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EFFECT OF Pb^{2+} IONS ON THE SYNTHESIS OF THE EXOPOLYSACCHARIDE COMPLEX BY *GORDONIA RUBRIPERTINCTA*

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Today, the issue of biosphere contamination with heavy metal salts is one of the key challenges for the safety and preservation of ecosystems. Among these, lead is particularly notable as a toxic metal that enters water and soil on a massive scale through industrial emissions, mineral extraction, and the consequences of armed conflicts. Lead does not degrade over time but accumulates continuously in the environment. High concentrations of this metal have a detrimental effect on living organisms. Conventional physicochemical approaches to soil and water detoxification typically require significant financial investment and are accompanied by the generation of hazardous secondary waste, which complicates their disposal. In light of this, bioremediation technologies based on the ability of specific microorganisms to accumulate or neutralize ecotoxicants are emerging as a promising alternative. Bacteria capable of surviving in extreme conditions are of particular interest.

This study investigated the effect of lead loading (specifically, lead ions at concentrations up to 0.6 mg/L) on the adaptive responses of the actinobacterium G. rubripertincta.

It was found that the bacteria exhibit extremely high tolerance to lead ions. The presence of the toxicant at concentrations up to 600 µg/L does not inhibit culture growth, ensuring stable biomass accumulation and colony formation. At the same time, a dose-dependent intensification of exopolysaccharide synthesis was observed. At the maximum toxic load, the concentration of cell-bound exopolysaccharides increased by nearly 1.5-fold, and that of free exopolysaccharides by 5-fold. The extracellular exometabolites of the studied microorganism appear to form a reliable protective barrier that ensures intensive binding and immobilization of Pb2+on the cell surface. An assessment of the dynamics of this process showed that when 6 µg/L and 60 µg/L of the toxicant were introduced into the medium, the bacteria removed 66% and 76% of the lead, respectively. The results confirm the high bioremediation potential of G. rubripertincta under conditions of severe chemical stress. In particular, the identified mechanisms of microbial adaptation open up promising prospects for the development of effective new-generation biopreparations. Such environmentally safe preparations can be successfully applied for the full restoration of agricultural soils and the purification of industrial wastewater from heavy metal ions. Further fundamental research will logically focus on a detailed study of the genetic determinants of metal resistance in this valuable strain and the optimization of conditions for its large-scale cultivation.

Keywords: Gordonia rubripertincta, lead ions, exopolysaccharides, bioremediation, heavy metals

Introduction. Lead is a heavy metal that is a natural component of the lithosphere, but intense anthropogenic pressure – driven by its widespread use due to specific physicochemical properties like softness, malleability, and high corrosion resistance – leads to its dangerous and uncontrolled accumulation in biogeocenoses. The main sources of environmental lead pollution are the mining industry, metallurgy, battery production, as well as the incineration and disposal of electronic waste (Tchounwou, 2012; Zhou, 2020). In addition, a significant amount of lead has already accumulated in soils since the days of active use of leaded gasoline and lead-based paints (Needleman, 2004). In the context of modern armed conflicts, munitions remnants and destroyed industrial infrastructure also become major sources of pollution, leading to localized extreme contamination of soils and groundwater with heavy metals (Certini, 2013).

Due to its inability to biodegrade, this element forms long-term reservoirs in the pedosphere, hydrosphere, and bottom sediments, gradually migrating through food chains (Kumar, 2020). Its presence in the soil disrupts the normal functioning of the microbiome, reduces fertility, and significantly inhibits plant growth and development (Pourrut, 2011; El Khattabi, 2025). For animals and humans, lead is a highly toxic element. It can accumulate in bone tissue, cause severe neurological disorders, disrupt the functioning of the cardiovascular and urinary systems, and negatively affect reproductive function (Wani, 2015).

The cytotoxicity of Pb^{2+} is largely due to their structural similarity to biogenic metals, which allows them to replace the latter in metabolic pathways. It binds strongly to the sulfhydryl (-SH) groups of proteins, which inevitably leads to their denaturation and complete inactivation of enzymes (Flora, 2012).Additionally, lead ions trigger the formation of excessive amounts of free radicals. This causes severe oxidative stress, which damages cell membrane lipids and DNA molecules (Ercal, 2001; Matović, 2015).

