Plasmid-mediated Chloramphenicol Resistance in *Staphylococcus hyicus*

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A small plasmid of 3.95 kb, encoding resistance to chloramphenicol (Cm) was detected in three of 33 *Staphylococcus hyicus* strains. The plasmid in each of the three strains was indistinguishable by Southern-blot hybridization and restriction enzyme analysis. It was shown by curing and by transformation to specify resistance to Cm. A preliminary restriction map of the plasmid, designated pSC2, is presented. Chloramphenicol acetyltransferase was demonstrated by enzyme assay and by SDS-PAGE of cell-free lysates of pSC2 transformants.

INTRODUCTION

Staphylococci are frequently isolated from skin infections of domestic animals. In pigs, *Staphylococcus hyicus* causes exudative epidermitis, which affects mainly suckling piglets and is often promoted by abrasions of the skin due to scratches, bites, rough bedding or ectoparasites (Eich, 1985; Gyles & Thoen, 1986; Rolle & Mayr, 1984). Exudative epidermitis causes great economic losses by retarding the growth of the piglets (Eich, 1985). Antibiotics are commonly used to control this infection (Eich, 1985; Rolle & Mayr, 1984), but the staphylococci are often resistant to antibiotics. The existence of plasmids in *S. hyicus* has been reported (Kloos *et al.*, 1981; Noble *et al.*, 1988; Schwarz & Blobel, 1989), but a causal relationship to antimicrobial resistance could not be definitely determined (Kloos *et al.*, 1981; Schleifer, 1986). Therefore, further studies were made of the antimicrobial resistance and plasmid content of *S. hyicus* strains isolated from piglets suffering from exudative epidermitis, with particular reference to their chloramphenicol resistance.

METHODS

Bacterial strains. A total of 33 strains of *S. hyicus* isolated from pigs with exudative epidermitis from different farms in several geographical areas of Germany between 1983 and 1986 were used. Most of the strains were provided by G. Amtsberg, Institut für Mikrobiologie und Tierseuchen, Hannover, FRG. The *S. hyicus* reference strain NCTC 10350 was included. All strains were identified according to Schleifer (1986).

Growth conditions and culture media. Stock cultures of *S. hyicus* were prepared by overnight incubation at 37 °C on sheep blood agar (blood agar base, Gibco, supplemented with 5%, v/v, sheep blood) and subsequent storage at 4 °C. Single colonies from these stock culture plates were inoculated into brain heart infusion broth (BHI, Merck) and grown for 18 h at 37 °C on a rotary shaker (75 r.p.m.) for plasmid isolation, antibiograms and chloramphenicol acetyltransferase assays. Antibiograms were obtained from ASS-agar plates (Merck). In curing experiments Trypticase-Soy-Agar plates (TSA, Oxoid) were used, and for protoplast transformation experiments osmotically stable media were prepared as described by Chang & Cohen (1979).

Plasmid isolation. For large-scale preparation of plasmid DNA, the three relevant *S. hyicus* strains were subjected to a modification of the alkaline lysis procedure of Maniatis *et al.* (1982). After overnight incubation in 500 ml BHI, containing 15 µg chloramphenicol ml⁻¹, the staphylococci were harvested by centrifugation for 10 min at 6000 g. Each pellet was resuspended in 10 ml glucose-TE buffer (50 mM-glucose, 25 mM-Tris/HCl, 10 mM-EDTA, pH 8.0), supplemented with 40 µg lysostaphin ml⁻¹ (Sigma). After 2 h incubation at 37 °C, 20 ml alkaline lysis solution, consisting of 1% (w/v) sodium dodecyl sulphate (SDS) in 0.2 M-NaOH, was carefully added.

Abbreviations: CAT, chloramphenicol acetyltransferase; Cm, chloramphenicol.

The suspension was stored on ice for 5 min, neutralized by the addition of 15 ml 3 M sodium acetate pH 4.8 and kept on ice for 1 h. The cell debris was then pelleted by centrifugation for 15 min at 6000 *g* and the plasmid-containing supernatant mixed with 2.5 vols ice-cold ethanol. The mixture was stored for 2 h at -70°C to precipitate plasmid DNA, which was then sedimented by centrifugation for 15 min at 6000 *g*. The supernatant was discarded. The sedimented plasmids were dried, resuspended in 3 ml TE-CsCl buffer (5.3 g CsCl per 5 ml TE-buffer), and 60 μl of an aqueous solution of ethidium bromide (8 mg ml^{-1}) was added. The plasmids were purified by density-gradient centrifugation for 16 h at 175000 *g*. Finally, the plasmid suspension was washed three times with an equal volume of butanol to remove ethidium bromide, dialysed for 20 h against 10 mM-Tris/HCl pH 8.0 and stored at -20°C . This protocol was used to isolate plasmid DNA for transformation experiments and restriction enzyme analysis. Small-scale preparation of plasmids, used for screening transformants and cured clones, was carried out as previously described (Schwarz *et al.*, 1989*a, b*).

