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Short Communication

*Tn*1721-induced mutation in an isoprenol degrading plasmid from *Pseudomonas putida*

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SUMMARY

Transformation experiments using pRO1825 containing the tetracycline resistance transposon *Tn*1721 were conducted to induce mutations in plasmid pSRQ50, which coded for acyclic isoprenol degradation. Transformants that were tetracycline resistant were examined for their ability to utilize geraniol. Strains unable to utilize geraniol were observed to have a 0.6 kb addition on the C fragment of pSRQ50.

INTRODUCTION

Pseudomonads have been shown to utilize various acyclic isoprenoid compounds. *Pseudomonas citronellolis,* a soil pseudomonad, has been observed to utilize citronellol, geraniol or farnesol as its sole source of carbon and energy [9].

In a previous report we described the isolation of a *Pseudomonas putida* strain from a citrus pulping facility [12]. This isolate was capable of utilizing acyclic isoprenol compounds as the sole source of carbon and energy. We also described the presence of a 50 Mdal transmissible plasmid pSRQ50 which encoded for the enzymes involved in the utilization of geraniol, an isoprenol compound.

Transposable elements that encode drug resistance are useful in microbial genetics. The use of drug-resistant transposable elements has led to the construction of deletion mutants, which has been helpful in understanding the physiology of degradative plasmids [4].

In this report we describe the isolation of mutations in the pSRQ50 plasmid, caused by the insertion of the *Tn*1721 tetracycline resistance determinant [1].

MATERIALS AND METHODS

Strains, plasmids and growth conditions

The organisms and plasmids used in this study are listed in Table 1. *Pseudomonas putida* PP0208(pSRQ50) was grown at 25°C on (TGY) medium containing: tryptone 5 g/l (Difco, Detroit, MI), yeast extract 2.5 g/l (Oxoid, Basingstoke, Hampshire, U.K.), glucose 1.0 g/l (Sigma, St. Louis, MO), sodium chloride 8.5 g/l (Sigma, St. Louis, MO) and agar 20 g/l (Gibco, Madison, WI).

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Table 1

Bacterial strains and plasmids used

Tc, tetracycline; ger, geraniol; trp, tryptophan; met, methionine; ser, serine.

Strain	Designation	Relevant characteristics	Reference or source
P. putida			
PPO208(pSRQ50)	None	Tc^s, ger^+, trp^-	[12]
PPO208(pRO1825/pSRQ50/ΔpSRQ50)	PPO208.1	Tc^r, ger^-, trp^-	This study
PPO208(pRO1825/pSRQ50/ΔpSRQ50)	PPO208.2	Tc^r, ger^+, trp^-	This study
P. aeruginosa			
PAO2(pRO1825)	None	Tc^r, ser^-	R.H. Olsen[a]
PAO2178	None	Tc^s, ger^-, met^-	R.H. Olsen[a]

[a] University of Michigan.

Nutritional studies were completed on minimal media (mm_0) previously described [10].

Transformation

P. putida PP0208(pSRQ50) was transformed according to the method of Davis et al. [3]. The transformants were selected on (TGY) medium containing tetracycline at 25 μg/ml. The transformants were further examined for their ability to grow on minimal salts medium containing tryptophan, tetracycline and geraniol as sole carbon and energy source. *P. aeruginosa* PA02178 was transformed according to the method of Mercer and Loutit [7].

DNA preparation, analysis, cloning and hybridization

Plasmid DNA was isolated from the strains using the method of Ish-Horowitz and Burke [5]. The plasmid DNA was further purified by ultracentrifugation in the presence of cesium chloride-ethidium bromide as described by Vandenbergh and co-workers [11,12].

Restriction endonuclease digestions were performed according to the manufacturer's specifications. Ligation reactions with pBR322 and pSRQ50, previously digested with *Pst*I, were performed at 17°C for 18 h. The ratio of vector DNA to plasmid pSRQ50 DNA was approximately 1:2.

Biotin label hybridization experiments were performed according to the specifications of the manufacturer.

Plasmid mini-screens were performed on *P. putida* and *P. aeruginosa* transformants using the method previously described [12].

RESULTS AND DISCUSSION

The strain *P. putida* PP0208 (pSRQ50) was transformed with a 12.4 Mdal plasmid, pRO1825 [8]. The plasmid pRO1825 contains the tetracycline transposon *Tn*1721 [1]. The transformants that appeared at a frequency of 2.2×10^4 transformants per μg of DNA were further examined for their ability to grow on a minimal salts medium (mm_0) containing geraniol as sole carbon and energy source. Two types of tetracycline-resistant transformants were observed in the experiments: *P. putida* PPO208.2, which was able to utilize geraniol as sole carbon and energy source, and *P. putida* PP0208.1, which was unable to utilize geraniol (Table 1). Restriction endonuclease digestion analysis of purified plasmid DNA from strain *P. putida* PP0208.1 with *Pst*I demonstrated a 0.6 kb addition to the C fragment of pSRQ50 (Fig. 1). This 0.6 kb insertion in the C fragment was also observed in hybridization experiments using biotin-labeled pRO1825. An additional plasmid was also observed in isolate PP0208.1, ΔpSRQ50, a 5.2 kb deletion plasmid of pSRQ50 (Fig. 2). Extrachromosomal DNA from tetracycline-resistant transformants that could utilize geraniol was subjected

