Inefficient transformation of *Streptomyces clavuligerus* protoplasts by DNA from the plasmid pIJ702, isolated from *S. lividans*, was attributed to restriction in view of the observation that efficient transformation was observed using modified pIJ702 (isolated from *S. clavuligerus*). The restriction system could be partially inhibited by treating protoplasts at 45 °C prior to transformation. This treatment increased the transformation frequencies of pIJ702 DNA by 100-fold and was used to introduce other plasmids into *S. clavuligerus*.

In *S. griseofuscus*, for example, lack of restriction of phage development was accompanied by efficient plasmid transformation (Cox & Baltz, 1984). Certain streptomycetes appear to be restriction-free. In *S. griseofuscus*, for example, lack of restriction of phage development was accompanied by efficient plasmid transformation (Cox & Baltz, 1984; Larson & Hershberger, 1984). In two restricting species, selection of restriction-deficient mutants has been achieved (Chater & Wilde, 1980; Hunter & Friend, 1984).

In this communication we demonstrate the presence of a restriction and modification system in *S. clavuligerus*, a species which produces several β-lactam antibiotics, including the β-lactamase inhibitor clavulanic acid (Reading & Cole, 1977). We also show the partial inhibition of *in vivo* restriction activity using heat treatment.

**METHODS**

*Bacterial strains.* These were *Streptomyces clavuligerus* ATCC 27064 and *Streptomyces lividans* 66 (John Innes Institute stock no. 1326).

*Media and buffers.* The compositions of YEME, P medium and R2YE (protoplast regeneration medium for *S. lividans*) are given in Chater et al. (1982). R5, the protoplast regeneration medium for *S. clavuligerus*, is described by Bailey et al. (1984). TSB is tryptone soya broth (Oxoid).

*Preparation and transformation of protoplasts.* Techniques for *S. lividans* were as described by Chater et al. (1982). The following modifications to the procedure were made for *S. clavuligerus*. Spores were inoculated into 25 ml TSB, in a 250 ml flask, and grown for 48 h at 26 °C, then 1 ml was transferred to a further 250 ml flask (with a coiled spring to disperse mycelial clumps) containing 10 ml TSB supplemented with 0.5% (w/v) glycine and 15 ml YEME + 34% (w/v) sucrose + 5 mM-MgCl₂ + 0.5% (w/v) glycine. After 24 h, mycelium was harvested and protoplasts prepared according to Chater et al. (1982). Transformations were carried out using 10⁶ protoplasts and 25% (w/v) polyethylene glycol 1000 in P medium. Protoplasts were allowed to regenerate on R5 medium. Transformants were selected from protoplast regeneration plates and purified as described by Chater et al. (1982).
Short communication

Table 1. Comparison of transformation frequencies in *S. clavuligerus* and *S. lividans*

The experiment was repeated on six occasions. Although absolute transformation frequencies varied (up to 10-fold) the relative differences within each experiment were constant. Representative data are given.

<table>
<thead>
<tr>
<th>pIJ702 isolated from</th>
<th>Recipient</th>
<th>Transformants per µg pIJ702</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>S. lividans</em></td>
<td><em>S. lividans</em></td>
<td>2.4 × 10⁶</td>
</tr>
<tr>
<td><em>S. lividans</em></td>
<td><em>S. clavuligerus</em></td>
<td>2.3 × 10²</td>
</tr>
<tr>
<td><em>S. clavuligerus</em></td>
<td><em>S. lividans</em></td>
<td>1.4 × 10⁶</td>
</tr>
<tr>
<td><em>S. clavuligerus</em></td>
<td><em>S. clavuligerus</em></td>
<td>1.1 × 10⁶</td>
</tr>
</tbody>
</table>

Table 2. Effect of protoplast pre-incubation temperature on transformation frequency

The experiment was repeated on four occasions. Although absolute transformation frequencies varied (up to 10-fold) the relative differences within each experiment were constant. Representative data are given.