Antibiograms. Antimicrobial resistance patterns of all *S. hyicus* strains were obtained by the agar diffusion method (Barry & Thornsberry, 1985). Discs containing, respectively, 25 μg ampicillin, 30 μg cephalothin, 30 μg chloramphenicol, 10 μg clindamycin, 15 μg erythromycin, 10 μg gentamicin, 30 μg kanamycin, 100 μg nitrofurantoin, 10 μg streptomycin, 30 μg tetracycline or 1.25 μg trimethoprim plus 23.75 μg sulfamethoxazole (Oxoid) were used. The antibiograms were evaluated after incubation for 18–24 h at 37°C (Barry & Thornsberry, 1985).

Curing. Single colonies of the *S. hyicus* strains were incubated for 48–72 h at 42°C in BHI, supplemented with 15 μg ethidium bromide ml^{-1} . The bacteria were then inoculated onto TSA plates and, after overnight incubation at 37°C , replica-plated onto selective TSA plates, containing 15 μg chloramphenicol ml^{-1} . Clones that appeared to be 'cured' were screened for plasmid content.

Plasmid transformation procedure. A modification of the method of Chang & Cohen (1979) was applied for the transfer of the *S. hyicus* plasmids. *Staphylococcus aureus* RN4220, a derivative of *S. aureus* 8325 (Novick, 1967), served as a recipient. Two millilitres of an overnight culture of *S. aureus* RN4220 were added to 48 ml BHI and incubated on a rotary shaker at 37°C until the culture reached an OD_{600} of 0.8. The staphylococci were then harvested by centrifugation for 10 min at 5000 *g* and resuspended in 5 ml SMMP-buffer (Chang & Cohen, 1979). Lysostaphin was added to a final concentration of 40 μg ml^{-1} , followed by incubation for 2 h at 37°C . The protoplasts were pelleted (10 min, 5000 *g*, 15°C) and resuspended in 5 ml SMMP-buffer. Transformation was carried out by mixing 500 μl of the protoplast suspension with 10–50 μl of a plasmid suspension, containing 1–5 μg DNA. Then 1.5 ml of a filter sterilized 40% (w/v) polyethylene glycol solution (PEG; approx. M_r 6000, Sigma) was added. After 2 min of gentle shaking, 5 ml SMMP-buffer was added and the protoplasts centrifuged again for 10 min at 5000 *g*. Each pellet was resuspended in 1 ml SMMP-buffer and incubated for 2 h at 37°C to allow phenotypic expression. Then 100 μl volumes of the protoplast suspension were applied to DM3-regeneration plates (Chang & Cohen, 1979), supplemented with 15 μg chloramphenicol ml^{-1} . Transformants appeared after 48–72 h at 37°C . SMMP-buffer was used instead of the plasmid suspension as a control. A previously described chloramphenicol resistance (Cm^R) plasmid from *Staphylococcus intermedius* (Schwarz *et al.*, 1989*a*) served as a positive control.

Restriction endonuclease analysis. Restriction enzyme digestions with the endonucleases *AccI*, *BamHI*, *BglII*, *BstEII*, *ClaI*, *EcoRI*, *HaeIII*, *HindIII*, *HpaII*, *KpnI*, *PvuII*, *MboI* and *TaqI* (Boehringer Mannheim) were carried out (Schwarz *et al.*, 1989*b*). Mapping of the different cleavage sites was performed by double-enzyme digestions. The sizes of the DNA fragments were determined by electrophoresis in 0.7–2.0% (w/v) agarose gels; electrophoresis was conducted for 3 h at 6 V cm^{-1} in Tris/acetate buffer, pH 7.8 (Maniatis *et al.*, 1982). Very small DNA fragments (50–300 bp) were analysed in 8% (w/v) polyacrylamide gels using Tris/borate buffer pH 8.0 (Maniatis *et al.*, 1982); these gels were run for 5 h at 10 V cm^{-1} . Both agarose and polyacrylamide gels were stained with ethidium bromide (10 μg ml^{-1}). The plasmid DNA was visualized by UV-illumination. Photographs were taken on Polaroid type 667 film. The sizes of the plasmid fragments were deduced from logarithmic plots by comparison with marker DNA which consisted of *HaeIII*-digested λdv1 DNA (Kröger *et al.*, 1984) and PB1 DNA cleaved separately with *PstI* (5664 bp), *HindIII* (4133, 845, 686 bp) and *PstI/BamHI* (3136, 2528 bp).

