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Copper and lead removal from aqueous solutions by bacterial consortia acting as biosorbents

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ABSTRACT

A bacterial consortium was selected in the presence of Cu from sediment samples taken from Sepetiba Bay, Brazil, which is a site historically contaminated by metals. Bacteria were exposed to 0, 1, 6, 12.5, 25 and 50 μg·mL^{-1} Cu, Pb and Cu + Pb for 11 days of bioassay. Results showed *Alcanivorax* dominance (81%) and cell counts of 10^8 cells·mL^{-1}. However, a reduction in dehydrogenase activity was observed from the fifth day of exposure for all Cu, Pb, and Cu + Pb concentrations tested. Esterase activity tended to increase, indicating higher energy demand to complete the bacterial lifecycle. Pb concentrations in the filtered culture medium (0.2 μm) were below the detection limit, indicating biosorption, whereas concentrations of Cu were close to the tested concentrations, indicative of efflux. Results suggest the need for biomarkers, such as esterase and dehydrogenase enzymatic activity, in the assessment of resistance and tolerance of communities previously exposed to stressors.

1. Introduction

Some metals are essential, in small amounts, for plant and animal optimum growth, although many are toxic even at very low concentrations. Chromium, lead, zinc and others are not only cytotoxic, but also carcinogenic and mutagenic in nature (Chen et al., 2016; Hogle et al., 2016; Morel and Price, 2003; Zhuang et al., 2014). As urbanization and industry has expanded, indiscriminate release and accumulation of heavy metals in the environment has increased, becoming a major concern that demands sustainable remediation technologies to rectify and reestablish natural conditions worldwide. There are several techniques to remediate contaminated water bodies and soils, but physicochemical methods, such as chemical precipitation and electrochemical treatment, are sometimes ineffective or expensive (Ahluwalia and Goyal, 2007). Heavy metal accumulation by extracellular polymeric substances (EPS) secreted by microorganisms is an innovative technology for removal of contaminants from the environment, in a process termed biosorption (Costa et al., 1995; D'Acunto et al., 2015; Rao, 2011). Biosorption includes a number of passive accumulation processes, such as ion exchange, complexation, microprecipitation, absorption and desorption, allowing its use as an effective alternative strategy for metal removal and recovery (Wong et al., 2001).

Management of polluted aquatic ecosystems requires an ecological risk assessment in which not only environmental factors (such as chemical and physicochemical factors), but also biological and ecotoxicological factors, must be considered in order to obtain a classification of polluted areas and to support actions for pollution control (Bidone and Lacerda, 2004; Fiori et al., 2013; Hakanson, 1980; Long and Chapman, 1985). Bioassays using organisms exhibiting bioaccumulation can measure contaminant bioavailability, its effect on biota (e.g. mortality), track its route through food chains, and its risks to human health (USEPA, 1991). Guidelines for analyzing the behavior and effects of pollutants on biota have been proposed for temperate and cold climate ecosystems (i.e. FDEP 1994; Long et al. 1995; MDEPQ 2007). These guidelines have been adopted by other countries without considering differences with the climates and ecology of the biomes the guidelines were developed for. Another crucial point is that these guidelines focus on specific contaminants individually, even though many different contaminants may pollute sediments simultaneously and, therefore, represent multiple exposures (van Gestel et al., 2011). In addition, antagonisms and synergies between contaminants are rarely identified in the guidelines. Typically, about 10^{10} bacterial cells g^{-1} d.w. colonize sediments, which are arranged in biofilms consisting of EPS where dissolved and particulate matter can be metabolized by several electron acceptors (Decho, 2000; Flemming and Wingender, 2010; Meyer-Reil, 1994). Products of one bacterial metabolic group frequently constitute the substrate for others, forming bacterial consortia that are highly mutually interdependent (Demaison and Moore, 1980; Meyer-Reil and Köster, 2000; Relexans et al., 1992). Biofilm cell subpopulations coexist at different growth stages, and when exposed to sub-inhibitory

