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Survey of Metal Tolerance in Moderately Halophilic Eubacteria

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The tolerance patterns, expressed as MICs, for 250 moderately halophilic eubacteria to 10 heavy metals were surveyed by using an agar dilution method. The moderate halophiles tested included 12 culture collection strains and fresh isolates representative of *Deleya halophila* (37 strains), *Acinetobacter* sp. (24 strains), *Flavobacterium* sp. (28 strains), and 149 moderately halophilic gram-positive cocci included in the genera *Marinococcus*, *Sporosarcina*, *Micrococcus*, and *Staphylococcus*. On the basis of the MICs, the collection strains showed, overall, similar responses to silver, cobalt, mercury, nickel, lead, and zinc. All were sensitive to silver, mercury, and zinc and tolerant of lead. The response to arsenate, cadmium, chromium, and copper was very heterogeneous. The metal susceptibility levels of the 238 freshly isolated strains were, in general, very heterogeneous among the four taxonomic groups as well as within the strains included in each group. The highest toxicities were found with mercury, silver, and zinc, while arsenate showed the lowest activity. All these strains were tolerant of nickel, lead, and chromium and sensitive to silver and mercury. *Acinetobacter* sp. strains were the most heavy-metal tolerant, with the majority of them showing tolerance of eight different metal ions. In contrast, *Flavobacterium* sp. strains were the most metal sensitive. The influence of salinity and yeast extract concentrations of the culture medium on the toxicity of the heavy metals tested for some representative strains was also studied. Lowering the salinity, in general, led to enhanced sensitivity to cadmium and, in some cases, to cobalt and copper. However, increasing the salinity resulted in only a slight decrease in the cadmium, copper, and nickel toxicities. Reduction in the yeast extract concentration resulted in an increased sensitivity to all metals, especially when this component was lowered to 0.01% (wt/vol). In contrast, a higher concentration only slightly lessened the toxicities of nickel and zinc.

Microorganisms can be classified into different categories on the basis of their optimal growth rates at different salinities. Kushner (9) has suggested the following groupings: (i) nonhalophilic microorganisms, with optimal growth in media containing less than 0.2 M salt; those which can tolerate high concentrations of salts are called halotolerant; (ii) slight halophiles, which grow best in media containing 0.2 to 0.5 M salt; (iii) moderate halophiles, which grow optimally in media containing 0.5 to 2.5 M salt; (iv) borderline extreme halophiles, which grow best in media with 1.5 to 4.0 M salt; and (v) extreme halophiles, which grow best in media containing 2.5 to 5.2 M (saturated) salt. Besides the extreme halophiles, the moderate halophiles are the most important group of microorganisms adapted to live in hypersaline habitats and constitute a very heterogeneous group which includes a great variety of bacteria. Moderately halophilic bacteria include mainly eubacteria (21), although some archaebacteria have been recently reported (11, 13, 25).

The majority of studies concerning this group of microorganisms have been focused on their physiology and taxonomy, and with respect to the former, these have been referred almost exclusively to one representative, *Vibrio costicola* (10), classically isolated from cured meats (19) and more recently found also in hypersaline waters (22) and soils (8).

Very few studies on interactions of moderate halophiles with heavy metals have been reported (7, 12). Such information might be desirable, as some of these metal-resistant halophilic bacteria could be used as bioassay indicator organisms in saline aquatic polluted environments (20).

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In the present study, we report for the first time the natural metal tolerance levels of a large number of moderately halophilic eubacteria, including both culture collection strains and freshly isolated strains representing a very heterogeneous group of moderate halophiles. An additional aim of this study was to investigate the possible influence of different compositions of the test medium on the bacterial responses to the heavy metals tested.

MATERIALS AND METHODS

Microorganisms and growth conditions. A total of 250 moderately halophilic bacterial strains were examined, 12 from culture collections (Table 1) and 238 from solar salterns and hypersaline soils located in Cádiz, Huelva, Alicante, Mallorca, and the Canary Islands (Spain). The latter strains have been characterized and identified as *Deleya halophila* (37 strains) (15) and members of the genera *Acinetobacter* (24 strains) (14) and *Flavobacterium* (28 strains) (14), as well as moderately halophilic cocci (149 strains) tentatively assigned to the genera *Marinococcus*, *Sporosarcina*, *Micrococcus*, and *Staphylococcus* (M. C. Marquez, Ph.D. thesis, University of Seville, Seville, Spain, 1988).