Since traditional physicochemical methods for removing lead from the environment (such as soil washing or chemical precipitation with reagents) are economically costly and often irreversibly destroy the natural structure of ecosystems, modern science is actively seeking alternative solutions (Dixit, 2015). This is why the use of microorganisms capable of surviving under conditions of extreme lead stress and binding this toxic metal is becoming one of the most promising and environmentally safe approaches to bioremediation (Dixit, 2015).

Therefore, the aim of our study was to investigate the adaptive responses of *Gordonia rubripertincta* bacteria to the toxic effects of lead ions by assessing the intensity of exopolysaccharide synthesis.

Materials and Methods. The study utilized a pure culture of *Gordonia rubripertincta*, kindly provided by the D. K. Zabolotny Institute of Microbiology and Virology of the National Academy of Sciences of Ukraine.

To obtain seed material, *G. rubripertincta* was cultured in a standard liquid medium – meat-peptone broth (MPB) – for 24 hours at 37 °C.

Primary incubation was carried out in 250-ml glass flasks with a working liquid volume of 50 ml. For this purpose, 45 ml of nutrient medium (MPB) and 5 ml (10% of the volume) of a one-day-old bacterial inoculum were added to each flask. Cultivation was carried out for 3 days at 37 °C with periodic stirring (100 rpm). The experiments were conducted in 4 biological replicates.

To comprehensively study the response of *G. rubripertincta* to xenobiotic stress, a solution of lead nitrate was added to the experimental flasks across a wide range of concentrations – from ecologically relevant to extremely toxic levels. In general, the tested variants were divided into the following groups: a baseline group with the maximum permissible concentration for fishery water bodies (Snizhko, 2021) of 6 μg/L Pb^{2+}, a group with a moderate concentration of 60 μg/L Pb^{2+},and high – 600 μg/L Pb^{2+}.

The differential centrifugation method was used to separate bacterial biomass and extracellular metabolites. The culture medium was transferred to centrifuge tubes. To break up massive conglomerates of bacteria and lead crystals (bioflocs), warm saline solution (0.9% NaCl) was added to the samples, and the samples were subjected to vigorous vortex mixing. This was followed by low-speed centrifugation at 1000 rpm for 5 minutes to precipitate heavy lead salt crystals. The resulting supernatant was carefully collected and centrifuged again at 4000 rpm for 15 min to precipitate *G. rubripertincta* cells. The cell-free culture supernatant was used for further analysis of exometabolites.

Quantification of exopolysaccharides (EPS) was performed using a colorimetric assay with anthrone reagent (Dreywood, 1946). After cooling the samples in cold water, the optical density of the solutions was measured on a spectrophotometer at a wavelength of 620 nm. The EPS concentration was calculated using a calibration curve.

The concentration of residual Pb^{2+} in the culture medium was determined using atomic absorption spectrophotometry.

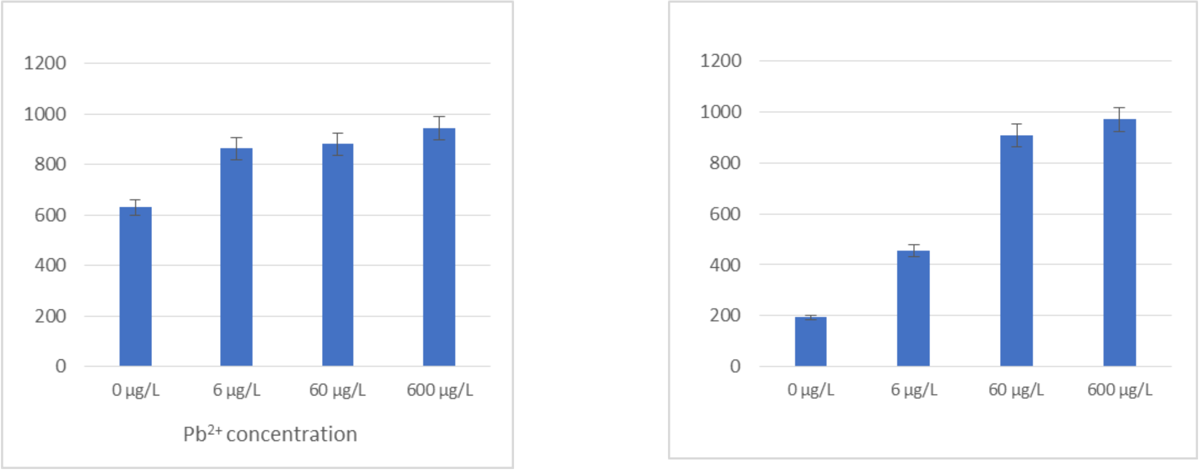
Statistical analysis of the data was performed using licensed Microsoft Excel software.

