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F'-Plasmid Transfer from *Escherichia coli* to *Pseudomonas fluorescens*

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Various F' plasmids of *Escherichia coli* K-12 could be transferred into mutants of the soil strain 6.2, classified herein as a *Pseudomonas fluorescens* biotype IV. This strain was previously found to receive F*lac* plasmid (N. Datta and R. W. Hedges, J. Gen. Microbiol. **70**:453-460, 1972). *ilv, leu, met, arg,* and *his* auxotrophs were complemented by plasmids carrying isofunctional genes; *trp* mutants were not complemented or were very poorly complemented. The frequency of transfer was 10^{-5}. Subsequent transfer into other *P. fluorescens* recipients was of the same order of magnitude. Some transconjugants were unable to act as donors, and these did not lose the received information if subcultured on nonselective media. Use of F' plasmids helped to discriminate metabolic blocks in *P. fluorescens*. In particular, *metA, metB,* and *argH* mutants were so distinguished. In addition, F131 plasmid carrying the *his* operon and a *supD* mutation could partially relieve the auxotrophy of *thr, ilv,* and *metA13* mutants, suggesting functional expression of *E. coli* tRNA in *P. fluorescens*. In *P. fluorescens metA* Rif^r mutants carrying the F110 plasmid, which carried the *E. coli metA* gene and the *E. coli rif*^{s} allele, sensitivity to rifampin was found to be dominant at least temporarily over resistance. This suggests interaction of *E. coli* and *P. fluorescens* subunits of RNA polymerase. *his* mutations were also complemented by composite P plasmids containing the *his-nif* region of *Klebsiella pneumoniae* (plasmids FN68 and RP41). *nif* expression could be detected by acetylene reduction in some *his*$^+$ transconjugants. The frequency of transfer of these P plasmids was 5×10^{-4}.

Many plasmids have been found to cross taxonomic boundaries. The first observations in this sense were made in *Pseudomonas* and enteric bacteria. A well-known case is the P class of plasmids (RP1, RP4, R1822, R6845) found to be transferable from *Pseudomonas aeruginosa, P. putida,* or *Escherichia coli* to an impressive variety of gram-negative strains, including species of the genera *Acinetobacter, Agrobacterium, Alcaligenes, Azotobacter, Neisseria, Rhizobium, Rhodopseudomonas, Rhodospirillum,* and *Vibrio* (5, 11, 12, 28). These P plasmids may also mobilize the chromosome of their host, as did RP4 in *Acinetobacter calcoaceticus*, R6845 in *Rhizobium leguminosarum* (5) and *Rhizobium meliloti* (23), and RP1 in *Pseudomonas glycinea* (20).

Versatile carriers may be used to study heterospecific transfer and expression of chromosomal genes such as those controlling the biosynthesis of amino acids, proteins, RNA, and DNA. Obviously, this operation requires a genetic tool which combines plasmid-coded genes for transfer and replication with the chromosomal genes, for which expression in a foreign cytoplasm is examined. Examples of such plasmids are (i) F' episomes, (ii) some "in vitro" recombined plasmid molecules, and (iii) transpositions of chromosomal genes onto such plasmids as RP1 (15, 18). The latter category of plasmids appears particularly promising in contrast to F' plasmids, whose host range until recently seemed to be restricted to *Enterobacteriaceae*.

Failure of F' transfer was observed by Datta and Hedges (11) in every *Pseudomonas, Rhizobium,* or *Agrobacterium* strain tested. Lack of transfer was also observed in *R. leguminosarum* (J. Beringer, personal communication), *Agrobacterium tumefaciens* strains A6, B6, and 5-GLY, *P. aeruginosa,* and *R. meliloti* (unpublished data).

However, some rare positive cases of F'-plasmid transfer into bacteria other than *Enterobacteriaceae* have been reported. (i) F *lac* has been transferred to a strain of *P. putida* (J. M. Amelink, Ph.D. thesis, University of Leiden, Leiden, The Netherlands; P. Van de Putte, personal communication). (ii) Datta and Hedges (11) reported another case of F *lac* transfer in *Rhizobium lupini* strain 6.2. Other F' plasmids have been transferred to the same strain (24).

As a strain unrelated to *E. coli*, the case of strain 6.2 seemed to be unique and therefore appeared to deserve further investigation. In doing so, we reconsidered the taxonomic status of strain 6.2 and have reclassified it among the fluorescent pseudomonads. This strain appears suitable for observations concerning regulatory interactions between *E. coli* and *Pseudomonas* genes.

MATERIALS AND METHODS

Strain. Strain 6.2 is included in the IBP catalog of *Rhizobium* strains (2) and was used by De Ley and Rassel (14). Lyophilized stocks of strain 6.2 were stored in the Department of Microbiology, State Faculty of Agronomical Sciences, Gembloux, Belgium. We used four lyophilized samples of strain 6.2 prepared in 1962 in Gembloux. All of them gave clones indistinguishable in their taxonomic and genetic characteristics. Strains of *P. fluorescens* are listed in Table 1.

Mutagenesis. Mutants were obtained by the action of *N*-methyl-N'-nitro-*N*-nitrosoguanidine according to Adelberg et al. (1) with tris(hydroxymethyl)-aminomethane maleic buffer (pH 6), a mutagen concentration of 300 μg/ml, and an incubation time of 30 min at room temperature without shaking. Colonies were directly replicated on minimal medium, and auxotrophs were scored on plates supplemented with mixtures of metabolites following the grid of Holliday (9, 19).

