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

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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*:H_2O 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, fengyncin, 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*:H_2O 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*:H_2O 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*:H_2O at a ratio of 1:5.

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