых культур, передача семенами, вирус штриховой мозаики ячменя.

UDK 578.865

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OCCURRENCE OF *CUCUMBER MOSAIC VIRUS* IN VEGETABLE CROPS IN UKRAINE

Symptomatic plants of Cucurbitaceae and Solanaceae families collected in Ukrainian agriecosystems used for commercial cultivation of vegetables have been analyzed. According to the ELISA results, 38 samples (of 126 samples in total, i.e., 30%) have been infected with Cucumber mosaic virus. CMV is widespread in Vinnytsia, Zaporizhzhia, Kyiv, Odessa, Poltava and Cherkasy regions. We have obtained a cDNA of 500 bp corresponding to the coat protein gene of Ukrainian CMV isolate.

Key words: Cucurbitaceae and Solanaceae families collected, ELIS.

Introduction. Viral infections cause considerable economic losses to farms engaged in vegetable growing. Usually large-scale assessment of such damages is conducted visually. However, the similarity of symptoms on plants caused by pathogenic agents of different nature, such as viruses, bacteria, fungi, in practice makes impossible to use effective means of treatment and prevention without well-defined identification of pathogens [1].

The only efficient way of controlling viral diseases is their timely diagnostics and putting in place respective preventive measures with regard to eliminate vectors and reservoirs of viral antigens, introducing resistant cultivars of plants and obtaining virus-free material [2].

Cucumber mosaic virus (CMV) belongs to genus *Cucumovirus* of family *Bromoviridae*. CMV infects about 200 species of plants belonging to 60 families, but most known for damaging cucumbers cultivated in the open field conditions [3]. CMV cause severe symptoms on plants and fruits, and greatly reduces the yield of the pumpkin crops. In recent years new data confirmed the expansion of this viral disease onto new territories together with spread of mixed and latent infections, emergence of new strains with altered pathogenicity.

The purpose of the work was to establish the distribution of *Cucumber mosaic virus* in vegetable crops in Ukraine.

Materials and methods. The samples were selected following the visual examination of virus symptoms. For virus detection, plant material was homogenized in 0.1M phosphate buffered saline (PBS), pH 7.4, 1:2 (m/v). Plant components were removed by centrifugation at 5.000 g for 20 minutes at +4˚C using centrifuge PC-6 [4]. The supernatant was taken for further using ELISA. ELISA was performed according to the recommendations of test-system manufacturer (DAS-ELISA) using 96-well plates ("Labsystem"). The results were read at the wavelength of 405/630 nm using microplate reader Termo Labsystems Opsis MR (USA) with software Dynex Revelation Quicklink [5]. Loewe test-system were used in ELISA for CMV detection. Morphology of purified virions was assessed under electron microscope JEM-1400 using 2% uranyl acetate as contrasting agent [6]. Total RNA extraction was carried out using RNeasy Plant Mini kit (Qiagen, UK). The results were checked by electrophoresis of nucleic acids in 1.5% agarose gel. Reverse transcription reaction (RT-PCR) was performed using primers specific to the coat protein gene of CMV:

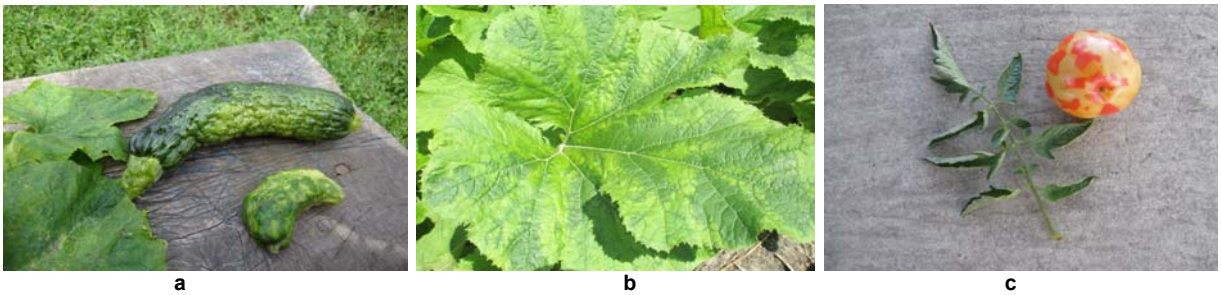
forward primer –
5' TATGATAAGAAGCTTGTTTCGCGCA-3'
reverse primer – 5'
TTTTAGCCGTAAGCTGGATGGACAACCC-3'

PCR products were analyzed by electrophoresis in 1.5% agarose gel using markers Gene Ruller 100 bp DNA Ladder plus (Fermentas, USA) [7].

Results and discussion. Approximately 126 plant samples belonging to the *Cucurbitaceae* and *Solanaceae* families were selected and tested for CMV. Plant samples were collected from following regions of Ukraine: Autonomic Republic of Crimea, Vinnytsia, Zaporizhzhia, Kyiv, Kirovohrad, Odessa, Poltava, Cherkasy and Chernihiv regions. Plants of *Cucurbitaceae* family (cucumber, squash, pumpkin, and zucchini) showed puckering, distortion, vein banding, yellowing, filamentary, yellow mosaic on leaf blade; dark green spots of different size, knobs and malformations on fruits (Fig.1). Plants of *Solanaceae* family (tomato, pepper, eggplant) showed disease symptoms in month after seedtime during flowering. The first symptoms were yellow spots and vein clearing on young leafs followed by systemic yellow and green mosaics, chloroses and local necroses (Fig.1).

Visual observation of external symptoms is an unreliable method for detection and identification of viral infection, because the appearance of viral infection mainly depends on interaction between a virus and a host. Besides, the strains of the same virus can often cause a variety of symptoms changing from hypersensitive to asymptomatic reaction on plants of the same species. The growing conditions and the presence of mixed infection can also effect the development of symptoms. For example, sometimes only mixed infection of pepper may lead to the appearance of mosaics and mottling. That's why the diagnosis of viral infection should be confirmed by specific methods of examination and identification of viruses, particularly by serological tests.

Thus, for detection of viral antigens in plant samples DAS-ELISA was carried out with using commercial test-system of Loewe (Germany). 126 samples of vegetable crop were checked and 38 of them were found to be infected by CMV. Virus-infected plants were detected in agriecosystems of Vinnytsia, Zaporizhzhia, Kyiv, Odessa, Poltava and Cherkasy regions. On plants of *Cucurbitaceae* family, CMV was found in Vinnytsia, Kyiv, Odessa, Poltava and Cherkasy regions. Virus was found in Vinnytsia, Kyiv, Odessa, Poltava and Cherkasy regions on plants of *Solanaceae* family. *Cucumber mosaic virus* was mostly detected as the single agent of infection with several cases of mixed infection. For example, CMV was found with *Watermelon mosaic virus 2* and *Zucchini yellow mosaic virus*. In accordance with ELISA results, we may conclude on the significant spread of the viral infection caused by contamination of healthy seed tissues and coats. In addition, CMV can be transmitted from the weeds (reservoir of infection) and through the soil as plant residues may contain virus.



a b c

Figure 1. Viral symptoms on plants of *Cucurbitaceae* and *Solanaceae* families induced by *Cucumber mosaic virus*:
a – dark green blistering on fruit coat of squash and cucumber caused by CMV;
b – dark green vein banding of leaf blade on squash caused by CMV;
c – leaf blade deformation (rolling) and yellow spots (discoloration) on tomato fruits

Altogether, obtained results point on rather serious situation with CMV spread in Ukrainian agriecosystems.

For direct detection of CMV, indication of its morphology, size of viral particles and confirmation the results of ELISA electron microscopy was used. As a result of these studies of highly purified and concentrated preparation spherical particles 29 nm in diameter were detected. According to the literature data they are typical for *Cucumovirus* genus, in particular for *Cucumber mosaic virus* (Fig.2).

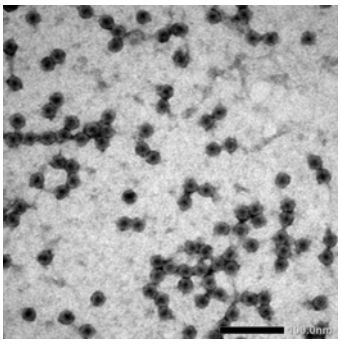


Figure 2. Electron micrograph of *Cucumber mosaic virus*

Thus, the presence of CMV in plant samples was confirmed by electron microscopy.

Further, we have extracted a total RNA preparation from CMV-infected squash plants to use the resulting product in RT-PCR (Fig. 3).

Total RNA extraction was carried out RNeasy Plant Mini kit (Qiagen, UK) following the manufacturer's recommendations. The results were checked by electrophoresis of nucleic acids in 1.5% agarose gel using TBE buffer.

The next step was the RT-PCR using primers specific to the coat protein gene of CMV.

Figure 3. Results of RT-PCR for CMV:
M – marker (100bp, Fermentas);
1 – cDNA of capsid protein gene of CMV №8;
1 – cDNA of capsid protein gene of CMV №13

We have obtained the cDNA of 500 bp which was further used for sequencing and construction of phylogenetic tree.

Conclusions. Symptomatic plants of *Cucurbitaceae* and *Solanaceae* families collected in Ukrainian agriecosystems used for commercial cultivation of vegetables have been analyzed. According to the ELISA results, 38 samples (of 126 samples in total, i.e., 30%) have been infected with *Cucumber mosaic virus*. CMV is widespread in Vinnytsia, Zaporizhzhia, Kyiv, Odessa, Poltava and Cherkasy regions. We have obtained a cDNA of 500 bp corresponding to the coat protein gene of Ukrainian CMV isolate. Purified CMV preparation has been obtained and included in the collection

of plant virus isolates at the Department of Virology, and have been used for comparison with other CMV isolates.

References

1. Gallitelli D. The ecology of Cucumber mosaic virus and sustainable agriculture // Virus Res. – 2000. – №71. – P. 9-21.
2. Rudneva T.O. Virus diseases of Cucurbitaceae plants on the territory of Ukraine / T.O. Rudneva., T.P. Shevchenko, I.G. Budzanivska, O.V. Shevchenko, V.P. Polischuk // Plant science. – 2006. – № 6. – P. 508–510.
3. Virus taxonomy. Eight report of the International Committee on Taxonomy of Viruses [M. Fauquet, M.A. Mayo, J. Maniloff et al.] – London: Academic Press, 2012. – 1259 pp.
4. Dijkstra J. Practical Plant Virology: Protocols And Exercises / J. Dijkstra, Cees P. de Jager. – Berlin; – Springer.Verlag and Heidelberg GmbH & Co, 1998. – 459 p.
5. Crowther J.R. (Ed.) ELISA. Theory and practice, Humana Press, N.Y. – 1995. – P. 223.
6. Hill S.A. Methods in Plant Virology, Alden Press, Oxford, Great Britain, – 1984. – P.164.
7. Finetti-Siaber M., Cillo F., Barbarossa L., Gallitelli D. Differentiation of Cucumber mosaic virus subgroups by RT-PCR // Plant Pathol. – 1999. – №81. – P.145-148.

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ПОШИРЕННЯ ВІРУСУ ОГІРКОВОЇ МОЗАЇКИ НА ОВОЧЕВИХ КУЛЬТУРАХ В УКРАЇНІ

Аналізували відібрані в агроценозах різних регіонів України рослини родин Cucurbitaceae та Solanaceae з вірусоподібними симптомами на наявність вірусу огіркової мозаїки (ВОМ). За результатами імуноферментного аналізу встановлено, що серед 126 тестованих зразків овочевих культур – 38 зразків містили антигени ВОМ. ВОМ широко розповсюджений в агроценозах Вінницької, Запорізької, Київської, Одеської, Полтавської та Черкаської областей. Було отримано кДНК гену капсидного білку українського ізоляту ВОМ розміром 500 bp.

Ключові слова: родини Cucurbitaceae та Solanaceae, імуноферментний аналіз, вірус огіркової мозаїки.

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РАСПРОСТРАНЕНИЕ ВИРУСА ОГУРЕЧНОЙ МОЗАИКИ НА ОВОЩНЫХ КУЛЬТУРАХ В УКРАИНЕ

Анализировали отобраные в агроценозах различных регионов Украины растения семейств Cucurbitaceae и Solanaceae с вирусоподобными симптомами на наличие вируса огуречной мозаїки (ВОМ). По результатам иммуноферментного анализа установлено, что среди 126 тестируемых образцов овощных культур – 38 образцов содержали антигены ВОМ. ВОМ широко распространены в агроценозах Винницкой, Запорожской, Киевской, Одесской, Полтавской и Черкасской областей. Было получено кДНК гена капсидного белка украинского изолята ВОМ размером 500 bp.

Ключевые слова: семейства Cucurbitaceae и Solanaceae, иммуноферментный анализ, вирус огуречной мозаики.

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CYTOTOXICITY AND ANTIVIRAL ACTIVITY OF NOVEL FLUORIC COMPOUNDS

The cytotoxicity and antiviral nucleoside activity of fluoric compounds in EBV model systems were investigated in vitro. The apoptosis-stimulating effect was found for compound SBIO-6, that makes it perspective for further research in the area of antitumor analysis. The results can be used in computer modeling of the structure-biological activity relationships of substances and will be used for development of new highly efficient antiviral agents.

Key wods: antiviral nucleoside activity, compound SBIO-6, antitumor analysis.

Introduction. Epstein-Barr virus (EBV) belonging to *Gammaherpesvirinae* subfamily, *Herpesviridae* family, is a lymphotropic DNA virus able to infect cells of lymphatic system [13]. Etiological role of EBV has been confirmed for such clinical human diseases as infectious mononucleosis (initial stage of infection), Burkitt's lymphoma, nasopharyngeal carcinoma, lymphoproliferative disorders [8, 9, 11]. After onset of the initial infection in human organism, herpesviruses are capable of latent infection and may be reactivated when effected by various factors, most commonly in case of immune deficiency state of a human organism. Having successfully invaded human body, the virus induces life-long persistence in its cells. Disorders of human immune system lead to virus activation with following manifestation of clinical symptoms. Prominent increase in occurrence of herpetic diseases among adults and children necessitates comprehensive research of herpetic infections and development of efficient methods of prophylaxis and treatment.

Today, chemotherapy using acyclic nucleosides is most developed. This group of preparations includes synthetic analogues of natural nucleosides forming DNA molecules of every biological species on our planet. Four preparations (acyclic analogues of nucleosides) sharing similar structure are known as main antiherpetic medicine agents: acyclovir, valacyclovir, penciclovir and famciclovir. Their efficiency has been tested and confirmed in randomized clinical trials. Every such preparation interrupts viral DNA synthesis during virus reproduction but has no effect on latent virus and extracellular virus particles, virions [1, 3, 12]. Therapy of herpetic infections remains challenging for physicians of various specializations. Novel potent preparations and therapy schemes which are being designed not only should be efficient and safe in the long term treatment, but also reasonably priced and available for wide rage of patients.

This work was aimed at cytotoxic and antiviral nucleoside activity of fluoric compounds in EBV model system *in vitro*.

Materials and methods. Epstein-Barr virus (EBV). Suspension of lymphoblastoid cells B95-8, obtained from the Institute of Virology of RAMS (Mocsow) in 1991, was used as a source of the virus. For virus accumulation, producer cells were cultivated without changing the media with the suspension density of $1x10^6$ cells/ml for 10 days. TPA (12-O-tetradecanoylphorbol-13-acetate) (Sigma, USA) was used as EBV inducer and added to B95-8 cell culture according to the manufacturer's recommendations. The virus was isolated from cells by Walls-Crawford method [4]. We have used following established cell cultures from European Collection of Animal Cell Cultures: 1) B95-8 (leucocytes of marmoset) cells which are transformed by Epstein-Barr virus (EBV) and produce it chronically were used as the source of EBV; and 2) Raji – non-differentiated lymphoblastoid human B cells isolated from Burkitt's lymphoma. Lymphoblastoid cells were

thermostatically cultivated in 50 ml and 250 ml plastic culture matrasses at 5% of CO_2. Growth media contained RPMI 1640 (90%), embryonic bovine serum (10%), antibiotic (streptomycin + penicillin, 100 mcg/ml each), and L-glutamine (2 mmol/l). Cells were passaged each 3 days by adding fresh media to the initial cell culture down to planting dose of 500000 cells per 1 ml.

We studied 4 compounds synthesized at the Institute of Organic Chemistry of NASU. Their structural formulas are given on Figure 1.

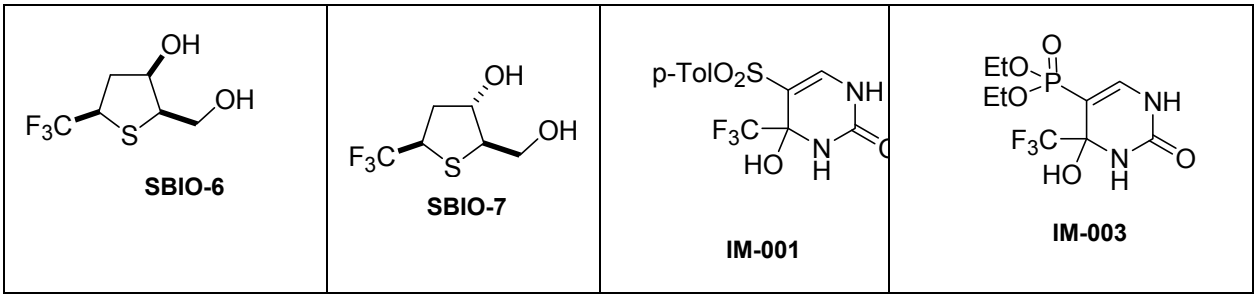

Figure 1. Structure of studied compounds

'Cymevene' (ganciclovir) (Hoffmann-La Roche, Switzerland) was used as a reference preparation. Studied preparations were initially diluted with DMSO. Working dilutions of the preparations were made using growth media containing RPMI-1640 and L-glutamine (Sigma, USA), embryonic bovine serum (Sigma, USA), penicillin (100 mcg/ml), and streptomycin (100 mcg/ml). samples were filtered through syringe membrane filters (Sarstedt, Germany) with pores of 0,22 mcm in diameter. Vital capacity of cells was assessed using MTT method [10]. MTT substrate (Sigma, USA) was diluted with sterile phosphate buffer (pH 7,2) at room temperature to the concentration of 5 mg/ml. 20 mcl of filtered MTT solution were deposited in wells of 96-well plates and incubated with cells for 2-4 hours at 37°C. After the incubation, the media was removed and 150 mcl of 96% ethanol was added into each well with cells to dilute the formazan crystals. Optical density was determined spectrophotometrically at the wavelength of 570 nm using 'Dynatech' plate reader (Switzerland). Further, linear regression algorithm of Microsoft Excel for Pentium Pro [2] was used when establishing the preparation concentration which inhibited vital capacity of cells by 50% (CC_{50}) as compared to the control. Polymerase chain reaction was used to determine the reproduction rate of Epstein-Barr virus in analyzed cells. The reaction was carried out by 'DNA Laboratory' company using 'AmpliSens-100-R' PCR test systems. To visualize apoptotic cells, they were stained with Hoechst 33342 dye (Sigma, USA). Hoechst 33342 dye is characterized with DNA tropism and able to bind DNA at A-G pairs, allowing to determine apoptotic cells in just 6-8 hours after receiving apoptotic stimulus. For this analysis, the cells were washed using buffered physiological solution by centrifuging at 1,500 rpm for 5 min. Further, cell precipitate was incubated with 100 mcl of Hoechst 33342 dye at final dilution of 0,1 mg/ml for 30 min at 37°C. Cells previously washed in buffered physiological solution were then resuspended in 50% glycerin with 4% paraformaldehyde and deposited onto glass slides. Further, the cell preparation was covered with cover glass and soldered with paraffin. Analysis for apoptotic cells in these preparations were carried out using fluorescent microscope ML-2 (Lumam, Russia) at x900 magnification.

Results and discussion. Cytotoxic effect of studied compounds in Raji cell culture was determined by applying the compounds into the cells at certain concentrations. Each concentration was repeated four times with obligatory use of control samples where the compounds were missing. The compounds were used at concentrations 2000, 1000, 500, 250, 125 and 62,5 mcg/ml. Plates with cell culture were incubated at 37°C and 5% of CO_2. After 48 hour exposure to a compound, the percentage of viable cells in all samples was determined by staining with trypan blue and by using MTT method as described above. The resulting effects of the compounds are presented in Table 1. When analyzing these results spectrophotometrically, optical density for wells with 'control' cells (not treated with the compounds) was used as 100%. In turn, rates of decrease of vital capacity of cells relative to control values are given in Table 1. These figures are directly proportional to a percentage of dead cells.