When possible, auxotrophs were subclassified by responses to intermediary metabolites. In the case where there responses led to clear-cut analogies with *E. coli* mutants, the mutations were named according to *E. coli* nomenclature (4). Strain PMG13 was used for mutagenesis to produce double auxotrophic mutants. Strains PMG13, PMG30, and PMG54 were scored for additional auxotrophies found as comutations in Rif^r clones induced by *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine (3). A majority of His$^-$ and Ilv$^-$ clones were obtained as comutations of Rif^r clones. Auxotrophic mutations found in the different mutagenesis experiments include: (i) 16 *met* mutations, all of them able to grow with homocysteine or cystathionine and not responsive to homoserine; (ii) 10 *his* mutations, all but one responding to L-histidinol (the latter one was named *hisD28*); (iii) 12 *ilv* mutations, all requiring both isoleucine and valine; (iv) 3 *leu* mutations; (v) 1 *arg* mutation, responding only to arginine and not to either ornithine or citrulline; (vi) 9 *trp* mutations, four mutants responding to anthranilate and subsequently called *trpE* (the growth of the 5 other mutants is stimulated but at distinctly different degrees by indole [see Results]); (vii) 4 *thr* mutations; (viii) 1 *cys* mutation responding equally well to methionine; and (ix) 2 *aro* mutations.

F' plasmids. F' (21) and other plasmids are listed in Table 2 and were obtained through the courtesy of B. Bachmann, J. Beringer, F. Cannon, and N. Glansdorff. Map positions are given in Fig. 1. F' plasmids were stored on minimal medium either in stabs or in aliquots supplemented with 10% glycerol and frozen at −90°C. Before use, each *E. coli* merodiploid was checked for every marker for sensitivity to phage MS2. The RecA phenotype was tested by UV sensitivity.

TABLE 1. *Strains of P. fluorescens*

Strain	Marker(s)
PMG4	*his-4*
PMG4	*his-5*
PMG27	*his-27*
PMG28	*hisD28*
PMG29	*his-29*
PMG36	*his-36*
PMG11	*metA11*
PMG13	*metA13*
PMG14	*metA14*
PMG18	*metA18*
PMG19	*metA19*
PMG20	*metA20*
PMG22	*metA22*
PMG23	*metA23*
PMG45	*metB45*
PMG46	*metB46*
PMG6	*ilv-6*
PMG12	*ilv-12*
PMG30	*ilv-30*
PMG32	*ilv-32*
PMG9	*leu-9*
PMG24	*leu-24*
PMG25	*leu-25*
PMG51	*metA13 trp-51*
PMG52	*metA13 trpE52*
PMG53	*metA13 trpE53*
PMG54	*metA13 trpE54*
PMG55	*metA13 trp-55*
PMG64	*trp-64*
PMG65	*trp-65*
PMG73	*thr-73*
PMG74	*thr-74*
PMG113	*metA13 trp-113*
PMG114	*ilv-30 his-114*
PMG122	*metA13 trpE54 argH122*
PMG124	*metA13 trpE54 his-124*
PMG270	*his-27 str-270*
PMG280	*hisD28 str-280*
PMG360	*his-36 str-360*
PMG130	*metA13 str-130*
PMG220	*metA22 str-220*
PMG230	*metA23 str-230*
PMG131	*metA13 rpo-131*
PMG1221	*metA13 trpE54 argH122 rpo-1221*

Media. *E. coli* strains were grown in Davis and Mingioli medium (13). Strain 6.2 grew satisfactorily in this medium, but a better balanced growth occurred in a medium designed by Rigaud (33) and called medium 36. The composition is as follows (per liter): sodium glutamate, 1 g; NaCl, 0.2 g; K_2HPO_4, 0.5 g; $MgSO_4 \cdot 7H_2O$, 0.2 g; mannitol, 10 g; $CaSO_4 \cdot 2H_2O$, 0.1 g; and $CaCO_3$, 0.1 g. As carbon source, mannitol may be replaced by lactate. Glutamate must be replaced by NH_4Cl in taxonomic experiments using different carbon sources. For maintenance in slants and stabs, medium 36 supplemented with yeast extract was found to be the most convenient. *E. coli* broth medium 869 (10 g of tryptone, 5 g of yeast extract, 5 g of NaCl, and

TABLE 2. *E. coli strains carrying plasmids*[a]

Strain	Plasmid	Chromosomal markers	Plasmid markers
KLF1/AB2463	F101	*thr-1 leu-6 thi-1 argE3 his-4 proA2 recA13 mtl-1*	*thi*$^+$ *leu*$^+$
KLF4/AB2463	F104	*thi-1 thr-1 leu-6 argE3 his-4 proA2 recA13 mtl-1*	*thr*$^+$ *leu*$^+$ *pro*$^+$
KLF5/AB2463	F105	*thr-1 leu-6 thi-1 argE3 his-4 proA2 recA13 mtl-1*	*metB*$^+$ *argECBH*$^+$
KLF10/JC1553	F110	*argG6 metB1 his-1 leu-6 recA1 mtl-2*	*argH*$^+$ *metA*$^+$ *metB*$^+$
KLF33/JC1553	F133	*argG6 metB1 his-1 leu-6 recA1 mtl-2*	*argH*$^+$ *metB*$^+$ *ilv*$^+$
AB3519	F25	*thi-1 ilvD188 his-4 trp-3 proA2 mtl-1*	*ilvD*$^+$ *ilvE*$^+$
AB1528	F16	*thi-1 ilvC7 argE3 his-4 proA2*	(*ilvEDAC*)$^+$ *ilvB*?
AB1526	F216	*thi-1 ilvD16 metB1 argH1 his-1 mtl-2*	(*ilvEDA*)$^+$
KLF2/JC1553	F102	*argG6 metB1 his-1 leu-6 recA1 mtl-2*	*argG*$^+$
KLF2/KL110	F122	*argG6 metB1 his-1 leu-6 thyA23 recA1 mtl-2*	*argG*$^+$ *metC*$^+$
KLF31/JC1553	F131	*argG6 metB1 his-1 leu-6 recA1 mtl-1*	*his*$^+$ *supD43*
Mx383	F196	*trp-49 his-90 arg-47 recA1*	*his*$^+$ *supD32*
DFF1/JC1556	F150	*argG6 metB1 his-1 leu-6 recA mtl-2*	*his*$^+$
KLF23/KL181	F123	*thi-1 pyrD34 his-68 trp-45 recA1 mtl-2*	*trp*$^+$
KLF25/KL181	F125	*thi-1 pyrD34 his-68 trp-45 recA1 mtl-2*	*trp*$^+$ *pyrD*$^+$
SB18d	FN68	*Δhis*	*bla*$^+$ *his*$^+$ *nif*$^+$
JC1553	RP41	*pro met*	*tet*$^+$ *kan*$^+$ *amp*$^+$ *bla*$^+$

