Stability, frequency and multiplicity of transposon insertions in the pyoverdine region in the chromosomes of different fluorescent pseudomonads

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Tn5 mutagenesis of different fluorescent pseudomonads was achieved by conjugational transfer of the suicide vector pSUP 10141. Pyoverdine negative (Pvd⁻) mutants were detected by the absence of fluorescence on King's B medium and by their inability to grow in the presence of the iron chelator EDDHA [ethylenediamine di(*o*-hydroxyphenylacetic acid)]. In *P. fluorescens* ATCC 17400 and three rhizosphere isolates (one *P. putida* and two *P. fluorescens*), the percentage of Pvd⁻ mutants ranged between 0 and 0.54%. In a *P. chlororaphis* rhizosphere isolate, this percentage was higher (4%). In these mutants both of the Tn5 antibiotic resistances (Km and Tc) were stable and the transposon could be detected by hybridization. In Pvd⁻ mutants of *P. fluorescens* ATCC 17400, the transposon was found to be inserted twice in the chromosome while single insertions were detected in the DNA of other, randomly tested mutants. In *P. aeruginosa* PAO1, where 13.1% of the mutants were Pvd⁻, both antibiotic resistances were rapidly lost and accordingly no transposon insertion could be detected by hybridization. However, the Pvd⁻ phenotype was generally stable in these mutants. The plasmid pNK862 containing a mini-Tn10 transposon was introduced by electroporation into *P. aeruginosa* PAO1 and Km^r mutants were recovered, 89% of which were Pvd⁻ and confirmed to be *P. aeruginosa* by PCR amplification of the *P. aeruginosa* lipoprotein gene. The mini-Tn10 insertions were also found to be unstable in PAO1.

Introduction

Transposon mutagenesis of Gram-negative bacteria by conjugational transfer of suicide vectors is a very powerful technique presenting many advantages compared to other techniques (UV irradiation and chemical mutagenesis) such as single gene inactivation, and stability and possibility of cloning the mutated gene (Kleckner, 1981). Among the different available transposons, Tn5 has been the most widely used for obtaining mutants in different Gram-negative bacteria (Berg & Berg, 1983). The main advantages of Tn5 are its relative randomness of insertion (Berg, 1977) and its ability to

function in different hosts. There have already been several reports on the successful use of Tn5 for generating mutants, in particular Pvd⁻ mutants in several fluorescent pseudomonads (Marugg *et al.*, 1985; O'Sullivan & O'Gara, 1990), including a rhizosphere strain of *P. aeruginosa* (Höfte *et al.*, 1991). However, Goldberg *et al.* (1990) recently described the instability and precise excision of Tn5 in *P. aeruginosa*. These authors demonstrated that only the long terminal inverted repeats were needed to cause the excision, without involvement of the transposase. We were interested to see whether this instability was specific for Tn5 or whether, as suggested by these authors, it is the result of an adaptation of *P. aeruginosa* to eliminate composite transposons from its genome. High affinity iron uptake in fluorescent pseudomonads is mediated by pyoverdine or pseudobactin, a complex fluorescent

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Abbreviation: EDDHA, ethylenediamine di(*o*-hydroxyphenylacetic acid).

siderophore made of a catecholate chromophore and a peptide arm of variable length and composition containing unusual amino acids (Wendenbaum *et al.*, 1983; Hohnadel & Meyer, 1988). Both catechol groups from the chromophore and hydroxamate or hydroxycarboxylate groups provided by the unusual amino acids δ -N-hydroxyornithine or β -hydroxyaspartic acid respectively (depending on the pyoverdine) participate in complexing Fe(III) (Hohnadel & Meyer, 1988). Mutants in the biosynthesis of the siderophore are easy to detect either by their lack of fluorescence (Flu⁻) in iron-limited media and/or by their inability to grow (EDDHA⁻) in the presence of the iron chelator ethylenediamine di(o-hydroxyphenylacetic acid). Our interests focus on the regulation of pyoverdine biosynthesis in different fluorescent pseudomonads and on the role of pyoverdine in the antagonism shown by some strains in this group of bacteria against fungal pathogens like *Pythium* and *Fusarium* (Leong, 1986). We show here that in *P. aeruginosa*, Tn5 and mini-Kan Tn10 are unstable and yield an unusually high percentage of Pvd⁻ mutants.

