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SHORT COMMUNICATIONS

Tolerance of Marine Proteobacteria of the Genera *Pseudoalteromonas* and *Alteromonas* to Heavy Metals

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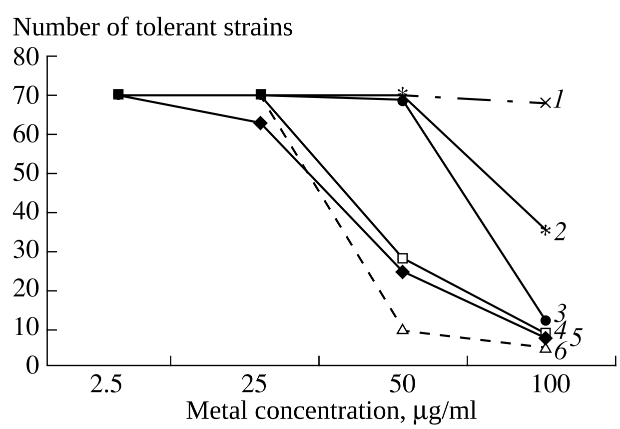
Most of metals [Li(I), Be(II), Al(III), V(IV), Cr(III), Mn(II), Fe(II), Co(II), Ni(II), Cu(II), Zn(II), Sr(II), Mo(VI), Ag(I), Cd(II), Sn(IV), Sb(III), Cs(I), Hg(II), Pb(II), U(VI), Pu(III)], which are abundant in the hydrosphere and atmosphere, occur in low concentrations (< 1 mmol/m^3) in freshwater and seawater [1]. The release of metals into the environment through the waste products generated by the metal-working industries leads to their accumulation in the hydrosphere and atmosphere and may affect the human health. Aquatic microbial communities, which are capable of self-regulation and adaptation to altered environments, may serve as suitable objects to assess the contamination level at which microorganisms still maintain their homeostasis [2, 3].

The aim of the present work was to study the heavy metal tolerance of marine proteobacteria of the genera *Alteromonas* and *Pseudoalteromonas*, typical inhabitants of aquatic ecosystems.

Experiments were performed with 70 strains of aerobic proteobacteria of the genera *Alteromonas* and *Pseudoalteromonas*, which were isolated from seawater, the bivalved mollusks *Patinopecten yessoensis* and *Crenomytilus grayanus*, and the eelgrass *Zostera marina*. The samples of seawater, mollusks, and eelgrass were collected in the Trinity Bay of the Sea of Japan in the summer months of 1985–1994. The isolation, identification, cultivation, and maintenance procedures were described elsewhere [4, 5]. The strains *Alteromonas macleodii* ATCC 27126^T, *Pseudoalteromonas atlantica* IAM 12927^T, *P. carrageenovora* ATCC 12662^T, *P. elyakovii* KMM 162^T (=ATCC 700519^T), *P. haloplanktis* ATCC 14393^T, *P. nigrifaciens* IAM 13010^T, *P. piscicida* ATCC 15057^T, and *P. tetraodonis* IAM 14160^T used in this work were obtained from the American Type Culture Collection, from the Institute of Molecular and Cell Biology (Japan), and from U. Simidu and M. Akagawa–Matsushita.

Bacteria were cultivated at 28°C on an agar medium (pH 7.8) containing (g/l) peptone, 5.0; yeast extract, 2.0; glucose, 1.0; K_2HPO_4, 0.2; $MgSO_4$, 0.05; and agar, 20.0; in a mixture of distilled and seawater (1 : 1). Pure cultures were maintained on soft agar rods of the same composition (except that the concentration of agar was 5 g/l). The rods were stored under mineral oil at 4°C and in a liquid medium with 20% glycerol at −80°C. Phenotypic characteristics were studied using standard methods [6] as described earlier [4, 5]. The tolerance of proteobacteria to potentially hazardous metals was assessed by growing them on agar plates supplemented with one of the following salts: $CuSO_4 \cdot 5H_2O$, HgCl, $CdCl_2$, LiCl, $Pb(C_2H_3O_2)_2$, and $NiCl_2 \cdot 6H_2O$, each taken at concentrations of 2.5, 25, 50, and 100 μg/ml. After the inoculation of bacteria by streaking, the agar plates were placed in a thermostatic chamber and incubated for two to five days at 28°C. The bacterial growth was then visually assessed.

