

## ABSTRACT&amp;REFERENCES

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EXTRACTS OF POMEGRANATE, PERSIMMON, NETTLE, DILL, KALE AND *SIDERITIS* SPECIFICALLY MODULATE GUT MICROBIOTA AND LOCAL CYTOKINES PRODUCTION: *IN VIVO* STUDY

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**Tamara Meleshko**, Senior Lecturer, Junior Researcher, Department of Clinical and Laboratory Diagnostics and Pharmacology, RDE Centre of Molecular Microbiology and Mucosal Immunology, State Higher Educational Institution «Uzhhorod National University», Narodna sq., 3, Uzhhorod, Ukraine, 88000

E-mail: meleshkotv@ukr.net

ORCID: <http://orcid.org/0000-0003-4046-1509>

**Oleksandra Pallah**, Assistant, Junior Researcher, Department of Clinical and Laboratory Diagnostics and Pharmacology, RDE Centre of Molecular Microbiology and Mucosal Immunology, State Higher Educational Institution «Uzhhorod National University», Narodna sq., 3, Uzhhorod, Ukraine, 88000

E-mail: ssarvash@gmail.com

ORCID: <http://orcid.org/0000-0003-3636-6621>

**Viktor Petrov**, PhD, Assistant, Department of Clinical Disciplines, State Higher Educational Institution «Uzhhorod National University», Narodna sq., 3, Uzhhorod, Ukraine, 88000

E-mail: petrovviktor.uzh@gmail.com

ORCID: <http://orcid.org/0000-0001-9450-9884>

**Nadiya Boyko**, Doctor of Biological Sciences, Professor, Head of Department, Director of Center, Department of Clinical and Laboratory Diagnostics and Pharmacology, RDE Centre of Molecular Microbiology and Mucosal Immunology, State Higher Educational Institution «Uzhhorod National University», Narodna sq., 3, Uzhhorod, Ukraine, 88000

E-mail: nadiya.boyko@gmail.com

ORCID: <http://orcid.org/0000-0002-2467-7513>

The **aim** of the work was to use the *in vivo* model to reveal main changes in gut microbiota of immune competent mice in dynamic and find out the specificity of immunomodulation action (activity) of traditional foods ingredients - edible plants extracts on local mucosal cytokines to its oral administration.

**Materials and methods.** In this study, seven groups of immunocompetent BALB/c mice were formed. All experimental mice had been fed orally by plants' extracts (15mg/200 µl/mouse) for the 14th days. The extracts of edible plants – ingredients of traditional food such as kale leaves, persimmon, pomegranate, dill, *Sideritis scardica*, and nettle were obtained as

described and orally administrated to experimental animals. For microbiological analysis of gut microbiota changes, the colon content has been investigated, the key microbial representatives were isolated by plating of its serial dilution on selected chromogenic medium, identified serologically and biochemically. The production of cytokines in different gut compartments and gut associated lymphoid tissues (GALT) were detected by Enzyme-linked immunosorbent assay (ELISA).

**Results.** In experiments in mice, the ability of Kale, Dill and *Sideritis* extracts, when administered orally, selectively inhibit the content of *E. coli*, *K. pneumoniae*, *E. faecalis*, *L. acidophilus* in the colon of mice had been demonstrated, and increasing of *B. bifidum* had been observed. Nettle extract leads to an increase in *E. coli*, and persimmon extract – to an increase in levels of *E. faecalis*, *Bifidobacterium* and a decrease in the content of *Candida* spp. Pomegranate extract specifically stimulates the growth of *Bifidobacterium*. There are sufficient differences in produced cytokines in fragment culture and serum of mice fed with different plants extracts. TNF- $\alpha$ , and IL-2 increased both systemically and locally in the different gut compartments by Dill extract, Nettle and *Sideritis* extracts only at mucosal sites. IL-2, but also IL-10 and IL-12, IFN- $\gamma$ , and IL-17 but not TNF- $\alpha$  were stimulated in different levels by Pomegranate, Persimmon and Kale extracts: both systemically and locally.

**Conclusions.** No harmful influence of tested plants had been observed. The most beneficial properties are inherent to Persimmon extract and slightly less detected in Pomegranate and Kale extracts. *Sideritis* extract rather show no significant influence on all the studied indices while the Nettle and Dill extracts are acting pro-inflammatory

**Keywords:** edible plants extracts, immunomodulation action, gut microbiota

## References

1. Woodcock, M. E., Hollands, W. J., Konic-Ristic, A., Glibetic, M., Boyko, N., Koçaoğlu, B., Kroon, P. A. (2013). Bioactive-rich extracts of persimmon, but not nettle, *Sideritis*, dill or kale, increase eNOS activation and NO bioavailability and decrease endothelin-1 secretion by human vascular endothelial cells. *Journal of the Science of Food and Agriculture*, 93 (14), 3574–3580. doi: <http://doi.org/10.1002/jsfa.6251>
2. Konić-Ristić, A., Srdić-Rajić, T., Kardum, N., Aleksić-Veličković, V., Kroon, P. A., Hollands, W. J. et al. (2013). Effects of bioactive-rich extracts of pomegranate, persimmon, nettle, dill, kale and *Sideritis* and isolated bioactives on arachidonic acid induced markers of platelet activation and aggregation. *Journal of the Science of Food and Agriculture*, 93 (14), 3581–3587. doi: <http://doi.org/10.1002/jsfa.6328>
3. Pallah, O. V., Meleshko, T. V., Bati, V. V., Boyko, N. V. (2019). Extracts of edible plants as beneficial microorganisms growth stimulators. *Biotechnologia Acta*, 12 (3), 67–74. doi: <http://doi.org/10.15407/biotech12.03.067>

4. Carbonell-Capella, J. M., Barba, F. J., Esteve, M. J., Frígola, A. (2013). Quality parameters, bioactive compounds and their correlation with antioxidant capacity of commercial fruit-based baby foods. *Food Science and Technology International*, 20 (7), 479–487. doi: <http://doi.org/10.1177/1082013213492523>

5. Moyer, R., Hummer, K., Wrolstad, R. E., Finn, C. (2002). Antioxidant compounds in diverse ribes and rubus germplasm. *Acta Horticulturae*, 585, 501–505. doi: <http://doi.org/10.17660/actahortic.2002.585.80>

6. Correia, R. T., Borges, K. C., Medeiros, M. F., Genovese, M. I. (2012). Bioactive compounds and phenolic-linked functionality of powdered tropical fruit residues. *Food Science and Technology International*, 18 (6), 539–547. doi: <http://doi.org/10.1177/1082013211433077>

7. Perez-Gregorio, R., Simal-Gandara, J. (2017). A Critical Review of Bioactive Food Components, and of their Functional Mechanisms, Biological Effects and Health Outcomes. *Current Pharmaceutical Design*, 23 (19), 2731–2741. doi: <http://doi.org/10.2174/1381612823666170317122913>

8. Lankelma, J. M., Nieuwdorp, M., de Vos, W. M., Wiersinga, W. J. (2015). The gut microbiota in internal medicine: implications for health and disease. *The Netherlands journal of medicine*, 73 (2), 61–68.

