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Suppressor Mutation in *Pseudomonas aeruginosa*

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Suppressor mutations were identified in *Pseudomonas aeruginosa*, and a comparison was made with *Escherichia coli* suppressor systems. A suppressor-sensitive (sus) derivative of a plasmid, RP4 *trp*, and several Sus mutants of IncP-1 plasmid-specific phages, were isolated by using *E. coli*. Plasmid RP4 *trp*(sus) was transferred to *P. aeruginosa* strains carrying *trp* markers which did not complement RP4 *trp*(sus), and Trp^+ variants were selected. Some, but not all such revertants, could propagate PRD1 Sus phages, and these mutants were found to be suppressor positive. Plating efficiencies of various Sus phages on these strains were compared with those on *E. coli* strains carrying known suppressor genes. The results suggested that the *Pseudomonas* suppressors were probably amber suppressors. In addition, some Sus phages (PRD1*sus-55*, PRD1*sus-56*) were obtained which, although apparently of the amber type for *E. coli*, were able to propagate equally well on sup^+ or *sup* strains of *P. aeruginosa*. On the other hand, several mutants of phage PRR1 which were suppressed in *E. coli* were not suppressed by the *P. aeruginosa* suppressors. Suppressor-sensitive mutants were also isolated with *P. aeruginosa* bacteriophages E79 and D3.

Nonsense mutations and their suppressors are very useful for genetic and other studies of bacteria and bacteriophages. However, the use of such systems has not been well established in *Pseudomonas*. Watson and Holloway (20) identified a suppressor gene in *P. aeruginosa* PAT after isolating a mutant in which two different auxotrophic mutations were simultaneously suppressed. They also isolated suppressor-sensitive (Sus) derivatives of phage E79 and suppressor-sensitive (sus) derivatives of R plasmid R18. However, they claimed that their suppressor was different from the amber or ochre suppressors of *Escherichia coli*. Mindich et al. (10) introduced amber mutations in ampicillin and tetracycline genes of RP1 and transferred that plasmid to various *Pseudomonas* species. They succeeded in isolating suppressor mutants from variants which showed simultaneous resistance to ampicillin and tetracycline. Their suppressor strain suppressed amber mutants of PRD1. They used this system for the study of bacteriophage $\phi 6$ morphogenesis in *P. pseudoalcaligenes*.

The purpose of the present study was to identify and characterize suppressor systems in *P. aeruginosa* and compare them with those in *E. coli*. A hybrid R plasmid, RP4 *trp*, which carries the whole tryptophan operon of *E. coli*, was constructed by Nagahari et al. (11, 12), and the *trp* genes of this plasmid were found to be expressed in *P. aeruginosa*. We have obtained a mutant of the RP4 *trp* plasmid which carries a sus character in the *trp* operon of RP4 *trp*. We have also isolated several Sus mutants of PRD1 and PRR1 bacteriophages which plate on bacterial strains carrying IncP-1 and other plasmids. Using the above-mentioned plasmid and phages, we could isolate suppressors in *P. aeruginosa* which may correspond to the amber suppressors of *E. coli*. Several Sus mutants of *P. aeruginosa* bacteriophages E79 and D3 were also isolated. This paper describes the isolation and characterization of the sus plasmid, Sus phages, and their suppressors.

MATERIALS AND METHODS

Media. Minimal medium for *E. coli* was that of Vogel and Bonner (18). For *P. aeruginosa*, G medium was used (6). Amino acids were added as necessary at a concentration of 50 μg/ml; thiamine was added at 20 μg/ml. Nutrient broth and agar have been previously described (4).

Drugs were used at the following concentrations: for *E. coli*, carbenicillin (Cb, Fujisawa Pharmaceuticals Co.), 500 μg/ml; kanamycin (Km, Meiji Seika Co.), 10 μg/ml; neomycin (Nm, Nippon Kayaku Co.), 10 μg/ml; tetracycline (Tc, Lederle Japan Ltd.), 25 μg/ml; nalidixic acid (Nd, Sigma Chemical Co.), 25 μg/ml, unless otherwise indicated. For *P. aeruginosa*, the drug concentrations were: carbenicillin, 500 μg/ml; kanamycin and neomycin, each 250 μg/ml; tetracycline, 100 μg/ml; streptomycin (Sm, Meiji Seika), 250 μg/ml.

Bacterial strains. The bacterial strains used in this study are listed in Table 1. Strains carrying plasmid RP4 are designated as C600(RP4) and PAO3016(RP4).

