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SHORT COMMUNICATION

Crude Lysates of *Staphylococcus aureus* Can Transform *Bacillus subtilis*

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Plasmids can be transferred from *Staphylococcus aureus* to *Bacillus subtilis* by crude lysates prepared with penicillin or lysostaphin. These lysates mediate drug-resistance plasmid transformation in competent *B. subtilis* at an efficiency paralleling that of purified DNA.

INTRODUCTION

Purified plasmid DNA from *Staphylococcus aureus* coding for drug resistance can transform, replicate and express its genetic information in *Bacillus subtilis* (Ehrlich, 1977). The regimen employed in the laboratory involved extensive purification of the plasmid DNA. We show here that heterospecific transformation of *B. subtilis* can occur using crude lysates of plasmid-harbouring cells, a regimen much more likely to be encountered under natural conditions.

Initially, a variety of mixed cultures of the two organisms were tested for plasmid transfer but without success. It is known that competent cells are biosynthetically latent and relatively insensitive to killing by penicillin G (Nester, 1963). Therefore, a penicillin-induced lysate of plasmid-harbouring *S. aureus*, when added to competent *B. subtilis*, should have minimal effect in killing the recipient bacteria but at the same time could promote plasmid transfer. Another lytic agent with selective killing effects on the donor *S. aureus* is lysostaphin. Crude lysates made with lysostaphin were also mixed with competent cells and found to mediate plasmid transfer.

METHODS

Strains. Staphylococcus aureus donor strains bearing Cm^r plasmids were as follows (Novick, 1976): RN154(pC223), RN1305(pC221), RN2438(pUB112) and SA231(pC194). The plasmids were all originally transduced by Novick into the same plasmid-free host strain, designated RN450, resulting in an isogenic host background. The *B. subtilis* recipient strain SB863, derived from SB168, had the Sm^r (*str-2*) gene as well as the *aroB1*, *trpC2*, *tyrA*, *hisA*, *cys-1* and *leu-1* markers (Stanford collection). Bacteria were grown in Luria broth (Miller, 1972). Resistant organisms were selected on Difco nutrient agar supplemented with antibiotics [chloramphenicol (Cm) at 15 μg ml^{-1}; streptomycin (Sm) at 30 μg ml^{-1}].

DNA preparations. Plasmids were purified from *S. aureus* by the low-salt lysis of stationary phase cultures using clear lysis followed by CsCl–ethidium bromide density gradient centrifugation (Novick, 1976), and from *B. subtilis* by a rapid screening method using 0·7% (w/v) agarose gel electrophoresis (Meyers *et al.*, 1976). Lysostaphin lysates of *S. aureus* were made by growing cultures to mid-exponential phase (about 2×10^8 ml^{-1}), centrifuging, resuspending the cell pellet in 1 ml 20 mM-Tris/HCl containing 2 mM-EDTA, pH 8·0 (TE buffer) and adding lysostaphin (Schwarz-Mann) to a final concentration of 50 μg ml^{-1}; lysis occurred after incubation at 37 °C for 15 min. Penicillin G lysates were made by adding 0·6 μg penicillin

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G ml^{-1} to the culture and allowing growth to continue for 3 h; the culture was then divided into two and penicillinase was added to one half to inactivate the antibiotic.

Competence procedure and transformation. Bacteria were made competent by using C1 medium at 37 °C for growth and C2 medium at 30 °C for induction of competence (Stewart, 1969); 0·8% of the viable cells could be transformed for a single marker using prototrophic DNA. For transformation, 0·5 ml of one of the *S. aureus* lysates was added to 5 ml of competent *B. subtilis* (approximately 10^8 organisms) and incubated at 37 °C for 30 min. Then 2 vol. L broth was added and the bacteria were grown for a further 2 h before concentration and plating.

RESULTS AND DISCUSSION

To test for *in vivo* plasmid transfer from *S. aureus* to *B. subtilis*, three mixed culture experiments were performed. The *B. subtilis* recipients were chromosomally Sm^r, while the *S. aureus* donors had plasmid-coded Cm^r. Growing the two cultures separately and mixing them in broth, or growing the two cultures together in liquid or on non-selective plates, prior to plating on selective plates, all gave uniformly negative results. The only colonies growing on doubly selective media were *S. aureus* Sm^r mutants, arising at a frequency of 10^{-8} to 10^{-10}. No plasmid transfer was detected.