9. Oriach, C. S., Robertson, R. C., Stanton, C., Cryan, J. F., Dinan, T. G. (2016). Food for thought: The role of nutrition in the microbiota-gut-brain axis. *Clinical Nutrition Experimental*, 6, 25–38. doi: <http://doi.org/10.1016/j.yclnex.2016.01.003>

10. Chung, H., Pamp, S. J., Hill, J. A., Surana, N. K., Edelman, S. M., Troy, E. B. et. al. (2012). Gut Immune Maturation Depends on Colonization with a Host-Specific Microbiota. *Cell*, 149 (7), 1578–1593. doi: <http://doi.org/10.1016/j.cell.2012.04.037>

11. Duan, J., Chung, H., Troy, E., Kasper, D. L. (2010). Microbial Colonization Drives Expansion of IL-1 Receptor 1-Expressing and IL-17-Producing  $\gamma\delta$  T Cells. *Cell Host & Microbe*, 7 (2), 140–150. doi: <http://doi.org/10.1016/j.chom.2010.01.005>

12. Atarashi, K., Tanoue, T., Shima, T., Imaoka, A., Kawanishi, T., Momose, Y. et. al. (2010). Induction of Colonic Regulatory T Cells by Indigenous Clostridium Species. *Science*, 331 (6015), 337–341. doi: <http://doi.org/10.1126/science.1198469>

13. Manohar, M., Baumann, D. O., Bos, N. A., Cebra, J. J. (2001). Gut Colonization of Mice with ActA-Negative Mutant of *Listeria monocytogenes* Can Stimulate a Humoral Mucosal Immune Response. *Infection and Immunity*, 69 (6), 3542–3549. doi: <http://doi.org/10.1128/iai.69.6.3542-3549.2001>

14. Zhu, W., Lin, K., Li, K., Deng, X., Li, C. (2018). Reshaped fecal gut microbiota composition by the intake of high molecular weight persimmon tannin in normal and high-cholesterol diet-fed rats. *Food & Function*, 9 (1), 541–551. doi: <http://doi.org/10.1039/c7fo00995j>

15. George, N. S., Cheung, L., Luthria, D. L., Santin, M., Dawson, H. D., Bhagwat, A. A., Smith, A. D. (2019). Pomegranate peel extract alters the microbiome in mice and dysbiosis caused by *Citrobacter rodentium* infection. *Food science & nutrition*, 7 (8), 2565–2576. doi: <http://doi.org/10.1002/fsn3.1106>

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## COMPARATIVE ANALYSIS OF THE COMPOSITION OF INTESTINAL MICROBIOME IN PATIENTS WITH LIVER DISEASES

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**Vladyslav Martynov**, Department of Microbiology, Virology and Biotechnology, Oles Honchar Dnipro National University, Gagarina ave., 72, Dnipro, Ukraine, 49010

E-mail: [v.martynov1844@gmail.com](mailto:v.martynov1844@gmail.com)

ORCID: <http://orcid.org/0000-0002-8602-6679>

**Viktoriia Havryliuk**, PhD, Associate Professor, Department of Microbiology, Virology and Biotechnology, Oles Honchar Dnipro National University, Gagarina ave., 72, Dnipro, Ukraine, 49010

E-mail: [microviro@ukr.net](mailto:microviro@ukr.net)

ORCID: <http://orcid.org/0000-0001-5982-2808>

**Tetiana Skliar**, PhD, Associate Professor, Department of Microbiology, Virology and Biotechnology, Oles Honchar Dnipro National University, Gagarina ave., 72, Dnipro, Ukraine, 49010

E-mail: [microviro@ukr.net](mailto:microviro@ukr.net)

ORCID: <http://orcid.org/0000-0003-2707-5240>

**Iryna Sokolova**, PhD, Associate Professor, Department of Microbiology, Virology and Biotechnology, Oles Honchar Dnipro National University, Gagarina ave., 72, Dnipro, Ukraine, 49010

E-mail: [microviro@ukr.net](mailto:microviro@ukr.net)

*Aim:* to analyze the composition of the intestinal microbiome in patients with liver diseases of various etiologies.

*Materials and methods:* 128 patients of different sexes with pathological liver lesions were examined. The diagnosis of diseases was carried out using laboratory and instrumental methods of research. The study of the composition of microbiota in qualitative and quantitative indicators was carried out using standard bacteriological methods. The statistical analysis was performed using Student's t-test. Differences in indicators were considered significant at  $P < 0.05$ .

*Results:* the comparative analysis of the composition of the intestinal microbiocenosis of patients with various liver diseases showed significant drops in the titers of symbiotic bacteria: lactobacilli to  $10^0$ – $10^4$  CFU/ml, bifidobacteria to  $10^5$ – $10^8$  CFU/ml, enterococci to  $10^5$ – $10^6$  CFU/ml and typical *E. coli* to  $10^3$ – $10^6$  CFU/ml, as well as an increase in the number of opportunistic microorganisms: *Candida* to  $10^4$ – $10^9$  CFU/ml, hemolytic *E. coli* to  $10^7$ – $10^9$  CFU/ml, *Staphylococcus spp.* to  $10^4$ – $10^6$  CFU/ml, *Klebsiella spp.* and *Enterobacter spp.* to  $10^6$ – $10^8$  CFU/ml. According to the degree of colonization of associative and conditionally pathogenic microbiota of the intestinal tract, significant fluctuations in the deviations of indicators were recorded in women and men with non-alcoholic steatohepatitis, alcoholic hepatitis and hepatitis C.

**Conclusions:** *the study revealed the significant imbalance of microbiome of the gastrointestinal tract: there was a tendency to increase the quantitative and qualitative indicators of the content of representatives of the opportunistic microbiota against the background of decreasing titers of symbiotic microorganisms. The most significant deviations in the composition of the intestinal microbiome were observed in patients with hepatitis C. The differences in the microbial landscape of the intestine of patients of different sex with liver diseases are shown, which was mentioned in the changing relationship of individual members of the intestinal microbiota*