[a] All *E. coli* strains were obtained from B. Bachmann except the last two listed, which were obtained from F. Cannon.

1 g of glucose per liter) is convenient for culture only if supplemented with 2 mM Ca^{2+}. Slants stored at 4°C are very stable.

Crosses between strain 6.2 mutants and *E. coli* F′ plasmids. Different procedures were utilized and gave similar results. (i) *E. coli* merodiploids were grown overnight in minimal medium, diluted in the same medium for 2 h, and mixed with an equal volume of recipient cells at an equal turbidity. The mixture was shaken for 2 to 8 h, centrifuged, washed, and plated on selective media. Viable counts were performed on minimal plates to counterselect the other partner. (ii) Crosses were also carried out by replicating perpendicular streaks of *E. coli* merodiploids and *P. fluorescens* 6.2 mutants. Crossed streaks can be either immediately replicated on selective plates or replicated on rich medium before being replaced again on selective plates. (iii) Another successful method is to streak both donor and recipient together on rich plates. In the case of plate crossing, loops of fully grown, crossed streaks were resuspended in 0.5 ml of a dilution fluid plated after appropriate dilution and incubated at 29°C. In general, counterselection of the donor strains was through multiple auxotrophies (two or four) and on the Mtl$^-$ character. Donor FN68 was counterselected with streptomycin or rifampin, using appropriate resistant derivates of strain 6.2 recipients. Purified prototrophic transconjugants were tested for the presence of nonselected markers of strain 6.2, inability to grow at 37°C, and production of fluorescent pigment.

Acetylene reduction for Nif detection. His$^+$ exconjugants were grown in tubes containing 5 ml of medium 36 without nitrogen source. Either ordinary tubes or Pankhurst tubes with pyrogallol were used (29). However, growth under anerobic conditions was extremely poor. Since shaken cultures did not allow any nitrogenase expression, cells were grown on minimal selective plates (plus glutamate but lacking histidine), bacteria were scraped and inoculated in ordinary tubes provided with cotton plugs, and growth was allowed for one night; tubes were then capped with Suba seals, and 0.2 ml of acetylene was injected. Optical density was followed in a Beckman C spectrophotometer. No shaking was allowed during the whole experiment. Gas samplings were taken first after 8 h and then every 24 h for 6 days. Ethylene production was measured by injecting 0.2-ml gas samples into a 5700 A Hewlett-Packard gas chromatograph with a 1.50-m Porapak N column (5-mm ID) at 37°C, using N_2 as carrier gas at a flow rate of 50 ml/min (29).

RESULTS

Taxonomic status of strain 6.2. Strain 6.2 produces a light-green diffusible pigment. This

pigment is strongly fluorescent and is by no means common among rhizobia. Besides, since the different isolates repeatedly failed to induce nodules in lupine roots (unpublished data) and grew much faster than *R. lupini* strains are expected to do, we started reassessing the classification of this strain in the direction of fluorescent pseudomonads.

Taxonomic tests were performed according to Stanier et al. (34) and Doudoroff and Palleroni (16). Sixteen discriminating tests were proposed to characterize a fluorescent pseudomonad (34). Thirteen were available to us.

Strain 6.2 produced a fluorescent pigment and was able to use D-glucose, L-arginine, spermine, sarcosine, β-alanine, 2-ketogluconate, pelargonate, and *p*-hydroxybenzoate as carbon sources. It was unable to use D-fucose, starch, cellobiose, and *m*-hydroxybenzoate. This is in complete conformity with the properties of fluorescent pseudomonads as described by Stanier et al. (34). No genus shares these 13 properties with fluorescent pseudomonads. In addition, the strain can use leucine, isoleucine, aspartate, glutamate, ornithine, histidine, and proline as carbon sources and cannot use threonine and rhamnose. These nine characters are shared by the majority of fluorescent pseudomonads.

Ten characters allow differentiation between *P. aeruginosa* and *P. fluorescens*. Seven tests were available to us (Table 3). These seven observations were in complete conformity with the pattern exhibited by *P. fluorescens* and definitely excluded *P. aeruginosa* (34). Five characters distinguished *P. fluorescens* from *P. putida*; four of them were applied (Table 3). Strain 6.2 liquefied gelatin slowly, the reaction becoming evident after 2 weeks of incubation. These results allocate strain 6.2 to the *P. fluorescens* group, which includes biotypes I to IV of *P. fluorescens, P. aureofaciens,* and *P. chlororaphis*. The tests shown in Table 4 establish that strain 6.2 belongs to *P. fluorescens* biotype IV. Strain 6.2 responded positively to every one of 55 carbohydrates, fatty acids, dicarboxylic acids, and amino acids reported to support the growth of biotype IV (34). Minor discrepancies were slow growth with valerate and inability to use 2,3-butylene glycol, citraconate, or ethanolamine.

