Resistance of Actinobacteria of the genus *Gordonia* to potentially toxic metals

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Abstract

Effects of potentially toxic metals (Cd, Cr, Cu, Mo, Ni, Pb, Zn) on the strains of alkanotrophic Actinobacteria of the genus *Gordonia* were studied. The strains were isolated from the samples of oil-contaminated soils and wastewater. Collection strains of *Gordonia* spp. highly resistant to potentially toxic metals (up to 40.00 мM) were selected. These strains are recommended for use in technologies for cleaning wastewater and water bodies contaminated with potentially toxic metals.

Keywords: *Gordonia*, Actinobacteria, bioremediation, potentially toxic metals

Introduction

According to N. Reimer's classification, metals with a density of more than 8 g/cm^3 and a weight of more than 50 atomic units should be considered as potentially toxic metals (PTMs). Environmental pollution by PTMs is one of the most common and environmentally hazardous types of anthropogenic pollution for living organisms. The production of many industries is accompanied by the uncontrolled supply of non-degradable and highly toxic wastes containing high concentrations of PTMs into natural ecosys-tems (Beretta 2018).

Industrial wastewater from chemical, textile, engineering, electrotechnical plants, non-ferrous metal-lurgy and other industries is contaminated with salts of non-ferrous and potentially toxic metals (Zaynutdinova 2013). Wastewater is most frequently polluted with zinc, cadmium, copper, chromium, nickel, mercu-ry, iron compounds, less often with cobalt and manganese. Contaminated wastewater generally con-tains a mixture of several PTMs (Berengarten 2008; Varaeva 2016).

Traditional ways of wastewater treatment from PTMs are based on the use of physicochemical methods (Sokolov 2015), which are not environmentally safe and do not provide complete removal of PTM ions from wastewater (Elizarova 2016). Modern approaches involve the use of biological methods of puri-fication, such as aquatic plants, and bioreactors combined with immobilized microorganisms (Erum 2015; Shekhar 2015). In this connection, researchers are attracted by Actinobacteria with a wide range of functional capabilities, like utilization of natural and anthropogenic hydrocarbons as a carbon source, degradation of pharma pollutants, oil products and other emergent pollutants, as well as the production of active biosurfactants and complexes that facilitate mobilization and desorption of PTM ions.

The purpose of this work is to analyze the resistance of alkanotrophic Actinobacteria of the genus *Gordonia* to potentially toxic metals.

Methods

The working collection included 98 strains of Actinobacteria from the Regional Specialized Collection of Alkanotrophic Microorganisms (www.iegmcol.ru) representing the species *Gordonia alkanivorans* (2 strains), *G. amicalis* (8 strains), *G. rubripertincta* (59 strains), and *G. terrae* (29) (tab. 1). The strains were previously isolated from oil- and PTM contaminated soils and waters. The following salts were used in this work:

$CuSO_4x5H_2O$, $(CH_3COO)_2Cdx2H_2O$, K_2CrO_4, $(NH_4)_6Mo_7O_{24}x4H_2O$, Ni-SO_4x7H_2O, $Pb(NO_3)_2$, $ZnSO_4x7H_2O$. Salt concentrations ranging from 0.08 to 80.00 mM in terms of pure metal. The degree of Actinobacterial resistance to PTMs was evaluated based on the minimum inhibitory concentrations (MICs) determined by the microwell method (Kuyukina 2001). For this, 100 µl of nutrient broth were aseptically applied to each well of a 96-well immunological plate, and aque-ous solutions of PTM salts were serially diluted. As inocula, 48 h cultures of Actinobacteria pre-grown on the meat infusion agar were used. Bacterial suspensions (from 1.0 to 2.0 × 10^9 cells/ml) were pre-pared in a sterile saline solution (0.5% NaCl solution) and added to the wells containing the limiting dilutions of HMs. The plates were incubated in a thermostat at 28° C for 48 h. Once the incubation period was over, 50 µl of 0.2% iodonitrotrozolium chloride aqueous solution (Sigma, United States) was added to each well. This dye is known to act as a competitive oxygen acceptor of electrons in the electron transport chain of aerobic organisms. When the dye is introduced into the medium, it is re-duced to water-insoluble formazan, which manifests itself in a few minutes as red-violet staining only in the presence of actively respiring microorganisms. The controls used were (1) a bacterial culture grown in the nutrient medium without adding the metals, and (2) a non-inoculated nutrient medium. The experiments were carried out in quadruplicates.

Results

According to our data, all cultures retained the ability to grow in the presence of PTM ions. MIC values ranged from 0.08 to 40.0 mM.

Figure 1 presents the data obtained by the cluster analysis. They illustrate the distribution of the bacte-rial cultures in clusters depending on the degree of resistance to PTMs. As seen from the dendrogram, the strains tested are combined into four groups. Group D consists of strains with intragroup similarity up to 5 standard units, and the level of resistance ≤ 1.25 mM. Group C includes strains resistant to HMs in the range of 1.25 – 2.50 mM. Group B strains resist to PTM concentrations from 2.50 to 5.00 mM. Strains with the highest resistance are combined into group A (MIC ≥ 5.00 mM).

