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Plasmid-Determined Resistance to Boron and Chromium Compounds in *Pseudomonas aeruginosa*

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Plasmids determining resistance to arsenic, mercury, silver, and tellurium compounds in *Escherichia coli* and *Pseudomonas aeruginosa* were tested for resistance to 40 other metal compounds. Resistance to trivalent boron and hexavalent chromium compounds was a property of certain *P. aeruginosa* plasmids.

In addition to antibiotic resistance, R plasmids are known to provide resistance to toxic heavy-metal ions. Penicillinase plasmids of *Staphylococcus aureus* confer resistance to antimony, arsenic, bismuth, cadmium, lead, mercury, and zinc compounds (3, 11, 13). Lactose plasmids of group N streptococci provide resistance to compounds of arsenic and chromium (4). R plasmids of enterobacteria determine resistance to arsenic, cobalt, mercury, nickel, silver, and tellurium salts (5, 9, 14–17), and resistance to compounds of mercury and tellurium in *Pseudomonas aeruginosa* is plasmid mediated (1, 16).

This study was undertaken to determine whether plasmids of gram-negative bacteria determine resistance to metal compounds other than those already known. Unusual resistances were not detected in enterobacterial plasmids, but resistance to boron and chromium compounds was found to be determined by certain plasmids in *P. aeruginosa*.

MATERIALS AND METHODS

Bacterial strains and plasmids. The source for most of the plasmids has been described previously (7, 16). RP4-pMG5 is a hybrid plasmid formed by recombination between incompatibility group P-1 (IncP-1) plasmid RP4 and IncP-2 plasmid pMG5 (8). Plasmid pMG6 was found in an unusually antibiotic-resistant *P. aeruginosa* isolate from the sputum of a patient transferred to the Massachusetts General Hospital from Athens, Greece. It is incompatibile with the CAM plasmid and hence belongs to IncP-2 (7). Plasmid pMG18 was detected in *P. putida* Ps5227-75 from H. Matsumoto, Shinshu University, Matsumoto, Japan. pMG18 is incompatible with R2 and hence belongs in IncP-9 (8). Plasmids were tested for resistance either in *Escherichia coli* J53 (F⁻ *pro-22 metF63*), *P. aeruginosa* PU21 (FP⁻ *ilvB112 leu-1 str⁺rif⁺*), or *P. aeruginosa* PAO303 (FP⁻ *arg-18*) (2, 6).

Chemicals. Chemicals were obtained from standard commercial sources except for the following (obtained from the Alfa Products Div., of Ventron Corp., Danvers, Mass.): $BeCl_2$, $Ga_2(SO_4)_3$, Na_2SeO_3, Na_2SeO_4, SeS_2, Na_2MoO_4, $InCl_3$, $NaSbO_3$, K_2OsO_4, $OsCl_3$, $IrCl_3$, and $TlNO_3$.

Determination of metal ion resistance. Brain heart infusion agar (Difco) plates containing graded concentrations of inhibitors (typically 10, 5, and 1 mM) were spotted with 10^4 to 10^5 organisms from an overnight culture in L broth (for *E. coli*) or nutrient broth (Difco) containing 4 mg of potassium nitrate per ml (for *P. aeruginosa*). Resistance to silver nitrate was tested with tryptone-yeast extract agar plates without added sodium chloride (9). The minimal inhibitory concentration was determined as the lowest concentration of inhibitor preventing growth after overnight incubation at 37°C. In many cases, resistance was also determined qualitatively by a gradient plate technique (16).

Survival of colony-forming activity on inhibitor plates was determined by the method of Novick and Roth (11).