The buoyant density of the DNA of strain 6.2 was 1.719 g/cm^3 (14; P. Charles, personal communication), which corresponds to 62% guanine plus cytosine in total conformity with biotype IV (which contains the fluorescent pseudomonads with the lowest guanine-plus-cytosine content) and again allowed exclusion of *P. aeruginosa, P. chlororaphis*, and *P. aureofaciens* (22).

Transfer of *E. coli* F' plasmids to *P. fluorescens* 6.2. About 15 F' factors representing

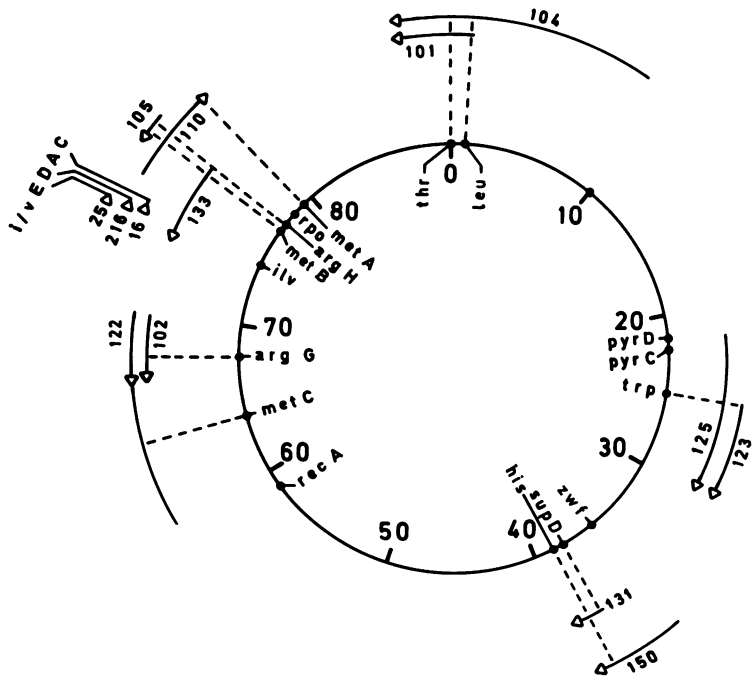

FIG. 1. *Genetic map of E. coli K-12 genome covered by F' plasmids used in this study (21).*

portions equivalent to 30% of the *E. coli* chromosome were used in this work (Table 2). In addition, two plasmids containing the *his* and *nif* (nitrogen fixation) genes from *K. aerogenes* were also used in parallel with F′ *his* plasmids. These two plasmids, FN68 and RP41 (7, 15), are transpositions on P plasmids coding for antibiotic resistance. Table 5 reports results of transfer attempts for a variety of mutants. Prototrophs after crosses with F′ plasmids were easily recovered at frequencies ranging from 10^{-6} to 10^{-4} per donor or recipient cell (donor and recipient being equally mixed).

TABLE 3. *Diagnostic tests for taxonomic characterization of strain 6.2:[a] strain 6.2 belongs to the P. fluorescens group*

Character	Strain 6.2	*P. aeruginosa*	*P. fluorescens*	*P. putida*
Pyocyanine production	−	+	−	
Growth				
At 4°C	+	−	+	
At 41°C	−	+	−	
Utilization of:				
Geraniol	−	+	−	
Acetamide	−	+	−	
Trehalose	+	−	+	−
Inositol	+	−	+	−
Gelatin liquefaction	+		+	−
Utilization of two or more of the following nitrogenous compounds:				
Benzylamine, creatine, glycine, and hippurate	−[b]		−	+

[a] Characters of strain 6.2 are compared with those of *Pseudomonas* type strains as reported by Stanier et al. (34).
[b] Negative for each of the four products tested.

Prototrophs were easily obtained with the following mutants: 8 histidine, 12 methionine, 3 leucine, 1 arginine, and 5 isoleucine-valine requirers. No prototrophs were obtained with one *ilv* mutant and two *thr* mutants. Complementation attempts for Trp^- mutants were negative for five mutants and repeatedly gave tiny colonies for two others. Thus, histidine auxotrophy of mutants *his-36, -04, -05, -27, -28, -29,* and *-113* was suppressed by plasmids F131, F196, F150, FN68, and RP41, all containing the histidine operon, and not by F110, F104, F133, and F25, which do not contain any gene coding for the biosynthesis of histidine. Similarly, mutants *met-11, -13, -14, -22, -45,* and *-46* responded to F110 or F105 but not to F25, F102, F150, and F196. One interesting exception is the *leu-25* mutation, which was suppressed by the F131 plasmid (but with resulting low growth). This plasmid contains the histidine operon and the *supD* allele, and no structural genes for leucine biosynthesis are known on this portion of the *E. coli* chromosome.

