Transposition of Tn917 in *Bacillus megaterium*†

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Transposon Tn917, carried on plasmid pTV1, was introduced into *Bacillus megaterium* and transposed efficiently and apparently randomly. Insertional mutations included at least eight different auxotrophic loci, two carbon source loci, and sporulation loci. One trp::Tn917 mutation was further verified as an insertion by both reversion and transduction.

Transposon Tn917 from *Streptococcus faecalis* (12) is presently the most useful transposon for *Bacillus subtilis* genetic manipulation. It is 5.3 kilobases in length, confers incomplete erythromycin resistance, and has terminal inverted repeats very closely related to the Tn3 family of transposons of gram-negative organisms (11). Youngman et al. (16) constructed a Tn917-carrying plasmid, pTV1, that is temperature sensitive for replication and demonstrated that Tn917 could transpose efficiently at 48°C in *B. subtilis*. They also constructed plasmids capable of making transpositional lacZ or cat-86 fusions or both and of cloning genes carrying Tn917 insertions into a pBR322 derivative (14, 15, 17). These plasmids are presently being used to analyze the temporal expression of developmentally regulated sporulation and germination genes (18) as well as DNA-damage-inducible (din) loci (10).

Our laboratory has been developing genetic and cloning techniques for *B. megaterium* (3, 5, 7, 13). The introduction of a transposon would greatly enhance the genetic versatility of this species. Here we report the successful introduction and transposition of Tn917 in *B. megaterium*.

Plasmid pTV1 carrying Tn917 was isolated from *B. subtilis* PY143 (16) by use of a modified alkaline-sodium dodecyl sulfate rapid plasmid preparation (7) and was transformed into *B. megaterium* PV204 protoplasts with selection for chloramphenicol resistance (5 μg/ml). Strain PV204 is a prototrophic derivative of strain QM B1551 that has been cured of six of seven resident plasmids by growth in sublethal concentrations of ethidium bromide. It retains only the 8.0-kilobase pVY105 plasmid (7). Growth conditions were as previously described (2), except that Luria-Bertani (LB) medium was used (9). Minimal-glucose medium (2) was used for transductions and auxotrophic selection. The presence of pTV1 in *B. megaterium* was verified by chloramphenicol resistance, inducibility by erythromycin and resistance to macrolide, lincosamide, and streptogramin (MLS) antibiotics; and visualization of the plasmid by agarose gel electrophoresis. Resistance was induced with 0.15 μg of erythromycin per ml and was always selected with both 5 μg of erythromycin per ml and 500 μg of lincomycin per ml.

To test the stability of pTV1 and to verify its temperature sensitivity in *B. megaterium*, strain PV307 (PV204 with pTV1) was grown in broth without antibiotics at various temperatures to the mid-logarithmic phase (A660 = 0.6 to 0.8). Cells were then diluted, plated for single colonies on LB agar with inducing levels of erythromycin, incubated overnight, and then replica plated to selective levels of MLS antibiotics (with or without chloramphenicol) to score plasmid loss. Stability on solid medium was also measured by plating a PV307 broth culture grown with chloramphenicol and MLS antibiotics for single colonies on LB agar with only inducing levels of erythromycin. Plates were incubated overnight, replica plated onto LB agar with erythromycin and lincomycin (with or without chloramphenicol), and scored for plasmid loss. As in *B. subtilis*, pTV1, was stable to 37°C but exhibited temperature-sensitive replication, resulting in an 80 to 100% loss from *B. megaterium* at 46°C (Fig. 1).

