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Genetics

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 SA231 (pC194) Cm^r, RN154 (pC223) Cm^r, RN1305 (pC221) Cm^r, RN1777 (pS177) Sm^r, RN1801 (pT127) Tc^r, RN1953 (pK545) Km^r/Nm^r, and RN2438 (pUB112) Cm^r from R. Novick (2). *B. subtilis* strains SB634 *thy⁻ aroB tyr-1* and SB748 *his-2 trypC2 thy⁻*, 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 (3).

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 lysis 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).

*Eco*RI endonuclease and T4 ligase were purified and used as described (5, 6). *Hin*dIII was a commercial preparation (BioLabs).

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

Electrophoresis and Electron Microscopy. Horizontal agarose slab gels were used as described (8). Electron microscopy was also performed (9).

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: 10^7 cells of SB634 (competence level 0.2%) exposed to 0.1 μg of pT127 DNA yielded 10 Tc^r 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⁻aroB⁻tyr⁻*, to the Tc^r 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^r colonies was chosen for further study. A profile of the cesium chloride/ethidium bromide density gradient for its cleared lysis supernatant is displayed in Fig. 1. Two peaks of radioactivity can be seen. Identically treated-parental Tc^s cells yielded only the lower density peak (Fig. 1) composed of linear DNA molecules.

The heavier peak, detected in the extract of Tc^r transformants, contains supercoiled circular DNA molecules, as revealed by electron microscopy. These match the pT127 DNA molecules extracted from *S. aureus*, by the following criteria: (*i*) the two intact circular DNA preparations have identical electrophoretic mobilities; (*ii*) they are both resistant to *Eco*RI endonuclease and are cleaved by the *Hin*dIII 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^r *Bacillus* colonies, as well as with the pT127 DNA isolated from *S. aureus*.

Chloramphenicol resistance plasmids

Four *S. aureus* Cm^r 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^r plasmid, above.

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

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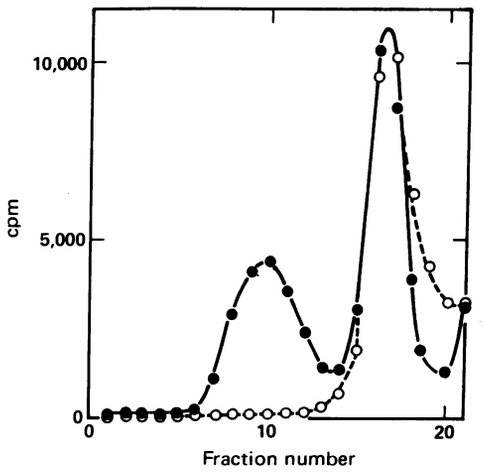

FIG. 1. Cesium chloride/ethidium bromide gradient of *B. subtilis* cleared lysates. We used a cleared-lysis procedure (see *Materials and Methods*) on 200 ml of a tritium-labeled *B. subtilis* culture (1 μCi of [^{3}H]thymidine per ml). The concentrated supernatant was centrifuged for 36 hr at 36,000 rpm in a Spinco 50 rotor. Tubes were punctured at the bottom, and 0.22-ml fractions were collected. A 5-μl aliquot of each fraction was assayed for radioactivity. The solid line corresponds to a Tc^r transformant; dashed line corresponds to the parental strain. Density increases from right to left. Specific activity of DNA was 5000 cpm/μg.

(*i*) *B. subtilis* transformants resistant to 50 μg/ml of chloramphenicol (parental strains are sensitive to 5 μg/ml) were obtained at about the same frequency as the Tc^r transformants, upon exposing competent cells to plasmid DNAs. Growth without antibiotic for 20 generations led to the loss of resistance in less then 2% of cells transformed with pC194, about 10% in those transformed with pUB112, and about 20% for the other two plasmids.

(*ii*) Cm^r transformants contain plasmid DNA indistinguishable from that isolated from the corresponding *S. aureus* strains as indicated by (*a*) electrophoretic mobility of intact DNAs and (*b*) response toward *Eco*RI and *Hin*dIII restriction enzymes. The number of restriction sites detected in various plasmids is indicated in Table 1, together with their molecular weights.

(*iii*) Plasmid DNAs isolated from the transformed *B. subtilis* cells carry the information specifying chloramphenicol resistance and can transform other *B. subtilis* cells for the same genetic determinant.