Another characteristic of the crosses is that large F′ plasmids such as F101, F104, F150, and F133 give tiny transconjugant colonies often difficult to transplant or subclone. With clones complemented by shorter *E. coli* plasmids such as F25 and F131, there was no difficulty in

TABLE 4. *Diagnostic tests for taxonomic characterization of strain 6.2:[a] strain 6.2 belongs to P. fluorescens biotype IV*

Characteristic	Strain 6.2	*P. fluorescens* biotype:				*P. aureofaciens*	*P. chlororaphis*
		I	II	III	IV		
Utilization of:							
Ethanol	−	−	+	+	−	−	+
Butanol	−	−	+	+	−	−	+
Isobutanol	−	−	+	+	−	−	+
Yellow pigment	−				−	+	
Denitrification	+	−			+	−	
Utilization of:							
Benzylformate	−				−	+	
Butylamine	−				−	+	
Arabinose	+			−	+	−	−
Sorbitol	+		+		+	−	−
Hydroxymethylglutarate	−	+			−		
Adonitol	−	+			−		
Xylose	−	+			−		
Propyleneglycol	−		+		−		
Trigonelline	−		+		−		
Propionate	−		+		−		
Isovalerate	−		+		−		

[a] Characters of strain 6.2 are compared with those of *Pseudomonas* type strains as reported by Stanier et al. (34).

TABLE 5. *Transfer of F' plasmid from E. coli K-12 to P. fluorescens*[a]

Recipient	Markers	Donor plasmid	Selection	Transconjugant frequency	No. of expt
PMG122	*trpE53 metA13 argH122*	F105	Arg^+	1.8×10^{-5}	2
			Met^+	5×10^{-8}	2
		F110	Arg^+	1.2×10^{-5}	2
			Met^+	1.6×10^{-5}	2
		F102	Arg^+	1.7×10^{-7}	2
			Met^+	6×10^{-8}	2
		F122	Arg^+	7×10^{-7}	2
			Met^+	4×10^{-7}	2
PMG13	*metA13*	F110	Met^+	5×10^{-5}	6
		F105	Met^+	4×10^{-7}	6
		F133	Met^+	3×10^{-7}	2
PMG14	*metA14*	F110	Met^+	6×10^{-6}	1
PMG22	*metA22*	F110	Met^+	5×10^{-5}	2
		F105	Met^+	6×10^{-8}	2
PMG45	*metB45*	F110	Met^+	10^{-5}	4
		F105	Met^+	6×10^{-5}	4
PMG46	*metB46*	F110	Met^+	2×10^{-5}	1
		F105	Met^+	8×10^{-5}	1
		F133	Leu^+	10^{-5}	1
PMG24	*leu-24*	F101	Leu^+	2×10^{-5}*	1
PMG25	*leu-25*	F101	Leu^+	10^{-5}*	3
		F104	Leu^+	6×10^{-6}*	1
PMG30	*ilv-30*	F25	Ilv^+	3.5×10^{-6}	4
		F16	Ilv^+	2.5×10^{-5}	4
		F216	Ilv^+	3.7×10^{-5}	4
		F133	Ilv^+	1.3×10^{-5}	4
		F196	Ilv^+	3×10^{-9}	1
PMG28	*hisD28*	F131	His^+	3.5×10^{-5}	3
PMG36	*his-36*	F131	His^+	4×10^{-5}	5
		F196	His^+	8×10^{-6}	1
		F150	His^+	3×10^{-5}*	2
		F110	His^+	10^{-7}	2
PMG27	*his-27*	F131	His^+	2×10^{-5}	2
		F150	His^+	3×10^{-5}*	1
PMG270	*his-27 str-270*	F131	His^+	5×10^{-5}	3
		RP41	His^+	5×10^{-4}	3
			His^+ Kan^r	5×10^{-4}	3
			Kan^r	5×10^{-4}	3
PMG270	*his-27 str-270*	FN68	His^+	5×10^{-4}	3
PMG114	*his-114 ilv-30*	F131	His^+	5×10^{-5}	1
PMG124	*his-24 metA13*	F131	His^+	4×10^{-5}	1
PMG51	*trp-51 metA13*	F123	Trp^+	3×10^{-5}*	6
PMG55	*trp-55 metA13*	F123	Trp^+	10^{-7}	4
PMG53	*trpE53 metA13*	F123	Trp^+	2×10^{-7}	2
PMG64	*trp-64 metA13*	F123	Trp^+	10^{-8}	2

[a] Transconjugant frequency is expressed per recipient cell. Values differing significantly from the spontaneous reversion frequencies are underlined. Experiments in which transconjugants were found as tiny colonies are indicated by an asterisk.

maintenance or storage on minimal selective medium. One can assume that *Pseudomonas* clones carrying F' plasmids that are too large receive for the donor genetic information leading to an impairment of growth or that maintenance of large plasmids is more difficult.

Stability and conservation of the transconjugants. Transconjugants to be further studied were purified several times on minimal selective media. If grown on broth agar, the transconjugants quickly gave rise to the original recipient characters by plasmid loss. PMG(F25), PMG36(F131), and PMG(F110) transconjugants were remarkably stable for 3 years on minimal slants stored at 4°C or stabs stored at room temperature. Recovery from slants, in any case, is better than for *E. coli*. However, F105 was rather poorly maintained in *E. coli*, was unstable in strain 6.2, and was difficult to maintain if not purified weekly.

Identification of the metabolic blocks of methionine auxotrophs of *P. fluorescens* by

using F′ plasmids. Every tested methionine auxotroph of our collection grew in the presence of methionine, homocysteine, or cystathionine. Two metabolic steps in *E. coli* are known to correspond to these growth responses: homoserine-*O*-transsuccinylase and cystathionine synthetase, respectively, controlled by the *metA* and *metB* genes. The F110 plasmid carries these linked but not close genes. *metB* is also found on F105 and F133 (Fig. 1).

