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Zinc bioaccumulation by microbial consortium isolated from nickel smelter sludge disposal site

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Abstract

Heavy metal pollution is one of the most important environmental issues of today. Bioremediation by microorganisms is one of technologies extensively used for pollution treatment. In this study, we investigated the heavy metal resistance and zinc bioaccumulation by microbial consortium isolated from nickel sludge disposal site near Sereď (Slovakia). The composition of consortium was analyzed based on MALDI-TOF MS of cultivable bacteria and we have shown that the consortium was dominated by bacteria of genus *Arthrobacter*. While consortium showed very good growth in the zinc presence, it was able to remove only 15 % of zinc from liquid media. Selected members of consortia have shown lower growth rates in the zinc presence but selected isolates have shown much higher bioaccumulation abilities compared to whole consortium (up to 90 % of zinc removal for NH1 strain). Bioremediation is frequently accelerated through injection of native microbiota into a contaminated area. Based on data obtained in this study, we can conclude that careful selection of native microbiota could lead to the identification of bacteria with increased bioaccumulation abilities.

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Introduction

Elevated concentrations of heavy metals are introduced into the environment through metalliferous mining, metal smelting, metallurgy industry activities, and waste disposal sites. This global environmental problem has developed over the past few decades due to the rapid increase in industrialization and urbanization (Dixit *et al.* 2015).
Some heavy metals are essential elements for the existence of living organisms because they perform several functions in biological systems. These heavy metals such as Ca, Co, Cr, Cu, Fe, K, Na, Ni, and Zn are essential nutrients; some of heavy metals are relatively harmless but can be toxic in larger amounts or certain forms. Essential metals function as catalysts for biochemical reactions are stabilizers of protein structures and bacterial cell walls and serve in maintaining osmotic balance. Many other metals have no biological role (e.g. Ag, Al, Cd, Au, Pb, and Hg) are non-essential and potentially toxic to living organisms, especially microorganisms (Hussein *et al.* 2005). Heavy metals toxicity leads to decreases in biodiversity and productivity and thereby, results changes in structure and function of ecosystems (Mayor *et al.* 2013).

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Heavy metals polluted environments are however a unique source of poly-extremotolerant microorganisms for modern biotechnology and bioremediation applications (Pristas *et al.* 2015).
The available physicochemical methods of metal removal are not only expensive but also produce secondary sludge and are, therefore, not eco-friendly. Hence, the need to remove hazardous trace metals with eco-friendly method is acute (Singh *et al.* 2012).
The opposite of these physicochemical strategies is bioremediation. This process use microbes to transform pollutants in environment into less toxic substances or immobilization in order to reduce their bioavailability. Bioremediation gained more attention recently to clean up the polluted environment and ensures a safe and economical alternative to commonly used physicochemical methods (Guo *et al.* 2010). The activity of microorganisms may lead to adsorption and precipitation of potentially toxic elements by biosorption or bioaccumulation, but also to increase their solubility or formation of volatile compounds with mechanisms such as biovolatilization or bioleaching (Boriová *et al.* 2015).
It is well known that certain species, such as *Pseudomonas* sp., *Escherichia* sp., *Bacillus* sp., *Acinetobacter* sp., and *Enterobacter* sp. can effectively remove metals in pure cultures, but multispecies consortia can better withstand extreme conditions often encountered with industrial waste like spikes of pH or high metal concentration. They provide a microenvironment, which could be very beneficial for metal precipitation. The positive interaction between constituent species may also facilitate the survival of sensitive strains. In view of these facts, the applicability of consortia in bioremediation processes, offer promising solution for combating heavy-metal pollution in the environment (Carpio *et al.* 2016; Malik, 2004).
This work investigates influence of heavy metals on bacterial growth, metabolic activity and compares zinc bioaccumulation by pure cultures and microbial consortium isolated from heavy metals contaminated nickel sludge disposal site.