***Table* 1. Influence of studied compounds on vital capacity of Raji cells**

Concentration, mcg/ml	Studied compounds							
	SBIO-6		SBIO-7		IM-001		IM-003	
	% of dead cells							
	Trypan blue	*MTT test*	*Trypan blue*	*MTT test*	*Trypan blue*	*MTT test*	*Trypan blue*	*MTT test*
62,5	9	13	0	0	9	0	13	0
125	21	21	6	0	12	0	12	5
250	46	58	12	1	14	2	13	7
500	78	100	11	5	50	50	11	15
1000	75	100	55	56	89	80	23	35
2000	92	100	90	100	100	100	54	71
Correlation coefficient	0,819	-0,98	0,979	-0,98	0,923	-0,90	0,956	-0,99
CC_{50} (mcg/ml)	600	200	1000	1000	750	800	1900	1400
CC_{50} (averaged for two methods) (mcg/ml)	400		1000		775		1650	

Results obtained using two different methods demonstrate a difference in the cytotoxic effect of the preparations. Cytotoxicity index represented as a percentage of dead cells by trypan blue staining was generally higher than that provided by MTT method. It may be explained by a fact that mitochondrial dehydrogenase system of a dead cell stops

converting the substrate following a decrease of cell's functional activity and alterations of internal balance. The cell membrane, however, at that point is still intact and impermeable for trypan blue dye. Using correlation analysis, we have established a compound concentration decreasing vital capacity of cell population by 50% – i.e., index of cytotoxic concentration, CC_{50} [6]. Results presented in the table show that SBIO-6 compound was characterized with CC_{50} of 592 (trypan blue staining) and 220 mcg/ml (MTT method) and thus was the most toxic. CC_{50} values for IM-001 and IM-003 compounds exceeded 1000 mcg/ml providing evidence that these compounds were non-toxic for Raji cell culture and were potent agents for assessing their antiEBV activity.

Antiviral activity of test agents was determined by a level of inhibition of EBV reproduction in Raji cells using semiquantitave PCR for a range of compound concentrations (10, 50, 100 mcg/ml), where each was repeated three times. Ganciclovir was used a reference preparation.

For infection with EBV, cells were precipitated in sterile tubes by centrifuging at 1,500 rpm for 10 min. The obtained precipitate was washed twice using RPMI 1640 media without serum for maximum removal of embryonic bovine serum which prevents virus adsorption on a cell. Resultant precipitate was diluted in minimal volume of media, and then virus preparation was added. Virus adsorption was carried out at 37°C for 1 h. Afterwards the cell were washed twice as described above and the growth media with embryonic bovine serum (5%) was added down to planting dose of 500000 cells per 1 ml.

After virus adsorption and dilution of cell culture to 500000 cells/ml, studied compounds were added at concentrations 10, 50, 100 mcg/ml (in RPMI 1640 media). Studied samples were collected in 48 h. This time interval is considered optimal for both growth dynamics of Raji cell culture and EBV reproduction cycle. Virus DNA accumulation levels in infected cells treated with the compounds (applied at a range of concentration) was determined in comparison to control infected cells where virus DNA accumulation constituted 100% (Fig.2).

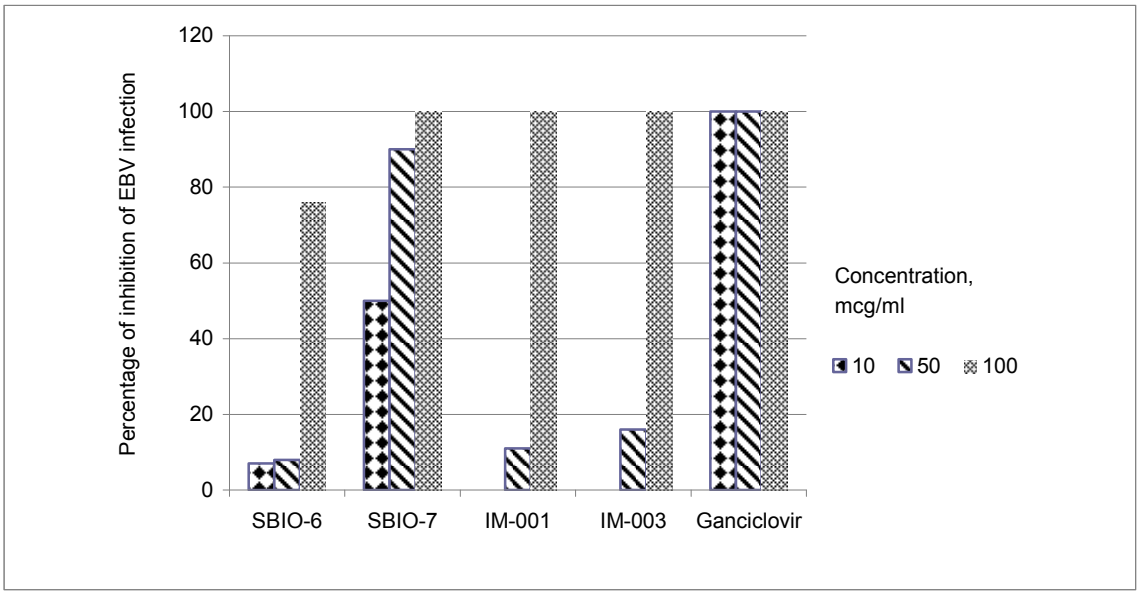

Figure 2. Activity of fluoric compounds against Epstein-Barr virus

Presented results allow considering studied compounds as perspective antiviral substances active against Epstein-Barr virus.

We have also evaluated the ability of studied compounds of inducing apoptosis in EBV-transformed Raji cells. Advances in fundamental knowledge on molecular mechanisms of virus-induced oncogenesis, functions and interactions among viral and cellular oncogenes, mechanisms for evading apoptotic death of virus-infected cells leading to tumor growth favored the development of novel approach for selection of antiviral preparations capable of not only efficient inhibition of virus reproduction but also stimulating recovery or elimination of infected cell, in particular by apoptosis, for treating virus-associated neoplasms [5, 7, 14].

One of the approaches to therapy of EBV-positive tumors presumes 'switching' the latent form of EBV infection (which is typical for the majority of EBV-positive tumor cells) into cytolytic form. This approach may be clinically beneficial as lytic EBV infection leads to cell destruction. Another therapeutical advantage is based on the induction of expression of EBV 'lytic' genes (viral thymidine kinase and DNA polymerase) which are expressed exclusively during the lytic cycle of the virus and are capable of converting nucleoside analogues into their respective cytotoxic forms. For instance, many various chemotherapeutical preparations (arginine butyrate, 5-fluorouracil (5-FU), cis-platinum, taxol, gemcitabine, doxorubicin, and sodium butyrate) have been tested in combination with ganciclovir for their ability to 'switch' EBV infection from latent form to lytic cycle in tumor cells. Gemcitabine and doxorubicin activated transcription from promoters of two pre-early virus genes, *BZLF1* and *BRLF1*, in EBV-negative B cells. Ganciclovir led to an increase in cell death initially induced by gemcitabine or doxorubicin in virus-transformed lymphoblastoid cells, and prevented the development of lytic form of viral infection [15].

Research conducted on the determination of apoptotic cell after application of studied compounds at the range on concentrations confirmed apoptosis-stimulating effect for SBIO-6 compound. The results are presented on Fig.3.

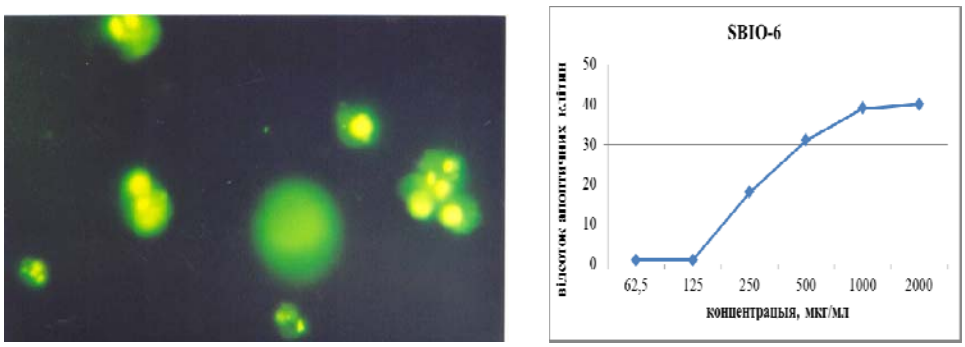

Figure 3. Detection of apoptotic Raji cells after adding SBIO-6 compound at 1000 mcg/ml (Hoechst 33342 dye)

As can be seen from Fig.3, initial 'active' concentration for this compound was 250 mcg/ml which resulted in 18% of apoptotic cells. Further increase of compound concentration led to subsequent increase in number of apoptotic cells.

Apoptosis modulators influence tumor cells and hence represent efficient mean for treating cancer patients. This approach is promising because it allows increasing tumor cells' sensibility to effects of cytotoxic factors. In turn, this allows decreasing doses for chemo- or radiotherapy and reducing death rates for normal (non-tumor) cells. In addition, apoptosis modulators may induce lethal damage and elimination of tumor cells resistant to conducted therapeutical agents. There are grounds to assume that only combined (complex) therapy of tumor growth may 'guarantee' maximum damage of malignant transformed cells, diminish their resistance to chemotherapy agents, prevent dissemination of tumor (metastasis), and also reduce toxic side effects of preparations/means of chemo-, radiotherapy and other ways of cancer treatment.

Fluoric nucleoside analogues are used as chemotherapeutic preparations for combating many viral infections induced, in particular, by Hepatitis C virus and HIV. However, use of such preparations for treating EBV infection is not developed at the time. This work describes novel concept in treating EBV-associated disorders, i.e. use of fluoric nucleoside analogues for preventing virus replication and subsequent virus liquidation. In spite of unknown mechanisms of action of the preparations under study, their indexes of antiviral activity and low cytotoxicity *in vitro* make them perspective agents for designing antiviral compounds targeting EBV infections.

Conclusions. Here we confirmed cytotoxic effect of studied compounds SBIO-6, SBIO-7, IM-001, and IM-003 in lymphoblastoid Raji cell culture. Value of CC_{50} for these compounds constituted 350-3700 mcg/ml. We have assessed antiherpetic activity of studied compounds toward Herpes simplex virus type 1 (HSV-1) and type 2, and Epstein-Barr virus. Analysis of antiviral activity of the compounds demonstrated absent or negligibly low activity against HSV-1 and notable activity against EBV. When applied at the concentration of 100 mcg/ml, SBIO-7, IM-001, and IM-003 compounds totally (100%) inhibited EBV reproduction. Determination of apoptotic cells resulting from the application of studied compounds at a range of concentrations revealed apoptosis-stimulating effect for SBIO-6 which may be perspective for future research as an antitumor agent. Obtained results may be used for computer-assisted modeling of structure functional relations for recommended compounds and for prognostic design of novel highly active antiviral agents.

References

1. Ершов Ф.И., Коваленко А.Л., Романцов М.Г., и др. Герпетическая инфекция: вопросы патогенеза, методические подходы к терапии. Руководство для врачей / Под ред. Романцова М.Г., Голубева С.Ю. – М., 1997. – 97 с.

2. Лопач С.Н., Чубенко А.В., Бабич П.Н. Статистические методы в медико-биологических исследованиях с использованием Excel. – 2001. – 320 с.

3. Посібник з хіміотерапії вірусних інфекцій: Навчально методичний посібник для лікарів /за ред. Дзюблик І.В. – К: Авіцена, 2004. – 176 с.

4. Уоллз Э., Крофорд. Д. Культивирование клеток В95-8 // Лимфоциты. Методы – М: Мир, 1990. – С.230-249.

5. Фильченко А. А., Стойка Р. С. Апоптоз: краткая история, молекулярные механизмы, методы выявления и возможное значение в онкологии // Экспер. Онкол. – 1996, Т.18. – С.435-448.

6. Щербинська А.М., Дяченко Н.С., Рибалко С.Л. та ін. Вивчення антивірусної дії потенційних лікарських засобів // Доклінічні дослідження лікарських засобів (методичні рекомендації). – Київ. – 2001. – с. 371-390.

7. De Clercq E. Antivirals and antiviral strategies // Nat. Rev. Microbiol. – 2004. – 2, N 9. – P. 704–720.

8. Jung-Chung Lin. Antiviral Therapy for Epstein-Barr Virus-Associated Diseases// Tzu Chi Med J. – 2005. – 17, No 1 – P. 379-385.

9. Kutok J.L. and Wang F. Spectrum of Epstein-Barr Virus-Associated Diseases.// Annu.Rev.Pathol.Mech.Dis. – 2006. –Vol. 1. – P. 375-404.

10. Mosmann T. Rapid colorimetric assay for cellular growth and survival:application to proliferation and cytotoxicity assays//J Immunol. Methods. – 1983. – 65. – P.55-63.

11. Murray P. G., Young L. S. The Role of the Epstein-Barr Virus in Human Desease//Front Biosci. – 2002. – Vol.2, N 3. – P. 519-540.

12. Pagano J.S., Gershburg E. Epstein-Barr virus infections: prospects for treatment // J. Antimicrobial Chemotherapy. – 2005. – 56, N 2. – P. 277–281.

13. The Herpesviruses. Volume 1 /Ed.: Bernard Roizman. – New York and London:Premium Press. – 1982. – 445 p.

14. Scaffidi C., Kirchhoff S., Krammer P.H., Peter M. E. Apoptosis signaling in lymphocytes // Curr. Opin. Immunol. – 1999. – Vol. 11. – P.277-285.

15. Spender L.C., Cannell E.J., Hollyoake M., Wensing B., Gawn J.M. Control of cell cycle entry and apoptosis in B lymphocytes infected by Epstein-Barr virus. // Journal of virology. – 1999. – V.73, No.6. – P.4678-4686.

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ЦИТОТОКСИЧНІСТЬ ТА ПРОТИВІРУСНА АКТИВНІСТЬ НОВИХ ФТОРОВМІСНИХ СПОЛУК

Досліджена цитотоксичність та антивірусна активність фторовмісних нуклеозидних сполук у модельній системі ВЕБ в системі in vitro. Виявлено апоптозстимулюючий ефект для сполуки SBIO-6, що може бути перспективою для подальших досліджень його в напрямку протипухлинного аналізу. Отримані результати можуть бути використані при комп'ютерному моделюванні взаємозв'язку між структурою і біологічною активністю речовин і будуть застосовуватися для створення нових високоактивних противірусних засобів.

Ключові слова: фторовмісні нуклеозидні сполуки, сполука SBIO-6, протипухлинний аналіз.

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ЦИТОТОКСИЧНОСТЬ И ПРТИВОВИРУСНАЯ АКТИВНОСТЬ НОВЫХ ФТОРСОДЕРЖАЩИХ СОЕДИНЕНИЙ

Исследована цитотоксичность и антивирусная активность фторсодержащих нуклеозидных соединений в модельной системе ВЭБ в системе in vitro. Выявлено апоптозстимулирующий эффект для соединения SBIO-6, что может быть перспективой для дальнейших исследований его в направлении противоопухолевого анализа. Полученные результаты могут быть использованы при компьютерном моделировании взаимосвязи между структурой и биологической активностью веществ, и будут применяться для создания новых высокоактивных противовирусных средств.

Ключевые слова: фторсодержащие нуклеозидные соединения, соединение SBIO-6, противоопухолевый анализ.

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LOOKING FOR KILLERS: BACTERIOPHAGES AGAINST PHYTOPATOGENIC BACTERIA

The samples of rotten tomatoes and papers were collected from different regions of Ukraine. Phytopathogenic microorganisms – causative agents of plant diseases were extracted and introduced into culture for strain identification. The presence of bacteriophages in the samples was determined by using agar overplayed method and TEM (transmission electron microscopy).

Key words: phytopathogenic microorganisms, bacteriophages.

Introduction. Bacterial pathogens are significant factors which reduce yields of agriculturally important plants worldwide. Among these bacteria the most important are *Pseudomonas syringae*, *Pectobacterium carotovorum,* and bacteria from *Xanthomonadaceae* family.

A variety of approaches have been developed to minimise the impact of bacterial plant diseases on the quantity and economy of food production. Currently, phytopathogens are controlled through management programs, which mostly relay on application of bactericides (copper-based). However, irrational use of these compounds leads to evolution of bacteria and emergence of new, highly resistant forms of phytopathogens. Besides chemical compounds are often accumulated in plants/soils and pose environmental hazards. Antibiotics (e.g., tetracycline and streptomycin) have been utilized in agriculture to control phytopathogenic bacteria also. Extensive use of antibiotics in agriculture has led to selection of resistant bacterial strains [11]. Moreover, resistance genes have been spread to other bacteria, including human and animal pathogens or nonpathogenic bacteria present in the environment [7].

Due to these agrichemical disadvantages, biological control of plant bacteria has attracted attention of many scientists and bacteriophages propose more advantages then other biological agents [6]. Bacteriophages are very specific, even to bacterial pathovars and strains; they don't cause lysis of microbial cells, represented on plants and don`t influence on soil normoflora. Besides, phages are natural components of ecosystems and always persist in host population [4]. In nature bacteriophages coexist with the host microorganisms in balance, so there is no necessity of searching them elsewhere or produce them in the laboratory by synthesis *de novo*. We just need to isolate bacteriophages from environment were specific host is present, investigate their biological properties and convert these viruses into the weapon against their hosts. Bacteriophages can also be coupled with the application of other control strategies (antagonistic bacteria, biocides etc.) for increased pressure on the pathogen [16].

The first works, that showed the potential of bacteriophages in control of phytopathogenic microorganisms, were published in 1924. Mollman and Hemstreet demonstrated that phages lysates prevent rotting of cabbage, caused by pathogenic microorganism *Xanthomonas campestris pt. campestris* [13]. Then many scientists explored phages antimicrobial activity on important agricultural plants, such as rice, pepper, tomatoes and etc. [9]. Despite the promising early works, phage therapy preparation did not prove to be a reliable and effective means of controlling phytobacteria. The main reason of this is the development of antibiotics and biocides. During the last decades of of the 20th century, bacteriophages were re-evaluated as antimicrobial agents [6]. In 2005 fist commercial phage preparation was recommended in the US for usage on crops to control infection caused with two phytopatogenic bacteria – *Xanthomonas campestris pt. vesicatoria* and *Pseudomonas syringae pt. tomato* [8].

Success of application of "AgriPhage^{TM}" (OmnyLytics) stimulated the development of new phage-based preparation against the most harmful phytobacteria worldwide. Ukraine, as agricultural country, faced with problem of crop yield losses due to bacterial infections also [5] and is interested in the development of bacteriophage preparations. However, situation is complicated with the absence of information about distribution of phytopathogenic bacteria in Ukraine. Isolation and identification of actual bacterial strains should be conducted prior to the development of bacteriophage preparations. Hence, the objectives of this study were isolation of bacteria and their bacteriophages from samples of infected plants.

Materials and methods. Samples of rotten vegetables – tomato (*Solanum lycopersicum* L.) and sweet pepper (*Capsicum anuum* L.) were collected from distinct regions of Ukraine (15 samples of tomato and 10 samples of sweet pepper from Kirovograd-, Cherkassy-, Sumy-, Kherson-, Kiev regions). Liquid medium of Luria-Bertani (baktotrypton – 1%, yeast extract – 0,5%, NaCl – 1%) was used for bacteria cultivation and bacteriophage enrichment. Miller agar (peptone – 1%, yeast extract 0,5%, NaCl – 1%, agar 1.4%) was used for propagation of bacteriophages and their hosts, whereas 1,4% agar and 0,7% agar were used for the hard and soft-agar layers, respectively, in phage plating. All bacterial isolates were maintained on clippings of Miller's agar [1].

The samples of tomatoes and sweet peppers with symptoms of rotting were sterilized by 72% ethanol. Then small pieces of diseased tissues were cut off with sterile knife and placed into LB-broth for enrichment of bacteriophages.For bacteria isolation, the sap was taken from cut surface using microbial loop and plated on Miller's [2].

