Differentiation of Staphylococcal Species and Strains by Ribosomal RNA Gene Restriction Patterns

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Staphylococcal DNA was digested with endonucleases and probed with labelled ribosomal RNA (rRNA) from *Escherichia coli*. Reproducible restriction patterns containing between seven and 22 bands were obtained for seven different species of staphylococci. These profiles were species-specific with different strains of a particular species sharing an identical or similar restriction pattern. The results reported here indicate that rRNA gene restriction pattern analyses have an application in the taxonomy of staphylococci.

INTRODUCTION

Classification of coagulase-positive and coagulase-negative staphylococci has been based primarily on biochemical tests (Kloos & Schleifer, 1975). Other methods that have been applied include plasmid profile analysis (Archer *et al.*, 1984; Gelmi *et al.*, 1987), phage typing (de Saxe *et al.*, 1981; Vindel *et al.*, 1987), DNA base composition (Kocur *et al.*, 1971) and antibiotic susceptibility (Schito & Varaldo, 1988). However, these methods are not always able to discriminate usefully between species.

More recently, whole-cell polypeptide analysis by SDS-PAGE demonstrated that each of four staphylococcal species gave a characteristic and reproducible banding pattern (Clink & Pennington, 1986). Immunoblotting of exported proteins can also be used for typing strains of *Staphylococcus aureus* (Thomson-Carter & Pennington, 1989). However, due to variability between different gels these techniques have limited application for taxonomic purposes.

Analysis of restriction fragment length polymorphisms of ribosomal RNA (rRNA) genes may have potential as a taxonomic technique since rRNAs are both ubiquitous and highly conserved (Grimont & Grimont, 1986). Studies on a variety of micro-organisms including *Haemophilus influenzae* (Irimo *et al.*, 1988), *Providencia stuartii* (Owen *et al.*, 1988) and *Candida* spp. (Magee *et al.*, 1987) have demonstrated that rRNA gene restriction pattern analyses provide a novel means of distinguishing among isolates. This study was therefore initiated to investigate the usefulness of rRNA gene restriction pattern analysis in staphylococcal species and strain differentiation.

METHODS

Bacterial strains. A total of 22 strains representing seven different species were used in this study. The isolation and identification of these strains is described elsewhere (Clink & Pennington, 1986). All strains included had previously shown good correlation with type strains of the same species (data not shown). Species or individual strains are listed in the legends to Figs 1 and 2.

Gel electrophoresis of endonuclease-cleaved DNA. Staphylococcal DNAs were prepared by the method of Johnson (1985). DNA samples (20 µg) were cleaved with *Hind*III or *Eco*RI (2 units per µg of DNA) following the manufacturer's instructions (BCL). After 5 h incubation the enzyme reaction was stopped by ethanol

precipitation. Horizontal agarose gel electrophoresis was done overnight as described by Maniatis *et al.* (1982) using a 0.8% (w/v) agarose (type II medium EEO; Sigma) gel in Tris/borate buffer (0.089 M-Tris/borate, 0.089 M-boric acid, 0.002 M-EDTA, pH 8.3). Phage λ DNA, cleaved by *Hind*III, was used as a fragment size marker.

Radioactive labelling of rRNA. Ribosomal 16 + 23S rRNA from *Escherichia coli* (BCL) was end-labelled to a specific activity of 1.3×10^6 c.p.m. μg^{-1} using [γ - ^{32}P]ATP [Amersham; 5000 Ci mmol^{-1} (185 TBq mmol^{-1}), 10 mCi ml^{-1} (370 MBq ml^{-1})] and a 5'-DNA terminus labelling kit with T4 polynucleotide kinase (BCL) following the exchange reaction procedure.

Southern transfer and hybridization. The electrophoresed gel was washed and blotted by the method of Southern (1975). The nitrocellulose (Hybond-C; Amersham) was pre-hybridized and then hybridized with radioactively labelled 16 + 23S rRNA (3×10^5 c.p.m. ml^{-1}) then washed and autoradiographed, all as described by Grimont & Grimont (1986).

RESULTS AND DISCUSSION

Reproducible rRNA gene restriction patterns were obtained from the seven species studied. The DNA of each species reacted with the radioactively labelled rRNA probe to produce a characteristic banding pattern (Fig. 1). Digestion with *Hind*III produced fewer fragments than *Eco*RI cleavage. However, both endonucleases generated reproducible restriction patterns.

Cleavage by *Hind*III yielded an average of nine fragments per lane with the sizes varying from >23 kb to 0.75 kb (Fig. 1*a*). Fragments larger than 10 kb are included for qualitative comparisons only. A total of 11 fragments, from 7.6 kb to 0.75 kb, were common to more than one species but not to all species, e.g. a 4.7 kb fragment was apparent in lanes 3, 4 and 6.

DNAs from the same samples cleaved by *Eco*RI gave an average of 15 fragments per lane, with sizes varying from >23 kb to 0.6 kb (Fig. 1*a*). A greater number of common fragments were observed, 28 in all, from the 11.5 kb band in lanes 1, 3, 5 and 7 to the 0.8 kb band in lanes 1, 2, 3 and 4. Again, none of these bands were common to all species. Therefore, although some similarities were apparent each species had its own characteristic banding pattern.