Based on the intraspecific degree of resistance to PTM ions, they can be presented in a row: *G. amicalis* > *G. terrae* > *G. rubripertincta* > *G. alkanivorans*, where the most resistant are *G. amicalis, less re-sistant are G. alkanivorans.*

Table 2 presents the average data on intraspecific resistance to PTMs (mM).

Table 1 *Strains of Gordonia spp. used in the experiments.*

Species	Strains
G. alkanivorans	IEGM 748, 1269
G. amicalis	IEGM 726, 768, 1266, 1273, 1274, 1275, 1277, 1279
G. rubripertincta	IEGM 95T, 96, 97, 98, 99, 100, 101, 102, 103, 104, 105, 106, 109, 110, 111, 112, 113, 114, 115, 116, 117, 118, 119, 120, 121, 122, 123, 124, 126, 127, 128, 129, 131, 132, 133, 134, 135, 137, 138, 139, 140, 141, 142, 518, 721, 722, 723, 724, 725, 728, 730, 731, 732, 733, 734, 747, 749, 761, 1136
G. terrae	IEGM 108, 130, 136, 143T, 144, 145, 146, 147, 148, 149, 150, 151, 152, 153, 154, 155, 156, 157, 158, 159, 160, 161, 162, 163, 164, 586, 735, 751, 1123

Table 2 *Resistance of Gordonia species to PTMs.*

Species	Cd^{2+}	CrO_4^{2-}	Cu^{2+}	MoO_4^{2-}	Ni^{2+}	Pb^{2+}	Zn^{2+}
G. alkanivorans	0.40± 0.24	2.82± 2.19	2.50± 0.0	1.57± 0.94	1.88± 0.63	10.00± 0.0	0.67± 0.59
G. amicalis	0.92± 0.44	4.69± 2.32	3.59± 1.46	2.34± 0.41	4.06± 1.21	16.25± 6.5	5.12± 3.37
G. rubripertincta	0.51± 0.58	2.94± 1.85	2.34± 1.19	2.32± 1.36	2.21± 1.77	6.86± 6.0	1.42± 1.12
G. terrae	1.20± 3.58	3.90± 7.13	1.55± 0.61	2.13± 0.88	3.49± 7.03	4.21± 2.26	2.20± 7.18