Other bacteria (*Pectobacterium carotovorum, Pectobacterium amylovorum, Pseudomonas syringae pt. tomato, Xanthomonas campestris pt. campestris 117* and *125, Serratia marcescens IMBG291*) tested for phage sensitivity were obtained from culture collection of Laboratory of mi-

crobial ecology of The Institute of Molecular Biology and Genetics of NASU. Tubes and Petri dishes with samples were incubated at 27°C for 12 hours. After incubation the LB-broth with phages was centrifuged at of 5000 rev / min. for 25 minutes [15]. Supernatants were collected in sterile tubes and treated with chloroform to remove opportunistic microorganisms. Bacterial colonies on agar after 12 h of incubation were described and plated on Miller's agar clippings for further strain identification.

In the next step, the samples were analyzed for the presence of bacteriophages by double-agar technique. For this purpose 0,3 ml of target bacteria and 1 ml of the sample were added to soft agar. Isolated plagues were described and transferred to sterile ependorfs with normal saline (1 ml) [3].

Some portion of enriched bacteriopages were subjected to differential centrifugation (5000 rev. / min 20 min, 24,000 rev / min (~ 51,000 g) 120 min centrifuge UCP-65, RCS-50 rotor), the precipitate obtained after UHSC was resuspended in sterile normal saline (200 ml) [15].

The morphology of isolated viruses was studied using a transmission electron microscope (model JEM-1400, Laboratory for biophysical studies at the Institute of Microbiology and Virology named by D.K. Zabolotniy National Academy of Sciences of Ukraine). For reticula-substrates we used 0.1% formvar solution in chloroform. Phages were contrasted with 2% solution of uranilatcetate [15].

Results and discussion. Under our investigations, in general 25 samples of rotten vegetables (fig. 1) were collected from different Ukrainian regions (Kirovograd-, Cherkassy, Sumy-, Kiev regions) and 22 microorganisms were isolated for further researches.

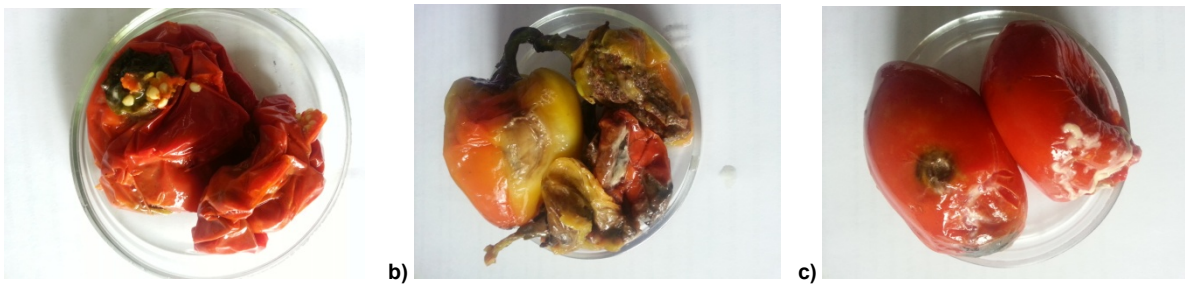


Figure 1. Some samples of peppers and tomatoes, infected with phytopathogenic microorganisms:
a) sample XXII, b) sample XXIV, c) sample XXV

As a first step, enriched samples (I-XXII) were mixed and then plated on 19 bacterial isolates. As a result we observed the plague formation on 11 bacterial isolates and the number of PFU varied from one to thousands per Petri dishes (fig. 2). The morphological properties of plague were very heterogeneous depending on host bacteria. Some microorganisms were totally lysed after fist plating, following experiment confirmed high phages concentration in the samples.

Figure 2. Morphology of plagues on bacterial lawn:
a) bacteria VII – one giant and hundreds of small plagues; b) at least 3 different types of plagues on bacteria X;
c) totally lysed bacterial layer and secondary growth on bacteria XII

On the next stage, we collected 3 new vegetable samples from Kherson and Kiev region (named XXIII-XXV) and isolated 3 new microorganisms. These 3 samples were also mixed and plated on all isolated bacteria (I-XXV). The sample gave positive result on 2 own bacteria (XXIII and XXIV) and 10 isolated previously (tab.1). Vise versa the sample I-XXII did not contain lytic agents against bacteria XXIII-XXV. The most of plagues were small (1 mm) in size, only phages, that infected bacteria XXII, gave large (4 mm) plagues with halo (fig. 3).

Fifure 3. Morphology of plagues on bacterial lawn after addition sample XXIII-XXV:
a) hundreds of very small colonies on bacteria I; b) two types of plagues on bacteria XV – middle, d~2 mm and small, d~1 mm;
c) big plagues with halo on bacteria XXII

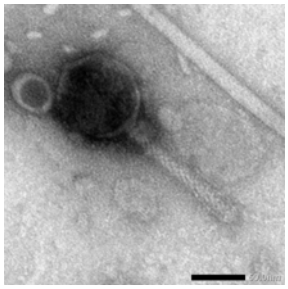
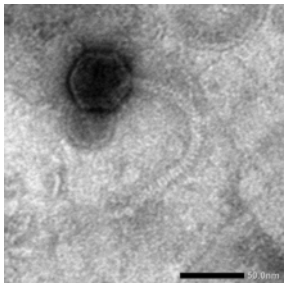
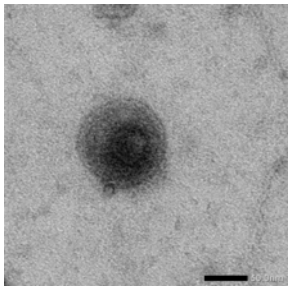
To determine the host range of the lytic phages, that were isolared previously, we tested their ability to produce plaques on laboratory pathovars of phytophathoganic bacteria from culture collection – *Pectobacterium atrosepticum*, *Pectobacterium* cat*orovorum*, *Xanthomonas campestris pt. campestris* 117 and 125, *Serratia marcescens IMBG291*, *Pseudomonas syringae pt. tomato*. Among the tested plant-pathogenic strains only one microorganism – *Serratia marcescens IMBG291* was phage-sensitive (fig. 4). Results of three experiments are summarized in table 1:

Figure 4. Plagues on bacteria lawn of *Serratia marcescens IMBG291* after addition of sample I-XXV ("Total")

***Table* 1. Results of sample plating on isolated bacteria**

Bacteria sample and region of isolation	Reaction on phage after addition of the sample I-XXII	Reaction on phages after addition of the sample XXIII-XXV	Morphology of plagues after addition of the sample I-XXII	Morphology of plagues after addition of the sample XXIII-XXV
I (tomato, Kirov. r.)	+	+	Middle, d~3 mm	Small, d~1 mm
II (tomato, Kirov. r.)	+	-	Very small, d<1 mm	
III (tomato, Kirov. r.)	-	-		
V (tomato, Kirov. r.)	-	-		
VI (tomato, Kirov. r.)	+	+	Totally lysed	Middle, d~2 mm
VII (sweet pepper, Kirov. r.)	+	+	Big, d~5 mm & small, d~1 mm	Very small, d<1 mm
IX (sweet pepper, Kirov. r.)	-	-		
X (sweet pepper, Kirov. r.)	+	+	Totally lysed	Big, d~4 mm & small, d~1 mm
XI (tomato, Cherkas. r.)	+	+	Ghostly, d~2 mm	Small, d~1 mm
XII (tomato, Cherkas. r.)	+	+	Totally lysed	Small, d~1 mm
XIV (tomato, Cherkas. r.)	+	+	3 types – big (4 mm), middle (2 mm) and small (1 mm)	Small, d~1 mm
XV (tomato, Cherkas. r.)	+	+	Totally lysed	Middle, d~2 mm & small, d<1 mm
XVI (tomato, Cherkas. r.)	-	-		
XVII (sweet pepper, Cherkas. r.)	+	+	Totally lysed	Small, d<1 mm
XVIII (sweet pepper, Cherkas. r.)	-	-		
XIX (sweet pepper, Cherkas. r.)	-	-		
XX (sweet pepper Sumy r.)	-	-		
XXI (tomato, Sumy r.)	-	-		
XXII (sweet pepper, Cherkas. r.)	+	+	Totally lysed	Big, d~4 mm
XXIII (tomato, Kiev r.)	Not tested	+		Small, d~2 mm
XXIV (sweet pepper, Khers. r.)	Not tested	+		Totally lysed
XXV (tomato, Khers. r.)	Not tested	-		

Data obtained with an electron microscope for mixed samples indicate the presence of many different phages, members of 3 families – *Myoviridae*, *Siphoviridae* and *Podoviridae*. Most of observed myoviruses belonged to A1 morphotype. Morphology of siphoviruses capsids dimensionally matched the data for B1 morphotype. While, podoviruses from samples were presented with big, about 105 nm in diameter, spherical particles with short tales and belonged to C1 morphotype. The prevalence of "tailed" phages from *Myoviridae* and *Siphoviridae* families corresponds to observed small sizes of plagues formed on the bacterial lawn. Typical for representatives of family *Podoviridae* plague morphology (pic. 5) was described only in cases of four bacteria (VII, X, XIV and XXII).

a)  b)  c) 

Picture 5. Bacteriophages morphology, obtained after TEM:
a) member of *Myoviridae* family, type A1, head ~ 85X80 nm, tail ~ 130 nm; b) member of *Siphoviridae* family, type B1, head ~ 50X45 nm, tail ~ 145 nm; member of *Podoviridae* family, type C1, head ~ 105X105 nm, tail ~ 20 nm

This is the fist our attempt to isolate phytopathogenic bacteria from diseased plants together with their specific bacteriophages. Newly isolated bacteria are probably members of family Xanthomonadaceae that include common pathogenes of tomatoes and peppers according to data of other scientists [10]. Our results are speculative, but we can found a lot of viruses to potentially phytopathogenic microorganisms, isolated from rotten tomatoes and sweet papers. In future researches we intend to identify microorganisms to species and pathovars and confirm their influence on plants *in vitro* and *in vivo*.

Readable results were observed during second trials, phages from 2 samples formed plagues on 10 phytopathogenic microorganisms from different regions, it means that isolated viruses are probably polyvalent or their hosts are relatives and have the same receptors. We plan to investigate all isolated phages in details after identification of target microorganisms.

Noteworthy is also the fact of insensitivity of laboratory bacterial strains to newly isolated bacteriophages. These results may be explained in two ways. The first explanation is the absence of bacterial strain related to laboratory strains in samples that were collected. According to second suggestion laboratory strains have lost susceptibility to mostly bacteriophages due to the numerous passages.

Conclusions. In this survey 22 isolates of bacteria were plated from infected tomato and sweet pepper. Identification of these bacteria is in progress. Bacteriophages, specific to the pathogenic microorganisms, were isolated, accumulated and examined by the method of electron microscopy. Three distinct groups of bacteriophages, based on their virion morphology, were identified.

References

1. Атабеков И.Г. Практикум по общей вирусологии. – М.: Издательство Московского университета. – 1981. – 192 с.

2. Cappuccino J. G., Sherman N. Microbiology: a laboratory manual. 4-th edition. – Menlo-park, CA: The Benjamin-Cumming Publishing Company Inc. – 1990. – 186 p.

3. Carlson K., Kutter E., Sulakvelidze A. Bacteriophages: biology and applications. Appendix: Working with bacteriophages: Common techniques and methodological appproaches. – Boca Raton: CRC Press. – 2005. – PP. 437-494.

4. Clockie M.R.J., Millard A.D., Letarov A.V., at all. Phages in nature. // Bacteriophage. – 2011. – Vol.1. – № 1. – P. 31-45.

5. Гвоздяк Р.І., Пасічник Л.А., Яковлева Л.М. та ін. Фітопатогенні бактерії. Т 1. Бактеріальні хвороби рослин. – К.: ТОВ "НВП Інтерсервіс". – 2011. – 444 с.

6. Ho K. Bacteriophage therapy for bacterial infections. Rekindling a memory from the pre-antibiotics era. // Perspect. Biol. Med. – 2001. – Vol. 44. – P. 1–16.

7. Jarvis W.R. Preventing the emergence of multidrug-resistant microorganisms thought antimicrobial use controls: the complexity of the problem. // Inf. Con. And Hosp. Epidem. – 1996. – Vol. 17. – № 8. – P. 10-15.

8. Jones J.B., Iriarte F.B., Obradovic A., at all. Management of bacterial spot on tomatoes with bacteriophages. // Proc. Int. Symp. Biol. Control Bact. Plant Dis., Land-Forstwirtsch: Mitt. Biol. Bundesanst. – 2006. – № 408. – P. 154.

9. Jones J.B., Jackson L.E., Balogh B., at all. Bacteriophages for plant disease control. // Annu. Rev. Phytopathol. – 2007. – Vol. 45. – P. 245-262.

10. Jones J.B., Lacy G.H., Bouzar. H., at all. Reclassification of the Xanthomonads associated with bacterial spot disease of tomato and paper // Systematic and Applied Microbiology. – 2004. – Vol. 27. – P. 755-762.

11. Khachatourians G.G. Agricultural use antibiotics and the evolution and transfer of antibiotic-resistant bacteria. // CMAJ – 1998. – vol. 159. – № 9. – P. 1119-1120.

12. Loper J.E., Henkels M.D., Roberts R.G., at all. Evaluation of streptomycin, oxytetracycline and copper resistance of Erwinia amylovora isolated from pear orchards in Washington State. // Plant Dis. – 1991. – Vol. 75. – P. 287-290.

13. Mallmann W.L., Hemstreet C.J. Isolation of an inhibitory substance from plants. // Agric. Res. – 1924. – P. 599–602.

14. Миронов А. А., Комиссарчик Я. Ю., Миронов В.А. Методы электронной микроскопии в биологии и медицине: Методологическое руководство. – СПб.: Наука, 1994. – 400 с.

15. Остерман Л.А. Методы исследования белков и нуклеиновых кислот: электрофорез и ультрацентрифугирование. – М.: Наука, 1981. – 288 с.

16. Tanaka H., Negishi H., Maeda H. Control of tobacco bacterial wilt by an avirulent strain of Pseudomonas solanacearum M4S and its bacteriophage. // Ann. Phytopathol. Soc. Jpn. – 1990. – Vol. 56. – P. 243–246.

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У ПОШУКАХ ВБИВЦЬ: БАКТЕРІОФАГИ ПРОТИ ФІТОПАТОГЕННИХ МІКРООРГАНІЗМІВ

Зразки перців і томатів із симптомами бактеріальної гнилі були відібрані у різних регіонах України. Фітопатогенні мікроорганізми – збудники хвороб рослин були виділені із овочів і введені в культуру для подальшого визначення до штамів. Присутність вірусів до виділених мікроорганізмів в отриманих зразках було підтверджено за допомогою методу агарових шарів та методу електронної мікроскопії.

Ключові слова:фітопатогенні мікроорганізми, бактеріофаги.

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В ПОИСКАХ УБИЙЦ: БАКТЕРИОФАГИ ПРОТИВ ФИТОПАТОГЕННЫХ МИКРООРГАНИЗМОВ

Образцы перцев и томатов с симптомами бактериальной гнили были отобраны в разных регионах Украины. Фитопатогенные микроорганизмы – возбудители болезней растений были выделены из овощей и введены в культуру для дальнейшего определения штаммов. Присутствие вирусов к выделенным микроорганизмам в полученных образцах были подтверждены с помощью метода агаровых слоев и метода электронной микроскопии.

Ключевые слова: фитопатогенные микроорганизмы, бактериофаги.

UDK 578.85/86

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ADAPTATION OF BIOTESTING METHOD FOR DETECTION OF *PBCV-1* IN WATER SAMPLES

Here we report on the adaptation of biotesting method for detection of algae viruses in samples of water and bottom sediments. We have shown that the modification involving the use of two media layers of different density with application of samples previously enriched in aeration box proved to be most efficient. Here we also describe that the water samples collected from technical water reservoirs of the National Exhibition Center of Ukraine demonstrate lytic activity towards test culture of symbiotic algae Chlorella sp. (ACKU 95-02). The stage of initial accumulation of a virus has been carried out.

Key words: algae viruses, Chlorella sp (ACKU 95-02), PBCV-1.

Introduction. Viruses are typical for any water system. These organisms are vastly abundant; their content may reach over 10 millions of particles per milliliter of water [1, 2]. The viruses are thought to influence great part of genetic and species biodiversity in seas and oceans [3]. Despite wide range of virus species found in water reservoirs,

only a small part of these (118 virus species) may directly infect algae [4]. This phenomenon is uncommon, especially in the light of strict virus-host specificity and wide diversity of algae – potent hosts for viruses. Only *Chlorophyta* division contains approximately 20-25 thousand species of algae typical for fresh-water and hyperhalic reservoirs, seas, oceans and terrestrial biotopes [5].

Research on ecology of algae viruses has scientific, fundamental and applied value. Lack of food resources and energy sources is a major social issue of modern living, and microalgae represent one of the few practical solutions for this challenge. They are considered as a potent source of nutrients and biofuel. In turn, algae viruses cause raw material waste and consequential significant financial losses. It is also known that lysis of about 25% of water (phytoplankton) bloom is attributed to virus-induced lysis [6,7]. Such viruses are thought of as candidates for biological control of water blooms. On the other hand, algae viruses (whether directly or indirectly) are connected with global warming due to the generation of dimethyl sulfoxide (DMSO) when the algae are lysed. This gas is a major biogenic source of sulfur. Generation of dimethyl sulfoxide leads to progressive accumulation of greenhouse gases [8, 9].

We also know that some algae viruses are involved in horizontal gene transfer, contributing to genetic diversity of microalgae associations [10, 11].

Nearly 60 years have passed since the first report of virus-like particles (VLPs) in the cells of microalgae [12]. However, research of algae viruses still remains at the beginning descriptive stage. This may be explained by the difficulties in virus isolation from the environment using traditional methods of virology. Many algae viruses may be detected by modern molecular biological methods but still lack information on susceptible algae cultures, preventing virus isolation and accumulation [13].

Ambiguous results of interaction of a virus with susceptible cell pose another issue of algae virus research. High concentration of algae culture and small amount of virus typically lead to nearly complete lysis of susceptible test culture. In turn, small numbers of algae cultures in the media coupled with specific conditions may lead to cytoproliferative effect. In addition, routine cultivation of a test culture of microalgae needs certain skills, time-consuming and costly.

In spite of said above we turned to adaptation of biotesting method for detection of PBCV-1 in samples from fresh water reservoirs.

Materials and methods. The samples were collected following general recommendations pursuing virus preservation in a sample and preventing sample contamination with secondary microflora. We have used different methods when sampling water for detection of algae viruses: washing out mantle cavity of bivalved mollusks, sampling bottom sediments, and direct sampling of water from reservoir. Further, collected and prepared samples were kept in a fridge at +4^{o}C. For initial virus detection we have collected 74 water samples plus 32 samples of soil and washouts/swabs from the foulings of water-immersed objects.

Samples of unicellular fresh-water green algae *Chlorella sp.*(ACKU 218-03), *Chlorella sp.* (ACKU 531-02), *Chlorella sp.* (ACKU 532-03) and symbiotic culture of *Chlorella sp.* (ACKU 95-02) were used as test objects. These cultures were kindly provided by the Department of Botany, Taras Shevchenko' National University of Kyiv

Different modifications of biotesting were used for virus detection in the collected samples. PBCV-1 was used as a positive control.

Results and Discussion. Biotesting is a classical approach in virology. The method comes to virus detection using test objects. Alterations of vital functions of a test object (or its death) serve as a signal of virus presence. Biotesting approach is widely used due to its simplicity, quickness and availability. Biotesting is a historically proven technique in virology and forms a part of Koch's triad – necessary element for confirmation of the infectious nature of any pathogen.

However, biotesting approach has several limitations when used for algae viruses. Firstly, many detected algae viruses still have no established susceptible microalgae cultures. Secondly, virus interaction with sensitive cells does not always follow cytopathic pathway. Cytoproliferative effect is also possible. Thirdly, the algae demonstrate varying degree of susceptibility to virus infection at different stages of their life cycle. We should also point that microalgae are rather problematic to cultivate, they often require specific media and regimes of light and temperature. However it is impossible to refuse using this method totally, and therefore there's a need for its modification and selecting optimal conditions for each test culture and the virus.