**Keywords:** *intestinal dysbiosis, nonalcoholic fatty liver disease, alcoholic liver disease, hepatitis C*

## References

1. Compare, D., Coccoli, P., Rocco, A., Nardone, O. M., De Maria, S., Carteni, M., Nardone, G. (2012). Gut–liver axis: The impact of gut microbiota on non alcoholic fatty liver disease. *Nutrition, Metabolism and Cardiovascular Diseases*, 22 (6), 471–476. doi: <http://doi.org/10.1016/j.numecd.2012.02.007>
2. Trukhan, D. I., Viktorova, I. A., Safonov, A. D. (2019). *Bolezni pecheni*. Saint-Peterburg, 239.
3. Loomba, R., Sanyal, A. J. (2013). The global NAFLD epidemic. *Nature Reviews Gastroenterology & Hepatology*, 10 (11), 686–690. doi: <http://doi.org/10.1038/nrgastro.2013.171>
4. Aron-Wisnewsky, J., Gaborit, B., Dutoir, A., Clement, K. (2013). Gut microbiota and non-alcoholic fatty liver disease: new insights. *Clinical Microbiology and Infection*, 19 (4), 338–348. doi: <http://doi.org/10.1111/1469-0691.12140>
5. Gangarapu, V., Yildiz, K., Tuzun Ince, A., Baysal, B. (2014). Role of gut microbiota: Obesity and NAFLD. *The Turkish Journal of Gastroenterology*, 25 (2), 133–140. doi: <http://doi.org/10.5152/tjg.2014.7886>
6. Miele, L., Valenza, V., La Torre, G., Montalto, M., Cammarota, G., Ricci, R. et. al. (2009). Increased intestinal permeability and tight junction alterations in nonalcoholic fatty liver disease. *Hepatology*, 49 (6), 1877–1887. doi: <http://doi.org/10.1002/hep.22848>
7. Zhu, L., Baker, S. S., Gill, C., Liu, W., Alkhoury, R., Baker, R. D., Gill, S. R. (2013). Characterization of gut microbiomes in nonalcoholic steatohepatitis (NASH) patients: A connection between endogenous alcohol and NASH. *Hepatology*, 57 (2), 601–609. doi: <http://doi.org/10.1002/hep.26093>
8. Lieber, C. S. (1993). Aetiology and pathogenesis of alcoholic liver disease. *Baillière's Clinical Gastroenterology*, 7 (3), 581–608. doi: [http://doi.org/10.1016/0950-3528\(93\)90003-b](http://doi.org/10.1016/0950-3528(93)90003-b)
9. Hartmann, P., Seebauer, C. T., Schnabl, B. (2015). Alcoholic Liver Disease: The Gut Microbiome and Liver Cross Talk. *Alcoholism: Clinical and Experimental Research*, 39 (5), 763–775. doi: <http://doi.org/10.1111/acer.12704>
10. Aly, A. M., Adel, A., El-Gendy, A. O., Essam, T. M., & Aziz, R. K. (2016). Gut microbiome alterations in patients with stage 4 hepatitis C. *Gut Pathogens*, 8 (1). doi: <http://doi.org/10.1186/s13099-016-0124-2>
11. EASL–EASD–EASO Clinical Practice Guidelines for the management of non-alcoholic fatty liver disease. (2016). *Journal of Hepatology*, 64 (6), 1388–1402. doi: <http://doi.org/10.1016/j.jhep.2015.11.004>
12. EASL Clinical Practical Guidelines: Management of Alcoholic Liver Disease (2012). *Journal of Hepatology*, 57 (2), 399–420. doi: <http://doi.org/10.1016/j.jhep.2012.04.004>
13. Brenner, D. J., Krieg, N. R., Staley, J. T. (Eds.). (2005). *Bergey's Manual of Systematic Bacteriology*. Boston: Springer. doi: <http://doi.org/10.1007/0-387-29298-5>
14. Backhed, F., Ding, H., Wang, T., Hooper, L. V., Koh, G. Y., Nagy, A. et. al. (2004). The gut microbiota as an environmental factor that regulates fat storage. *Proceedings of the National Academy of Sciences*, 101 (44), 15718–15723. doi: <http://doi.org/10.1073/pnas.0407076101>
15. Drenick, E. J., Fisler, J., Johnson, D. (1982). Hepatic Steatosis After Intestinal Bypass—Prevention and Reversal by Metronidazole, Irrespective of Protein-Calorie Malnutrition. *Gastroenterology*, 82 (3), 535–548. doi: [http://doi.org/10.1016/s0016-5085\(82\)80403-4](http://doi.org/10.1016/s0016-5085(82)80403-4)
16. Cope, K., Risby, T., Diehl, A. M. (2000). Increased gastrointestinal ethanol production in obese mice: Implications for fatty liver disease pathogenesis. *Gastroenterology*, 119 (5), 1340–1347. doi: <http://doi.org/10.1053/gast.2000.19267>
17. Baker, S. S., Baker, R. D., Liu, W., Nowak, N. J., Zhu, L. (2010). Role of Alcohol Metabolism in Non-Alcoholic Steatohepatitis. *PLoS ONE*, 5 (3), e9570. doi: <http://doi.org/10.1371/journal.pone.0009570>
18. Lichtman, S. N., Keku, J., Schwab, J. H., Sartor, R. B. (1991). Hepatic injury associated with small bowel bacterial overgrowth in rats is prevented by metronidazole and tetracycline. *Gastroenterology*, 100 (2), 513–519. doi: [http://doi.org/10.1016/0016-5085\(91\)90224-9](http://doi.org/10.1016/0016-5085(91)90224-9)
19. Yokota, A., Fukiya, S., Islam, K. B. M. S., Ooka, T., Ogura, Y., Hayashi, T. et. al. (2012). Is bile acid a determinant of the gut microbiota on a high-fat diet? *Gut Microbes*, 3 (5), 455–459. doi: <http://doi.org/10.4161/gmic.21216>
20. Devkota, S., Wang, Y., Musch, M. W., Leone, V., Fehlner-Peach, H., Nadimpalli, A. et. al. (2012). Dietary-fat-induced taurocholic acid promotes pathobiont expansion and colitis in Il10<sup>-/-</sup> mice. *Nature*, 487 (7405), 104–108. doi: <http://doi.org/10.1038/nature11225>
21. Turnbaugh, P. J. (2012). Fat, bile and gut microbes. *Nature*, 487 (7405), 47–48. doi: <http://doi.org/10.1038/487047a>
22. Machado, M. V., Cortez-Pinto, H. (2012). Gut microbiota and nonalcoholic fatty liver disease. *Annals of Hepatology*, 11 (4), 440–449. doi: [http://doi.org/10.1016/s1665-2681\(19\)31457-7](http://doi.org/10.1016/s1665-2681(19)31457-7)
23. Tremaroli, V., Bäckhed, F. (2012). Functional interactions between the gut microbiota and host metabolism. *Nature*, 489 (7415), 242–249. doi: <http://doi.org/10.1038/nature11552>
24. Abu-Shanab, A., Quigley, E. M. M. (2010). The role of the gut microbiota in nonalcoholic fatty liver disease. *Nature Reviews Gastroenterology & Hepatology*, 7 (12), 691–701. doi: <http://doi.org/10.1038/nrgastro.2010.172>
25. Casafont Morencos, F., de Las Heras Castaño, G., Martín Ramos, L., López Arias, M. J., Ledesma, F., Pons Romero, F. (1996). Small bowel bacterial overgrowth in patients with alcoholic cirrhosis. *Digestive Diseases and Sciences*, 41 (3), 552–556. doi: <http://doi.org/10.1007/bf02282340>
26. Mutlu, E., Keshavarzian, A., Engen, P., Forsyth, C. B., Sikaroodi, M., Gillevet, P. (2009). Intestinal Dysbiosis: A Possi-



ble Mechanism of Alcohol-Induced Endotoxemia and Alcoholic Steatohepatitis in Rats. *Alcoholism: Clinical and Experimental Research*, 33(10), 1836–1846. doi: <http://doi.org/10.1111/j.1530-0277.2009.01022.x>

27. Canesso, M. C. C., Lacerda, N. L., Ferreira, C. M., Gonçalves, J. L., Almeida, D., Gamba, C. et al. (2014). Comparing the effects of acute alcohol consumption in germ-free and conventional mice: the role of the gut microbiota. *BMC Microbiology*, 14 (1). doi: <http://doi.org/10.1186/s12866-014-0240-4>

28. Bajaj, J. S., Heuman, D. M., Hylemon, P. B., Sanyal, A. J., White, M. B., Monteith, P. et al. (2014). Altered profile of human gut microbiome is associated with cirrhosis and its complications. *Journal of Hepatology*, 60 (5), 940–947. doi: <http://doi.org/10.1016/j.jhep.2013.12.019>

29. Leclercq, S., Matamoros, S., Cani, P. D., Neyrinck, A. M., Jamar, F., Stärkel, P. et al. (2014). Intestinal permeability, gut-bacterial dysbiosis, and behavioral markers of alcohol-dependence severity. *Proceedings of the National Academy of Sciences*, 111 (42), E4485–E4493. doi: <http://doi.org/10.1073/pnas.1415174111>

30. Bajaj, J. S., Ridlon, J. M., Hylemon, P. B., Thacker, L. R., Heuman, D. M., Smith, S. et al. (2012). Linkage of gut microbiome with cognition in hepatic encephalopathy. *American Journal of Physiology-Gastrointestinal and Liver Physiology*, 302 (1), G168–G175. doi: <http://doi.org/10.1152/ajpgi.00190.2011>

31. Liu, Q., Duan, Z. P., Ha, D. K., Bengmark, S., Kurtovic, J., Riordan, S. M. (2004). Synbiotic modulation of gut flora: Effect on minimal hepatic encephalopathy in patients with cirrhosis. *Hepatology*, 39 (5), 1441–1449. doi: <http://doi.org/10.1002/hep.20194>

32. Gupta, A., Dhiman, R. K., Kumari, S., Rana, S., Agarwal, R., Duseja, A., Chawla, Y. (2010). Role of small intestinal bacterial overgrowth and delayed gastrointestinal transit time in cirrhotic patients with minimal hepatic encephalopathy. *Journal of Hepatology*, 53 (5), 849–855. doi: <http://doi.org/10.1016/j.jhep.2010.05.017>

33. Chari, S., Teysse, S., Singer, M. V. (1993). Alcohol and gastric acid secretion in humans. *Gut*, 34 (6), 843–847. doi: <http://doi.org/10.1136/gut.34.6.843>

34. Dinoso, V. P. (1972). Gastric secretion and gastric mucosal morphology in chronic alcoholics. *Archives of Internal Medicine*, 130 (5), 715–719. doi: <http://doi.org/10.1001/archinte.130.5.715>

35. Shindo, K., Machida, M., Miyakawa, K., Fukumura, M. (1993) A syndrome of cirrhosis, achlorhydria, small intestinal bacterial overgrowth, and fat malabsorption. *American Journal of Gastroenterology*, 88, 2084–2091.