The ability of Tn917 to transpose in *B. megaterium* was determined. The effects of temperature, liquid versus solid media, and the number of high-temperature incubations on transposition were tested (Table 1). Two successive incubations at 46°C provided the most efficient transposition in *B. megaterium*, as in *B. subtilis*, in both broth and solid media. Transposition procedures were as described by Youngman et al. (16), except that the primary 46°C broth culture incubation was for only 4 to 8 h instead of overnight because of the heat sensitivity of *B. megaterium*. Cultures were diluted, plated for single colonies, incubated overnight at 30°C, and screened by replica plating. A control PV307 culture was incubated at 46°C without antibiotics for 4 to 8 h and then plated onto LB agar with 0.15 μg of erythromycin per ml before each transposition experiment. After overnight incubation, colonies were counted and replica plated to LB agar with erythromycin and lincomycin (with or without chloramphenicol) to verify that no previous transposition had occurred. The Tn917 transposition frequency was determined as described by Youngman et al. (17), except that colonies were incubated at 30 and 46°C instead of 33 and 48°C. When the frequency of transposition was measured (MLS' colonies at 46°C per MLS' colonies at 30°C), the average frequency from three experiments was 3.8 \times 10^{-4}. The addition of mitomycin C (10 ng/ml) increased transposition 40-fold to 1.5 \times 10^{-2}. The frequency of transposition in *B. megaterium* even without mitomycin C was approximately five-fold higher than the 2 \times 10^{-5} to 9 \times 10^{-5} reported in *B. subtilis* for Tn917 on pTV1 and some derivative plasmids (16).

The efficiency of transposition and the recovery of mutants with two successive incubations at 46°C either on agar or in broth are shown in Table 2. It is evident that a high percentage of transposition could be achieved under both conditions and that 10 ng of mitomycin C per ml further increased transposition, as has been observed in *B. subtilis* (S. Zahler, personal communication). Auxotrophs were detected by a lack of growth on minimal medium, character-

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† This paper is dedicated to Nicholas A. Bohall, Jr., who died tragically during its writing.
selected following transposition of Tn917. Sporulation mutants were detected by replica plating 48 to 72 h chloramphenicol-sensitive (Cm\(^+\)) MLS\(^+\) colonies onto duplicate LB agar plates with erythromycin and lincomycin and then exposing one set of plates to heat (80°C for 20 min) before incubation. Approximately 1% of the transposition clones were heat sensitive. Each clone was tested for auxotrophy to screen out indirect sporulation-defective colonies caused by auxotrophy or defective citric acid cycle enzymes (4). The colonies were then streaked onto LB agar with erythromycin and lincomycin, incubated for 48 to 72 h, and compared with a wild-type culture by phase-contrast microscopy to further verify the spo designation. Both asporogenous (<1% spores) and oligosporogenous (10 to 20% spores) mutants were found. In most transposition experiments, bright-yellow, oligosporogenous colonies were also recovered. These constituted 11% of the sporulation mutants isolated and may represent a specific mutational block. Transposition mutagenesis has allowed screening for sporulation mutants in a species which does not have an easily recognizable Spo\(^-\) phenotype.

To show that Tn917 was indeed causing the mutations by insertion into the chromosome, reversion of a trp::Tn917 mutation to Trp\(^+\) and cotransduction of the transposon with the genetic lesion were tested. A trp::Tn917 mutation was grown to an \(A_{600}\) of approximately 0.7 in liquid minimaltryptophan medium, and dilutions were plated on LB agar and minimal medium plates to determine the frequency of Trp\(^+\) revertants. The reversion rate observed in these experiments was approximately \(8 \times 10^{-8}\), and every revertant was MLS\(^+\). Precise excision with loss of Tn917 has not been reported in any Bacillus species previously to our knowledge. Phage MP13 is a transducing phage isolated in our laboratory (13), and preparation of MP13 lysates and transduction procedures have been described previously (2). The cotransduction frequency of trp and hisH is approximately 50% in B. megaterium (1). The trp::Tn917 mutation was transduced into strain PV3 (hisH21). Of 385 His\(^+\) transductants, 188 were found to be Trp\(^+\), and all of these were also MLS\(^+\). While there is a possibility that the Trp\(^+\) phenotype may have been caused by a polar effect of an insertion upstream of the affected trp gene, the insertion was linked to trp by greater than 99.5% and is most probably within a trp gene.