All strains were grown in a saline medium with a final total salt concentration of ca. 100 g/liter supplemented with 5 g of yeast extract (Difco Laboratories, Detroit, Mich.) per liter. The composition of the salt solution (referred to as SW-10) was as follows (grams per liter): NaCl, 81; $MgCl_2$, 7; $MgSO_4$, 9.6; $CaCl_2$, 0.36; KCl, 2; $NaHCO_3$, 0.06; NaBr, 0.026 (22). However, other concentrations of salts and yeast extract were also used in experiments in which we studied the effect of different salinity and nutrients of the medium on the bacterial susceptibility to the metals tested. The pH of all

TABLE 1. MICs of 10 metal ions tested against 12 collection strains of moderately halophilic eubacteria used in this study

Microorganism	MIC (mM) of[a]:									
	Ag	As	Cd	Co	Cr	Cu	Hg	Ni	Pb	Zn
"*Chromobacterium marismortui*" ATCC 17056	0.05	5	0.5	**2.5**	1	1	0.01	**2.5**	**5**	1
Deleya halophila CCM 3662	0.05	10	1	**2.5**	**2.5**	1	0.05	**5**	**5**	0.5
Flavobacterium halmephilum CCM 2831	0.05	10	**2.5**	1	**2.5**	0.5	0.05	**2.5**	**2.5**	0.5
Halomonas elongata ATCC 33173	0.05	**20**	**5**	**2.5**	1	**2.5**	0.05	**2.5**	**5**	0.5
Halomonas subglaciescola UQM 2926	0.05	5	**5**	**2.5**	**2.5**	**2.5**	0.05	**2.5**	**2.5**	0.5
Marinococcus albus CCM 3517	0.05	1	0.5	0.5	0.5	1	0.05	1	**2.5**	0.1
Marinococcus halophilus NRCC 14033	0.05	1	0.5	1	1	0.5	0.05	**2.5**	**2.5**	0.5
Micrococcus halobius CCM 2591	0.05	5	0.5	1	**5**	**2.5**	0.05	**5**	**2.5**	1
"*Micrococcus varians* var. *halophilus*" CCM 3316	0.05	5	1	1	**5**	**2.5**	0.05	**2.5**	**5**	0.5
Paracoccus halodenitrificans CCM 286	0.05	10	0.5	1	**5**	**2.5**	0.01	**5**	**2.5**	0.5
"*Pseudomonas halosaccharolytica*" CCM 2851	0.05	**20**	1	**2.5**	**2.5**	**2.5**	0.05	**5**	**5**	0.5
Sporosarcina halophila DSM 2226	0.05	5	0.5	1	**5**	1	0.05	**2.5**	**2.5**	0.5

[a] Boldface type indicates concentrations involving tolerance of the corresponding metal ion.

media was adjusted to 7.2. When necessary, the medium was solidified by adding 20 g of Bacto-Agar (Difco) per liter. The cultures were incubated at 37°C in an orbital shaker (New Brunswick Scientific Co., Inc., Edison, N.J.) at 200 strokes per minute.

Chemicals. The heavy metals tested were purchased from Sigma Chemical Co. (St. Louis, Mo.) as sodium arsenate, lead acetate, silver nitrate, cadmium chloride, cobalt chloride, cupric sulfate, mercuric chloride, nickel sulfate, and zinc sulfate, and from E. Merck AG (Darmstadt, Federal Republic of Germany) as potassium chromate. Stock solutions were made in distilled water and were sterilized by filtration through 0.22-μm-pore-size membrane filters (Millipore Corp., Bedford, Mass.). These solutions were kept at 4°C for no longer than 1 day.