In the course of this work we have tried several biotesting techniques: 1) biotesting in liquid media; 2) biotesting on solid media; 3) biotesting on solid media using two media layers of different density. Every technique was tried with both native and synchronized test cultures (Fig.1). We have also used water samples filtered via bacterial filter (Millex GV, 0,22) and enriched filtered samples previously enriched in a special aeration box.

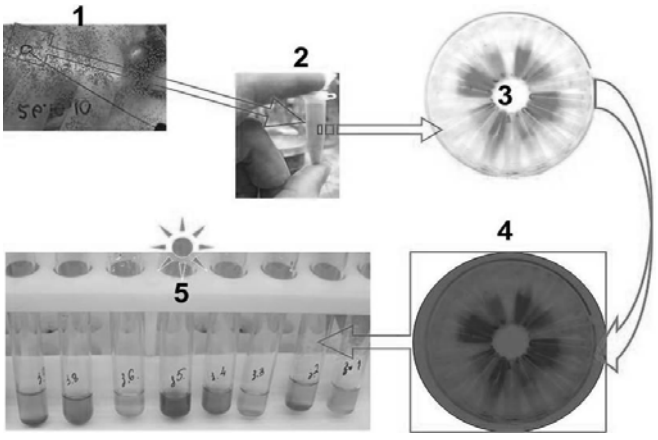

Fig.1. Scheme for synchronization of microalgae test culture:
1-10-14-day lawn of test cultures; 2,3 – cultures resuspended in 1,5 ml of liquid media; 4 – cultures kept in the dark, 12-hour incubation; 5 – 2,5 ml of liquid media is added to the culture which is further cultivated at normal regime of light

The modification of biotesting method involving the use of two media layers of different density with application of water samples previously enriched in aeration box proved to be most efficient in our work.

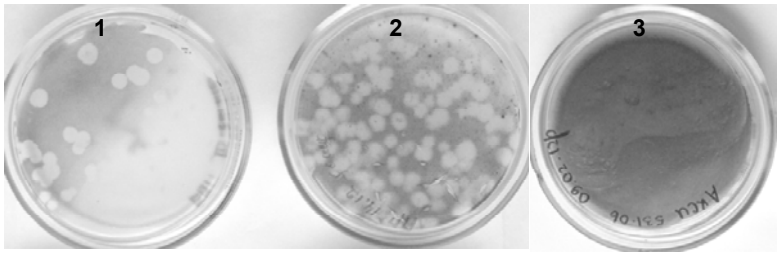

Fig.2. Results of biotesting in the modification involving the use of two media layers of different density with application of water samples previously enriched in aeration box:
1 – positive control контроль (PBCV-1); 2 – experimental samples; 3 – control of test culture

Conclusion. We have demonstrated that water samples collected from technical water reservoirs of the National Exhibition Center of Ukraine (50.370651; 30.474002) showed lytic activity towards symbiotic algae *Chlorella sp.* (ACKU 95-02) and induced lysis zones of approximately 1 cm in diameter (Fig.2). We have also carried out the stage of initial accumulation of the virus. The modification of biotesting method involving the use of two media layers and water samples previously enriched proved to be most efficient. Other modifications were less efficient or provided no results at all. However we need to mention that chosen modification is more time-consuming and requires specialized equipment.

References

1. Weinbauer, M., Rassoulzadegan, F., 2004. Are viruses driving microbial diversifica-tion and diversity? Environ. Microbiol. 6, 1–11.

2. Sobecky, P.A., Hazen, T.H., 2009. Horizontal gene transfer and mobile genetic ele-ments in marine systems. Methods Mol. Biol. 532, 435–453.

3. Suttle, C.A., 2005. Viruses in the sea. Nature 437, 356–361.

4. Virus Taxonomy. Classification and Nomenclature of Viruses Ninth Report of the International Committee on Taxonomy of Viruses Editors, Andrew M.Q. King, Michael J. Adams, Eric B. Carstens, and Elliot J. Lefkowitz.

5. Костіков І.Ю., Джаган В.В. та ін. Ботаніка. Водорості та гриби.

6. Keizo Nagasaki, Yuji Tomaru, Noriaki Katanozaka, Yoko Shirai, Kensho Nishida, Shigeru Itakura, and Mineo Yamaguchi. Isolation and Characterization of a Novel Single-Stranded RNA Virus Infecting the Bloom-Forming Diatom Rhizosolenia setigera // Applied and Environmental Microbiology, February 2004, p. 704-711, Vol. 70, No. 2.

7. Castdery T. Algal viruses: Characteristics and ecological effects. – PY: Dep.of Micr.,University of Bergen, 2001. – 204 p.

8. Hill, R.W.,White, B.A., Cottrell,M.T.,Dacey, J.W.H., 1998.Virus-mediatedtotal release of dimethylsulfoniopropionate frommarine phytoplankton: a potential climate process. Aquat. Microb. Ecol. 14, 1–6.

9 Wilson, W.H., Turner, S., Mann, N.H., 1998. Population dynamics of phytoplankton and viruses in a phosphate-limited mesocosm and their effect on DMSP and DMS production. Estuar. Coast Shelf Sci. 46, 49–59.

10. Sobecky, P.A., Hazen, T.H., 2009. Horizontal gene transfer and mobile genetic ele-ments in marine systems. Methods Mol. Biol. 532, 435–453.

11. Weinbauer, M., Rassoulzadegan, F., 2004. Are viruses driving microbial diversifica-tion and diversity? Environ. Microbiol. 6, 1–11.

12. Spencer, R., 1955. A marine bacteriophage. Nature 175, 690–691;

13. Suttle, C.A., Fuhrman, J.A., 2010. Enumeration of virus particles in aquatic or sed-iment samples by epifluorescence microscopy. In: Wilhelm, S.W., Weinbauer, M.G., Suttle, C.A. (Eds.), Manual of Aquatic Viral Ecology. American Society of Limnology and Oceanography, Waco, TX, USA, pp. 145–153.

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АДАПТАЦІЯ МЕТОДИКИ БІОТЕСТУВАННЯ ДЛЯ ВИЯВЛЕННЯ *PBCV-1* У ПРОБАХ ВОДИ

Проведено адаптацію метода біотестування для детекції альговірусів у пробах води та придонних осадах. Встановлено, що найефективнішою є модифікація методу біотестування з використанням двох шарів середовища різної щільності та додаванням попередньо збагачених в аераційній камері проб. Показано здатність зразків води відібраних з технічних озер Національного виставкового центру України лізувати тест культуру симбіотичної водрості Chlorella sp (ACKU 95-02). Проведено первинне накопичення вірусу.

Ключові слова: альговіруси, симбіотична водорость Chlorella sp (ACKU 95-02), PBCV-1.

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АДАПТАЦИЯ МЕТОДИКИ БИОТЕСТИРОВАНИЯ ДЛЯ ДЕТЕКЦИИ *PBCV-1* В ПРОБАХ ВОДЫ

Проведено адаптацию метода биотестирования для детекции альговирусов в пробах воды и придонных осадках. Установлено, что наиболее эффективной является модификация метода биотестирования с использованием двух слоев среды различной плотности и добавлением предварительно обогащенных в аэрационной камере проб. Показана способность образцов воды отобранных из технических озер Национального выставочного центра Украины лизировать тест культуру симбиотической водрости Chlorella sp (ACKU 95-02). Проведено первичное накопление вируса.

Ключевые слова: альговирусы, симбиотическая водоросль Chlorella sp (ACKU 95-02), PBCV-1.

UDK: 611.118+616.34-008.87+616.006

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EFFECT OF THEMULTIPROBIOTIC THERAPY ON THE HYPOTHALAMIC-PITUITARY-ADRENAL SYSTEM AND CYTOKINE PROFILE UNDER CONDITIONS OF STRESS

It was studied the influence of multistrain probiotic (MP) on the stress hormones content (adrenocorticotropic hormone (ACTH) and cortisol) and the content of proinflammatory (interleukin (IL) 1β and IL-12Bp40) and antiinflammatory (IL-4 and IL-10) cytokines in conditions of erosive and ulcerative lesions caused water immersion restraint stress (WIRS). Established that MP significantly accelerated recovery of functioning of the hypothalamic-pituitary-adrenal system in terms of the stress action, that was confirmed by a more rapid return of ACTH and cortisol concentrations to values of intact rats. Also MP decrease proinflammatory (IL-1β and IL-12B p40) and increase antiinflammatory (IL-4 and IL-10) cytokines content in the rats serum after WIRS. These data suggest that one of the mechanism of the therapeutic effect of MP on lesions in the gastric mucosa caused by stress is the impact on the stress system and cytokine profile.

Keywords: adrenocorticotropic hormone, cortisol, cytokines, stress, multistrain probiotic.

Physiological response to stress is a compensatory reaction that eliminates or reduces the degree of homeostasis alteration [1]. A mobilization of all systems is the basis of this response. Due to the activation of the hypothalamic-pituitary-adrenal system (stress system) levels of glucocorticoids grows, which enables increased blood glucose as the main energy source for the adaptation to stress [2]. Under conditions of excessive exposure to stressful factors processes of immune reactivity trigger to protect against possible infections [3, 4]. The result is anexcretion of proinflammatory cytokines (interleukin-1 (IL-1), IL-6, IL-12, tumor necrosis factor α). Today it is known that inflammatory mediators engaged in a stimulating impact on the stress systemthat is detected by the increased synthesis of corticotropin-releasing hormone and glucocorticoids, which in turn suppress the immune response by negative feedback [5-8]. When adaptation to excessive or prolonged stress exposure is insufficient, pathological changes in organism emerge. And first of all, significant release of stress hormones damages the gastric mucosa (GM).

Under prolonged stress action endocrine glands (pituitary and hypothalamus) are depleted, resulting in fall of glucocorticoids levelbelow the physiological. To maintain the integrity of the GM, homeostatic cortisol level is essential, as both too high and too low content of this hormone damages the GM [9]. It is observed disbalance in self-regulation of the "stress system – immune system" loop under the depletion of the hypothalamic-pituitary-adrenal system. Significant release of proinflammatory cytokines in these conditions aggravates the lesions of the GM [10].

Today, more and more data suggest the relationship between symbiotic microflora of the intestine, nervous system and stress system [11]. An immune system plays not the least role in this interaction. In our previous study, we found that the therapeutic administration of multistrain probiotic (MP) "Symbiter ® acidophilic concentrated" reduced erosive and ulcerative lesions under conditions of stress action [12]. To reveal the mechanisms of such influence the aim of current work was to determine the effects of MP on the content of stress hormones (cortisol and adrenocorticotropic hormone(ACTH)) and the content of proinflammatory (interleukin (IL) 1β and IL-12Bp40) and antiinflammatory (IL-4 and IL-10) cytokines under conditions of erosive and ulcerative lesions in GM induced by water immersion restraint stress (WIRS).

Methods

The study was carried on 70 male rats in accordance with the standards of the Convention on Bioethics of the Council of Europe's, 1997, European Convention for the Protection of Vertebrate Animals used for Experimental and Other Scientific Purposes and the Law of Ukraine from 21.02.2006 № 3447-IV "On Protection of Animals from Abuse".

Animals were divided into 10 groups of 7 rats each (Table 1).

***Table* 1. The deWIRSion of rats on the research groups**

Group number	The number of therapeutical injections	The substance that was injected	Duration (hours) between stress exposure and measuring of hormones and cytokines in serum
1	–	–	intact rats (not subjected to stress)
2	–	–	stress control (hormones and cytokines level was measured immediately after stress)
3	2	water (control)	24
4	2	multistrain probiotic	24
5	4	water (control)	48
6	4	multistrain probiotic	48
7	6	water (control)	72
8	6	multistrain probiotic	72
9	8	water (control)	96
10	8	multistrain probiotic	96

Rats were subjected to 3-hour WIRS by Takagi et al., 1964 [13]. One day prior to the experiment, the rats were not fed, but they had free access to water. For immobilization rats were placed in a perforated metal camera that was putdown vertically into the water for 3 hours so that the water level reaches the jugular fossa of animals. Water temperature was 22-23° C.

After the stress animals of 3-10 groups were treated with water or aqueous solution of MP in a volume of 0.5 ml/200 g orally twice a day. MP containing 14 probiotic strains genera *Lactobacillus*, *Lactococcus*, *Bifidobacterium*, *Propionibacterium*, was administered at a dose of 140 mg/kg (1.4×10^{10} CFU/kg). Treatment was started in an hour after 3-hour WIRS.

Intact and exposed to stress animals were sacrificed by cervical dislocation after a specified time after the WIRS. Rats blood was collected from the heart into centrifuge tubes without anticoagulant and leaved for 20-30 minutes at room temperature to complete the formation of a clot. Then, blood samples were centrifuged at 1000 g for

15 minutes and the supernatant (serum) were harvested in separate disposable microtubes, frozen at -20° C and used for further studies. Serum ACTH and cortisol content were determined at 1st-3rd days after WIRS, the antiinflammatory cytokines –at 1st-4th days. ACTH and cortisol were determined by ELISA using commercial kits of DRG International Inc. (USA) and SRL LLC "Granum" (Kharkiv, Ukraine)production. Their contents were expressed in pg/ml and nmol/l accordingly. The content of IL-1β was measured by ELISA using commercial kits of GE Healthcare production (Amersham, IL-1β Rat Biotrak ELISA System) and expressed as pg/ml. Content of IL-4, 10, 12B p40 was measured by ELISA using specific polyclonal antibodies (Sigma). Their expression are expressed in units of optical density. All samples were analyzed in two repetitions.

Statistical analysis of data was carried out by the "Statistica 8.0" software package. For the analysis of the data distribution type Shapiro-Wilk`sW criterion was used. Since the obtained results were normally distributed, t-Student test for independent samples was used for the comparison of data. Mean of value (M) and standard error of the mean (m) were calculated. Significant difference was considered at $p \le 0,05$.

Results. It was found that the concentration of ACTH in the serum of intact rats was 23 ± 9,7 pg/ml, and the concentration of cortisol – 27 ± 8,3 nmol/l. As a result of stress cortisol levels increased by 2.2 times ($p<0.001$), while the concentration of ACTH decreased by 7.9 times ($p<0.001$), that confirmed the negative feedback between the level of cortisol in the blood and the level of secretion of ACTH by pituitary gland (Fig. 1a, b) [14]. After 24 hours from the WIRS concentration of cortisol in the blood serum of rats decreased by 5.7 times($p<0.001$) compared with intact controls, which may indicate adrenal depletion under the influence of stress factors and the enter to the third phase of the general adaptation syndrome – the stage of depletion (fig. 1a, b). ACTH concentration after 24 hours was reduced by 3.2-fold ($p<0.01$) compared with intact rats, which may indicate depletion of the pituitary gland. The concentration of ACTH was 2.5 times higher ($p<0.05$) compared with measured immediately after WIRS (Fig. 1a, b). For the next 2 days after WIRS it was established a gradual recovery of the level of ACTH and cortisol to the intact control values. So, after48 hours from the WIRS concentration of ACTH was 1.9 times lower ($p<0.05$), and cortisol – 3.4 times lower ($p<0.001$) compared with intact controls. Within 72 hours after the WIRS ACTH concentration in serum of rats treated with water did not differ significantly from that of intact rats, but cortisol concentration was lower by 1.6-fold the level of the intact control ($p<0.05$) (Fig. 1a, b). So, for 3 days after the stress concentration ACTH and cortisol plasma levels were reduced compared with intact rats, indicatingthe depletion of the endocrine glands, and gradually restored to normal levels.

MP significantly accelerated recovery of functioning of the hypothalamic-pituitary-adrenal system under stress conditions, which was confirmed by a more rapid return of ACTH and cortisol concentrations to values of intact rats (Fig. 1a, b). The level of ACTH under the treatment of MP did not differ from the intact control on the 1st day after the WIRS and the cortisol concentration was restored to the level of intact animals in 3 days after stress exposure. Thus, the effect of MP on the content of stress hormones is one of the mechanisms of its gastroprotective effect. Indeed, we have found significant erosive and ulcerative lesions of the GM on the 1st-3rd days after WIRS despite ofsmall level of ACTH and cortisol in the blood of rats. And the ulcer area in 3 days after WIRS sighnificantly exceeded the registered immediately after stress. Therapy with MP facilitated the restoration of basal levels of ACTH and corticosteroids, which correlated with acceleration ofthe stress-induced lesions healing in the GM.

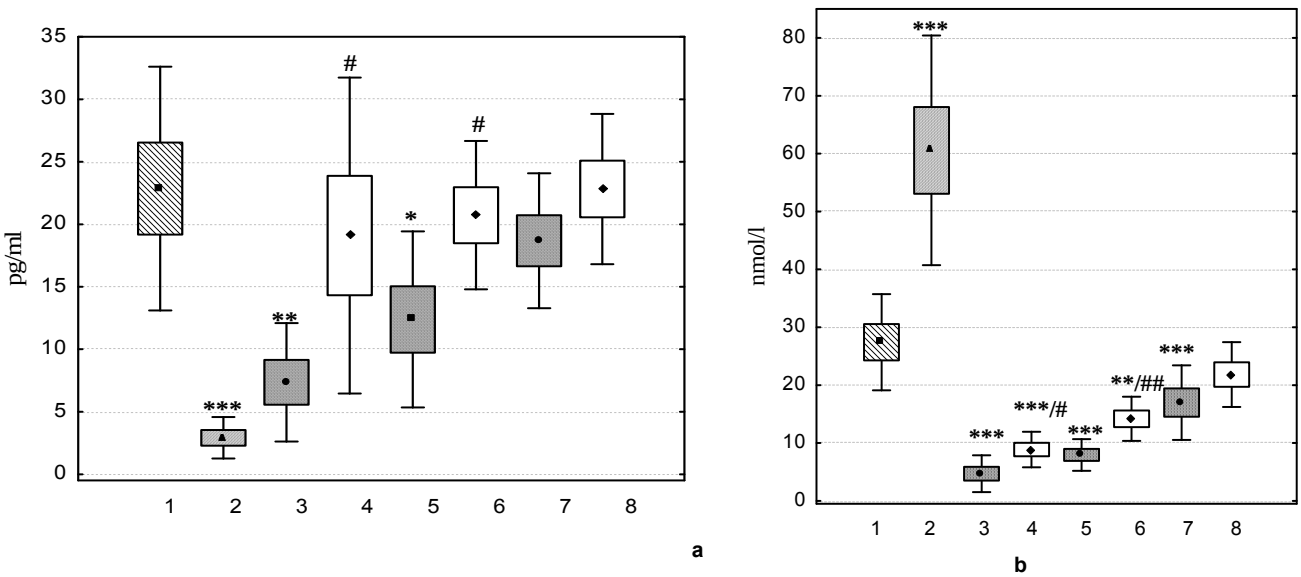

Figure 1. Content of adrenocorticotropic hormone (a) and cortisol (B) in serum of rats after water immersion restraint stress and under the conditions of therapeutic administration of multistrain probiotic (n = 7 per group) (M; box: SD; whiskers: m)
1 – intact control; 2 – immediately after stress; 3, 5, 7 – 1, 2, 3 days after stress in rats who were treated with water;
4, 6, 8, 1, 2, 3 days after stress in rats which were treated with probiotic

*, **, *** – $p<0.05$, $p<0.01$, $p<0.001$ compared with intact control, #, ## – $p<0.05$, $p<0.01$ compared with corresponding groups of rats treated with water.

Study of immunomodulatory properties of MP under conditions of stress-induced lesions of the GM showed that after the application of stress in serum of rats which were injected with water the concentration of proinflammatory cytokines IL-1β and IL-12B p40 significantly increased and remained higher compared to intact animals within 4 days

after WIRS (Table 2). The concentration of antiinflammatory IL-4 was also higher compared to control at all the days of observation after the WIRS, indicating a compensatory function of the immune system in terms of the stress. At the same time, the content of IL-10 in serum of rats after stress did not change.

