

**PHYTOTESTING OF TOXICITY OF CULTURAL FLUID
OF BACTERIA *BACILLUS VELEZENSIS***

N. Tkachuk¹, L. Zelena², S. Krapyvnyi³, Y. Podterher¹

¹*T.H. Shevchenko National University "Chernihiv Colehium", Chernihiv, Ukraine,
nataliia.smykun@gmail.com*

²*Danylo Zabolotny Institute of Microbiology and Virology of National Academy of Sciences of
Ukraine, Kyiv, zelenalyubov@gmail.com*

³*Chernihiv Lyceum 32, Chernihiv, Ukraine, sk0982463946@gmail.com*

Representatives of the genus *Bacillus* are actively studied as controlling agents of biodamage, in particular some metals [1]. *Bacillus velezensis* strains (heterotypic synonyms of which are species names of *B. amyloliquefaciens* subsp. *plantarum*, *B. methylotrophicus*, *B. oryzicola*, *B. methylotrophicus* subsp. *plantarum* [2]) deserve attention, as they have significant potential in counteracting biological damage, which is determined by both antimicrobial properties [3] and the ability to inhibit the formation of biofilms of corrosive bacteria on the surface of the material [4]. Previously, we isolated and identified strains of *B. velezensis* NUChC C1 and NUChC C2b with the ability to inhibit the development of biofilms of sulfate-reducing bacteria [4]. For the practical use of the cultural fluids, their low toxicity is important [5]. Onions (*Allium cepa* L.) are considered as a standard test object for determining toxicity [6-8]. Therefore, the aim of this work was to study the test parameters of *A. cepa* under the influence of culture fluid of bacteria *B. velezensis* NUChC C2b.

A 5-day pure culture of *B. velezensis* strain NUChC C2b [4] was used in the experiments. Incubation was performed in meat-peptone broth (MPB) under aerobic conditions and at a temperature of 29 ± 2 °C. The culture grown in MPB and with a cell count of 1×10^8 cells/ml (0.5 McFarland) was used in the following variants: C2b_{1:9} - culture of *B. velezensis*:distilled water 1:9; C2b_{9:1} - *B. velezensis* culture:distilled water 9:1. MPB solution:distilled water 1:9 was used as a control.

The test plant was selected onion (*A. cepa*), variety Chalcedony. The seeds of the plant in the phytotest were grown in Petri dishes on filter paper moistened with a suitable solution in the dark at a temperature of 23.0 ± 2.0 °C. The experiment was laid down in triplicate. On the 5th day, the energy of seed germination and the biometric parameter - the length of the roots - were determined.

The root length index (RLI), which describes the phytotoxicity index, was calculated by the formula (1),

$$RLI = \frac{L_T(i) - L_C}{L_C} \quad (1)$$

where $L_T(i)$ and L_C are the mean root lengths in the test (i) and in the control, respectively. Based on the published empirical value of risk assessment [9-11], phytotoxicity can be divided into four classes, such as:

weak ($-0.25 \leq RLI < 0$),

average ($-0.5 \leq RLI < -0.25$),

high ($-0.75 \leq RLI < -0.5$),

extreme toxicity ($-1 \leq RLI < -0.75$).

The phytotoxic effect of solutions (PhTE) was calculated by formula (2) [12],

$$PhTE = \frac{L_C - L_T(i) \times 100}{L_C} \quad (2)$$

where $L_T(i)$ and L_C are the average root lengths in the test (i) and in the control, respectively.

Statistical processing of the obtained data was carried out using the statistical module of Microsoft Office Excel 2010. We used the methods of descriptive statistics for calculation of the arithmetic mean (M) and the standard error of the arithmetic mean (m) [13]. The Student's significance criterion (t) was calculated, and the 95% probability of differences ($p \leq 0.05$) was considered statistically significant.

The results of the study of phytotoxicity of culture fluid solutions *B. velezensis* are presented in Table 1.

Table – Test-indicators and interpretation of the results

A variant of the experiment	Germination energy relatively control, %	The length of the roots relatively control, %	RLI	PhTE	Interpretation of phytotest results	Comments
C2b _{1:9}	121±4	139±25	0,387	-38,70	No toxicity	Weak growth stimulation
C2b _{9:1}	67±22	24±3*	-0,764*	76,40	Extreme toxicity	Growth inhibition by more than 50%

Note: RLI - root length index; PhTE - phytotoxic effect of solutions; * - differences from control are significant at $p \leq 0.05$

It was found that according to phytotoxic indices, the culture solution of *B. velezensis*:H₂O 1:9 (variant C2b_{1:9}) did not show toxicity and had a stimulating effect on the growth of the test plant. But statistical processing of the results indicates that the differences between the test indicators in the experiment and control are not statistically significant.

With an increase in the concentration of liquid culture *B. velezensis* (variant C2b_{9:1}), a significant inhibition of the growth of the test plant was observed - the solution turned out to be extremely toxic. The statistical processing of the results indicates the statistical significance of the differences between the test indicators in the experiment and control. The obtained results are determined by the composition of the culture fluid of the studied bacteria. Thus, it is known that depending on the strain, metabolites of *B. velezensis* are bacilysin, bacillibactin, difficidin, fengycin, bacillaen, macrolactin, surfactin, plantathizolicin, subtilin, mersacidin, bacillomycin, locilomycin, amylocyclicin, nematicide [14-16].