Phages. IncP-1 plasmid-specific RNA phage PRR1

TABLE 1. *Strains used*[a]

Strain	Character	Derivation	Source/reference
E. coli			
CA274-1	HfrC *trp*(Am) *lac*(Am) *ϕ80psu-3(ts-6) sus-2*		K. Saigo (see 13)
CA274-1(RP4 *trp*)	CA274-1 carrying RP4 *trp*		This study
CA274-1(RP4 *trp*[sus])	CA274-1 carrying RP4 *trp*(sus)		This study
C600	*leu thi thr lacY tonA supE*		K. Saigo
C600-2	*leu thi thr trp lacY tonA supE res mod*		K. Nagahari
C600-2(RP4 *trp*)	C600-2 carrying RP4 *trp*		This study
W3110	*thi*		K. Saigo
W3110-2	*thi Δ(trpA-E) nal tonB*		K. Nagahari
W3110-2(RP4 *trp*[sus])	W3110-2 carrying RP4 *trp*(sus)		This study
W3110-64	Suppressor-positive derivative of W3110-2		This study
K37	*supD* (amber suppressor)		T. Shiba
BE900	*supE* (amber suppressor)		T. Shiba
BE102	*supF* (amber suppressor)		T. Shiba
K133	*supG* (ochre suppressor)		T. Shiba
CA168	*supB* (ochre suppressor)		T. Shiba
CA169	*supC* (ochre suppressor)		T. Shiba
Ymel	*mel supE supF*		K. Saigo
P. aeruginosa			
PML14	Prototroph	Formerly P14	4
PML1516	*trp-304* (Ant$^-$ Ind$^+$), *str-301*	Formerly P15-16	4
PAT458	*trp-3458* (Ant$^-$ Ind$^+$), *str-1110*		2-458 of J. A. Waltho (19)
PAO1	Prototroph, *cml-2*		B. W. Holloway
PAO1819	*leu-9001 trpF9008*[b] (Ant$^-$ Ind$^+$)		H. Matsumoto
PAO3012	*trp-6*[c] (Ant$^-$ Ind$^+$), *cml-301*	Formerly M12	6
PAO3016	*leu-38 str-302* FP2	Formerly M16	6
PAO3088	*leu-38 trp-302* (Ant$^+$ Ind$^+$), *str-302*		This study
PAO3271	*ilvB112 leu-38 rif-301 str-304* RP4		PU21(RP4) of G. A. Jacoby (5)
PAO3281	*met-9020 trp-325* (Ant$^-$ Ind$^+$), *catA9001 narA9011 pca-9001*	Mutagenesis of PAO2210	H. Matsumoto
PAO3282	*met-9020 trp-314* (Ant$^-$ Ind$^+$), *catA9001 narA9011 pca-9001*	Mutagenesis of PAO2210	H. Matsumoto
PAO3288	*trp-305* (Ant$^-$ Ind$^+$)	Mutagenesis of PAO1	This study
PAO3301	*met-9020 trp-325 catA9001 narA9011 pca-9001 sup-51*	Spontaneously from PAO3281	This study
PAO3304	*met-9020 trp-304 catA9001 narA9011 pca-9001 sup-52*	Mutagenesis of PAO3282	This study
PAO3305	*met-9020 trp-304 catA9001 narA9011 pca-9001 sup-53*	Mutagenesis of PAO3282	This study

[a] Abbreviations: *leu, met, thi, thr,* and *trp,* leucine, methionine, thiamine, threonine, and tryptophan requirements, respectively; *catA,* catechol 1,2-oxygenase; *lac,* lactose fermentation; *mel,* melibiose fermentation; *nar,* nitrate reduction; *pca,* protocatechuate utilization; *cml, nal, rif, str,* and *ton,* resistance to chloramphenicol, nalidixic acid, rifampin, streptomycin, and phage T1, respectively; Am, amber; sus, suppressor sensitivity; *sup,* suppressor activity; FP2, *Pseudomonas* fertility factor; Ant and Ind, growth response to anthranilate and indole, respectively; *res,* restriction; *mod,* modification.

[b] *trpF9008* is a late marker.

[c] *trp-6* maps at 33 min.

(15) was donated by G. A. Jacoby, and DNA phage PRD1 (14) was donated by R. H. Olsen. T4 wild type, T4*am*$E_{1140}N_{82}$, λcI, λcI*sus*S_7, and *sus*N_7 were obtained from K. Saigo. *P. aeruginosa* phages E79, D3, F116L, and G101 were provided by B. W. Holloway (8, 9, 16).

Genetic nomenclature. The following designations are used throughout: Su$^+$, permissive for suppressible mutation; Su$^-$, nonpermissive for suppressible mutation; and Sus, suppressible phenotype. Suppressibility of a gene is indicated by "sus."