Experimental

Sample collection, isolation, growth, and identification of bacteria

Sludge samples were obtained from sludge waste disposal site of Nickel smelter near Sereď (Slovakia). Samples from the surface and 10 cm depth at three different locations were aseptically collected, transferred to the laboratory and stored in the cold (4 °C) prior to microbiological analysis.
To the 0.5 g of solid sample 10 mL of sterile PBS solution was added and after 20 min of intensive mixing 0.5 mL aliquot was transferred to the liquid diluted LB medium (LB medium (Becton Dickinson, USA) diluted 1 : 3 with distilled water) and cultivated aerobically at room temperature. After 24 h of cultivation the bacterial culture was transferred to the fresh diluted LB medium and after another 24 h of cultivation used in subsequent analyses. This mixed bacterial culture is referred as consortium in further.
To analyse the composition of consortium, aliquots of consortium was spread on non-selective agar medium (TSA – Tryptone Soya Agar, Oxoid, USA) and cultivated under aerobic conditions at laboratory temperature for 48 h. The individual colonies were selected on the basis of cell and colony morphology and used for further analyses. After repeated cultivation and control for the purity, the isolates were typed and identified by the MALDI TOF MS approach (Kopcakova *et al.* 2014).

The impact of heavy metals on metabolic activity of consortium

For evaluation of heavy metal influence on metabolic activity of consortium we have used BIOLOG EcoPlates™, which contain 31 different carbon sources and a blank in triplicate (Insam, 1997). Each well of the BIOLOG EcoPlate™ was inoculated with 100 µL of inoculum (overnight consortium culture diluted 1 : 50 in phosphate buffered saline, Thermo Fisher Scientific, USA) and incubated at 25 °C. Filter sterilized $NiCl_2$

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and $CoCl_2$ were added at appropriate concentrations. Absorbance readings were taken periodically (after 24, 48, and 144 h) at 590 nm with a microplate reader (Synergy 2, BioTek, USA).
The metabolic response (MR) of consortium was expressed as average well optical density of all carbon sources ($MR=\Sigma OD_i/31$; Gryta *et al.* 2014).

Bioaccumulation of zinc

The bioaccumulation of zinc was tested in liquid diluted LB medium. Fresh overnight culture of consortium or selected isolates was inoculated (1 : 100) into diluted LB medium supplemented with 10 mM $ZnCl_2$ and cultivated for 18 h at room temperature. Following cultivation, the cell biomass was removed by centrifugation at 3 000 g, the wet weight of biomass was measured and residual zinc concentration in the spent liquid medium was determined spectrophotometrically using dithizone method (Ahmed *and* Alam, 2003). All experiments were done at least in duplicates.

Results and Discussion

Heavy metals pollution is one of the most serious environmental issues, nowadays. Heavy metals significantly impact soil bacterial community due to their toxic effect. To transform pollutants into less toxic substances or immobilization in order to reduce their bioavailability, bioremediation by microorganisms is frequently used (Guo *et al.* 2010). In several countries e.g. in USA, 12 % of the *in situ* projects and 11 % of the *ex situ* projects have used bioremediation (US EPA, 2010). The activity of microorganisms led to adsorption and precipitation of potentially toxic elements by mechanism biosorption or bioaccumulation. Bioremediation is frequently accelerated through injection of native microorganisms into a contaminated area.
The aim of our study was to analyse the native microbiota of nickel sludge disposal site near Sereď. This landfill belongs to the most serious ecological burdens in Slovakia and is composed of nickel sludge with extremely high heavy metal content: Fe (50–80 %), Cr_2O_3 (2.5–3.5 %), SiO_2 (6–8 %), Al_2O_3 (6–8 %), CaO (2.5–3.5 %), Ni (0.17 %), P_2O_3 (0.6 %) (Michaeli *et al.* 2012). To the best our knowledge of the microbiota of the sludge was never analyzed. Recently, Remenár *et al.* (2014) analysed cultivable bacteria from nickel and cobalt contaminated agricultural soils adjacent to the landfill.
In our experiment, the bacterial consortium was isolated from the mixed sludge samples. The consortium showed relatively good growth in the presence of several heavy metals (data not shown). To test influence of nickel and cobalt on metabolic activity of obtained consortium BIOLOG EcoPlates™ were used. Both metals showed strong inhibition of metabolic activity of consortia (Fig. 1). At both concentrations tested, cobalt exerted much stronger inhibition effect on consortium compared to nickel. There are no general rules on the relative toxicity of nickel and cobalt because published data are often contradictory, even for similar type of microbial systems (Gikas 2008). Several authors however, reported stronger inhibitory effect of cobalt over nickel. Cobalt is more potent inhibitor than nickel of the growth of *Escherichia coli* and *Pseudomonas putida* (Wu *et al.* 1994). Schmidt *and* Schlegel (1989) who studied the effects of Ni(II) and Co(II) on several bacteria isolated from metal contaminated soils, have also reported that bacterial resistance to Co(II) is lower compared to Ni(II) resistance. *Arthrobacter butzleri* has been reported to be more sensitive to Ni(II) than to Co(II) (Otth *et al.* 2005).