36. Yan, A. W., E. Fouts, D., Brandl, J., Stärkel, P., Torralba, M., Schott, E. et al. (2010). Enteric dysbiosis associated with a mouse model of alcoholic liver disease. *Hepatology*, 53 (1), 96–105. doi: <http://doi.org/10.1002/hep.24018>

37. Hartmann, P., Chen, P., Wang, H. J., Wang, L., McCole, D. F., Brandl, K. et al. (2013). Deficiency of intestinal mucin-2 ameliorates experimental alcoholic liver disease in mice. *Hepatology*, 58 (1), 108–119. doi: <http://doi.org/10.1002/hep.26321>

38. Hetta, H. F. (2014). Gut immune response in the presence of hepatitis C virus infection. *World Journal of Immunology*, 4 (2), 52. doi: <http://doi.org/10.5411/wji.v4.i2.52>

39. Mantis, N. J., Rol, N., Corthésy, B. (2011). Secretory IgA's complex roles in immunity and mucosal homeostasis in the gut. *Mucosal Immunology*, 4 (6), 603–611. doi: <http://doi.org/10.1038/mi.2011.41>

40. Haro, C., Rangel-Zúñiga, O. A., Alcalá-Díaz, J. F., Gómez-Delgado, F., Pérez-Martínez, P., Delgado-Lista, J. et al. (2016). Intestinal Microbiota Is Influenced by Gender and Body Mass Index. *PLOS ONE*, 11 (5), e0154090. doi: <http://doi.org/10.1371/journal.pone.0154090>

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**QUORUM SENSING AUTOINDUCERS BIOSYNTHESIS BY BIOFILM CULTURES OF PSEUDOMONAS AERUGINOSA STRAINS WITH DIFFERENT LEVELS OF THE CYCLIC DIGUANOZINMONOPHOSPHATE**

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**Mykola Galkin**, PhD, Associate Professor, Department of Microbiology, Virology and Biotechnology, Odessa I. I. Mechnikov National University, Dvoryanska str., 2, Odessa, Ukraine, 65082

E-mail: [kgalkin@onu.edu.ua](mailto:kgalkin@onu.edu.ua)

ORCID: <http://orcid.org/0000-0002-4957-7148>

**Anastasia Semenets**, Junior Researcher, Biotechnology Science-Education Center, Odessa I. I. Mechnikov National University, Dvoryanska str., 2, Odessa, Ukraine, 65082

E-mail: [asems@ukr.net](mailto:asems@ukr.net)

ORCID: <http://orcid.org/0000-0002-6223-9506>

**Boris Galkin**, Doctor of Biological Science, Professor, Director of Center, Biotechnology Science-Education Center, Department of Microbiology, Virology and Biotechnology, Odessa I. I. Mechnikov National University, Dvoryanska str., 2, Odessa, Ukraine, 65082

E-mail: [bgalkin@onu.edu.ua](mailto:bgalkin@onu.edu.ua)

ORCID: <http://orcid.org/0000-0002-3391-0938>

**Tetiana Filipova**, Doctor of Biological Science, Professor, Head of Department, Department of Microbiology, Virology and Biotechnology, Odessa I. I. Mechnikov National University, Dvoryanska str., 2, Odessa, Ukraine, 65082

E-mail: [tphilippova@ukr.net](mailto:tphilippova@ukr.net)

ORCID: <http://orcid.org/0000-0002-7034-3223>

*The aim of the work is to establish relationships between content of the cyclo-di-GMP and the ability of P. aeruginosa to form biofilm and synthesis of the quorum sensing system autoinducers.*

*Materials and methods of research.* Wild-type strain *P. aeruginosa* PA01 and *P. aeruginosa* strains with low (PA01p-JN2133) and high (PA01ΔwspF) levels of cyclic diguanosine monophosphate were used. Cultivation was performed in 24-well flat-bottomed plates Nuclon at 37 °C in LB medium. Biofilm mass was determined in CV-test. The measurements were performed on a Smart Spec Plus spectrophotometer

(Bio-Rad, Hungary) at a wavelength of 592 nm. Acyl-homoserin lactones were extracted with acidified ethyl acetate and quantified by GC/MS. The cyclo-di-GMP content was determined using a Seattle reporter plasmid by measuring the fluorescence intensity of cells in biofilms.

**Results of the research.** It was found that the strain of *P. aeruginosa* PA01 pJN2133, the intracellular content of cyclo-di-GMP in which was 4 times less than that in wild type strain, forms biofilms with mass in 3.5 times lower compared to *P. aeruginosa* PA01. *P. aeruginosa* PA01  $\Delta$ wspF exceeds *P. aeruginosa* PA01 in 1.5 times and 33 %, respectively. Even more significant difference were found mutant strains was compared. The level of cyclo-di-GMP in *P. aeruginosa* PA01  $\Delta$ wspF was in 5.9 times higher than in the PA01 pJN2133 strain cells, and five times higher in biofilm weight. The highest amount of quorum sensing system signalling molecules were synthesized by a strain with a low level of secondary messenger.

**Conclusions.** There is a directly proportional relationship between the intracellular content of cyclic-di-GMP and the ability to form a biofilm: the higher content of the secondary messenger; leads to increased mass of the biofilm. The concentration of QS autoinducers in the medium is inversely related to the intracellular content of cyclic-di-GMP: it is increased in the strain with a low content of the secondary messenger and decreased in the strain with its increased level, compared to the parent strain

**Keywords:** *Pseudomonas aeruginosa*, quorum sensing, biofilms, cyclic-di-GMP, QS autoinducers

## References

- Bjarnsholt, T., Jensen, P. O., Fiandaca, M. J., Pedersen, J., Hansen, C. R., Andersen, C. B. et. al. (2009). *Pseudomonas aeruginosa* biofilms in the respiratory tract of cystic fibrosis patients. *Pediatric Pulmonology*, 44 (6), 547–558. doi: <http://doi.org/10.1002/ppul.21011>
- Gellatly, S. L., Hancock, R. E. W. (2013). *Pseudomonas aeruginosa*: new insights into pathogenesis and host defenses. *Pathogens and Disease*, 67 (3), 159–173. doi: <http://doi.org/10.1111/2049-632x.12033>
- Sadikot, R., Bedi, B., Maurice, N. (2018). Microarchitecture of *Pseudomonas aeruginosa* biofilms: A biological perspective. *Biomedical and Biotechnology Research Journal*, 2 (4), 227–236. doi: [http://doi.org/10.4103/bbrj.bbrj\\_98\\_18](http://doi.org/10.4103/bbrj.bbrj_98_18)
- Ha, D.-G., O'Toole, G. A. (2015). C-di-GMP and its Effects on Biofilm Formation and Dispersion: a *Pseudomonas Aeruginosa* Review. *Microbial Biofilms*, 301–317. doi: <http://doi.org/10.1128/9781555817466.ch15>
- Valentini, M., Filloux, A. (2016). Biofilms and Cyclic di-GMP (c-di-GMP) Signaling: Lessons from *Pseudomonas aeruginosa* and Other Bacteria. *Journal of Biological Chemistry*, 291 (24), 12547–12555. doi: <http://doi.org/10.1074/jbc.r115.711507>
- Simm, R., Morr, M., Kader, A., Nimtz, M., Römling, U. (2004). GGDEF and EAL domains inversely regulate cyclic di-GMP levels and transition from sessility to motility. *Molecular Microbiology*, 53 (4), 1123–1134. doi: <http://doi.org/10.1111/j.1365-2958.2004.04206.x>
- Römling, U., Galperin, M. Y., Gomelsky, M. (2013). Cyclic di-GMP: the First 25 Years of a Universal Bacterial Second Messenger. *Microbiology and Molecular Biology Reviews*, 77 (1), 1–52. doi: <http://doi.org/10.1128/mmbr.00043-12>
- Seshasayee, A. S. N., Fraser, G. M., Luscombe, N. M. (2010). Comparative genomics of cyclic-di-GMP signalling in bacteria: post-translational regulation and catalytic activity. *Nucleic Acids Research*, 38 (18), 5970–5981. doi: <http://doi.org/10.1093/nar/gkq382>
- Hickman, J. W., Tifrea, D. F., Harwood, C. S. (2005). A chemosensory system that regulates biofilm formation through modulation of cyclic diguanylate levels. *Proceedings of the National Academy of Sciences*, 102 (40), 14422–14427. doi: <http://doi.org/10.1073/pnas.0507170102>
- Lee, J., Zhang, L. (2014). The hierarchy quorum sensing network in *Pseudomonas aeruginosa*. *Protein & Cell*, 6 (1), 26–41. doi: <http://doi.org/10.1007/s13238-014-0100-x>
- Bjarnsholt, T., Tolker-Nielsen, T., Hoiby, N., Givskov, M. (2010). Interference of *Pseudomonas aeruginosa* signalling and biofilm formation for infection control. *Expert Reviews in Molecular Medicine*, 12. doi: <http://doi.org/10.1017/s1462399410001420>
- Pearson, J. P., Gray, K. M., Passador, L., Tucker, K. D., Eberhard, A., Iglewski, B. H., Greenberg, E. P. (1994). Structure of the autoinducer required for expression of *Pseudomonas aeruginosa* virulence genes. *Proceedings of the National Academy of Sciences*, 91 (1), 197–201. doi: <http://doi.org/10.1073/pnas.91.1.197>
- Pesci, E. C., Milbank, J. B. J., Pearson, J. P., McKnight, S., Kende, A. S., Greenberg, E. P., Iglewski, B. H. (1999). Quinolone signaling in the cell-to-cell communication system of *Pseudomonas aeruginosa*. *Proceedings of the National Academy of Sciences*, 96 (20), 11229–11234. doi: <http://doi.org/10.1073/pnas.96.20.11229>
- Yang, L., Nilsson, M., Gjermansen, M., Givskov, M., Tolker-Nielsen, T. (2009). Pyoverdine and PQS mediated sub-population interactions involved in *Pseudomonas aeruginosa* biofilm formation. *Molecular Microbiology*, 74 (6), 1380–1392. doi: <http://doi.org/10.1111/j.1365-2958.2009.06934.x>
- Barken, K. B., Pamp, S. J., Yang, L., Gjermansen, M., Bertrand, J. J., Klausen, M. et. al. (2008). Roles of type IV pili, flagellum-mediated motility and extracellular DNA in the formation of mature multicellular structures in *Pseudomonas aeruginosa* biofilms. *Environmental Microbiology*, 10 (9), 2331–2343. doi: <http://doi.org/10.1111/j.1462-2920.2008.01658.x>
- Christensen, G. D., Simpson, W. A., Younger, J. J., Badour, L. M., Barrett, F. F., Melton, D. M., Beachey, E. H. (1985). Adherence of coagulase-negative staphylococci to plastic tissue culture plates: a quantitative model for the adherence of staphylococci to medical devices. *Journal of Clinical Microbiology*, 22 (6), 996–1006. doi: <http://doi.org/10.1128/jcm.22.6.996-1006.1985>
- Cataldi, T. R. I., Bianco, G., Frommberger, M., Schmitt-Kopplin, P. (2004). Direct analysis of selected N-acyl-L-homoserine lactones by gas chromatography/mass spectrometry. *Rapid Communications in Mass Spectrometry*, 18 (12), 1341–1344. doi: <http://doi.org/10.1002/rcm.1480>
- Palmer, G. C., Schertzer, J. W., Mashburn-Warren, L., Whiteley, M. (2010). Quantifying *Pseudomonas aeruginosa* Quinolones and Examining Their Interactions with Lipids. *Quorum Sensing*, 207–217. doi: [http://doi.org/10.1007/978-1-60761-971-0\\_15](http://doi.org/10.1007/978-1-60761-971-0_15)