Mutagenization of bacteriophages. Phage PRD1 was mutagenized with hydroxylamine (17) as

follows. A phage suspension (0.05 ml, 10^9 plaque-forming units) was added to a mixture of 0.55 ml of 0.1 M sodium phosphate buffer (pH 6.0) containing 10^{-3} M EDTA and 0.4 ml of 1 M $NH_2OH \cdot HCl$ (pH 6.0); the solution was incubated at 37°C for 24 or 48 h, diluted 1:100 with buffer (0.2 M NaCl, 0.01 M Tris [pH 7.5] containing 0.1% gelatin), and kept at 4°C until use. Detection of Sus mutants of PRD1 was done on plates with two indicators (Su^- and Su^+), and turbid plaques were sought. Nutrient agar plates were first overlaid with soft agar containing W3110-2(RP4) Su^-. After the soft-agar layers solidified, they were covered with soft agar containing C600(RP4) *supE* and portions of the mutagenized-phage solution. Survival after hydroxylamine mutagenesis was approximately 40% after 24 h and approximately 15% after 48 h. Approximately 16,000 plaques were examined, and 150 were found to be turbid on the double-indicator plates. Most were turbid on both indicators, but six were obtained which gave clear plaques on C600(RP4) but none on W3110-2(RP4). These were designated PRD1*sus-51* through PRD1*sus-56*, respectively. These phages were prepared on C600(RP4) by extraction from confluent-lysis plates.

Pseudomonas phages E79 and D3 were mutagenized in the same way. Selections were made with strains PAO3012 and PAO3301.

PRR1 mutagenesis was done with nitrous acid. The phage was treated with 0.2 M $NaNO_2$ in 0.06 M sodium acetate buffer (pH 4.6) for 2 h at 37°C. Mutagenized phages were plated on *E. coli* Su^+ strain Ymel(RP4) or C600(RP4). The survival ratio was about 3×10^{-4} on both strains. Plaques were transferred onto a lawn of W3110(RP4) Su^- to test their response. One of 1,200 plaques on Ymel(RP4) and 1 of 10^4 plaques on C600(RP4) did not show plaques on W3110(RP4). These phages were named *sus-51* and *sus-52*, respectively. The phage stocks were prepared on C600(RP4).

Plasmids. RP4, a self-transmissible IncP-1 plasmid conferring Cb^r Km^r/Nm^r Tc^r was obtained from G. A. Jacoby. RP4 *trp* was constructed by Nagahari et al. (11, 12). It consists of RP4 and the part of the phage λ chromosome that includes immunity, the φ80 attachment site, and the whole tryptophan operon (*A-E*) of *E. coli*. It has retained the activities of the original plasmid, such as drug resistance, transmissibility, and phage sensitivity.

Plasmid transfer. Conjugative transfer of plasmids was carried out as reported by Chandler and Krishnapillai (1). When plasmid RP4 *trp*(sus) was isolated, the procedure was as follows. CA274-1 was mixed with *E. coli* C600-2(RP4 *trp*) in a minimal medium supplemented with leucine, threonine, and thiamine; incubated at 30°C for 2 h; and then plated on minimal agar containing 500 μg of carbenicillin at 30°C, on which the colonies of plasmid-containing cells of CA274-1 appeared. The frequency of transfer of the plasmid was 2×10^{-3} per donor.

RESULTS

Isolation of RP4 *trp*(sus). *E. coli* CA274-1 has two amber mutations: *trp*(Am) and *lac*(Am). It is lysogenized by phage φ80*psu-3*(*ts-6*)*sus-2*, which carries the temperature-sensitive suppressor F and *sus-2* (13). This suppressor can suppress *trp*(Am) and *lac*(Am) on the chromosome as well as *sus-2* on the phage at 30°C, but not at 40°C, so that *E. coli* CA274-1 is Trp^+ at 30°C and Trp^- at 40°C. RP4 *trp* was introduced into this strain in the hope that RP4 *trp*(Am) might be obtained as a result of recombination at the *trp*(Am) region of the chromosome. Among the colonies of plasmid-containing strain CA274-1, a search was made for those which grew at 30°C but not at 40°C on minimal agar containing carbenicillin. From over 2,200 colonies tested, one such colony was found. This strain showed the following characteristics. It required tryptophan or indole for growth at 40°C but not at 30°C. It was resistant to carbenicillin, kanamycin, and tetracycline and was sensitive to plasmid-specific phages PRR1 and PRD1, but resistant to λ (PR4 *trp* carries λ immunity). It produced phages after UV induction at 30°C, but not at 40°C, which had the characteristics of φ80*psu-3*(*ts-6*)*sus-2*. These properties are compatible with the idea that the strain carries RP4 *trp*(sus).

