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Occurrence of a Class II Restriction Endonuclease in *Staphylococcus aureus*

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The occurrence of class II restriction endonucleases (enzymes that both recognize and cleave a specific nucleotide sequence in deoxyribonucleic acid [DNA]) in *Staphylococcus aureus* has been investigated by analysis of crude extracts obtained from different propagating strains of the International Phage Typing System. Of the four main groups of strains in the International System, only extracts of group II strains were found to contain class II restriction endonucleases. The identical cleavage patterns obtained by incubation of different DNAs with cell extracts of group II strains suggest that these enzymes all recognize and cleave the same nucleotide sequence. This recognition site has been determined to be $\begin{matrix}5'\text{-G-A-T-C-}3'\\3'\text{-C-T-A-G-}5'\end{matrix}$ for the prototype of these enzymes, *Sau*3AI (J. S. Sussenbach et al., Nucleic Acids Res. 3:3192-3202, 1976). Evidence is presented that the classification of group II strains is based on restriction modification and is correlated with the presence of a class II restriction endonuclease that recognizes and cleaves the above sequence.

In *Enterobacteriaceae*, restriction and modification systems have been described extensively (1, 2, 5). According to the model of Arber and Dussoix (1), two enzymes are involved. A restriction endonuclease recognizes specific nucleotide sequences in invading foreign deoxyribonucleic acid (DNA) and subsequently cleaves this DNA. A modification enzyme recognizing the same nucleotide sequences is able to modify these sequences by methylation. The modified DNA is protected against cleavage.

In 1968, Meselson and Yuan (7) isolated the first restriction enzyme from *Escherichia coli*. This enzyme proved to belong to the restriction endonucleases of class I, which cleave the DNA at some nonspecific site outside the recognition sequence. Restriction endonucleases of class II, cleaving the DNA in or at the end of the recognition sequence, have also been isolated from *E. coli*.

In *Haemophilus influenzae*, four restriction and modification systems have been described which are correlated with four of the six serological groups. Smith and Willcox (9) isolated a class II enzyme from *H. influenzae* serotype *d* that has proven to be very useful for dissecting DNAs from different sources. Recently, a great number of class II restriction endonucleases have been described from various bacterial species, mainly gram-negative bacteria (8). The rigidity of the cell wall and the high internal osmotic pressure of gram-positive bacteria might be responsible for the difficulties in isolating enzymes from these organisms.

Restriction and modification systems have also been described for *Staphylococcus aureus*. Williams and Rippon (12) have developed the International Phage Typing System as an epidemiological tool to characterize different strains of *S. aureus*. According to their phage patterns, the strains can be divided in four groups (Fig. 1). Though the phages are not related, these groups are not fortuitous. Group I and III strains are often resistant to various antibiotics, whereas group II strains are isolated from skin infections. It has been shown that differences in phage patterns between groups are mainly due to restriction and modification (4, 10). Several workers isolated restriction-deficient mutants of various strains of *S. aureus* (6, 10). Only in one case was a distinct modification-deficient mutant also isolated (6).

After treatment of propagating strain (PS) 3A (NCTC 8319) with lysostaphin, we were able to isolate the first specific restriction endonuclease in *S. aureus* (11). This enzyme, called *Sau*3AI, belongs to the class II enzymes and is absent in a restriction-deficient mutant, 3AR$^-$ (11).

The purpose of this study was to investigate whether other strains of *S. aureus* also contain class II restriction endonucleases.