Southern blot hybridization. This was performed as a capillary blot procedure (Gene Screen Plus catalog N.NEF 976, 1985), using a 'Gene Screen Plus' hybridization transfer membrane (DuPont). Prehybridization, radioactive labelling and hybridization were carried out as previously described (Schwarz *et al.*, 1989*b*). The Cm^R plasmid of *S. hyicus* reisolated from a transformed *S. aureus* clone, served as a probe.

Bioassay for the enzymic inactivation of chloramphenicol. A modification of the method of Burns *et al.* (1985) was used to demonstrate enzymic inactivation of Cm . A lawn of chloramphenicol-sensitive *S. aureus* RN4220 was streaked on an ASS plate. Discs 9 mm in diameter were cut of Whatman no. 1 filter paper, and inoculated with 20 μl suspensions of each chloramphenicol-resistant *S. hyicus* strain and a representative chloramphenicol-resistant *S. aureus* RN4220 transformant. The original, plasmidless *S. aureus* RN4220 served as a negative control and a chloramphenicol-resistant *S. intermedius* strain as a positive control. Then a disc containing 30 μg chloramphenicol was placed in the middle of the inoculated filter paper. After overnight incubation at 37°C the zones of inhibition were measured. If the Cm passed in its active form through the filter paper, the growth of the

susceptible *S. aureus* RN4220 would be inhibited. No, or significantly reduced, inhibition would be observed if the Cm was inactivated by the bacteria on the filter paper.

Rapid chloramphenicol acetyltransferase (CAT) assay. The method of Azemun *et al.* (1981) was modified to demonstrate CAT activity in the original chloramphenicol-resistant *S. hyicus* strains and their *S. aureus* RN4220 transformants. The original chloramphenicol-sensitive *S. aureus* RN4220 was used as negative control. The cultures were grown overnight at 37 °C in 2 ml BHI and harvested by centrifugation for 10 min at 5000 g in 1.5 ml reaction cups (Eppendorf). Each pellet was resuspended in 200 µl of a buffer containing 1 M-NaCl and 0.01 M-EDTA pH 8.0. Lysostaphin was added to a final concentration of 40 µg ml⁻¹ and the suspension was incubated for 30 min at 37 °C. Duplicate tubes were prepared of each culture with 100 µl 5 mM-Tris/HCl pH 8.0 or 100 µl 5 mM-acetyl coenzyme A (Boehringer Mannheim). Then 200 µl 5 mM-Cm was pipetted into each assay tube and the same volume of distilled water into the control tubes. Finally, 20 µl 0.2 mM-5,5'-dithiobis-(2-nitrobenzoic acid) (DTNB, Sigma) in 0.1 M-Tris/HCl pH 8.0 was added to all tubes. After 5 min at 37 °C, the colour in each assay tube was compared with that in the respective control tube.

Detection of CAT. (a) *Induction of CAT.* The expression of all known CAT variants from staphylococci is inducible by Cm or certain analogues. However, Cm itself serves as substrate for this enzyme. This leads, depending on the basal CAT activity, to a rapid inactivation of the inducer by acetylation and consequently to reduced induction rates. In order to optimize CAT induction, acetylated Cm must be replaced by fresh Cm. Therefore Cm was added to a final subinhibitory concentration of 75 µg ml⁻¹ after each doubling of the bacteria. This was estimated by turbidity measurements (Shaw, 1975). Thus, pSC2 transformants were grown in 50 ml BHI to OD₆₀₀ 0.65 (mid-exponential phase). After a final addition of Cm, the staphylococci were allowed to grow for another 30–60 min at 37 °C, to enable maximum CAT induction before harvest (Shaw, 1975). For determinations of the basal level of CAT expression without induction, pSC2 transformants were treated in the same manner, except that the medium was not supplemented with Cm. As a negative control, untransformed *S. aureus* RN4220 was grown under the same conditions, but also without Cm.