To test whether penicillin-lysed *S. aureus* could transform *B. subtilis*, four Cm^r *S. aureus* strains were grown separately to mid-exponential phase (about 2×10^8 ml^{-1}) and then divided into two portions for penicillin or lysostaphin lysis. Three preparations (lysostaphin

Table 1. *Plasmid transfer from crude lysates of S. aureus to B. subtilis*

Competent (0·8%) and non-competent (late-exponential phase culture not treated by the competence-induction protocol) *B. subtilis* SB863 were used as recipients for *S. aureus* crude lysates prepared by treatment with (i) lysostaphin (Lyso), (ii) penicillin followed after 3 h by penicillinase (Pen'ase) or (iii) penicillin alone (Pen).

S. aureus strain and lysate		Competence of SB863*	Cm^r colonies/ $10^{-9} \times$ viable colonies	Frequency†
SA231(pC194)	Lyso	+	0/3	0
	Lyso	−	0/3	0
	Pen'ase	+	0/1·4	0
	Pen'ase	−	0/3·9	0
	Pen	+	1/0·03	3×10^{-8}
	Pen	−	0/5·2	0
RN154(pC223)	Lyso	+	0/2·6	0
	Lyso	−	0/5·6	0
	Pen'ase	+	0/2·1	0
	Pen'ase	−	0/4·8	0
	Pen	+	0/0·02	0
	Pen	−	0/4·9	0
RN1305(pC221)	Lyso	+	18/3·6	5×10^{-9}
	Lyso	−	0/6·4	0
	Pen'ase	+	70/2·5	$2{\cdot}8 \times 10^{-8}$
	Pen'ase	−	0/5·9	0
	Pen	+	4/0·15	$2{\cdot}7 \times 10^{-8}$
	Pen	−	0/4·9	0
RN2438(pUB112)	Lyso	+	0/1·5	0
	Lyso	−	0/5·8	0
	Pen'ase	+	0/1·4	0
	Pen'ase	−	0/4·9‡	0
	Pen	+	0/0·01	0
	Pen	−	0/2·9	0
SB863 control		+	0/1·9	0

* +, Competent; −, non-competent.
† (No. of transformants ml^{-1})/(no. of viable cells ml^{-1}).
‡ There was one Sm^rCm^r *S. aureus* on this plate.

lysate, penicillin plus penicillinase lysate, and penicillin lysate) were used in transformation experiments with both competent and non-competent *B. subtilis*. It is evident (Table 1) that only cells subjected to the competence regime were transformed. Strain RN1305, harbouring plasmid pC221 (Novick, 1976), gave a positive result, with transformation of competent *B. subtilis* to Cm^r occurring at a frequency of 5×10^{-9} to 3×10^{-8}. This frequency is comparable to that using purified *S. aureus* plasmid DNA, about 5×10^{-9} colonies per genome equivalent (Ehrlich, 1977).

Since the donor *S. aureus* strains were isogenic except for the presence of plasmids, the transfer frequency into *B. subtilis* is a property of the plasmid and not of the donor genetic background. The molecular weights of the plasmids do not strongly influence the transformation efficiency, as pC221, pC223 and pUB112 are all 3×10^6 daltons, while pC194 is $1{\cdot}8 \times 10^6$ daltons (Ehrlich, 1977).

Plasmids were extracted from cultures grown from two randomly selected colonies from each *B. subtilis* transformant class, except for the unique transformant generated from the SA231 lysate. All the plasmids were found to have the same molecular weight as the *S. aureus* donor plasmids by agarose gel electrophoresis, within the resolution of the technique (Meyers *et al.*, 1976).

These experiments demonstrate that plasmids present in the lysostaphin- or penicillin-induced lysates of *S. aureus* can enter *B. subtilis* competent cells and donate their chloramphenicol resistance phenotype to the sensitive recipients. Since any *B. subtilis* culture normally contains a small proportion of competent bacteria (Young & Spizizen, 1961), it is likely that plasmids from *S. aureus* or other bacteria can transform *B. subtilis* or other bacteria in nature at a low frequency and have substantial evolutionary importance. Two recent reports on high-frequency genetic exchange between strains of *B. subtilis* inoculated into sterile soil indicate that homospecific genetic exchanges can occur naturally in soil (Graham & Istock, 1978, 1979). We have shown that purified DNA is not necessary for plasmid transformation between widely divergent bacterial species and thus offer no basis to doubt that heterospecific gene transfers can occur in nature.

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