Heavy-metal toxicity testing. For the determination of tolerance to heavy metals, an agar dilution method (23) with a multiple inoculator system (Don Whitley Scientific Ltd., West Yorkshire, United Kingdom) was chosen. Tubes containing 20 ml of melted standard SW-10 yeast extract agar and different concentrations of metal were poured onto plates on the day of the experiment. The range of concentrations for all heavy metals tested, similar to the one previously used in studies on metal tolerance of eubacteria (7, 17, 20), was as follows (millimolar): 0.005, 0.01, 0.05, 0.1, 0.5, 1, 2.5, 5, 10, 20, and 40. The agar plates were dried at 37°C for 30 min and then inoculated with 21 spots, each containing 10^4 to 10^5 microorganisms from exponentially growing cultures. Similar experiments with 10 g of glucose per liter and 2 g of ammonium chloride per liter, different yeast extract concentrations (10, 1, or 0.1 g/liter), or different salinities (70 or 130 g/liter) of the testing medium were also performed but only with cadmium, cobalt, copper, nickel, or zinc as the toxic agent. Controls consisting of media without metals and inoculated with the test microorganisms were carried out in all experiments.

Plates were read after incubation at 37°C for 2 days. The lowest concentration of metal that completely prevented growth was termed the MIC. The MICs for all strains were the same when tested in four different experiments.

On the other hand, it is well-known that unlike antibiotic resistance, which is evaluated with therapeutic doses, there are no standard acceptable metal concentrations used by all researchers to specify metal resistance. Indeed, metal salts and microbiological medium components can interact in ways which make data interpretation difficult. For this purpose, we chose those concentrations that have been employed in testing media containing yeast extract in previous studies carried out with eubacteria (7, 17, 20). Thus, those strains which were not inhibited by 10 mM As, 1 mM Ag, Cd, Co, Cr, Cu, Ni, Pb, and Zn, and 0.1 mM Hg were considered tolerant.

RESULTS

Response to heavy metals. The tolerance levels of the aerobic, heterotrophic, moderately halophilic species available from culture collections to the 10 heavy metals tested, expressed as MICs, are shown in Table 1. On the basis of the MICs, these 12 strains showed similar susceptibilities to silver, cobalt, mercury, nickel, lead, and zinc ions, except *Marinococcus albus* CCM 3517, for which the MICs of cobalt, chromium, nickel, and zinc were the lowest of all collection strains. On the contrary, the response to arsenate, cadmium, chromium, and copper was very heterogeneous. All these collection strains were sensitive to silver, mercury, and zinc. Only two strains (*Halomonas elongata* ATCC 33173 and "*Pseudomonas halosaccharolytica*" CCM 2851) were tolerant of arsenate, and only three strains (*Flavobacterium halmephilum* CCM 2831, *Halomonas elongata* ATCC 33173, and *Halomonas subglaciescola* UQM 2926) were tolerant of cadmium. Indeed, the two latter strains showed the highest tolerance of cadmium of all collection strains. On the other hand, all strains demonstrated tolerance of lead, and the majority of them were also tolerant of nickel (11 strains) and chromium (9 strains). Otherwise, only six strains were tolerant of copper and only five were tolerant of cobalt.

In Fig. 1 are shown the susceptibility levels of the 238 freshly isolated strains of moderately halophilic bacteria (included in the four different groupings) expressed as percentages of strains for which the MICs of the 10 heavy metals tested in this study were the same. Overall, the response was very heterogeneous among the four different groupings as well as within the strains belonging to the same group, with the following exceptions: *D. halophila* and moderately halophilic cocci, for silver; *Acinetobacter* sp. and *D. halophila*, for arsenate; *D. halophila*, for cobalt; *Flavobacterium* sp. and moderately halophilic cocci, for nickel; *D. halophila*, for lead; and *Flavobacterium* sp. and moderately halophilic cocci, for zinc. In contrast, the response to mercury was very homogeneous except for the group of cocci.

The percentages of tolerance for the 238 strains at the standard levels of the 10 heavy metals tested are presented in Table 2. All strains demonstrated tolerance of nickel, and a great fraction of them were also tolerant of lead and chro-