Results and Discussion. As a toxicant, lead is capable of causing a wide range of adverse effects. Throughout the experiment, the overall effect of the stressor on the physiological activity of the bacterial culture was investigated. The intensity of cell proliferation and biomass accumulation was assessed by daily measurement of the optical density of the culture medium at a wavelength of 540 nm. As the experimental data show, the bacteria demonstrated extremely high tolerance to heavy metal ions. Notably, the addition of lead nitrate to the culture medium did not inhibit growth. It was observed that even under conditions of significant toxic load at 100 MPC (600 μg/L), the optical density of the culture at the final stage of cultivation was practically indistinguishable from control values. Individual screening measurements at extremely high concentrations (up to 60,000 μg/L) also confirmed the culture's ability to grow.

The viability of cells under conditions of pollutant exposure was confirmed by counting colony-forming units (CFU/mL). It was noted that in the control variant, the indicator averaged 8.52×10^6 CFU/mL. Under conditions of increasing toxic load, the cell count remained consistently high within the range of $8.22\text{-}8.29 \times 10^6$ CFU/mL, indicating the absence of a pronounced bactericidal effect of lead on this culture.

Clearly, such a high level of resistance is due to the effective functioning of the microorganism's defense systems. As researchers note (Arenskötter, 2004; Álvarez, 2017), primary resistance to xenobiotics is largely ensured by the specific architecture of the cell wall and the presence of mycolic acids, which form a dense hydrophobic barrier. This barrier significantly slows the diffusion of toxic lead cations into the intracellular space, giving the bacteria time to activate the synthesis of the exopolysaccharide matrix (Gupta, 2017; Tiquia-Arashiro, 2018).

When assessing the level of cell-bound EPS (Fig. A), a pronounced dose-dependent effect was observed – their concentration increased from 1.37 (in the presence of 6 μg/L Pb^{2+} in the culture medium) to 1.5 (with 600 μg/L Pb^{2+} in the culture medium) of the control values. Presumably, thickening of the polysaccharide capsule serves as the basic defense mechanism. As a result, the cells are able to physically and chemically immobilize heavy metal ions directly on their surface, preventing them from entering the cell.



A ***B***

Fig. Level of G. rubripertincta exopolysaccharides under conditions of Pb2+addition to the culture medium

Note: A – cell-bound EPS; B – free EPS

To gain a comprehensive understanding of the strain's adaptive response, the fraction of free EPS (Fig. B) – which the bacteria secrete directly into the culture medium – was studied in parallel. Under conditions of 6 μg/L Pb^{2+} addition, this parameter increased 2.35-fold, and with a further increase in lead concentration, it increased fivefold compared to control values.

The obtained data suggest that under conditions of increasing stress, the bacterium hypersecretes mucus into the environment, creating a kind of buffer zone to bind lead cations at a distance, significantly reducing their bioavailability and the overall toxicity of the solution. These results are fully consistent with current scientific understanding of the mechanisms of microbial adaptation to conditions of chemical stress. It is known from the literature that in response to the appearance of heavy metal salts in the environment, bacteria specifically increase the secretion of exopolysaccharides (Gupta, 2017). Due to their high content of negatively charged functional groups (in particular carboxyl and phosphate groups), these polymeric molecules act as powerful biosorbents (Gupta, 2017). They effectively bind positively charged lead cations directly in the extracellular matrix, preventing damage to the plasma membrane (Gupta, 2017).