Methods

Bacterial strains and plasmids. *Pseudomonas aeruginosa* PAO1 and *Pseudomonas fluorescens* ATCC 17400 were obtained from J. M. Meyer (Strasbourg, France). *Pseudomonas chlororaphis* BTP9, *Pseudomonas putida* BTP1 and the unidentified fluorescent *Pseudomonas* strain BTP20 were rhizosphere isolates from the Centre Wallon de Biologie Industrielle (University of Liège, Belgium). *Pseudomonas fluorescens* strain 47 is an isolate from the rhizosphere of poplar (S. Hettiarachchi, Vrije Universiteit Brussel).

E. coli strain S17-1 (*thi pro hsdR⁻ hsdM⁺ recA* RP4 2-Tc::Mu-Km::Tn7 Tp Sm) was given by Simon *et al.* (1983), together with the plasmid pSUP10141 (a pACYC184 derivative with Tn5 inserted into its Tc^r gene and a 4 kb RP4 *Sau3A* fragment containing a Tc^r gene cloned in the Tn5 *Bam*HI site). Plasmid pMMB33 (IncP-4 Mob⁺ cos λ Km) was from Frey *et al.* (1983). Plasmid pNK862 is a derivative of pBR333 containing an *Eco*RI-*Pvu*II *ptac* promoter fragment ligated to a blunt made *Bcl*I site of IS10 right next to a 1.7 kb *Hind*III Km^r fragment from Tn903 (Way *et al.*, 1984).

Growth media. Bacteria were grown in LB medium. For the screening of Pvd⁻ mutants, modified King's B (MKB) medium was used (Proteose peptone 5 g l⁻¹, MgSO₄·7H₂O 1.5 g l⁻¹, K₂HPO₄ 1.2 g l⁻¹, glycerol 2 ml l⁻¹). The mutants were further characterized in Casamino acids (CAA) medium (Casamino acids 5 g l⁻¹, K₂HPO₄ 0.9 g l⁻¹, MgSO₄·7H₂O, 0.25 g l⁻¹). Antibiotics were used at the following concentrations (ml⁻¹): ampicillin (Ap) 100 µg; chloramphenicol (Cm) 25 µg for *E. coli*; streptomycin (Sm) 10 µg for *E. coli*; tetracycline (Tc) 15 µg for *E. coli* and 50 µg for *Pseudomonas*; kanamycin (Km) 100 µg for *E. coli* and 400 µg for *Pseudomonas*.

Tn5 mutagenesis. The mutagenesis was done by conjugational transfer of the suicide vector pSUP10141 (pACYC184::Tn5::RP4-Tc^r) from *E. coli* S17-1, which contains genes for plasmid mobilization (Simon *et al.*, 1983). *Pseudomonas* cells were grown in 3 ml of LB medium at 28 °C (37 °C for *P. aeruginosa*) to an OD₆₀₀ of 0.3–0.5; *E. coli*

S17-1(pSUP10141) was similarly grown at 37 °C in the presence of Sm (to select for the presence of chromosomally integrated mobilization genes), Cm (resistance of the vector) and Km (one of the two resistance genes of Tn5). The *Pseudomonas* cultures were subsequently kept for 1 h at 37 °C (42 °C for *P. aeruginosa*) in order to lower the efficiency of the restriction system. Both cultures were centrifuged for 2 min and washed three times with LB medium before mixing in 1 ml of LB medium. After a final centrifugation, the mixed cells were resuspended in 50 µl of LB medium. The cells were plated on a sterile nitrocellulose filter put on a pre-warmed LB plate and incubated overnight at 28 °C (37 °C for *P. aeruginosa*). The cells were washed out of the filter in 10 ml of LB medium and plated undiluted or 10× diluted on LB plates containing Ap (to counterselect *E. coli*) and Km; control plates were inoculated with the *E. coli* cells or the *Pseudomonas* cells alone. The plates were incubated for 2 d at 28 °C (37 °C for *P. aeruginosa*).