Characterization of RP4 *trp*(sus). To study the nature of the above-described plasmid more precisely, its transfer to a strain carrying a deletion for the tryptophan operon was tried. CA274-1(RP4 *trp*[sus]) was mixed with W3110-2 Δ*trp*(*A-E*) *nal* at 37°C for 1 h and plated on nutrient agar containing 25 μg of nalidixic acid per ml, 10 μg of tetracycline per ml, and 7.5 μg of kanamycin per ml. Transconjugants were obtained which showed the following characteristics: Cb^r, Tc^r, Km^r, Nd^r, Trp^-, and Lac^+ at any temperature (tryptophan could be replaced by indole but not by anthranilate), $T4^s$, λ^r, $PRD1^s$. One such clone, W3110-2(RP4 *trp*[sus]), was used in the experiment in which Trp^+ variants were sought. Apparent Trp^+ revertants appeared spontaneously at a frequency of about 2×10^{-7}, whereas with the original W3110-2 no revertants ($< 8 \times 10^{-10}$) were detected since it carried Δ*trp*. Some of these revertants from W3110-2(RP4 *trp*[sus]) showed suppressor activity. Thus, 12 of 68 revertants were permissive to T4 amber mutants. One such strain was named W3110-64. These results indicate that the plasmid thus obtained is RP4 *trp*(sus), carrying a suppressible mutation in the *trp* operon.

Characterization of PRD1 Sus phages. Complementation analysis of six PRD1 Sus phages was performed with *E. coli* W3110-2(RP4). Two phages (one loopful of each phage suspension [10^8/ml]) were mixed on a lawn on nutrient agar. By this method the phages were classified into three complementation groups: *sus-51*, *sus-52*, and *sus-53*; *sus-54*; *sus-55* and *sus-56*.

The efficiency of plating (EOP) of the six PRD1 Sus phages on several *E. coli* Su^- and Su^+ strains is shown in Table 2. Wild-type PRD1 showed essentially an equal number of plaques on every indicator. Six Sus phages, *sus-51* through *sus-56*, plated well on *E. coli* Su^+ strains but showed very few plaques on Su^- strains. CA274-1(RP4 *trp*[sus]) carries the temperature-sensitive *supF* gene, which is effective at 30°C but not at 40°C. The EOP of the Sus phages on this strain was fairly good at 30°C but much reduced at 40°C. *E. coli* W3110-64(RP4 *trp*-[sus]), which was described in the previous section, was found to be permissive for all PRD1 Sus phages.

These phages were tested on *Pseudomonas* strain PAO3016(RP4) (Table 1). Whereas wild-type PRD1 showed an EOP slightly greater than that on *E. coli* strains, four Sus phages (*sus-51* through *sus-54*) were very much restricted, similar to the EOP on *E. coli* Su^- strains. On the other hand, two mutant phages (*sus-55* and *sus-56*) grew very well on this *Pseudomonas* strain, at about the same levels as on *E. coli* Su^+ strains. (Strain PAO3301 shall be described later.)

Various suppressors have been identified in *E. coli*, and Sus phages with known responses to these suppressors are available for T4 or λ. To determine the nature of the mutations in our PRD1 Sus phages, their growth responses to several amber and ochre suppressors were investigated by comparison with known mutants of T4 and λ (Table 3). PRD1*sus-51* through PRD1*sus-54* behaved quite similarly. They grew very well on amber-suppressor strains containing either *supD*, *supE*, or *supF* but not on suppressor-negative or ochre-suppressor strains. The responses of PRD1*sus-55* and PRD1*sus-56* were similar to those of the above-mentioned phages, except that they were weakly suppressed by *supC*, an ochre suppressor. The behavior of the *E. coli* strains with known T4 and λ mutants was as expected. It can be concluded from these results that the Sus phages are most probably amber mutants.

Characterization of PRR1 Sus phages. The difference in EOPs of PRR1 phages on Su^+ and Su^- strains was not so great as those on DNA phages. The EOP ratio of PRR1 wild-type phage on Ymel:W3110:*P. aeruginosa* PAO3271 was 1.0:0.3:2.1, whereas that of PRR1*sus-51* was $1:10^{-5}:5 \times 10^{-3}$. The EOPs on various *E. coli* strains are shown in Table 3. PRR1*sus-51* was equally suppressed by amber suppressors (*D*, *E*, or *F*), but not by ochre suppressors. PRR1*sus-52* behaved similarly (data not shown). These results suggest that PRR1 Sus phages are amber in nature.

