SHORT COMMUNICATION

Transposon Mutagenesis in *Methylobacterium AM1 (Pseudomonas AM1)*

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A method of transposon mutagenesis using Tn5 has been developed for the facultative methylotroph *Methylobacterium AM1* using the IncP-10 plasmid R91-5. Auxotrophic mutants and mutants involving the metabolism of methanol have been isolated.

INTRODUCTION

One of the difficulties of methylotroph genetics is the isolation of mutants. In various organisms, including *Methylobacterium organophilum* XX (O'Connor & Hanson, 1978) and *Methylophilus methylotrophus* AS1 (Holloway, 1981), chemical mutagens such as ethyl methane sulphonate and *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine are not effective, while the frequency of mutants following ultraviolet radiation is very low. The effectiveness of transposon mutagenesis in a variety of bacteria (Berg & Berg, 1983; Kleckner, 1981) encouraged us to look for a transposon mutagenesis system which could be generally applicable to a variety of C₃-utilizing organisms.

METHODS

**Strains.** The strains of *Pseudomonas aeruginosa* and *Methylobacterium AM1* and plasmids used in this study are shown in Table 1.

**Media.** The media used for *P. aeruginosa* were as described previously (Haas & Holloway, 1976). For *Methylobacterium AM1* MacLennan's medium was used (MacLennan et al., 1971), with appropriate carbon source supplementation.

**Construction of pMO75.** *P. aeruginosa* PA0764(R91-5), which has Tn5 inserted into the chromosome, was mated to *P. aeruginosa* PAO11 and selection imposed for transfer of kanamycin resistance to PAO11. One transconjugant was selected which carried R91-5 loaded with Tn5 and this plasmid was denoted pMO75.

RESULTS AND DISCUSSION

We selected a derivative of the IncP-10 plasmid R91 as a suitable suicide vector. The parent plasmid, R91, was found originally in a clinical isolate of *P. aeruginosa* and has a replicative host range confined to *P. aeruginosa* (Chandler & Krishnapillai, 1974; V. Krishnapillai, personal communication). It confers carbenicillin resistance through the possession of TnJ. A variant, R91-5, was isolated which shows a high transfer frequency between *P. aeruginosa* strains (Chandler & Krishnapillai, 1974, 1977). In matings between *P. aeruginosa* PAO(R91-5) and *P. putida*, no carbenicillin-resistant transconjugants are found, but in such matings if R91-5 is loaded with a transposon such as Tn501, transconjugants are found which are both resistant to carbenicillin and express the mercury resistance carried by the transposon. Such transconjugants have the entire plasmid including the additional loaded transposon inserted into the chromosome, are highly efficient bacterial chromosome donors and have been used to map the *P. putida* chromosome (Dean & Morgan, 1983).
**Table 1. Bacterial strains and plasmids**

<table>
<thead>
<tr>
<th>Characteristics*</th>
<th>Reference†</th>
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<tbody>
<tr>
<td><strong>Pseudomonas aeruginosa</strong></td>
<td></td>
</tr>
<tr>
<td>PAOI826</td>
<td>leu-9001 thr-9001 pur-9012 met-9018</td>
</tr>
<tr>
<td>PAO764</td>
<td>trp-54 argA49 rif-5 fon-1::Tn5</td>
</tr>
<tr>
<td>PAO11</td>
<td>trp-54 nal-19 fon-1</td>
</tr>
<tr>
<td><strong>Methylobacterium AM1</strong></td>
<td></td>
</tr>
<tr>
<td>Wild-type</td>
<td></td>
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<tr>
<td><strong>Plasmids</strong></td>
<td></td>
</tr>
<tr>
<td>R9-5</td>
<td>Cb' Tra Dps Phi(G101) IncP-10</td>
</tr>
<tr>
<td>pMO75</td>
<td>R9-5::Tn5</td>
</tr>
</tbody>
</table>

* Genotype symbols are the same as those used by Bachmann (1983) for *Escherichia coli* except for the following: fon, resistance to bacteriophage F116L; nal, resistance to nalidixic acid. Phenotype symbols used are Cb', carbenicillin resistance; Tra, transfer ability; Dps, sensitive to donor-specific bacteriophages; Phi(G101), inhibitory to bacteriophage G101.