19. Rybtke, M. T., Borlee, B. R., Murakami, K., Irie, Y., Hentzer, M., Nielsen, T. E. et. al. (2012). Fluorescence-Based Reporter for Gauging Cyclic Di-GMP Levels in *Pseudomonas aeruginosa*. *Applied and Environmental Microbiology*, 78 (15), 5060–5069. doi: <http://doi.org/10.1128/aem.00414-12>

20. Lapach, S. N., Chubenko, A. V., Babych, P. N. (2001). *Statystycheskye metody v medyko-byolohycheskykh yssledovaniyakh s yspolzovanyem Excel*. Kyiv: Moryon, 260.

21. Halkin, M. B., Semenets, A. S., Finohenova, M. O., Halkin, B. M., Filipova, T. O. (2017). Biofilm formation and motility of bacteria *Pseudomonas aeruginosa* with different c-di-GMP level. *Microbiology&Biotechnology*, 2 (38), 40–50. doi: [http://doi.org/10.18524/2307-4663.2017.2\(38\).105020](http://doi.org/10.18524/2307-4663.2017.2(38).105020)

22. Lin Chua, S., Liu, Y., Li, Y., Jun Ting, H., Kohli, G. S., Cai, Z. et. al. (2017). Reduced Intracellular c-di-GMP Content Increases Expression of Quorum Sensing-Regulated Genes in *Pseudomonas aeruginosa*. *Frontiers in Cellular and Infection Microbiology*, 7. doi: <http://doi.org/10.3389/fcimb.2017.00451>

23. Turkina, M. V., Vikström, E. (2018). Bacteria-Host Crosstalk: Sensing of the Quorum in the Context of *Pseudomonas aeruginosa* Infections. *Journal of Innate Immunity*, 11 (3), 263–279. doi: <http://doi.org/10.1159/000494069>

24. Singh, S., Singh, S. K., Chowdhury, I., Singh, R. (2017). Understanding the Mechanism of Bacterial Biofilms Resistance to Antimicrobial Agents. *The Open Microbiology Journal*, 11 (1), 53–62. doi: <http://doi.org/10.2174/1874285801711010053>

25. Schuster, M., Peter Greenberg, E. (2006). A network of networks: Quorum-sensing gene regulation in *Pseudomonas aeruginosa*. *International Journal of Medical Microbiology*, 296 (2-3), 73–81. doi: <http://doi.org/10.1016/j.ijmm.2006.01.036>

26. Papenfort, K., Bassler, B. L. (2016). Quorum sensing signal-response systems in Gram-negative bacteria. *Nature Reviews Microbiology*, 14 (9), 576–588. doi: <http://doi.org/10.1038/nrmicro.2016.89>

27. Galkin, M. B., Mukhlis Abedalabas, I., Pachomova, E. Yu., Filipova, T. O. (2014). The effect of *Pseudomonas aeruginosa* signal quinolone on the rhamnolipids biosynthesis and rhamnosyltransferase 2 activity. *European Scientific Journal*, 3, 223–228.

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## UNCARINA ROEOSLIANA RAUH ONTOMORPHOGENESIS IN EARLY STAGES OF DEVELOPMENT

p. 29-35

**Maryna Gaidarzhy**, Doctor of Biological Sciences, Leading Researcher, Educational and Research Center “Institute of Biology and Medicine” Taras Shevchenko National University of Kyiv, Volodymyrska str., 64/13, Kyiv, Ukraine, 01601  
E-mail: [gaidarzhy@ukr.net](mailto:gaidarzhy@ukr.net)

ORCID: <http://orcid.org/0000-0001-8860-552X>

**Oksana Futorna**, PhD, Educational and Research Center “Institute of Biology and Medicine” Taras Shevchenko Na-

tional University of Kyiv, Volodymyrska str., 64/13, Kyiv, Ukraine, 01601

E-mail: [oksana\\_drofa@yahoo.com](mailto:oksana_drofa@yahoo.com)

ORCID: <http://orcid.org/0000-0002-3713-6644>

**Nataliia Nuzhyna**, PhD, Educational and Research Center “Institute of Biology and Medicine” Taras Shevchenko National University of Kyiv, Volodymyrska str., 64/13, Kyiv, Ukraine, 01601

E-mail: [nuzhynan@gmail.com](mailto:nuzhynan@gmail.com)

ORCID: <http://orcid.org/0000-0002-4404-4502>

**The aim.** To find out the morphology of the seeds and the features of its germination and to study the development of *Uncarina roeoesliana* Rauh (*Pedaliaceae*) plants in the early stages of ontogeny in connection with the problem of a seed germination and their cultivation under introduction.

**Materials and methods.** The latent and pregenerative periods of the ontomorphogenesis of *Uncarina roeoesliana* plants under growing conditions in the protected soils of the Botanical garden named after acad. O. V. Fomin were investigated. Biomorphological, introducing, histological methods were used in this work.