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concentrations of metals and antibiotics, surviving cells become less sensitive, with subsequent generations exhibiting resistance and tolerance (Harrison et al., 2005; Harrison et al., 2007; Lewis, 2007). The natural process of phenotypic diversification of bacterial consortia in biofilms facilitates the ability of microbes to either continue growing (i.e. develop resistance) or just survive (i.e. develop tolerance) in the presence of multiple toxic metals. Phenotypic changes also imply alterations to community metabolism and energy demands (Odum, 1969, 1985), which are ecologically-relevant indicators of environmental pollution (van Beelen and Doelman, 1997).

Here, by combining the concepts of two models-the stressed ecosystem development model (Odum, 1969, 1985) and the time- and dose-dependent biofilm multimetal resistance and tolerance model (Harrison et al., 2007)-we designed bioassays to bacteria consortia response to Cu and Pb exposure. The consortia was selected in from Sepetiba Bay sediments, which are historically contaminated mainly by metals (Herms and Lanzillota, 2012; Lacerda and Molisani, 2006). Microbial energy demand was measured by esterase enzymatic activity, as these enzymes play a crucial role in organic matter decomposition and, consequently, in ecosystem energy and nutrient cycling (Flemming and Wingender, 2010). Microbial cell viability and energy generation (adenosine triphosphate, ATP) was evaluated by dehydrogenase enzymatic activity. Although both these types of enzymes are closely correlated with the cell density and energy demand of pure and mixed microbial cultures (Stubberfield and Shaw, 1990), we also evaluated microbial community size by enumerating the numbers of autotrophic and heterotrophic bacterial cells. Different concentrations of Cu, Pb, and Cu + Pb solutions were tested to determine antagonistic or synergistic responses of the consortia to exposure to these metals.

2. Material and methods

2.1. Study area

Sepetiba Bay (22°54′ - 22°04′SE, 43°34′ - 44°10′ W) is a semi-enclosed water body connected to the Atlantic Ocean in the east, with an average depth of 6 m and covering an area of 500 km^2. It is surrounded by Rio de Janeiro city and its municipalities. The human population in this area has almost tripled since the late 1970s, from approximately 600,000 to 1.7 million people by the end of 2010, with consequent increased urbanization, industrialization and harbor activities (INEA, 2014; Molisani et al., 2004). Although the main source of metal contamination into the bay has ceased operation, many other industries in the surrounding areas continue to degrade this ecosystem. In particular, several metallurgical industries are in operation, degrading the environment through metal contamination and damaging the natural resources of this location (Herms and Lanzillota, 2012; Lacerda and Molisani, 2006; Marques et al., 2006; Rodrigues et al., 2009).

2.2. Bacterial consortia isolation and bioassay elaboration

Bacterial consortia were isolated from sequentially-autoclaved culture media. Culture media were made according to Madigan et al. (2003), with a mixture (1:1 v/v) of filtered seawater (Millipore, Cellulose, 0.45 μm), in order to remove suspended matter (Weber, 1973), and deionized water in Erlenmeyer flasks. The bacterial carbon source was 2 $g \cdot L^{-1}$ of yeast extract and 2 $g \cdot L^{-1}$ of urea. As a first step, fresh sediment samples from Sepetiba Bay were inoculated in this medium and incubated for ten days at 37 °C until10^8 cells $\cdot mL^{-1}$ was attained. Then, one aliquot of 5 mL was inoculated into new sterile culture medium to acclimate the bacterial consortia at room temperature. To select bacterial consortia resistant to metal, 5 mL of the latter medium was inoculated into new sterile culture medium with 50 μg Cu $\cdot mL^{-1}$, and incubated (37 °C) for ten days to attain 10^8 cells $\cdot mL^{-1}$. This bacterial culture was maintained for the bioassays and an aliquot was extracted for DNA sequence analyses.

