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Plasmid Involvement in Acyclic Isoprenoid Metabolism by *Pseudomonas putida*

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An organism identified as *Pseudomonas putida* was found to utilize citronellol or geraniol as the sole carbon and energy source. The ability to degrade these acyclic isoprenols was associated with pSRQ50, a 50-megadalton transmissible plasmid.

The involvement of naturally occurring plasmids in the degradation of many aliphatic, aromatic, and haloaromatic compounds has been previously described (1, 7, 10). Specifically, studies by Benson and Shapiro (1) have described the OCT plasmid from *Pseudomonas putida*. This plasmid has been shown to code for the initial steps of *n*-alkane assimilation.

Pseudomonads have also been shown to utilize various acyclic isoprenoid compounds. *P. citronellolis*, a soil pseudomonad, has been observed to utilize citronellol, geraniol, or farnesol as its sole source of carbon and energy (8).

In this report, we describe the isolation of a *P. putida* strain which is able to utilize citronellol or geraniol as the sole source of carbon and energy and demonstrate the presence of a transmissible plasmid that specifies degradation of these compounds.

Soil samples were obtained from an area surrounding a waste treatment lagoon at a citrus pulping facility. The soil samples were inoculated into a minimal salts medium (mmo) (9) for liquid culture enrichment and incubated for 72 h at 25°C. The medium contained an acyclic isoprenol as the carbon source (0.2%) and yeast extract (0.05%). After incubation, portions of the enrichments were plated onto mmo agar containing an acyclic isoprenol as the carbon source. A strain was obtained that was able to utilize either citronellol or geraniol as its sole carbon and energy source. The strain was purified and identified as *P. putida* PPU2. (Table 1).

P. putida PPU2. was studied for its extrachromosomal DNA content by the procedure of Hansen and Olsen (4). The isolate was observed to contain 80- and 50-megadalton (Mdal) resident plasmids, which were designated pSRQ80 and pSRQ50, respectively. Molecular size determination was based on the method of Hansen and Olsen (5).

Conjugal mating experiments were accomplished by the method of Olsen (6). The donors were auxotrophs obtained through mutagenesis with 1-methyl-3-nitro-1-nitrosoguanidine (Sigma Chemical Co., St. Louis, Mo.) by a method to be described elsewhere (P. A. Vandenbergh, C. F. Gonzalez, A. M. Wright, and B. S. Kunka, Appl. Environ. Microbiol., in press).

The strains *P. putida* PPU2.4(pSRQ80/pSRQ50) and PPU2.6(pSRQ80/pSRQ50) were plate mated with recipients *P. putida* PPO2O8 and PPO2011, respectively (Table 1). The recipients are plasmid-free strains which are unable to utilize either citronellol or geraniol as the sole source of carbon and energy. The initial plate matings to directly select for cit^+ or ger^+ transconjugants were unsuccessful, as were broth matings.

P. putida PPU2.6(pSRQ80/pSRQ50) was transformed with 5.4-Mdal plasmid pRO1742 by the procedure of Davis et al. (3). pRO1742 contains the streptomycin resistance transposon Tn*904* (W. R. McCombie, J. B. Hansen, G. J. Zylstra, B. Mauer, and R. H. Olsen, J. Bacteriol., in press). The transformants [*P. putida* PPU2.6(pRO1742/pSRO80/pSRQ50)], which appeared at a frequency of $10^3/\mu g$ of DNA, were checked for expression of all markers. They were used as donors in plate matings with *P. putida* PPO2011.

Plate mating experiments were accomplished at 25°C with mmo medium supplemented with histidine, streptomycin, and, as the sole carbon and energy source, citronellol. The transconjugants from these plate matings [*P. putida* PPO2011(pRO1742/pSRQ80/pSRQ50)] were also able to utilize geraniol as the sole carbon and energy source (Table 1). These strains were examined for their extrachromosomal DNA content and were observed to contain pSRQ80, pSRQ50, and a 10.6-Mdal dimer of pRO1742 (Fig. 1). The 10.6-Mdal plasmid coded for streptomycin resistance and was observed to have

TABLE 1. Nutritional properties of various pseudomonads

Strain	Phenotype[a]	Origin
P. putida		
PPU2.(pSRQ80/pSRQ50)	Prototroph, Cit^+ Ger^+ [b]	This study
PPU2.4(pSRQ80/pSRQ50)	His auxotroph, Cit^+ Ger^+	This study
PPU2.6(pSRQ80/pSRQ50)	Trp auxotroph, Cit^+ Ger^+	This study
PPO2011(pRO1742/pSRQ80/pSRQ50)	His auxotroph, Cit^+ Ger^+	This study
PPO208(pSRQ50)	Trp auxotroph, Cit^+ Ger^+	This study
PPO2011	His auxotroph, Cit^- Ger^-	R. H. Olsen[c]
PPO208	Trp auxotroph, Cit^- Ger^-	R. H. Olsen
P. aeruginosa		
PAO2(pRO1742)	Ser auxotroph	R. H. Olsen

[a] Cit, Citronellol; Ger, geraniol; His, histidine; Trp, tryptophan; Ser, serine; +, growth; −, no growth.
[b] Volatile carbon sources were incorporated directly into the medium as well as into the vapor phase in a sealed container. Incubation was for 72 h at 25°C.
[c] University of Michigan, Ann Arbor.

the same *Sst*I cleavage sites as pRO1742 (data not shown).

