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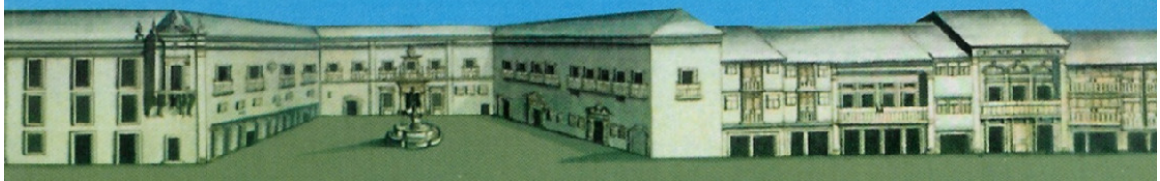

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Selection of indigenous acidophilic bacteria for the bioleaching of two SOMINCOR concentrates

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Keywords: bioleaching, acidophiles, chalcopyrite, lead concentrates

Biometallurgy, an interdisciplinary field involving geomicrobiology, microbial ecology, microbial biochemistry, and hydrometallurgy, is a novel promising technology for recovering valuable metals from industrial waste materials and for detoxifying these materials for environmentally safe deposition. Biometallurgy can be defined as the field of applications resulting for the control of natural (biochemical) processes of interactions between microbes and minerals to recover valuable metals (Morin et al., 2006).

Bioleaching exploits the ability of microbes that thrive in high acid environments, require inorganic food and energy sources and frequently display resistance to heavy metals. These unique chemolithoautotrophs are generally employed to leach sulphide ores such as chalcopyrite, an abundant but refractory copper sulphide mineral.

This work concerns to the screening of the bioleaching potential of a indigenous microbial consortium (Achada Sludge – LA), collected at the abanboned S. Domingos copper mine, for using on metals bioleaching and recovery of two different mineral concentrates: lead-zinc concentrates (Pb-Zn concentrates) and rich copper concentrates (chalcopyrite), supplied by Somincor (Sociedade Mineira de Neves-Corvo SA, Portugal).

The screening of iron-oxidising acidophiles, which are known to have a key role in the bio-oxidation of the sulphide minerals regenerating the oxidant ferric iron, was made by enrichment techniques (Johnson, 1995) at 35 °C, pH ≈ 2.0 and 150 rpm, with different concentrations of substrate (lead concentrates or chalcopyrite).

The best bioleaching results for Pb-Zn concentrates by Achada Sludge were obtained for 2% substrate, with a % metals recovery of 44% Fe, 22% Cu and 100% Zn after 2 weeks of enrichment, and reaching a recovery of 65% Fe, 82% Cu and 100% Zn after 2 months of enrichment. In the abiotic controls the % metals recovery ranged from 0% (Cu, Fe) to 10% (Zn) after 2 months. For copper concentrates, the bioleaching results in % metals recovery for 2% chalcopyrite were 41% Fe, 30% Cu and 75% Zn after 2 weeks of enrichment, reaching a recovery of 77% Fe, 76% Cu and 100% Zn after 1 month of enrichment. In the abiotic controls with chalcopyrite, the % metals recovery was 3% to Fe, 14% to Cu and 62% to Zn after 1 month.

LA consortium presented an high bioleaching potential for metal-recovery from both mineral concentrates tested. Biometallurgy processes applied for Cu-recovery in copper concentrates (chalcopyrite) and for Zn-recovery in Pb-Zn concentrates can be an interesting tool to improve the added value of products. In the case of Cu concentrate this process would allow the implementation of a copper bio-hydrometallurgy to produce copper cathodes instead of selling a concentrate. For the Pb-Zn concentrates, this type of treatment could improve the economic interest of products, which constitutes a residual stream.

Furthermore, the three LA consortia, the original LA and the enriched samples either with Pb-Zn concentrates or chalcopyrite, are being used to assess the effect of combined contamination of heavy metals on soil bacterial communities using genetic community fingerprinting by 16S rDNA profiles and heavy-metal resistant genomes.

Acknowledgements
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References

Morin, D., Lips, A., Pinches, T., Huisman, J., Frias, C., Norberg, A., Forssberg, E. (2006). BioMinE – Integrated project for the development of biotechnology for metal-bearing materials in Europe. *Hydrometallurgy*, 83, 69-76.

Johnson, D.B. (1995). Selective solid media for isolating and enumerating acidophilic bacteria. *Journal of Microbiological Methods*, 23, 205-218.

Functional screening of a metagenomic library obtained from soils and water of S. Domingo's mine

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Keywords: metagenome, heavy-metal resistance, 16S rDNA

Despite the dominance of microorganisms in the biosphere, relatively little is known about the majority of environmental microorganisms, largely because of their resistance to culture under standard laboratory conditions. As such, alternative approaches are required to assess the large amount of information in the environmental metagenome.

