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OCCURRENCE OF CU^{2+} -MICROORGANISMS ON ANTARCTIC ISLAND GALINDES

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Abstract. Copper-resistance of Antarctic microbial communities selected from different samples (sediments, plants etc.) was investigated at Cu^{2+} concentration 5, 25, 100 ppm. It was shown that all samples contain Cu^{2+}-resistant microorganisms in large amount about 10^4–10^7 CFU/g of sample. There were found 15 morphological types of bacteria, 3 types of actinomycetes, 5 types of fungi. For microorganisms of major part of samples (65%) 100 ppm Cu^{2+} did not show inhibition effect.
Key words: copper-resistance, Antarctic microbial communities.

Резюме. Було досліджено стійкість антарктичних мікробних угруповань, виділених з різних типів проб (осади, рослинність та ін.) до катіону Cu^{2+} у концентраційному діапазоні 5, 25 та 100 мг/л. Показано, що у вивчених пробах стійкі до міді мікроорганізми мають великий титр, який становить 10^4–10^7 колонійутворюючих одиниць/г проби. Розмаїття мідьрезистентних морфотипів для бактерій складає 15, актиноміцетів – 3, грибів – 5. Для мікроорганізмів більшості проб (65%)концентрація 100 мг/л Cu^{2+} не проявляла бактерицидної дії.

Introduction

Copper is one of the most toxic heavy metals for microorganisms. For such strains as Micrococcus sp. Rhodospirillum photometrcum (Андреюк, 1992) 0.02 – 0.1 ppm Cu^{2+} causes strong growth inhibition and for Pseudomonas putida, (Mishra, 1999) Klebsiella sp. (Rudd, 1983) concentration of Cu^{2+} above 0.5 ppm leads to germinate effect. One of the exceptions is Cu-resistant Thiobacillus which use sulfur Cu-containing sulfide minerals as the source of electrons (Панин, 1982, Rodricuez, 1985). Microbial communities are more resistant to influence of copper then isolated strains because they realize a complex of protective mechanisms; Microorganisms of industrial ecosystems are more resistant to Cu because of adaptation effects (Андреюк, 2001).

During the 8^{th} seasonal expedition to the Ukrainian Antarctic Station 'Academic Vernadsky', which is situated on Galindes island 63 samples (soils, stones, ice, water, plants etc.) were selected. As far as Antarctic microorganism exist in extreme conditions (low temperature during major part of the year, absence of free water) we decided to characterize their resistant to other extreme factors. In addition, it is known that rocks which form Antarctic island contain high concentrations of heavy metals, including copper (Войткевич, 1977). The objective of our study was to investigate the influence of Cu^{2+}, one of the most toxic heavy metals, on growth and homeostasis of Antarctic island (but not marine) microbial communities, find Cu^{2+}-resistant microorganisms. Information acquired in this way is important for development of our still very limited knowledge of complex characteristic of Antarctic microbial communities.

Materials and methods

Getting samples

Samples were selected from the most typical for the Antarctic islands landscapes: at the top of the rocks, soils, that contain high concentration of organic compounds, small lakes, lichens and moss fields. Samples were dried at 18–20^0C and packed in hermetic plastic bags.

Inoculate preparation

1 g of every dried sample was homogenized and suspended in 100 ml of sterile saline solution (0.9% NaCl) and vigorously shaken for 5 min. Than sample solution was put in 20 sterile flacks (V=5 ml) and stored in refrigerator at –25^0C until inoculation. The necessity of storing Antarctic samples solution in refrigerator can be explained that they contain a large number of psychrophyllic and psychrotolerant microorganisms. Storing inoculate at higher temperature could cause succession in microbial communities that would lead to results which are not correct.

Preparation of Cu^{2+} solution

For preparation of Cu^{2+} solution we used chemical pure $CuSO_4$ and suspended it in sterile distillate water in such amount that final concentration of Cu^{2+} was 1000 ppm. Solution was sterilized by boiling 30 min., than cooled to 25°C and added to nutrient medium (meat broth with 2% of agar) to final concentration 5, 25 and 100 ppm of Cu^{2+}.

