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Westfälische Wilhelms-Universität Münster

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Metals in Biology: Microbial Metallome

TBM01 **Effect of Cu on Pigmentation and Survival of *Pseudomonas Stutzeri***
Azma, R., Karachi/PK, Hayyat, A., Karachi/PK

TBM02 **Understanding the Mercury Effects on tRNA Processing in *Bacillus Subtilis***
Campos, J., Querétaro/MEX, Sánchez-Millan, E., Querétaro/MEX, Esquivel-Naranjo, E. U., Querétaro/MEX, Cervates-Chavéz, J. A., Querétaro/MEX, Cruz-Hernández, A., Querétaro/MEX, Garcia-Martinez, R., Querétaro/MEX, Pastrana-Martínez, X., Querétaro/MEX, Arvizu-Gómez, J. L., Irapuatop/MEX, Jones, G., Atlanta/USA

TBM03 **Functional Analysis of CopVTMK Proteins and their Involvements in Resistance to Heavy-metals in *Cupriavidus Metallidurans* CH34**
Derock, J., Mons/B, Wattiez, R., Mons/B

TBM04 **A Simple Functional Assay for Evaluation of Heavy Metal Mutagenicity in Eukaryotes**
Eleutherio, E., Rio de Janeiro/BR

TBM05 **7-(4'-Piperazino)carbonyl Derivatives of Ciprofloxacin: Synthesis and Antibacterial Activities**
Hu, M., Taipei/RC, Chen, P.-T., Taipei/RC

TBM07 **Conformational Changes Associated with the Removal of Zinc Ions from the Cyanobacterial Metalloregulator SmtB**
Kondrat, F. D. L., Coventry/GB, Scarff, C. A., Coventry/GB, Blindauer, C. A., Coventry/GB, Scrivens, J. H., Coventry/GB

TBM08 **Copper and Silver Bacterial Resistances: Functional Study of CopB in *Cupriavidus metallidurans* CH34**
Montante, C., Mons/B, Wattiez, R., Mons/B

TBM09 **Quercetinase of *Streptomyces* sp. FLA, a 'Cambialistic' Dioxygenase with Preference for Nickel Ions**
Nianios, D., Münster/D, Fetzner, S., Münster/D

TBM10 **Sulfate Assimilation Mediates Tellurite Reduction and Toxicity in *Saccharomyces Cerevisiae***
Ottosson, L. G., Gothenburg/S, Logg, K., Gothenburg/S, Ibstedt, S., Gothenburg/S, Sunnerhagen, P., Gothenburg/S, Käll, M., Gothenburg/S, Blomberg, A., Gothenburg/S, Warringer, J., Gothenburg/S

TBM11 **Control of Copper Tolerance in *Rhodobacter Capsulatus***
Rademacher, C., Bochum/D, Moser, R., Bochum/D, Lackmann, J.-W., Bochum/D, Masepohl, B., Bochum/D

TBM12 **Uptake and Distribution of Cadmium by Strain *Pseudomonas Aeruginosa* San Ai**
Rikalovic, M. G., Belgrade/SRB, Gojgic-Cvijovic, G., Belgrade/SRB, Vrvic, M. M., Belgrade/SRB, Karadzic, I., Belgrade/SRB