Isolation of suppressor mutants of *P. aeruginosa*. Plasmid RP4 *trp*(sus) was transferred from W3110-2(RP4 *trp*[sus]) to several strains of *P. aeruginosa* carrying *trp* mutations. PML1516, PAT458, PAO3012, PAO3088, and PAO1819 all became Trp^+ upon introduction of RP4 *trp*(sus), whereas the Trp^- character of PAO3281, 3282 and 3288 was not complemented by this plasmid. These strains became Trp^+ when the original plasmid, PR4 *trp*, was introduced. Cells carrying RP4 *trp*(sus) were isolated from these strains, and then mutants which became phenotypically Trp^+ were selected. They were then tested for sensitivity to PRD1 Sus phages. Those which could propagate Sus phages were considered to be suppressor positive. The frequency of spontaneous reversion to Trp^+ of PAO3281(RP4 *trp*[sus]) was 3×10^{-7}. Among 133 spontaneous Trp^+ mutants of PAO3281(RP4 *trp*[sus]), one was sensitive to PRD1*sus-51* and was named PAO3301. No suppressor-positive clones were found among 416 spontaneous Trp^+ mutants of PAO3282(RP4 *trp*[sus]) (the reversion rate was 10^{-7}). However,

TABLE 2. *EOP of PRD1 mutants*[a]

Phage	EOP on *E. coli* strain:					EOP on *P. aeruginosa* strain:	
	C600 (RP4)	W3110-64(RP4 *trp*[sus])	CA274-1(RP4 *trp*[sus]) 30°C	CA274-1(RP4 *trp*[sus]) 40°C	W3110-2(RP4)	PAO3016 (RP4)	PAO3301 (RP4 *trp*-[sus])
PRD1, wild	1	1.3	0.5	0.49	1.3	2.0	1.3
PRD1, *sus-51*	1	1.0	0.5	0.01	3×10^{-7}	3×10^{-7}	1.1
PRD1, *sus-52*	1	0.95	0.24	0.006	4×10^{-7}	7×10^{-7}	0.92
PRD1, *sus-53*	1	0.83	0.36	0.04	4×10^{-7}	4×10^{-7}	0.94
PRD1, *sus-54*	1	0.72	0.47	0.08	4×10^{-7}	8×10^{-7}	1.1
PRD1, *sus-55*	1	1.3	0.4	0.001	1×10^{-5}	1.0	1.0
PRD1, *sus-56*	1	1.0	0.5	0.001	5×10^{-5}	0.3	1.0

[a] Phages propagated on C600(RP4) (10^{10} to 10^{11} plaque-forming units per ml) were employed. The number of plaques on each indicator was compared with that on C600. Incubation temperature was 37°C except for CA274-1(RP4 *trp*[sus]).

TABLE 3. *Response of mutant phages to various E. coli suppressors*[a]

E. coli strain	PRD1							PRR1		T4		λcI	λ*sus*N$_7$	λ*sus*S$_7$
	Wild	*sus-51*	*sus-52*	*sus-53*	*sus-54*	*sus-55*	*sus-56*	Wild	*sus-51*	Wild	Amber			
W3110(RP4) Su$^-$	+	−	−	−	−	−	−	+	−	+	−	+	−	−
C600(RP4) *supE*	+	+	+	+	+	+	+	+	+	+	+	+	+	−
K37(RP4) *supD*	+	+	+	+	+	+	+	+	+	+	+	−	−	−
BE900(RP4) *supE*	+	+	+	+	+	+	+	+	+	+	+	+	+	−
BE102(RP4) *supF*	+	+	+	+	+	+	+	+	+	+	+	+	+	+
K133(RP4) *supG*	+	−	−	−	−	−	−	+	−	+	−	−	−	−
CA168(RP4) *supB*	+	−	−	−	−	−	−	+	−	+	−	+	−	−
CA169(RP4) *supC*	+	−	−	−	−	±	±	+	−	+	−	+	−	−
W3110-64(RP4 *trp*[sus]) Su$^+$	+	+	+	+	+	+	+	+	+	+	+	−	−	−

[a] Experiments were performed at 37°C. Symbols: +, EOP = 1 to 0.1; −, EOP < 10^{-5} for PRD1 and T4, = 10^{-2} to 10^{-3} for PRR1 *sus-51*, and < 10^{-3} for λ; ±, EOP = ca. 10^{-2}. Phages PRD1 and PRR1 were prepared on C600(RP4); phages T4 and λ were prepared on *E. coli* K-12 substrain Ymel (*supE supF*).

after mutagenesis with ethyl methane sulfonate by the method of Watson and Holloway (20), 2 of 142 Trp$^+$ clones tested propagated PRD1*sus-51*. These were named PAO3304 and PAO3305. In the case of PAO3288(RP4 *trp*[sus]), 1 of 421 spontaneous Trp$^+$ clones and 3 of 153 ethyl methane sulfonate-induced Trp$^+$ clones could suppress PRD1*sus-51*. However, all of the *sup* derivative from PAO3288 grew very poorly on nutrient agar at 30 or 37°C.

