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HEAVY METAL RESISTANCE OF MICROORGANISMS ISOLATED FROM COAL MINING ENVIRONMENTS OF SANTA CATARINA

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ABSTRACT

The coal mining activity is characterized by the generation of large amount of by-products. One of them is pyrite, which tends to acidify the water, solubilizing heavy metals. As a consequence the environment becomes acid and rich in heavy metals, selecting microorganisms able to survive in this condition, which are of great interest as bioremediation agents. This work describes the isolation and characterization of microorganisms from a coal mining area in Santa Catarina. These microorganisms comprised bacteria, fungi and yeasts resistant to zinc, nickel and cadmium.

Key words: heavy metal resistance, coal mining, acidophilic microorganisms, bioremediation.

INTRODUCTION

The acid mine drainage is one of the main problems associated with coal mining activities. One of the effects is the increase in heavy metal solubility, which results in the accumulation of these toxic elements in the environment. In consequence these sites become inhospitable and just those microorganisms able to tolerate the acidity and the high concentration of heavy metals can survive. Thus, the bioprospection of these natural selected organisms represents an important strategy in order to obtain agents for bioremediation processes (5). The present work describes the isolation and characterization of the heavy metal resistance of microorganisms from coal mining environments of Santa Catarina.

MATERIALS AND METHODS

Sampling and Sample Preparation

Samples of water, sediment, soil and plants were collected from pyrite contaminated sites in Capivari de Baixo and Criciúma (SC - Brazil) and kept in sterilized flasks under refrigeration until processing in laboratory.

Isolation of Microorganisms

The methods of isolation are summarized in Table 1. Inoculated plates were incubated up to two weeks at 30ºC. Different colonies of bacteria and fungi were selected and purified.

Enrichment experiments using GYA-pH 3 media were also conducted in increasing metal concentrations (up to 100 mM of Zn, Ni, Cd and Cu), for selective isolation of metalophilic microorganisms. Incubations were conducted at 30ºC and 150 rpm by one-week intervals between each metal concentration treatment.

Resistance test

The isolates were tested for heavy metals ($ZnCl_2$, $NiCl_2$, $CdCl_2$ and $CuSO_4$) resistance as described by Malik and Jaiswal, 2000 (7), using GYA media (pH 4.0 for acidophilics and 5.5 for the remainders) for bacteria and yeast, and MEA media for filamentous fungi. The concentrations tested were 1, 5, 10 and

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20 mM, except in the filamentous fungi, which was only 10mM, keeping, in all cases, the same incubation conditions as in the isolation procedure.

Lineages Characterization

Cellular and colony morphology of the isolated strains were characterized using a phase contrast microscope.

RESULTS

Ninety-six strains of bacteria and fungi were isolated. The bacteria were separated in two groups neutrophilic (growth at neutral pH) and acidophilic (growth at acidic pH). The neutrophilic group comprised mainly Gram-positive bacteria with four distinct morphologies, while the acidophilic group was composed by Gram-negative bacilli. Yeasts were isolated in acid medium, showing a great morphology diversity. The filamentous fungi were isolated in both acid and neutral medium without any morphological difference.

Distinct patterns of heavy metal resistance were evidenciated (Tables 1 and 2), being the Zn and Ni resistance the most widespread. Only the fungi were Cd resistant, and none of the isolates presented resistance to Cu. The incidence of resistant strains was higher among fungi.

Table 1. Methods for sample processing and isolation of microorganisms. TSA, Tryptic Soy Agar; SA, Saboraud Agar; GYA, Glucose Yeast Extract Agar; MEA, Malt Extract Agar.

Sample	Processing	Culture Media
Water	Dilution and plating	TSA (pH 7.0), SA (pH 5.5) and GYA (pH 2.5 and 4.0)
Sediment and soil	Ressuspension, dilution and plating	TSA (pH 7.0), SA (pH 5.5) and GYA (pH 2.5 and 4.0)
Plants	Maceration/fragmentation and plating	MEA (pH 5.5) and GYA (pH 2.5 and 4.0)

*All strains were resistant to $ZnCl_2$ and sensitive to $CuSO_4$.

Table 2. Resistance of bacteria and yeast strains to different heavy metals.

