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ISOLATION OF BACTERIA THAT DEGRADE ORGANOMETALLIC COMPOUNDS FROM METALLIC WASTES

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In this report we describe the isolation and identification procedure for heterotrophic bacteria found in wastes from Polish copper mines. The identification procedures included microscopic investigations, and metabolic tests API50CHB and ID32GN.

Key words: bioleaching, isolation of bacteria, heterotrophic bacteria, organometallic ores

INTRODUCTION

Economic development causes a huge increase of demand of metals, whose natural supplies quickly diminish. In addition, exploitation and enrichment of poorer and poorer layers of ores leave behind vast quantity of mining wastes. Traditional methods of ore enrichment including hydrometallurgical ones are approaching economical limits. However, the considerable quantities of old wastes are frequently richer in the demanded metals than many natural ore beds. Therefore the hydrometallurgical methods are presently often supplemented by bio-hydrometallurgical methods, which in the recent years became very popular, especially in mining of gold (Schnell et al., 1997; Nicholson et al., 2001; Karaś et al., 2002).

The presently used bio-hydrometallurgical methods are mostly base on bioleaching of sulfometallic ores in acid solutions. Metals in some ores, however, are present also in organometallic chelate compounds, some of them containing cyclic hydrocarbons. The content of the cyclic hydrocarbon chelates is rather small but due to the high

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demand for metals the exploitation of such ores (using the bioleaching methods) is worth considering.

The bioleaching of organometallic ores has to be done with heterotrophic bacteria, which can grow on organic material and degrade compounds such as proteins, fats and carbohydrates. Our previous studies showed that some heterotrophic bacteria in the absence of complex organic compounds can utilize even simple hydrocarbons. This finding suggests that these bacteria may also efficiently degrade other difficult to exploit organometallic ores (Farbiszewska et al.; 1995; Sudoł et al., 1997)

GOAL OF THE STUDIES

The aim of this work was isolation and characterization of autochthonous heterotrophic bacteria living in metallic flotation wastes. Our preliminary experiments indicate that the autochthonous bacteria may be very suitable for the exploitation processes.

BACTERIAL MATERIALS

The investigative material consisted of 17 test samples taken from stored flotation wastes. Some samples were taken from the presently used storage place called “Żelazny Most” and the other samples were from the recultivated waste storage called “Konrad”

CULTURE MEDIA

1. Agar TF

standard agar	- 9,4 g
glucose	- 3,5 g
glycerol	- 3,0 ml
polyvinyl alcohol 2,5%	- 3,0ml (our modification)
L-asparagine	- 0,2 g
agar (BTL)	- 5,0 g
distilled water	- 400,0 ml
pH 7	
2. Agar TSA:

bio-Trypcase	- 15,0 g
bio-Soyase	- 5,0 g
NaCl	- 5,0g
agar	- 20,0 g
polyvinyl alcohol 2,5%	- 5,0 ml
distilled water	- 1000,0 ml
pH	- 7,2

3. Mineral medium:

(NH ₄) ₂ SO ₄	- 2,0 g
K ₂ HPO ₄	- 3,0 g
KH ₂ PO ₄	- 2,0 g
MgSO ₄ ·7H ₂ O	- 0,5 g
Na ₂ CO ₃	- 1,0 g
distilled water	- 1000,0 ml
pH	- 7
4. Medium with resazurin and CN⁻ ions:

pepton proteose	- 3,0 g
NaCl	- 5,0 g
KH ₂ PO ₄	- 0,225 g
Na ₂ HPO ₄	- 5,64 g
resazurin 0,2%	- 10,0 ml
CN ⁻ 0,5% aqueous solution	- 15,0 ml
distilled water	- 1000,0 ml
pH	- 7,7
5. Medium with milk,
6. Medium with tributyrin,
7. Medium with blood,
8. Waksman's medium,
9. Simons's medium,
10. Kligler's medium,
11. Davis's medium.

ISOLATION OF BACTERIA

The primary cultures of the heterotrophic bacteria were grown on the agar containing blood and agar TF at 30°C for 72h. The dominant colonies grown on the agar with blood were collected at 24h, 48h and 72h time points. We also collected colonies that were able to limit growth of other bacteria and fungus. The next passages were done on agar TF and agar TSA. The bacteria were stained using Gram's method (Burbianka, 1983; Kędzia, 1990).

MICROSCOPIC INVESTIGATION

Microscopic investigations were carried out at a magnification of 2000. We determined the shape and size of the microorganisms, the presence of areola, and classified the bacteria as the Gram(+) or Gram(-) types (Sobczak, 1978).

INVESTIGATION OF METABOLISM

In the first step we tested the presence of enzymes such as catalase, oxidase, protease, lipase, amylase and hemolysin.

Next we tested:

- the ability of using citrate as a source of carbon (Burbianka, 1983; Kędzia, 1990),
- the ability of production of H₂S (Burbianka 1983, Kędzia 1990),
- the growth on agar containing Mn, Mg, Zn, Ag and Cu sulfates (Bergey's, 1984),
- the growth on Davis, ST6 and Rh medium (Bergey's, 1984),
- the growth on agar with Al₂O₃,
- the growth on mineral medium with Cu₂S, NiS and Ag₂S (Burbianka, 1983),
- the growth on medium with resazurin and Co, Ni and Cu phthalocyanine,
- the growth on mineral medium with Co, Ni and Cu phthalocyanina.

The isolated bacterial strains were also analyzed using the ID32GN and API 50CHB tests (Kluczek, 1999).

The isolated strains were resistant to toxic substances like Co, Ni, Cu, Ag and Mn phthalocyanine and could easily grow on glycerol, glycerol with polyvinyl alcohol, resazurin, or phthalocyanine as a sole source of carbon.

IDENTIFIED STRAINS OF BACTERIA

Based on the microscopic observations and the biochemical tests we identified the following autochthonous strains (Kluczek, 1999):

- *Pseudomonas fluorescens*,
- *Pseudomonas stutzeri*,
- *Acinetobacter johnsonii*,
- *Aeromonas hydrophila*,
- *Brevibacillus brevis*,
- *Bacillus pumilus*,
- *B. mycoides*,
- *B. sphaericus*,
- *B. circulans*,
- *B. amyloliquefaciens*.

Samples of the identified strains are stored in the Department of Molecular and Experimental Biology, University of Opole.

CONCLUSIONS

We isolated autochthonous bacteria from flotation waste of Polish cupriferous ores. These strains are able to degrade organometallic compounds such as phthalocyanines and therefore may be suitable for industrial applications. Preliminary tests of

bioleaching of these materials at neutral pH, with *B.mycooides* and *B.amyloliquefaciens* (from our collection) showed copper enrichment from 1,58% to 2,8% (Farbiszevska-Kiczma et al., 2004). We are now starting to prepare the bacterial composites optimal for bioleaching of the studied ores.

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W pracy przedstawiono sposób izolacji bakterii heterotroficznych z odpadów poflotacyjnych, pochodzących z osadników polskiego okręgu miedziowego oraz metodykę ich identyfikacji na podstawie badań mikroskopowych, własności metabolicznych i testów identyfikacyjnych ID32GN, API50CHB przeprowadzonych na mini analizatorze API.