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Resistance to cadmium in a marine bacterium

G. N. Flatau, R. L. Clément, F. Mahdyoun and M. J. Gauthier

Institut National de la Santé et de la Recherche Médicale, U. 303 "Mer et Santé"
1 avenue Jean Lorrain, F-06300 Nice, France

Introduction

In some bacteria, the intracellular content of cadmium seems to depend on their resistance to the metal. In a strain of *Staphylococcus aureus*, a plasmid encoding for the resistance to β-lactams also provided resistance to zinc, cadmium, mercury, arsenic and lead (DYKE et al. 1970, EL-SOHL and EHRLICH 1982). In other strains of this species, resistance to cadmium can be afforded by an efflux system encoded by a resistance plasmid (TYNECKA et al. 1981a, b) or by a chromosomic determinant (PERRY and SILVER 1982).

Gram negative bacteria as well as Gram positive ones can develop a resistance to metals (TREVORS et al. 1985). Except for some species however (MERGEAY et al. 1985, HORITSU et al. 1986), resistance to cadmium is generally not due to plasmid markers, but rather to metal trapping mechanisms, which seem to be more frequent than efflux or exclusion mechanisms (HIGHAM et al. 1985). Exopolymers, through their ability to adsorb cadmium, can be considered as the first step of different resistance processes (BITTON and FRIEHOFER 1978). Detoxication of cadmium can be achieved through its complexation by specific proteins, whose synthesis is induced by cadmium (KHAZAELI and MITRA 1981, HIGHAM and SADLER 1984) or by its trapping as granules of sulfides or phosphates (AIKING et al. 1984).

Those results come from investigations on terrestrial bacteria. But how is it with marine bacteria, which are Gram negative halophilic microorganisms, for which availability of cadmium largely depends on environmental parameters?

This work deals with different mechanisms of resistance to cadmium exerted by a marine bacterial strain sensitive to cadmium (MIC, 10 mg/l) and able to take up as much as 0.15 % of cadmium (w/w) dissolved in a saline solution.

Material and methods

The test strain was collected in a non polluted sediment in the Bay of Villefranche-sur-Mer (Alpes-Maritimes, France). It belonged to the *Pseudomonadaceae*. It was obligate aerobe, halophilic, and constituted by 1 x 2-3 µm motile rods. Its growth was inhibited by 10 mg Cd/l (as chloride).

Cells were mass-produced by incubating Marine Agar (Difco Lab. Mich.) plates for 18 h at 27 °C after inoculation with a previous culture incubated in Marine Broth (Difco) for 7 h at 27 °C. Mureinoplasts and protoplasts were prepared as previously described (FLATAU et al. 1986).

Cells were adapted by step by step cultures of the strain in progressively increasing concentration of cadmium dissolved in MB (FLATAU et al. 1987a). Sulfides were analysed by the method of KING and MORRIS (1967).

The role of the different cellular envelopes in the repartition of Cd was investigated by preparing mureinoplasts and protoplasts from previously intact cells exposed to 1 mg Cd/l dissolved in a saline solution (SM) (FLATAU et al. 1987b).

The role of glucose was studied by adding this carbohydrate to the SM (final concentration 2 g/l) in which were suspended cells of the test strain.

Results and discussion

Influence of glucose: When cells were exposed for 3 h to 1 mg Cd/l dissolved in SM, cells took up 0,96 ± 0.04 mg Cd/g (dry wt) (n = 4). When SM was supplemented with glucose the uptake decreased to 0.70 ± 0.02 mg Cd/g (n = 4), corresponding to a significant decrease of 27 % ($p \leq 0.01$) and suggesting an inhibitory effect due to the carbohydrate.

Role of different envelopes of the cell wall: After 24 h exposure to 1 mg Cd/l dissolved in SM cells had taken up 1.98 mg Cd/g. After the removal of the exopolymer coating, cellular content of cadmium was 1.72 mg Cd/g, i.e. a decrease of 13 % ($p \leq 0.05$). Mureinoplasts contained only 1.14 mg Cd/g corresponding to a decrease of 42 % relative to intact cells ($p \leq 0.05$). However, the difference in Cd content between mureinoplasts and protoplasts was not significant (Fig. 1).