**Results.** A method of accelerating the germination of freshly harvested seeds has been developed. *Uncarina roeoesliana* seeds are large (about 7x5 mm), mostly wide-triangular; brown in colour with little-noticed wing around the perimeter of the seed. The dorsal side forms folds, the hem is on the micropillary part of the seed. The periclinal walls are convex and often with papillae. The embryo is large, occupies most of the seed, the endosperm has a lot of lipids, which is typical for the representative of the same family *Sesamum indicum* L. The presence of papillae probably contributes to the moisture accumulation to increase the enzymatic activity when the seeds are swell. All of these features of seed germination are consistent with the environmental conditions of *Uncarina*'s natural habitats: high temperatures, low rainfall and significant dry periods. Probably the plants of this species belong to macrobiotics, that is, they can retain seed germination for a long time. The germination is aboveground. During the 24 weeks of development, the plants reach the virginal stage of development: they form radish roots, 5–6 pairs of leaves, of which only two pairs remain in the young plants at this stage, and a thickened basal part of the stem.

**Conclusions.** Comparing *Uncarina roeoesliana* with other caudiciform plants, we can conclude that this plant has a high potential for survival in arid conditions due to the ability of seeds to germinate only in conditions of considerable moisture and the ability of the plant to accumulate moisture in the basal part of the stem and in fleshy roots. In this case, the plant is adapted to exist both in the mode of a dormancy and in the mode of an active growth, but in the latter case only in the presence of sufficient moisture in the soil

**Keywords:** seeds, germination, early stages of ontogeny, caudiciform plant, endemic of Madagascar



## References

1. Egli, U., Albers, F.; Egli, U. (Ed.) (2002). Illustrated Handbook of succulent plants. Dicotyledones. New York, Berlin, Heidelberg: Springer-Verlag, 547.
2. Jacobsen, H. (1970). Das sukkulentenlexicon. Jena: VEB Gustav Fischer Verlag, 642.
3. Mirey, S., Kiani, S. (2016). Bioactivity of *Sesamum indicum*: a review study. *Der Pharmacia Lettre*, 8 (6), 328–334.
4. Maksymov, Y. M. (Ed.) (1990). Madahaskar. Moscow: Prohress, 280.
5. Gaidarzhii, M., Nuzhina, N. (2019). Morphology, phenology and fruiting of *Uncarina roeoesliana* Rauh in culture. *Visnyk of Lviv University. Biological Series*, 80, 59–66. doi: <http://doi.org/10.30970/vlubs.2019.80.07>
6. Danilova, M. F. (1996). Semeistvo Crassulaceae 11. *Sravnitelnaia anatomiiia semian. Vol. 5. Dvudolnye. Rosidae. Saint Petersburg: Mir i semia*, 25–34.
7. Takhtadzhian, A. L. (1987). *Sistema magnoliofitov. Leningrad: Nauka*, 438.
8. Corner, E. J. H. (1976). The seeds of dicotyledons. Vol. 1. Cambridge: Cambridge University Press, 311.
9. Zhmylev, P. Iu., Alekseev, Iu. E., Karpukhina, E. A., Balandin S. A. (2005). *Biomorfologiiia rastenii: illiustrirovannii slovar. Moscow: Grif*, 256.
10. Shyrobokova, D. N., Nikitina, V. V., Haidarzhii, M. M., Bahlai, K. M. (2003). *Kaktusy ta inshi sukulentni roslyny. Kyiv: Ukrainski propilei*, 108.
11. Barthlott, W. (1981). Epidermal and seed surface characters of plants: systematic applicability and some evolutionary aspects. *Nordic Journal of Botany*, 1 (3), 345–355. doi: <http://doi.org/10.1111/j.1756-1051.1981.tb00704.x>
12. Barthlott, W. (1984). Microstructural features of seed surfaces. *Syst. Assoc. Spec.*, 25, 95–105.
13. Brown, K. A., Parks, K. E., Bethell, C. A., Johnson, S. E., Mulligan, M. (2015). Predicting Plant Diversity Patterns in Madagascar: Understanding the Effects of Climate and Land Cover Change in a Biodiversity Hotspot. *PLOS ONE*, 10 (4), e0122721. doi: <http://doi.org/10.1371/journal.pone.0122721>
14. Callmander, M. W., Phillipson, P. B., Schatz, G. E., Andriambololonera, S., Rabarimanarivo, M., Rakotonirina, N. et. al. (2011). The endemic and non-endemic vascular flora of Madagascar updated. *Plant Ecology and Evolution*, 144 (2), 121–125. doi: <http://doi.org/10.5091/plecevo.2011.513>
15. Morat, P., Lowry, P. P. (1977). Floristic richness in the Africa-Madagascar region: a brief history and properties. *Adansonia*, 19 (1), 101–115.
16. Gaidarzhii, M. N. (2014). *Botanicheskaia ekskursiia na ostrov Madagaskar. Sokhranenie bioraznoobrazzia i introdukciia rastenii. Kharkiv*, 19–24.
17. Haidarzhii, M. M., Nikitina, V. V., Bahlai, K. M., Kalashnyk, S. O. (2011). Adaptatsiini stratehii sukulentnykh roslyn u preheneratyvnyi period. *Vidnovlennia porushenykh pryrodnykh ekosystem. Donetsk*, 90–92.
18. Scherbakov, V. G., Lobanov, V. G. (2003). *Biokhimiia i tovarovedenie maslichnogo syria. Moscow: Kolos*, 360.
19. Avekin, Ya., Haidarzhii, M. (2017). *Adenium obesum (Forssk.) Roem. & Schult. (Apocynaceae): development of vegetative organs in the early stages of ontogenesis. Ukrainian Journal of Ecology*, 7 (2), 173–183. doi: [http://doi.org/10.15421/2017\\_34](http://doi.org/10.15421/2017_34)
20. Haidarzhii, M., Nikitina, V., Bahlai, K., Kalashnyk, S. (2015). *Kaudeksni sukulentni roslyny v kolektsii Botanichnoho sadu. Visnyk Kyivskoho Natsionalnoho Universytetu imeni Tarasa Shevchenka. Introduktsiia ta zberezhennia roslynnoho riznomanittia*, 33, 11–14.

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## RESISTANCE OF LINES OF WINTER BREAD WHEAT CREATED WITH THE PARTICIPATION OF SPRING-WINTER HYBRIDS TO LEAF STEAD DISEASES

p. 36-41

**Ruslan Solomonov**, PhD, Senior Researcher, Scientific and Technological Department Development and Implementation of Innovative Technologies to Intensify Production, Odessa State Agricultural Experiment Station of National Academy of Agrarian Sciences of Ukraine, Maiatska Doroha str., 24, Khlebodar, Odessa region, Ukraine, 67667

E-mail: [rusolomonov@ukr.net](mailto:rusolomonov@ukr.net)ORCID: <http://orcid.org/0000-0002-6186-4676>

**Kryvenko Anna**, Doctor of Agricultural Sciences, Associate Professor, Deputy Director of Research, Odessa State Agricultural Experiment Station of National Academy of Agrarian Sciences of Ukraine, Maiatska Doroha str., 24, Khlebodar, Odessa region, Ukraine, 67667

E-mail: [kryvenko35@ukr.net](mailto:kryvenko35@ukr.net)ORCID: <http://orcid.org/0000-0002-2133-3010>

*When studying the collection of spring soft wheat of different genetic origin, individual samples that are resistant to pathogens and can be used as sources of resistance to pathogens in their hybridization with winter varieties were selected.*

*The aim of our research was to study the level of resistance to pathogens of leaf-stem diseases of spring-winter hybrids of different generations depending on the winter and spring components of crossbreeding and their genetic origin, as well as the effectiveness of selection on artificial infectious and natural backgrounds to obtain disease-resistant, with a set of economically valuable features and properties.*

*Materials and methods. The main method of creating a new hybrid material is intraspecific hybridization of spring and winter soft wheat, followed by selection of stable genotypes of different generations on natural (against powdery mildew) and artificial infectious (against brown and stem rust) backgrounds.*

*The source of material for the research was a collection of samples of spring soft wheat of different genetic and ecological-geographical origin in the amount of 101 pcs, Lines F<sub>3</sub> (18 pcs.), F<sub>4</sub> (141 pcs.) and F<sub>5</sub> (66 pcs.), Created from combinations crossing of spring samples with varieties of the Breeding and Genetic Institute - National Center for Seed Science and Variety Research, different in biological properties. Evaluation of collection varieties and lines was*

performed according to the generally accepted methods of variety testing.