Character of suppressor mutants. The nature of the suppressor of PAO3301(RP4 *trp*[sus]) was investigated. This strain showed the same degree of efficiency of propagating PRD1 Sus phages as did *E. coli* C600(RP4) (Table 2), whereas its parent, PAO3281(RP4 *trp*[sus]), did not support the multiplication of PRD1*sus-51* through PRD1*sus-54*. The EOPs of wild and mutant PRD1 on PAO3281(RP4 *trp*[sus]) were all lower than those on PAO3016(RP4) by about 30%. The sensitivity-resistance patterns of the permissive strain to several other phages (D3^s, E79^s, F116^r, G101^s) and to antibiotics were the same as its parent. Although it showed an apparent Trp$^+$ character, it retained the chromosomal *trp* marker as well as *trp*(sus) on the plasmid. The reasons for this are as follows. (i) Strain PAO3301(RP4 *trp*-[sus]) could transfer its R plasmid to other strains, such as PAO3012 *trp-6* or PAO3288 *trp-305*. After such transfer, PAO3012 became Trp$^+$, whereas PAO3288 remained Trp$^-$. As described above, *trp-6* of PAO3012 complements RP4 *trp*(sus), but *trp-305* of PAO3288 does not. If RP4 *trp*(sus) had changed to RP4 *trp* in PAO3301, then both PAO3012 and PAO3288 should have shown a Trp$^+$ phenotype upon acceptance of the plasmid. (ii) Variants of PAO3301(RP4 *trp*[sus]) which had lost the R plasmid were sought. First, PRD1-resistant colonies were isolated, and then those which were sensitive to carbenicillin, kanamycin, and tetracycline were identified. Twenty such variants, which were considered to lack the plasmid, were all found to be Trp$^-$. This indicates that the chromosomal marker had not been lost. (iii) Reintroduction of RP4 *trp*(sus) into such a strain resulted in a Trp$^+$ phenotype.

The above results indicate that PAO3301 has a suppressor which suppresses mutations of RP4 *trp*(sus) and PRD1 Sus phages. This suppressor was designated *sup-51*. By the same method, two suppressor-positive clones were obtained from ethyl methane sulfonate-treated PAO3282 carrying an independently isolated *trp* mutation. They were named *sup-52* (PAO3304) and *sup-53* (PAO3305), respectively. Both PAO3304(RP4 *trp*[sus]) and PAO3305(RP4 *trp*[sus]) propagated PRD1 Sus phages with high efficiency, at the same level as *sup-51*. Plasmid-deprived variants obtained as PRD1 insensitive and drug sensitive all became Trp$^-$ again. Suppressor-positive derivatives obtained from PAO3288 were not studied because of their poor growth.

Isolation and characterization of *P. aeruginosa* Sus phages. Phage E79 gives clear distinct plaques on PAO strains. Mutagenized phages were plated on agar layers of PAO3012

Su^- and PAO3301 Su^+, and turbid plaques were sought. The survival after 25 h of mutagenesis was 15% and that after 48 h was 1.5%. Among 2,100 plaques examined, 2 showed clear plaques on the Su^+ strain but did not plaque on the Su^- indicator. These were named E79*sus-51* and E79*sus-52*. Phage preparations of these were made on PAO3301.

The EOP of E79*sus-51* and E79*sus-52* on Su^- strains (PAO3012, PAO3281, PAO3282) was about 10^{-5} and 10^{-6}, respectively, compared with that on PAO3301 Su^+. PAO3304 and PAO3305 were permissive, like PAO3301. E79*sus-51* and E79*sus-52* did not complement each other on strain PAO3012.

Phage D3 is a temperate phage which gives turbid plaques on PAO strains. Conditional clear-plaque mutants of phage D3 were sought. The mutagenized phage sample was first plated on PAO3012 Su^-, and clear plaques were picked up. These clear mutants were then plated on PAO3301 Su^+, and those which gave turbid plaques were isolated. The survival after 25 h of mutagenesis was 0.2%. Among 12,400 plaques on the Su^- indicator, 246 were clear, 7 of which gave turbid plaques on PAO3301. These were designated as D3*sus-51*(C) through D3*sus-57*(C). For the preparation of phage stocks, a strain of PAO3301 lysogenized with each one of these phages was isolated. Cultures of these lysogens were treated with mitomycin C (1 μg/ml) to induce the phage production. Lysates containing more than 10^{10} plaque-forming units per ml were obtained.