Citronellol and geraniol were separately used as substrates for whole-cell oxygen uptake studies comparing parental strain *P. putida* PPU2.(pSRQ80/pSRQ50) with transconjugant *P. putida* PPO2011(pRO1742/pSRQ80/pSRQ50). Oxygen consumption was measured at 25°C with a model 53 YSI biological oxygen monitor and a Clark fixed-voltage polarographic probe (both from Yellow Springs Instrument Co., Yellow Springs, Ohio). The results indicated similar rates of utilization for the parental and transconjugant strains (Table 2).

P. putida PPO2011(pRO1742/pSR80/pSRQ50) was used as the donor for plate matings with *P. putida* PPO208. From these matings, a transconjugant [*P. putida* PPO208(pSRQ50)] was obtained. The transconjugant was a tryptophan auxotroph that was able to grow in minimal medium containing citronellol or geraniol as the sole carbon and energy source (Table 1). Extrachromosomal DNA content profiles of transconjugant *P. putida* PPO208(pSRQ50) revealed the presence of 50-Mdal plasmid pSRQ50 (Fig. 1).

TABLE 2. Oxidation of acyclic isoprenols by suspensions of whole cells[a]

P. putida strain	O_2 uptake (μl/h per mg [dry wt])[b]	
	Citronellol	Geraniol
PPU2.(pSRQ80/pSRQ50)	8.5	9.8
PPO2011(pRO1742/pSRQ80/pSRQ50)	9.0	6.0
PPO208(pSRQ50)	7.5	4.0

[a] Reaction mixtures contained 20 μM substrate.
[b] These values were corrected for endogenous oxygen uptake by subtracting the value for a control without either substrate.

Whole-cell oxygen uptake studies of *P. putida* PPO208(pSRQ50) on citronellol or geraniol as the substrate revealed rates of utilization similar to those of the other strains listed in Table 2.

In this study, strains PPO2011 and PPO208 acquired the ability to utilize citronellol or geraniol as the sole carbon and energy source through conjugation with *P. putida* PPU2.6(pRO1742/pSRQ80/pSRQ50). These re-

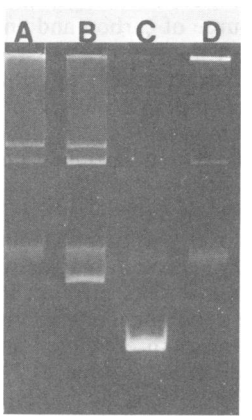

FIG. 1. Agarose gel electrophoresis of DNA preparation purified with cesium chloride-ethidium bromide. The agarose concentration was 0.7%, and migration was from top to bottom. Contents of lanes and bands (from top to bottom) are as follows. (A) Parental strain PPU2.(pSRQ80/pSRQ50); bands: 80-Mdal plasmid covalently closed circular (CCC) DNA, 50-Mdal plasmid CCC DNA, and fragmented chromosomal DNA. (B) PPO2011(pRO1742/pSRQ80/pSRQ50); bands: 80-Mdal plasmid CCC DNA, 50-Mdal plasmid CCC DNA, fragmented chromosomal DNA, and pRO1742 10.6-Mdal dimer. (C) PAO2(pRO1742); bands: faint fragmented chromosomal DNA and 5.4-Mdal plasmid (pRO1742) CCC DNA. (D) PPO208(pSRQ50); bands: 50-Mdal plasmid CCC DNA and faint fragmented chromosomal DNA.

sults suggest that the strains acquired the degradation ability from a transmissible plasmid (pSRQ50).

After purification with cesium chloride-ethidium bromide and digestion with *Sst*I, it was found that the pSRQ50 plasmid from *P. putida* PPO208(pSRQ50) was the same as that from the parental strain, *P. putida* PPU2. (data not shown).

Our whole-cell oxygen uptake studies comparing parental and transconjugant strains demonstrated slow utilization of these compounds. Similar results were initially observed by Seubert with *P. citronellolis* (8). Previous work has suggested that the failure of bacteria to utilize acyclic isoprenols is due to the toxic effect of the alcohols. Studies have shown that β-alkyl branching of the linear alkyl skeletons blocks the β-oxidation pathway, thus reducing biodegradation of these compounds, which are recalcitrant to environmental degradation (2).

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