***Table* 2. The content of proinflammatory and antiinflammatory cytokines in rats serum under water immersion restraint stress and therapeutic administration of multistrain probiotic (M ± m, n = 7 per group)**

index / Groups of rats	Interleukin 1β	Interleukin 12Bp40	Interleukin 4	Interleukin 10
1 (Intact rats)	314±71	0,105±0,015	0,076±0,009	0,199±0,018
2 (immediately after stress)	403±68*	0,268±0,025***	0,076±0,007	0,222±0,015
3 (Control, 1 day after WIRS)	449±66*	0,394±0,056***	0,172±0,040*	0,235±0,008
4 (Multistrain probiotic, 1 day after WIRS)	414±57*	0,311±0,034***	0,270±0,054***/#	0,374±0,063*/#
5 (Control, 2 days after WIRS)	563±51***	0,233±0,035**	0,141±0,023*	0,145±0,035
6 (Multistrain probiotic, 2 days after WIRS)	409±61***/#	0,179±0,032*/#	0,084±0,020#	0,171±0,013
7 (Control, 3 days after WIRS)	511±66**	0,293±0,016***	0,116±0,021	0,211±0,025
8 (Multistrain probiotic, 3 days after WIRS)	319±53##	0,258±0,030***	0,087±0,023	0,190±0,016
9 (Control, 4 days after WIRS)	471±54*	0,137±0,023	0,122±0,017*	0,214±0,020
10 (Multistrain probiotic, 4 days after WIRS)	334±41#	0,082±0,006#	0,072±0,007##	0,174±0,012

*, **, *** – p <0.05, p <0.01, p <0.001 compared with intact controls, # # # – p <0.05, p <0.01 compared with corresponding groups of rats treated with water.

Treatment with MP significantly reduced the concentration of proinflammatory IL-1β and IL-12B p40 after stress. For example, under the MP administration content of IL-1β and IL-12B p40 after WIRS did not differ from the level of intact controls at 3rd and4th day accordingly. It was found a strong effect of MP on the concentration of antiinflammatory cytokines. MP elevatedthe IL-4 concentration by 57% (p<0.05) and IL-10 by 59% (p<0.05) compared with the group of rats treated with water at 1st day after WIRS. In the following days, the concentration of antiinflammatory cytokines IL-4 and IL-10 in the group of rats administered with MP did not differ from that of intact animals. The results indicate an anti-inflammatory effectof MP under conditions of stress-induced lesions of the GM.

These results are consistent with other studies that have shown that probiotic strains reduce the concentration of proinflammatory cytokines under various pathologies of the digestive system [15-17]. Thus, Lin-Lin Chen et al. (2009) found that probiotics can reduce the content of IL-1β levels in experimental colitis [15]. Rodes et al. (2013) revealed that *Bifidobacterium longumsubsp. infantis* reduces the concentration of tumor necrosis factor-α and increases the concentration of anti-inflammatory IL-4 in a model of human intestinal microbiota[16]. Bermudez-Brito et al. (2013) demonstrated a reduction in proinflammatory cytokines level produced by human dendritic cells infected with *Salmonella typhi*, under the influence of *Bifidobacterium breve* CNCM I-4035 [17].

Summing up the results, we can conclude that the probiotic strains reduce the immune response and eliminate stress hyperactivation under stress creating favorable conditions for stress-induced lesions healing in the GM.

Conclusions.

1. MP restored basal levelof ACTH and corticosteroids in conditions of WIRS.

2. MP possessed the antiinflammatory effect under stress action, which was confirmed by a decrease of proinflammatory and increase of antiinflammatory cytokines in the serum of rats.

3. Effect of MP on the system stress and the immune system is one of the mechanisms of thestress-induced lesions healing in GM.

References

1. Veissier I. Stress and welfare: two complementary concepts that are intrinsically related to the animal's point of view / I. Veissier, A. Boissy // Physiol Behav. – 2007. – Vol.92, № 3. – P.429-433.

2. Compensatory gastroprotective role of glucocorticoid hormones / L. P. Filaretova [et al.]// Ross Fiziol Zh Im I M Sechenova. – 2007. – Vol.93, № 11. – P.1217-1228.

3. Involvement of interleukin-1 in immobilization stress-induced increase in plasma adrenocorticotropic hormone and in release of hypothalamic monoamines in the rat / F. Shintani [et al.] // J Neurosci. – 1995. – Vol.15, № 3 Pt 1. – P.1961-1970.

4. Campisi J. Stress-induced extracellular Hsp72 is a functionally significant danger signal to the immune system / J. Campisi, T. H. Leem, M. Fleshner // Cell Stress Chaperones. – 2003. – Vol.8, № 3. – P.272-286.

5. Acute stress modulates the histamine content of mast cells in the gastrointestinal tract through interleukin-1 and corticotropin-releasing factor release in rats / H. Eutamene [et al.] // J Physiol. – 2003. – Vol.553, № Pt 3. – P.959-966.

6. Corticotropin-releasing factor-producing neurons in the rat activated by interleukin-1 / F. Berkenbosch [et al.] // Science. – 1987. – Vol.238, № 4826. – P.524-526.

7. Release of multiple hormones by a direct action of interleukin-1 on pituitary cells / E. W. Bernton [et al.] // Science. – 1987. – Vol.238, № 4826. – P.519-521.

8. Interleukin-1 stimulates the secretion of hypothalamic corticotropin-releasing factor / R. Sapolsky [et al.] // Science. – 1987. – Vol.238, № 4826. – P.522-524.

9. From Gastroprotective to Ulcerogenic Effects of Glucocorticoids: Role of Long-Term Glucocorticoid Action / L. Filaretova [et al.] // Curr Pharm Des. – 2013. – [Epub ahead of print].

10. Accelerated Ulcer Healing and Resistance to Ulcer Recurrence with Gastroprotectants in Rat Model of Acetic Acid-induced Gastric Ulcer / Oh T. Young [et al.] // J Clin Biochem Nutr. – 2008. – Vol.42, № 3. – P.204-214.

11. The microbiome: stress, health and disease / Moloney R. D. [et al.] // Mamm Genome. – 2013. – [Epub ahead of print].

12. Вірченко О. В. Дослідження лікувально-профілактичного впливу мультипробіотика "Симбітер" при розвитку гострих уражень в слизовій оболонці шлунка щурів / О. В. Вірченко, Т. В. Берегова, О. І. Цирюк // Вісник проблем біології і медицини. – 2010. – № 1. – P.41-46.

13. Takagi K. Studies on the Drugs for Peptic Ulcer. A Reliable Method for Producing Stress Ulcer in Rats / K. Takagi, Y. Kasuya, K. Watanabe // Chem Pharm Bull (Tokyo). – 1964. – Vol.12, № – P.465-472.

14. Relations of plasma ACTH and cortisol levels with the distribution and function of peripheral blood cells in response to a behavioral challenge in breast cancer: an empirical exploration by means of statistical modeling / G. van der Pompe [et al.] // Int J Behav Med. – 1997. – Vol.4, № 2. – P.145-169.

15. Therapeutic effects of four strains of probiotics on experimental colitis in mice / L. L. Chen [et al.] // World J Gastroenterol. – 2009. – Vol.15, № 3. – P.321-327.

16. Effect of probiotics Lactobacillus and Bifidobacterium on gut-derived lipopolysaccharides and inflammatory cytokines: an in vitro study using a human colonic microbiota model / L. Rodes [et al.] // J Microbiol Biotechnol. – 2013. – Vol.23, № 4. – P.518-526.

17. Cell-free culture supernatant of Bifidobacterium breve CNCM I-4035 decreases pro-inflammatory cytokines in human dendritic cells challenged with Salmonella typhi through TLR activation / M. Bermudez-Brito [et al.] // PLoS One. – 2013. – Vol.8, № 3. – P.e59370.

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ВПЛИВ ТЕРАПЕВТИЧНОГО ВВЕДЕННЯ МУЛЬТИПРОБІОТИКА НА ГІПОТАЛАМО-ГІПОФІЗАРНО-НАДНИРКОВУ СИСТЕМУ І ЦИТОКІНОВИЙ ПРОФІЛЬ В УМОВАХ СТРЕСУ

Вивчали вплив мультиштамного пробіотика (МП) на вміст гормонів стресу (адренокортикотропний гормон (АКТГ) та кортизол) та вміст прозапальних (інтерлейкін (ІЛ) 1β та 12В р40) та антизапальних (ІЛ-4 та ІЛ-10) цитокінів за умов ерозивно-виразкових уражень, викликаних водно-іммобілізаційним стресом (ВІС). Встановлено, що МП суттєво прискорював відновлення функціонування гіпоталамо-гіпофізарно-надниркової системи за умов дії стресу, що відображалося у більш швидкому поверненні концентрації АКТГ та кортизолу до значень інтактних щурів. МП зменшував вміст прозапальних (ІЛ-1β та ІЛ-12В р40) та підвищував вміст антизапальних (ІЛ-4 та ІЛ-10) цитокінів в сироватці крові щурів після ВІС. Отримані дані свідчать, що одним з механізмів лікувального ефекту МП на ураження в слизовій оболонці шлунка, викликані стресом, є вплив на систему стресу та цитокіновий профіль.

Ключові слова: адренокортикотропний гормон, кортизол, цитокіни, стрес, мультиштамний пробіотик.

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ВЛИЯНИЕ ТЕРАПЕВТИЧЕСКОГО ВВЕДЕНИЯ МУЛЬТИПРОБИОТИКА НА ГИПОТАЛАМО-ГИПОФИЗАРНО-НАДПОЧЕЧНИКОВУЮ СИСТЕМУ И ЦИТОКИНОВЫЙ ПРОФИЛЬ В УСЛОВИЯХ СТРЕССА

Изучали влияние мультиштамного пробиотика (МП) на содержание гормонов стресса (адренокортикотропный гормон (АКТГ) и кортизол) и содержание провоспалительных (интерлейкин (ИЛ) 1β и 12В р40) и антивоспалительных (ИЛ-4 и ИЛ-10) цитокинов в условиях эрозивно-язвенных поражений, вызванных водно-иммобилизационном стрессом (ВИС). Установлено, что МП существенно ускорял восстановление функционирования гипоталамо-гипофизарно-надпочечниковой системы в условиях действия стресса, что отражалось в более быстром возврате концентрации АКТГ и кортизола до значений интактных крыс. МП сни жал содержание провоспалительных (ИЛ-1β и ИЛ-12В р40) и повышал содержание антивоспалительных (ИЛ-4 и ИЛ-10) цитокинов в сыворотке крови крыс после ВИС. Полученные данные свидетельствуют, что одним из механизмов лечебного эффекта МП на поражения в слизистой оболочке желудка, вызванные стрессом, является воздействие на систему стресса и цитокиновый профиль.

Ключевые слова: адренокортикотропный гормон, кортизол, цитокины, стресс, мультиштаммный пробиотик.

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THE EFFECT OF ACETIC ZINC ON SERUM ZINC LEVEL AND INTERLEUKIN 1β AND 6 LEVEL IN RATS EXPOSED TO ALCOHOL FOR 21 DAYS

The aim of this study was to examine the effect of acetic zinc supplementation on dynamics of zinc level and inflammatory cytokines (IL-1β and IL-6) production in serum of rats subjected to ethanol exposure for 21 days.The zinc level in serum was determined by flame atomic-absorption spectrophotometry. The level of IL-1β and IL-6 in serum was measured by enzyme-linked immunosorbent assay (ELISA) kits ("Sigma", США). A significant gradual decrease of serum zinc level and elevation of the levels of circulating IL-1β and IL-6 were seen in the ethanol-fed animals been maximal on 16^{th} and 21^{th}. The changes of zinc level and IL-1 β and IL-6 production in ethanol-intoxicated rats were completely corrected after acetic zinc supplementation that was more evident at prolonged ethanol exposure. Zinc level in such animals has been demonstrated to increase and exceed the control by 4.2 and 4.9 times on 16^{th} and 21^{st} day of alcoholization. The IL-1β and IL-6 level diminished and normalized also at these stages of study. Our results suggest that acetic zinc supplementation recovers zinc pool in blood and normalizes inflammatory cytokine production that may be due to reduction of zinc deficiency and attenuating of oxidative stress thus leading to inhibition of inflammation.

Key words. Ethanol, chronic alcohol intoxication, zinc deficiency, inflammatory cytokines, interleukin 1β, interleukin 6, acetic zinc.

Introducton

Ethanol has a variety of detrimental effects on immune system including effects on cell mediated and humoral immune response. It decreases neutrophil infiltration and phagocytic capability, inhibits lymphocyte activation following antigen stimulation, and alteres cytokine production by T cells and macrophages [1]. Prolonged ethanol exposure can directly and indirectly lead to the suppression of immunity and increased susceptibility to infections. The alcohol effects are dose-dependent, long-term ethanol consumption has been associated with inflammation [2]. Alcoholic liver disease is a result of a pro-inflammatory effect of chronic ethanol exposure [3].

Ethanol affects the production of cytokines that involved in inflammatory responses in plasma and a variety of tissues including lung, liver, and very importantly brain [4]. Cytokines are regulatory proteins playing a key role in immune and inflammatory response to infection by pathogens and oncogenesis. Excessive alcohol abusers have increased circulating levels of the inflammatory cytokines such as TNF-α (tumor necrosis factor-α), IL-1β (interleukin 1) and IL-6 (interleukin 6) [5]. A significantly increased production of IL-1β, IL-6, IL 12, and TNF-α by unstimulated peripheral blood monocytes was demonstrated in patients with alcoholic liver disease [6].

Severe zinc deficiency has been observed to develop in patients who chronically abuse alcohol, it's is one of the most consistent biochemical observation in alcoholic liver disease (ALD) [7]. Zinc affects the function of immune system, because it is one of the most highly proliferative organs. Zinc is crucial for normal growth and function of T and B cells, macrophages, neutrophils, and NK cells [8]. Zinc directly influences on blood mononuclear cell, diminishing the production of cytokines (IL-1, IL-6, TNF-α and IFN-γ) [9]. Various immune disorders are associated with zinc deficiency [10]. Decreased serum zinc level is observed in chronic inflammatory or infectious diseases [9, 11].

Zinc has been successfully used to restore impaired immune functions in diseases accompanied by diminished plasma zinc levels (rheumatoid arthritis, acrodermatitis enteropathica, hemodialysis patients, elderly individuals) [10]. A dietary zinc supplement has been proposed as possibly being an efficient method to palliate zinc deficiency in alcoholism [12]. Studies using animal models have demonstrated that Zn treatment prevents alcohol-induced liver injury under both acute and chronic alcohol exposure conditions [13, 14]. Zn has a high potential to be used in the prevention and treatment of ALD [12]. It may be a completely new therapeutic tool for the selective suppression of lymphocyte functions [10] and inhibition of inflammation. In

comparison to conventional immunosuppressive drugs, zinc has the advantage of being most nontoxic, even in dosages well exceeding the recommended dietary intake [15]. Acetic zinc has the lowest toxicity among zinc salts.

However, the molecular basis of zinc effects on immunity in alcoholism is largely unknown. Altogether, these observations would support the importance of studying of acetic zinc effect on the production of pro-inflammatory cytokines in blood of rats chronically consumed alcohol. The circulating cytokines now may contribute to diagnostic biomarkers of excessive alcohol intake and alcoholism [4].

This study was undertaken to examine the effect of acetic zinc supplementation on dynamics of zinc level and inflammatory cytokines (IL-1β and IL-6) production in serum of rats subjected to ethanol exposure for 21 days.

Materials and Methods

The research was conducted on white nonlinear rats (males) with body weight ranging from 180 to 200 g. Rats were kept under standard conditions with free access to animal chow and tap water. Animals were divided into 3 groups (n=10 per group), namely: (1) control; (2) chronic alcohol intoxication (animals were intragastrically treated with 40% ethanol (2 ml/100 g); one time per day for 21 days); (3) chronic alcohol intoxication and acetic zinc treatment (animals were simultaneously intragastrically treated with ethanol and acetic zinc (0.2 g /100 g that was considerably less than LD_{50}=0,278±0,049 for white rats) for 21 days). The development of chronic alcohol intoxication in rats was performed as described by M.H. Halilov and S.A. Zackirhodjayev [16].

Rats were sacrificed by cervical dislocation on next day after treatment with ethanol and acetic zinc for 4, 7, 11, 16 and 21 days. The protocol of animal experiment was approved by the European Convention for the Protection of Vertebrate Animals used for Experimental and Other Scientific Purposes (Strasbourg, 18.III.1986). Blood samples were collected for further analyses. Serum was collected by centrifugation of whole blood sample at 1000×g for 10 min at 4°C and stored at – 80°C.

The zinc level in serum samples was determined by flame atomic-absorption spectrophotometer C115-M1 ("SELMICHROM", Ukraine) with deuterium background correction and digital analytical complex CAS-120 [17].

The level of cytokines (IL-1β and IL-6) was measured in serum samples by commercially available enzyme-linked immunosorbent assay (ELISA) kits ("Sigma", США). Samples were processed according to the manufacturer's instructions. Optical densities of each well were analyzed using a microplate reader. Samples were processed in triplicates, and were analyzed in batches to minimize inter-assay variability. The level of cytokines was determined by calibration curve plotted using the IL-1β and IL-6 standards.

All analyses were performed using the Microsof Exel 2007 software package, version 12 (Microsoft Corporation, USA). The Student's t-test was used for the evaluation of the statistical significance of the differences observed between two or more groups, respectively. P values less than 0.05 were considered statistically significant.

Results and discussion

Serum zinc level has been measured in rats intragastrically treated with ethanol for 4, 7, 11, 16 and 21 days. Zinc level progressively diminished at all stages of study and was less than control by 32%, 2 and 2.75 times on 4 th, 7 th, 11th day of ethanol exposure, respectively. The most essential decrease was observed on 16th and 21st day of ethanol exposure (by 3.2 and 3.7 times in comparison to the control) (Figure 1).

Simultaneous introduction of acetic zinc and ethanol to rats led to elevation of serum zinc level which exceeded the control at all stages of study. Results shown in Figure 1 indicate that zinc level increased by 2, 4.4. and 8.5 times on 4 th, 7 th, 11th day of alcoholization, respectively, comparative to untreated animals. An elevation was most evident on 16th and 21st day and made 12.9 and 18 times similarly to that observed in rats exposed to alcohol only. In this case zinc level exceeded the control by 4.2 and 4.9 times.

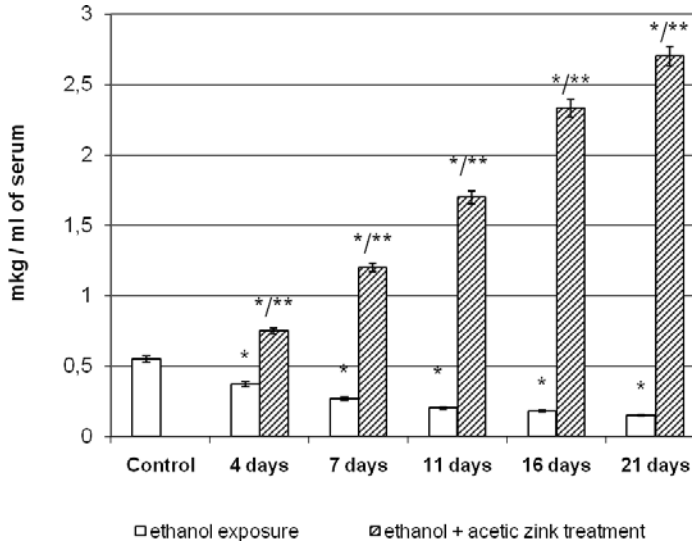

Figure 1. Serum zinc level in rats exposed to ethanol and acetic zinc for 21 day

* – P ≤ 0,05 in comparison to the control group, ** – P ≤ 0,05 in comparison to the group, treated with ethanol only

It seems clear that ethanol causes a notable fall in the level of zinc in serum of rats. We may assume that zinc deficiency develops in alcoholized rats and deepens after prolonged ethanol exposure. Our results are in agreement with findings of other authors. Chronic alcohol feeding associated with inflammation is characterized by low plasma zinc levels or a noticeable zinc deficiency [18, 7]. Even the shot-term ethanol treatment (for 5 days) has been shown to decrease the zinc level in serum of rats [19].

The physiologic plasma zinc concentration represents a very mobile and immunologically important pool [10]. The observed by us decrease of zinc level in ethanol-intoxicated rats likely reflects a redistribution of serum zinc into the liver. Such redistribution according to the findings of Singh et. al., occurs within the inflammation, caused by increased production of inflammatory cytokines, mainly IL-1 and IL-6, and the subsequent induction of zinc-binding metallothionein in hepatocytes [20].

