Pseudomonas mendocina AS302 A BACTERIUM WITH A
NON SELECTIVE AND VERY HIGH METAL BIOSORPTION CAPACITY

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ABSTRACT

Pseudomonas mendocina AS302 was selected out of some 80 different strains by using a
metal enrichment-screening test. The strain is highly resistant against mercury, antimony,
arsenate, arsenite and thallium. AS302 shows a high biosorption capacity for several metals
without any specificity. The biosorption could be described by Freundlich adsorption
isotherms. Electrophoretic mobility measurements indicated the presence of some amino
groups presumably on proteins.
INTRODUCTION

A large screening of soils contaminated by heavy metals, due to mineral processing or mining activities, revealed many different strains with high resistances to heavy metals. One group of bacteria belonged to Alcaligenes eutrophus (1) containing at least two large plasmids on which a DNA fragment was found that hybridizes with czc CBAD of A. eutrophus CH34 (2). Several other bacteria were selected and could be divided in different groups on basis of heavy metal resistance, plasmid pattern, protein pattern and carbon source profile. Some representative strains of A. eutrophus and representatives of each other group, together about 80 different strains, were used in a metal enrichment test. This test is based on a visualization of microbially enriched metal by means of precipitation reactions on agar plates (3). An estimation of the metal uptake capacities of the elements silver, nickel, palladium and thallium could be made, which enabled to select the best bacterial strains for further investigation. One of these selected strains was Pseudomonas mendocina AS302.

PRESENTATION OF PSEUDOMONAS MENDOCINA AS302

Pseudomonas mendocina AS302 was isolated from a waste heap in Likasi South in the Shaba province in Zaire. The waste contained high concentrations of Cd (51 ppm), Co (2350 ppm), Cu (17500 ppm), Pb (2270 ppm), Zn (2780 ppm), Ni (33 ppm), Cr (23 ppm) and Hg (33 ppm). The sample had a high water soluble Co concentration (42 ppm). The strain was on minimal medium highly resistant against mercury (0.2 mM), antimony (0.2 mM), arsenate (20 mM), arsenite (2 mM) and thallium (1 mM). Plasmid extraction showed at least one plasmid (pMOL219). A possible role of the plasmid in metal resistance is not clear.

BIOSORPTION

AS302 showed a high biosorption capacity for several metals without any specificity where other biomass showed a specific binding in favour of certain metals. A biosorption capacity between 300 and 400 μmol/g dry biomass was measured for Ti, Mn, Fe, Co, Ni, Cu, Zn, Pd, Cd, Pb and Cr. Silver biosorption capacity was around 700 μmol/g dry biomass. Figure 1 shows the biosorption capacity for several metals.

Biosorption isotherms

The biosorption of the metals Ni, U, Y, Pd and Ag follows the Freundlich adsorption isotherm model (3):

\[ \log q = \log k + \frac{1}{n} \log C_e \]

in which

\( q \) = biomass biosorption uptake capacity (mg metal/g dry biomass)
\( C_e \) = equilibrium metal concentration (mg metal/l)
\( k \) = metal biosorption uptake capacity at an equilibrium concentration of 1 mg metal/l
\( \frac{1}{n} \) = slope of the isotherm

Figure 2 shows a high biosorption of Ag, U, Pd and Y and a lower sorption of Ni. Compared to the highest metal biosorption capacity found for other bacteria in the literature (Table I), AS302 has a high biosorption capacity for U, Pd, Y and Ni.
Figure 1. Biosorption capacity and specificity of *P. mendocina* AS302: metal biosorption capacity; $C_e$: metal equilibrium concentration.

Figure 2. Biosorption isotherms of Ag, Ni, U, Pd and Y.
$q = $ biosorption capacity (μmol metal/g dry cell weight) with an initial metal concentration of 1 mM.
Biosorption was in decreasing order for Ag, Pd, U, Y and Ni for a same metal equilibrium concentration.

Table 1. Comparison of the metal biosorption capacity of AS302 with other bacteria in the literature.

<table>
<thead>
<tr>
<th>Metal</th>
<th>Metal biosorption capacity of AS302 mg/g biomass</th>
<th>Bacteria from literature</th>
<th>Metal biosorption capacity of these bacteria mg/g biomass</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ag</td>
<td>75</td>
<td>Bacillus subtilis</td>
<td>90</td>
<td>4</td>
</tr>
<tr>
<td>Ni</td>
<td>24</td>
<td>Bacillus licheniformis</td>
<td>30.5</td>
<td>5-6</td>
</tr>
<tr>
<td>Ni</td>
<td>24</td>
<td>Bacillus subtilis</td>
<td>37</td>
<td>4</td>
</tr>
<tr>
<td>Pd</td>
<td>108</td>
<td>Bacillus subtilis</td>
<td>30</td>
<td>7</td>
</tr>
<tr>
<td>Y</td>
<td>38</td>
<td>Bacillus subtilis</td>
<td>123</td>
<td>7</td>
</tr>
<tr>
<td>U</td>
<td>188</td>
<td>Methylobacillus</td>
<td>180</td>
<td>8</td>
</tr>
</tbody>
</table>

Electrophoretic mobility

Electrophoretic mobility (EPM) measurements (Figure 3) showed a change of the cellular charge from -2.5 to +1.4 in the pH range between 2 and 4. This high positive charge at low pH indicates the presence of some amino groups presumably on proteins. Acid base titrations revealed that, compared with some other strains, AS302 exhibits a surplus of negative and a lack of positive charges. This is also reflected in the same acidic isoelectric point (pH 3.1).

Figure 3. Electrophoretic mobility (EPM) in function of pH for P. mendocina AS302.

Transmission Electron Microscopy

Figure 4 shows a transmission electron microscopy photo of AS302 with Ag (A). Electron dense dots could be observed on the surface of the cell. Yttrium (B) is localized at the cellular membrane. Palladium (results not shown) is localized in some dark areas inside AS302.
Figure 4 Transmission electron microscopy of *P. mendocina* AS302 with biosorbed Ag(A) and Y(B).

CONCLUSIONS

The heavy metal resistant strains *Pseudomonas mendocina* AS302 showed a very high biosorption capacity comparable with the best strains from literature. Metals seem to be sorbed at the cell surface and amino groups could be involved as functional binding groups. The high biosorption capacity and low specificity makes AS302 as an interesting candidate for the removal of metals from waste water.
ACKNOWLEDGEMENTS

This project was supported by the CEC program BRITE-EURAM BE-5350.

REFERENCES