Ten methionine auxotrophs of *P. fluorescens* 6.2 responded to plasmid F110, but only two of them were also suppressed by plasmids F105 and F133 (Table 4). No mutant responded to F102 containing *metC* (responsible for cystathioninase). These observations allow discrimination and classification of Met$^-$ mutants, assuming a correspondence with *E. coli* loci: *metB46, metB46, metA11, metA13, metA14,* and *metA22.*

Identification of the metabolic block of the Arg$^-$ *P. fluorescens* mutant by using F′ episomes. Mutation *arg-122* was obtained in the strain containing the mutations *metA13* and *trpE52. arg-122* responded to arginine but not to ornithine or citrulline, which suggests a block in *argG* or *argH* if we assume a correspondence with *E. coli* enzymes and metabolic blocks. *arg-122* was suppressed by the episomes F105 and F110, both containing the gene cluster *arg-ECBH* of *E. coli*, and not by F102 and F122 (containing *argG*) (Table 5), thus implying that the denomination *argH122* is correct.

Such a mutant should be defective in argininosuccinate lyase. This was confirmed by enzymatic assays in crude extracts of this mutant (M. Mergeay, A. Boyen, C. Legrain, and N. Glansdorff, manuscript in preparation). All *arg*$^+$ clones were also *met*$^+$ if suppressed by F110, but they were *met* if suppressed by F105. This confirmed the discrimination of the *met* mutants in *metB* and *metA* mutants and strongly reinforces the suggestion of isofunctional blocks of DNA in *E. coli* and *P. fluorescens* 6.2.

Expression of the *E. coli rpo*$^+$ allele in 6.2 mutants resistant to rifampin. The *rpoA* and *rpoB* genes are carried by plasmid F110 (4, 21). Mutations affecting the locus coding for the β subunit of RNA polymerase are generally selected by screening rifampin-resistant clones. Spontaneous mutants of strains PMG13 and PMG122 resistant to rifampin were used in crosses with the F110 plasmid. These strains carried, respectively, the following markers: *metA13, rpo-131* (strain PMG131), and *metA13, argH122, trpE54,* and *rpo-1221* (strain PMG1221). Mutation *rpo-1221* allowed a rather poor growth on minimal medium supplemented with requirements and 50 μg of rifampin per ml, whereas mutation *rpo-131* allowed normal growth in the presence of 100 μg of rifampin per ml.

Scoring for rifampin resistance or sensitivity in transconjugants must be performed on minimal selective plates. On broth plates, strong segregation back to the original auxotrophic recipients occurred. Crosses between F110 and 6.2 strains carrying *rpo* mutations were made by selecting *met*$^+$ or *arg*$^+$ and looking for the Rif phenotype among *met*$^+$ and *arg*$^+$ clones. In strain PMG1221 every *met*$^+$ or *arg*$^+$ is sensitive to rifampin (50 μg/ml) after 4 days of incubation following replica plating and thus exhibited dominance of the *E. coli rpo*$^+$ allele on the *Pseudomonas rpo* allele. Longer incubation periods allow the appearance of resistant clones by spotting out. In strain 131 (resistant to higher concentrations of rifampin), among 200 replicated *met*$^+$ clones, 70 clearly exhibited rifampin sensitivity and again dominance of the *E. coli* wild-type allele. However, clones resistant to rifampin emerged in the Rif^s patches at frequencies up to 1%.

From these observations, it appears that the *E. coli rpo*$^+$ allele of RNA polymerase is expressed in *P. fluorescens* 6.2. The *rpo*$^+$ allele is normally dominant in *E. coli.* Dominance suggests a temporary association of RNA polymerase subunits from both donor and recipient and therefore a rather conservative evolution of both *P. fluorescens* and *E. coli* RNA polymerases.

The appearance of Rif^r clones among recipient clones and subsequent segregation of Rif^r clones among Rif^s recipient clones may be due to one of the following: (i) spontaneous deletions in the F′ plasmid (spontaneous deletions and loss of rifampin sensitivity of F110 were indeed obtained [25]); (ii) integration of only the *E. coli met*$^+$ region into the chromosome (some *metB*$^+$ Rif^r occurring spontaneously at frequencies of 1% from Rif^s-complemented clones became extremely stable and did not revert to the original *met*$^-$ if subcultured in rich medium, a normally efficient process of curing with most of the transconjugants receiving the F110 plasmid; a genetic transfer mechanism specific to *P. fluorescens* 6.2 is needed to check this hypothesis of integration); (iii) reassociation of *Pseudomonas* subunits conferring resistance to rifampin; (iv) modifications in RNA polymerase regulation.

Expression of *his* genes from *E. coli* and *K. aerogenes* in *P. fluorescens*. Every tested *his* mutant (*his-4, -5, -27, -29, -36, -114, -124* and *hisD28*, the only one not to respond to histidinol) was complemented by F′ plasmid F131, F196, or F150 (Table 2).

His$^+$ transconjugants obtained from F150, a large episome, were tiny and difficult to sub-

clone. His^+ obtained from F131, F196, RP41, and FN68 grew faster and were stable and easy to store on slants, stabs, or frozen cultures supplemented with 10% glycerol. They were formed at frequencies up to 5×10^{-5} with F' plasmids and 10^{-3} with RP41. These results also show that both *E. coli* and *K. aerogenes* regions coding for histidine biosynthesis can be expressed in *P. fluorescens* 6.2.