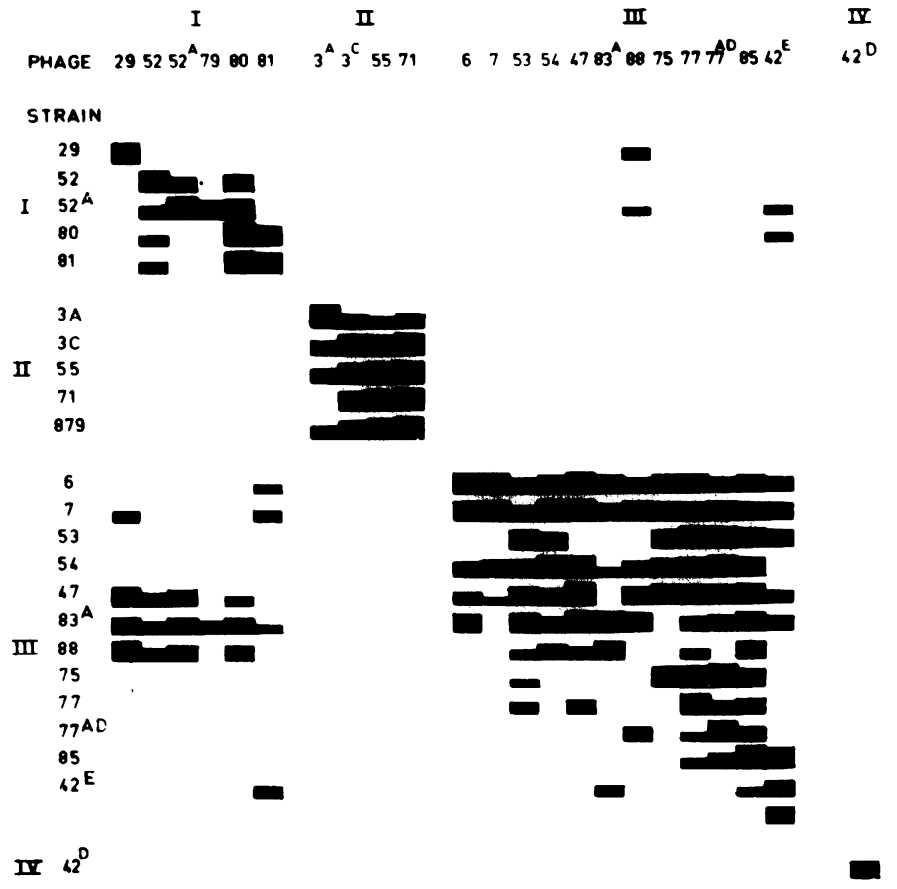

FIG. 1. *Sensitivity of S. aureus strains of the International Phage Typing System for the phages of the system (4). The log titers of the phages on the strains are represented by the height of the black columns. The log titer on the propagating strains is 8. Absence of a black column indicates insensitivity of the strain for the particular phage. Based on the titration results, the strains and phages are divided in four groups, I, II, III, and IV. Group II strains are only sensitive to group II phages and are insensitive to phages of the other groups. The sensitivity patterns of group I and III strains are more complex.*

MATERIALS AND METHODS

Strains of bacteria and bacteriophages. The following strains of *S. aureus* were examined for class II restriction endonucleases. Group I: The cured strains 29C and 80C derived from propagating strains (PS) 29 (NCTC 8331) (10) and 80 (NCTC 9789) (3). Group II: PS 3A (NCTC 8319), 3C (NCTC 8327), 55 (NCTC 8358), 71 (NCTC 9315), and the nonlysogenic strain 879 (13). Group III: PS 47 (NCTC 8325). Group I/III: The nonlysogenic strain 57 (14). Group IV: PS 42 D (NCTC 10033).

A wild-type group II strain (1030) was isolated from a patient and also tested for class II enzymes. The restriction-deficient mutant 879 R4 was derived from 879 after ethyl methane sulfonate treatment. The culture of bacteria and the preparation and purification of phage stocks have been described previously (11). DNA was prepared from phage 3A (NCTC 8408), phage 6 (NCTC 8403), phage 71 (NCTC 9316), phage 80 (NCTC 9788), and human adenovirus type 5 (Ad5) as described before (11).