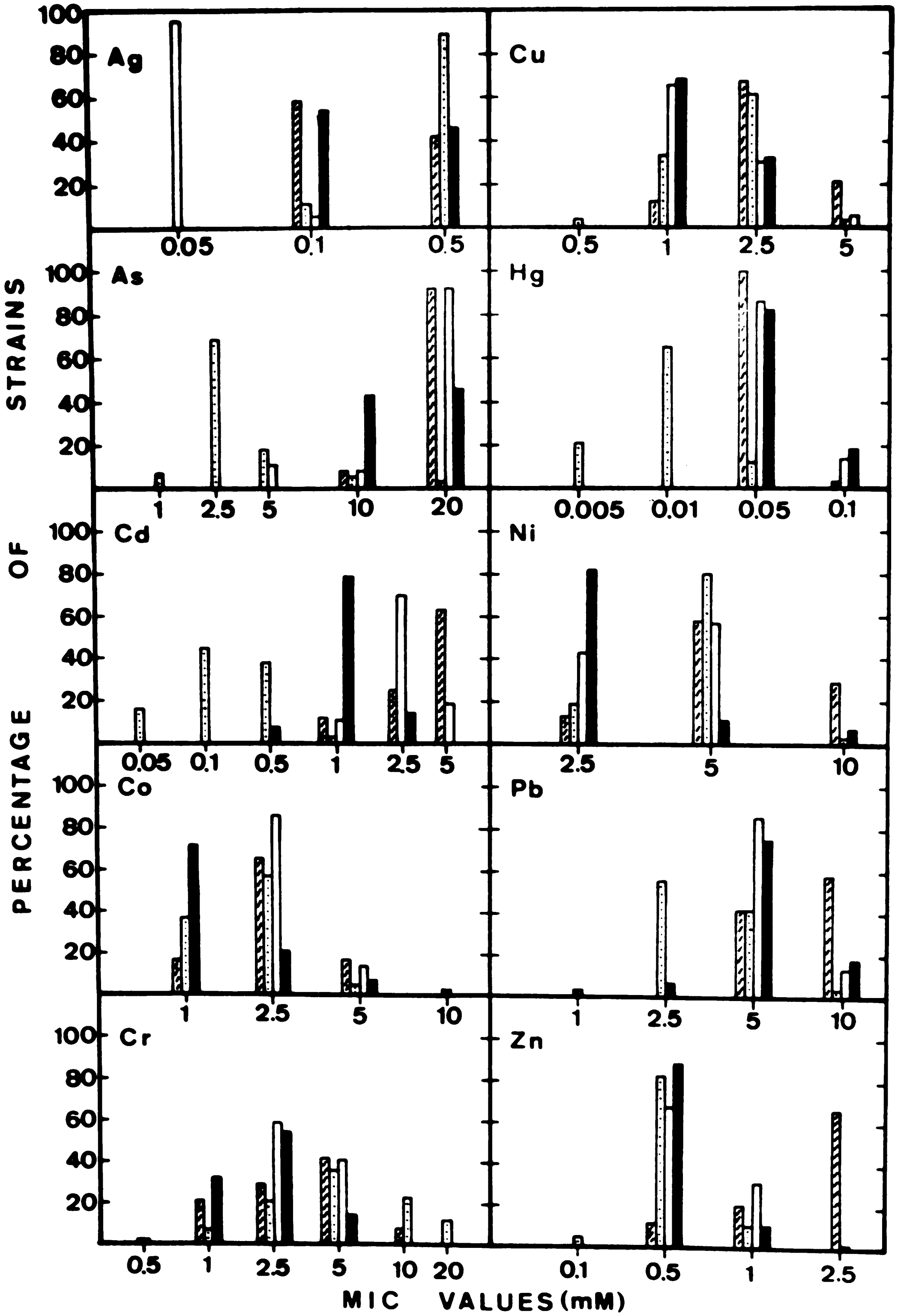

FIG. 1. Susceptibility of 238 moderately halophilic strains belonging to *D. halophila* (▭), *Acinetobacter* sp. (▨), *Flavobacterium* sp. (■), and gram-positive cocci (▤), expressed as percentages of strains with the same MICs, to 10 heavy metals.

mium (more than 65% of strains for this latter metal), while none were tolerant of silver or mercury. However, the frequencies of tolerance of the other five heavy metals were quite variable.