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Using the plasmid pMO75 (R9-5::Tn5), we have developed a procedure for transposon mutagenesis of the well-characterized facultative methylotroph *Methylobacterium AM1* (formerly *Pseudomonas AM1*) (Peel & Quayle, 1961; Green & Bousfield, 1983).

*P. aeruginosa* PAOI826(pMO75) was used as the Tn5 donor. It was grown overnight in nutrient yeast broth (NYB) at 37 °C to give a cell density of $1 \times 10^9$ ml⁻¹ and then mated to *Methylobacterium* MBM1 which had been grown in MacLennan’s liquid minimal medium containing 0.4% methanol for 48 h at 28 °C to give a cell density of $1 \times 10^8$ ml⁻¹. The mating was carried out on a cellulose acetate membrane (Gelman, pore size 0.45 μm, 25 mm diameter) using 2 ml of recipient and 1 ml of donor. The membrane carrying the cells was placed for 6 h at 28 °C on prewarmed agar plates of MacLennan’s medium containing 0.2% succinate and 5% (v/v) nutrient yeast broth (MSBS). The cells were then washed off the membrane into 1 ml saline and 0.2 ml samples plated onto MSBS plates containing kanamycin (100 μg ml⁻¹) and rifampicin (200 μg ml⁻¹). These plates were incubated for 4–5 d at 28 °C and control plates carrying each parent separately were incubated for the same period.

Colonies from the mating occurred at a frequency of $10^{-7}$ per donor cell. These colonies were patched onto the same medium and replica plated onto MacLennan’s medium containing either succinate or methanol as sole carbon source, each with kanamycin (100 μg ml⁻¹) and rifampicin (200 μg ml⁻¹). Failure to grow on the succinate medium indicated a potential auxotrophic mutation, and failure to grow on the methanol medium indicated a mutation in a gene of the C₁-utilization pathway.

From nine separate matings, 4070 kanamycin-resistant transconjugant colonies were examined. Of these, six were mutants for methanol utilization (cou, C-one utilization) and six were auxotrophs, giving an overall mutant recovery of 0.3%. Of the auxotrophs, all non-siblings, three required thiamin and two required tyrosine; the requirements of the sixth could not be identified. The six cou mutants isolated are currently being characterized and will be described elsewhere.

The relative ease of isolation of these mutants points to the success of this procedure for Tn5 transposon mutagenesis in *Methylobacterium*. Tn5 was chosen because of its established low specificity of insertion (Berg & Berg, 1983). We have also shown that Tn5-induced auxotrophic mutants of *E. coli* could be readily isolated following matings of *E. coli* K12 with *P. aeruginosa* (pMO75) donors, using a similar procedure to that described above for *Methylobacterium* (data not shown). We have also tested Tn2521 (Sinclair & Holloway, 1982) loaded onto R9-5 for mutagenesis but did not detect any transposition into *Methylobacterium* AM1. The present results support the view that R91-5 has a wide host range of conjugation but a narrow host range of replication and hence this transposon vector system should be applicable to other C₃-utilizing organisms. It is not possible to determine without physical analysis whether the mutants
obtained resulted only from insertion of Tn5 or by insertion of the whole pM075 plasmid. From mutagenesis experiments using pM075 in *P. putida* and *P. syringae* (A. Morgan & R. Hirst, unpublished data; R. Nordeen & B. Holloway, unpublished data) it is known that the frequency of insertion of the whole plasmid into the bacterial chromosome is 1000-fold less than that of the transposon alone. With *Methylobacterium* AM1, it is not possible to score for the presence of carbenicillin resistance encoded by the TnI of pM075, due to the low level of expression of resistance for this antibiotic.

The limited range of auxotrophs found may reflect a site specificity of insertion of Tn5 into the AM1 chromosome. More likely, it may result from selection imposed by the isolation procedure, for example inhibition by amino acids in the MSBS medium on which transconjugants were first plated. The growth of wild-type methylotrophs can be inhibited by individual amino acids, and such inhibitions could be exacerbated in methylotroph auxotrophs. Perhaps the isolation of auxotrophs in methylotrophs could be made easier by searching for only one requirement at a time, substituting a single amino acid in place of the nutrient yeast broth in the MSBS medium on which exconjugant cells are placed to select for transposition.

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**REFERENCES**