In the present study, acetic zinc administration to ethanol-intoxicated rats restored zinc level in serum and it even exceeded the control value. The effect of this drug was more evident in rats exposed to ethanol for a longer time (for 16 and 21 days). We can assume that acetic zinc treatment restores the reserves of zinc in organism and might be a tool for alleviating of zinc deficiency.

Zinc is considered to be an antioxidant, membrane-stabilizing and anti-inflammatory agent. It has a role in the prevention of free radical-induced injury during inflammatory processes, decreasing reactive oxygene species (ROS) generation via inhibition NADPH oxidase [9]. It also inhibits NF-kB activation and this results in a decrease in production of inflammatory cytokines [8].

Chronic alcohol exposure has been reported to augment secretion of inflammatory cytokines by PBMC [6]. Several studies in human and animal models have confirmed that increases in the production of inflammatory cytokines such as IL-1, IL-6, TNF-α and IFN-γ are associated with decreased zinc status [9].

In line with this, the circulating level of cytokines IL-1β and IL-6 was measured in ethanol-intoxicated rats treated with acetic zinc. It was found that the ethanol-fed rats produced much higher levels of IL-1β and IL-6 than the control rats, and this increase was progressively intensified by prolonged ethanol exposure. The elevation of IL-1β serum level was insignificant in animals treated with ethanol for 4 and 7 days, it averaged 12% and 20%, respectively, in comparison to control animals (Figure 2). The level of this cytokine increased more significantly after more long ethanol exposure for 11, 16 and 21 days and its value exceeded the control by 58%, 71% and 76%, respectively.

The serum level of IL-6 weakly increased at early stages of alcohol intoxication (for 4 and 7 days), exceeding the control by 19% and 35%, respectively (Figure 3). In contrast to IL-1β, IL-6 level dramatically rose by 2, 2.3 and 2.6 times in comparison to control in rats exposed to ethanol for 11, 16 and 21 days, respectively.

Simultaneous treatment of rats with ethanol and acetic zinc led to gradual reduction of IL-1β and IL-6 level in serum at all stages of study. The observed decrease was insignificant in animals treated with zinc for a few days, but this preparation evoked more substantial effect when has been introduced for a longer time (16 and 21 days).

When rats were treated with acetic zinc and ethanol for 4, 7 and 11 days, the serum level of IL-1β decreased by 12%, 23% 34%, respectively, in comparison to animals exposed to alcohol only (Figure 2). Treatment with drug for 16 and 21 days diminished IL-1β level by 40% and 43%, respectively. It should be noticed that level of this cytokine came to normal at last stage of study.

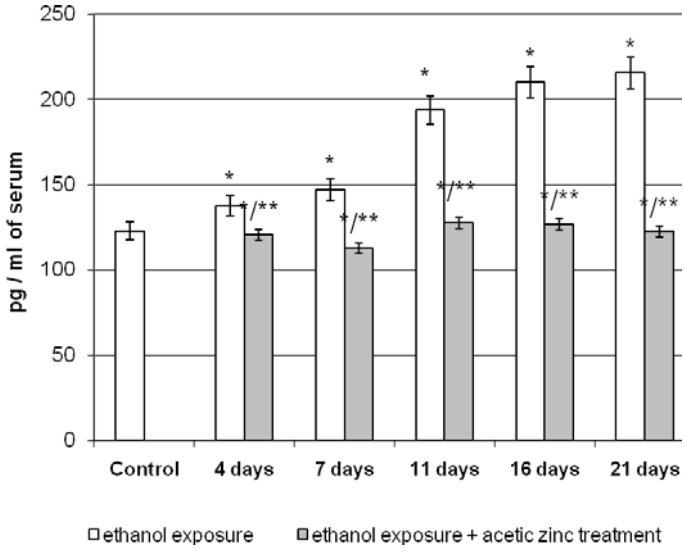

Figure 2. Serum level of IL-1β in rats exposed to ethanol and acetic zinc for 21 day

* – P ≤ 0,05 in comparison to the control, ** – P ≤ 0,05 in comparison to the group, treated with ethanol only

Short-term treatment of rats with acetic zinc and ethanol (for 4 and 7 days) hasn't been observed to alter the serum IL-6 level significantly. In the first case it didn't differ from such in alcoholic animals, in the second case it decreased by 11% (Figure 3). The level of this cytokine diminished by 45%, 53% and 60% in rats subjected to zinc administration for 11, 16 and 21 days, respectively, in comparison to alcoholic animals that haven't been treated with preparation. Thus, the diminishing of Il-6 level was most evident in rats treated with acetic zinc for a longer time (16 and 21 days), similarly to IL-1β. Both IL-1β and Il-6 levels normalized on 16th and 21st day of zinc treatment.

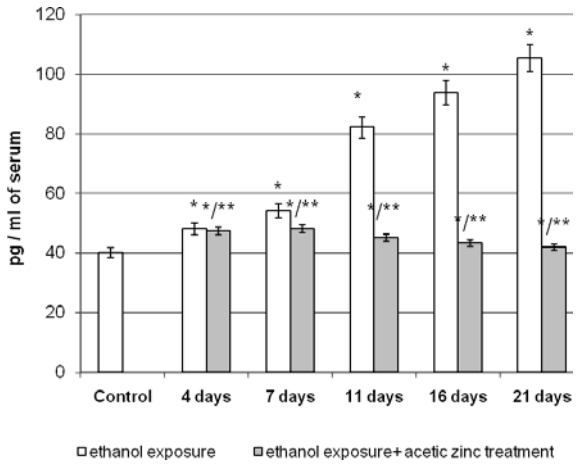

Figure 3. Serum level of IL-6 in rats exposed to ethanol and acetic zinc for 21 day

* – P ≤ 0,05 in comparison to the control, ** – P ≤ 0,05 in comparison to the group, treated with ethanol only

Studies in experimental human model provided a possible mechanism by which zinc deficiency may affect cell-mediated immunity adversely [8]. It was observed that the production of inflammatory cytokines (IFN-γ, TNF-α and IL-1β) by activated monocytes/macrophages is increased as a result of zinc deficiency [9]. Such augmentation leads to the generation of increased amounts of reactive oxygen species (ROS) and developing of the oxidative stress. Chronic alcoholism is also associated with an increased intracellular production of inflammatory cytokines [5, 6].

As could be expected, ethanol induced a significantly increased production of IL-1β and IL-6 analyzed in a group of rats with alcohol intoxication. This observation indicates that secretion of inflammatory cytokines by peripheral blood monocytes of alcoholic rats could be increased. Such effect could be related to the activation of these cells. This is consistent with observations about the activation of human monocytes and macrophages in chronic alcoholic individuals [21]. Such activation could represent a risk factor for the development of systemic inflammatory syndrome [22].

An increase of the production of inflammatory cytokines IL-1β and IL-6 in ethanol-intoxicated rats may reflect the cell-mediated immune dysfunctions and might be due to decrease of zinc level in blood. The observed effect was adversely affected in long-term alcohol exposure that is associated with amplification of zinc deficiency.

We have demonstrated the ability of acetic zinc to inhibit the production of inflammatory cytokines IL-1β and IL-6 in blood, which was simulated by alcohol. This preparation is more efficient when introducing to animals subjected to prolonged ethanol exposure (for 16 and 21 days).

Zinc is considered to affect directly the cytokine production [10, 11]. This preparation could also attenuate oxidative stress in alcoholic animals that is in line with findings concerning the antioxidant properties of this microelement. Zinc was demonstrated to induce the production of metallothionein, which is a scavenger of ˙OH [8]. It also competes with iron and copper ions for binding to cell membrane, thus decreasing the production of ˙OH, inasmuch as these ions catalyze the production of ˙OH from H_2O_2.

The beneficial effect of zinc preparation is associated with reducing of oxidative stress and inhibition of the production of inflammatory cytokines. Dietary zinc supplementation has been observed to prevent hepatocyte apoptosis in mice subjected to long-term ethanol exposure [13]. The action of zinc was assumed as suppression of oxidative stress and death receptor-mediated pathways (NF-R1, FasL, Fas, Fas-associated factor-1, and caspase-3).

Oral administration of zinc chloride has antioxidant effect on stomach and intestine of rats treated with ethanol [12]. This preparation prevented and reversed alterations of thiobarbituric acid reactive substance and reactive species levels, total protein SH content, superoxide dismutase and catalase activity induced by ethanol [14].

Oral administration of Zn(II)-curcumin complex has been reported to adjust the inflammatory cytokine-mediated oxidative damage to the gastric mucosa in the rats exposed to ethanol [23]. This drug prevented formation of ulcer lesions induced by ethanol, inhibited TNF-α and IL-6 expression, increased the activity of SOD and reduced MDA levels in gastric mucosa.

We can assume that acetic zinc inhibits inflammation in alcoholic animals via restoring zinc pool in organism and thus removing the outcomes of zinc deficiency. The observed augmentation of zinc level in blood of alcoholic animals subjected to acetic zinc treatment is evidence of such assumption.

Altogether, our results suggest that acetic zinc supplementation has some benefit by recovery of zinc pool in blood and diminishing the secretion inflammatory cytokines thus restoring impaired immune function in alcoholism.

Conclusions

Alcoholization led to zinc deficiency in blood and augmentation of the production of inflammatory cytokines IL-1 β and IL-6 in rats that is most evident at long-term ethanol exposure (for 21 days). Acetic zinc supplementation recovers zinc pool in blood and normalizes cytokine production that may be due to reduction of zinc deficiency and attenuating of oxidative stress thus leading to inhibition of inflammation. This preparation had beneficial effect when it has been used in prolonged alcohol intoxication.

These results support and extend on previous observations [12], suggesting the high potential of zinc be developed as an effective agent in the prevention and treatment of alcoholism.

References

1. Messingham K.A., Faunce D.E., Kovacs E.J. Alcohol, injury, and cellular immunity // Alcohol. – 2002. – Vol. 28(3). – P.137-149.

2. Goral J., Karavitis J., Kovacs E.J. Exposure-dependent effects of ethanol on the innate immune system // Alcohol. – 2008. – Vol. 42(4). – P.237-247.

3. Nagy L.E. Recent insights into the role of the innate immune system in the development of alcoholic liver disease // Experimental Biology and Medicine. – 2003. – Vol. 228. – P.882-890.

4. Achur R.N., Freeman W.M., Vrana K.E. Circulating cytokines as biomarkers of alcohol abuse and alcoholism // J. Neuroimmune Pharmacol. – 2010. – Vol.5(1). – P.83-91.

5. Khoruts A., Stahnke L., McClain C.J., Logan G., Allen J.I. Circulating tumor necrosis factor, interleukin-1 and interleukin-6 concentrations in chronic alcoholic patients // Hepatology. – 1991. – Vol. 13(2). – P.267-276.

6. Laso F.J., Vaquero J.M., Almeida J., Marcos M., Orfao A. Production of inflammatory cytokines by peripheral blood monocytes in chronic alcoholism: relationship with ethanol intake and liver disease // Cytometry B. Clin. Cytom. – 2007. – Vol. 72(5).- P.408-415.

7. Zhou Z. Zinc and alcoholic liver disease // Dig. Dis. – 2010. – Vol. 28(6). – P.745-750.

8. Prasad A.S. Zinc in human health: effect of zinc on immune cells // Mol. Med. – 2008. – Vol. 14(5-6). – P.353-357.

9. Prasad A.S. Zinc: role in immunity, oxidative stress and chronic inflammation // Curr. Opin. Clin. Nutr. Metab. Care. – 2009. – Vol. 12(6). – P.646-652.

10. Rink L., Kirchner H. Zinc-altered immune function and cytokine production // J. Nutr. – 2000. – Vol. 130, 5S Suppl. – P.1407S-1411S.

11. Fukada T., Yamasaki S., Nishida K., Murakami M., Hirano T. Zinc homeostasis and signaling in health and diseases: Zinc signaling // J. Biol. Inorg. Chem. – 2011. – Vol. 16, No 7. – P.1123-1134.

12. Kang Y. J., Zhou Z. Zinc prevention and treatment of alcoholic liver disease // Mol. Aspects Med. – 2005. – Vol. 26, No 4–5. – P.391–404.

13. Zhou Z., Liu J., Song Z., McClain C.J., Kang Y.J. Zinc supplementation inhibits hepatic apoptosis in mice subjected to a long-term ethanol exposure // Exp. Biol. Med. (Maywood). – 2008. – Vol. 233(5). – P.540-548.

14. Ineu R.P., Oliveira C.S., Oliveira V.A., Moraes-Silva L., da Luz S.C., Pereira M.E. Antioxidant effect of zinc chloride against ethanol-induced gastrointestinal lesions in rats // Food Chem. Toxicol. – 2013. – Vol. 58. – P.522-529.

15. Roberts E.A. Zinc toxicity: from "no, never" to "hardly ever" // Gastroenterology. – 2011. – Vol. 140(4). – P.1132-1135.

16. Halilov M.Ch, Zakirchodjaev Sh.Ya. Characteristics of some pathochemical alterations in blood, liver and brain tissue in experimental alcoholic intoxication // Problems of Alcoholism Clinic: Collected articles, Tashkent, 1983. – P.38-41.

17. Solchaga M., Montoro R., De La Guardia M. Flame absorption spectroscopic determination of cadmium, copper, iron, lead, and zinc in mussels // J. Assoc. Off. Anal. Chem. – 1986. – Vol. 69(5). – P.874-876.

18. Beregova T.V., Grigorova N.V., Eschenko Yu, V. et al. The effect of acute and chronical alcoholization on zinc content in blood // Experimental and Clinical Physiology and Biochemistry. – 2008. – Vol. 1(41). – P.49-53.

19. Florianczyk B. Zinc level in selected tissues of ethanol and morphine intoxicated mice // Med. Sci. Monit. – 2000. – Vol. 6(4). – P.680-683.

20. Singh, A., Smoak, B. L., Patterson, K. Y., LeMay, L. G., Veillon, C. & Deutster, P. A. Biochemical indices of selected trace elements minerals in men: effect of stress // Am. J. Clin. Nutr. – 1991. – Vol. 53. – P.126–131.

21. Zhang Z., Bagby G.J., Stoltz D., Oliver.P, Schwarzenberger P.O., Kolls J.K. Prolonged ethanol treatment enhances lipopolysaccharide/phorbol myristate acetate-induced tumor necrosis factor-α production in human monocytic cells // Alcohol Clin. Exp. Res. – 2001. – Vol. 25. – P.444–449.

22. Moss M., Parsons P.E., Steinberg K.P., Hudson L.D., Guidot D.M., Burnham E.L., Eaton S., Cotsonis G.A. Chronic alcohol abuse is associated with an increased incidence of acute respiratory distress syndrome and severity of multiple organ dysfunction in patients with septic shock // Crit. Care Med. – 2003. – Vol. 31. – P.869–977.

23. Mei X., Xu D., Xu S., Zheng Y., Xu S. Novel role of Zn(II)-curcumin in enhancing cell proliferation and adjusting proinflammatory cytokine-mediated oxidative damage of ethanol-induced acute gastric ulcers // Chem. Biol. Interact. – 2012. – Vol. 197(1). – P.31-39.

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ВПЛИВ ОЦТОВОКИСЛОГО ЦИНКУ НА РІВЕНЬ ЦИНКУ ТА ІНТЕРЛЕЙКІНІВ 1β І 6 У СИРОВАТЦІ КРОВІ ЩУРІВ, ПІДДАНИХ ДІЇ АЛКОГОЛЮ УПРОДОВЖ 21 ДОБИ

Метою роботи було вивчити дію оцтовокислого цинку на динаміку рівню цинку та продукції прозапальних цитокінів (IL-1β і IL-6) у крові щурів, підданих дії етанолу упродовж 21 доби.Рівень цинку у сироватці крові було визначено атомно-адсорбційною спектрофотометрією. Рівень IL-1β і IL-6 було визначено з використанням наборів для імуноферментного аналізу (ELISA) ("Sigma", США).Було встановлено значне поступове зниження рівню цинку та підвищення вмісту IL-1β і IL-6 у сироватці крові тварин, яким вводили етанол, що набувало максимуму на 16-у і 21-у добу. Зміни рівню цинку та продукції IL-1β і IL-6 у тварин з алкогольною інтоксикацією повністю коректувалися після введення цинку, що було найбільш вираженим при тривалій дії етанолу. Показано, що рівень цинку у таких тварин зростав і перевищував контроль у 4,2 і 4,9 рази на 16-у і 21-у добу алкоголізації. Вміст IL-1β і IL-6 знижувався і нормалізувався на цих же етапах експерименту. Наші результати свідчать про те, що введення оцтовокислого цинку відновлює пул цього металу у крові і нормалізує продукцію прозапальних цитокінів, що може бути наслідком усунення цинкового дефіциту і пригнічення оксидативного стресу і, як наслідок, зменшення запалення.

Ключові слова. Етанол, хронічна алкогольна інтоксикація, цинковий дефіцит, прозапальні цитокіни, інтерлейкін 1β, інтерлейкін 6, оцтовокислий цинк.

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ВЛИЯНИЕ УКСУСНОКИСЛОГО ЦИНКА НА УРОВЕНЬ ЦИНКА И ИНТЕРЛЕЙКИНОВ 1β I 6 В СЫВОРОТКЕ КРОВИ КРЫС, ПОДВЕРГНУТЫХ ВОЗДЕЙСТВИЮ АЛКОГОЛЯ В ТЕЧЕНИЕ 21 СУТОК

Целью работы было изучить действие уксуснокислого цинка на диамику уровня цинка и продукции провоспалительных цитокинов (IL-1β и IL-6) в крови крыс, которых подвергали действию этанола в течение 21 суток. Уровень цинка в сыворотке крови был определен атомно-адсорбционной спектрофотометрией. Уровень IL-1β и IL-6 был определен с использованием наборов для иммуноферментного анализа (ELISA) ("Sigma", США). Установлено значительное постепенное снижение уровня цинка и повышение содержания IL-1β и IL-6 в сыворотке крови животных, которым вводили етанол, что достигало максимума на 16-е и 21-е сутки. Изменения уровня цинка и продукции IL-1β и IL-6 у животных с алкогольной интоксикацией полностью корректировались посля введения цинка, что было наиболее выраженным при длительном действии этанола. Показано, что урівень цинка у таких животных возростал и превышал контроль в 4,2 и 4,9 раза на 16-е и 21-е сутки алкоголизации. Содержание IL-1β и IL-6 снижалось и нормализировалось на этих ж етапах эксперимента. Наши результаты свидетельствуют о том, что введение уксускокислого цинка восстанавливает пул этого металла в крови и нормализирует продукцію провоспалительных цитокинов, что может быть результатом устранения цинкового дефицита и угнетения оксидативного стресса и, как следствие, уменьшение воспаления.

Ключевые слова: этанол, хроническая алкогольная интоксикация, цинковый дефицит, пртивовоспалительные цитокины, интерлейкин1β, интерлейкин 6, уксуснокислый цинк.

UDK 591.1+612.3+616-092

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MOTOR ACTIVITIES OF THE RAT STOMACH AFTER LONG-TERM INJECTION OF OMEPRAZOLE AND OMEPRAZOLE WITH MULTIPROBIOTIC "APIBACT"

Changes in spontaneous and carbaholine-stimulated gastric motor activity was studied in rats after 28 days administration of omeprazole (proton pump inhibitor) and after 28 days simultaneous administration of omeprazole and multiprobiotic "Apibact". It was found that omeprazole inhibited spontaneous and stimulated gastric motor activity. Multiprobiotic "Apibact" significantly prevented the development of changes in gastric motility caused by omeprazole.

Keywords: gastric motor activity, proton pump inhibitor, Omeprazole, "Apibact".

