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Bacterial strains resistant to inorganic and organic forms of mercury isolated from polluted sediments of the Orbetello Lagoon, Italy, and their possible use in bioremediation processes

M. Pepi[1], S. Focardi[2], A. Tarabelli[1], M. Volterrani[1] and S. E. Focardi[1]

[1] Department of Environmental Sciences, University of Siena, Via P. A. Mattioli, 4; 53100, Siena, ITALY, milva.pepi@unisi.it
[2] Department of Chemistry, University of Siena, Via A. Moro, 2; 53100, Siena, ITALY, silvia.focardi@unisi.it

Abstract. Bacteria are able to adapt to heavy metals in contaminated environments, by developing specific mechanisms of resistance. A mercury (Hg)-resistant bacterial community was isolated from polluted sediments of the Orbetello Lagoon, Italy. The members of the Hg-resistant bacterial community showed high levels of resistance both to the inorganic and to the organic forms of Hg. 16S rRNA gene sequencing showed the presence of different genera, and a bacterial strain resistant to Hg belonging to the genus *Luteimonas* was evidenced for the first time. The *merA* gene coding for mercury-reductase conferring resistance to inorganic Hg, and the *merB* gene coding for mercury-lyase enzymes, for resistance to the organic forms of Hg, were detected in the tested bacterial strains. The community showed the presence of bacteria belonging to the genera *Pseudomonas* and *Psychrobacter* that highlighted the capability to reduce Hg^{2+} to the volatile form of Hg^0. Experiments carried out with immobilized cells of the Hg-resistant strains removed 96% of Hg in sediments leachates in a bioremediation laboratory scale pilot plant. A methylating activity of the sulphate-reducing bacteria of the same polluted sediments was moreover evidenced. These results evidenced the presence of microbial communities highly adapted to the presence of Hg into the sediments of the lagoon. The use of the isolated autochthonous bacterial strains for bioremediation of the native sediments contaminated by Hg is suggested.

Key words: Mercury, bacteria, bacterial resistance, sediments, bioremediation.

Introduction

Microorganisms are ubiquitous and able to adapt to extreme environments. Among microorganisms, bacteria show different mechanisms of resistance to heavy metals. Interactions of bacteria with heavy metals could change the valence states of the latter, thus modifying their fate in the environment. Some of the interactions bacteria-heavy metals could represent useful processes for toxic elements removal from the environment.

The biogeochemical cycle of mercury (Hg) is well known. Hg in its ionic form can possibly produce the most toxic form of this metal: methyl mercury (Me-Hg). This organic form of Hg originates from the methylating activity of sulphate-reducing bacteria and is able to be bioaccumulated and biomagnified in the trophic chain (Barkay et al., 2003).

Bacteria have evolved mechanisms of resistance to deal with high levels of different forms of Hg in the environment, in virtue of the presence of genes *merA* and *merB*. Bacteria achieve detoxification of Hg by reduction of Hg^{2+} to Hg^0 and diffusional loss of Hg^0 from the cell (Barkay et al., 2003).

Hg contamination of lagoon sediments is a well-known problem and the relative biogeochemical cycle has been characterized in various places (Han et al., 2007). Sediments of the Orbetello Lagoon, show mercury contamination due to geologic characteristics and to the nearby Monte Amiata mercury mines.

A study on Hg-resistant bacteria was undertaken in order to characterize bacterial strains able to cope with mercury in sediments of the Orbetello Lagoon, and to assess their possible use in bioremediation of this contaminated site.

Materials and Methods

Orbetello Lagoon, on the Tyrrhenian coast of central Italy

(42° 26' 34" N, 11° 13' 29" E), covers an area of about 2,300 ha with a water depth of about 1 metre. Superficial sediment sampling was carried out using a grab sampler. Sterile portions for microbiological analyses were maintained at 4°C in the dark until arrival at the laboratory. Enrichment cultures arranged in Nelson's medium (NeM), in the presence of 10 μg ml^{-1} of mercury chloride ($HgCl_2$) allow isolation of Hg-resistant bacterial strains. Isolates were stored at -80°C in 30% sterile glycerol. For 16S rDNA sequencing of isolated bacterial strains, a single colony was suspended in 50 μl double-distilled water and treated for 5 min at 100°C. Amplification of the 16S rRNA gene was accomplished. Sequencing was carried out at the Bact 16S biomolecular research service (CRIBI Biotechnology Centre, University of Padua, Italy). Resistance to inorganic mercury added as $HgCl_2$ and to organic mercury added as CH_3HgCl in isolated bacterial strains was assessed by MIC tests. The presence of *merA* and *merB* genes in the isolates was investigated. Three of the isolated Hg-resistant bacterial strains were tested for their capacity to volatilize inorganic mercury ($HgCl_2$) and organic mercury (CH_3HgCl). The presence of methylating activity in anaerobic cultures of sulphate-reducing bacteria originated from the same polluted sediments was moreover investigated.