Bioassays were conducted in Erlenmeyer flasks (125 mL) at37 °C, each of them with 5 mL of the selected bacteria consortia resistant to metal and 50 mL of sterile culture medium (Madigan et al., 2003). The culture medium was mixed with sterile Cu and Pb standard stoke solutions of 6, 12.5, 25 and 50 $\mu g \cdot mL^{-1}$ Cu, Pb and Cu + Pb.·The lowest concentration of 6 $\mu g \cdot mL^{-1}$ is the maximum allowed content of Pb (the most toxic metal tested) in human blood according to Brazilian laws (Brasil, 2016). The maximum concentration of 50 $\mu g \cdot mL^{-1}$ is the Pb content found in the Sepetiba Bay sediments, according to Ribeiro (2014), and Abuchacra et al. (2015). Enzyme activities and bacterial biomass were assessed over eleven days of exposure, on days 1, 3, 5, 7 and 11.

2.3. Classification of bacterial consortia

DNA extraction of 30-mL cultures was performed using the PureLink ™ Genomic DNA Mini Kit (Invitrogen), according to the manufacturer's instructions. Extracted DNA was analyzed in 1% agarose gels and quantified with a Nanodrop™ spectrophotometer (Thermo Fisher Scientific, Inc.).

Small subunit rRNA genes were amplified by polymerase chain reaction (PCR) using the following primer pair: 27F-1401R (Lane, 1991. All PCR amplifications were made in 25 μL reaction volumes using the GoTaq® Green Master Mix (Promega). PCRswererun in a Veriti® Thermal Cycler (Life Technologies) under the following conditions: 95 °C for 5 min; 30 denaturation cycles at 95 °C for 1 min, annealing at 55 ° C for 1 min, and extension at 72 °C for 5 min. All amplicons were analyzed in 1.5% SYBR Safe-stained agarose gels and were cloned with a TOPO TA cloning kit (Invitrogen), according to the manufacturer's instructions. Cloning products were reamplified by PCR with the M13F-1401Rprimer pair. PCR amplified vector inserts of the correct size were purified with the PureLink™ PCR Purification Kit (Invitrogen). A total of 26 cloning products of each sample were sequenced using the BigDye Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems) by Genomic -Engenharia Molecular (São Paulo, Brazil) using the T7 universal primer.

DNA sequences were assembled with the Bio-Edit Sequence Alignment Editor, and trimming, clustering and classification were performed in the Mothur software (Schloss et al., 2009). Sequences were compared using the BLASTX algorithm against the National Center for Biotechnology information database (NCBI, (http://www.ncbi.nlm.nih.gov) and the Ribosomal DataBase Project (RDP) (Cole et al., 2009; Wang et al., 2007).

2.4. Biomass quantification

Cell biomass quantifications were carried out on 2 mL aliquots from each Erlenmeyer flask, which were vacuum-filtered through a sterile Millipore membrane (0.22 μm pore diameter) (Bloem et al., 1986) and stained with acridine orange fluorochrome. Only healthy cells were enumerated under epifluorescent microscopy at 1000× magnification (Axioskop 1, Zeiss, triple filter Texas Red – DAPI – fluorescein isothiocyanate), in accordance with (Kepner and Pratt, 1994). When this staining method is used with UV light, the bacterial physiologically healthy cell will emit green light (Carlucci et al., 1986; Mirrett, 1982). The data obtained was converted to the total number of bacterial cells (cell $\cdot mL^{-1}$) following the equations proposed by Kepner and Pratt (1994). The detection limits of epifluorescent technique were 10^5 bacterial cells mL^{-1} (Hobbie et al., 1976).

2.5. Dehydrogenase activity quantification

Colorimetric methods based on INT reduction provide an accurate assay of dehydrogenase activity under both anaerobic and aerobic conditions (Lenhard, 1968; Mosher et al., 2003; Trevors, 1982; von Mersi and Schinner, 1991). The INT assay relies upon the close-coupling of the succinate dehydrogenase enzyme complex, which oxidizes