SHORT COMMUNICATION

Transfer of Plasmids among Bacilli

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Plasmids were transferred from *Staphylococcus aureus* to *Bacillus subtilis* and between *B. subtilis* and several other species of *Bacillus* by protoplast fusion and regeneration. The plasmids replicate and express themselves normally in all bacilli examined.

INTRODUCTION

When protoplast preparations from two different strains are fused, genetic exchange between the chromosomes may result. This has been demonstrated for *Bacillus subtilis* (Schaeffer *et al.*, 1976), *B. megaterium* (Fodor & Alföldi, 1976) and *Streptomyces* spp. (Hopwood *et al.*, 1977). The method relies on fusion promoted by polyethyleneglycol or calcium ions followed by regeneration of the cell wall on a solid medium. In addition, Chang & Cohen (1979) showed that protoplasts of *B. subtilis* in the presence of polyethyleneglycol could be transformed by plasmids prepared from *Staphylococcus aureus*. The plasmids were able to replicate and express themselves in *B. subtilis* (Ehrlich, 1977) and have been suggested as possible cloning vehicles. Considerable progress has been made in understanding the interaction between plasmids and their new host (Shivakumar & Dubnau, 1978; Gryczan *et al.*, 1978, 1980).

I have used similar methods of protoplast fusion to those of Schaeffer *et al.* (1976) in interspecific crosses between *S. aureus* and *B. subtilis* and between different *Bacillus* species. Little, if any, interspecific chromosomal DNA exchange occurred, but *S. aureus* plasmids specifying chloramphenicol resistance were transferred at quite high frequency. This provides a quick and easy method for introducing *S. aureus* plasmids into widely varying strains of bacilli and from one species of *Bacillus* to another.

METHODS

Strains. *Staphylococcus aureus* 8325-4 derivatives containing Cm^R (chloramphenicol-resistance) plasmids pCW6, pCW8 and pC221 (Wilson & Baldwin, 1978) were used. The *Bacillus subtilis* strains employed were MB21 (*leu-8 metC3*), MB24 (*trpC2 metC3 rif-2*) and SB634 (*aroB tyr-1 thyA thyB* pC221). The plasmid in strain SB634 had been inserted by transformation of competent cells by a preparation from *S. aureus* 8325-4 (pC221) (W. Shaw, personal communication). Other bacilli used were *B. licheniformis* NCTC 6346, *B. polymyxa* ATCC 2459, *B. megaterium* KM (sporogenic) and *B. megaterium* KM (asporogenic). Both strains of *B. megaterium* were obtained from Dr K. McQuillen and are sensitive to lysozyme.

Growth of strains. Bacteria were grown in Oxoid brain–heart infusion broth, supplemented, as necessary, with chloramphenicol (Cm) at 5 µg ml⁻¹ and rifampicin (Rif) at 5 µg ml⁻¹. Rif-resistant mutants were selected by plating about 10¹⁰ organisms on Oxoid nutrient agar supplemented with Rif at 5 µg ml⁻¹, incubating at 37 °C for 24 h and purifying the resistant clones.

Fusion procedure. A modification of the method of Schaeffer *et al.* (1976) was used. Bacteria were grown to about 100 µg dry wt equivalent ml⁻¹; then 15 ml of each culture was centrifuged and the pellet was

suspended in 3 ml tryptone-yeast extract-sucrose broth (TYE-sucrose; Elliott *et al.*, 1975) containing lysozyme at 200 $\mu\text{g ml}^{-1}$ (or Sigma lysostaphin at 100 $\mu\text{g ml}^{-1}$ for *S. aureus*). The cultures were shaken gently at 37 °C until all the cells had formed protoplasts. Equal portions (1 ml) were removed from each culture and mixed, and this mixture and the two parent protoplast preparations were centrifuged. The pellets were taken up in 40 % (w/v) polyethyleneglycol 6000 in TYE-sucrose (PEG-TYE-sucrose) or 0.5 M-sucrose, 0.02 M-maleate, 0.02 M-MgCl₂, pH 6.5 (PEG-SMM; Schaeffer *et al.*, 1976) and plated on a rich regeneration agar derived from that of Wyrick & Rogers (1973). The agar medium consisted of (% w/v): Oxoid casein hydrolysate, 0.5; K₂HPO₄, 0.35; KH₂PO₄, 0.15; sodium succinate, 20; MgCl₂, 0.4; glucose, 0.5; and Difco agar, 2.5. Chloramphenicol (5 $\mu\text{g ml}^{-1}$) and rifampicin (5 $\mu\text{g ml}^{-1}$) were included when necessary.

Plasmid isolation and agarose electrophoresis. Plasmids were isolated from *S. aureus* by the method of Stiffler *et al.* (1974) and from *B. subtilis* by the method of Gryczan *et al.* (1978). The preparations were electrophoresed in 0.8 % (w/v) agarose gels by the method of Wilson & Baldwin (1978).