(b) *Preparation of cell-free extracts.* Harvested cells of transformed and untransformed *S. aureus* RN4220 were washed with 5 ml of buffer containing 0.05 M-Tris/HCl and 0.15 M-NaCl, pH 7.5. The bacteria were pelleted and resuspended in 1 ml of the same buffer. Then 40 µl lysostaphin (2 mg ml⁻¹) was added. After 15 min at 37 °C, 20 µl deoxyribonuclease (1 mg ml⁻¹, Boehringer Mannheim) was added, followed by another 15 min incubation at 37 °C. The cell debris was pelleted by centrifugation for 20 min at 15000 g and the clear supernatant used for SDS-PAGE.

(c) *SDS-PAGE.* Twenty microlitres of the clear supernatant were mixed with 50 µl loading buffer, heated for 10 min at 100 °C and analysed in 11% (w/v) polyacrylamide gels (Maniatis *et al.*, 1982). Electrophoresis was carried out for 6 h at 10 V cm⁻¹. Coomassie brilliant blue was used to stain the gels. SDS-7-marker (Sigma), containing trypsin inhibitor from soybean (M, 20100), trypsinogen (PMSF treated) (24000), carbonic anhydrase from bovine erythrocytes (29000), glutaraldehyde-3-phosphate dehydrogenase from rabbit muscle (36000), albumin from egg (45000), and bovine serum albumin (66000), served as standards.

RESULTS

Plasmid content and chloramphenicol resistance

Cm^R occurred in only three of the 22 plasmid-positive and in none of the 11 plasmid-negative *S. hyicus* strains. Although these three chloramphenicol-resistant strains were not related epidemiologically, they exhibited a common plasmid band in agarose gel electrophoresis (Fig. 1, lanes 1, 3, 5), which was not detectable in the plasmid profiles of the remaining 19 plasmid-containing *S. hyicus* strains.

Curing and protoplast transformation

In curing experiments, clones were obtained from all three strains which had become sensitive to Cm and lost all their plasmids. For the interspecific transformation of protoplasts of *S. aureus* RN4220 the complete plasmid content of the three chloramphenicol-resistant *S. hyicus* strains was used. In all three cases chloramphenicol-resistant *S. aureus* RN4220 transformants were obtained. Fifteen clones from each of the three transformations were analysed for plasmid content. All of them contained a small plasmid of the same size as that in the *S. hyicus* strains (Fig. 1, lanes 2, 4, 6).

Fig. 1

Fig. 1. Agarose gel electrophoresis of uncleaved plasmids of the three chloramphenicol-resistant *S. hyicus* strains (lanes 1, 3, 5) and their respective chloramphenicol-resistant *S. aureus* RN4220 transformants (lanes 2, 4, 6). The presumptive Cm^R plasmid is marked with an arrow; chr, chromosomal DNA.

Fig. 2

Fig. 2. Agarose gel electrophoresis of single restriction enzyme digests of the Cm^R plasmid pSC2 from *S. hyicus*. Sizes of the marker DNA are shown on the left, sizes of the plasmid fragments on the right. Lanes: 1, pSC2 cleaved with *AccI*; 2, pSC2 cleaved with *BstEII*; 3, pSC2 cleaved with *HpaII*; M, PB1/ λ dvl marker DNA; 4, pSC2 cleaved with *MboI*; 5, pSC2 cleaved with *TaqI* (this yielded six fragments; only the three largest fragments are visible).

Restriction endonuclease analysis

The plasmids of the chloramphenicol-resistant transformants were compared on the basis of restriction enzyme digests. For this we used the following endonucleases, known to cleave Cm^R plasmids of *S. aureus* or *S. intermedius* (Gillespie & Skurray, 1988; Schwarz *et al.*, 1989a): *AccI*, *BamHI*, *BglII*, *BstEII*, *ClaI*, *EcoRI*, *HaeIII*, *HindIII*, *HpaII*, *KpnI*, *PvuII*, *MboI* and *TaqI*. The transformed Cm^R plasmids of the three *S. hyicus* strains had the same distribution of restriction endonuclease cleavage sites. None of them had recognition sites for *BamHI*, *ClaI*, *EcoRI*, *HaeIII*, *HindIII*, *KpnI* or *PvuII*. In all cases linearization was achieved with *AccI*, *BstEII* and *HpaII* (Fig. 2). The linear plasmid was approximately 3.95 kb in size. Digestion with *MboI* resulted in three fragments, of 2.05, 1.10 and 0.80 kb. *TaqI* cleaved the plasmid into six fragments, of 2.68, 0.48, 0.32, 0.30, 0.11 and 0.05 kb. A preliminary restriction enzyme map of the Cm^R plasmid from *S. hyicus*, designated pSC2, was constructed by double restriction enzyme digests. This restriction map was used to compare pSC2 with other staphylococcal Cm^R plasmids such as pC223, pC194, pC221 and pUB112 (Fig. 3). A high degree of conformity between pSC2, pC221 and pUB112 was found in the regions which encoded the resistance mechanism and replication functions in the latter two plasmids. In contrast, there were only minor structural relationships between pSC2 and pC223 or pC194.

