SHORT COMMUNICATION

Constitutive Erythromycin Resistance Plasmid in Staphylococcus aureus

By M. C. F. BASTOS, M. C. BONALDO AND E. G. C. PENIDO*

Departamento de Microbiologia Geral,
Instituto de Microbiologia da U.F.R.J., Ilha do Fundão, 21941,
Rio de Janeiro, Brazil

(Received 23 June 1980; revised 23 July 1980)

A plasmid with a molecular mass of $1.4 \times 10^6$ daltons has been identified in Staphylococcus aureus. This plasmid determines constitutive resistance to erythromycin and lincomycin and is the smallest naturally occurring element coding for antibiotic resistance in this species.

INTRODUCTION

Erythromycin resistance in Staphylococcus aureus is often inducible (Bronson & Pattee, 1972; Ono et al., 1975; Lacey, 1977) but may be constitutive (Garrod, 1957). Inducible resistance can be determined by plasmid genes (Novick & Bouanchaud, 1971; Dornbusch & Dahlstrom, 1973; Lindberg & Rudin, 1975). Iordanescu (1976) has described such a plasmid with a molecular mass of $2.4 \times 10^6$ daltons. Constitutive macrolide resistance may be determined by a penicillinase plasmid (Mitsuhashi et al., 1965; Novick, 1967). Integration of genes coding for constitutive resistance into the chromosome can occur (Novick, 1967; Lindberg & Rudin, 1975).

We describe here constitutive macrolide resistance coded for by a small plasmid (pRJ5) of only $1.4 \times 10^6$ daltons.

METHODS

Strains. The Staphylococcus aureus strains used were isolated from patients in Brazil; all were coagulase-positive and DNAase-positive. They were purified in Chapman (BBL) medium and stored on nutrient agar slants at room temperature. Strain A-443, which is resistant to penicillin (Pc), tetracycline (Tc), erythromycin (Em) and lincomycin (Lm), was used as the donor of drug resistance. Strains SCC-5, SCC-8 and SCC-10, which are sensitive to Tc, Em and Lm and marked chromosomally with resistance to rifampicin (Rif), were used as recipients.

Media. Nutrient broth, nutrient agar (Difco) and phage buffer (Novick, 1963) were used. CaCl$_2$ (final concentration 0.01 M) was added for the phage work.

Determination of drug resistance. The minimal inhibitory concentration (m.i.c.) was determined by an agar plate dilution method (Lacey & Grinstead, 1973).

Preparation of phage lysates. Ultraviolet-induced lysates were prepared by the method of Schwesinger & Novick (1975) by exposure to a GE-germicidal lamp (giving a dose of 0.93 J m$^{-2}$ s$^{-1}$) for 20 s. The titre of lysates was determined by the method of Adams (1959).

Transduction. A suspension of recipient cells, grown overnight statically in nutrient broth at 37 °C, was mixed with an equal volume of lysate supplemented with CaCl$_2$ in a sterile centrifuge tube. A low multiplicity of infection was used (from 0.002 to 0.005). After incubation at 37 °C for 30 min, sodium citrate (final
concentration 0·03 m) was added and the mixture was centrifuged for 15 min at 12000 g. The cells were resuspended in 4·5 ml nutrient broth supplemented with sodium citrate (final concentration 0·01 m) and incubated statically at 37 °C for 2 h. The cells were again centrifuged and resuspended in nutrient broth to concentrate them 5 to 20 times. Then, 0·1 ml was seeded on nutrient agar supplemented with Tc (10 μg ml⁻¹), Em (10 μg ml⁻¹) or Lm (10 μg ml⁻¹). The plates were incubated at 37 °C for 48 h to detect transductants. Simultaneous transfer of two or more drug resistance markers was analysed by replica-plating (Lederberg & Lederberg, 1952).

Loss of antibiotic resistance. About 10⁶ colony-forming units from a broth overnight culture of strain A-443 were inoculated into two flasks containing 10 ml nutrient broth. One culture was grown at 43 °C and the other at 37 °C, both with the same vigorous shaking for 6 h. Dilutions of each culture were then plated on nutrient agar, incubated for 48 h at 37 °C and replica-plated to nutrient-antibiotic agar; loss of antibiotic resistance was recorded after 24 h at 37 °C.

Isolation of plasmid DNA. The method of Meyers et al. (1976) was used with modifications for S. aureus strains. Cells grown overnight in 40 ml Tryptic Soy Broth were washed and resuspended in 1·5 ml TE buffer (10 mM-Tris, 1 mM-EDTA; pH 8·0). Lysostaphin was added to a final concentration of 500 μg ml⁻¹ and the mixture was held for 15 min at 37 °C; 200 μl EDTA (250 mM; pH 8·0) and 200 μl SDS (10%, w/v, in 50 mM-Tris, 5 mM-EDTA; pH 8·5) were then added and the mixture was kept on ice for 3 h. Cleared lysates were centrifuged for 40 min at 39100 g. After RNA digestion, phenol extraction and centrifugation were done to obtain a clear aqueous phase. The aqueous phase was brought to 90% with ethanol and the other at 37 °C, from 12.0 to 52.6

RESULTS AND DISCUSSION

The resistances to Tc and Em-Lm of donor strain A-443 were transduced at high frequencies (from 1·4 x 10⁻⁴ to 7·4 x 10⁻⁴) to all three recipient strains (SCC-5, SCC-8, SCC-10). There was 100% cotransduction of Em and Lm resistance; this was expected because strain A-443 showed constitutive resistance to the macrolide antibiotics (data not shown).