Expression of an *E. coli sup* gene carried by the F131 episome. Most of the auxotrophic mutations tested in this work responded to episomes carrying isofunctional genes. Some mutations, however, also responded to episomes not carrying isofunctional genes. Plasmid F131 seemed to be rather effective in this sense: mutation *leu-25* is suppressible by F131. Mutations *metA13* and *ilv-30* became bradytrophic when F131 was introduced into strains PMG114 and PMG124 to suppress the *his-114* and *his-124* mutations, respectively. This partial suppression was not observed using RP41 for *his* complementation. Mutation *thr-74* did not respond to F104 or F101 episomes (large episomes carrying the *thr-leu* region of *E. coli*) but was suppressible by F131 also. F131 carries *supD*, a suppressor of amber mutations (4). The observed effect of *supD* is compatible with the mutagenic origin of most of our mutations (*N*-methyl-*N'*-nitro-*N*-nitrosoguanidine). The credibility of the expression of a mutated tRNA is supported by the variety of responding mutations. The present experiments therefore strongly suggest that the mutated tRNA gene of *E. coli supD* is expressed in *P. fluorescens*.

***nif* expression in *his*$^+$ transconjugants of *P. fluorescens* obtained from plasmids carrying *Klebsiella* genes.** FN68 (8) and RP41 (15) are plasmids containing the *his-nif* region of *K. pneumoniae*. This enteric bacterium is able to fix free nitrogen. FN68 is a complex plasmid made by the transposition of the *bla* determinant (coding for resistance to carbenicillin) of a P plasmid on an F' *his-nif* plasmid of a *Klebsiella-E. coli* hybrid (10). RP41 was formed by transposing the *bla-his-nif* region of FN68 into RP4 DNA (15). Transfer of RP41 to *his* mutants of different recipients was observed in *E. coli* (7), *Salmonella typhimurium* (31), *A. tumefaciens* (15), *R. meliloti* (15), and *R. leguminosarum* (J. Beringer, personal communication) but not in *P. aeruginosa* (15). *his* mutants of strain 6.2 are efficiently complemented by FN68 and RP41 (Table 5). Among FN68-induced his^+ transconjugants, eight clones were purified four times before assaying nitrogen fixation, as described in Materials and Methods. Four clones exhibited ethylene production under aerobic conditions. Figure 2 shows the evolution of ethylene in parallel with the optical density. If the inocula came from shaken precultures, no reduction appeared. Compared with known *nif* expressions in enteric bacteria, the levels observed here are low but the conditions were far from being optimal, due to the presence of some oxygen and to the fact that nitrogen competes with acetylene in the reduction process. Among the eight his^+ (FN68) clones tested, four did not exhibit any acetylene reduction. This could be explained by spontaneous deletion of the *nif* genes. This suggestion is supported by the fact that the four nif^+ his^+ clones lost the acetylene reduction capacity after two to five further single-colony purifications.

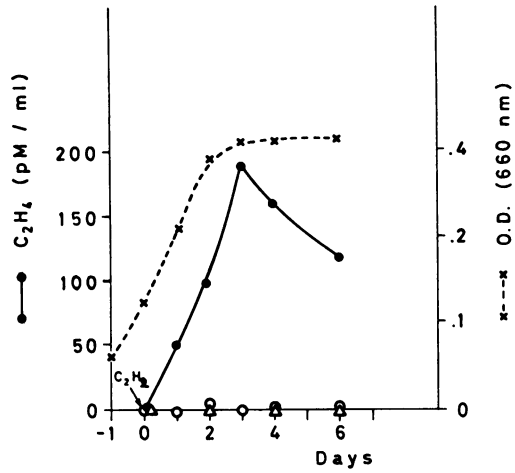

FIG. 2. *Evolution of acetylene reduction in cultures of the P. fluorescens 6.2 his^+ transconjugant PMG270(FN68). Inoculation was done on day −1. On day 0, cultures were injected with 0.5 ml of C_2H_2. C_2H_4 production was measured in a gas chromatograph daily. Culture (a) was unshaken: (×) optical density; (●) C_2H_4 content. Culture (b) was unshaken, with oxygen trapped by addition of pyrogallol in the side arm of a Pankhurst tube at the time of inoculation: (○) C_2H_4 content. Growth was strongly inhibited by anaerobic conditions (not shown). Culture (c) was shaken and also grew very slowly (not shown): (△) C_2H_4 content.*

Spontaneous deletions in plasmids have been observed with RP41-induced his^+ clones. RP41 contain determinants for tetracycline and kanamycin resistance which were transferred to *P. fluorescens* 6.2 with the same efficiency as the his^+ marker. But his^+ clones purified once or twice on minimal medium spontaneously lose nonselected markers. Thirty-five his^+ clones were thrice cloned. After the third cloning 10 clones were Tet^s, 12 were Kan^s, 5 were Tet^s and Kan^s, and 8 were still resistant to both drugs. Acetylene reduction in liquid medium was observed in two clones from six tested and disap-

peared in the next purification. However, when *his*$^+$ transconjugants obtained with RP41 and FN68 plasmids were replicated or streaked onto plates lacking histidine and nitrogen sources, they grew very slowly, but significantly better after 7 to 10 days than F131-induced (not containing *nif*) *his*$^+$ clones, which did not grow at all. Thus, appropriate selection in controlled microaerophilic conditions could improve and maintain the *nif* expression in this aerobic pseudomonad. The deletion of nonselected markers of RP41 was also observed in selected Kan^r or Tet^r clones. Many of them lost *his*$^+$ independence in the absence of selection after a few purifications. This situation is in contrast with observations made in *S. typhimurium* and *E. coli*, where RP41 is extremely stable as a whole set of markers (30). On the other hand, if RP41 is transferred to *R. meliloti* 41-A_1, Tet^r or *his*$^+$ transconjugants appear to acquire deletions of unselected markers, a situation similar to our case (15). *nif* genes are not expressed in *R. meliloti*, although they can be detected after retransfer to *E. coli* (15).