Fig. 3. Restriction maps of the staphylococcal Cm^R plasmids pC223 (Novick, 1976), pC194 (Horinouchi & Weisblum, 1982), pC221 (Brenner & Shaw, 1985), pUB112 (Brückner *et al.*, 1984) and pSC2. Restriction enzyme cleavage sites are: Ac, *AccI*; BgI, *BglI*; Bg, *BglII*; Bs, *BstEII*; C, *ClaI*; E, *EcoRI*; Ha, *HaeIII*; H, *HindIII*; Hp, *HpaII*; M, *MboI*; Pv, *PvuII*; Sc, *SacI*; T, *TaqI*. Scales (kb) are shown below each map; regions encoding replication functions (REP) and chloramphenicol acetyltransferase (CAT) are indicated below the maps of pC223, pC194 and pC221 (the arrows indicate the direction of transcription of the REP- and CAT genes). In pSC2, the correct positions of the $\hat{\text{T}}$ - and $\check{\text{T}}$ -cleavage sites could not be determined exactly; the two possible positions of each are both marked.

Southern blot hybridization

The Cm^R plasmid, reisolated from a transformed clone and radioactively labelled, served as a probe for Southern blot hybridization. The probe specifically recognized the respective Cm^R plasmid of the three *S. hyicus* strains, leading to identical hybridization patterns (Fig. 4). The additional plasmids occurring in two of the three *S. hyicus* strains were not recognized. The upper signals visible in the autoradiograph are due to open circular forms of pSC2 which, because of their small quantities, are not visible in the less sensitive ethidium-bromide-stained agarose gel.

Mechanism of chloramphenicol resistance

In the modified bioassay the three chloramphenicol-resistant *S. hyicus* strains, a representative chloramphenicol-resistant *S. aureus* RN4220 transformant and the chloramphenicol-resistant *S. intermedius*-strain differed significantly from the chloramphenicol-sensitive *S. aureus* RN4220. All the chloramphenicol-resistant staphylococcal strains inactivated Cm, abolishing its inhibitory effects on the growth of the chloramphenicol-sensitive *S. aureus* RN4220. The chloramphenicol-sensitive *S. aureus* RN4220 did not inactivate Cm.

Fig. 4

Fig. 5

Fig. 4. Southern blot hybridization. (a) Agarose gel, stained with ethidium bromide, showing the plasmid content of the three chloramphenicol-resistant *S. hyicus* strains; (b) autoradiography of the gel after hybridization with labelled Cm^r plasmid isolated from a transformed clone of *S. aureus* RN4220.

Fig. 5. Coomassie-blue-stained gel following SDS-PAGE of cell-free lysates of a Cm -induced pSC2 transformant of *S. aureus* RN4220 (lane 1), the original chloramphenicol-sensitive *S. aureus* RN4220 (lane 2) and an uninduced pSC2 transformant (lane 3). The presumptive CAT band is marked by an arrow. M, marker proteins.

All the chloramphenicol-resistant *S. hyicus* strains, and their chloramphenicol-resistant *S. aureus* RN4220 transformants, produced CAT, as determined in the rapid CAT assay, but CAT activity was not detectable in the original chloramphenicol-sensitive *S. aureus* RN4220. Furthermore, CAT could also be demonstrated by SDS-PAGE in cell-free extracts from the chloramphenicol-resistant transformants, but not in extracts from the original chloramphenicol-sensitive *S. aureus* RN4220. The presumptive CAT band appeared in SDS-PAGE with an M_r of approximately 23000 Da (Fig. 5). Great differences in the level of CAT expression were observed between uninduced and Cm -induced pSC2 transformants, suggesting that the CAT of pSC2 was an inducible enzyme.