Cultivation of microorganisms

Microbial growth was observed by determination of CFU (colony forming units) on solid medium meat broth with 2% of agar in Petri dishes at 15^0C. Results were counted at 3, 5 and 7 days of cultivation. We used medium for organoheterotrophic microorganisms because it can show the largest diversity of microorganisms that form microbial community. Cu^{2+} was used as selective factor to find it's germinate concentration for Antarctic microorganisms.

Bacteriological counts

A number of Cu^{2+}-resistant microorganisms was counted by formula:

$M=N \cdot 1^n \cdot K$,

where M – number of colony forming units (CFU) per 1g of sample, N – number of CFU on Petri dishes, n – dilution of sample, K – humidity coefficient of sample (as far as samples were dried, $K = 1{,}02$).

Results and discussions

It has been shown that all investigated samples contain a large diversity of Cu^{2+}-resistant heterotrophic microorganisms (Fig. 1, 2) (Fig. 1-4 see the color paste). There were found 15 morphological types of bacteria, 3 types of actinomycetes, 5 types of fungi.

It was observed that there growing concentrations of Cu^{2+} can have different impact on diversity of Antarctic microorganisms. For 30% of samples growing concentrations of Cu^{2+} caused decreasing of microbial diversity (Fig 3.). For example for microorganisms selected from sample T19 growing at 5 ppm Cu^{2+} microbial diversity 4 morphological types while at 25 and 100 ppm Cu^{2+} there is only one type. For 70% of samples different concentrations of Cu^{2+} do not influence on microbial diversity (Fig. 4). In sample T35 bacteria are represented with 1 morphological type at all concentrations of Cu^{2+}.

It was shown that 34 investigated Antarctic samples contain Cu^{2+} -resistant microorganisms in large amount. It doesn't depend on the type of sample and is about 10^4–10^7 CFU/g of sample (Table).

Table.

Bacteriological counts of Cu^{2+}-resistant Antarctic microoganisms

Sample №	Cu^{2+}-resistant CFU/ g of sample, Cu^{2+} concentration in growth medium			Sample type
	5 ppm	25 ppm	100 ppm	
T19	$5{,}6 \cdot 10^5$	$1{,}6 \cdot 10^5$	$1 \cdot 10^4$	sludge
T43	$1{,}3 \cdot 10^5$	$1{,}1 \cdot 10^4$	$1{,}1 \cdot 10^4$	soil
T44	$1{,}1 \cdot 10^5$	$8 \cdot 10^4$	$1{,}1 \cdot 10^4$	soil
T48	$6{,}9 \cdot 10^5$	$4{,}3 \cdot 10^5$	$1{,}6 \cdot 10^5$	soil
T50	$4 \cdot 10^4$	$2 \cdot 10^4$	absent	sludge
T51	$7{,}3 \cdot 10^5$	$5{,}4 \cdot 10^5$	$2{,}9 \cdot 10^5$	sludge
T52	$1{,}8 \cdot 10^5$	$2{,}1 \cdot 10^5$	absent	sludge
T53.1	$3{,}5 \cdot 10^5$	$3{,}4 \cdot 10^5$	$1{,}1 \cdot 10^4$	sludge
T56.1	$6{,}0 \cdot 10^5$	$3{,}0 \cdot 10^5$	$2{,}1 \cdot 10^4$	lichens
T60	$3{,}8 \cdot 10^5$	$3{,}7 \cdot 10^5$	$2{,}1 \cdot 10^4$	moss
T63	$2{,}0 \cdot 10^5$	$6{,}1 \cdot 10^4$	absent	moss
T20	$2{,}1 \cdot 10^7$	$2{,}1 \cdot 10^7$	$2{,}1 \cdot 10^7$	soil
T32	$2{,}1 \cdot 10^7$	$2{,}1 \cdot 10^7$	$2{,}1 \cdot 10^7$	moss
T33	$2{,}1 \cdot 10^7$	$2{,}1 \cdot 10^7$	$2{,}1 \cdot 10^7$	sludge
T54	$2{,}1 \cdot 10^7$	$2{,}1 \cdot 10^7$	$2{,}1 \cdot 10^7$	lichens
T57	$2{,}1 \cdot 10^7$	$2{,}1 \cdot 10^7$	$2{,}1 \cdot 10^7$	lichens
T36	$7{,}1 \cdot 10^4$	$1{,}6 \cdot 10^5$	$2{,}2 \cdot 10^7$	soil
T59	$8{,}1 \cdot 10^4$	$1{,}0 \cdot 10^5$	$2{,}3 \cdot 10^7$	soil