The analysis of the results obtained in this study showed that most of the marine aerobic proteobacteria were tolerant to all of the metal salts studied when they were used at concentrations from 2.5 to 25 μg/ml. $CdCl_2$, $CuSO_4$, HgCl, and $NiCl_2$ were most toxic when present in the medium at a concentration of 100 μg/ml: only a small number of strains (from 3 to 17% of the total number of the strains tested) could tolerate this



The tolerance of *Pseudoalteromonas* and *Alteromonas macleodii* strains to different concentrations of (*1*) LiCl; (*2*) $Pb(C_2H_3O_2)_2$; (*3*) $NiCl_2$; (*4*) HgCl; (*5*) $CuSO_4$; and (*6*) $CdCl_2$.

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Marine strains tolerant to high concentrations of heavy metals

Strain	Source of isolation	$CuSO_4 \cdot 5H_2O$		HgCl		CdCl		$NiCl_2 \cdot 6H_2O$	
		50 μg/ml	100 μg/ml	50 μg/ml	100 μg/ml	50 μg/ml	100 μg/ml	50 μg/ml	100 μg/ml
Pseudoalteromonas citrea KMM 157	Mollusk *Patinopecten eyssoensis*	+*	+	+	+	±	–	+	–
Pseudoalteromonas citrea KMM 461	Seawater	+	–	+	±	+	±	+	+
Pseudoalteromonas nigrifaciens KMM 155	Mussel *Crenomytilus grayanus*	+	–	+	–	–	–	+	+
Pseudoalteromonas sp. KMM 174	The same	+	–	+	+	+	±	+	–
Pseudoalteromonas sp. KMM 291	"	+	–	+	–	±	–	+	±
Pseudoalteromonas sp. KMM 124	"	+	–	+	+	+	–	–	–
Pseudoalteromonas sp. KMM 48	Eelgrass *Zostera marina*	+	+	–	–	–	–	+	+
Pseudoalteromonas sp. KMM 121	Seawater	–	–	+	–	–	–	+	+
Pseudoalteromonas sp. KMM 180	The same	±	–	+	–	+		+	+
Pseudoalteromonas sp. KMM 1352	"	+	–	–	–	–	–	+	±
Pseudoalteromonas sp. KMM 469	"	+	+	±	–	±	–	+	–
Pseudoalteromonas sp. KMM 183	"	+	–	–	–	–	–	+	+
Pseudoalteromonas sp. KMM 1362	"	+	+	+	+	–	–	–	–
Pseudoalteromonas sp. KMM 1359	"	±	–	+	+	+	±	–	–
Pseudoalteromonas sp. KMM 176	"	–	–	–	–	+	±	+	–
Pseudoalteromonas sp. KMM 734	"	+	±	+	±	+	±	+	–

Note: "+," "–," and "±" stand for "good growth," "no growth," and "poor growth," respectively.

salt concentration (see figure and table). $Pb(C_2H_3O_2)_2$ at a concentration of 100 μg/ml inhibited the growth of half of the tested strains. All the strains except *A. macleodii* ATCC 27126^T and *P. atlantica* IAM 12927^T were tolerant to 100 μg/ml LiCl. This can be explained by a relatively weak toxic effect of Li ions on aerobic bacteria, since lithium is known to inhibit primarily the glycolytic enzymes [1]. The type strains were less tolerant to the metal salts under study than the marine isolates: the growth of the former was inhibited by metal salts present in the medium at a concentration of 25 μg/ml. The high metal tolerance of the marine isolates can probably be explained by the fact that many of them are encapsulated strains. Knowing the role of polysaccharides in the sorption of heavy metal ions [1, 3], this suggestion seems quite reasonable. Interestingly, the percentage of the metal-resistant strains isolated from seawater (50% of the strains studied) was higher than the percentage of such strains isolated from the mollusks and seaweed (26 and 11%, respectively). The above table lists the strains that can tolerate (without preadaptation) the concentrations of heavy metals five- to tenfold higher than their concentrations in seawater [1] and gives the sources of their isolation. Analysis of the tabulated data suggests that the tolerance of bacteria to a particular metal does not always correlate with their tolerance to other metals. This may be due to the existence of different mechanisms responsible for bacterial tolerance to heavy metals [8].

To conclude, the data obtained in this study show that the marine proteobacteria of the genera *Alteromo-*

nas and *Pseudoalteromonas* can tolerate the concentrations of heavy metals 2.5 times as great as their concentrations in seawater. However, higher concentrations of heavy metals inhibited the growth of the marine bacteria and, hence, can irreversibly impair the homeostasis of aquatic microbial communities.

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