Uptake and distribution of cadmium by strain *Pseudomonas aeruginosa* san ai

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Miroslav M. Vrvic, Belgrade/SRB, Gordana Gojgic-Cijovic, Belgrade/SRB, Ivanka Karadzic, Belgrade/SRB,
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The bioremediation of heavy metals using microorganisms has received a great deal of attention for its potential application in industry. Among the most common heavy metals used and the more widespread contaminants of the environment is mercury, cadmium, lead and copper. The mechanisms associated with metal removal by microorganisms are complex and occur as: biosorption of metal ions on the cell surface, intracellular uptake of metal ions or chemical transformation of metal ions by microorganisms. Biosorption is a passive uptake of metals based upon physicochemical interactions between metal and functional groups of the cell wall. Intracellular metal uptake, sometimes called active biosorption is slow and nutrient dependent process. This way of uptake is accumulation of metal cations by living cells, where they, dye to their metabolically pathways, were modified and used for microbial needs or in form that is less/no toxic, and returned in environment. Various microbial species, mainly *Pseudomonas,* have been shown to be efficient in removal of the different heavy metals from polluted effluents, using combinations of mentioned mechanisms. Present study represents trial to characterize and determine Cd^{2+} uptake by strain *P. aeruginosa* san ai. In order to evaluate tolerance of the strain, MIC for cadmium were determined, afterwards the metal uptake was monitored. Preliminary results showed that MIC for Cd was 2 mM. More than 80% of cadmium added to the culture grown in submerged conditions was found to be associated with biomass. Investigation of distribution of metal in cell fractions, as well in protein, lipid and polysaccharide fractions is underway.

Acknowledgment: This work was supported by the project III 43004 of the Ministry of Science and Technological Development of Serbia. Also, the authors are grateful to Professor Dr. N. Fujiwara from the Institute for Technological Research (TRI), Osaka, Japan for his kind donation of strain.

Uptake and distribution of cadmium by strain *Pseudomonas aeruginosa* san ai

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Abstract: The bioremediation of heavy metals using microorganisms has received a great deal of attention for its potential application in industry. Among the most common heavy metals used and the more widespread contaminants of the environment is mercury, cadmium, lead and copper. The mechanisms associated with metal removal by microorganisms are complex and occur as: biosorption of metal ions on the cell surface, intracellular uptake of metal ions or chemical transformation of metal ions by microorganisms. Biosorption is a passive uptake of metals based upon physicochemical interactions between metal and functional groups of the cell wall. Intracellular metal uptake, sometimes called active biosorption is slow and nutrient dependent process. This way of uptake is accumulation of metal cations by living cells, where they were modified and used for microbial needs or in form that is less/no toxic, and returned in environment. Various microbial species, mainly *Pseudomonas,* have been shown to be efficient in removal of the different heavy metals from polluted effluents, using combinations of mentioned mechanisms. This study represents trial to characterize and determine Cd2+ uptake by strain *P. aeruginosa* san ai. In order to evaluate tolerance of the strain, MIC for cadmium were determined, afterwards the metal uptake was monitored. Preliminary results showed that MIC for Cd was 2 mM. More than 80% of cadmium added to the culture, grown in submerged conditions, was found to be associated with biomass. Investigation of distribution of metal in cell fractions, as well in protein, lipid and polysaccharide fractions is underway.

Introduction

The pollution of the environment with toxic heavy metals is spreading throughout the world along with industrial progress. Metal toxicity can be divided into three categories: blocking the essential biological functional groups of molecules, displacing the essential metal ion in biomolecules and modifying the active conformation of biomolecules.[1] The toxicity effects greatly depend on the bioavailability of the toxicant meaning the proportion of the contaminant present in the environment in the forms that can be assimilated by organism.[2] The bioremediation of heavy metals using dead or viable biomass of bacteria, fungi and algae or plant (fito remediation) has received a great deal of attention in recent years, not only as a scientific novelty but also for its potential application in industry.[3,4]

The mechanisms associated with metal removal by microorganisms are rather complex compared with those associated with chemical absorbents and can be divided into three categories: (1) biosorption of metal ions on the cell surface, (2) intracellular uptake of metal ions, and (3) chemical transformation of metal ions by microorganisms.[5] Process where non-living biomass is involved in removal metal ions is called biosorption. On the other hand bioaccumulation is an intracellular metal accumulation by living cells.[6] Various microbial species, mainly *Pseudomonas,* have been shown to be relatively efficient in biosorption and bioaccumulation of the different heavy metals (Cr, Cu, Zn, Pb, Cd) from polluted effluents.[7]

Materials and methods

Microorganism and cultivation conditions

P. aeruginosa san ai was isolated from industrial mineral metal cutting oil.[8] The strain was activated in nutrient agar (Torlak, Serbia) at 30°C for 24h.