Introduction. The proton pump inhibitor (PPI) is widely used for the treatment of gastroesophageal reflux disease, peptic ulcer diseases, and functional dyspepsia [10]. A systematic literature search has been performed, showing that the delaying effect of PPIs on gastric emptying of solid meals is consistent [2, 11]. Early we established that after 28-days of gastric acid secretion depression by H^+-K^+-ATPase blocker omeprazole application in the stomach the inflammation, dysbacteriosis [12] and motility disturbances were developed [9]. For correction of dysbacteriosis we used multiprobiotic "Symbiter® acidophilic" concentrated. We have shown that after simultaneous administration of omeprazole and multiprobiotic "Symbiter® acidophilic" concentrated spontaneous gastric motility was the same as after omeprazole only but gastric motility stimulated by carbochol was increased. As it didn't reach the control means we proposed to use another probiotic and namely multiprobiotic "Apibact®". Multiprobiotic "Symbiter® acidophilic" concentrated and multiprobiotic "Apibact®" are unique composition of multicomponent symbiotic probiotic bacteria. One dose (10 cm^3) of multiprobiotics contains not less than 10^{12} live cells of probiotic bacteria. In contrast to multiprobiotic "Symbiter® acidophilic" concentrated multiprobiotic "Apibact®" contains 2.5% propolis extract. Propolis is a resinous substance collected by *Apis mellifera* from various tree buds which they then use to coat hive parts and to seal cracks and crevices in the hive [3]. Recently, numerous biological properties of propolis have been reported including cytotoxic, antiherpes, free radical scavenging, antimicrobial, and anti-HIV activities [1, 4-8, 13].

In connection with this the aim of the study was to investigate the spontaneous and stimulated gastric motility in the rats after 28-days of simultaneous administration of omeprazole and multiprobiotic "Apibact®".

Matherials and Methods. The study was conducted with the approval of the ethics committee of our institution. All animals were treated in a humane manner in full compliance with our instition. Thirty white non-linear rats (180-200 g) were randomly divided into 3 groups. Animals of the 1st group (control) during 28 days were injected with 0,2 ml of H_2O intraperitoneally (i.p.) and 0,5 ml of H_2O (per os). Animals of the 2nd group during 28 days were injected with inhibitor of H^+-K^+-ATPase OM (14 mg/kg, diluted in 0,2 ml of H_2O, i.p.) ("Sigma-Aldrich", USA) and 0,5 ml of H_2O (per os). The rats of the 3rd group during 28 days were injected with the same dose of OM and multiprobiotic "Apibact®" (limited company "O.D.Prolisok") in dose 0,14 ml/kg (per os).

In a day after last introduction of omeprazole, omeprazole + multiprobiotic "Apibact®" or water, an experiment was carried out to determine the spontaneous and stimulated gastric motility. Each experiment started in the morning, on an empty stomach, after a day of starvation and free access to water. Animals were anesthetized with urethane (Sigma, USA) in dose 1.1 g/kg (i.p.). Gastric motility was studied using the balloonographic method. Graphic record of investigated parameters was performed on the computer connected via pressure gauge "Rhytm" (Kiev, Ukraine) with the sensor. In a 2 hours of spontaneous motility recording the rats were i.p. injected with agonist of acetylcholine receptors carbachol in dose 10 mkg/kg.

Rat euthanasia was carried out through introduction of lethal narcosis dose.

Normal distribution of studied parameter for each sampling was checked using Shapiro-Wilks W test. Average value (M) error and standard deviation (SD) were calculated to discover significant changes of motility indices. Sampling comparison was performed using the unpaired Student's t-test. Differences among values were considered statistically significant if $p<0.05$.

Results and discussion. In the result of our investigations it was established that in the rats of control group the frequency of spontaneous contractions in the stomach was 3,5 contractions per minute (fig.1A). Long-term introduction rats omeprazole didn't influence on the frequency of spontaneous contractions in the stomach but it caused the decrease of amplitude and motility index by 77% ($p<0,01$) and **77**% ($p<0,01$) respectively (fig.1B). In this group of rats the motility activity evoked by carbachol also was dramatically suppressed. In group of rats which were injected with omeprazole and multiprobiotic "Apibact®" the frequency, amplitude and motility index of spontaneous contractions in the stomach were the same as in group of rats after long-term injection of omeprazole (fig. 1C). As to gastric motility stimulated by carbachol, multiprobiotic "Apibact®" greatly enhanced the amplitude and the motility index in the stomah compared with the group of rats treated with omeprazole alone.

We hypothesized that the elimination of dysbiosis in the colon, restoring the content of chort-chain fatty acids in it, which is one of the main reasons for the recovery motility in the colon, which in turn leads to a shortening braking cologastric reflex. Also we need to take into account the anti-inflammatory action of propolis. Propolis diminished the inflammation in the stomach that also helped to restore gastric motility.

Thus, multiprobiotic "Apibact®" can be used in patients which are long-term treated with omeprazole for prevention of gastric motility disturbances.

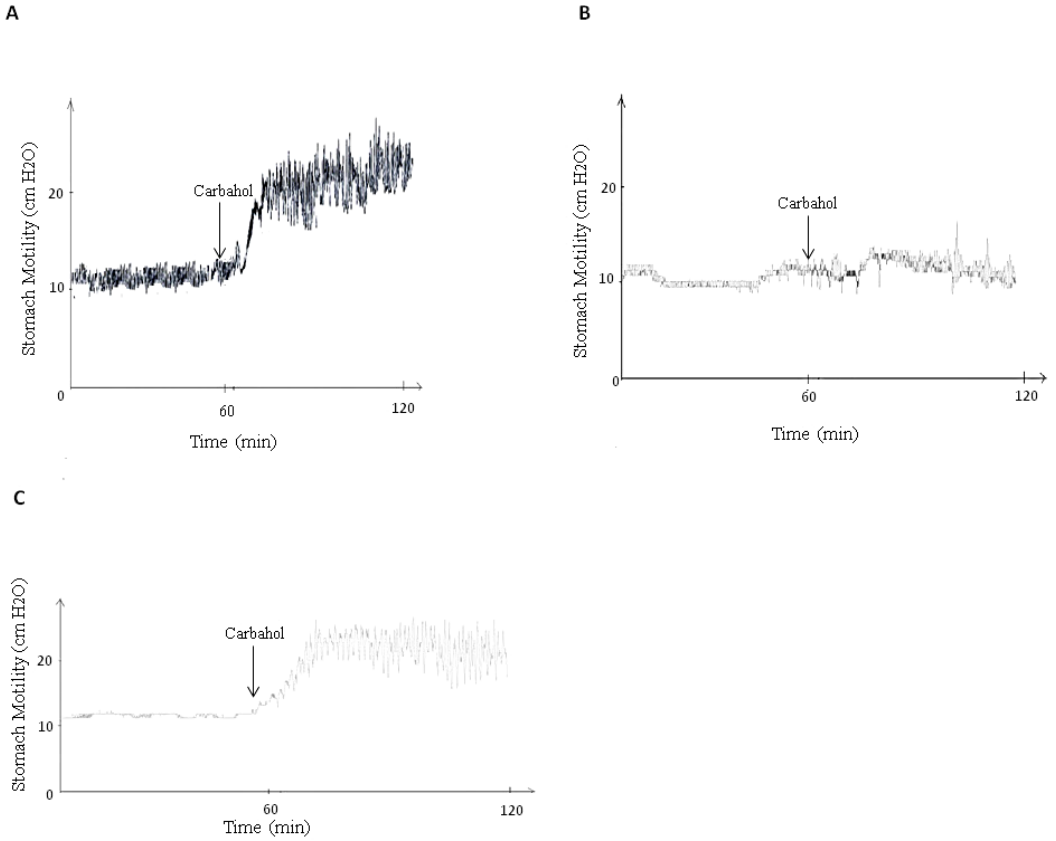

Figure 1. Representative recording showing the effects of various drugs on the spontaneous and stimulated by carbahol gastric motility in rats.
A – control group of rats, B – group of rats in a day after 28-days of omeprazole (14 mg/kg) administration,
C – group of rats in a day after 28-days of simultaneous administration of omeprazole (14 mg/kg) and multiprobiotic "Apibact®" (0,14 ml/kg)

References

1. Basnet P., Matsuno T., Neidlein R. Potent free radical scavenging activity of propol isolated from Brazilian propolis // Zeitschrift für Naturforschung C. – 1997. – Vol. 52, № 11-12. – P. 828–833.
2. Cowan A., Earnest D.L., Ligozio G., Rojavin M.A. Omeprazole-induced slowing of gastrointestinal transit in mice can be countered with tegaserod // Eur J Pharmacol. – 2005. – Vol. 517, № 1-2. – P. 127-131.
3. Greenaway W., Scaysbrook T., Whately F.R. The composition and plant origin of propolis: a report of work Oxford // Bee World. – 1990. – Vol. 71, № 3. – P. 107–118.
4. Ito J., Chang F.R., Wang H.K. et al. Anti-AIDS agents. 48. Anti-HIV activity of moronic acid derivatives and the new melliferone-related triterpenoid isolated from Brazilian propolis // Journal of Natural Products. – 2001. – Vol. 64, №10. – P. 1278–1281.
5. Kimoto T., Aga M., Hino K. et al. Apoptosis of human leukemia cells induced by Artepillin C, an active ingredient of Brazilian propolis // Anticancer Research // 2001. – Vol. 21, № 1A. – P. 221–228.
6. Kimoto T., Arai S., Kohguchi M. et al. Apoptosis and suppression of tumor growth by Artepillin C extracted from Brazilian propolis // Cancer Detection and Prevention. – 1998. – Vol. 22, № 6. – P. 506–515.
7. Matsuno T., Matsumoto Y., Saito M., Morikawa J. Isolation and characterization of cytotoxic diterpenoid isomers from propolis // Zeitschrift für Naturforschung C. – 1997. – Vol. 52, № 9-10. – P. 702–704.
8. Park Y.K., Koo M.H., Abreu J.A.S. et al. Antimicrobial activity of propolis on oral microorganisms // Current Microbiology. –1998. – Vol. 36, №1. – P. 24–28.
9. Pilipenko S.V., Shelyuk O.V., Tseysler Yu.V., Nurischenko N.Ye., Beregova T.V., Yankovskyi D.S., Dyment G.S. // Motor function of stomach in rats after long-term administration of omeprazole and its correction by multiprobiotic // Bulletin of problems of biology and medicine – 2013. – Vol. 1 (102), issue 3. – P. 162-166.
10. Sanaka M., Yamamoto T., Kuyama Y. Effects of proton pump inhibitors on gastric emptying: a systematic review // Dig Dis Sci. – 2010. – Vol. 55, № 9. – P. 2431-2440.
11. Tougas G., Earnest D.L., Chen Y. et al. Omeprazole delays gastric emptying in healthy volunteers: en effect prevented by tegaserod // Aliment Pharmacol Ther. – 2005. – Vol. 22, № 1. – P. 59-65.
12. Tsyryuk O.I., Beregova T.V. Effect of multiprobiotic "Symbiter® acidophilic" concentrated on morphofunctional changes in stomac evoked by 28-days introduction of omeprazole // Journal of Pre-Clinical and Clinical Research. – 2012. – Vol. 4, № 1. – P. 52-56.
13. Vynograd N., Vynograd I, Sosnowski Z. A comparative multi-centre study of the efficacy of propolis, acyclovir and placebo in the treatment of genital herpes (HSV) // Phytomedicine. – 2000. – Vol. 7, №1. – P. 1–6.

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МОТОРНА АКТИВНІСТЬ ШЛУНКА ПІСЛЯ ТРИВАЛОГО ВВЕДЕННЯ ОМЕПРАЗОЛУ З МУЛЬТИПРОБІОТИКОМ "АПІБАКТ"

Досліджено зміни спонтанної та стимульованої карбахоліном моторної активності шлунка у щурів після 28-денного введення блокатора протонної помпи омепразолу, а також після 28-денного одночасного введення омепразолу та мультипробіотика "Апібакт". Виявлено, що омепразол пригнічував спонтанну та стимульовану моторну активність шлунка. Мультипробіотик "Апібакт" суттєво запобігав розвитку змін в моториці шлунка, викликаних омепразолом.

Ключові слова: моторна активність шлунка, блокатори протонної помпи, Омепразол, Апібакт.

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МОТОРНАЯ АКТИВНОСТЬ ЖЕЛУДКА КРЫС ПОСЛЕ ДЛИТЕЛЬНОГО ВВЕДЕНИЯ ОМЕПРАЗОЛА С МУЛЬТИПРОБИОТИКОМ "АПИБАКТ"

Исследовано изменения спонтанной и стимулированной карбахолином моторной активности желудка у крыс после 28-ми дней введения блокатора протонной помпы омепразола, а также после 28-ми дней введения омепразола и мультипробиотика "Апибакт". Установлено, что омепразол угнетал спонтанную и стимулированную моторную активность желудка. Мультипробиотик "Апибакт" существенно предотвращал развитие изменений в моторике желудка, вызванных омепразолом.

Ключевые слова: моторная актавность желудка, блокаторы протонной помпы, Омепразол, Апибакт.

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BIOINFORMATICAL SEARCH FOR TRANSCRIPTION FACTORS BINDING SITES IN *MBL1* PROMOTER OF *RATTUS NORVEGICUS*

In the current work we have analyzed the Mbl1 (mannose-binding lectin) promoter for the presence and functional specifity of transcription factors binding sites (TFBS). We have utilized the weight-position matrices from MatrixFamily Library Version 9.0 and JASPAR CORE Vertebrata databases and two programs – MatInspector and Jaspar. Phylogenetical analysis of rat Mbl1 promoter in comparison with Mus musculus gene-ortholog and the search for functional modules of cooperating transcription factors were conducted with DiAlignTF program. Thus we defined 3 regions in Mbl1 promoter, enriched with conservative transcription factors binding sites, and 6 modules of potentially cooperating TFs.

Key words: mannose binding lectin, weight-position matrices, transcription factor binding sites.

Introduction. Mbl1 refers to the complement system, sometimes called the "complement cascade". It is a complex network of plasma (blood) proteins that cooperate to provide antimicrobial defense and maintain healthy tissue. It's part of the immune system, and *complements* the antibody- and cell-driven responses to infection by pathogens. There are three ways that the complement system becomes activated: the classic complement pathway triggered by antigen-antibody complexes; the lectin pathway triggered by molecular patterns, which are present on many pathogens or exposed neoepitopes on apoptotic, senescent and some tumor cells and/or injured/ inflamed tissues [1 – 4]. These molecular patterns contain sugars, such as D-mannose, fucose and N-acetyl-D-glucosamine; the third way is the unique alternative complement pathway that is always activated at a low level, and doesn't require a microbial surface to trigger it. All these pathways converge on convertase 3 (C3), the central molecule of complement system, that continues the cascade of proteolytic reactions with eventual opsonisation of particles, release of inflammatory peptides and enhanced engulfment by phagocytes of above-mentioned cells as well as cell debris. Whenever MBL is bound to specific molecular patterns it associates with MBL-associated serine proteases (MASPs) and via MASPs initiates complement pathway activation [5] and thrombin-like activity [6]. A number of membrane receptors for MBL, including C1q phagocytic receptor (C1qRp), calreticulin (also known as C1qR), and CR1(CD35), have been described. Interactions with these receptors may also be important in stimulating phagocytosis by MBL [7; 8].

Thereby MBL1 is in the first line of defense in preimmune host. It is produced primarily in the liver and also in kidney, lung and testis [9, 10]. It also refers to acute phase proteins (APP).

There are some evidences that the genes of complement system are regulated by transcription factors including C/EBP (CAAT – binding protein), GR (glucocorticoid receptor), ISGF (interferon – stimulated growth factor) and AP1 (the activator protein 1) [11]. However, the regulation of *Mbl1* expression still remains unknown. The *in silico* search for transcription factors binding sites in promoter of *Mbl1* has revealed the potential regulators of its transcription partly coinciding with its known functions.

Methods. The sequence of 2007 bp (from -1934 to + 73 bp), containing adjacent 5'UTR, was chosen for the search of potential transcription factor binding sites (TFBSs). Two software tools were utilized – MatInspector (http://www.genomatix.de/online_help/help_matinspector/matinspector_help.html) [12] and Jaspar (http://jaspar.gener-eg.net/) [13]. Their open-access databases contain 907 and 217 weight matrices (PWMs) of TFBSs for vertebrates respectively in Matrix Family Library Version 9.0 (August 2012) and JASPAR CORE Vertebrata. PWM represents the complete nucleotide occurrence probabilities and information content evaluation of each matrix position. It allows the quantification of the matrix similarity between the weight matrix and a potential TFBS detected in the target sequence. Similar and/or functionally related transcription factor binding sites are grouped in MatInspector into so-called matrix families (186 for Vertebrates).

The selection of TFBSs was started at recommended thresholds – 0.8/individual families and 0.8/individual matrices for MatInspector and 80 % threshold and minimal 8.000 similarity score for Jaspar. The identified individual matrix matches were sorted on the basis of their corresponding TFs association or not association with liver or hepatocytes. The phrases "transcription factor and liver", "transcription factor and hepatocytes" were used for literature mining with the help of IHOP service (http://www.ihop-net.org/UniPub/IHOP/). Hereafter the selected TFBSs were subjected to phylogenetic fingerprinting – the search of evolutionary conserved TFBSs with regard of their positions by alignment of *Rattus norvegicus* and *Mus musculus Mbl1* promoters with the help of DiAlign TF program (http://www.genomatix.de/online_help/help_dialign/dialign_TF. html). The following settings of similarity scores were used – >0.85 for core similarity and optimized threshold for the matrix similarity. The "core sequence" of a matrix is defined as the usually 4 highest conserved positions of the matrix. The optimized threshold for the matrix similarity allows a maximum of three matches in 10 000 bp of non-regulatory test sequences (1.5 million bp of coding sequences, excluding first exons and genomic repeats).

Further we have analyzed the identified matches of TFBSs for the presence of the cis-regulatory modules using the library Vertebrate_Modules Version 5.6 and the DiAlignTF option. Modules represent the association of at least two TFBSs which are shown to act synergistically or antagonistically. The visualization of results was made with Dia 0.97.2 (25/10/12) program (http://dia.en.softonic.com/).

Results and discussion. In the current work we searched for the potential transcription factors binding sites within *Mbl1* gene promoter of *Rattus norvegicus.* At the first stage of our study we obtained 494 and 270 matrix matches of TFBSs with the help of MatInspector and Jaspar programs respectively. The application of the further filter – the tissue-specificity notably the association of transcription factors with the liver and hepatocytes has cut off their number to 108 and 112 obtained with MatInspector and Jaspar correspondingly.

As transcriptional regulation is mostly evolutionary conserved we have applied phylogenetic footprinting and compared *Mbl1*promoter of *Rattus norvegicus* with its ortholog in *Mus musculus* with the help of DiAlign TF software. We

defined 3 conservative regions enriched with transcription factors (Fig. 1). The first one (-15 – 500 bp) contains binding sites of ISGF (Interferon stimulated growth factor), PEA3 (Polyomavirus enhancer activator 3 or ETS translocation variant 4), Spi-B (Spi-1/PU.1 related transcription factor), 2 TFBSs of HNF-3/Fkh Homolog 2 (FOXD3), HNF6 – Hepatocyte nuclear factor 6 (Onecut- 2), c-Maf (V-maf musculoaponeurotic fibrosarcoma oncogene homolog), C/EBP (Ccaat-enhancer-binding protein), SOX6 (sex-determining region Y (Sry) box-containing protein 6); the second region (- 730 – 955 bp) contains TFBSs of GR (Glucocorticoid receptor), 3 NFAT (Nuclear factor of activated T-cells), Hoxb1 (Homeobox protein Hox-B1), 2 C/EBP, Oct-1(Octamer-binding protein 1) and 2 SRF (Serum response factor) and the third one (- 1670 – 1900 bp) consists of 2 GR, GABP (GA binding protein transcription factor), GATA, 2 Oct-1, HNF6, AP2 (Activator protein 2), STAT3 and STAT5 (Signal transducer and transcription activator), CREB (Cyclic AMP-responsive element-binding protein) and Foxa2 (Forkhead box protein A2) (Fig 2).

For each family of transcription factors within conservative regions of Mbl1 promoter, we searched for Modules of TFs from library Vertebrate_Modules Version 5.6, using DiAlignTF option (Table 1).

