Plasmid Involvement in Linalool Metabolism by *Pseudomonas fluorescens*

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Received 17 April 1986/Accepted 8 June 1986

A bacterial strain was isolated from a wastewater lagoon and identified as *Pseudomonas fluorescens*. This isolate was able to utilize linalool as a sole carbon and energy source. The ability was found to be encoded on a 60-megadalton transmissible plasmid, pSRQ60. The plasmid was also mated to a commercial waste treatment strain, which expanded its ability to utilize other isoprenoid compounds.

Pseudomonads have been shown to utilize acyclic isoprenoid compounds. *Pseudomonas citronellolis* and *Pseudomonas incognita* have been previously described as being able to utilize citronellol, geraniol, and linalool (3, 6). Recently, a 50-megadalton (MDa) plasmid has been associated with the degradation of geraniol (9).

In this report we describe the isolation of a *Pseudomonas fluorescens* strain capable of utilizing linalool as a sole carbon and energy source and demonstrate the presence of a transmissible plasmid that specifies its degradation.

Soil samples were obtained from the area surrounding a waste treatment lagoon from a turpentine processor. The soil samples were inoculated into a minimal salts medium (7) for liquid culture enrichment and incubated for 48 h at 25°C. The medium contained linalool (0.3%) and yeast extract (0.05%). After incubation, portions of the enrichments were plated onto minimal medium containing linalool as the sole carbon and energy source. A strain was obtained that was able to utilize linalool. The strain was purified and identified as *P. fluorescens* PFL7. (Table 1).

*P. fluorescens* PFL7 was examined for its extra-chromosomal DNA content by the procedure of Ish-Horowitz and Burke (2). The isolate was observed to contain 60- and 41-MDa resident plasmids, which were designated pSRQ60 and pSRQ41, respectively (Fig. 1). Molecular size determination was based on a method previously described (9).

Mating experiments were accomplished by the method of Olsen (4). The donors were auxotrophs obtained through mutagenesis with 1-methyl-3-nitro-1-nitrosoguanidine (Sigma Chemical Co., St. Louis, Mo.) by the procedure described by Vandenbergh et al. (8).

The strains *P. fluorescens* PFL7.1(pSRQ60, pSRQ41) and PFL7.2 (pSRQ60, pSRQ41) were mated with recipient *Pseudomonas putida* PPO208. *P. putida* PPO208 is a plasmid-free strain which is unable to utilize linalool. The transconjugant *P. putida* PPO208(pSRQ60) was then successfully mated with *P. putida* PPU2 (pSRQ50, pSRQ80). The recipient in this mating is a commercial strain that degrades geraniol, a component of CITROX (Microlife Technics), and is unable to utilize linalool. Selection of linalool-utilizing transconjugants was successful at frequencies ranging from $2 \times 10^6$ to $5 \times 10^6$ transconjugants per donor. These strains were checked for the expression of linalool metabolism and other markers.

The isolates were examined for their plasmid content and were observed to contain the 60-MDa plasmid, pSRQ60 (Fig. 1). Growth studies were begun to check for the utilization of other C_{10}H_{18}O substrates (Table 1). The transconjugants had acquired the ability to utilize linalool; however, they were unable to utilize nerol, limonene, or citronellal. In broth studies with minimal salts medium containing 0.3% linalool the following growth rates (generations per hour) were observed: *P. fluorescens* PFL7. (pSRQ60, pSRQ4), 0.18; *P. putida* PPU2.9(pSRQ60, pSRQ50, pSRQ30), 0.14; and *P. putida* PPO208(pSRQ60), 0.10. A sample of the broth was

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**TABLE 1. Nutritional properties of bacterial strains**

<table>
<thead>
<tr>
<th>Strain</th>
<th>Relevant characteristicsa</th>
<th>Origin</th>
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<tbody>
<tr>
<td><em>P. fluorescens</em></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PFL7 (pSRQ60, pSRQ41)</td>
<td>Prototroph, Lin&quot;+</td>
<td>This study</td>
</tr>
<tr>
<td>PFL7.1 (pSRQ60, pSRQ41)</td>
<td>Ura auxotroph, Lin&quot;</td>
<td>This study</td>
</tr>
<tr>
<td>PFL7.2 (pSRQ60, pSRQ41)</td>
<td>Leu auxotroph, Lin&quot;</td>
<td>This study</td>
</tr>
<tr>
<td><em>P. putida</em></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PPU2. (pSRQ50, pSRQ80)</td>
<td>Prototroph, Ger&quot;-, Lin&quot;</td>
<td>Microlife Technics</td>
</tr>
<tr>
<td>PPU2.9 (pSRQ60, pSRQ50, pSRQ80)</td>
<td>Prototroph, Ger&quot;-, Lin&quot;</td>
<td>Culture Collection</td>
</tr>
<tr>
<td>PPO208</td>
<td>Prototroph, Trp auxotroph, Lin&quot;</td>
<td>This study</td>
</tr>
<tr>
<td>PPO208 (pSRQ60)</td>
<td>Prototroph, Trp auxotroph, Lin&quot;</td>
<td>This study</td>
</tr>
</tbody>
</table>

* Lin, Linalool; Ger, geraniol; Ura, uridine; Leu, leucine; Trp, tryptophan; +, growth; -, no growth.
* Volatile carbon sources were supplied in the vapor phase in a sealed container. Incubation was for 48 h at 25°C.
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The metabolism of linalool has been extensively investigated by Renganthan and Madyastha (5). Their isolate of Pseudomonas incognita grew slowly in the presence of 0.3% linalool as the sole carbon and energy source. The presence of the 60-MDa plasmid pSRQ60 enhances the degradative rate of our strain. The substrate range of a commercial strain has also been extended to include an additional isoprenoid, linalool.

Isoprenoid compounds generated from chemical manufacturers and citrus processors are often released into the environment. These compounds are not considered toxic; however, they are difficult for bacteria to degrade (1). Plasmids that encode specific isoprenoid metabolism will allow for more effective bacterial utilization of these compounds.

LITERATURE CITED

filter sterilized and checked for the residual linalool at the completion of the growth experiment. This was accomplished by gas chromatography on a Supelco column (model GP5%SP-2100/0.1%SP-401). From comparisons with linalool standards it was determined that 90% of the carbon source had been utilized.