Tn10 mutagenesis. Plasmid pNK862 is a pBR derivative containing a mini Tn10 transposon with a Tn903 Km^r gene flanked by the two IS ends of Tn10. The flanking transposase works *in cis* and does not transpose together with the resistance to Km (Way *et al.*, 1984). Since the transposase expression depends on the *tac* promoter, no transposition occurs in *E. coli* in the absence of IPTG. We decided to introduce this plasmid directly by electroporation into *Pseudomonas* since the plasmid is not mobilizable (due to deletion of the *bom/**nic* site). Electroporation was done using a Bio-Rad Gene Pulser and Pulse Controller (Smith & Iglewsky, 1989). Briefly, *P. aeruginosa* PAO1 cells were grown in LB medium to an OD₆₀₀ of 0.5, centrifuged, washed three times in 300 mM-sucrose and concentrated 100× in the sucrose solution. A 40 µl vol. of these cells was mixed with 1 µg of the plasmid DNA (pNK862 or pMMB33 as a control) and put on ice. The Gene Pulser was set at 25 µF and 1.6 kV, and the pulse generated was through a high power 400 Ω resistor. Prechilled cuvettes with 0.2 cm electrode gaps were used. Directly after electroporation, 1 ml of LB medium was added and the cells were grown for 2 h at 37 °C before plating on LB plates containing Km.

Screening of mutants. Mutants were picked with sterile toothpicks and inoculated in MKB medium in Microtiter plates. After 2 d growth at the appropriate temperature, the plates were examined under UV for fluorescence as an indication of pyoverdine biosynthesis and excretion. Non-fluorescent mutants (Flu⁻) were grown in CAA medium (3 ml) and CAA medium containing 0.5 mg EDDHA ml⁻¹.

PCR amplification. A 0.25 kb fragment corresponding to the open reading frame of the *P. aeruginosa* lipoprotein (Cornelis *et al.*, 1989), which is specific for fluorescent pseudomonads (unpublished results), was amplified in a New Brunswick PCR apparatus using 30 cycles of amplification. Each cycle comprised 1 min of denaturation at 94 °C, 1 min of annealing at 60 °C and 2 min of elongation at 74 °C. The first primer was a 30-mer extending from the ATG initiation codon and the second was a 21-mer, complementary to the end of the open reading frame, extending from the second stop codon.

Southern blots. A 1.6 kb *Sal*I fragment probe containing part of the Tc^r gene inserted in Tn5 from pSUP10141 was labelled with digoxigenin as described by the manufacturer (Boehringer Mannheim). For the Tn10 probe, a 1.7 kb *Hind*III fragment containing the Tn903 Km^r gene was similarly labelled. DNA from the wild-type and mutants was digested with *Eco*RI or *Bam*HI (for Tn5 and Tn10 probes) or with *Sma*I (Tn5 probe); after electrophoresis the DNA was transferred to a nylon membrane (Hybond N). After UV cross-linking, the membrane was prehybridized and hybridized in 5× SSC, 0.5% blocking reagent from the manufacturer, 0.1% Sarkosyl and 0.02% SDS. Washings were done according to the manufacturer's recommendations. Antibody incubation and signal detection with AMPPD (Boehringer Mannheim) were as described by Kreike *et al.* (1990).

Results

Spontaneous Pvd⁻ mutants

The level of spontaneous mutants was determined for *P. aeruginosa* PAO1 and *P. fluorescens* ATCC 17400 by analysing 1503 and 987 colonies respectively for their fluorescence on agar CAA medium. No spontaneous Pvd⁻ mutant (lack of fluorescence) could be detected, indicating that the level of spontaneous mutants must be lower than 0.1%.

Tn5 mutagenesis

The results of Tn5 mutagenesis with the conjugative suicide vector pSUP10141 are summarized in Table 1. The efficiency of transposition per recipient ranged from 1.1×10^{-6} for *P. aeruginosa* PAO1 to 6×10^{-4} for *P. putida* BTP1. The percentage of transconjugants depended on whether or not the cells were pre-incubated at higher temperatures to lower the host restriction system. For PAO1 no transconjugants were obtained when the cells were not preincubated for 1 h at 42 °C before conjugation. Tn5 mutagenesis yielded no Flu⁻ mutants in the case of *P. putida* BTP1. For *Pseudomonas* sp. BTP20, *P. fluorescens* 47 and *P. fluorescens* ATCC 17400 the percentage of Pvd⁻ mutants (Flu⁻ and EDDHA⁻) was very similar, ranging from 0.4% for strain 47 to 0.54% for ATCC 17400. This percentage was ten times greater in the case of *P. chlororaphis* (4%) and 30 times in the case of *P. aeruginosa* PAO1 (13.1%), indicating preferential insertion of Tn5 into the Pvd genes (Hohnadel *et al.*, 1986). Different types of Pvd⁻ mutants were found: Flu⁻EDDHA⁻; Flu⁺EDDHA⁻; and Flu⁺ only in the presence of EDDHA. These mutants are now being characterized. Only the first type of mutants was found for *Pseudomonas* strain BTP20; these mutants were very sensitive to EDDHA (results not shown).