RESULTS

Screening for resistance to metal compounds. Resistance to toxic metal compounds is often clustered on plasmids (11, 13, 16). Accordingly, 20 plasmids (Table 1) that determine resistance to arsenic, mercury, silver, or tellurium compounds in *E. coli* or *P. aeruginosa* were tested for resistance to the metal compounds listed (in order of increasing atomic number) below: $LiNO_3$, $BeCl_2$, $Na_2B_4O_7$, $Na_2B_2O_4$, $NaBO_3$, $MgCl_2$, $AlCl_3$, $CaCl_2$, $Cr(NO_3)_3$, $K_2Cr_2O_7$, Na_2CrO_4, $MnCl_2$, $FeSO_4$, $FeCl_3$, $CoCl_2$, $NiSO_4$, $CuSO_4$, $ZnSO_4$, $Ga_2(SO_4)_3$, Na_2HAsO_4, $NaAsO_2$, Na_2SeO_3, Na_2SeO_4, SeS_2, Na_2MoO_4, $(NH_4)_6Mo_7O_{24}$, $AgNO_3$, $CdSO_4$, $InCl_3$, $SnCl_2$, $SbCl_3$, $NaSbO_3$, $CsCl$, $BaCl_2$, Na_2WO_4, K_2OsO_4, $OsCl_3$, $IrCl_3$, $AuCl_3$, $Pb(C_2H_3O_2)_2$, $TlNO_3$, $Bi(NO_3)_3$, $UO_2(C_2H_3O_2)_2$. (Water of hydration has been omitted.) An additional 20 plasmids that determine resistance only to antibiotics were also screened.

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TABLE 1. *Plasmids tested for metal ion resistance*

Plasmid	Inc group	Phenotype[a]	Tested in:	
			E. coli	*P. aeruginosa*
RIP55	C (A-C), P-3	Cb Cm Gm Km Su Tm Hgr		+
R57b	C (A-C)	Ap Cm Gm Km Su Hgr	+	
JR211	C (A-C)	Ap Cm Gm Km Sm Su Hgr Pmr	+	
JJ1	FII	Cm Sm Sp Su Hgr	+	
R826	H2[b]	Ap Cm Gm Km Sm Tc Asr Hgr Pmr Ter	+	
R391	J	Km Hgr	+	
R830a	L	Km Sm Hgr Pmr	+	
R15	N	Sm Su Hgr	+	
R46	N	Cb Sm Su Tc Asr	+	+
R702	P, P-1	Km Sm Su Tc Hgr	+	+
R938	P, P-1	Cb Cm Km Sm Su Tc Hgr	+	+
pMG1	P-2	Gm Sm Su Hgr Ter Uv		+
pMG5	P-2	Ak Bt Km Su Tm Hgr Pmr Ter		+
RPL11	P-2	Cb Cm, Gm Sm Su Tc Hgr Pmr Ter		+
R38	P-2	Sm Su Tc Hgr Pmr Ter		+
R931	P-2	Sm Tc Hgr Ter Uv		+
R3108	P-2	Sm Su Tc Hgr Pmr Ter		+
CAM	P-2	Cam Ter Uv		+
FP2	P-8	Hgr Pmr		+
pMG101	?	Ap Cm Sm Su Tc Agr Hgr Ter	+	

[a] Phenotypic abbreviations (resistance): Ak, amikacin; Ap, ampicillin; Agr, silver nitrate; Asr, sodium arsenate; Bt, butirosin; Cb, carbenicillin; Cm, chloramphenicol; Gm, gentamicin; Hgr, mercuric chloride; Km, kanamycin; Pmr, phenylmercuric acetate; Sm, streptomycin; Sp, spectinomycin; Su, sulfonamide; Tc, tetracycline; Ter, potassium tellurite; Tm, tobramycin; Uv, ultraviolet irradiation. Phenotypic abbreviation (biodegradation): Cam, camphor.

[b] R826, originally assigned to incompatibility group S, has been reclassified to group H2 (18).

With four exceptions, no additional metal ion resistances were found. Either the test compound failed to inhibit R^- or R^+ bacteria at the highest concentration tested, or no difference was detected in the minimal inhibitory concentrations of R^- and R^+ strains.