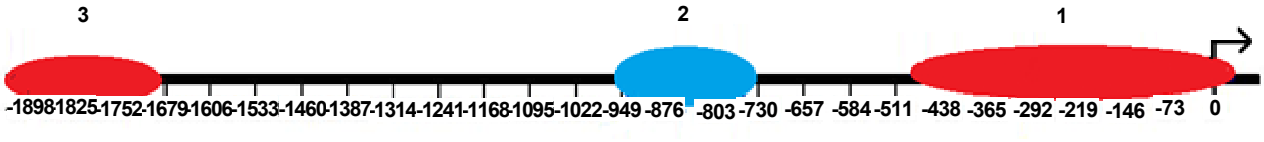

Fig. 1. **Localization of enriched regions containing evolutionary conserved transcription factor binding sites in *Mbl1* promoter of *Rattus norvegicus***

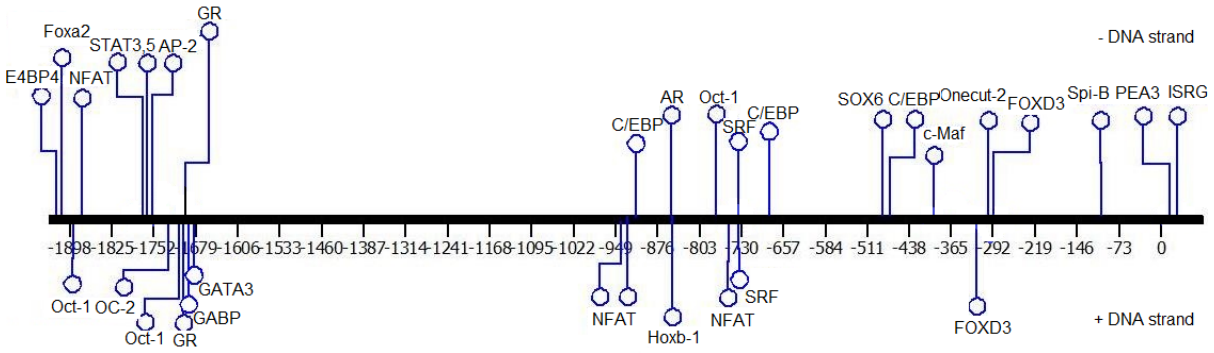

Fig. 2. **Transcription factors and their cognate binding sites within evolutionary conserved regions in *Mbl1* promoter (individual TFs are shown).**

Table 1. **Modules of associated transcription factors within conservative regions of *Mbl1* promoter**

Modules of associated TFs	DNA strand (+,-)	Distance between TFBSs (bp)	Position in promoter (anchor positions, bp)
ETS – ISGF	- , -	0	-44; -40
FKHD – FKHD	-, +	34 – 42	-290; -320
SRF – NFAT	+, +	3 – 11	-743;-753
C/EBP – NFAT	+, -	4 – 12	-901; -923
AP2- GR	-, -	47 – 55	-1686; -1747
STAT3 – STAT5	-, -	1 – 7	-1774; -1783

The transcription factor ISGF-3 that binds within the first region is an interferon-dependent transcription factor that is activated in cytoplasm in result of interferon α/β binding to its cognate receptors [14]. ISGF-3 activates the target genes and realizes antiviral, antiproliferative and immunomodulatory effects of IFNs. All ETS family members are identified through a highly conserved DNA binding domain, the ETS domain, which is a winged helox-turn-helix structure that binds to DNA sites with a central GGA(A/T) DNA sequence. As well as DNA-binding functions, evidence suggests that the ETS domain is also involved in protein-protein interactions. ETS factors act as transcriptional repressors, transcriptional activators, or both [15, 16].

The ETS transcription factors belong to the family of highly conserved sequence-specific DNA binding proteins, which in association with other transcription factors regulate a number of diverse cellular processes including proliferation and differentiation. The Fox proteins containing the Forkhead boxes are important regulators of liver metabolism, homeostasis and proliferative response [17]. The members of this family have crucial roles in various aspects of immune regulation [18]. The binding sites for HNFs (hepatocyte nuclear factors), are identified in each region within *Mbl1* promoter. These transcription factors regulate liver development, regeneration, hepatocyte proliferation and metabolism [19]. The binding sites for AP-1 are widely represented in promoters of liver-specific genes and mediate the regulatory role of AP-1 in the processes of liver development, homeostasis metabolism and proliferation [20,21]. C/EBP δ is associated with proliferation of liver cells [22] and the acute-phase response (APR) in which the liver plays a prominent role [23].

The C/EBP-δ transcription factor is known to be rarely expressed in the liver but significantly up-regulated at the early stage of the acute phase response [24]. The studies with C/EBP- knockout mice demonstrated that neonatal mice treated with purified bacterial lipopolysaccharide or recombinant IL-1 lack an acute phase response typical for wild-type

mice [25]. Sox [sex-determining region Y (Sry) box-containing] proteins are a family of transcription factors that is characterized by a homologous sequence called the HMG-box (for high mobility group). This HMG box is a DNA binding dimain. Accumulating evidence documents that Sox proteins play additional roles in adult tissue homeostasis and regeneration. Remarkably, their forced expression, in combination with other synergistic factors, reprograms differentiated cells into somatic or pluripotent stem cells [26]. However, despite their wide abundance among the tissues, their functions in liver still remain poorly investigated.

The proteins belonging to the NFAT (nuclear factor of activated T cells) family of transcription factors play a central role in inducible gene transcription during the immune response [27]. They rather frequently cooperate with AP-1 [27]. Serum response factor (SRF) is a transcription factor that binds to a CarG box motif within the serum response element (SRE) of genes that are expressed in response to mitogens. The SRF is essential for hepatocyte proliferation and survival. It controls the postnatal body growth and regulates expression of multiple genes in hepatocytes [28]. The POU-domain transcription factor Oct-1 is widely expressed in adult tissues and it modulates the activity of genes important for the cellular response to metabolic and stress signals [29, 30]. It is proposed that rather than acting as a primary trigger of gene activation or repression, Oct1 is a switchable stabilizer of repressed and inducible states [31]. Oct- 1 was shown to interact with HNF-1 in mutual regulation of liver-specific genes [32]. Glucocorticoid receptors (GR) together with C/EBP are the main activators of the synthesis of acute-phase proteins**.** The regulation of gene expression in hepatocytes by glucocorticoids is essential for normal liver homeostasis, metabolism and hepatocyte proliferation [33].

Transcription factor GATA is expressed in early fetal liver and is essential for organogenesis. It is also implicated in carcinogenesis in several endoderm-derived organs [34]. STAT-mediated intracellular signal cascade plays a critical role in antiviral defense, acute phase response, hepatic injury, repair, inflammation and transformation [35].

The search for the modules in *Mbl1* promoter has revealed six pairs of transcription factors acting in coordination and sometimes physically interacting with one another. There are two modules localized in the first region of *Mbl1* promoter. The ISGF and representatives of ETS family of TFs may interact with one another. For example, *PU.1,* a member of ETS family, interacts with ISGF/IRF in enhancers of *Igκ*, *Igλ, IL-1β 5* genes and in promoters of *CD20, gp91, Toll-like receptor 4* genes. This interaction is mediated by the proline-glutamate-serine-threonine-rich (PEST) domain of *PU.1* [36]. This interaction is significantly enhanced by phosphorylation of serine 148, a residue located within the PEST domain of *PU.1* [37].

The module between two representatives of FKHD TF family was found in -290; -328 position. Its functional role was investigated in promoter of transthyretin (TTR) gene [38]. It was revealed, that the presence of both HNF3-S binding site, located in TTR promoter (−106 to −94) and HNF3-W BS in TTR enhancer (−140 to −131) is essential for TTR transcription activation.

Next, we found SRF – NFAT module in -734; -762 position. The association of both transcription factors has been established in smooth muscle cells (SMCs) for intronic serum response factor (SRF)-binding CArG element in alpha-actin gene promoter [39].

C/EBP – NFAT module was defined in -894; -932 position. Interactions between C/EBP – NFAT was revealed in peroxisome proliferator-activated receptor – 2 gene (*Ppar2*). They form a composite enhancer complex to potentiate expression of the *Ppar2* gene [40].

AP2-GR module is located in -1686; -1747 positions. AP2 and GR binding sites are closely located in phenyl – ethanolamine N-methyltransferase (PNMT) gene promoter and cooperatively regulate its expression [41].

The majority of STAT transcription factors associate with each other to regulate gene expression. Two adjacent STAT binding sites were defined in -1774; -1783 positions. STAT3 forms heterodimer with STAT5 and both coregulate gene expression in response to Colony-stimulating factor (CSF-1) and other cytokines [42].

Thus, in the current work we predicted *in silico* the TFBSs in *Mbl1* promoter of *Rattus norvegicus*. The involvement of multiple transcription factors reveals the potential complex regulation of the processes at the first line of organism defence via *Mbl1* gene. The obtained results comprise the justiiified basis for taskoriented experimental validation.

References

1. Ogden CA, deCathelineau A, Hoffmann PR, at al. C1q and mannose binding lectin engagement of cell surface calreticulin and CD91 initiates macropinocytosis and uptake of apoptotic cells. // J Exp Med. – 2001. – Vol. 194(6). – P. 781-95.

2. Stuart LM, Takahashi K, Shi L, Savill J, Ezekowitz RA. Mannose-binding lectin-deficient mice display defective apoptotic cell clearance but no autoimmune phenotype. // J Immunol. – 2005. – Vol. 174(6). – P. 3220-6.

3. Takahashi K, Yamanaka S. Induction of pluripotent stem cells from mouse embryonic and adult fibroblast cultures by defined factors. // Cell. – 2006. – Vol. 126(4). – P. 663-76.

4. Tomaiuolo R, Ruocco A, Salapete C, at al. Activity of mannose-binding lectin in centenarians. // Aging Cell. – 2012. – Vol. 11(3). – P. 394-400.

5. Fujita T, Matsushita M, Endo Y. The lectin-complement pathway-its role in innate immunity and evolution. // Immunol Rev. – 2004. – Vol. 198. – P. 185-202.

6. Takahashi K, Saha D, Shattino I, at al. Complement 3 is involved with ventilator-induced lung injury. // Int Immunopharmacol. – 2011. – Vol. 11(12). – P. 2138-43.

7. Holmskov U, Thiel S, Jensenius JC. Collections and ficolins: humoral lectins of the innate immune defense. // Annu Rev Immunol. – 2003. – Vol. 21. – P. 547-78.

8. Fujita T, Endo Y, Nonaka M. Primitive complement system--recognition and activation. // Mol Immunol. – 2004. – Vol. 41(2-3). – P. 103-11.

9. Uemura K, Saka M, Nakagawa T, at al. L-MBP is expressed in epithelial cells of mouse small intestine. // J Immunol. – 2002. – Vol. 169(12). – P. 6945-50.

10. G. P. Wagner, C. Amemiya, F. Ruddle. Hox cluster duplications and the opportunity for evolutionary novelties // Proc Natl Acad Sci U S A. – 2003. – Vol. 100(25). – P. 14603–14606.

11. J. Volanakis. TRANSCRIPTIONAL REGULATION OF COMPLEMENT GENES // Annual Review of Immunology. – 1995. – Vol. 13. – P. 277-305.

12. Cartharius K, Frech K, Grote K, at al. MatInspector and beyond: promoter analysis based on transcription factor binding sites // Bioinformatics. – 2005. – Vol. 21(13). – P. 2933-42.

13. Sandelin A, Alkema W, Engström P, at al. JASPAR: an open-access database for eukaryotic transcription factor binding profiles // Nucleic Acids Res. – 2004. – Vol. 32(Database issue). – P. 91-94.

14. Schindler C, Fu XY, Improta T, at al. Proteins of transcription factor ISGF-3: one gene encodes the 91-and 84-kDa ISGF-3 proteins that are activated by interferon alpha. // Proc Natl Acad Sci U S A. – 1992. – Vol. 89(16). – P. 7836-9.

15. Sharrocks AD. The ETS-domain transcription factor family. // Nat Rev Mol Cell Biol. – 2001. – Vol. 2(11). – P. 827–37.

16. Hollenhorst Peter C.,1 Lawrence P. McIntosh,2 and Barbara J. Graves 3,4 Genomic and Biochemical Insights into the Specificity of ETS Transcription Factors. // Annual Review of Biochemistry. – 2001. – Vol. 80. – P. 437-471.

17. Le Lay J, Kaestner KH. The Fox genes in the liver: from organogenesis to functional integration. // Physiol Rev. – 2010. – Vol. 90(1). – P. 1-22.

18. Coffer PJ, Burgering BM.Forkhead-box transcription factors and their role in the immune system.// Nat Rev Immunol. – 2004. – Vol 4(11). – P. 889-99.

19. Schrem H, Klempnauer J, Borlak J. Liver-enriched transcription factors in liver function and development. Part I: the hepatocyte nuclear factor network and liver-specific gene expression. // Pharmacol Rev. – 2002. – Vol. 54(1). – P. 129-58.

20. Hasenfuss SC, Bakiri L, Thomsen MK, at al. The AP-1 transcription factor Fra-1 is dispensable for murine liver fibrosis, but modulates xenobiotic metabolism. // Hepatology. – 2013 (in press).

21. Stepniak E, Ricci R, Eferl R, at al. c-Jun/AP-1 controls liver regeneration by repressing p53/p21 and p38 MAPK activity. // Genes Dev. – 2006. – Vol. 20(16). – P. 2306-14.

22. Dinic S., Ivanovic-Matic S., Mihailovic M., Bogojevic D. and Poznanovi G. Expression of C/EBP δ in Rat Liver During Development and the Acute-Phase Response. // Gen. Physiol. Biophys. – 2004. – Vol. 23. – P. 499–504.

23. Yamada T, Tobita K, Osada S at al. CCAAT/enhancer-binding protein delta gene expression is mediated by APRF/STAT3. // J Biochem. – 1997. – Vol. 121(4). – P. 731-8.

24. Burgess-Beusse BL, Darlington GJ. C/EBPalpha is critical for the neonatal acute-phase response to inflammation. // Mol Cell Biol. – 1998. – Vol. 18(12). – P. 7269-77.

25. Gracz AD, Magness ST. Sry-box (Sox) transcription factors in gastrointestinal physiology and disease. // Am J Physiol Gastrointest Liver Physiol. – 2011. – Vol. 300(4). – P. 503-15.

26. Sarkar A, Hochedlinger K. The sox family of transcription factors: versatile regulators of stem and progenitor cell fate. // Cell Stem Cell. – 2013. – Vol. 3;12(1). – P. 15-30.

27. Rao A, Luo C, Hogan PG. Transcription factors of the NFAT family: regulation and function. // Annu Rev Immunol. – 1997. – Vol. 15. – P. 707-47.

28. Sun K, Battle MA, Misra RP, Duncan SA. Hepatocyte expression of serum response factor is essential for liver function, hepatocyte proliferation and survival, and postnatal body growth in mice. // Hepatology. – 2009. – Vol. 49(5). – P. 1645-54.

29. Tantin D., Schild-Poulter C., Wang V. et al. The octamer binding transcription factor oct-1 is a stress sensor. // Cancer Res. – 2005. – Vol. 65. – P. 10750-10758.

30. Wang P, Jin T Oct-1 functions as a sensor for metabolic and stress signals.// Islets. – 2010. – Vol. 2(1). – P. 46-8.

31. Shakya A, Kang J, Chumley J at al. Oct1 is a switchable, bipotential stabilizer of repressed and inducible transcriptional states.// J Biol Chem. – 2011. – Vol. 286(1). – P. 450-9.

32. D X Zhou, T S Yen. The ubiquitous transcription factor Oct-1 and the liver-specific factor HNF-1 are both required to activate transcription of a hepatitis B virus promoter. // Mol Cell Biol. – 1991. – Vol. 11(3). – P. 1353–1359.

33. C-J Chang, Y-L Chen, S-C Lee. Coactivator TIF1β Interacts with Transcription Factor C/EBPβ and Glucocorticoid Receptor To Induce α1-Acid Glycoprotein Gene Expression. // Mol Cell Biol. – 1998. – Vol. 18(10). – P. 5880–5887.

34. Soini T, Haveri H, Elo JM, at al. Transcription factor GATA-4 is abundantly expressed in childhood but not in adult liver tumors. // J Pediatr Gastroenterol Nutr. – 2012. – Vol. 54(1). – P. 101-8.

35. Gao B. Cytokines, STATs and liver disease. // Cell Mol Immunol. – 2005. – Vol. 2(2). – P. 92-100.36. Perkel, J. M., and M. L. Atchison. A two-step mechanism for recruitment of Pip by PU.1.J. Immunol. – 1998. – Vol. 160 (1). – P. 241-52.

37. Pongubala, J. M., S. Nagulapalli, M. J. Klemsz at al. PU.1 recruits a second nuclear factor to a site important for immunoglobulin k39enhancer activity. // Mol. Cell. Biol. – 1992. – Vol. 12(1). – P. 368-78.

38. Robert H. Costa and Dennis R. Grayson1. Site-directed mutagenesis of hepatocyte nuclear factor (HNF) binding sites in the mouse transthyretin (TTR) promoter reveal synergistic interactions with its enhancer region // Nucleic Acids Res. – 1991. – Vol. 19(15). – P. 4139-45.

39. Gonzalez Bosc LV, Layne JJ, Nelson MT, Hill-Eubanks DC. Nuclear factor of activated T cells and serum response factor cooperatively regulate the activity of an alpha-actin intronic enhancer. // J BiolChem. – 2005. – Vol. 280(28). – P. 26113-20.

40. Teddy T. C. Yang, Chi-Wing Chow. Transcription Cooperation by NFATC/EBP Composite Enhancer Complex // THE JOURNAL OF BIOLOGICAL CHEMISTRY. – 2003. – Vol. 278(18). – P. 15874 –15885.

41. Steven N. Ebert, Mary Beth Ficklin. Glucocorticoid-Dependent Action of Neural Crest Factor AP-2:Stimulation of Phenylethanolamine N-Methyl transferase Gene Expression // J Neurochem. – 1998. – Vol. 70(6). – P. 2286-95.

42. Rosenthal LA, Winestock KD, Finbloom DS. IL-2 and IL-7 induce heterodimerization of STAT5 isoforms in human peripheral blood T lymphoblasts. // Cell Immunol. – 1997. – Vol. 181(2). – P. 172-81.

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БІОІНФОРМАЦІЙНИЙ ПОШУК ДЛЯ САЙТІВ З'ЄДНАННЯ ТРАНСКРИПЦІЙНИХ ФАКТОРІВ В *Mbl1 ПРОМОТОРУ RATTUS NORVEGICUS*

Проведено біоінформатичний аналіз промотору щурячого гена Mbl1, який кодує манозозв'зуючий лектин, на наявність та функціональну специфічність сайтів зв'язування транскрипційних факторів. В роботі було використано позиційно-вагові матриці з баз данних Matrix Family Library Version 9.0 та JASPAR CORE Vertebrata, а також дві програми пошуку сайтів зв'язування – MatInspector та Jaspar. Було проведено філогенетичний аналіз з промотором гена-ортолога Mus musculus та здійснено пошук функціональних модулів знайдених транскрипційних факторів в програмі DiAlign TF. В результаті в промоторі гена Mbl1 щура нами було визначено 3 ділянки, збагачені консервативними сайтами зв'язування транскрипційних факторів, та знайдено 6 модулів потенційної кооперативної взаємодії між транскрипційними факторами.

Ключові слова: манозо-зв'язуючий лектин, позиційно-вагові матриці, сайти зв'язування транскрипційних факторів.

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БИОИНФОРМАЦИОННЫЙ ПОИСК ДЛЯ САЙТОВ СОЕДИНЕНИЯ ТРАНСКРИПЦИОННЫХ ФАКТОРОВ В *Mbl1* ПРОМОТОРА *RATTUS NORVEGICUS*

Проведен биоинформатический анализ промотора крысиного гена Mbl1, который кодирует маннозосвязывающий лектин, на наличие и функциональную специфичность сайтов связывания транскрипционных факторов. В работе были использованы позиционно-весовые матрицы из баз данных Matrix Family Library Version 9.0 и JASPAR CORE Vertebrata, а также две программы поиска сайтов связывания – MatInspector и Jaspar. Филогенетический анализ с промотром гена-ортолога Mus musculus и поиск функциональных модулей найденных транскрипционных факторов осуществлены в программе DiAlign TF. В результате в промоторе гена Mbl1 крысы нами было определено 3 участка, обогащенных консервативными сайтами связывания транскрипционных факторов, и найдено 6 модулей потенциального кооперативного взаимодействия между траснкрипционными факторами.

Ключевые слова: маннозо-связывающий лектин, позиционно-весовые матрицы, сайты связывания транскрипционных факторов.

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