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Investigating heavy metal resistance, bioaccumulation and metabolic profile of a metallophile microbial consortium native to an abandoned mine

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Abstract

Contaminated sites represent new ecological niches where historical pollution has originated an unusual microbial biodiversity. The knowledge of these microorganisms contributes to the discovery of new pathways and metabolic networks and may offer potential solutions for damaged areas. In the present work seven microbial consortia have been isolated from an abandoned mine of blend and galena (Ingurtosu, Italy) through a selection for resistance to zinc (tested up to 40 mM in solution). All the consortia were able to accumulate zinc and the best accumulator, named *Ing5*, has been studied for the following characteristics: resistance and accumulation of Zn, Cd, Hg, bioaccumulation mechanisms of Zn, and influence of Zn and Cd on the metabolic profile. The results indicate that the consortium *Ing5* bears resistance systems for Cd and Hg as well as Zn and that, for some of the 5 isolates belonging to *Ing5*, the resistance thresholds are higher in consortium than in pure culture. The prevalent mechanism for zinc accumulation can be reasonably considered to be metabolism-dependent, inducible and regulated by metal concentrations. The study on the metabolic profile, carried out by the Biolog system, shows that Zn exerts a very low influence on the metabolic profile and that this influence can also be positive; Cd has a stronger negative influence but that, despite this, the consortium is able to maintain a wide metabolic potential in the presence of heavy metals. These features of *Ing5* make it a good candidate for biotechnological applications and for further investigation of the degradation of organic pollutants in the presence of metals.

Keywords: Zinc; Cadmium; Biolog; Bioremediation

1. Introduction

The practical difficulties of sampling and the complexity to detect and grow microorganisms make it questionable whether the inventorying of the world's microbial diversity is practicable, but the importance of microbial function demands that our knowledge is extended (Convention on Biological Diversity, 2002). Microbiological explorations of new ecological niches can widen the knowledge of the microbial domain and help in finding a response to different research needs in several fields. Contaminated sites represent ecological niches where historical pollution has resulted in an unusual microbial biodiversity, exerting environmental pressure on fundamental ecological parameters such as abundance, diversity, nutrient recycling, and food

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chains. In these niches pathways and metabolic networks have been developed as adaptive responses to harsh conditions. These pathways and metabolic networks may represent potential solutions for polluted areas. They can help to overcome metabolic bottlenecks still existing in the biodegradation processes; among them, the inhibition that heavy metals can exert on the biodegradation of organic pollutants (Roane et al., 2001). The remediation of co-contaminated sites (heavy metals and organic pollutants) is a complex problem. Studies on this subject are not extensive, although co-contaminated sites represent a high percentage of those included in the national priority lists for remediation, for instance 40% in US (Sandrin et al., 2000). The studies carried out have been recently reviewed (Sandrin and Maier, 2003) and demonstrate that heavy metals have the potential to inhibit pollutant biodegradation under both aerobic and anaerobic conditions. Approaches to increase biodegradation in co-contaminated environments include inoculation with metal-resistant microorganisms, which are able to reduce the bioavailability of metals via sequestration (Roane et al., 2001). Another approach is to find the right conditions that enable the metal-resistant microorganisms to degrade organic pollutants in the presence of heavy metals.

Contaminated sites can be a preferential source of those microorganisms which represent, therefore, important material for both study and applications of bioremediation for differential targets (Malik, 2004). The term bioremediation for heavy metals may seem improper, since no process can degrade and thus eliminate inorganic elements (Barkay and Schaefer, 2001); nevertheless in some cases their immobilization, performed by microorganisms, may be the only feasible means to protect groundwater and food-chain from contaminations. In these cases remedial goals can be achieved in different ways: the precipitation, and thus the immobilization, through different biological processes of inorganic contaminants, the concentration and then reduction in volume of contaminated matrices and the compartmentalisation of metals to a part of the environment in which their toxicity is reduced. The importance of the bacterial effects in the removal of heavy metals is supported by many studies and Fein (2000) suggests the incorporation of bacteria in models of water–rocks interaction and contaminants transport. The measures evolved by microorganisms to respond to heavy metal stress have been reviewed (Nies, 1992, 1999; Ji and Silver, 1995; Nies and Silver, 1995) and the main processes are bioaccumulation, enzymatic reduction and complexation. Bioaccumulation can occur either by metabolism-independent (passive) biosorption or by intracellular, metabolism-dependent (active) uptake (Ledin, 2000). Some aspects of the subtle mechanisms by which bacteria perform these processes have to be further investigated: for instance, the thermodynamic parameters of biosorption, the composition of surface sites responsible for deprotonation and the role of the differences between different types of bacteria (Daughney et al., 1998). Most efforts have been put into the area of optimising the conditions for metal retention and on studying the mechanisms of bioaccumulation for pure cultures of microorganisms, but future work must consider multi-component systems (consortia) (Ledin, 2000). The present work represents an effort in this direction.

The site investigated is the abandoned mine of Ingurtosu in Sardinia, one of the most famous Italian mining archaeology monuments and one of the priority sites for intervention of restoration and remediation. Exhaustive geochemical studies (Zuddas et al., 1998; Podda et al., 2000; Zuddas and Podda, 2005) have reported the presence of minerals rich in Zn, Pb, Cd, Ni and Cu and a severe heavy metals contamination of the superficial natural water system and the sediments. From this site, seven microbial consortia have been isolated and tested for their capacity to bioaccumulate zinc. The most efficient consortium (*Ing5*) has been studied for the following characteristics: resistance and accumulation of Zn, Cd, Hg, mechanisms of zinc bioaccumulation, and the influence of heavy metals on the metabolic profile.

2. Materials and methods

2.1. Microbial consortia selection and culture conditions

Microbial material is native to Ingurtosu, an abandoned mine of blend and galena located in southwestern Sardinia (Italy, Central Mediterranean Sea) at 150 m s.l. and 5 km from the coast. Samples were collected from water, sediment and soil along the banks of Rio Naracauli, which flows through the mine. Aliquots from each sample were immediately inoculated in 5% mineral medium (MM) (Schmidt and Schlegel, 1989) + gluconate 0.5% as carbon source, containing 1.5 mM $ZnSO_4$.

Each sample was re-inoculated in laboratory, using the same solution in 50-ml flasks and incubated in a rotary shaker (100 rpm) at 28 °C for 2 weeks. The selection for zinc resistance was performed through enrichment procedure after Schmidt and Schlegel (1989) with some modifications: the microbial broth cultures were inoculated (10%) by sequential steps in