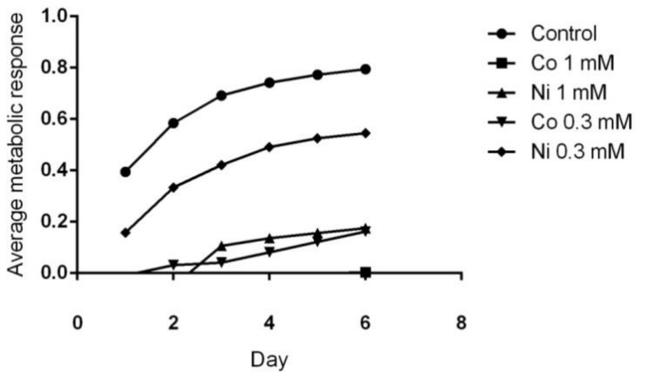

Fig. 1. Effect of heavy metals to metabolic activity of isolated consortium – expressed as average well colour optical density in each microplate.

Microbial consortium obtained was tested for the heavy metal bioaccumulation using zinc

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as a model. Upon cultivation in liquid zinc supplemented medium, the consortium was able to remove of about 15 % of zinc and bioaccumulation of zinc reached 0.18 mg/g of wet biomass weight (Fig. 2). Observed bioaccumulation abilities of isolated consortium are not very high and several authors reported much higher accumulation rates. Singh *et al.* (2012) reported that microbial consortium isolated from coal could efficiently remove over 80 % of metals, such as Ni, Zn, Cd, Cu and Cr, while the removal of Pb was nearly 45 %. According to Carpio *et al.* (2016) microbial consortium isolated from the contaminated water and sediment could remove more than 50 % of zinc from medium.

Bacterial consortia isolated from contaminated environments are usually genetically complex and consist of multiple bacterial taxa (Carpio *et al.* 2016) of different physiological and bio-accumulation properties.

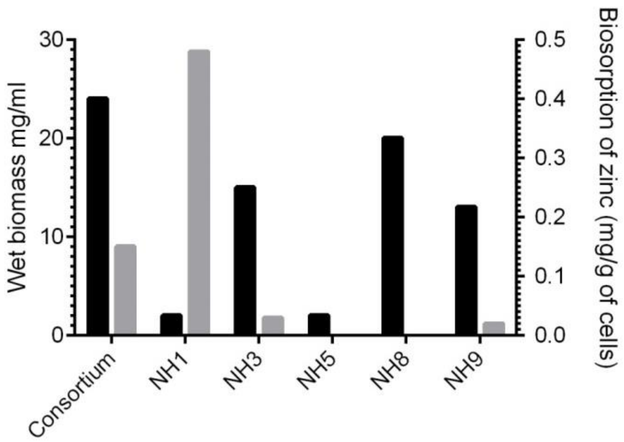

Fig. 2. Comparison of zinc removal from medium by bacterial strains and consortium.