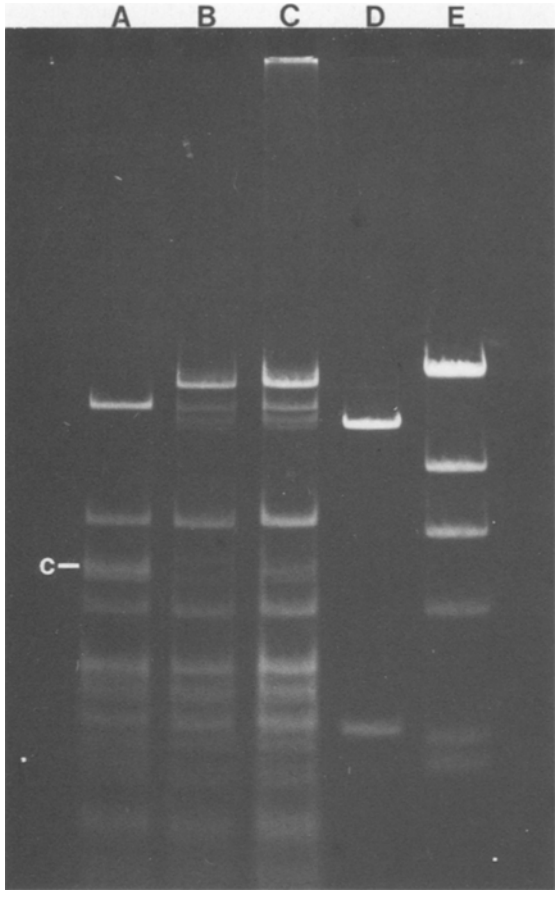

Fig. 1. *Pst*I digested CsCl-ethidium bromide-purified plasmid DNA from various *Pseudomonas* strains. The agarose concentration was 0.7% and migration was from top to bottom. Lanes: A, *Pst*I digest of plasmid DNA from PP0208(pSRQ50); B, *Pst*I digest of plasmid DNA from PP0208.1; C, *Pst*I digest of plasmid DNA from PP0208.2; D, *Pst*I digest of plasmid DNA from PA02(pR01825); E, Lamda *Hin*dIII digestion.

to restriction analysis using *Pst*I (Fig. 1). The 0.6 kb addition was not observed in the C fragment of pSRQ50 from *P. putida* PP0208.2. These results suggest that genes essential for the utilization of geraniol were located on this C fragment of pSRQ50.

Cloning experiments were accomplished in *P. aeruginosa* PA02178 using the vector pBR322 and the plasmid pSRQ50. The cells were then plated onto media containing tetracycline. The colonies were screened for the presence of plasmid DNA. The *Pseudomonas* replicator on pSRQ50 was determined to be located on the C fragment. The strains containing this plasmid were, however, unable to utilize geraniol as a sole carbon and energy source. This suggests that the other enzymes described in the pathway by Cantwell et al. [2] are located on other fragments of the plasmid.

Although many of these oils are $C_{10}H_{18}O$ isoprenoid molecules, their degradative pathways appear to be quite different [2,6]. Further work has shown that another plasmid, pSRQ60, is specific for the degradation of linalool (personal observation). The degradation of recalcitrant molecules in the environment is always of interest. Therefore, the use

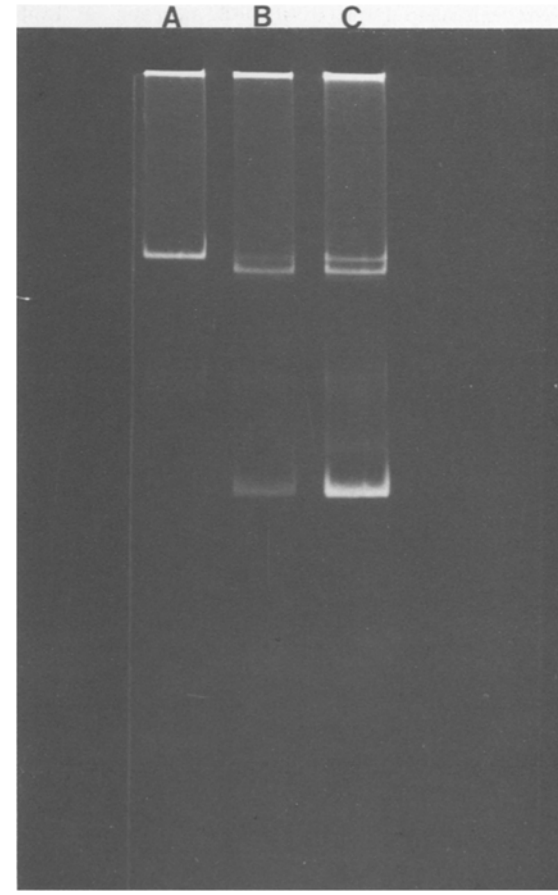

Fig. 2. Cesium chloride-ethidium bromide-purified plasmid DNA from various *Pseudomonas* strains. The agarose concentration was 0.7% and migration was from top to bottom. Lanes: A, plasmid DNA from PP0208(pSRQ50); B, plasmid DNA from PP0208.1; C, plasmid DNA from PP0208.2.

of transposons such as *Tn*1721 will assist us in the understanding of the structural organization of a plasmid coding for the degradation of isoprenol compounds.

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