Preparation of cell extracts. About 3 g of packed cells was treated with lysostaphin as reported elsewhere (11). After centrifugation, the cells were resuspended in 8 ml of 0.01 M tris(hydroxymethyl)aminomethane (Tris)-hydrochloride-0.01 M β-mercaptoethanol, pH 7.4. Subsequently, cells were disrupted by sonic treatment at 0°C and centrifuged for 1.5 h at 40,000 rpm in a Spinco SW41 rotor at 4°C. The supernatant was collected, and streptomycin sulfate was added for the precipitation of nucleic acids (2 ml of a 10% streptomycin sulfate solution in 0.01 M Tris-hydrochloride-0.01 M β-mercaptoethanol, pH 7.4). The precipitate was removed by centrifugation at 35,000 rpm for 30 min in a Spinco SW41 rotor at 4°C, and the supernatant was dialyzed against 0.01 M Tris-hydrochloride to 0.01 M β-mercaptoethanol, pH 7.4. The crude extract (about 10 ml) was tested for the presence of endonucleolytic activity as described below.

Endonuclease assay and gel electrophoresis. For the detection of specific endonucleases, 30 μl of the crude extract was incubated with 1 μg of DNA from

phage 3A, phage 6, phage 71, phage 80, and Ad5, respectively, in 15 mM $MgCl_2$, 6 mM Tris-hydrochloride (pH 7.5), 6 mM β-mercaptoethanol, and 60 mM NaCl at 30°C for 1 h. After incubation, sodium acetate was added to 0.1 M and the DNA was deproteinized by extraction with chloroform-isoamyl alcohol (24:1). The DNA was then precipitated by addition of 2.7 volumes of ethanol and centrifugation at −5°C in a Spinco SW65 rotor at 35,000 rpm for 30 min. The DNA pellet was dissolved in 20 mM Tris-hydrochloride (pH 7.5)-1 mM ethylenediaminetetraacetic acid and subjected to electrophoresis in 1.4% agarose gels (J. S. Sussenbach and M. G. Kuijk, Virology, in press).

RESULTS

Occurrence of class II restriction endonucleases in *S. aureus*. The discovery of a class II restriction endonuclease in *S. aureus* strain 3A (*Sau*3AI) raised the question of whether other *S. aureus* strains also contain class II endonucleases. This led us to investigate various strains of the International Phage Typing System for the presence of class II restriction endonucleases. The assay conditions indicated under Materials and Methods allow a broad screening for class II enzymes in *S. aureus*, since almost all known class II restriction endonucleases are able to cleave DNA under these conditions (8).

Cells extracts from various strains were tested with the large DNA of Ad5 as a general indicator and with the DNA of phages 3A, 6, 71, and 80 carrying different modifications (Table 1). It appears that class II enzymes are only detectable in group II strains (PS 3A, 3C, 55, 71, and 879) of the International Phage Typing System. No specific endonucleolytic activity was observed in extracts of strains of the other groups, not even when the pH of the assay solution was varied between 6.8 and 9.0 (results not shown).

TABLE 1. *Endonucleolytic activity of class II in extracts of strains of S. aureus*[a]

Group	Strain	DNA				
		3A	6	71	80	Ad5
I	29 C	−	−	−	−	−
I	80 C	−	−	−	−	−
II	3 A	−	+	−	+	+
II	3 C	−	+	−	+	+
II	55	−	+	−	+	+
II	71	−	+	−	+	+
II	879	−	+	−	+	+
II	879 R4	−	−	−	−	−
II	1030	−	+	−	+	+
III	47	−	−	−	−	−
IV	42 D	−	−	−	−	−
I/III	57	−	−	−	−	−

[a] DNA of phage 3A, phage 6, phage 71, phage 80, and Ad5 was incubated with extracts of different strains from the four groups of the International Phage Typing System. Incubations were performed as indicated in Materials and Methods. Symbols: +, The particular DNA was cleaved into specific fragments (see Fig. 2 and 3); −, absence of cleavage.

Characerization of endonucleases in group II. As shown above, all strains of group II of the International Phage Typing System contain class II restriction endonucleases. The presence of such enzymes is not restricted to the propagating strains of group II, since strain 1030, which was isolated from a patient and was characterized by phage typing to be a group II strain, also appeared to contain a class II restriction endonuclease.