**Result.** The results of studying the resistance of  $F_{3-5}$  wheat lines created on the basis of spring-winter hybrids to the main pathogens on a natural and artificially created infectious background are presented. The links between resistance to pathogens and the main economic and valuable traits in wheat lines have been identified. Wheat lines with complex resistance to leaf-stem pathogens were obtained.

**Conclusions.** Dedicated winter bread wheat lines which combine in their genotype relative disease resistance with high productivity. These lines are mainly created from crossing spring specimens of Mexican, Western European, Canadian and Russian origin with local winter varieties

**Keywords:** wheat lines, pathogens, resistance, elements of productivity, yield

### References

1. Alfimov, V. A., Bespalova, L. A., Puzyrnaia, O. IU. (2001). Ustoichivost sortov ozimoi pshenicy v sviazii s izmeneniami rasovogo sostava v populiacii buroi rzhavchiny Krasnodarskogo kraia. Pshenica i tritikale. Krasnodar, 306–317.
2. Krivchenko, V. I. (1979). Ispolzovanie genofonda v selekcii selskokhoziaistvennykh kultur na ustoichivost k vrednym organizmam. Problemy zaschity rastenii ot vreditelii, boleznei i sornikov. Moscow: «Kolos», 114–118.
3. Dmitriev, A. P. (2003). Osobennosti biologii vzaimootnoshenii v sisteme parazit-khoziaiin kak osnova vybora tipa ustoichivosti zernovykh kultur k rzhavchine. Tipy ustoichivosti rastenii k bolezniam. Saint Petersburg, 33–34.
4. Grigoreva, O. G. (1987). Donory effektivnykh genov ustoichivosti k stebelnoi rzhavchine pshenicy. Problemy ispolzovaniia genofonda v selekcii rastenii na immunitet k bolezniam i vrediteliam. Leningrad: VIR, 33–37.
5. German, S. E., Kolmer, J. A. (1992). Effect of gene Lr34 in the enhancement of resistance to leaf rust of wheat. Theoretical and Applied Genetics, 84 (1-2), 97–105. doi: <http://doi.org/10.1007/bf00223987>
6. Voronkova, A. A., Puchkov, Iu. M. (1977). Selekciiia pshenicy na ustoichivost k rzhavchine. Krasnodar: Krasnodarskoe kn. izd-vo, 56.
7. Mikhailova, L. A. (2003). Genetika ustoichivosti pshenicy k buroi rzhavchine. Tipy ustoichivosti rastenii k bolezniam. Saint Petersburg, 45–60.
8. Romanenko, A. A., Bespalova, L. A., Kudriashov, I. N., Ablova, I. B. (2005). Novaia sortovaia politika i sortovaia agrotehnika ozimoi pshenicy. Krasnodar, 224.
9. Khomenko, S. O., Solona, V. Y., Zvarun, T. V. (2011). Osoblyvosti selektsii pshenytsi yaroii v umovakh lisostepu Ukrainy. Selekciiia i nasynnytstvo, 100, 181–191.
10. Babaianc, O. V., Babaianc, L. T. (2014). Osnovy selekcii i metodologiiia ocenok ustoichivosti pshenicy k vobuditeliam boleznei. Odessa: VMV, 401.
11. Bazalii, V. V., Bazalii, H. H., Larchenko, O. V. (2008). Ekologichna plastychnist i stabilnist urozhainosti sortiv pshenytsi z riznym typom rozvytku. Faktory eksperymentalnoi evoliutsii orhanizmiv. Kyiv: Lohos, 5, 17–22.
12. Ablova, I. G. (2008). Principy i metody sozdaniia sortov pshenicy, ustoichivyykh k bolezniam i ikh rol v stanovlenii agrosistem. Krasnodar, 49.
13. Kyrychenko, V. V., Petrenkova, V. P., Cherniaeva, I. M. et al.; V. V. Kyrychenko, V. V., Petrenkova, V. P. (Eds.) (2012). Osnovy selektsii polovykh kultur na stiikist do shkidlyvykh orhanizmiv. Kharkiv: In-t roslynnytstva im. V. Ya. Yurieva, 320.
14. Tkachyk, S. O. (Ed.) (2014). Metodyka provedennia ekspertyzy sortiv roslyn hrupy zernovykh, krupianykh ta zernobovykh na prydatnist do poshyrennia v Ukraini (PSP). Kyiv: TOV «Nilan-LTD», 82.
15. Rybalka, O. I. (2008). Porivniannia ekspres-metodu sedimentatsii SDS30 pry vyznachenni yakosti zerna y boroshna pshenytsi. Zerno i khlib, 1.
16. Koishibaev, M., Shamanin, V. P., Morgunov, A. I. (2014). Skrining pshenicy na ustoichivost k osnovnym bolezniam. Ankara: FAO-SEK, 64.
17. Dermenko, O. P., Panchenko, Yu. S., Havryliuk, L. L. (2012). Zakhyst pshenytsi ozymoi vid buroi lystkovoi irzhi. Karantyn i zakhyst roslyn, 11, 4–7.
18. Oelke, L. M., Kolmer, J. A. (2005). Genetics of Leaf Rust Resistance in Spring Wheat Cultivars Alsen and Norm. Phytopathology, 95 (7), 773–778. doi: <http://doi.org/10.1094/phyto-95-0773>

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### PHYSIOLOGICAL ASPECTS OF RAT ACTIVITY, THEIR ANXIETY AND MEMORY AFTER ADMINISTRATION OF FULL GABA<sub>A</sub>-RECEPTOR COMPLEX AGONIST PROPOXAZEPAM

p. 42-48

**Mykola Golovenko**, Doctor of Biological Sciences, Professor, Chief Researcher, Academician of National Academy of Medical Sciences of Ukraine, Laboratory of Physico-Chemical Pharmacology, A. V. Bogatsky Physical-Chemical Institute of National Academy of Sciences of Ukraine, Liustdorf-ska doroha str., 86, Odessa, Ukraine, 65080

E-mail: [n.golovenko@gmail.com](mailto:n.golovenko@gmail.com)

ORCID: <http://orcid.org/0000-0003-1485-128X>

**Igor Belenichev**, Doctor of Biological Sciences, Professor, Head of the Department, Department of Pharmacology and Medical Prescriptions, Zaporizhia State Medical University, Maiakovskoho ave., 26, Zaporizhia, Ukraine, 69035

E-mail: [i.belenichev1914@gmail.com](mailto:i.belenichev1914@gmail.com)

ORCID: <http://orcid.org/0000-0003-1273-5314>

**Vitalii Larionov**, Doctor of Biological Sciences, Head of Laboratory, Laboratory of Physical-Chemical Pharmacology, A. V. Bogatsky Physical-Chemical Institute of National Academy of Sciences of Ukraine, Liustdorfska doroha str., 86, Odessa, Ukraine, 65080

E-mail: [lvb\\_78@ukr.net](mailto:lvb_78@ukr.net)

ORCID: <http://orcid.org/0000-0003-2678-4264>



**Anatoliy Reder**, PhD, General Director, “INTERCHEM” SLC, Liustdorfska doroha str., 86, Odessa, Ukraine, 65080, Senior Researcher, A. V. Bogatsky Physical-Chemical Institute of National Academy of Sciences of Ukraine, Liustdorfska doroha str., 86, Odessa, Ukraine, 65080

**E-mail:** reder@interchem.com.ua

**ORCID:** <http://orcid.org/0000-0002-1801-8378>

**Serhiy Andronati**, Doctor of Chemical Sciences, Professor, Head of Department, Academician of National Academy of Sciences of Ukraine, Department of Medicinal Chemistry, A. V. Bogatsky Physical-Chemical Institute of National Academy of Sciences of Ukraine, Liustdorfska doroha str., 86, Odessa, Ukraine, 65080

**E-mail:** office.physchem@nas.gov.ua

**ORCID:** <http://orcid.org/0000-0001-8451-6327>

*The action of 7-bromo-5 (o-chlorophenyl)-3-propoxy-1,2-dihydro-3H-1,4-benzodiazepin-2-one (propoxazepam) with prolonged administration in various doses on behavioural reactions, anxiety and the memory of rats, as well as their muscle tone was estimated, which is important paying attention to its main (anticonvulsant and analgesic) pharmacological effects.*

**The aim of the study** was a comparative assessment of the severity and duration of propoxazepam effect after its administration to rats at doses of 2, 5 and 10 mg/kg (10 days) on higher functions of the central nervous system according to indicators such as motor and exploratory activity, as well as reference and working memory.