In the final stage of the research, the concentration of lead remaining in the solution after cell separation was assessed. The experimental data obtained indicate that *G. rubripertincta* has high biotechnological potential for purifying the environment of heavy metal compounds. A comparative analysis of the initial lead concentrations and the amount remaining in the supernatant after cultivation allowed us to identify patterns in the Pb^{2+} ion removal process. The highest bioaccumulation efficiency is observed under low and medium loading conditions – the cells remove 66% (6 μg/L) and 76% of lead ions from the solution, respectively; thereafter, the purification efficiency percentage decreases slightly.

Conclusions. The results confirm that the exopolymer complex *of G. rubripertincta* acts as a powerful barrier that ensures bacterial stability and allows for the effective removal of Pb^{2+} even in heavily contaminated ecosystems. This opens up real prospects for the development of new, environmentally safe bioremediation technologies.

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ВПЛИВ ІОНІВ PB^{2+} НА СИНТЕЗ ЕКЗОПОЛІСАХАРИДНОГО КОМПЛЕКСУ *GORDONIA RUBRIPERTINCTA*

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На сьогодні питання забруднення біосфери солями важких металів є одним із ключових викликів для безпеки та збереження екосистем. Серед них особливе місце посідає свинець – токсичний метал, який масово потрапляє у воду та ґрунти через промислові викиди, видобуток копалин та наслідки збройних конфліктів. Свинець не розкладається з часом, а постійно накопичується у довкіллі. Високі концентрації цього металу згубно впливають на живі організми. Класичні фізико-хімічні підходи до детоксикації ґрунтів і вод зазвичай потребують значних фінансових вкладень та супроводжуються генерацією небезпечних вторинних відходів, що ускладнює їх утилізацію. З огляду на це, перспективною альтернативою стають технології біоремедіації, що базуються на здатності специфічних мікроорганізмів акумулювати або нейтралізувати екотоксиканти. Особливу увагу привертають бактерії, що здатні виживати в екстремальних умовах.

У роботі досліджено вплив свинцевого навантаження (зокрема, іонів плюмбуму у концентрації до 0,6 мг/л) на адаптивні реакції актинобактерій G. rubripertincta.

Встановлено, що бактерії проявляють надзвичайно високу толерантність до іонів плюмбуму. Наявність токсиканта у концентраціях до 600 мкг/л не інгібує ріст культури, забезпечуючи стабільне накопичення біомаси на рівні та формування колоній. При цьому констатовано дозозалежну інтенсифікацію синтезу екзополісахаридів. За максимального токсичного навантаження концентрація клітинно-зв'язаних екзополісахаридів зростає майже в 1,5 рази, а вільних – у 5 разів. Позаклітинні екзометаболіти досліджуваного мікроорганізму, очевидно, формують надійний протекторний бар'єр, який забезпечує інтенсивне зв'язування та іммобілізацію Pb2+ на поверхні клітин. Оцінка динаміки цього процесу засвідчила, що за умов внесення у середовище 6 мкг/л та 60 мкг/л токсиканта бактерії вилучають із середовища 66% і 76% плюмбуму відповідно. Отримані результати підтверджують високий біоремедіаційний потенціал G. rubripertincta за умов жорсткого хімічного стресу. Зокрема, виявлені механізми мікробної адаптації відкривають багатообіцяючі перспективи для розробки ефективних біопрепаратів нового покоління. Такі екологічно безпечні препарати можуть бути успішно застосовані для повноцінного відновлення сільськогосподарських ґрунтів та очищення стічних вод промислових підприємств від іонів важких металів. Подальші фундаментальні дослідження будуть логічно спрямовані на детальне вивчення генетичних детермінант металорезистентності цього цінного штаму та оптимізацію умов його масового культивування.

Ключові слова: Gordonia rubripertincta, іони плюмбуму, екзополісахариди, біоремедіація, важкі метали

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