Although PAO1 mutants were selected on 400 µg Km ml⁻¹, they rapidly lost this high level of resistance upon subculturing to reach the level of the wild-type (100 µg ml⁻¹). This loss of resistance to Km was paralleled by a similar decrease in resistance to Tc (from 100 µg ml⁻¹ to 20 µg ml⁻¹). However, except for some PAO1 mutants, the Pvd⁻ phenotype was very stable. The mutants from other fluorescent pseudomonads were all stable and kept their resistance to both Km and Tc even in the absence of selection.

Tn10 mutagenesis

The Tn10 mini-Kan plasmid was successfully introduced by electroporation in the two fluorescent pseudomonads

Fig. 1. PCR amplification of the *P. aeruginosa* lipoprotein gene from the DNA of PAO1 Tn10 Pvd⁻ mutants: lane 1, PAO1; lanes 2 to 9, Pvd⁻ mutants.

Table 1. Efficiency (per recipient) of Tn5 mutagenesis by conjugational transfer of pSUP10141 into different fluorescent pseudomonads and percentage of Pvd⁻ mutants

Strain	Efficiency	Pvd ⁻ / no. tested	Percentage Pvd ⁻
<i>P. aeruginosa</i> PAO1	1.14×10^{-6}	50/384	13.1
<i>P. chlororaphis</i> BTP9	2.35×10^{-6}	4/96	4.16
<i>P. fluorescens</i> 47	8×10^{-5}	8/1920	0.41
<i>P. fluorescens</i> ATCC 17400	4×10^{-6}	4/764	0.52
<i>P. putida</i> BTP1	6×10^{-4}	0/960	0
<i>Pseudomonas</i> sp. BTP20	4.4×10^{-5}	4/864	0.46

Table 2. Efficiency of electroporation of pMMB33 and of transposition of Tn10 mini-Kan (pNK862) in two *Pseudomonas* strains

Strain	pMMB33 (c.f.u. µg ⁻¹)	Tn10 (c.f.u. µg ⁻¹)
<i>P. aeruginosa</i> PAO1	2×10^7	5×10^3
<i>P. fluorescens</i> 47	1×10^6	2.5×10^3

tested (Table 2). The efficiency of electroporation with the control plasmid pMMB33 was 2×10^6 to 2×10^7 c.f.u. (µg plasmid DNA)⁻¹, in good agreement with results obtained by others (Smith & Iglewsky, 1989). We

Fig. 2. Detection of Tn5 insertions in *EcoRI*-digested DNA from different mutants of *P. fluorescens* ATCC 17440: lane 1, wild-type DNA; lane 2, Pvd⁺ mutant 1G9; lane 3, Pvd⁻ mutant 13D4; lane 4, Pvd⁻ mutant 5H1; lane 5, Pvd⁻ mutant 2G11; lane 6, Pvd⁻ mutant 3G6; lane 7, Pvd⁺ mutant 14H10; lane 8, λ HindIII, digoxigenin labelled.

Fig. 3. Map of the transposon Tn5 in pSUP10141 containing the Tc^r gene from RP4. The map shows the location of *Sma*I (Sm) and *Sal*I (S) sites and the sizes of the corresponding fragments in kb. B, *Bam*HI/*Sau*3A sites. The 1.6 kb *Sal*I fragment was used as a probe in hybridization experiments. The first fragment on the left (dashed line) was not detected by the probe.

expected a relatively high efficiency of transposition since the *tac* promoter is efficiently recognized by the RNA polymerase in *P. aeruginosa* (Bagdasarian *et al.*, 1983). The transposition efficiency was similar in both strains (2.5 to 5×10^3 c.f.u. μg^{-1}). In *P. aeruginosa*, 89% of the mutants were Pvd⁻ (Flu⁻ EDDHA⁻). In strain 47 (a *P. fluorescens* isolate), all the clones that were resistant to Km were Pvd⁻. As for Tn5 mutagenesis, PAO1 mutants were found to lose their resistance to Km upon subculturing but they retained their Pvd⁻ phenotype.

