# Bacillus *Velezensis Nuchc* C2b As a Promising Bioremediation Agent in The Environments Contaminated with Heavy Metals

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## ABSTRACT

It was shown that the use of *Bacillus velezensis* NUChC C2b liquid culturehas reduced the toxicity of an aqueous solution of $Pb(CH_3COOH)_2$ with a concentration of $Pb^{2+}$ 0.57 mg/L according to the test-parameters of *Lepidiumsativum*. The results obtained could be due to activity of sider ophorebacillibactin produced by *Bacillus velezensis* NUChC C2b. Concentrated liquid culture *B. velezensis* strain NUChC C2b is promising for using in bioremediation of the environment contaminated with $Pb^{2+}$, in combination with stronger bioremediation agents.

**Keywords:** bacillibactin; *Bacillus velezensis*; bioremediation; lead; *Lepidiumsativum*

## INTRODUCTION

Microbial bioremediation of heavy metals-polluted industrial effluents has been adopted as one of the most effective eco-friendly tool to cope up with the harmful effects of metals [1].At the same time, siderophore-producing bacteria deserve attention [2-4], because siderophores are chelating compounds that are effective for cleaning media from heavy metals [3, 5]. Previously, we isolated and identified strains of *Bacillus velezensis* bacteria with a high level of gene expression of siderophorebacillibactin [6]; the effect of liquid culture on the toxicity of the environment with a high content of lead has not been studied. Currently, the toxicity of various substrates is assessed by phytotesting using garden cress (*Lepidiumsativum* L.) as a test plant.

## OBJECTIVES

The aim of this study was to investigate the toxicity of the solution of the salt of lead according to the test-parameters of *L. sativum* in the presence of the liquid culture of bacillibactin-producing bacteria *B. velezensis* strain NUChC C2b.

## METHODOLOGY

Five-daypurecultureofbacillibactin-producing bacteria *Bacillus velezensis*strain NUChC C2b from the collection of the Department of Biology of the T.H. National University "Chernihiv Colehium" was used [6]. The strain was identified by the conventional microbiological method and 16S rDNA sequencing [6]. The nucleotide sequences were deposited in the GenBank with accession numbers MN749356.1 and MN749357.1.Incubation of bacteria was carried out in meat-peptone broth (MPB) under aerobic conditions and a temperature of 29 ± 2 °C.

Seeds of test plants *L. sativum*of 10 pieces were put in Petri dishes on filter paper moistened withappropriate solutions.Distilledwater with the addition of MPB (9 parts water: 1 part MPB) was used as a control (option I). The toxicant was lead salt $Pb(CH_3COOH)_2$ with a concentration of 0.57 mg $Pb^{2+}$ /L (option II), which exceeds the maximum allowable concentration of lead in drinking water (0.01 mg/L) according to the World Health Organization 57 times.

A culture of *B. velezensis* strain NUChC C2b grown in MPB with a cell count of $1 \times 10^8$ cells/ml (0.5 McFarland) was added to distilled water (option III) or to a solution of lead salt (option IV) to obtain the number of bacterial cells in a solution of $1 \times 10^7$ cells/ml.

The experiment was repeated three times. The incubation temperature of the Petri dishes was 23.0 ± 2.0°C. Seed germination energy (3rd day), seed germination and biometric-morphometric parameters (length of roots and aboveground part of seedlings) (5th day) were determined.

Seed germination index (SGI) and root length index (RLI) that exemplified phytotoxicity index were described in Eq. (1) and (2),

$$SGI = \frac{N_T(i) - N_C}{N_C} \quad (1)$$

$$RLI = \frac{L_T(i) - L_C}{L_C} \quad (2)$$

where $N_T(i)$ and $N_C$ represent the number of germinated seeds in test (i) and in control, and $L_T(i)$ and$L_C$ refer to the mean root length in test (i) and incontrol respectively.

The results were processed statistically using Excel 2010, determining: arithmetic mean and arithmetic mean error; significance of differences according to Student's t-test.

## RESULTS

The results of the study are presented in Table **1**.

**Table 1.Test-indicators of *L. sativum.***

| Option | Germination energy, % | Germination, % | Length, mm | | SGI | RLI |
|---|---|---|---|---|---|---|
| | | | Aboveground part | Roots | | |
| I (control) | 83.3 ± 6.7 | 90.0 ± 0.0 | 33.9 ± 1.9 | 10.2 ± 0.7 | 0.00 | 0.00 |
| II | 90.0 ± 5.8 | 90.0 ± 5.8 | 28.4 ± 2.1 | 7.4 ± 0.8* | 0.00 | − 0.28 |
| III | 83.3 ± 12.0 | 83.3 ± 12.0 | 32.2 ± 2.8 | 9.9 ± 1.3 | − 0.07 | − 0.04 |
| IV | 90.0 ± 0.0 | 90.0 ± 0.0 | 34.0 ± 1.3 | 8.9 ± 0.9 | 0.00 | − 0.13 |

Note: the differences are significant * compared to the control at $p \leq 0.05$

It was found that in the presence of lead salt, the growth of garden cress roots is significantly inhibited. The solution with $Pb^{2+}$ shows pronounced toxicity, inhibition of growth of almost 30%. The addition of *B. velezensis* strain NUChC C2b liquid cultureto such a solution reduces its toxicity. Thus, with the complex action of $Pb^{2+}$ and *B. velezensis* there is a slight toxic effect, a slight inhibition of the growth of the test plant. However, it should be noted that the differences between the test-indicators in options III and IV compared to the control are statistically insignificant, so the indicators are within

the control. It may be necessary to use a more concentrated *B. velezensis* strain NUChC C2b liquid culture, with higher concentration ofsiderophore, for greater binding efficiency of $Pb^{2+}$ ions. In addition, its effectiveness can be increased by combination with other bioremediation agents.

## CONCLUSION

The liquid culture of *B. velezensis* strain NUChC C2b has the ability to reduce the toxicity of a solution with a high concentration of $Pb^{2+}$, apparently due to the presence of sider ophore bacillibactin. Therefore, the concentrated liquid culture of *B. velezensis* strain NUChCC2b can be used to bioremediate environment contaminated with $Pb^{2+}$, in combination with stronger bioremediation agents.

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