Influence of salinity and composition of test medium on toxicity of metals to moderate halophiles. Tables 3 and 4 show that varying the salinity and yeast extract concentration of the medium affected the sensitivity of some bacteria to

TABLE 2. Comparison of the percentages of tolerance of the four different groups of freshly isolated moderately halophilic eubacteria tested in this study to 10 heavy metals

Taxonomic group	% Tolerance to the following heavy-metal ion:									
	Ag	As	Cd	Co	Cr	Cu	Hg	Ni	Pb	Zn
Acinetobacter spp. (24 strains)	0	92	88	83	79	88	0	100	100	67
Cocci (149 strains)	0	8	0	61	90	63	0	100	99	2
D. halophila (37 strains)	0	92	89	100	100	35	0	100	100	0
Flavobacterium spp. (28 strains)	0	46	14	28	68	32	0	100	100	0

heavy metals. All strains shown were more sensitive to cadmium at lower salinity (SW-7), and many were more sensitive to cobalt and copper. Raising the salinity did not, in general, increase metal tolerance; lowering the yeast extract concentration increased the sensitivity of all strains to all metals tested. A partial reduction in the toxicities of these five metals was observed when the concentration of yeast extract was raised.

DISCUSSION

Moderately halophilic eubacteria compose a heterogeneous group of microorganisms able to grow in a very wide range of salt concentrations (10), including hypersaline habitats (18). Most studies have been concerned with a few species, mainly *V. costicola* (10), and with knowledge of the ecology or physiology of moderate halophiles. We began metal resistance studies on moderate halophiles to determine the possible ecologic role of this group in heavy-metal biotransformations in natural environments.

For this purpose, we surveyed 250 strains of moderate halophiles for their natural patterns of heavy-metal tolerance. The behavior of these strains was not homogeneous with respect to all metals tested, even within the strains belonging to the same genus.

For both the 12 culture collection strains and the 238 fresh isolates, responses to the heavy metals tested were very heterogeneous, since at least three different MICs were detected for every metal ion (Fig. 1). In fact, six different MICs were found for cadmium and chromium. This fact suggests that moderately halophilic bacteria exert a very heterogeneous behavior in relation with their individual natural susceptibility levels to the 10 heavy metals tested in the present study. The strains of *D. halophila* were the most homogeneous of all the 238 strains since for more than 80% of them, the MICs of silver, arsenate, cobalt, mercury, and lead were the same, and for more than 70%, the MICs of cadmium, copper, and zinc were the same. In contrast, the strains belonging to the *Acinetobacter* sp. group seemed to be the most heterogeneous, since for only 80% of them were the MICs of arsenate and mercury the same. The response to the heavy metals tested should definitely not be used as a taxonomic feature in this group of moderately halophilic bacteria (Fig. 1).

When standard metal concentrations were used in this study for defining a possible metal tolerance, it was found that the 24 strains belonging to the genus *Acinetobacter* were the most metal tolerant of all the 238 strains tested, with more than 70% of them demonstrating tolerance of eight different metal ions. The *Flavobacterium* sp. strains were the most metal sensitive, with only three markedly different metal tolerances (Cr, Ni, Pb) exhibited by more than 60% of the strains.

All the fresh isolates showed uniform tolerance of chromium, nickel, and lead and an equal sensitivity to silver and mercury. These results agree with those previously reported for *V. costicola* strains (7), suggesting a general pattern of tolerance or sensitivity of moderately halophilic eubacteria to these heavy metals. However, *V. costicola* exhibited a higher sensitivity to copper and cobalt, and with respect to cadmium, the response was similar to that found for moderately halophilic cocci and the *Flavobacterium* sp. group. Onishi et al. (12) studied the cadmium tolerance of 41 strains of halophilic bacteria with different salt requirements, reporting that about half of the 31 moderate halophiles tested were cadmium tolerant. However, the concentration of cadmium chloride used by them to define metal tolerance

TABLE 3. Influence of salinity and composition of test media on the MICs of five heavy metals for five moderately halophilic rods from culture collections

