Replication and expression of plasmids from *Staphylococcus aureus* in *Bacillus subtilis*

(DNA/genetic transformation/molecular cloning/biohazards)

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ABSTRACT One *S. aureus* plasmid coding for tetracycline resistance, pT127, and four plasmids (pC194, pC221, pC223, and pUB112) coding for chloramphenicol resistance have been introduced by transformation into *B. subtilis*. The plasmids replicate in—and confer antibiotic resistance upon—their new host. These experiments show that the potential for genetic exchange between diverse bacterial species is greater than has been commonly assumed.

Most of the plasmids studied so far have a narrow host range. Some, however, can replicate in a wider host range. For example, plasmid RP4 from *Pseudomonas aeruginosa* can be transferred to other Gram-negative bacteria such as *Escherichia coli*, *Salmonella typhimurium*, *Klebsiella aerogenes*, *Rhizobium leguminosarum*, and *Agrobacterium tumefaciens* (1).

Another instance of plasmid-replicon transfer among bacterial species, perhaps even more widely separated, is reported in this work. Five *Staphylococcus aureus* plasmids, coding for tetracycline or chloramphenicol resistance, have been introduced by direct DNA transformation into *Bacillus subtilis*. The plasmids can replicate and express their genetic information (antibiotic resistance) in this new host.

**MATERIALS AND METHODS**

**Bacterial Strains.** *S. aureus* strains used were SA291 (pC194) Cm"<sup>+</sup>, RN154 (pC223) Cm"<sup>+</sup>, RN1305 (pC221) Cm"<sup>+</sup>, RN1777 (pS177) Sm"<sup>+</sup>, RN1801 (pT127) Tc"<sup>+</sup>, RN1953 (pK545) Km"<sup>+</sup>/Nm"<sup>+</sup>, and RN2438 (pUB112) Cm"<sup>+</sup> from R. Novick (2). *B. subtilis* strains SB634 thy"<sup>-</sup> araB <sup>-</sup> tyr-1 and SB748 his-2 trpC2 thy"<sup>-</sup> were derived from SB168, are from the Stanford collection.

**Media.** *B. subtilis* was grown in L and Penassay liquid media. Resistant bacteria were selected on L agar plates supplemented with antibiotics (tetracycline (Tc), 15 µg/ml; chloramphenical (Cm), 5 µg/ml; streptomycin (Sm), 30 µg/ml; kanamycin (Km), 3 µg/ml) *S. aureus* cells were grown in CY liquid media or on GL-agar plates.

**DNAs and Enzymes.** Plasmid DNAs were prepared from *S. aureus* strains essentially as described by Novick (2). Low-salt lysates of stationary phase cultures were clarified by centrifugation, concentrated with polyethylene glycol (molecular weight; 6000), and centrifuged to equilibrium in CsCl density gradients containing ethidium bromide. Cleared lys treatment of *B. subtilis* strains was essentially as described for *E. coli* (4). Lysates were then processed similarly to the *S. aureus* ones (see above).

EcoRI endonuclease and T4 ligase were purified and used as described (5, 6). *HindIII* was a commercial preparation (BioLabs).

Abbreviations: Tc, tetracycline; Cm, chloramphenicol; Sm, streptomycin; Km, kanamycin; Nm, neomycin.

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Transformation Procedure. Induction of competence and transformation of *B. subtilis* were as described (7).

**RESULTS**

**Tetracycline resistance plasmid pT127**

*S. aureus* strain RN1801 carries the plasmid pT127 that confers tetracycline resistance on its host (3). The plasmic DNA can transform *B. subtilis* strains to tetracycline resistance: 10<sup>7</sup> cells of SB634 (competence level 0.2%) exposed to 0.1 µg of pT127 DNA yielded 10 Tc<sup>+</sup> colonies. No colonies were observed if either the cells or the DNA was omitted. The typical appearance and phenotypic match of the SB634 parent strain, auxotrophic thy"<sup>-</sup>araB"<sup>-</sup> tyr"<sup>-</sup>, to the Tc<sup>+</sup> colonies confirmed that the colonies were *B. subtilis*.

The level of resistance of the transformants was higher than 25 µg/ml in L-broth, although at concentrations above 15 µg/ml some inhibition of growth could be seen. The parental *B. subtilis* strain is inhibited by 5 µg/ml of Tc. Resistance was found to be a stable trait: growth for some 20 generations in liquid medium devoid of antibiotic, followed by plating on the solid medium of the same type, resulted in less than 2% observed colonies sensitive to tetracycline, as revealed by replica-plating on medium supplemented with the drug.

One of the Tc<sup>+</sup> colonies was chosen for further study. A profile of the cesium chloride/ethidium bromide density gradient for its cleared lys supernatant is displayed in Fig. 1. Two peaks of radioactivity can be seen. Identically treated-parental Tc<sup>+</sup> cells yielded only the lower density peak (Fig. 1) composed of linear DNA molecules.

The heavier peak, detected in the extract of Tc<sup>+</sup> transformants, contains supercoiled circular DNA molecules, as revealed by electron microscopy. These match the pT127 DNA molecules isolated from *S. aureus*, by the following criteria: (i) the two intact circular DNA preparations have identical electrophoretic mobilities; (ii) they are both resistant to EcoRI endonuclease and are cleaved by the *HindIII* nuclease into three matching segments. These data are displayed in Fig. 2.

