The Journal of Microbiology, March 2002, p.51-54
Vol. 40, No. 1

Heavy Metal Biosorption and its Significance to Metal Tolerance of Streptomycetes

Jae-young Rho and Jae-heon Kim*

Department of Microbiology, College of Natural Sciences, Dankook University, Cheon-an, 330-714, Republic of Korea

(Received October 24, 2001 / Accepted February 18, 2002)

Heavy metal adsorptions of four streptomycetes were compared with each other. Among the test strains, *Streptomyces viridochromogenes* showed the most efficient metal binding activity, which was carried out by cell wall as well as freeze-dried mycelium. An order of adsorption potential (zinc > copper > lead > cadmium) was observed in single metal reactions, whereas this adsorption order was disturbed in mixed-metal reactions. The metal adsorption reactions were very fast, pH dependent and culture age-independent, suggestive of a physico-chemical reaction between cell wall components and heavy metal ions. The metal tolerant stains presented the weakest adsorbing activity, indicating that the metal biosorption was not the basis of the metal tolerance.

Key words: cell wall, freeze-dried mycelium, heavy metal adsorption, *Streptomycetes*

The surface envelopes of bacterial cells can adsorb various heavy metals by virtue of ionic bonds to their intrinsic chemical groups. The sites for metal binding are different according to bacterium species and metals. In *Pseudomonas fluorescens*, a Gram-negative bacterium, for example, the lipoplysaccharide of the outer membrane appears to be associated with platinum while gallium and uranium are bound over the whole cell envelope (Krueger *et al.*, 1993). The homogeneous distribution of adsorbed metals in cytoplasm has also been reported for metals like copper and lead in *Pseudomonas stutzeri* (Mattuschka *et al.*, 1994). Some metals such as lanthanide in *Escherichia coli* are precipitated principally in periplasmic space (Bayer and Bayer, 1991). Periplasmic metal accumulation was reported for *Citrobacter* sp., which produced metal phosphate by a cell-bound phosphatase (Jeong *et al.*, 1997).

Heavy metal adsorption, however, appears to be stronger in Gram-positive cell walls. The metal binding sites in the cell wall of *Bacillus subtilis* have been described (Beveridge and Murray, 1976). Anionic groups such as carboxylate and phosphate groups of peptidoglycan and teichoic acids are considered the major metal binding sites (Beveridge and Murray, 1980). The heavy metal adsorption by streptomycetes, also belong to Gram-positive bacteria, has been presumed to contribute a large heavy metal binding capacity and is considered an alternative method to recover metals from waste liquid. In this respect, uranium absorption by streptomycetes has been most frequently studied using whole cell or cell wall preparations. Uranium ions were bound to phosphodiester residues (Friis and Myers-Keith, 1986) and released by EDTA treatment (Horikoshi *et al.*, 1981). Further progress in uranium recovery using immobilized cells has been reported (Nakajima *et al.*, 1982). However, the commercial application of biosorption appears to be limited primarily due to competition with commercial ion-exchangers (Gadd, 2001).

In addition, some *Streptomyces* species exhibit multiple tolerance against different metals and metaloids whereas others are more sensitive to them (Abbas and Edwards, 1989), and streptomycetes can be sub-grouped according to their resistance to antimony, arsenate and arsenite (Hänel *et al.*, 1989). The relative effects of different heavy metals on growth and antibiotics production have been reported (Abbas and Edwards, 1990). Concerning the molecular mechanism of metal resistance, mercury resistance through the mercuric reductase system has been revealed for *S. lividans* (Nakahara *et al.*, 1985; Rother *et al.*, 1999).

We have isolated a lead-tolerant *Streptomyces* strain from mine soil samples and found that the extracellular precipitation of superoxide dismutases as a result of lead adsorption could be a major lead tolerance mechanism of the strain (So *et al.*, 2001). This report indicates that the metal adsorption by the cell mass of streptomycetes is not correlated to metal tolerance.