**Materials and methods.** The study was conducted on 40 Wistar rats weighing 220-290 g. The psychophysiological state of the animals was evaluated using the “open field” test, the formation of spatial working and long-term memory in a radial eight-arm maze, muscle relaxation, imbalance and movements coordination - using the “rotating rod” method. Statistical processing was performed in Microsoft Excel 2016 with AtteStat 12.

**The results of the study.** When comparing the test results of the “open field”, “radial labyrinth” and “rotarod” in groups of animals that were administered different doses of the compound, it was found that, in general, these parameters at doses of 2 mg/kg and 5 mg/kg statistically significantly different (increase) from control values, but are similar. At a dose of 10 mg/kg, most of the parameters (except for the rotarod test) for these animals were characterized by a downward trend.

**Conclusions.** The administration of the drug in doses of 2, 5 and 10 mg/kg leads to a decrease in anxious behaviour of animals, which is also accompanied by a pronounced dose-dependent negative effect on the endurance, coordination and memory of animals. The administration of the drug at a dose of 10 mg/kg impaired the learning ability of animals and reduced memory function. This should be considered in clinical studies of the compound

**Keywords:** propoxazepam, locomotor and exploratory activity, reference and working memory

## References

- Mamylyna, N. V., Pavlova, V. I. (2013). Fiziologicheskie aspekty povedencheskoi aktivnosti zhivotnykh v usloviakh emocionalnogo stressa. Cheliabinsk: «Cicero», 298.
- Golovenko, N. Y., Larionov, V. B., Reder, A. S., Valivodz', I. P. (2017). An effector analysis of the interaction of propoxazepam with antagonists of GABA and glycine receptors. *Neurochemical Journal*, 11 (4), 302–308. doi: <http://doi.org/10.1134/s1819712417040043>
- Golovenko, N. Ya., Voloshchuk, N. I., Andronati, S. A., Taran, I. V., Reder, A. S., Pashynska, O. S., Larionov, V. B. (2018). Antinociception induced by a novel benzodiazepine receptor agonist and bradykinin receptor antagonist in rodent acute and chronic pain models. *European Journal of Biomedical and Pharmaceutical Sciences*, 5 (12), 79–88.
- Golovenko, N. Y., Kabanova, T. A., Andronati, S. A., Halimova, O. I., Larionov, V. B., Reder, A. S. (2020). Anti-inflammatory effects of propoxazepam on different models of inflammation. *International Journal of Medicine and Medical Research*, 5 (2), 105–112. doi: <http://doi.org/10.11603/ijmmr.2413-6077.2019.2.10900>
- Voloshchuk, N. I., Reder, A. S., Golovenko, M. Ya., Taran, I. V., Pashynska, O. S. (2017). Farmakologichnyi analiz neyrohimichnih antinotsitseptivnih mehanizmiv dii propoksazepamu. *Farmakologiya ta likarska toksikologiya*, 1 (53), 3–11.
- Golovenko, M. Y., Larionov, V. B., Reder, A. S., Andronati, S. A., Valivodz', I. P., Yurpalova, T. O. (2018). Pharmacodynamics of Interaction between Propoxazepam and a GABA-Benzodiazepine Receptor-Ionofor Complex. *Neurophysiology*, 50 (1), 2–10. doi: <http://doi.org/10.1007/s11062-018-9711-9>
- Golovenko, N. Y., Kovalenko, V. N., Larionov, V. B., Reder, A. S. (2020). Dose and time-dependent acute and subchronic oral toxicity study of propoxazepam in mice and rats. *International Journal of Pharmacology and Toxicology*, 8 (1), 1. doi: <http://doi.org/10.14419/ijpt.v8i1.29531>
- Organisation for Economic Cooperation and Development (2008). OECD Guideline for Testing of Chemicals (TG 407). Repeated Dose 28-Day Oral Toxicity Study in Rodents. OECD/OECD.
- Voloshchuk, N. I., Taran, I. V., Reder, A. S., Golovenko, M. Y. (2018). Experimental study of ulcerogenic action of propoxazepam. *Reports of Vinnytsia National Medical University*, 22 (1), 6–9. doi: [http://doi.org/10.31393/reports-vnmedical-2018-22\(1\)-01](http://doi.org/10.31393/reports-vnmedical-2018-22(1)-01)
- Golovenko, M. Ya., Larionov, V. B., Reder, A. S. (2020). Investigation of safety profile of propoxazepam by salmonella/microsome test, Information, its impact on social and technical processes. SH SCW “NEW ROUTE”. Haifa, 162–165.
- Andronaty, S. A., Voronyna, T. A., Holovenko, N. Ya. (1992). Hydazepam. Kyiv: Naukova dumka, 196.
- Larionov, V. B., Holovenko, M. Ya., Valivodz', I. P., Reder, A. S. (2020). Psykhotropni vlastyivosti pokhidnoho 1,4-benzodiazepinu, potentsiinoho antykonvulsanta ta analhetyka iz polimodalnym mekhanizmom dii. *Medychna nauka ta praktyka na suchasnomu istorychnomu etapi*. Kyiv, 129–133.

13. Volokhova, H. A., Stoianov, A. N., Tokman, E. P. (2009). Vlyianyе solkoseryla na kohnytyvnie funktsyy pry yshemycheskom ynsulte. *Liky Ukrainy*, 4 (130), 110–114.
14. Nadel, L., Hardt, O. (2010). Update on Memory Systems and Processes. *Neuropsychopharmacology*, 36 (1), 251–273. doi: <http://doi.org/10.1038/npp.2010.169>
15. Wishaw, I. Q., Li, K., Wishaw, P. A., Gorny, B., Metz, G. A. (2008). Use of Rotorod as a Method for the Qualitative Analysis of Walking in Rat. *Journal of Visualized Experiments*, 22. doi: <http://doi.org/10.3791/1030>
16. Perez-Leighton, C. E., Boland, K., Billington, C. J., Kotz, C. M. (2013). High and low activity rats: Elevated intrinsic physical activity drives resistance to diet-induced obesity in non-bred rats. *Obesity*, 21 (2), 353–360. doi: <http://doi.org/10.1002/oby.20045>
17. West, C. H. K., Boss-Williams, K. A., Weiss, J. M. (1998). Motor activation by amphetamine infusion into nucleus accumbens core and shell subregions of rats differentially sensitive to dopaminergic drugs. *Behavioural Brain Research*, 98 (1), 155–165. doi: [http://doi.org/10.1016/s0166-4328\(98\)00064-3](http://doi.org/10.1016/s0166-4328(98)00064-3)
18. Seibenhener, M. L., Wooten, M. C. (2015). Use of the Open Field Maze to Measure Locomotor and Anxiety-like Behavior in Mice. *Journal of Visualized Experiments*, 96, 524–534. doi: <http://doi.org/10.3791/52434>
19. Belenichev, I., Burlaka, B., Puzyrenko, A., Ryzhenko, O., Kurochkin, M., Yusuf, J. (2019). Management of amnesic and behavioral disorders after ketamine anesthesia. *Georgian Medical News*, 9 (294), 141–145.
20. Rex, A. (1996). “Anxiolytic” action of diazepam and abecarnil in a modified open field test. *Pharmacology Biochemistry and Behavior*, 53 (4), 1005–1011. doi: [http://doi.org/10.1016/0091-3057\(95\)02121-3](http://doi.org/10.1016/0091-3057(95)02121-3)
21. Sereidenin, S. B., Blednov, Yu. A., Badyshtov, B. A., Gordey, M. L., Nagovitsina, Ya. (1990). Pharmacogenetic analysis of mechanisms of emotional stress: effects of benzodiazepines. *Annali dell’Istituto Superiore di Sanità*, 26 (1), 81–87.
22. King, D. J. (1992). Benzodiazepines, amnesia and sedation: Theoretical and clinical issues and controversies. *Human Psychopharmacology: Clinical and Experimental*, 7 (2), 79–87. doi: <http://doi.org/10.1002/hup.470070202>