The composition of isolated consortium was analysed using cultivation approach and subsequent identification of isolates by MALDI-TOF MS analysis. The analysis showed that consortium is composed by gram-positive bacteria only and it is dominated by actinobacteria of *Arthrobacter* genus (Fig. 3). Among 22 randomly selected isolates at least 16 were affiliated to this genus. Carpio *et al.* (2016) reported predominance of proteobacteria in microbial consortium isolated from the contaminated water and sediment. Remenár *et al.* (2014), who analysed microbiota of soils near nickel sludge in Sereď, reported the dominance of actinobacteria in these soils. According their research in nickel-contaminated soils dominate genus *Streptomyces* (18.1 %) followed by *Arthrobacter* (15.2 %), *Rubrobacter* (5.7 %), and *Glycomyces* (4.8 %) genera. The occurrence of *Arthrobacter* genus in heavy metal contaminated soil is not surprising and it was reported by several authors (Alvarez *et al.* 2017; Henne *et al.* 2009).

The phylum actinobacteria and genus *Arthrobacter* constitute some of the main and most diverse phyla within the domain Bacteria. These taxa exhibit diverse physiological and metabolic properties, such as production of extracellular enzymes and the formation of a wide variety of secondary metabolites. This versatility in secondary metabolite production makes them important tools for biotechnological applications such as bioremediation (Ludwig *et al.* 2012). The environmental prevalence of *Arthrobacter* may be due to its ability to survive long periods under stressful conditions, such as temperature shifts, oxygen radicals, and toxic chemicals, including heavy metals. *Arthrobacter* sp. is one of the most important genus in relation to tolerance to heavy metals and its potential use in bioremediation (Alvarez *et al.* 2017). Based on these data several isolates (NH3, NH5, NH8, and NH9) identified as *Arthrobacter* spp. By MALDI TOF analysis. No reliable identification was obtained for NH1 isolate nevertheless the isolate was identified as *Arthrobacter* spp. by 16S rRNA sequencing (data not shown).

The obtained results showed that growth in the presence of zinc and bioaccumulation abilities vary greatly among isolates. All isolates showed lower growth rates compared to consortium (Fig. 2) and no clear correlation was observed between growth rates and zinc bioaccumulation. While for the most isolates bioaccumulation ability was lower compared to consortium, NH1 isolate was able to eliminate the most of zinc from medium, despite the slowest growth rate. NH1 isolate is able to take up to 95 % of zinc (0.5 mg of zinc per 1 g of wet cell biomass) and therefore it is the most appropriate isolate for bioremediation use (Fig. 2).