DISCUSSION

Plasmids as carriers of resistance genes in staphylococci have long been known (Lyon & Skurray, 1987). For *S. hyicus*, plasmids and antimicrobial resistance patterns have also been reported (Kloos *et al.*, 1981; Noble *et al.*, 1988; Schwarz & Blobel, 1989). However, a causal relationship between these plasmids and specific resistance patterns had not been established (Kloos *et al.*, 1981; Schleifer, 1986). First attempts to identify antimicrobial resistance plasmids in *S. hyicus* by Noble *et al.* (1988) involved solely curing experiments. A plasmid encoding resistance to Cm could not be detected by this method. Furthermore, curing experiments alone might result in misleading information, particularly if the 'cured' derivative had lost two or more plasmids. Therefore, in addition to curing experiments we conducted the more specific protoplast transformations. Both procedures proved the extrachromosomal nature of Cm^R in the three *S. hyicus* strains and enabled us to identify a 3.95 kb plasmid, designated pSC2 as being responsible for this resistance. Moreover, the protoplast transformations resulted in transformants which carried only the plasmid encoding Cm^R and none of the other plasmids of the original *S. hyicus* strains. Thus, the Cm^R plasmid of the transformants proved suitable for further characterization by restriction endonuclease analysis. For this purpose enzymes were

chosen which had cleavage sites in the Cm^R plasmids of 'human' *S. aureus* and 'canine' *S. intermedius* cultures (Gillespie & Skurray, 1988; Schwarz *et al.*, 1989a). The restriction analyses resulted in the construction of a preliminary restriction map of pSC2, which was used as a basis for the comparison of pSC2 with other staphylococcal Cm^R plasmids.

In staphylococci, three distinct families of Cm^R plasmids have been identified on the basis of restriction endonuclease cleavage sites and sequence analyses of their CAT genes. The prototype Cm^R plasmids of these families are pC223, pC194 and pC221 (Gillespie & Skurray, 1988). The *S. hyicus* Cm^R plasmid pSC2 appears to be closely related to the members of the pC221 family. The criterion for classification of pSC2 in this plasmid family is the similarity of the restriction maps in the region of the REP (plasmid replication) and CAT genes. Members of the pC221 family have been isolated during the last 35 years (Gillespie & Skurray, 1988) from several staphylococcal species of human and animal origin in different geographical areas. A possible reason for the 'in toto'-spread of this highly conserved REP/CAT region could be the existence of specific sequences flanking the REP/CAT region and enabling recombinational events to occur (Gillespie & Skurray, 1988). Such a recombination site was mapped in the nucleotide sequence of pC221 (Novick *et al.*, 1984). In contrast to other observations (Lacey, 1980), the occurrence of this REP/CAT gene region in 'human' *S. aureus* and 'porcine' *S. hyicus* cultures also confirmed the *in vivo* gene transfer between different staphylococcal species from infections of humans and animals. Furthermore, the presence of homologous CAT regions indicates that the members of the pC221 family share a common resistance mechanism, namely the enzymic inactivation of Cm (Shaw & Brodsky, 1988) by the same CAT variant. This enzyme catalyses the transfer of acetyl groups from acetyl-CoA to Cm, resulting in 3-acetyl and 1,3-diacetyl derivatives (Shaw, 1967). These acetylated Cm derivatives are devoid of antibiotic activity (Shaw & Unowsky, 1968) and also of the ability to induce CAT gene expression (Brückner & Matzura, 1985). In contrast to enterobacteria, which produce CAT constitutively (Fitton *et al.*, 1978), CAT expression in staphylococci has to be induced (Winshell & Shaw, 1969) with Cm or its fluorinated derivatives (Brückner & Matzura, 1985). The inducibility of CAT from *S. hyicus* encoded by pSC2, could also be demonstrated since there were great differences in the levels of CAT expression in uninduced and Cm-induced pSC2 transformants. All Cm^R plasmids of staphylococci that have been investigated carry the genetic information for the CAT monomer, a polypeptide of varying *M_r* in the range of 22 500–26 200 (Zaidenzaig *et al.*, 1979; Horinouchi & Weisblum, 1982). Formerly, native CAT was considered to be tetrameric (Shaw, 1975), but recently, X-ray crystallography revealed CAT to be a trimer of identical subunits with a distinct protein fold (Leslie *et al.*, 1988). The *M_r* of a subunit of CAT from pSC2 was determined to be in the order of 23 000, according to its electrophoretic mobility in SDS-PAGE relative to that of the marker proteins. However, this size should be considered to be only approximate, because some CAT variants of staphylococci seem to migrate anomalously in SDS-PAGE, giving lower *M_r* values than expected from their amino acid sequences (Zaidenzaig *et al.*, 1979).

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