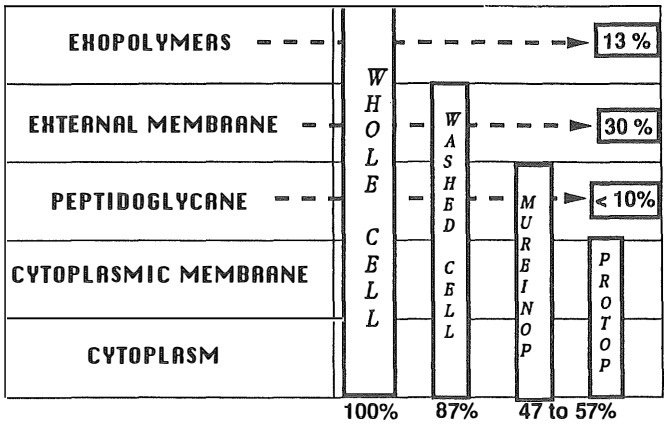

Fig. 1. Repartition of cadmium in the different layers of the cells (percentage relative to the whole cells). Cells exposed for 24 h to Cd were washed three times to eliminate exopolymers (washed cells). Mureinoplasts (mureinop) and protoplasts (protop) were prepared from these washed cells.

Adaptation of cells to cadmium: The length of the lag phase was not correlated with cadmium concentration in the medium. During their adaptation, cells were less and less able to utilize citrate, or to acidify saccharose, glucose, maltose and starch. Partial loss of motility and decrease of the length of cells were observed at 50 mg Cd/l. Cd uptake from MB was roughly linear with Cd concen-

trations in MB (r = 0.979) but the accumulation factor was a decreasing function of Cd concentration in MB (r = 0.979).

Cellular trapping of cadmium: In the presence of cadmium, neither new cellular protein nor an increase in phosphate level was detected, suggesting that the metal did not induce any de novo protein synthesis and was not trapped as phosphate. However, cellular sulfide content increased with increasing Cd concentration in MB (Fig. 2), indicating a probable precipitation as sulfide in or on the cells.

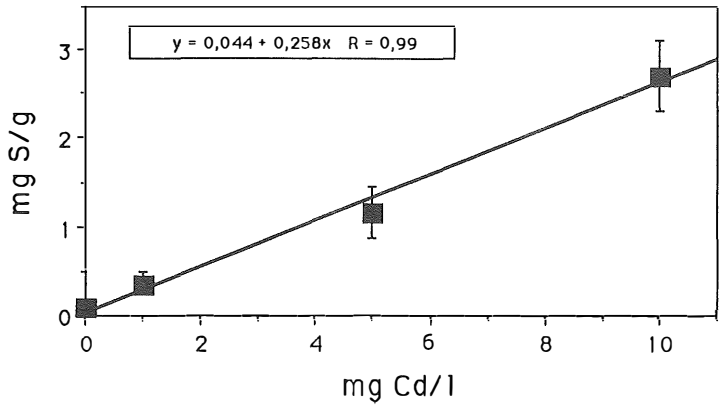

Fig. 2. Increase of sulphides in cells (mg S/g dry wt) with the increase of cadmium in the medium (MB) (mg Cd/l).

Three mechanisms providing a resistance to cadmium were observed: (i) the trapping of the metal by the exopolymers and the envelopes, at the level of the cell wall, (ii) an efflux mechanism and (iii) the detoxication of cadmium as sulfide which may take place at the level of the periplasmic space, the cytoplasmic membrane or the cytoplasm.

In this strain, cadmium transport was likely energy-dependent (FLATAU et al. 1989). Enrichment of SM by glucose could have provided a carbon source but also a higher level of energy which could have triggered efflux mechanisms, as it was assumed for other bacteria (BRYNHILDSEN et al. 1988). But the most efficient mechanism was probably the trapping of cadmium by exopolymers and outer membrane, since mureinoplasts contained roughly two times less cadmium than whole cells. As the cell wall adsorbs a large part of cadmium load and thus prevent its penetration into the deeper layers of the cell, it plays a leading part in the protection of cytoplasm against the entry of cadmium (and probably other metals) and its toxicity (FLATAU et al. 1987b). When cadmium enters the periplasmic space or reaches the cell membrane or the cytoplasm, other mechanisms take place. Cells have to adapt to cadmium which can impair cellular metabolism. The fact that the initial characteristics of the strain were restored after one subculture in a Cd-free medium indicated a physiological adaptation to cadmium rather than a selection of mutants (FLATAU et al. 1987a). The survival of cells with gradually increasing cellular content of cadmium suggests that they detoxify the metal by precipitating it as sulfide less soluble than as chloride, thus less available and less toxic.

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