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Susceptibility to heavy metals and antibiotics of eight deep-sea hydrothermal vent bacteria carrying a 51.7 kb plasmid

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Abstract

Eight Pseudomonas-like bacteria isolated from the tube of the deep-sea hydrothermal vent Polychaete *Alvinella pompejana* were found to carry a 51.7 kb plasmid. All isolates but one were resistant to zinc (3 mM or more) and arsenate ions (200 mM or more). The strains were resistant to penicillin and chloramphenicol.

Introduction

Microorganisms are known to employ a large variety of mechanisms for adaptation to the presence of heavy metal ions (GADD and GRIFFITHS 1978). In many organisms, the genes controlling metal resistance are carried on plasmids which provide the bacteria with a competitive advantage over other organisms when metals are present (TREVORS et al. 1985). In deep-sea hydrothermal vents, alvinellid Polychaetes are exposed to a wide range of temperatures and high concentrations of metal compounds. Alvinellid-associated bacteria have been found to display multiple heavy metal resistance (JEANTHON and PRIEUR 1990). A collection of 299 bacterial strains isolated from hydrothermal Polychaetes was screened to examine their plasmid content (unpublished data). In this study are presented the results obtained for 8 strains isolated from the tube of *Alvinella pompejana* (DESBRUYERES and LAUBIER 1980).

Material and methods

Samples were collected from the Parigo vent field on the East Pacific Rise (12°48'56"N, 103°56'72"W) at a depth of 2600 m during the *Hydronaut* cruise in November 1987. Bacterial strains were isolated from the tube of an individual of *A. pompejana* captured using the port manipulator of the submersible *Nautile* and placed in an insulated box for the trip to the surface. Upon recovery, the worm's tube was rinsed in sterile seawater and ground. Ground material was diluted and dilutions were plated onto metal amended medium. Metal amended medium was prepared by adding to an agar medium (OPPENHEIMER and ZO-

BELL 1952) either 100 µg Zn ml^{-1} or 90 µg Cu ml^{-1}. Plates were incubated at 1 atm (101.29 kPa) until returned to the laboratory. Colonies were selected at random and streaked for purity.

Plasmids were isolated using the method of KADO and LIU (1981). Samples were run in 0.8 % agarose gels in acetate buffer (MANIATIS et al. 1982). After 5 h, gels were submerged in ethidium bromide solution (1 µg ml^{-1}) for 25 min, visualized with a transilluminator and photographed with a Polaroid 667 film. Plasmids (238 kb and 163 kb) from *Alcaligenes eutrophus* CH 34 (MERGEAY et al. 1985), plasmid pULB113 (68 kb) from *Escherichia coli* CM 214 (LEJEUNE et al. 1983), plasmid RP4 (60 kb) from *E. coli* CM 140 (DATTA et al. 1971), plasmid pSS50 (50 kb) from *A. eutrophus* A5.1 (SHIELDS et al. 1985), plasmid pUCD615 (17.5 kb) from *E. coli* CM 600 (ROGOWSKY et al. 1986), plasmid pBR 325 (5.9 kb) from *E. coli* HB101 (BOLIVAR 1978) were isolated as size standards.

Strains were observed for morphology, motility, flagellation, and Gram staining and subjected for twenty-seven biochemical tests (JEANTHON and PRIEUR 1990).

Minimal inhibitory concentrations to cadmium, zinc, copper, cobalt, nickel, and arsenate were determined in Tris medium (ECHOLS et al. 1961) supplemented with 0.2 % succinate. Solidified media contained 15 g of agar per liter. For mercury, added as merbromin, TY medium containing 10 g of Bacto-tryptone (Difco), 5 g of yeast extract, 5 g of NaCl, 1 g of glucose and 0.03 g of cysteine in 1 l of water, was used. Antibiotic resistance testing was performed with Mueller-Hinton agar plates and antibiotic discs (Diagnostic Pasteur; Marnes-la-Coquette, France) using the procedure described by the manufacturer. The antibiotics tested were ampicillin (10 µg), chloramphenicol (30 µg), erythromycin (15 U.I), gentamycin (10 U.I), kanamycin (30 U.I), penicillin (10 U.I), streptomycin (10 U.I), and tetracycline (30 U.I). Analytical grade salts $CdCl_2x2.5H_2O$, $ZnCl_2$, $CuCl_2x2H_2O$, $CoCl_2x6H_2O$, $NiCL_2x6H_2O$ and $Na_2HAsO_4x7H_2O$ were used to prepare stock solutions in distilled water. They were sterilized by filtration through 0.22-µm-pore membrane filters (Nuclepore Corp., Pleasanton, CA) and stored into sterile flasks in the dark at 4 °C.