Bacteria		Yeasts	
Strain	**Resistance**	**Strain**	**Resistance**
B2	1 mM $ZnCl_2$ and 1 mM $NiCl_2$	**B12**	20 mM $ZnCl_2$ and 1 mM $NiCl_2$
B6	1 mM $NiCl_2$	**B15**	20 mM $ZnCl_2$ and 1 mM $NiCl_2$
YE-05b	1 mM $NiCl_2$	**B16**	20 mM $ZnCl_2$ and 1 mM $NiCl_2$
YE-16	1 mM $ZnCl_2$ and 1 mM $NiCl_2$	**YE-02**	10 mM $ZnCl_2$ and 1 mM $NiCl_2$
YE-18	1 mM $ZnCl_2$ and 5 mM $NiCl_2$	**YE-05a**	10 mM $ZnCl_2$
CV-11	20 mM $ZnCl_2$	**YE-07**	5 mM $ZnCl_2$
CV-24	20 mM $ZnCl_2$	**YE-14**	10 mM $ZnCl_2$, 10 mM $NiCl_2$ and 1 mM $CdCl_2$
CV-25	20 mM $ZnCl_2$	**YE-19**	10 mM $ZnCl_2$, 10 mM $NiCl_2$ and 1 mM $CdCl_2$
CV-26	10 mM $ZnCl_2$	**YE-20**	1 mM $ZnCl_2$ and 1 mM $NiCl_2$
CV-33	20 mM $ZnCl_2$	**YE-22**	1 mM $ZnCl_2$
CV-56	1 mM $ZnCl_2$	**YE-23**	1 mM $ZnCl_2$
		YE-24	1 mM $ZnCl_2$

Table 3. Resistance of filamentous fungi to different heavy metals*.

Strain	$NiCl_2$	$CdCl_2$	Strain	$NiCl_2$	$CdCl_2$	Strain	$NiCl_2$	$CdCl_2$
11	+	-	**34**	-	-	**60**	-	-
17	-	-	**38**	-	-	**61**	+	-
18	-	-	**39**	+	+	**71**	-	-
24	+	-	**49**	+	-	**10M**	+	-
26	+	-	**50**	+	-	**51P**	-	-
30	-	-	**59**	-	-	**D**	-	+

*All strains were resistant to $ZnCl_2$ and sensitive to $CuSO_4$.

DISCUSSION

Heavy metal resistance is a widespread attribute among microorganisms isolated from mining environments. Bacteria from *Acidiphilium* and *Acidocella* genera are able to resist to levels as high as 1M Cd, Zn, Ni and Cu, with this resistance being plasmid- mediated (3,6).

Filamentous fungi and yeasts can also show high levels of metals and metalloids resistance, being this resistance associated to the capacity to accumulate these elements (1,2,4,8). This work evaluated a great number of strains of different microorganisms, and in general, the incidence of heavy metal resistance was higher among fungi. However, except for Cd, the resistance levels were similar to those of bacteria. For fungi, the detected resistance levels were similar to those related by Durán *et al*., 1999 (2), except in the case of Zn, which was lower.

Results of this study showed that heavy metal resistance among bacteria is widespread. The strains isolated by enrichment procedures showed an extreme tolerance (up to 100mM) to the tested metals (except Cu), which is in agreement with results of other studies.

The high incidence of heavy metal resistance detected in this work indicates the potential of these microorganisms as bioremediation agents.

RESUMO

Resistência a metais pesados em microrganismos isolados de ambientes da mineração do carvão de Santa Catarina

A atividade de mineração do carvão é responsável pela geração de diferentes sub-produtos. Entre esses, está a pirita que acidifica a água e acelera o processo de solubilização de metais. Como conseqüência, o ambiente torna-se ácido e rico em metais pesados, os quais selecionam os microrganismos capazes de sobreviver nestas condições. Esses microrganismos podem, por sua vez, serem empregados como agentes para a biorremediação de áreas contaminadas com metais pesados. No presente trabalho é descrito o isolamento e a caracterização de bactérias, fungos e leveduras resistentes aos metais zinco, níquel e cádmio.

Palavras-chave: resistência a metais pesados; mineração do carvão; microrganismos acidofílicos; biorremediação.

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