Resistance to borate, metaborate, chromate, and dichromate. Several plasmids belonging to IncP-2 of *P. aeruginosa* were found to determine resistance to borate. These plasmids also conferred resistance to metaborate but not to perborate.

Accordingly, a larger set of *Pseudomonas* plasmids was tested for borate and metaborate resistance. Chromate resistance was tested as well, since several IncP-2 plasmids gave equivocal results on initial screening.

Of 12 IncP-2 plasmids tested, 8 conferred resistance to borate and metaborate (Table 2). IncP-5 plasmid Rms163 and IncP-9 plasmid pMG18 had this property as well. Resistant strains showed a two- to fourfold increase in resistance to borate and a two- to fivefold increase in resistance to metaborate. A 5- to 10-fold increase in resistance to chromate was found for plasmid pMG6, another member of IncP-2. pMG6 gave a twofold increase in resistance to dichromate but did not enhance resistance to chromic nitrate. None of the other naturally occurring *Pseudomonas* plasmids tested affected susceptibility to boron or chromium compounds, but hybrids formed by recombination between IncP-1 plasmid RP4 and IncP-2 plasmids pMG5 or RPL11, which determine borate resistance, express this resistance in *P. aeruginosa* as well as other IncP-2 determinants. Enhanced borate resistance by RP4-pMG5 or RP4-RPL11 was not detectable in *E. coli*.

The ability of R^+ and R^- strains to form individual colonies on media containing sodium borate or sodium arsenate is shown in Fig. 1. By this test, pMG1 and pMG6 enhance resistance to borate and chromate, respectively, three- to fourfold.

DISCUSSION

Two new resistances were detected in *Pseudomonas* plasmids: resistance to the trivalent compounds of boron, borate and metaborate, and resistance to the hexavalent compounds of chromium, chromate and dichromate. We suggest the designations Bor and Crr for these phenotypes (10). Bor is determined by plasmids of the IncP-2 group, which also determine resistance to mercury and tellurium compounds, by IncP-9 plasmid pMG18, which determines mercuric ion resistance, and by IncP-5 plasmid Rms163, which carries no other known metal

TABLE 2. *Borate, metaborate, and chromate susceptibility of plasmids in P. aeruginosa*

Plasmid	Inc group	Phenotype[a]	MIC[b] (mM) for:		
			$Na_2B_4O_7$	$NaBO_2$	Na_2CrO_4
RP4	P-1	Cb Km Tc	5	10	5
R30	P-1	Cb Km Tc	5	10	5
R68	P-1	Cb Km Tc	5	10	5
R527	P-1	Cb Cm Gm Km Sm Su Tc Hgr	5	10	5
R702	P-1	Km Sm Su Tc Hgr	5	10	5
R839	P-1	Cb Km Sm Su Tc Hgr	5	10	5
R1033	P-1	Cb Cm Gm Km Sm Su Tc Hgr	5	10	5
RP4-pMG5	P-1	Cb Km Su Tc Tm Hgr	*20*	*50*	5
RP4-RPL11	P-1	Cb Cm Gm Km Sm Su Tc	*20*	*50*	5
pMG1	P-2	Gm Sm Su Hgr Ter Uv	*10*	*50*	5
pMG2	P-2	Gm Sm Su Hgr Ter Uv	5	10	10
pMG5	P-2	Km Su Tm Hgr Pmr Ter	*20*	*50*	5
pMG6	P-2	Cm Gm Km Sm Su Tm Hgr Ter	5	10	*50*
RPL11	P-2	Cb Cm Gm Sm Su Tc Hgr Pmr Ter	*20*	*50*	5
R38	P-2	Sm Su Tc Hgr Pmr Ter	*10*	*50*	10
R39	P-2	Sm Su Tc Hgr Pmr Ter	*10*	*50*	10
kR61	P-2	Cm Sm Su Tc Hgr Pmr Ter	*20*	*50*	5
Rms159	P-2	Cm Sm Tc Hgr Pmr Ter	*10*	*20*	5
R931	P-2	Sm Tc Hgr Ter Uv	5	10	5
R3108	P-2	Sm Su Tc Hgr Pmr Ter	*10*	*50*	5
CAM	P-2	Cam Uv Ter	5	10	5
pMG7	P-3	Gm Km Su Tm Hgr	5	10	5
RIP64	P-3	Cb Cm Gm Su Tm Hgr	5	10	5
R679	P-4	Sm Su	5	10	5
R1162	P-4	Sm Su	5	10	5
R5265	P-4	Sm Su	5	10	5
Rms163	P-5	Cm Su Tc	*10*	*50*	5
Rms149	P-6	Cb Gm Sm Su	5	10	5
Rms148	P-7	Sm	5	10	5
FP2	P-8	Hgr Pmr	5	10	5
R2	P-9	Cb Sm Su Uv	5	10	5
pMG18	P-9	Cb Gm Km Sm Su Hg	*20*	*50*	5
R91	?	Cb	5	10	5
RP1-1	?	Cb	5	10	5
RP8	?	Cb	5	5	5
R716	?	Sm Hgr	5	10	5