Streptomycin and kanamycin/neomycin plasmids

The results obtained with pS177Sm^r and pK545Km^r-Nm^r plasmids differ from those obtained with the Tc^r and Cm^r. We did not observe *B. subtilis* transformants resistant to streptomycin or kanamycin. A number of explanations could be put forward—these plasmids might fail to replicate or to express their genetic information in the new host. Alternatively, a particular regimen of selection might be required to detect the transformants, because in both cases *B. subtilis* colonies resistant to antibiotic, due to endogenous mutations, were observed at a frequency of about 10^{-7} for Km, 10^{-6} for Sm.

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

Plasmid	Resistance marker	M_r,† millions	No. of *Hin*dIII sites
pC194	Cm	1.8	1
pC221	Cm	3.0	1
pC223	Cm	3.0	1
pUB112	Cm	3.0	1
pT127	Tc	2.9	3‡

* None of the plasmids has an *Eco*RI-sensitive site.
† From Novick (2) and confirmed here by comparing cleaved DNAs with the *B. subtilis* phage Phi-3-T *Eco*RI segments (5). M_r, molecular weight.
‡ Sizes of the segments are 1.5, 0.9, and 0.4 million, respectively.

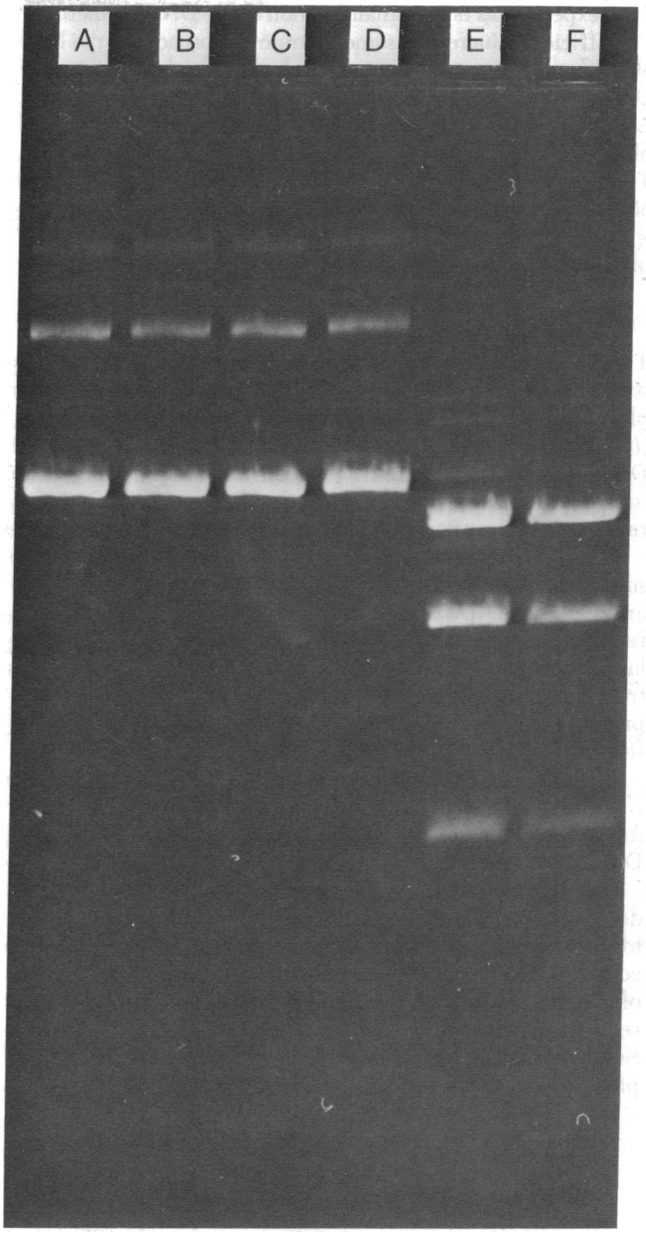

FIG. 2. Agarose gel electrophoresis of plasmid pT127 DNA. Lanes A, C, and E contain DNA extracted from *S. aureus;* lanes B, D, and F contain DNA from *B. subtilis.* Untreated DNA is in lanes A and B, *Eco*RI-treated DNA in lanes C and D, and *Hin*dIII-cleaved DNA is in lanes E and F.

Efficiency of transformation of *B. subtilis* with plasmid DNAs

B. subtilis strains of competence greater than 0.1% could be transformed with *S. aureus* plasmids at an efficiency of about 10^{-9} colonies per genome equivalent, that is, close to 100 colonies per μg of DNA. Colonies per genome equivalent are expressed on the basis of *input* DNA. (Under the conditions of

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 (10^4 colonies per μg 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 *Hin*dIII 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.

*Hin*dIII 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 *Hin*dIII 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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