Overall, the results show that transposon Tn917 has been successfully introduced into another Bacillus species besides B. subtilis and is able to function efficiently. Plasmid pTV1, on which the transposon is carried, exhibits temperature-

**TABLE 2. Tn917 transposition in B. megaterium growing on agar or in broth**

<table>
<thead>
<tr>
<th>Medium and exp*</th>
<th>No. of colonies screened</th>
<th>No. (%) of MLS(^+) Cm(^+) clones</th>
<th>No. of auxotrophs</th>
<th>No. of Spo(^-) clones</th>
</tr>
</thead>
<tbody>
<tr>
<td>Agar 1</td>
<td>1,998</td>
<td>1,902 (95.2)</td>
<td>10</td>
<td></td>
</tr>
<tr>
<td>Agar 2</td>
<td>1,766</td>
<td>1,438 (81.4)</td>
<td>8</td>
<td>5</td>
</tr>
<tr>
<td>Agar 3</td>
<td>2,282</td>
<td>1,091 (47.8)</td>
<td>9</td>
<td>9</td>
</tr>
<tr>
<td>Broth 4</td>
<td>433</td>
<td>372 (85.9)</td>
<td>6</td>
<td>4</td>
</tr>
<tr>
<td>Broth 5</td>
<td>3,078</td>
<td>2,799 (90.9)</td>
<td>25</td>
<td>12</td>
</tr>
<tr>
<td>Broth 6</td>
<td>1,390</td>
<td>1,380 (99.3)</td>
<td>11</td>
<td>13</td>
</tr>
</tbody>
</table>

* Two successive 46°C incubations were done, the first for 4 to 8 h and the second overnight.

b Mitomycin C (10 ng/ml) was present.

**TABLE 1. Optimum conditions for transposition of Tn917 in B. megaterium**

<table>
<thead>
<tr>
<th>Medium</th>
<th>No. of incubation cycles</th>
<th>% MLS(^+) Cm(^+) clones at indicated temp (°C)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Agar</td>
<td>42</td>
<td>10.8</td>
</tr>
<tr>
<td>Broth</td>
<td>1</td>
<td>18.9</td>
</tr>
<tr>
<td>Broth plus 10 ng of mitomycin C</td>
<td>2</td>
<td>98.6</td>
</tr>
</tbody>
</table>

FIG. 1. Effect of temperature on the stability of pTV1 in B. megaterium. Plasmid stability was measured by scoring chloramphenicol sensitivity following incubation for 16 h at the indicated temperatures in liquid (○) or solid (●) LB media.

ized by growth on the vitamin, amino acid, and base pools of Holliday (6), and then verified. Carbon source mutants were isolated on minimal medium without sodium citrate and with the appropriate sugar substituted for glucose. Auxotrophs were recovered at frequencies ranging from 0.53 to 1.6% of the population cured of pTV1; the broth procedure yielded a slightly higher frequency. The frequency of auxotrophs generated in B. megaterium was less than the 3 to 8% observed in B. subtilis but was similar to the frequency observed for Tn10 transposition in Salmonella typhimurium, an organism in which no insertional hot spot is present (8). When the 69 auxotrophs obtained from the transpositions were tested for their nutritional requirements, eight different classes of auxotrophs as well as seven mutants of undetermined requirements were found. These auxotrophs included 15 cysC, 2 cysA or cysB, 11 trp, 10 his, 9 ilv, 1 pyr, 5 rib, and 9 gly isolates. In addition, when carbon source mutants were screened in one experiment, one mutant unable to use fructose and one mutant defective in lactose, maltose, and sucrose utilization were isolated. None of the classes of mutants was found to occur in a disproportionate abundance. Based upon the frequency and distribution of auxotrophs observed, it is probable that in B. megaterium transposon Tn917 does not have an insertional hot spot such as the glt locus of B. subtilis (16).

Mutations that affected the sporulation process were also
sensitive replication similar to that demonstrated in *B. subtilis*.

We have recently learned that two other researchers have introduced pTV1 into *B. megaterium*. Ann Moir (personal communication) has transformed the plasmid into strain KM, and Curtis Thorne (personal communication) has transformed pTV1 into strain QB1551 (wild type) with the hope of using *B. megaterium* as a restriction-compatible intermediary to introduce Tn917 into *B. anthracis*. To our knowledge, neither has extensively tested Tn917 transposition properties in *B. megaterium*. In summary, we are encouraged by the apparent randomness of Tn917 insertions into the *B. megaterium* chromosome. These random insertions should enable us to obtain almost any type of nonessential mutant for which a screening procedure exists.

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LITERATURE CITED