* To whom correspondence should be addressed.
(Tel) 82-41-550-3452; (Fax) 82-41-551-9229
(E-mail) jaehkim1@dankook.ac.kr

Materials and Methods

Microorganisms and culture condition

Streptomyces subrutilus P5, *Streptomyces* sp. C1, *S. subrutilus* KCTC 9086 and *S. viridochromogenes* KCTC 9009 were used throughout this study. The first two strains are lead tolerant, isolated from soil samples of closed gold mines located in Cheon-an and Kong-ju, Korea, survivable at more than 1.5 mM of lead ions. Their taxonomic positions have been determined mainly based on 16S rRNA sequence homologies (GenBank AF401982 for *S. subrutilus* P5 and GenBank AF403713 for *Streptomyces* sp. C1). Liquid yeast extract-malt extract (YEME) medium (ISP No.2), composed of 2 g glucose, 2 g yeast extract and 5 g malt extract in 1 L deionized water, pH 7.2, was used as the culture medium to obtain biomass. Spores were inoculated into a 1 L Erlenmeyer flask containing 300 ml YEME broth to the final concentration of ca. 10^6 spores/ml, and incubated for 6 days at 30°C with shaking at 150 rpm. Mycelium was harvested by centrifugation for 15 min at 15,000 × g and washed twice with distilled water. The mycelium was freeze-dried without addition of protective agents and subjected to adsorption tests.

Cell walls were prepared from freeze-dried mycelium by treating with boiling sodium dodecyl sulphate, and eventually enzymes, to remove remaining nucleic acids and proteins (Hancock, 1994).

Metal binding assay

The binding of heavy metals was measured for freeze-dried mycelium and cell wall preparation. The test was started by adding 10 mg of freeze-dried mycelium into 10 ml of a heavy metal solution prepared by dissolving Zn $(NO_3)_2$, Cu $(NO_3)_2$, Cd $(NO_3)_2$ or Pb$((NO_3)_2$ into deionized water (initial concentration, 150 μg/ml). During the reaction, the metal-mycelium mixture was kept dispersed by continuous shaking on a tumbler mixer. At the specific reaction times indicated in the text, the reaction mixture was filtered to remove mycelium using a nylon membrane (Whatman, pore size; 0.45 μm) and the residual metal concentration was measured by an atomic absorption spectrometry using a Pye-Unicam SP9 spectrometer. The difference between initial and residual concentrations divided by biomass was calculated as the specific amount of adsorbed heavy metal.

To investigate the pH effect, the initial concentration was decreased to 100 μg/ml for each metal to avoid possible precipitation, and pH values of metal solutions were adjusted to 3 and 6 using 0.1 N HCl or NaOH, before the addition of the biomass.

The metal binding test was also carried out for the mixed metal solution in which $NiCl_2$ and the four metal salts listed above were dissolved together to give the final concentration of 1 mM for each metal ion. 10 mg of freeze-dried mycelium or 3 mg of cell wall preparation was dispensed into the mixed metal solution and incubated for two hours. Biomass was removed by filtration as above and the residual metal concentrations for each metal were measured using an inductively coupled plasma optical emission spectrophotometer (Unicam 701). All experiments were carried out at least twice and the representative results are given in this report.

Results and Discussion

Single-metal system

The metal adsorbing activities of the streptomycetes were determined for cadmium, copper, and lead using freeze-dried mycelium. Table 1 shows that, although each heavy metal was adsorbed to various extents into the biomass, the same order of absorption of all strains was followed: zinc > copper > lead > cadmium. Of these, zinc and copper were highly adsorptive showing more than 0.94 μmols/mg biomass, so that almost all metal ions dissolved initially in the reaction mixtures were retained in the biomass. Lead was moderately adsorptive and, on an average, about 0.4 μmols/mg biomass were adsorbed. Cadmium was least adsorbed by the mycelium (0.24-0.47 μmole/mg biomass). Among the test strains, *S. viridochromogenes* was the most effective for all heavy metals. Its superior metal adsorbing capacity might be due to the relatively high phosphorus content in the cell wall (Rho, 1997), because it is known that the major metal binding site of the Gram-positive bacteria is the teichoic acid moiety (Beveridge and Murray, 1980; Rho, 1997). The time course of metal adsorption showed that the metal adsorption occurred very rapidly and reached equilibrium after ca. 30 sec for all test strains. The lead adsorption according to incubation time is shown in Fig. 1. We noticed the higher metal binding capacity of *S. viridochromogenes* once again, because this strain reached equilibrium at a higher level of metal adsorption than the other strains. The adsorption of other heavy metals followed the same pattern (data not shown). No reproducible changes according to age of mycelium were observed for metal adsorption. Thus, in the lead adsorption of *S. subrutilus* P5, the mycelium from three-day and nine-day cultures presented sim-