An often-cited estimate is that as much as 99% or more of microbial life remains unculturable, and therefore cannot be studied and understood in a way that microbial ecologists have become accustomed to over the past century. Although the portrait of the microbial world was revolutionized by analysis of 16S rRNA genes, such studies yielded only a phylogenetic description of community membership, providing little insight into the genetics, physiology, and biochemistry of the members. Metagenomics provides a second tier of technical innovation that facilitates study of the physiology and ecology of environmental microorganisms. The composition of microbial populations of tailings waste at the abandoned São Domingo's copper mine was investigated, and the ability of pure and mixed cultures of indigenous microorganisms to reduce sulphates was examined. These approaches revealed new genes and gene products that may have a major role in biomining processes.

Materials and Methods

Samples of soil and water from an abandoned cupric pyrite mine (Mina de S. Domingos, Southeast Alentejo, Portugal) were used in batch assays to study the sulphate reducing ability of microbial population in presence of zinc, copper and iron. The inoculum (OI) (200 ml) was grown 15 days in (g l^{-1}): 1.2 Na_2SO_4; 0.16 $CuSO_4.5H_2O$; 1.245 $FeSO_4.7H_2O$; 0.33 $ZnSO_4.7H_2O$; 0.3 $Na_3C_6H_5O_7$, pH 6.0, final volume 600ml (BI). This culture (200 ml) was then subjected to a double concentration of the metals (same volume) used previously, during 1.5 months (BII).

Separation of whole DNA from samples of each batch assay including original inoculum (OI, BI, and BII) was performed using UltraClean soil DNA isolation Kit (MO BIO).

After isolation of gDNA, PCR was performed using 16S rDNA primers for bacteria and archaea, primers 1510R and 1492R respectively, (Hugenholtz and Goebel, 2001). The amplicons were inserted in pJET plasmid (CloneJET PCR cloning kit, Fermentas) and a metagenomic library was constructed with recombinant DH5a cells with clones with partial 16S rDNA gene that were sequenced by an ABI PRISM 310 sequencer (Applied Biosystems).

In order to assess the effect of combined contamination of heavy metals on soil bacterial communities using genetic community fingerprinting by 16S rDNA profiles and heavy-metal resistant genomes, PCR with primers specific for heavy-metal resistance genes was carried ou - sections of mercury resistance determinant on the transposon Tn501, *mer*1, the *czc* gene cluster of plasmid pMOL30, that ensures resistance to Cd^{2+}, Co^{2+} and Zn^{2+}, *czc*A,B, system of copper sequestration, with copper metallothionein *CuBP* and the copper resistance determinant on the plasmid pRJ1004, *pco*A and finally the genetic system for arsenate resistance, arsenate reductase, arsC.

Results

Results obtained showed the amplification of genes correspondent to *CuBP* and *ars*C in all batch assays. However, analysis the sequences obtains from the metagenomic library constructed with each pool of DNA revealed that microbial population suffers adaptive events that have reduced the strain heterogeneity of the community. These results can be explained by the effect of selective pressure caused by metal concentration of culture media, in the dynamics of microbial population. Analysis of sequence data is still being processed.

Samples	Primers		Homology (%)
BI	*arsC*	*Nitrobacter hamburgensis*	89
		Delftia acidovorans	80
		Ralstonia pickettii	81
	CuBp	*Novosphingobium aromaticivorans*	92
		Sphingomonas sp.	88
		Pseudomonas sp.	79
		Burkholderia multivorans	78
BII	*arsC*	*Ralstonia pickettii*	82
	CuBp	*Pseudomonas sp*	75
		Gluconacetobacter diazotrophicus	
BI	*1510R*	*Syntrophus aciditrophicus*	92
	1492R	*Mannheimia haemolytica*	79
BII	*1492R*	*Desulfovibrio desulfuricans*	82

Sá-Pereira, P., Rodrigues, M., Castro, I. V., Simões, F. Identification of an arsenic resistance mechanism in strains of *Rhizobium*. World Journal of Microbiology and Biotechnology, (2007). 23(10): 1351-1356

Christopher G. Bryan, Kevin B. Hallberg and D. Barrie Johnson. Mobilisation of metals in mineral tailings at the abandoned São Domingos copper mine (Portugal) by indigenous acidophilic bacteria. Hydrometallurgy, 83(1-4):184-194, September 2006, 16th International Biohydrometallurgy Symposium

Isabel Videira e Castro, Paula Sá Pereira, Fernanda Simões, José Matos and Eugénio Ferreira. Use of Lotus/Rhizobium symbiosis in regeneration of polluted soils. (2007) Lotus Newsletter 37(2): 87-88.

Hugenholtz, P. & Goebel, B. M. (2001). The polymerase chain reaction as a tool to investigate microbial diversity in environmental samples. In *Environmental Molecular Microbiology: Protocols and Applications*, pp. 31–42. Edited by P. A. Rochelle. Wymondham, UK: Horizon Scientific Press 2001