These mutants gave essentially the same number of plaques on either Su^- or Su^+ strains, although they were clear on PAO3012, PAO3281, or PAO3282 and turbid on PAO3301, PAO3304, or PAO3305. They were classified into two complementation groups: *sus-51*, *-52*, *-53*, *-54* and *-57* in one group (CI), and *sus-55* and *-56* in the other (CII). Egan and Holloway (3) studied clear-plaque mutants of D3, but we do not know whether our classifications CI and CII correspond to theirs.

EOP of Sus phages on various *P. aeruginosa* strains. As PRD1, E79, and D3 Sus phages had been obtained, various *P. aeruginosa* strains of different origin were investigated for their response to these phages, to determine whether any strain might have a suppressor activity (Table 4). Three Su^+ strains behaved similarly, plating all tested phages equally well. None of the other strains tested was permissive for any Sus phages except PRD1*sus-55* and PRD1*sus-56*. PML14 and PML1516, which had been used as pyocin indicators (4), a PAT strain, and several PAO sublines were all similar in this respect. Besides the strains shown in Table 4, PAO1, PAO3012, PAO3016, PAO3271, and PAO3282 were all found to be Su^-. PRD1*sus-55* and PRD1*sus-56*, which grew very well on the amber suppressor strain (*supD,E,F*), but not on Su^- strains, of *E. coli* (Table 3), showed plaques on all of the *Pseudomonas* strains with essentially the same efficiency. Besides the phage mutants shown in Table 4, PRR1*sus-51* and PRR1*sus-52*, which were suppressed by amber suppressors in *E. coli* (Table 3), did not grow on either Su^- or *sup-51*, *-52*, or *-53* strains of *Pseudomonas*, the EOP being $<10^{-3}$ for PRR1*sus-51* and $<10^{-2}$ for PRR1*sus-52*, compared with an EOP of 1 for Su^+ *E. coli* strains.

DISCUSSION

Suppressor mutations were obtained in *P. aeruginosa* which could suppress certain mutations in a plasmid and in several phages. The suppressor-sensitive mutations in the plasmid used for the isolation of their mutations seemed to be of the amber type for the following reasons. The mutation in the *trp* operon of RP4 *trp*(sus) was suppressed by the known temperature-sensitive amber suppressor *supF*(Ts) at 30°C but not at 40°C. The Trp^+ phenotype of this plasmid was also expressed in W3110-64 Su^+ isolated in this study, which was able to suppress a known amber mutant, T4*am* $E_{1149}N_{82}$, at high efficiency (Table 3). Sus λ phages could not be used in this case, because the strain was not sensitive to λ phages. Other suppressors listed in Table 3 were not tested, since *trp* mutants were not available for them. Plasmid RP4 *trp*(sus) was isolated on strain CA274-1, which carried *trp*(Am) on the chromosome. The mutant plasmid could have originated by recombination at the homologous site, as at first expected, or by the occurrence of an independent, spontaneous mutation. This point has been left unsolved.

PRD1 and PRR1 Sus phages were first isolated on *E. coli* C600, a *supE* strain, or on *E. coli* Ymel, a *supE supF* strain. They were all suppressed very well with *supD*, *supE*, or *supF*, the amber suppressors. These *E. coli* suppressors behaved as expected for the Sus phages T4 and λ (Table 3). Therefore, from these data PRD1 and PRR1 phage mutants seemed to be of the amber type.

Mutants which could suppress RP4 *trp*(sus) were isolated from *P. aeruginosa* PAO3281 and PAO3282. These Su^+ strains (RP4 derivatives of PAO3301, PAO3304, PAO3305) were also able to suppress PRD1 Sus phages as efficiently as *E. coli* amber suppressors. Therefore, these suppressors may be regarded as amber suppressors. Although three suppressors were isolated independently, they may be of the same kind since they behaved quite similarly. However, differ-

TABLE 4. *Response of various P. aeruginosa strains to mutant phages*[a]

Bacterium	PRD1							E79			D3		
	Wild	*sus-51*	*sus-52*	*sus-53*	*sus-54*	*sus-55*	*sus-56*	Wild	*sus-51*	*sus-52*	Wild	CI *sus-57*	CII *sus-55*
PML14	+	−	−	−	−	+	+	+	−	−	−	−	−
PML1516	+	−	−	−	−	+	+	+	−	−	−	−	−
PAT458	+	−	−	−	−	+	+	+	−	−	−	−	−
PAO3281	+	−	−	−	−	+	+	+	−	−	+, T	+, C	+, C
PAO3301 *sup-51*	+	+	+	+	+	+	+	+	+	+	+, T	+, T	+, ST
PAO3304 *sup-52*	+	+	+	+	+	+	+	+	+	+	+, T	+, T	+, ST
PAO3305 *sup-53*	+	+	+	+	+	+	+	+	+	+	+, T	+, T	+, T