[a] Phenotypic abbreviations as for Table 1.
[b] Enhanced minimal inhibitory concentrations (MICs) are italicized.

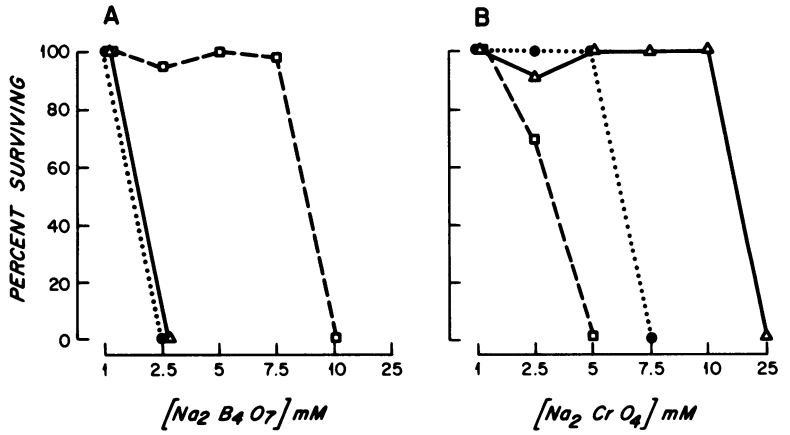

FIG. 1. *Survival of colony-forming ability of strains PU21 (●), PU21(pMG1) (□), and PU21(pMG6) (△) as a function of sodium borate (A) or sodium chromate (B) concentrations.*

resistance markers. Crr is determined by IncP-2 plasmid pMG6, which carries resistance to mercury and tellurium compounds, but other mercury- and tellurium-resistant IncP-2 plasmids lack chromium resistance. Hence, both Bor and Crr appear to be unique resistance markers. The biochemical mechanisms for resistance to boron and chromium compounds is not yet known. It is worth noting that for chromium, at least, the hexavalent compounds to which plasmids confer resistance are much more toxic and mutagenic for bacteria than are trivalent compounds (12).

Resistance to compounds other than those of arsenic, boron, chromium, mercury, silver, and tellurium was not found in this survey of *E. coli* and *P. aeruginosa* plasmids. In particular, resistance was not detected to compounds of antimony, bismuth, cadmium, lead, or zinc, which occurs in plasmids of gram-positive organisms (3, 11, 13), nor was resistance to cobalt or nickel found as described by Smith on plasmids of *E. coli* (15). A marginal increase in resistance could have been overlooked at the inhibitor concentrations chosen, and plasmids other than those tested may carry additional metal resistance markers.

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