There were found differences in germinate concentrations of Cu^{2+} for microorganisms selected from different samples. By criteria of germinate concentration of copper samples can be divided into 4 groups. For the first group germinate concentration of Cu^{2+} is 5 ppm. This group is represented with 1 sample (3% from total amount of samples). Second group – germinate concentration 25 ppm (7% of samples); 3rd – germinate concentration 100 ppm (25%). The fourth group was the biggest one represented with 23 samples (65%) – concentration 100 ppm Cu^{2+} had no influence on microbial growth. So, microorganisms selected from majority samples were resistant to 100 ppm Cu^{2+}. Dispersion of sensitive and resistant microorganisms is shown on Fig.5.

Usually, number of samples that contain metal-resistant microorganisms decreases with increasing of metal concentration (Fig. 5A). Unlike these, major part of Antarctic samples contain Cu^{2+}-resistant microorganisms (67%), while sensitive are in minor (in general – 23%) (Fig 5 B).

Conclusion

1. Copper-resistance is wide spread for heterotrophic microorganisms of Galindes island – Cu^{2+}- resistant microorganisms are selected from almost all samples.

2. Antarctic microbial communities show strong copper-resistance, wide diversity and large amount at Cu^{2+}-resistant CFU/g of sample concentration Cu^{2+} concentration 5–100 ppm.

It can be concluded that such data perform scientific interest and need further investigation.

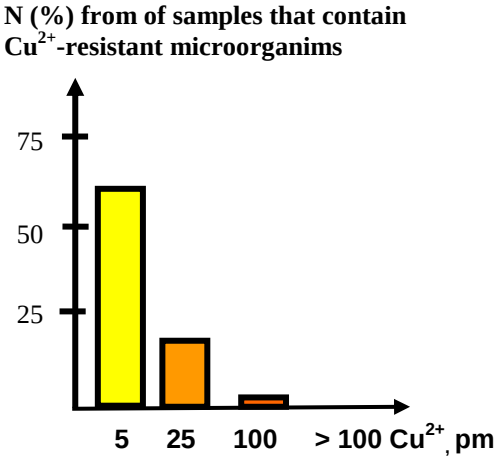

Fig.5A. Typical dispersion of Cu^{2+}-resistant microorganisms

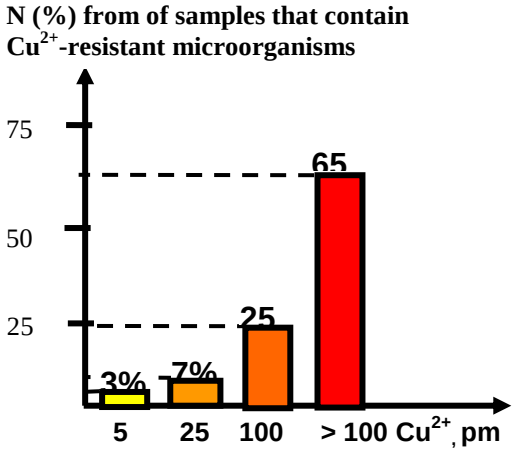

Fig.5B. Dispersion of Cu^{2+}-resistant microorganisms selected from Antarctic samples

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