Prefermentation was carried out in LB medium[13] at 30° C for 24 h and fermentation in LB medium with addition of 100 mmol sterile $CdCl_2$, at 30 °C for five days.

Minimal inhibitory concentration for cadmium (MIC)

MIC was determined calorimetrically at 550 nm, for cadmium concentration ($CdCl_2$) in range of 0.5 to 10 mM.

Dynamic of cadmium uptake

Dynamic of cadmium uptake was monitored five days and metal concentration in fermentation broth and biomass was determined by atomic absorption spectrometry .

SDS PAGE electrophoreses

SDS PAGE was performed on (7.5% acrylamide running gel) to compare protein profile in fermentation broth and biomass of strain growth in LB medium with cadmium and in a control.

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Results and discussion

Cadmium belongs to group high toxic metal together with Hg, Pb, Ag, Sb and U and is human carcinogen. Effect on cells is oxidative stress by changing Fe and Zn cofactors. Free Fe then generate reactive hydroxyl and the final result Is peroxidation of membrane lipids.[9]

Minimal Inhibitory Concentration (MIC)

MIC's for cadmium was determined to be concentration of 2 mM cadmium, so it could be concluded that strain is tolerant to this metal. This MIC value in comparison with MICs for native strains reported in literature (from 0.95 to 6 mM) is somewhere in the middle (*Table 1.*).[10, 11]

Table. 1. Experimental cadmium MIC for *P. aeruginosa* san ai and reported MIC range for different *P. aeruginosa* strains from literature

Metal	MIC	Litherature*
Cd	2 mM	from 0.95 to 6 mM

*native reported strains

Dynamic of cadmium uptake

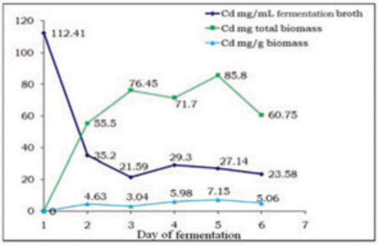

Fig. 1. Dynamic of cadmium uptake by *P. aeruginosa* san ai on LB medium with $CdCl_2$

As *Fig. 1.* shows that the most intensive uptake of metal is during first 24 h of fermentation. In that time period concentration of cadmium in fermentation broth is reduced four times. It could be concluded that process of cadmium uptake is associated with bioaccumulation, i.e. it is made distribution of metal in the cytosol and cell structures .

SDS PAGE electrophoreses

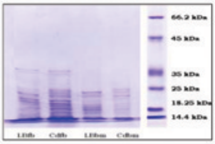

Fig. 2. Protein profile of fermentation broth and biomass of *P. aeruginosa* san ai growth with cadmium and in control: LBfb-fermentation broth from LB without $CdCl_2$,Cdfb-fermentation broth from LB with CdCl2, LBbm-biomass from LB without $CdCl_2$, Cdbm-biomass with $CdCl_2$.

Fig. 2. shows protein profile (SDS electrophoreses) of fermentation broth (fb) and biomass (bm) of *P. aeruginosa* san ai growth on LB medium without and with presence of cadmium. It could be seen that molecular weights of proteins bands are below 45 kDa and there are differences in presence of protein bans and in their intensity in samples. This is result of stress caused by exposure of bacterial culture to cadmium.

Conclusion

1. *P. aeruginosa* san ai is tolerant to cadmium with MIC of 2 mM.
2. Concentration of cadmium in fermentation broth was reduced four times during first 24 hours of fermentation in presence of metal, which indicate that mechanism of cadmium uptake is probably bioaccumulation.
3. Protein profiles of fermentation broth and biomass of microbe grown with cadmium, and control showed significant differences, caused by stress with heavy metal.
4. Our results indicate that P. *aeruginosa* san ai has potential to be used in bioremediation.