Table 1. Heavy metal biosorption capacities (μmols/mg biomass) after two-hour incubation in single metal suspension of freeze-dried streptomycetes

Metals	P5	C1	Ss	Sv
Cadmium	0.32	0.24	0.27	0.47
Copper	1.15	0.94	1.08	1.40
Lead	0.41	0.39	0.39	0.55
Zinc	1.61	1.45	1.50	1.89

P5; *S. subrutilus* P5, C1; *Streptomyces* sp. C1, Ss; *S. subrutilus* KCTC9086, Sv; *S. viridochromogenes*

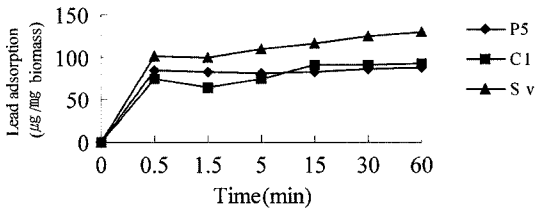

Fig. 1. Time course of the lead adsorption by streptomycetes, P5; *S. subrutilus* P5, C1; *Streptomyces* sp. C1, Sv; *S. viridochromogens*

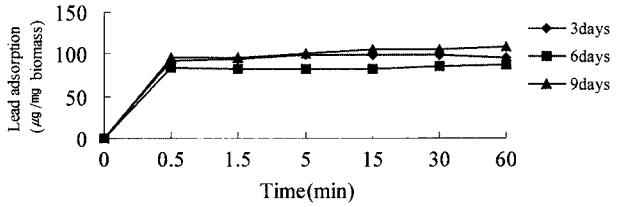

Fig. 2. Effect of cell age on the lead adsorption of *S. subrutilus* P5.

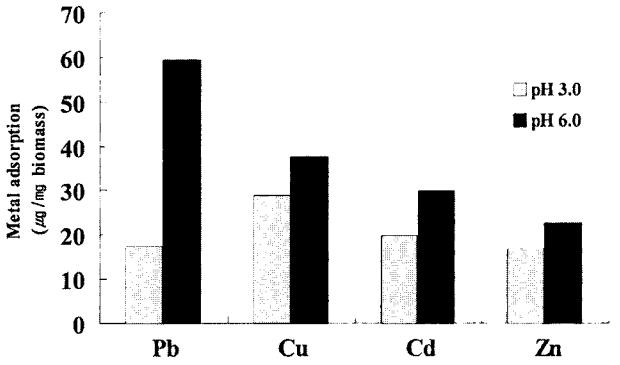

Fig. 3. Effect of pH on the absorption of heavy metals after two-hour incubation in each metal suspension (100 μg/ml) by *S. subrutius* P5.

ilarly higher adsorption levels than that from six-day cultures (Fig. 2). The metal adsorption was a pH dependent reaction as the pH increase of the reaction mixture from pH 3.0 to pH 6.0 accompanied a simultaneous increase in metal adsorption (Fig. 3). This pH-dependent increase was most evident in lead adsorption in the case of *S. subrutilus* P5. The lower level of lead adsorption (60 μg/mg) in this case, compared with those of previous results (ca. 80 μg/mg), might be due to the lower initial lead concentration used for this pH test. A similar concentration dependency of biosorption was reported for *Streptoverticillum cinnamoneum*, although we did not measure it in this study (Puranik and Paknikar, 1997). The reason for less evident pH dependent increases in adsorption of other heavy metals is not clear, but it might be correlated to that different binding sites with different ionizing patterns contribute to metal adsorption for each heavy metal (Beveridge and Murray, 1980).

The biosorption capacities determined in this study match well with the biosorption range of other actinomycetes (Friis and Myers-Keith, 1986; Nakajima and Sakaguchi, 1986; Mattuschka *et al.*, 1994; Puranik and Paknikar, 1997), among which *S. viridochromogenes* exceeded other actinomycete strains in lead adsorption (164 μg/mg or 0.79 μmols/mg). Significantly, lead adsorption was much higher for various fungi including *Mucor rouxii* (769 mg/g) (Lo *et al.*, 1999). In many studies, heavy metal binding was found to be a result of physico-chemical reaction and metals were rapidly adsorbed by surface structures of the cell. Negatively charged sites on the cell wall of Gram-positive bacteria, most of them phosphate groups of teichoic acids, are the major metal binding sites. Our results on pH-dependence and rapid equilibrium time of metal biosorption are in accord with the contribution of the cell wall in metal biosorption.