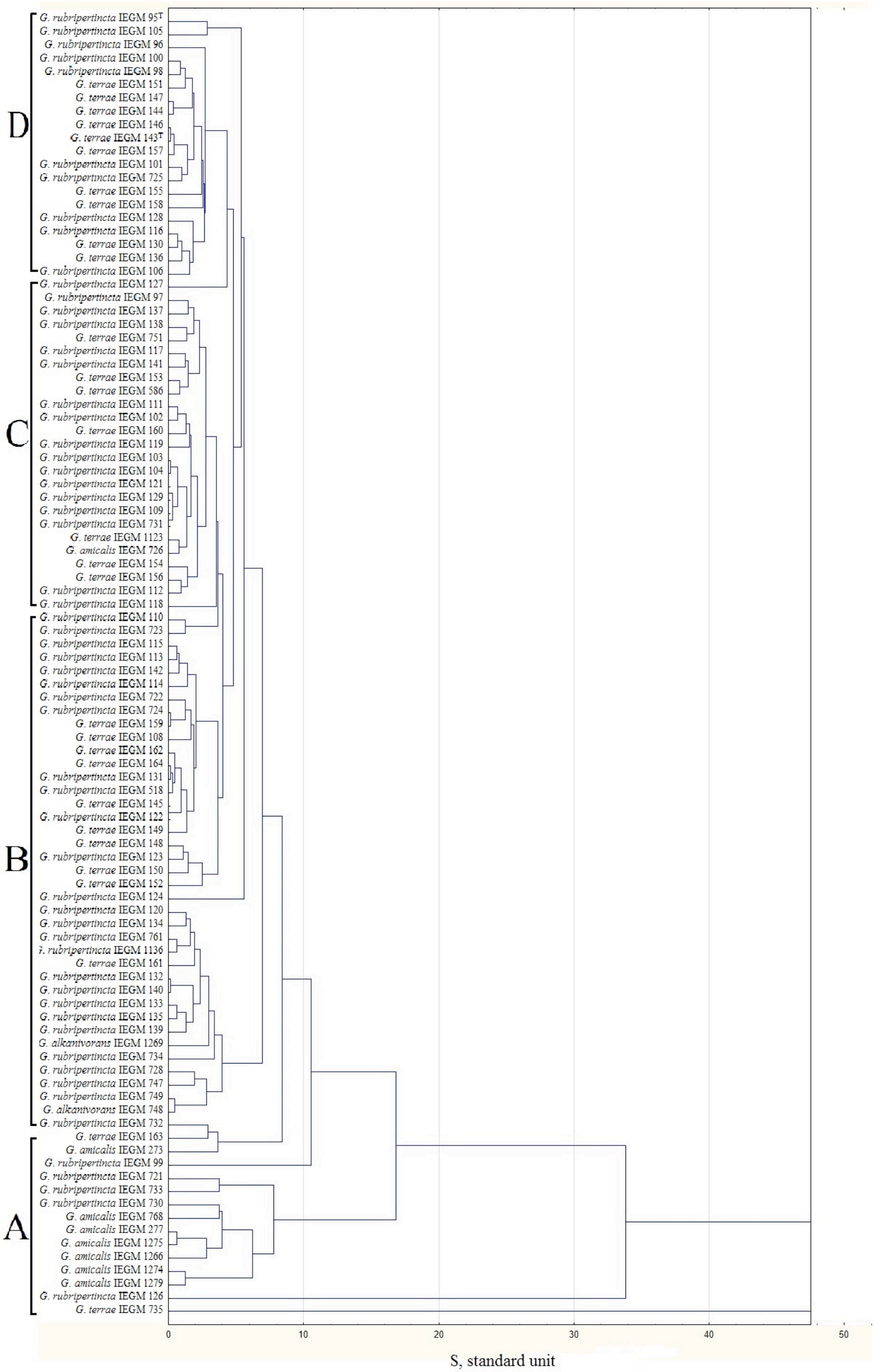

Figure 1 *Dendrogram showing the resistance of the genus Gordonia strains under study to HMs.*

Among the actinobacteria of the genus *Gordonia*, *G. terrae* strains had the highest resistance to PTMs. Compared with *G. rubripertincta*, their resistance to cadmium, chromium, and zinc was 8, 4, and 8 times higher, respectively (tab. 3).

Of 59 *G. rubripertincta* strains, *G. rubripertincta* IEGM 99, 730, and 733 showed high resistance to PTMs. Their MIC values were on average ≥ 10 mM (tab. 4).

Low resistance to PTMs was detected in strains belonging to *G. amicalis and G. alkanivorans*. Their resistance was significantly lower (from 2 to 32 times), compared with *G. terrae* strains. The most resistant strains of these species are presented in Tables 5–6.

Conclusions

In this study, the resistance of *Gordonia* spp. to PTMs was investigated. According to the degrees of toxic effects on the strains studied, PTM ions can be arranged in the following order: Cd^{2+} > MoO_4^{2-} > Zn^{2+} > Cu^{2+} > Ni^{2+} >

***Table 3** Resistance of G. terrae to HMs.*

Strains	PTM	MIC, mM
IEGM 735	$Cd2^{+}$	20
IEGM 735	CrO_4^{2-}	40
IEGM 143^T, 149, 154, 159, 735, 751, 1123	Cu^{2+}	2.5
IEGM 735	MoO_4^{2-}	5
IEGM 735	Ni^{2+}	40
IEGM 161, 735	Pb^{2+}	10
IEGM 735	Zn^{2+}	40

***Table 4** Resistance of G. rubripertincta to HMs.*

Strains	PTM	MIC, mM
IEGM 126, 730, 733	$Cd2^{+}$	2.5
IEGM 732	CrO_4^{2-}	10
IEGM 95T, 105, 730,733, 734, 747, 749	Cu^{2+}	5
IEGM 99	MoO_4^{2-}	10
IEGM 721,733	Ni^{2+}	10
IEGM 126	Pb^{2+}	40
IEGM 99, 124, 730	Zn^{2+}	5

***Table 5** Resistance of G. amicalis to HMs.*

Strains	PTM	MIC, mM
IEGM 1266, 1274, 1275, 1277, 1279	$Cd2^{+}$	1.25
IEGM 1273	CrO_4^{2-}	10
IEGM 1266, 1273, 1274, 1279	Cu^{2+}	5
IEGM 726, 768, 1273, 1274, 1275, 1277, 1279	MoO_4^{2-}	2.5
IEGM 768, 1266, 1273, 1274, 1277, 1279	Ni^{2+}	5
IEGM 768, 1266, 1274, 1275, 1277, 1279	Pb^{2+}	20
IEGM 1266, 1274	Zn^{2+}	10

***Table 6** Resistance of G. alkanivorans to HMs.*

Strains	PTM	MIC, mM
IEGM 748	$Cd2^{+}$	0.63
IEGM 748	CrO_4^{2-}	5
IEGM 748, 1269	Cu^{2+}	2.5
IEGM 748	MoO_4^{2-}	2.5
IEGM 748	Ni^{2+}	2.5
IEGM 748, 1269	Pb^{2+}	10
IEGM 748	Zn^{2+}	1.25

$CrO_4^{2-} > Pb^{2+}$, where cadmium is the most toxic, and lead is the least toxic. The collection strains of *G. alkanivorans* IEGM 748; *G. amicalis* IEGM 1266, 1274; *G. rubripertincta* IEGM 99, 126, 733; and *G. terrae* IEGM 149, 735 with high resistance levels to PTMs were selected. These strains are promising for the development of efficient processes for biotechnological treatment of wastewater and soil contaminated with PTMs.

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