Results and discussion

Plasmid analysis

All strains harbor a plasmid band of 51.7 kb and two of them (isolates 813 and 862) carry a second plasmid band of 4.6 kb. Both plasmids presented here were not found in 291 other strains isolated on metal amended media from the dorsal integument of hydrothermal vent Polychaetes (unpublished data).

Characterization of isolates

Strains 797, 801, 813, 815 and 818 were isolated on copper amended medium and strains 862, 881 and 885 were isolated on zinc amended medium. All isolates are Gram-negative, oxidase- and catalase-positive rods, which produce H_2S from cysteine, degrade gelatin, do not fermente glucose to acid, and do not possess a constitutive arginine dihydrolase. Motile strains are polarly flagellated. Based on results of the taxonomic tests, isolates were assigned to *Pseudomonas*-like bacteria. Phenotypic traits distinguishing the isolates are reported in Table 1. Strains 801 and 818 were phenotypically identical on the basis of 30 separate characters.

Table 1. Phenotypical traits distinguishing the eight isolates from *A. pompejana*'s tube carrying the 51.7 kb plasmid.

Strain	Motility	Growth in 0 % NaCl	Nitrate reduction	Acid from lactose	starch	sorbitol	mannose	glycerol
797	+	+	-	-	-	-	+	-
801	-	+	-	+	+	+	+	+
813	+	+	-	+	+	+	+	-
815	+	+	+	+	+	-	+	+
818	-	+	-	+	+	+	+	+
862	+	+	-	+	-	+	-	+
881	+	-	-	-	-	-	-	-
885	-	+	-	+	+	+	+	-

Table 2. Minimal inhibitory concentrations of 7 metals and susceptibility to 8 antibiotics of the eight isolates carrying the 51.7 kb plasmid. MICs are expressed as millimolar concentrations. R: resistant; i: moderate; s: sensitive.

Strain	Cd	Zn	Cu	Co	Ni	As	Hg	P	C	GM	K	E	TE	AM	S
797	1	5	2	1	1	300	0.25	R	R	s	s	R	R	i	s
801	1	5	2	1	1	300	0.25	R	R	s	s	R	i	i	s
813	1	5	1	1	1	400	0.25	R	R	s	s	R	R	i	s
815	1	4	1	1	1	400	0.25	R	R	s	s	R	R	i	s
818	1	5	1	1	1	300	0.25	R	R	s	s	i	R	i	s
862	1	5	2	1	1	300	0.25	R	R	s	s	i	i	i	s
881	0.4	0.5	4	1	1	200	0.1	R	R	s	s	R	i	i	s
885	1	5	1	1	1	300	0.25	R	R	s	s	R	i	i	s

P: penicillin (10 U.I); C: chloramphenicol (30 µg); GM: gentamycin (10 U.I); K: kanamycin (30 U.I); E: erythromycin (15 U.I); TE: tetracycline (30 U.I); AM: ampicillin (10 µg); S: streptomycin (10 U.I).

Susceptibility to heavy metals and antibiotics

MICs of cadmium, zinc, copper, cobalt, nickel, arsenate and mercury and susceptibility to penicillin, chloramphenicol, gentamycin, kanamycin, erythromycin, tetracycline, ampicillin and streptomycin for the isolates are reported in Table 2. With the exception of one isolate, all strains display high resistance to zinc and arsenate ions. Strain 881 is resistant to large amounts of copper. Arsenic, zinc and copper were detected in *A. pompejana* epidermal cells as well as in the associated bacteria and their matrices and arsenic and zinc appeared to be the most abundant elements (GAILL et al. 1984). Moreover, high concentrations of zinc and copper in undiluted spring fluid were measured (EDMOND and von DAMM 1985). Heavy metal resistance of isolates may be thus linked to the abundance of these elements in their environment. All isolates were resistant to penicillin and chloramphenicol and sensitive to gentamycin, kanamycin and streptomycin. Both antibiotic and metal resistance are known to be located on plasmids (KONDO et al. 1974). Further experiments may determine the role of the 51.7 kb plasmid.

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