PCR amplification of the *P. aeruginosa* lipoprotein gene

Fig. 1 shows the amplification of the *P. aeruginosa* PAO1 lipoprotein gene in Tn10 Pvd⁻ mutants. In all mutants, a fragment of the lipoprotein gene (*lpp*) of the correct size was amplified except for one Tn10 mutant (lane 8), which was probably a contaminant. Surprisingly, in lane 9, two bands were amplified, one with a molecular mass identical to the control lipoprotein gene and one with a

slightly higher molecular mass. As yet, this observation remains unexplained.

Southern blot for the detection of insertions

Fig. 2 shows the hybridization signals for Tn5 mutants of *P. fluorescens* ATCC 17400 cut with *EcoRI*. In *P. fluorescens* ATCC 17400, double insertions were visible in lanes 4, 5 and 6, corresponding to Pvd⁻ mutants, while single insertions were seen for the non-pyoverdine mutants 1G9 and 14H10 (lanes 2 and 7). For the Pvd⁻ mutant 13D4 (lane 3), a double band could be seen when the membrane was colour-developed in place of the AMPPD detection (results not shown). Single insertions were also detected in Pvd⁻ Tn5 mutants of *P. chlororaphis* and *P. fluorescens* 47 (results not shown). To confirm the double insertions observed in the *P. fluorescens* ATCC 17400 Pvd⁻ mutants, the DNA from these mutants was digested with *Sma*I, which cuts once in the transposon and twice in the inserted RP4 Tc^r gene

Fig. 4. Confirmation of double Tn5 insertions in *Sma*I-digested DNAs from *P. fluorescens* ATCC 17400 Pvd⁻ mutants: lane 1, λ HindIII, digoxigenin-labelled; lanes 2 to 5, Pvd⁻ mutants 3G6, 5H1, 2G11, 13D4; lane 6, wild-type.

(Fig. 3). With the 1.6 kb *Sa*II fragment probe, which includes part of the Tc^r gene (Fig. 3), we detected a double band at 0.6 kb, corresponding to the two *Sma*I fragments in the transposon and two bands, each corresponding to the right-end of the transposon plus adjacent *P. fluorescens* DNA, confirming a double insertion for these four *P. fluorescens* Pvd⁻ mutants (Fig. 4). In Southern blots with PAO1 DNA, we failed to detect any insertion of Tn5 or mini-Kan in the chromosome, confirming the observation that the transposon-borne antibiotic resistance was lost (results not shown).

Discussion

Tn5 is the most frequently used transposon for mutagenesis of various Gram-negative bacteria, including fluorescent pseudomonads. Tn5 is supposed to insert almost randomly in the genome, although there are reports describing 'hot spots' (Berg *et al.*, 1983; Lodge *et al.*, 1991). Upon insertion, the transposon duplicates 9 bp of the target sequence and analysis of these target sequences has revealed that Tn5 prefers sequences containing GC pairs at their extremities (Lodge *et al.*, 1988). Other factors such as supercoiling of the DNA may also contribute to a preferential insertion of Tn5 (Lodge &

Berg, 1990). In order to study the randomness of Tn5 insertions in different fluorescent pseudomonads we looked for Pvd⁻ mutations, since these are easy to screen for (absence of fluorescence on iron-limited media such as succinate, King's B or CAA, or lack of growth in the presence of the strong synthetic iron chelator EDDHA). A minimum of 35 genes in five complementation units have been shown to be necessary for the complementation of pseudobactin mutants in *P. putida* WCS358 (Marugg *et al.*, 1985). Similarly four clusters of 12 genes were found to be a minimum for the complementation of all pseudobactin mutants of the plant-growth-promoting *Pseudomonas* strain B10 (Moores *et al.*, 1984). If one considers an average of 3 kb per gene, then a minimum of 105 to 144 kb would be necessary to contain all the information needed for the biosynthesis of pyoverdine/pseudobactin. If the size of the *P. aeruginosa* chromosome (5850 kb) (Holloway *et al.*, 1990; Rmling *et al.*, 1989) is taken as representative of the chromosome size of other fluorescent pseudomonads, then random insertions of Tn5 would yield about 1.8 to 2.5% Pvd⁻ mutants. By UV or chemical mutagenesis of *P. aeruginosa* PAO1, Hohnadel *et al.* (1986) found about 0.2% Pvd⁻ mutants, but their selection was based only on the absence of fluorescence. This low percentage of Pvd⁻ mutants after UV mutagenesis is an indication that the level of spontaneous mutants must be very low, precluding any possibility that our PAO1 Pvd⁻ mutants could be spontaneous. We found that some mutants were Flu⁺ EDDHA⁻; we consider that these could be deficient in the synthesis of the peptide arm of the siderophore or in the uptake of ferripyoverdine (unpublished results). The percentages of Pvd⁻ mutants for *P. fluorescens* ATCC 17400, *P. fluorescens* 47 and *Pseudomonas* sp. BTP20 were in good agreement with this prediction, and could be interpreted as the result of a random insertion of Tn5 in the chromosome. Single Tn5 insertions were observed in all fluorescent pseudomonads examined except in *P. fluorescens* ATCC 17400 Pvd⁻ mutants, where double insertions were detected in all mutants tested. Non-pyoverdine mutants, however, contained only single insertions. To our knowledge, such a phenomenon has never been described before. No Pvd⁻ mutants could be detected after mutagenesis of *P. putida* BTP1, although a total of 1865 mutants were tested and replicated on MKB and MKB EDDHA medium, indicating a lower frequency of insertion of Tn5 in this region. Slightly higher than expected values were obtained for *P. chlororaphis* BTP9 (4% Pvd⁻ mutants or about twice the expected value) and especially for *P. aeruginosa* PAO1 (13% or about five times the expected value and twenty times the value observed for other strains). These results suggest that, in *P. aeruginosa*, Tn5 inserts preferentially into one region where clusters of