Comparison of the cleavage patterns of phage 6 and Ad5 DNAs obtained after digestion of these DNAs with extracts of the six strains of group II revealed that all these strains contain a class II restriction endonuclease with the same specificity (Fig. 2 and 3). Previously, it was shown that one of these strains, PS 3A, contains a restriction endonuclease that recognizes the sequence $\begin{matrix}5'\text{-G-A-T-C-}3'\\3'\text{-C-T-A-G-}5'\end{matrix}$ (11). This implies that all group II strains contain a class II enzyme that recognizes and cleaves the same sequence.

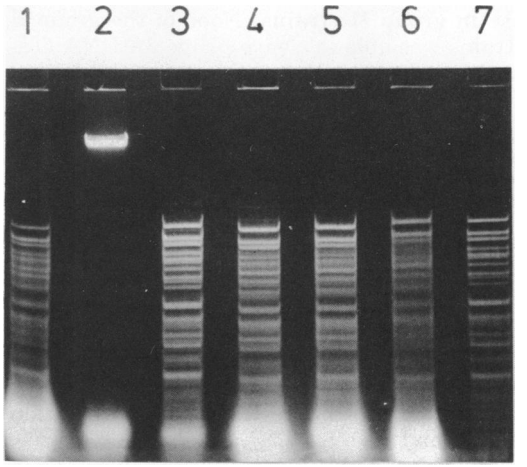

FIG. 2. *Digestion of Ad5 DNA with crude extracts of group II strains of S. aureus. Incubations were performed as described in Materials and Methods. After digestion and deproteinization, DNA was subjected to gel electrophoresis in 1.4% agarose gels as described elsewhere (Sussenbach and Kuijk, in press). The bright spot at the bottom of each lane is a contaminant present in the cell extract that binds ethidium bromide. Lane 1 represents digestion with cell extracts from strain 1030; lane 2 with 879 R4; lane 3 with 879; lane 4 with PS 71; lane 5 with PS 55; lane 6 with PS 3C; and lane 7 with PS 3A.*

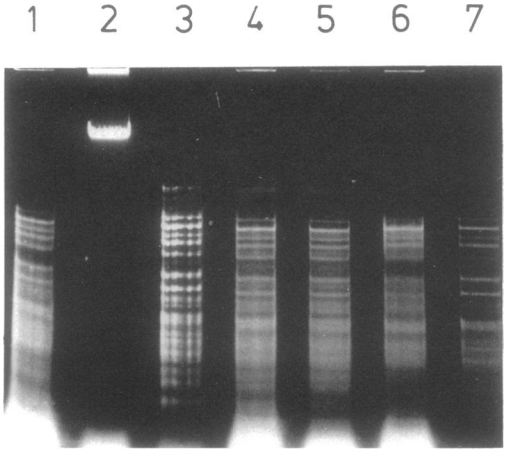

FIG. 3. *Digestion of phage 6 DNA with crude extracts of group II strains of S. aureus. Incubations were performed as described in Materials and Methods. After digestion and deproteinization, DNA was subjected to gel electrophoresis in 1.4% agarose as described elsewhere (Sussenbach and Kuijk, in press). The bright spot at the bottom of each lane represents a contaminant in the cell extract that binds ethidium bromide. Lane 1 represents digestion with strain 1030; lane 2 with 879 R4; lane 3 with 879; lane 4 with PS 71; lane 5 with PS 55; lane 6 with PS 3C; and lane 7 with PS 3A.*

Correlation between restriction modification and the presence of a specific endonuclease in group II strains. None of the group II strains was able to cleave phage 3A DNA (Table 1). Since this DNA has been synthesized in PS 3A, it is most likely that phage 3A DNA contains modified $\begin{matrix}5'\text{-G-A-T-C-}3'\\3'\text{-C-T-A-G-}5'\end{matrix}$ sequences, which are not accessible anymore to the *Sau*3AI type of enzymes present in group II strains. It was shown before that phage 6 propagated on 3A is equally insensitive to the *Sau*3AI enzyme (11).