When the transductants were selected by Em or Lm, 16·9 to 19·1% of the tested clones were also TcR, but when selected by Tc, only 1·3 to 2·4% of the colonies were also EmR-LmR. The pigmentation and phage type of the transductant strains were identical to those of the recipients and their resistance was similar to that of the donor strain A-443.

Following growth at 37 °C, Em resistance was lost from 12·1 to 31·0% of the colonies and at 43 °C, from 12·0 to 52·6%. Resistance to Pc or Tc was lost only occasionally. In rare cases, there was simultaneous loss of resistance to Pc and Em (0·48 to 1·60%), to Tc and Em (0·06 to 0·27%) or to Pc and Tc (0·06 to 0·22%).

The above results indicate that in strain A-443 the genes coding for PcR, TcR and EmR-LmR are probably carried by different extrachromosomal elements.

The results of plasmid isolation are shown in Fig. 1. In column A, the lower band (diffuse) corresponds to residual chromosomal matter and the upper band to plasmid DNA (62 x 10⁶ daltons); although the sample is Escherichia coli strain A-252 F⁻ Lac⁺, it may indicate a plasmid other than F. Column B shows the bands obtained with DNA extracted from strain A-443; these correspond, from top to bottom, to chromosomal matter and to plasmids of 5·8 x 10⁶, 2·8 x 10⁶, 2·4 x 10⁶ and 1·4 x 10⁶ daltons, respectively. In column C, which represents DNA from recipient strain SCC-8 RifR-PcR, no plasmid bands are visible. In column D, representing DNA from transductant T121 (SCC-8 RifR-PcR-TcR), there are two bands corresponding to plasmids of 5·8 x 10⁶ and 2·8 x 10⁶ daltons. Column E, representing DNA from cotransductant T124 (SCC-8 RifR-PcR-TcR-EmR-LmR), shows the same four plasmid bands as in column B. In column F, representing DNA from
transductant E129 (SCC-8 Rif² Pe⁰ Ec² Em² Lm²), two bands corresponding to plasmids of 2.4 × 10⁶ and 1.4 × 10⁶ daltons are visible. Column G, showing DNA from cotransductant E119 (SCC-8 Rif² Pe⁰ Ec² Em² Lm²), again has the same four plasmid bands as in column B. Columns H to L contain reference plasmids.

Thus, strain A-443 harbours at least two different plasmids, one of 2.8 × 10⁶ daltons and the other of 1.4 × 10⁶ daltons. Like other small plasmids of S. aureus (Grubb et al., 1972; Kono & Sasatsu, 1976; Iordanescu, 1977), they can be cotransduced. However, they segregate separately in the cotransductant strains.

As the plasmids of 5.8 × 10⁶ and 2.8 × 10⁶ daltons were also present in the extracts of transductant T121 (Te²) and the cotransductants T124 and E119 (both Te² Em² Lm²), we conclude that these plasmids correspond, respectively, to the open circular and covalently closed circular forms of the Te plasmid. The size of this plasmid is in accordance with literature data (Novick & Bouanchaud, 1971; Chopra et al., 1973; Stiffler et al., 1974).

The plasmids of 2.4 × 10⁶ and 1.4 × 10⁶ daltons were always present in the DNA preparations obtained from transductant E129 (Em² Lm²) and from cotransductants E119 and T124 (both Te² Em² Lm²). Thus, these plasmids represent, respectively, the open circular and covalently closed circular forms of the Em plasmid.

As far as we are aware, this is the first report of a plasmid coding only for constitutive
erythromycin resistance in \textit{S. aureus}. Novick \textit{et al.} (1979) showed that the \textit{ermB} gene (coding for constitutive \textit{Em}^R) included in the penicillinase plasmid pI258 is a transposable element of $3.5 \times 10^6$ daltons.

Our plasmid, designated pRJ5, differs from plasmid pE194 isolated by Iordanescu (1976) in that it is smaller and codes for constitutive resistance. Further work is required to fully characterize this plasmid.

We are indebted to Dr Carlos Solé-Vernin for phage typing our strains, to Dr Diógenes S. Santos for helping with plasmid isolation and to Dr R. W. Lacey for his criticisms and suggestions. This work was performed with the financial support of Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) and Plano Integrado de Genética.

\textbf{REFERENCES}

\begin{itemize}
\item \textbf{IORDANESCU, S.} (1976). Three distinct plasmids originating in the same \textit{Staphylococcus aureus} strain. \textit{Archives roumaines de pathologie expérimentale et de microbiologie} \textbf{35}, 111–118.
\item \textbf{LINDBERG, M.} \& \textbf{RUDIN, L.} (1975). Thymineless bacteriophage induction in \textit{Staphylococcus aureus}.
\end{itemize}