Mixed metal system

We carried out the adsorption test in reaction mixtures where biomass was exposed to the heavy metal ion mixture. In this mixed metal reaction, we did not see the sequential binding order found in the single metal reactions (Table 2). Thus, compared with the single metal reactions, the decrease in zinc adsorption was notable for all strains, while the lead adsorption was not changed significantly. The copper adsorption also decreased for all strains except *S. subrutilus* P5. Therefore, it seems that, while lead occupied its binding sites where adsorption of other metals took place relatively poorly, the other four metal ions were competing for metal binding sites. The cadmium adsorption pattern was also changed; while cadmium was less adsorptive to *S. subrutilus* P5 and *Streptomyces* sp. C1, more cadmium was adsorbed by *S. subrutilus* KCTC 9086 and *S. viridochromogenes*. These

Table 2. Heavy metal biosorption capacities (μmols/mg) after two-hour incubation in mixed metal suspension of freeze-dried streptomycetes

Metals	P5	C1	Ss	Sv
Cadmium	0.23	0.10	0.36	0.64
Copper	1.18	0.42	0.47	0.63
Lead	0.49	0.31	0.67	0.79
Zinc	0.63	0.40	0.21	0.47
Nickel	0.10	0.10	0.24	0.51

Abbreviations: see Table 1

Table 3. Heavy metal biosorption capacities (μmols/mg wall) after two-hour incubation in mixed metal suspension of cell wall preparations of streptomycetes

Metals	P5	C1	Ss	Sv
Cadmium	0.06	0.04	0.2	0.87
Copper	0.54	1.08	1.16	1.54
Lead	0.83	0.59	1.51	1.86
Zinc	1.04	1.04	0.17	0.69
Nickel	0.16	0.2	0.32	0.66

Abbreviations: see Table 1

two strains presented also higher lead and nickel adsorption capacities, which was not tested in the single metal reaction.

Cell wall preparations of the test strains exhibited more effective metal adsorption compared with the freeze-dried mycelia (Table 3). In other words, most metals were deposited in the cell wall, except the cadmium adsorption by *S. subrutilus* P5, *Streptomyces* sp. C1 and *S. subrutilus* KCTC 9086 and the copper adsorption by *S. subrutilus* P5. Sometimes, heavy metal adsorption occurs mainly in the interior part of the mycelium (live or dead); copper in *S. pilosus* (Golab *et al.*, 1991) and uranium in *S. longwoodensis* (Friis and Myers-Keith 1986) are examples. It seems that cell walls might not be important adsorbents in these cases. As a whole, *S. viridochromogenes* was the most effective adsorbent also in these mixed metal reactions where cadmium-, nickel- and lead adsorption occurred more extensively than other strains. Zinc and copper, on the other hand, showed moderate affinity to *S. viridochromogenes*. These differential metal bindings of cell walls might be due to the differences in binding strength or to the binding selectivity of cell wall components as is known for *B. subtilus* (Beveridge and Murray, 1976). The role of cell wall functional sites on the lead adsorption by a streptomycete strain has been revealed by electron microscopic studies which showed the metal deposit area located in the cell wall structures (Golab *et al.*, 1991). In another study, *S. albus* adsorbed copper and gold as well as lead in its cell wall (Mattuschka *et al.*, 1994).

Interestingly, when we compared lead tolerances of the test strains, *S. viridochromogenes*, the strongest heavy metal adsorbent, was the most sensitive strain that was not able to survive 1.0 mM of lead in the medium. On the other hand, *S. subrutilus* P5 and *Streptomyces* sp. C1 could tolerate lead of more than 1.5 mM, although their lead adsorption was weaker than *S. viridochromogenes*. Therefore, the metal biosorption that occurs as a result of chemical binding between metal ions and cell material, live or dead, should not be a factor in metal tolerance.

Acknowledgments

The authors appreciate the support by the Brain Korea 21 Project.

References

Abbas, A. and C. Edwards. 1989. Effects of metals on a range of *Streptomyces* species. *Appl. Environ. Microbiol.* 55, 2030-2035.

Abbas, A. and C. Edwards. 1990. Effects of metals on *Streptomyces coelicolor* growth and actinorhodin production. *Appl. Environ. Microbiol.* 56, 675-680.

Bayer, M.E. and M.H. Bayer. 1991. Lanthanide accumulation in the periplasmic space of *Escherichia coli* B. *J. Bacteriol.* 173, 141-149.