RESULTS AND DISCUSSION

Transfer of plasmids from S. aureus to B. subtilis

When protoplasts of *B. subtilis* MB21 and *S. aureus* carrying either pCW6, pCW8 or pC221 were exposed to PEG-TYE-sucrose and plated, a few colonies appeared after 2 d on CM-supplemented regeneration plates that had received undiluted, mixed protoplast suspensions. On the succeeding 2 to 3 d more colonies appeared, all of which were Cm-resistant bacilli that were sporogenic and auxotrophic for methionine and leucine, i.e. derivatives of strain MB21. No colonies were ever found on CM-supplemented regeneration plates that had received the unmixed, control protoplast preparations, and no *S. aureus* were ever seen to regenerate. The maximum number of colonies (after 5 d) was about 200 for crosses involving pCW8 and about 50 for pCW6 and pC221. That the plasmid was conferring resistance was shown by agarose gel electrophoresis. Plasmid DNA isolated from the Cm-resistant *B. subtilis* was indistinguishable in molecular weight from that isolated from *S. aureus*, whereas no plasmid DNA was detected in extracts of the parent *B. subtilis* MB21. Preparations of pC221 from *B. subtilis* SB634 and from *B. subtilis* MB21 made Cm-resistant in the experiments above with *S. aureus* 8325-4 (pC221) appeared to be identical on agarose gels. This indicated that neither method of transfer (transformation or polyethyleneglycol treatment) caused physical modification of the plasmid. However, it is likely that the mode of transfer in the experiments with polyethyleneglycol was caused by lysis of the *S. aureus* protoplasts followed by transformation of the *B. subtilis* protoplasts. This would explain the asynchrony of appearance of resistant colonies. Also, if 1 μg deoxyribonuclease I ml^{-1} was included in the regeneration agar, then no resistant colonies appeared. When mixed cells or protoplasts were exposed to TYE-sucrose without polyethyleneglycol, Cm-resistant *B. subtilis* did not appear. Perhaps the polyethyleneglycol, which caused clumping of the protoplasts, put the two strains in intimate association.

Transfer of plasmids among bacilli

When *B. subtilis* SB634 and *B. megaterium* KM (sporogenic) protoplasts were exposed to PEG-SMM and plated on Cm-supplemented regeneration agar, the *B. subtilis* were unable to grow because of lack of thymidine. After 2 d at 42 °C many colonies (about 200 per plate receiving undiluted preparation) of *B. megaterium* appeared, whereas preparations that had not received polyethyleneglycol or that consisted of a single strain showed no colonies. The *B. megaterium* colonies were recognized by their colonial and microscopic appearance. The Cm-resistant *B. megaterium* derived in the cross just described was then used as a donor. It was fused with *B. subtilis* MB24 and plated on regeneration agar containing Cm and Rif. Colonies of *B. subtilis* developed that retained the genetic markers of MB24 but had acquired Cm-resistance (Table 1).

None of the crosses between *B. subtilis* and *B. megaterium* resulted in transfer of the chromosomal Rif^R marker from the former to the latter. Thus, although plasmids are

Table 1. *Plasmid transfer among bacilli*

Protoplasts of donor (plasmid-bearing) and recipient (plasmid-deficient) strains were mixed, fused and plated on selective regeneration agar. In each case, individual strains and unfused, mixed protoplasts gave no colonies.

Recipient	Donor	Counter-selection against donor	Cm ^R colonies/ 10 ⁻⁶ × regenerable colonies	Frequency*
<i>B. megaterium</i> KM (sporogenic)	<i>B. subtilis</i> SB634	Thy starvation	227/10	2.2 × 10 ⁻⁵
<i>B. subtilis</i> MB24	<i>B. megaterium</i> KM (sporogenic) (pC221)	Rif sensitivity	500/15	3.3 × 10 ⁻⁵
<i>B. megaterium</i> KM (sporogenic) (Rif ^R)	<i>B. subtilis</i> MB21 (pC221)	Rif sensitivity	163/12	1.3 × 10 ⁻⁵
<i>B. megaterium</i> KM (asporogenic)	<i>B. subtilis</i> SB634	Thy starvation	70/10	7.0 × 10 ⁻⁶
<i>B. licheniformis</i> 6346 (Rif ^R)	<i>B. subtilis</i> MB21 (pC221)	Rif sensitivity	109/8	1.3 × 10 ⁻⁵
<i>B. polymyxa</i> 2459	<i>B. subtilis</i> SB634	Thy starvation	436/18	2.4 × 10 ⁻⁵

* (No. of resistant recipients ml⁻¹)/(no. of colonies regenerated in the absence of Cm ml⁻¹).

readily transferred during fusion it seems that chromosomal markers are transferred very rarely, if at all.

By the method of protoplast fusion Cm-resistance was transferred from *B. subtilis* to *B. megaterium* KM (asporogenic), *B. licheniformis* and *B. polymyxa*. (Recognition of strains was based on colony morphology and microscopic appearance of the cells.) The frequency of transfer varied somewhat, though controls never showed spontaneous acquisition of resistance (Table 1). Transfer of the plasmid among bacilli was unaffected by inclusion of 10 µg deoxyribonuclease I ml⁻¹ in the regeneration agar, and treatment of protoplasts with polyethyleneglycol was obligatory for plasmid transfer. In all cases examined, the strain receiving the plasmid did not additionally acquire other markers and the strains were indistinguishable morphologically from the parents. In particular, the presence of the plasmid did not appear to affect the ability of the strain to sporulate. In the case of *B. polymyxa* the sporangia retained the swollen appearance characteristic of this species. It seemed that pC221 is included in the spore during sporulation because heat-treated (80 °C, 15 min) spores of Cm-resistant derivatives of *B. subtilis*, *B. megaterium* KM (sporogenic) and *B. licheniformis* were able to grow out normally on nutrient agar containing Cm.

These experiments indicate that protoplast fusion can be used to transfer plasmids among widely different bacilli so long as they are able to regenerate their cell walls. The Cm^R plasmids of *S. aureus* have attracted attention as potential cloning vehicles in *B. subtilis* (Ehrlich, 1977; Gryczan & Dubnau, 1978) and the observations described here may enable this to be extended to other bacilli.

The technique is, in effect, a simplification of that described by Chang & Cohen (1979) in that protoplasts instead of purified plasmid preparations are used as donors. This considerably reduces the time taken to do the crosses. The method described by Feitelson & Lederberg (1980) for transfer of *S. aureus* plasmids to *B. subtilis* uses crude lysates and competent *B. subtilis*. The procedure described here does not require that the *B. subtilis* be made competent.

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