It would be important to study the phytotoxicity of the solution of culture *B. velezensis*:H₂O at a ratio of 1:5, because it is at this concentration of culture fluid showed a decrease in biofilm formation of sulfate-reducing bacteria [4].

Thus, in the phytotest with onions, the phytotoxicity of the culture fluid of *B. velezensis*:H₂O at a ratio of 1:9 was not established, but the extreme toxicity at a ratio of 9:1 was reliably shown. The prospect of further research is to assess the phytotoxicity of the culture fluid of *B. velezensis*:H₂O at a ratio of 1:5.

References

1. Tkachuk N., Zelena L. The Impact of Bacteria of the Genus *Bacillus* upon the Bio-damage/Biodegradation of Some Metals and Extensively Used Petroleum-Based Plastics. *Corros. Mater. Degrad.* 2021. Vol. 2. P. 531–553.
2. Dunlap Ch.A., Kim S.-J., Kwon S.-W., Rooney A.P. *Bacillus velezensis* is not a later heterotypic synonym of *Bacillus amyloliquefaciens*; *Bacillus methylotrophicus*, *Bacillus amyloliquefaciens* subsp. *plantarum* and '*Bacillus oryzicola*' are later heterotypic synonyms of *Bacillus velezensis* based on phylogenomics. *International Journal of Systematic and Evolutionary Microbiology*. 2016. No 66. P. 1212–1217.
3. Dimopoulou A., Theologidis I., Benaki D., Koukounia M., Zervakou A., Tzima A., Diallinas G., Hatzinikolaou D.G., Skandalis N. Direct Antibiotic Activity of Bacillibactin Broadens the Biocontrol Range of *Bacillus amyloliquefaciens* MBI600. *mSphere*. 2021. Vol. 6, Issue 4. e0037621.
4. Tkachuk N., Zelena L., Lukash O., Mazur P. Microbiological and genetic characteristics of *Bacillus velezensis* bacillibactin-producing strains and their effect on the sulfate-reducing bacteria biofilms on the poly(ethylene terephthalate) surface. *Ecological Questions*. 2021. Vol. 32, Issue 2. P. 119-129.
5. Zain W.S.M., Salleh N.I.H., Abdullah A. Natural Biocides for Mitigation of Sulphate Reducing Bacteria. *International Journal of Corrosion*. 2018. P. 1-7.
6. Constantin M.J., Owen E.T. Introduction and perspectives of plant genetic and cytogenetic assay. A report of US EPA Gene-Tox programme. *Mutation Research*. 1982. Vol. 99. P.1–12.
7. Cauhan L.K.S., Saxena P.N., Gupta S.K. Cytogenetic effects of cypermethrin and fenvalerate on the root meristem cells of *Allium cepa*. *Environ. Exp. Bot.* 1999. Vol. 42. P.181–189.
8. Nilüfer A., Serap C., Senay S., Dilek Y., Özelm Ö. Evaluation of clastogenicity of 4,6-Dinitro-*o*-cresol (DNOC) in *Allium* root tip test. *J. Biol. Environ. SCL*. 2008. №2. P.59–63.
9. Bagur-González M.G., Estepa-Molina C., Martín-Peinado F., Morales-Ruano S. Toxicity assessment using *Lactuca sativa* L. bioassay of the metal(loid)s As, Cu, Mn, Pb and Zn in soluble-in-water sat-

urated soil extracts from an abandoned mining site. *Journal of Soils and Sediments*. 2011. Issue 11. P. 281-289.

10. Mtisi M., Gwenzi W. Evaluation of the phytotoxicity of coal ash on lettuce (*Lactuca sativa* L.) germination, growth and metal uptake. *Ecotoxicology and Environmental Safety*. 2019. Issue 170. P. 750-762.

11. Cai X., Ostroumov S.A.. Finding of toxicity of herbal shampoo to plant seedlings: phytotest of mixture product that contains membranotropic chemicals as components. *Ecologica*. 2021. Vol. 28, Issue 101. P. 6-10.

12. Багдасарян А.С. Биотестирование почв техногенных зон городских территорий с использованием растительных организмов: дис. канд.биол.наук: 03.00.16 / Багдасарян Александр Сергеевич. Ставрополь, 2005. 159 с.

13. Плохинский Н.А. Биометрия. Москва: Изд-во Московского ун-та, 1970. 368 с.

14. Pan H.Q., Li Q.L., Hu J.C. The complete genome sequence of *Bacillus velezensis* 9912D reveals its biocontrol mechanism as a novel commercial biological fungicide agent. *J. Biotechnol.* 2017. Vol. 247. P. 25-28.

15. Balderas-Ruíz K.A., Bustos P., Santamaria R.I., González V., Cristiano-Fajardo S.A., Barrera-Ortiz S., Mezo-Villalobos M., Aranda-Ocampo S., Guevara-García Á.A., Galindo E., Serrano-Carreón L. *Bacillus velezensis* 83 a bacterial strain from mango phyllosphere, useful for biological control and plant growth promotion. *AMB Express*. 2020. Vol. 10, Issues 1. P. 163.

16. Silva F.J., Ferreira L.C., Campos V.P., Cruz-Magalhães V., Barros A.F., Andrade J.P., Roberts D.P., de Souza J.T. Complete Genome Sequence of the Biocontrol Agent *Bacillus velezensis* UFLA258 and Its Comparison with Related Species: Diversity within the Commons. *Genome Biol Evol.* 2019. Vol. 11, Issues 10. P. 2818-2823.