[a] Experiments were performed at 37°C. +, EOP ~ 1; −, EOP ≤ 10^{-5}; T, turbid plaque; C, clear plaque; ST, slightly turbid. Bacteria harboring RP4 were used for PRD1. PRD1 phages were prepared on *E. coli* C600(RP4); E79 and D3 were prepared on PAO3301.

ences were found between the *P. aeruginosa* suppressors and *E. coli supD*, *supE*, and *supF*. PRD1*sus-55* and PRD1*sus-56*, which behaved apparently amber-like in *E. coli*, formed plaques efficiently on various *P. aeruginosa* strains irrespective of suppressor character. Moreover, PRR1*sus-51* and PRR1*sus-52*, which were permissive in *E. coli supD*, *supE*, and *supF*, were not suppressed by the pseudomonad suppressors. These results may be interpreted as indicating a difference in the amino acid which is inserted in response to the amber codon, just like λ*susS*$_7$ which is suppressible by *supF* but not by *supD* or *supE* (Table 3). Mindich et al. (10) reported a similar observation. Their PRR1 Sus phages which were permissive on *E. coli supD* were not suppressed by their *Pseudomonas* suppressors. The mechanism of apparent suppression of PRD1*sus-55* and PRD1*sus-56* in *P. aeruginosa* is not clear.

Using a strain of *Pseudomonas* carrying the *sup-51* mutation, we isolated several *sus* mutants of phages E79 and D3. The efficiency of suppression was very high in the case of E79 sus phages, and probably in D3*sus*(C) also, although precise estimation of efficiency of suppression of D3 clear plaques was not possible. The suppressible mutation of these phages could be of the amber type, but at present we have no direct method of testing this.

We have not isolated so far any effective suppressor from PAO3288, and the reason for this is unclear. However, suppressors could be readily isolated from PAO3281 and PAO3282. The reasons for these differences may be in the previous history of exposure to mutagens of these strains. This point may be solved by genetic analysis. Although we failed to transduce the Su^+ character by phage G101 to RP4-containing strains (data not shown), we were able to transfer the *sup* genes to several other *P. aeruginosa* strains by FP2-mediated conjugation. Further genetic analysis to locate the map position of these suppressors is now in progress.

The cistron of the *trp* operon carrying the amber mutation in *E. coli* CA274-1, in which RP4 *trp*(sus) was isolated, has not been identified. However, our results suggest that the suppressible mutation in the plasmid resides in the *trpA* gene. Growth of CA274-1 and CA274-1 (RP4 *trp*[sus]) at 37°C, or of W3110-2 which carries RP4 *trp*(sus) and which has a chromosomal deletion for the *trp* operon, was supported by indole but not by anthranilate. The same was true for the growth of *P. aeruginosa* Trp^- strains PAO3281, PAO3282, and PAO3288, which were not complemented by RP4 *trp*(sus). Therefore, the *trp* gene in question could be either *trpA*, *trpC*, *trpD*, or *trpF* (2). The *trpF* cistron can be excluded because RP4 *trp*(sus) could complement PAO1819 *trpF*. In addition, transduction experiments with G101 showed that the *trp* marker of PAO3281 was different from *trpF* of PAO1819 and *trp-6* of PAO3012 (unpublished data). The *trp-6* marker of PAO3012 is probably *trpC* or *trpD*, which are both located at 33 min from the origin of chromosomal transfer mediated by FP2 (7; H. Matsumoto, personal communication). It has been known that *trp* genes are separated into at least three regions on *P. aeruginosa* chromosome (*trpA,B*; *trpC,D,E*; and *trpF*), although they are all closely linked into one cluster in *E. coli* (2). Therefore, the most probable locus of the suppressible *trp* allele is *trpA*, which is located at about 23 min on the genetic map of *P. aeruginosa* PAO (H. Matsumoto, personal communication). Preliminary results from mating experiments support this view (data not shown).

In conclusion, nonsense mutations and their suppressors have been demonstrated in *P. aeruginosa*, and they may well be amber suppressors. Mindich et al. (10) and Watson and Holloway (20) have also reported a similar system in *Pseudomonas*. Mindich et al. claimed that their suppressors were unstable in *P.*

aeruginosa. Our suppressors, except those isolated from strain PAO3288, show stability. The suppressor isolated by Watson and Holloway was found to be located close to the *thr* markers on the strain PAT chromosome (20). Their suppressor did not correspond to *supC*, *supD*, or *supF*. It will be of interest to compare our system experimentally with those that have been previously described.

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