Beveridge, T.J. and R.G.E. Murray. 1976. Uptake and retention of metals by cell walls of *Bacillus subtilis*. *J. Bacteriol.* 127, 1502-1518.

Beveridge, T.J. and R.G.E. Murray. 1980. Sites of metal deposition in the cell wall of *Bacillus subtilis*. *J. Bacteriol.* 141, 876-887.

Friis, N. and P. Myers-Keith. 1986. Biosorption of uranium and lead by *Streptomyces longwoodensis*. *Biotechnol. Bioeng.* 28, 21-28.

Gadd, G.M. 2001. Microbial metal transformation. *J. Microbiol.* 39, 83-88.

Golab, Z., B. Orlowska, and R.W. Smith. 1991. Biosrption of lead and uranium by *Streptomyces* sp. *Water, Air and Soil Pollution* 60, 99-106.

Hänel, F., H. Krügel, and G. Fiedler. 1989. Arsenical resistance of growth and phosphate control of antibiotic biosynthesis in *Streptomyces*. *J. Gen. Microbiol.* 135, 583-591.

Hancock, I. C. 1994. Analysis of cell wall constituents of Gram-positive bacteria. pp. 63-84. *In* Goodfellow, M. and A. G. ODonnell (eds), Chemical methods in prokaryotic systematics. John Wiley & Sons, Chichester

Horikoshi, T., A. Nakajima, and T. Sakaguchi. 1981. Studies on the accumulation of heavy metal elements in biological systems XIX. Accumulation of uranium by microorganisms. *Eur. J. Appl. Microbiol. Biotechnol.* 12, 90-96.

Jeong, B.C., C. Hawes, K.M. Bonthrone, and L.E. Macaskie. 1997. Localization of enzymatically enhanced heavy metal accumulation by *Citrobacter* sp. and metal accumulation in vitro by liposomes containing entrapped enzyme. *Microbiol.* 143, 2497-2507.

Krueger, S., G.Y. Olson, D. Johnsonbaugh, and T.J. Beveridge. 1993. Characterization of the binding of Gallium, Platinum, and Uranium to *Pseudomonas fluorescens* by small-angle X-ray scattering and transmission electron microscopy. *Appl. Environ. Microbiol.* 59, 4056-4064.

Lo, W., H. Chua, K.M. Lam, and S.P. Bi. 1999. A comparative investigation on the biosrption of lead by filamentous fungal biomass. *Chemosphere* 39, 2723-2736.

Mattuschka, B., G. Straube, and J.T. Trevors. 1994. Silver, copper, lead and zinc accumulation by *Pseudomonas stutzeri* AG259 and *Streptomyces albus*: electron microscopy and energy dispersive X-ray studies. *BioMetals* 7, 201-208.

Nakahara, H., J.L. Schottel, T. Yamada, Y. Miyakawa, M. Asakawa, J. Harville, and S. Silver. 1985. Mercuric reductase enzymes from *Streptomyces* species and group B *Streptococcus*. *J. Gen. Microbiol.* 131, 1053-1059.

Nakajima, A.T. Horikoshi, and T. Sakaguchi. 1982. Recovery of uranium by immobilized microorganisms. *Eur. J. Appl. Microbiol. Biotechnol.* 16, 88-91.

Nakajima, A. and F. Sakaguchi. 1986. Selective accumulation of heavy metals by microorganisms. *Appl. Microbiol. Biotechnol.* 24, 59-64.

Puranik, P.R. and K.M. Paknikar. 1997. Biosorption of lead and zinc from solutions using *Streptoverticillum cinnamoneum* waste biomass. *J. Biotechnol.* 55, 113-124.

Rho, J-y. 1997. Taxonomy of heavy metal resistant streptomycetes isolated from old mines and characterization of the metal binding protein. Ph D. thesis, Dan-kook University, Republic of Korea.

Rother, D., R. Mattes, and J. Altenbuchner. 1999. Purification and characterization of MerR, the regulator of the broad-spectrum mercury resistance genes in *Streptomyces lividans* 1326. *Mol. Gen. Genet.* 262, 154-162.

So, N-w., J-y. Rho, J-y. Lee, I. C. Hancock, and J-h. Kim. 2001. A lead-absorbing protein with superoxide dismutase activity from *Streptomyces subrutilus*. *FEMS Microbiol. Lett.* 194, 93-98.