Equivalence of the two DNA preparations was verified by further genetic tests: two *B. subtilis* strains, SB634 and SB748, were transformed to tetracycline resistance with the DNA isolated from the Tc<sup>+</sup> *Bacillus* colonies, as well as with the pT127 DNA isolated from *S. aureus*.

**Chloramphenicol resistance plasmids**

Four *S. aureus* Cm<sup>+</sup> plasmids (pC194, pC221, pC223, and pUB112) were tested for their ability to transform *B. subtilis* to chloramphenicol resistance. The results were similar to those described for the Tc<sup>+</sup> plasmid, above.
The results determined that antibiotic-resistant transformants, upon exposure to competent cells, can transform other B. subtilis cells for the same genetic determinant.

Streptomycin and kanamycin/neomycin plasmids

The results obtained with pS177Smr and pK545Km's-Nmr plasmids differ from those obtained with the Tc' and Cm'. We

Table 1. Resistance markers, size, and HindIII sites of S. aureus plasmids*

<table>
<thead>
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<th>Plasmid</th>
<th>Resistance marker</th>
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<th>No. of HindIII sites</th>
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<tr>
<td>pC194</td>
<td>Cm</td>
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<td>1</td>
</tr>
<tr>
<td>pC221</td>
<td>Cm</td>
<td>3.0</td>
<td>1</td>
</tr>
<tr>
<td>pC223</td>
<td>Cm</td>
<td>3.0</td>
<td>1</td>
</tr>
<tr>
<td>pUB112</td>
<td>Cm</td>
<td>3.0</td>
<td>1</td>
</tr>
<tr>
<td>pT127</td>
<td>Tc</td>
<td>2.9</td>
<td>3‡</td>
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* None of the plasmids has an EcoRI-sensitive site.
† From Novick (2) and confirmed here by comparing cleaved DNAs with the B. subtilis phage Phi-3-T EcoRI segments (5).
‡ Sizes of the segments are 1.5, 0.9, and 0.4 million, respectively.

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these experiments only a small fraction of the DNA is taken up.) The efficiency increased some 50 times, approaching the level of \(10^{-7}\) colonies per genome equivalent (\(10^9\) colonies per \(\mu\)g of DNA) with plasmids isolated from transformed \(B.\) subtilis cells. Less than 0.5% of the efficiency remained after cleavage of plasmid DNAs with the \(HindIII\) restriction endonuclease. Treatment of cleaved DNAs with T4 ligase, resulting in about 50% recircularization (as revealed by electron microscopy inspection), restored 30–50% of the original efficiency for \(Cm^r\) plasmids, and less than 0.2% for the \(Tc^r\) one.

**DISCUSSION**

The experiments reported here indicate that (i) \(B.\) subtilis strains can be transformed to antibiotic resistance with \(S.\) aureus plasmid DNAs; (ii) the transformants acquire plasmid DNA; (iii) this DNA is indistinguishable from the \(S.\) aureus plasmid DNA by criteria of size, restriction enzyme pattern, and genetic information. This evidence shows that \(S.\) aureus plasmids can replicate and be expressed in \(B.\) subtilis.

The five \(S.\) aureus plasmids introduced in \(B.\) subtilis can be subdivided into three groups: \(Tc^r\) (pT127), \(Cm^r\) small (pC194) and \(Cm^r\) large (pC221, pC223, and pUB112, Table 1). Data reported by Novick (3) indicate that the three plasmids of the last group are not identical: they respond differently to various treatments which induce relaxation of supercoiled plasmid–protein complexes. It appears therefore that at least five different \(S.\) aureus plasmids can be maintained in \(B.\) subtilis.

The efficiency of interspecies transformation described here is high enough to allow the process to be demonstrated easily in the laboratory by employing competent cells and plasmid DNA.

\(HindIII\) restriction endonuclease cleavage of the plasmids decreases their biological activity to < 0.5%. This might be due to the possible presence of the restriction site within the gene coding for the antibiotic resistance and/or to the destruction of the circular structure of the plasmids necessary for their replication in the host. Another plasmid, pFT23, a hybrid between the pSC101 replicon and the \(thy\) gene of the \(B.\) subtilis phage Phi-3-T, did not lose any transforming efficiency when made linear by the action of \(Bam\) endonuclease (5). In that case, circular structure was not obligatory because the transforming \(thy\) gene could be integrated into another replicating structure: the chromosome of the host. Ligation of the cleaved \(Cm^r\) plasmids almost fully restored their biological activity, whereas the \(Tc^r\) plasmid was not reactivated, presumably because of a lower probability of correct reassembly of the three \(HindIII\) segments.

\(S.\) aureus plasmids introduced into \(B.\) subtilis are promising vectors for cloning in this new host because of their small size, easily selectable markers, and a small number of cleavage sites for certain restriction enzymes.

The demonstration that replicating plasmids are shared among species of bacteria as widely diverse as \(Staphylococcus\) and \(Bacillus\), or \(Escherichia\) and \(Agrobacterium\) [\(P.\) aeruginosa plasmid RP4, (1)] makes it likely that plasmid sharing occurs commonly in nature. This is pertinent to our views of natural microbial evolution and, in turn, to the uniqueness of constructing DNA recombinants in the laboratory, which is a premise of much policy discussion.

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