Test medium[a]	MIC																								
	"*C. marismortui*" ATCC 17056					*D. halophila* CCM 3662					*F. halmephilum* CCM 2831					*H. elongata* ATCC 33173					"*P. halosaccharolytica*" CCM 2851				
	Cd	Co	Cu	Ni	Zn	Cd	Co	Cu	Ni	Zn	Cd	Co	Cu	Ni	Zn	Cd	Co	Cu	Ni	Zn	Cd	Co	Cu	Ni	Zn
SW-10 + 0.5% YE	0.5	2.5	1	2.5	1	1	2.5	1	5	0.5	2.5	1	0.5	2.5	0.5	5	2.5	2.5	2.5	0.5	1	2.5	2.5	5	0.5
SW-7 + 0.5% YE	0.1	2.5	0.5	2.5	1	0.5	2.5	0.5	5	0.5	1	0.5	0.1	2.5	0.5	2.5	1	2.5	2.5	0.5	0.5	2.5	2.5	5	0.5
SW-13 + 0.5% YE	1	2.5	1	2.5	1	2.5	2.5	1	5	0.5	2.5	1	0.5	2.5	0.5	5	2.5	2.5	2.5	0.5	2.5	2.5	2.5	5	0.5
SW-10 + 0.01% YE	0.05	0.5	0.05	0.5	0.1	0.1	0.5	0.05	1	0.05	0.1	0.1	0.01	0.1	0.05	0.5	0.1	0.05	0.1	0.05	0.1	0.1	0.5	0.5	0.05
SW-10 + 0.1% YE	0.1	1	0.5	1	0.5	0.5	1	0.5	2.5	0.1	0.5	0.1	0.1	1	0.1	1	0.5	1	0.5	0.1	0.5	0.5	1	1	0.1
SW-10 + 1% YE	1	5	2.5	5	2.5	2.5	5	2.5	10	1	2.5	1	1	5	1	5	2.5	2.5	5	1	2.5	2.5	2.5	10	1
SW-10 + 0.2% NH_4Cl + 1% glucose	0.05	0.5	0.05	0.1	0.1	0.1	0.5	0.05	0.1	0.1	—[b]	—	—	—	—	0.5	0.05	0.05	0.05	0.1	0.1	0.1	0.1	0.1	0.05

[a] All media consisted of saline medium with a final total salt concentration of ca. 10% (SW-10), 7% (SW-7), or 13% (SW-13), supplemented with yeast extract (YE) or ammonium chloride and glucose.

[b] —, No growth.

TABLE 4. Influence of salinity and composition of test media on the MICs of five heavy metals for five moderately halophilic cocci from culture collections

Test medium[a]	MIC																								
	M. halobius CCM 2591					*M. halophilus* NRCC 14033					"*M. varians* var. *halophilus*" CCM 3316					*P. halodenitrificans* CCM 286					*S. halophila* DSM 2226				
	Cd	Co	Cu	Ni	Zn	Cd	Co	Cu	Ni	Zn	Cd	Co	Cu	Ni	Zn	Cd	Co	Cu	Ni	Zn	Cd	Co	Cu	Ni	Zn
SW-10 + 0.5% YE	0.5	1	2.5	5	1	0.5	1	1	2.5	0.5	1	1	2.5	2.5	0.5	0.5	1	2.5	5	0.5	0.5	1	1	2.5	0.5
SW-7 + 0.5% YE	0.1	1	2.5	5	1	0.1	1	1	2.5	0.5	0.5	1	2.5	2.5	0.5	0.1	1	2.5	5	0.5	0.1	1	1	2.5	0.5
SW-13 + 0.5% YE	0.5	1	2.5	10	1	0.5	1	1	2.5	0.5	1	1	2.5	2.5	0.5	0.5	1	2.5	10	0.5	0.5	1	2.5	2.5	0.5
SW-10 + 0.01% YE	0.05	0.1	0.5	1	0.05	0.05	0.1	0.1	0.5	0.05	0.1	0.1	0.1	0.5	0.05	0.05	0.01	0.1	1	0.01	0.05	0.5	0.1	0.5	0.05
SW-10 + 0.1% YE	0.1	0.5	1	2.5	0.1	0.1	0.5	0.5	1	0.05	0.5	0.5	0.5	1	0.05	0.1	0.5	0.5	2.5	0.05	0.1	0.5	0.5	1	0.05
SW-10 + 1% YE	0.5	2.5	2.5	10	2.5	0.5	2.5	2.5	5	1	1	2.5	2.5	5	1	0.5	2.5	2.5	10	1	0.5	2.5	2.5	5	1

[a] All media consisted of saline medium with a final total salt concentration of ca. 10% (SW-10), 7% (SW-7), or 13% (SW-13), supplemented with yeast extract (YE) or ammonium chloride and glucose. No bacterial growth was observed in SW-10 test medium supplemented with 0.2% NH_4Cl and 1% glucose.

was 50 μM/ml (0.23 mM); using our methods, only seven strains would have been scored as cadmium tolerant.