pyoverdine biosynthesis and/or regulation genes are present. Pvd mutations could be mapped in two regions of the chromosome of *P. aeruginosa* PAO1: one locus is in the 35' region between *argC* and *strA*, and the other one is in the 65' region between *catA* and *mtu-9002* (Hohnadel *et al.*, 1986). In the new recalibrated map of the PAO1 chromosome, these two loci would be at 25' and 45–50' respectively (O'Hoy & Krishnapillai, 1987). Krishnapillai *et al.* (1981) described the preferential insertions of transposon Tn1 into the same 60–65' region (old map) in the PAO1 chromosome. Tn5 was found to be stable in the fluorescent pseudomonads examined except in PAO1, where it was rapidly lost. Excision of Tn5 has already been described in *E. coli* (Egner & Berg, 1981). Two types of excisions can be distinguished: precise excision where the target 9 bp DNA repetition is also excised, and imprecise excision, where the target repetition remains, together with some transposon DNA (Lundblad *et al.*, 1984). In the first case the mutation reverts to the wild-type, in the second, it does not. The frequency of the two events in *E. coli* was found to be 10^{-6} for precise excision and 10^{-5} for imprecise excision of Tn5 from the *lac* gene in a F' *lac* strain (Egner & Berg, 1981). In some *P. aeruginosa* strains, including PAO1, Tn5 was found to be unstable (Stapleton *et al.*, 1984; Goldberg *et al.*, 1990), while in others the transposon was stably maintained (Höfte *et al.*, 1991). Goldberg *et al.* (1990) described the high frequency (10^{-3}) precise excision of Tn5 from plasmid DNA when this plasmid was transferred to PAO1. The mechanism of excision is poorly understood but is independent of the transposase and of the host RecA protein (Egner & Berg, 1981; Goldberg *et al.*, 1990). However, the terminal inverted repeats of the transposon seem to play an important role in this process (Lundblad *et al.*, 1984; Goldberg *et al.*, 1990). Mutations in alleles of RecBC were also found to increase the rate of excision in *E. coli* (Lundblad *et al.*, 1984). In our case, the transposon was found to excise almost completely since resistances to both Km and Tc were lost and no signal was found in hybridization experiments, even when the whole transposon was used as a probe (results not shown). However, with the exception of a few mutants, the rate of reversion was low indicating that imprecise excision occurred or that the transposon caused deletions in the chromosome.

We successfully introduced the Tn10 *ptac* mini-Kan into PAO1 and *P. fluorescens* 47. To our great surprise, 89% of the Km^r clones were Pvd⁻ in the case of *P. aeruginosa* and 100% in the case of *P. fluorescens* 47. PCR amplification of the *P. aeruginosa* lipoprotein gene for the *P. aeruginosa* mutants confirmed, however, that all but one were *P. aeruginosa*.

We conclude that pyoverdine gene cluster(s) can be hot spots for transposon insertion in some fluorescent

pseudomonads and that care should be taken to control the stability of a given transposon in fluorescent pseudomonads.

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