Extracts obtained from a restriction-deficient mutant of 879 (879 R4) were not able to cleave phage 6 or Ad5 DNA, indicating that the *Sau*3AI type of restriction endonuclease in 879 is responsible for the restriction in this strain (Fig. 2 and 3).

Phage 3A had a much lower titer on strain 879 than on PS 3A, but after propagation on strain 879 the phage was modified and was now restricted on its original host 3A (Fig. 4). We concluded that strain 879 contained a different restriction and modification system than strain 3A or possibly a second system of minor importance. The observation that extracts of strain 879 R4 do not cleave any of the DNAs tested suggests that the additional system either is not a class II enzyme or is not detected by our methods.

DISCUSSION

Screening of extracts of various strains of *S. aureus* belonging to different groups of the International Phage Typing System shows that only extracts of group II strains contain class II restriction endonucleases. All group II strains tested possess enzymes with the same specificity. Since it was previously shown that PS 3A contains an enzyme, *Sau*3AI, that recognizes and cleaves $\begin{matrix}5'\text{-G-A-T-C-}3'\\3'\text{-C-T-A-G-}5'\end{matrix}$, it can be concluded that all the class II enzymes detected in group II strains recognize and cleave the same sequence. The presence of *Sau*3AI-type enzymes in all group II strains and their absence in five strains of other groups agrees with the division of distinct groups in the International System. The classification of group II is obviously based on restriction correlated with the presence of a *Sau*3AI type of enzyme.

The observation that DNAs from phages propagated in group II strains (phage 3A propagated in PS 3A and phage 71 propagated in PS 71) are not cleaved by extracts of group II strains (Table 1) suggests that the presence of a *Sau*3AI type of restriction endonuclease in group II strains is accompanied by the presence of a modification enzyme that modifies $\begin{matrix}5'\text{-G-A-T-C-}3'\\3'\text{-C-T-A-G-}5'\end{matrix}$ sequences. On the other hand, the accessibility of phage 6 and phage 80 DNAs (propagated in PS 6 and PS 80, respectively) to group II enzymes indicates that modification enzymes modifying the above sequence are absent in group I and III strains.

It is remarkable that no class II-type enzymes could be detected in extracts of strains of

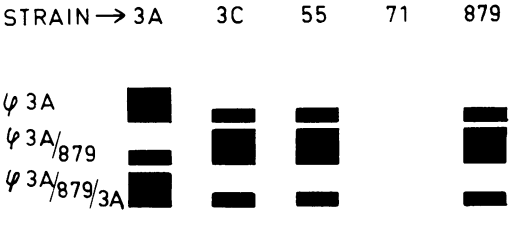

FIG. 4. *Sensitivity of group II strains for phage 3A containing different modifications. The log titers of phage 3A propagated on PS 3A (φ3A), of phage 3A propagated on 879 (φ3A/879), and of phage 3A propagated on 879 and then on PS 3A (φ3A/879/3A) are represented. The height of the black columns represents the log titer of the phages on each strain. The insensitivity of PS 71 for these phages is due to lysogenicity (10).*

other groups, though strong restriction systems are demonstrated (4, 10) in such strains (Fig. 1). It cannot be ruled out that class II enzymes are lost during extraction, e.g., by proteolytic breakdown or sonic treatment.

Our screening procedure does not allow detection of class I enzymes. In class I-type systems in *E. coli*, the frequency of modification deficiency among restriction deficient mutants is about 50% (15). The very low frequency of modification deficiency among restriction-deficient mutants in *S. aureus* argues against the presence of class I enzymes in *S. aureus*. The possibility of another class of enzymes is still open.

The restriction and modification behavior of phage 3A (Fig. 4) suggested a second modification system in strain 879. No class II enzyme could, however, be detected in the restriction-deficient mutant 879 R4 (Table 1). Again, this might be caused by loss during the preparation of extracts, to the presence of a class I enzyme, or to an enzyme of an unknown class.

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