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STUDY OF PHENOTYPIC PROPERTIES OF CHEMOLITHOTROPHIC ACIDOPHILIC BACTERIA ISOLATED FROM TECHNOGENIC WASTES

Изучены физиолого-биохимические и практически полезные свойства ацидофильных хемолитотрофных бактерий, изолированных из техногенних отходов углеобогащения с высоким содержанием тяжелых металлов. По культурально-морфологическим свойствам изученные штаммы практически не отличались, однако выявлены отличия в скорости роста, накоплении биомассы и окислению энергетических субстратов. Результаты изучения основних биологических свойств ацидофильных хемолитотрофных бактерий позволили предварительно отнести их к роду Acidithiobacillus, новым пердставителям Acidithiobacillus ferrooxidans и Acidithiobacillus thiooxidans. Все изученные штаммы были способны выщелачивать металлы из техногенних отходов; степень извлечения металлов зависела от штамма и метала. Отобранны наиболее эффективные штаммы для использования в процессах биовыщелачивания.

In biotechnological processes the leaching of metals using bacteria from different physiological groups; often this is chemolithotrophic acidophilic bacteria (ACB) as mesophilic as moderately thermophilic. They have a number of useful features such as the ability to use energy simultaneously several inorganic compounds (sulfur, compounds of sulfur, ferrous iron, natural and industrial raw materials containing metals), resistance to heavy metals, the ability to grow at low pH and in a wide range of temperatures.

The object of work is study the basic biological and practically useful properties ACB that extracted from the waste heaps of Central Processing Plant of Lvov-Volyn coal basin.

Materials and methods. It have been studied by using classic and modern microbiological and physico-chemical methods the basic properties of acidophilic chemolithotrophic bacteria assigned to base of their systematic – Gram strain - range and optimum of temperature and pH - relation to different energy sources (iron, sulfur, compounds of sulfur); - the ability to grow in autotrophic and mixotrophic conditions; - resistance to heavy metals; - the leaching of metals [Karavayko, 2006; Loginov, 2011]. The strains have been cultivated and supported on liquid and agar medium of 9K, as the energy used ferrous iron in concentrations 12,0 g/l and 44,5 g/l, and thiosulfate in concentration 5,0 g/l.

Results and discussion. Established that culture which studied almost haven't different from each other and had the appearance of small, short, straight sometimes slightly curved single, arranged in pairs or short chains of rod-shaped cells, spores haven't form, the Gram have stained negative. The base of taxonomy of thiobacteria have been founded on attitude to the energy sources. On this basis the culture studied were conditionally divided by 2 groups. The strains of 1 group oxidized ferrous iron, sulfur, thiosulphate; the culture of 2 group which used as a source of energy only sulfur and compounds of sulfur. The cultures 1 and 2 groups growing in autotrophic conditions with different energy sources, on nutrient media with different component and concentration composition - Silverman-Lundhrema 9K, Leten, Waxman, Vyshnyak.

The obtained results confirm the existing literature data that these bacteria have't exactly specified the need for mineral nutrition; they are able to grow in a highly mineralized environments, and at low salt loads. The installed capble of mesophilic ACB the 1 and 2 groups to growth in mixothrophic conditions with additional growth factors the carbohydrate and protein (glucose, molasses, yeast extract) origin in concentrations of 0,02%. But on the full organic media - MRS Gorbenko, FRS - these cultures haven't grown.

ACB cultures that isolated from man-made waste enrichment, resistance a high level of heavy metals. Established that the minimal concentrations of copper, lead, zinc, cadmium, nickel, in which bacteria is growth, several times their content in waste dumps were removed from these cultures. A significant lack of knowledge regarding chemolithotrophic tionic acidophilus bacteria refers to growth of some cultures. Bacteria of the genus *Acidithiobacillus* represent slow-growing microorganisms. This is so they use low energy inorganic substrate - sulfur or ferrous iron. Therefore, the selection of strains that differ at maximum speed of growth and the accumulation of a significant amount of biomass is important for their use in the processes of bacterial leaching of metals. Great importance is not only in amount of biomass, also oxidation activity of energy source is important too. Under the conditions of our research, amount of the biomass that were synthesized by researched bacteria, differ slightly. Comparative analysis of biomass accumulation and oxidation of iron by strains shows that biomass is not correlated with the degree of oxidation of iron. By results of these experiments were selected three most active cultures for use in biotechnological processes of leaching of metals.

All strains that were examined, had the ability to leach metals from waste of technogenic flotation of coal enrichment. The efficiency of leaching of metals depended on strain , metal and source of energy. So, while using ferrous iron ,regardless of the strain - manganese, cadmium and nickel almost completely passed from the solid phase to a solution ; transition of copper, zinc and lead was much smaller and depended on the strain. In the presence of thiosulfate degree of extraction was minimal [Blayda, 2015].

Thus, for the first time, were studied the important properties of mesophilic ACB, that were extracted from technogenic waste of coal flotation enrichment. It was established that the main distinctions were in the nature of strain diversity. Based on the data on use of sources of energy, we can assume identity of cultures that studied to the genus *Acidithiobacillus*. Bacteria, that oxidized sulphur (S^{+2}), thiosulfate ($Na_2S_2O_3$) and divalent iron compounds ($FeSO_4$) , conventionally assigned to *Acidithiobacillus ferrooxidans* (Group 1) and ,those that oxidized only sulfur and thiosulfate according to *Acidithiobacillus thiooxidans* (Group 2). Molecular genetic analyzes (PCR) are conducted for the additional detection of studied microorganisms and features of strains. Studied strains, joined unique collection of practically useful ACB bacteria, that actively leaches the metals from mineral technogenic origin. The results will allow to use active strains in the process of bioleaching in future and also in obtaining of bacterial preparation to improve the extraction of metals from minerals.

References

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