Response of tryptophan mutants to plasmids. *trp* mutants of *P. fluorescens* 6.2 were recognized by Irving Crawford (personal communication) as exhibiting a typical *Pseudomonas* regulation. It included repressibility of *trpE*, *-D* and *-C* genes, constitutivity of the *trpF* gene, and inducibility of *trpA* and *-B* genes (10, 17, 31, 32). Six *trp* mutants were tested in complementation attempts with F123 and F125 plasmids. Four phenotypic classes were recognized in these six mutants: *trpE52* and *-E53* were anthranilate responding and lacked anthranilate synthase (I. Crawford, personal communication); *trp-65* grew satisfactorily with 10 μg of indole per ml; *trp-55* responded only to very high concentrations of indole up to 50 μg/ml and accumulated anthranilate; *trp-51* and *trp-113* responded to 20 μg of indole per ml, did not accumulate indole glycerol phosphate, and accumulated anthranilate. Apparently we have no mutants corresponding to *trpA* and *trpB* coding for tryptophan synthetase.

The mutants of the first three groups were not complemented by F123 or F125. With *trp-51* and *trp-113* only, we repeatedly observed very tiny clones which grew to normal size only after 10 days. Recloned, they were again slow growing. Frequencies of obtaining exconjugants were similar to those observed in other complementations (Table 5). The strong divergence of regulation patterns may at least partly account for the apparent inefficient complementation and slow growth. The gene susceptible to complementation would not be *trpA* or *trpB*, since *trp-51* did not accumulate indole or indole glycerol phosphate, nor is it *trpE*, since it accumulated anthranilate. Among the remaining possibilities, *trpF* is a constitutive gene and *trpD* and *trpC* are repressible genes. It would be interesting to explore more deeply the relationship between the complementation and the regulatory status of the concerned gene.

Retransfer abilities of acquired F' plasmids. Table 6 reports some attempts of F' plasmid transfer from *P. fluorescens* 6.2 transconjugants to other PMG recipients. Frequency of retransfer of F131 and F110 is of the same order of magnitude as in *E. coli-P. fluorescens* crosses. Around 50% of the transconjugants recloned from stabs after 12 to 18 months did not transfer any more and, in fact, did not segregate towards original auxotrophy if grown on broth agar. This observation suggested the possibility of integration of parts of the plasmid into the genome of *P. fluorescens*.

DISCUSSION

These results showed that mutations of *P. fluorescens* 6.2 could be complemented by *E. coli* chromosomal genes carried on F' plasmids. Effective complementation involved structural genes of *E. coli* coding chiefly for the biosyntheses of arginine, isoleucine-valine, histi-

TABLE 6. *Transfer of F' plasmids between P. fluorescens strains*[a]

Recipient	Donor (transconjugant)	Selection	Spontaneous reversion (recipient)	Transconjugant frequency	No. of expt
PMG280	PMG36(F131)	His$^+$	10^{-7}	10^{-5}	1
PMG360	PMG36(F131)	His$^+$	8×10^{-8}	7×10^{-6}	3
PMG130	PMG22(F110)	Met$^+$	3×10^{-7}	2×10^{-5}	2
PMG130	PMG13(F110)	Met$^+$	4×10^{-7}	10^{-5}	2
PMG220	PMG13(F110)	Met$^+$	10^{-8}	4×10^{-6}	1
PMG220	PMG22(F110)	Met$^+$	10^{-8}	10^{-5}	1
PMG230	PMG23(F110)	Met$^+$	5×10^{-7}	2×10^{-5}	1
PMG114	PMG124(F131)	His$^+$	5×10^{-9}	6×10^{-5}	1

[a] *P. fluorescens* recipients and transconjugants were obtained from the experiments described in Table 5. Counterselection was performed by using streptomycin in the first seven crosses and by omitting the donor requirements in the last cross.

dine, leucine, and methionine and was useful in determining some metabolic blocks, allowing us to recognize *argH, metA, metB,* and *hisD* mutations of *P. fluorescens* 6.2. Complementation also involved a suppressor locus carried by F131, the plasmid complementing all *his* mutations. This locus is probably *supD. supD* exists in the mutant allele on F131 but not in plasmid RP41, FN68, or F150. Complementation also affects the β subunit of RNA polymerase since the sensitivity to rifampin carried by plasmid F110 was found to be dominant over two different alleles of rifampin resistance in *P. fluorescens* 6.2. Some *his*$^+$ clones obtained from plasmid RP41 or FN68 containing *nif* genes of *Klebsiella* can express nitrogenase. However, in the absence of direct *nif* selection, this property is progressively lost, likely by plasmid deletion. On the other hand, *E. coli rha* genes carried by the F110 plasmid and *ara* genes carried by F101 were not expressed. Complementation by chromosomal genes belonging to other taxa was reported to some extent in *E. coli.* For example, R′ plasmids containing *P. aeruginosa* tryptophan chromosomal genes complemented *E. coli trp* mutations (18). Tryptophan auxotrophy of an *A. tumefaciens* mutant was also suppressed by an RP4 plasmid on which ϕ80-*trp* transducing phage had been transposed (S. van den Elsacker and J. Schell, personal communication). Another RP4-*trp* plasmid constructed in vitro (27) was also transferred to a *trp* mutant of *P. aeruginosa* (26). Taken together, these complementations clearly indicate a rather broad cytoplasmic ability to express foreign gene products. From an evolutionary point of view, the case of RNA polymerase is interesting in that it implies interaction of *E. coli* and *P. fluorescens* subunits in the RNA polymerase enzyme complex. An interesting subject is how *E. coli* genes will respond to *Pseudomonas* regulatory mechanisms. RNA polymerase, arginine, tryptophan, and histidine biosyntheses are attractive systems in this respect.

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