Cells of the mercury-resistant bacterial strains ORHg1, ORHg4 and ORHg5 adhering to pumice particles were tested for their capacity to remove mercury from liquid solutions consisting of Nelson's medium with 10 μg ml^{-1} of Hg^{2+} (added as $HgCl_2$) or from leachate solutions containing mercury obtained from sediments treated with acidic solutions.

Results and Discussion

A microbial community Hg-resistant was isolated from sediments of the Orbetello Lagoon, Italy, where the presence of Hg contamination is well known. 16S rRNA gene sequences assigned the isolates to the genera *Bacillus*, *Pseudomonas*, *Psychrobacter*, *Halomonas*, *Luteimonas* and *Micrococccus*. Resistance to Hg in a bacterial strain belonging to the genus *Luteimonas* was evidenced for the first time. Levels of resistance to inorganic mercury, added as $HgCl_2$ ranged from 0.01 mM to 0.3 mM. Those of resistance to organic mercury, added as CH_3HgCl showed a range included from 0.0005 mM to 0.01 mM. Strain *Psychrobacter* sp. ORHg1 showed resistance to $HgCl_2$ at a value of 0.1 mM, and strains *Pseudomonas* spp. ORHg4 and ORHg5 showed levels of resistance of 0.075 mM in the presence of the same inorganic form of Hg. With respect to the organic form of mercury, ORHg1 showed higher levels of resistance to CH_3HgCl (0.005 mM), and strains ORHg4 and ORHg5 were resistant to 0.0025 mM CH_3HgCl. Levels of resistance of the isolates to inorganic and organic mercury revealed by MIC values were substantially similar to values in the literature for heterotrophic aerobic bacteria (Baldi et al., 1993). For all three tested bacterial strains, *Psychrobacter* sp. ORHg1, *Pseudomonas* sp. ORHg4 and *Pseudomonas* sp. ORHg5, the capacity to volatilize ionic mercury added as $HgCl_2$ was greatest within five-ten minutes of the contact between the induced bacterial cells and Hg^{2+} (Fig. 1). Levels of mercury removal detected in this work were similar to those previously reported for heterotrophic Hg-resistant bacterial strains (Capolino et al., 1997). Genes *merA* and *merB* were evidenced in bacterial strains of the Hg-resistant community isolated from sediments of the Orbetello Lagoon. Microbial diversity of a bacterial consortium for mercury detoxification could provide a reservoir of strains with complementary ecological niches, resulting in a better efficiency in eventual bioremediation processes (Wagner-Döbler, 2003). Methylating activities of Hg^{2+} by anaerobic suphate-reducing bacteria, from the same polluted sediments, were evidenced.

A laboratory scale bioreactor with fixed beds with isolates immobilized as biofilms showed good efficiency (96%) in mercury removal from sediments leachate solutions. An extended description of the pilot plant was reported (Pepi et al., 2011).

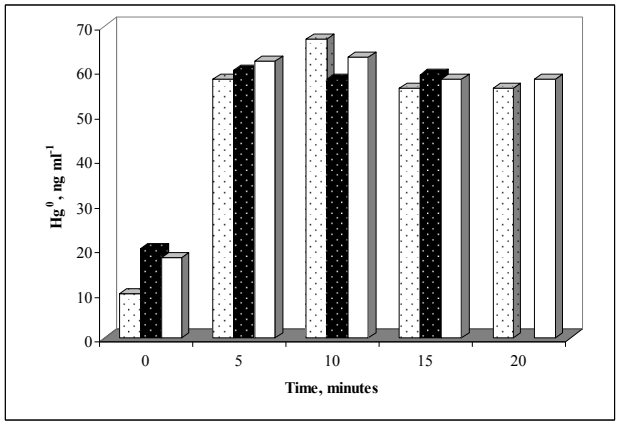

Fig. 1. Volatilization of Hg^{2+} to Hg^0 by the Hg-resistant bacterial strains *Psychrobacter* sp. ORHg1 (▒), *Pseudomonas* sp. ORHg4 (▓) and *Pseudomonas* sp. ORHg5, (□) in the presence of Hg^{2+} added to the dense bacterial culture as $HgCl_2$.

Conclusion

The isolated Hg-resistant bacterial strains could be used in bioremediation applications to remove mercury from sediments. This application could be especially important in sites where mercury contamination reaches high values, with the presence of methylmercury, where it would decrease the mercury content in portions of removed sediments before their safe storage, thereby decreasing managements costs.

The isolated Hg-resistant bacterial community represents a reservoir of bacterial strains to be maintained and characterized for applications of bioremediation.

Autochthonous adapted bacterial strains of contaminated sites could represent an opportunity for environmental friendly processes and for eventual

processes able to remove heavy metals, thus avoiding contacts with living organisms.

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