<table>
<thead>
<tr>
<th>pIJ702 isolated from</th>
<th>Recipient</th>
<th>Room temp.</th>
<th>42°C</th>
<th>45°C</th>
<th>48°C</th>
<th>50°C</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>S. clavuligerus</em></td>
<td><em>S. clavuligerus</em></td>
<td>4.2 × 10⁵</td>
<td>2.8 × 10⁵</td>
<td>1.5 × 10⁵</td>
<td>1.5 × 10²</td>
<td>3.0 × 10¹</td>
</tr>
<tr>
<td><em>S. lividans</em></td>
<td><em>S. clavuligerus</em></td>
<td>5.0 × 10¹</td>
<td>8.0 × 10²</td>
<td>5.4 × 10³</td>
<td>4.0 × 10¹</td>
<td>0</td>
</tr>
<tr>
<td><em>S. lividans</em></td>
<td><em>S. lividans</em></td>
<td>3.6 × 10⁶</td>
<td>1.2 × 10⁶</td>
<td>2.0 × 10⁵</td>
<td>7.1 × 10⁴</td>
<td>1.0 × 10³</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Protoplast regeneration (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>S. clavuligerus</em></td>
</tr>
<tr>
<td><em>S. lividans</em></td>
</tr>
</tbody>
</table>

selected by overlaying regeneration plates with 2.5 ml soft nutrient agar containing 50 µg thiostrepton ml⁻¹ and incubating for 48 h at 26 ºC to identify resistant colonies.

Heat treatment of protoplasts. This was done at the stage just prior to transformation. Protoplasts were pelleted and suspended in the small volume of P medium remaining after the supernatant was poured off. The tube was then incubated in a water bath for 10 min at the temperatures given in Table 2. Transformation was then carried out as above.

Isolation of plasmid DNA. The methods described by Kieser (1984) and Chater et al. (1982) were used.

RESULTS AND DISCUSSION

The 5.6 kb multicopy plasmid vector, pIJ702 (Katz et al., 1983), was used in these studies. Firstly, a comparison was made of transformation frequencies in *S. clavuligerus* and *S. lividans*, a species containing no defined restriction barriers (Chater & Bruton, 1983). The results in Table 1 show that using pIJ702 prepared from *S. lividans* the frequency of transformation in *S. clavuligerus* was very low (10⁴ fewer transformants than *S. lividans*). However, when pIJ702 was isolated from *S. clavuligerus* and used in transformation, the frequencies were high in both species. The most likely interpretation of these results is that *S. clavuligerus* possesses a restriction system, which leads to very inefficient transformation with foreign DNA, and a modification system, which allows DNA to avoid this restriction barrier. For self-cloning experiments in *S. clavuligerus*, the vector DNA must be modified (Bailey et al., 1984).

In order to use *S. clavuligerus* as a recipient for foreign DNA we attempted to inhibit the restriction system by heat treatment. Table 2 shows the effect of protoplast pre-incubation temperature on transformation frequencies. At 45 ºC there was a 10²-fold increase in frequency of transformation with pIJ702 prepared from *S. lividans*. At higher temperatures the viability of the protoplasts was severely impaired. Further experiments showed that heat treatment times up to 30 min gave no further increase in transformation frequency.

As there was no stimulation of transformation using modified DNA in *S. clavuligerus*, or in *S.
The heat treatment appeared to be affecting the ability of S. clavuligerus to restrict foreign plJ702 DNA. The effect is not specific to plJ702 DNA as the technique was also used to introduce into S. clavuligerus SCP2-derived low-copy-number vectors, such as plJ913 (Lydiate et al., 1985), and SLP1-derived vectors, such as plJ41 (Thompson et al., 1982). Under standard conditions, no transformants were found using plJ913 or plJ41, but following heat treatment up to \(1 \times 10^3\) transformants per \(\mu\)g were obtained.

There is now much interest in the production of new hybrid antibiotics by the transfer of cloned antibiotic biosynthetic genes isolated from one antibiotic producer into a recipient strain. We have demonstrated that in S. clavuligerus a potential barrier (an active restriction system) to this process can be inactivated by a simple procedure. This may allow the use of the industrially important strain, S. clavuligerus, as a recipient for ‘foreign’ antibiotic biosynthetic genes.

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REFERENCES