Several studies have demonstrated that metal toxicity can be heavily influenced by environmental factors such as pH, temperature, soluble organic matter, clay minerals, inorganic anionic and cationic components, etc. (2, 3, 6, 24). Moreover, some constituents of common growth media show high binding activity for metal ions and, hence, may reduce the concentrations of free ions (16). In the present study, we assessed the effect of salinity and yeast extract on the response of moderately halophilic bacteria to some of the heavy metals tested (Tables 3 and 4). When we tested the toxicity of these metals in a medium with lower salinity than the standard medium, a general enhancment of the cadmium toxicity for all the strains tested and, in some cases, of the cobalt and copper toxicities was observed. This could be due to osmotic cellular changes which result in a higher availability of the toxic ions for the bacteria or to the formation of complex metal species which exert higher toxicities than free metal cations. These results indicate that tolerances of cadmium, cobalt, and copper should be cautiously compared between moderately halophilic bacteria and marine bacteria.

However, increasing the salinity had only minimal effects. Decreased cadmium toxicity was observed in some strains of moderately halophilic rods, and decreased copper and nickel toxicities were seen for only two strains of cocci. These results partially agree with those reported by Onishi et al. (12), who observed a reduction in the toxicity of cadmium in a moderately halophilic *Pseudomonas* sp. when the NaCl concentration was increased from 1 to 3 M. In contrast with the results of Babich and Stotzky (1), no reduction in the toxicity of Zn was observed. However, the highest NaCl concentration tested by these authors was only 1 M.

A reduction in the concentration of yeast extract in the test medium caused a noteworthy increase in all metal toxicities, while a higher yeast extract concentration resulted in lessened toxicities of nickel and zinc and, for only some strains, the other three metals. Overall, these results are in agreement with previous reports that showed the influence of different components of the culture media on the availability of the toxic heavy-metal ions for the microbes (16). Thus, some important components of commonly used media such as peptone, tryptone, yeast extract, and Casamino Acids (Difco) share a high binding power to different metal ions and, hence, can prevent their toxicity (2, 4, 6). Indeed, it has been shown that copper is modified in the presence of agar (5), although currently no alternative procedures have been adapted by the scientific community. Nevertheless, it seems clear that the concentration of yeast extract in the test media must be carefully taken into account for future comparison purposes, at least in the group of moderately halophilic eubacteria.

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LITERATURE CITED

1. **Babich, H., and G. Stotzky.** 1978. Toxicity of zinc to fungi, bacteria, and coliphages: influence of chloride ions. Appl. Environ. Microbiol. **36:**906–914.
2. **Babich, H., and G. Stotzky.** 1980. Environmental factors that influence the toxicity of heavy metals and gaseous pollutants to microorganisms. Crit. Rev. Microbiol. **8:**99–145.
3. **Babich, H., and G. Stotzky.** 1983. Influence of chemical speciation on the toxicity of heavy metals to the microbiota, p. 1–46. *In* J. O. Nriagu (ed.), Aquatic toxicology. John Wiley & Sons, Inc., New York.
4. **Babich, H., and G. Stotzky.** 1985. Heavy metal toxicity to microbe-mediated ecologic processes: a review and potential application to regulatory policies. Environ. Res. **36:**111–137.
5. **Bird, N. P., J. G. Chambers, R. W. Leech, and D. Cummins.** 1985. A note on the use of metal species in microbiological tests involving growth media. J. Appl. Bacteriol. **59:**353–355.
6. **Gadd, G. M., and A. J. Griffiths.** 1978. Microorganisms and heavy metal toxicity. Microb. Ecol. **4:**303–317.
7. **Garcia, M. T., J. J. Nieto, A. Ventosa, and F. Ruiz-Berraquero.** 1987. The susceptibility of the moderate halophile *Vibrio costicola* to heavy metals. J. Appl. Bacteriol. **63:**63–66.
8. **Garcia, M. T., A. Ventosa, F. Ruiz-Berraquero, and M. Kocur.** 1987. Taxonomic study and amended description of *Vibrio costicola*. Int. J. Syst. Bacteriol. **37:**251–256.
9. **Kushner, D. J.** 1985. The Halobacteriaceae, p. 171–214. *In* C. R. Woese and R. S. Wolfe (ed.), The bacteria, vol. 8. Academic Press, Inc. (London), Ltd., London.
10. **Kushner, D. J., and M. Kamekura.** 1988. Physiology of halophilic eubacteria, p. 109–138. *In* F. Rodriguez-Valera (ed.), Halophilic bacteria, vol. 1. CRC Press, Inc., Boca Raton, Fla.