Comparisons of the restriction patterns obtained for *S. aureus* 161 and the methicillin-resistant *S. aureus* strain 81 (MRSA 81), by either *Hind*III or *Eco*RI cleavage, demonstrate that strains of the same species share similar profiles. *Hind*III cleavage of *S. aureus* 161 and MRSA 81 DNAs gave restriction patterns of ten and eight bands respectively (Fig. 1*a*, lanes 3 and 7). Six fragments were common to both strains: >23, 7.3, 3.5, 1.2, 0.9 and 0.8 kb. Similarly, *Eco*RI cleavage of the same samples gave nine common fragments: 11.5, 8.4, 6.3, 5.2, 4.8, 4.3, 3.9, 2.9 and 2.4 kb (Fig. 1*a*, lanes 1 and 5) from a total of 22 bands for *S. aureus* 161 and 16 bands for MRSA 81. Since these different strains of *S. aureus* shared similar but not identical restriction patterns other intraspecific patterns were studied.

DNAs of five strains from each of three clinically important species were analysed using both *Hind*III and *Eco*RI: *S. aureus*, *S. epidermidis* and *S. capitis* (Fig. 2). Characteristic banding patterns for each of the three species were apparent. The five constituent strains of each species shared identical or similar rDNA restriction patterns.

*Hind*III cleavage of five *S. capitis* strains and five *S. epidermidis* strains revealed only one difference among strains of either species: *S. capitis* 2 (Fig. 2*a*, lane 5) had a 3.4 kb fragment whereas strains 37, 21, 20 and 17 (lanes 1 and 4) had a 3.1 kb fragment; *S. epidermidis* 4 (Fig. 2*a*, lane 9) had an additional fragment of 1.8 kb.

S. aureus strains appeared to be more heterogeneous than either *S. capitis* or *S. epidermidis* strains. Only two strains were indistinguishable: *S. aureus* 154 and *S. aureus* 151 (Fig. 2*a*, lanes 12 and 13). Strain 159 (lane 11) had an additional fragment of 0.8 kb. Strain 150 and 103 differed by four fragments each in the 12.6 to 4.2 kb range.

The restriction patterns obtained by *Eco*RI cleavage of the same 15 samples were different from the *Hind*III patterns (2*b*). *S. capitis* 37 and all five *S. aureus* strains did not cleave satisfactorily, substantial amounts of high-molecular-mass DNA persisting (Fig. 2*b*, lanes 1 and 11–15). However, restriction patterns for *S. aureus* strains could be discerned: strains 154, 151 and 150 (lanes 12, 13 and 14) had 20 bands, strain 159 (lane 11) had 16 and strain 103 (lane 15) had 11. Only four fragments from 1.2–1.0 kb, were common to all five strains.

S. capitis strains 21, 20 and 17 (lanes 2–4) were indistinguishable, each having 14 matched fragments. Strain 2 (lane 5) with 20 bands shared eight fragments with these strains: 7.4, 3.9, 2.6,

Fig. 1. Autoradiograph of nitrocellulose after transfer of (a) *Hind*III restriction fragments or (b) *Eco*RI restriction fragments of the DNA from six staphylococcal species and hybridization with ^{32}P -labelled 16 + 23S rRNA. (a) Lane 1, *S. capitis*; 2, *S. cohnii*; 3, *S. aureus* 161; 4, *S. xylosus*; 5, *S. saprophyticus*; 6, *S. hominis*; 7, *S. aureus* MRSA 81. (b) Lane 1, *S. aureus* MRSA 81; 2, *S. hominis*; 3, *S. saprophyticus*; 4, *S. xylosus*; 5, *S. aureus*; 6, *S. cohnii*; 7, *S. capitis*.

2.0, 1.3, 1.2, 1.1 and 0.8 kb. *S. epidermidis* strains 14 and 8 (lanes 6 and 7) had 14 bands each, differing only by the heaviest pair of fragments in the range 10.0–8.7 kb. Strains 7, 4 and 1 (lanes 8, 9 and 10) with 13, 18 and 14 fragments respectively, were similar superficially but contained a total of 14 variable bands mostly in the range of >23 to 2.0 kb.

The observation that certain strains have identical rRNA gene restriction patterns may be attributed to their isolation. *S. epidermidis* and *S. capitis* strains were isolated from members of staff in the diagnostic laboratory indicating a possible common origin for these strains. In

Fig. 2. Autoradiograph of nitrocellulose after transfer of (a) *Hind*III restriction fragments or (b) *Eco*RI restriction fragments of the DNA from three staphylococcal species and hybridization with ^{32}P -labelled 16 + 23S rRNA. (a) and (b) lanes 1-5, *S. capitis* strains 37, 21, 20, 17 and 2; lanes 6-10, *S. epidermidis* strains 14, 8, 7, 4 and 1; lanes 11-15, *S. aureus* strains 159, 154, 151, 150 and 103.

contrast, the non-identical *S. aureus* strains 159, 150 and 103 were all obtained from clinical specimens from different sources. From this preliminary study sufficient interspecific differences exist to form the basis of a typing scheme. Intraspecific differences are not so clearly defined and analyses of larger numbers of strains are required.

The degree of intraspecific variation observed here was comparable with that reported by Grimont & Grimont (1986), where one or several rRNA gene restriction patterns were apparent within a species, using a 16S + 23S rRNA probe obtained from *E. coli*. Strains with identical patterns showed insignificant divergence in DNA hybridization studies, and different patterns corresponded to significant divergences in thermal stability studies of DNA/DNA hybrids. (Results of Grimont & Grimont, 1986).

Use of *E. coli* rRNA as a broad spectrum probe would allow application of the procedure described in this paper to the large numbers of isolates required in taxonomic studies.

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