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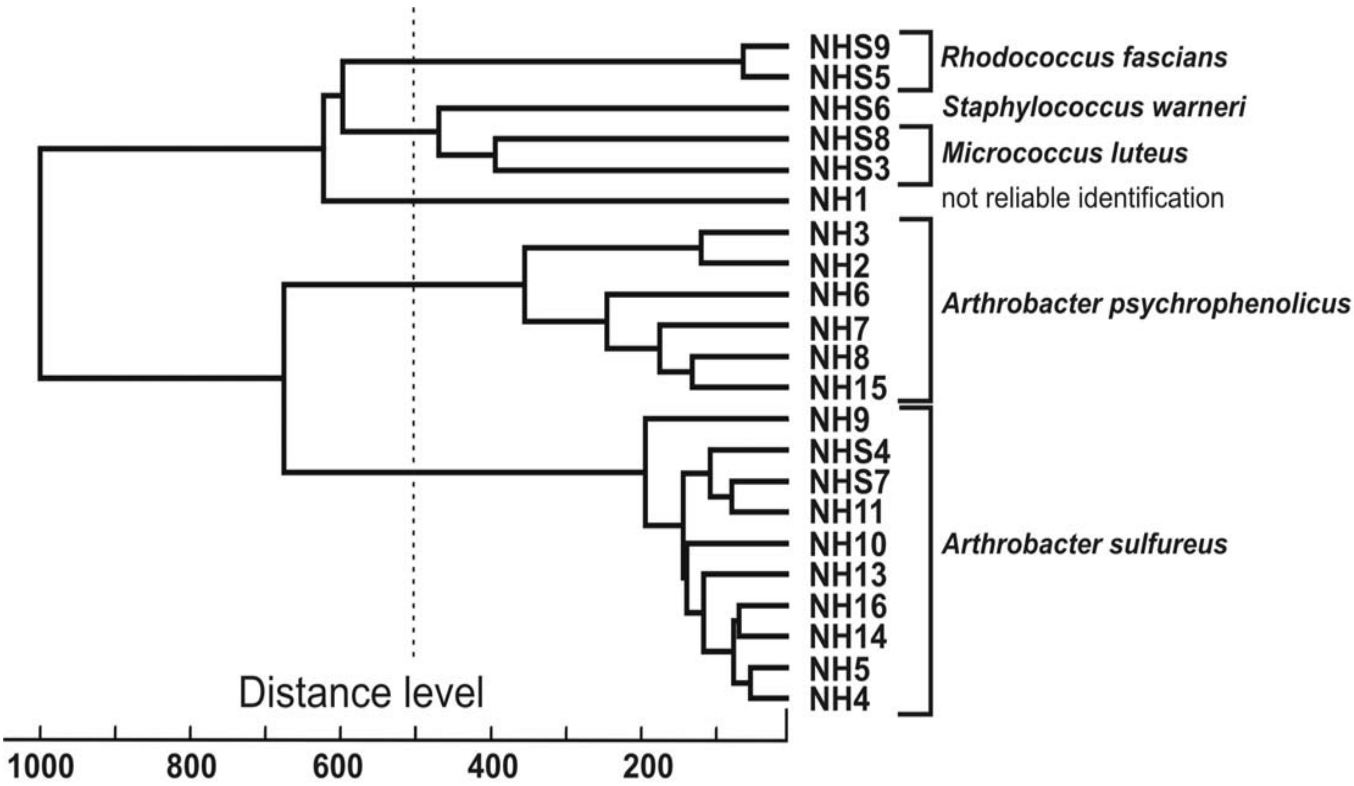

Fig. 3. MALDI TOF based similarity cluster analysis of isolates from indigenous microbiota of nickel sludge disposal site near Sered. The MALDI TOF based identification is shown next to the clades.

It has been suggested that stimulating the growth of indigenous microorganisms with metal bioaccumulation capacity may be a useful strategy for immobilizing metals in soils and preventing contamination of groundwater (Valentine *et al.* 1996). Several studies focused on microbial metal bioaccumulation involving multi- or single-component systems of pure cultures. The advantages of the individual components lies in the providing information on metal binding characteristics of individual organisms, but cannot be directly applied to the behaviour of metals in heterogeneous systems. Studies focused on the behaviour of multi-component systems, like a consortium of microorganisms, represent an approach, even if still simplified, closer to the real situations in which bacteria are found in natural systems (Ledin, 2000; Alvarez *et al.* 2017). These advantages mainly consist of synergistic interactions among members of the consortium. Our data suggest that analysis of contaminated environments could lead to the identification of bacteria with increased bioaccumulation abilities.

Conclusions

Indigenous microbiota of nickel sludge disposal site near Sereď have been found to be dominated by *Arthrobacter* spp. The microbiota was analysed from the point of metal bioaccumulation capabilities and while the highest growth rates were observed for mixed bacterial consortium, the highest zinc bioaccumulation rates were observed for individual strain. Despite extreme conditions, heavy metals contaminated sites could be source of autochthonous microflora, which could be used to concentrate, remove and recover metals from contaminated sites and thereby enhance the efficiency of treatment processes.

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