11. **Mathrani, I. M., D. R. Boone, R. A. Mah, G. E. Fox, and P. P. Lau.** 1988. *Methanohalophilus zhilinae* sp. nov., an alkaliphilic, halophilic, methylotrophic methanogen. Int. J. Syst. Bacteriol. **38**:139–142.
12. **Onishi, H., T. Kobayashi, N. Morita, and M. Baba.** 1984. Effect of salt concentration on the cadmium tolerant *Pseudomonas* sp. Agric. Biol. Chem. **48**:2441–2448.
13. **Paterek, J. R., and P. H. Smith.** 1988. *Methanohalophilus mahii* gen. nov., sp. nov., a methylotrophic halophilic methanogen. Int. J. Syst. Bacteriol. **38**:122–123.
14. **Quesada, E., M. J. Valderrama, V. Bejar, A. Ventosa, F. Ruiz-Berraquero, and A. Ramos-Cormenzana.** 1987. Numerical taxonomy of moderately halophilic Gram-negative nonmotile eubacteria. Syst. Appl. Microbiol. **9**:132–137.
15. **Quesada, E., A. Ventosa, F. Ruiz-Berraquero, and A. Ramos-Cormenzana.** 1984. *Deleya halophila*, a new species of moderately halophilic bacteria. Int. J. Syst. Bacteriol. **34**:287–292.
16. **Ramamoorthy, S., and D. J. Kushner.** 1975. Binding of mercury and other heavy metal ions by microbial growth media. Microb. Ecol. **2**:162–176.
17. **Riley, T. V., and B. J. Mee.** 1982. Susceptibility of *Bacteroides* spp. to heavy metals. Antimicrob. Agents Chemother. **22**: 889–892.
18. **Rodriguez-Valera, F., A. Ventosa, G. Juez, and F. Imhoff.** 1985. Variation of environmental features and microbial populations with salt concentrations in a multi-pond saltern. Microb. Ecol. **11**:107–115.
19. **Smith, F. B.** 1938. An investigation on a taint in rib bones of bacon. The determination of halophilic vibrios (n. spp.). Proc. R. Soc. Queensl. **49**:29–53.
20. **Trevors, J. T., K. M. Oddie, and B. H. Belliveau.** 1985. Metal resistance in bacteria. FEMS Microbiol. Rev. **32**:39–54.
21. **Ventosa, A.** 1988. Taxonomy of moderately halophilic heterotrophic eubacteria, p. 71–84. *In* F. Rodriguez-Valera (ed.), Halophilic bacteria, vol. 1. CRC Press, Inc., Boca Raton, Fla.
22. **Ventosa, A., E. Quesada, F. Rodriguez-Valera, F. Ruiz-Berraquero, and A. Ramos-Cormenzana.** 1982. Numerical taxonomy of moderately halophilic Gram-negative rods. J. Gen. Microbiol. **128**:1959–1968.
23. **Washington, J. A., II, and V. L. Sutter.** 1980. Dilution susceptibility test: agar and macro-broth dilution procedures, p. 453–458. *In* E. H. Lennette, A. Balows, W. J. Hausler, Jr., and J. P. Truant (ed.), Manual of clinical microbiology, 3rd ed. American Society for Microbiology, Washington, D.C.
24. **Wood, J. M., and H. K. Wang.** 1983. Microbial resistance to heavy metals. Environ. Sci. Technol. **17**:582–590.
25. **Yu, I. K., and F. Kawamura.** 1987. *Halomethanococcus doii* gen. nov. sp. nov.: an obligately halophilic methanogenic bacterium from solar salt ponds. J. Gen. Appl. Microbiol. **33**: 303–310.