Characterization of *Staphylococcus* Species by Ribosomal RNA Gene Restriction Patterns

By MARIE-LAURE DE BUYSER,¹† ANNE MORVAN,¹
FRANCINE GRIMONT² AND NÉVINE EL SOLH¹*

¹Centre National de Référence pour les Staphylocoques, Laboratoire des Staphylocoques et des Streptocoques, Institut Pasteur, 75724 Paris Cedex 15, France

²Unité des Entérobactéries, Institut National de la Santé et de la Recherche Médicale, Unité 199, Institut Pasteur, 75724 Paris Cedex 15, France

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The rRNA gene restriction patterns of 110 strains belonging to 12 staphylococcal species have been determined. The strains, isolated from various sources, were epidemiologically unrelated. Total DNA was cleaved with restriction enzymes *Hind*III and *Eco*RI, electrophoretically separated and probed with radiolabelled 16S rDNA from *Bacillus subtilis* inserted in a plasmid vector, pBR322. Forty-four distinct *Hind*III patterns and 44 distinct *Eco*RI patterns were observed. Strains belonging to different species had different patterns. Although distinct patterns were also observed within some species, a core of common bands could be discerned within each species or subspecies. Analysis of the patterns revealed two taxa in *Staphylococcus xylosus* which were not evident using phenotypic characteristics. Of 18 strains which were difficult to identify using phenotypic schemes, 15 showed patterns typical of known species. The three remaining atypical strains showed unusual patterns and may belong either to a known species, not included in the study, or to a new species. Since various patterns were observed within some species (e.g. *S. aureus* and *S. epidermidis*), rRNA gene restriction patterns may have epidemiological, as well as taxonomic interest.

INTRODUCTION

In the last 14 years, the taxonomy of the genus *Staphylococcus* has undergone extensive changes and 26 species have been delineated by DNA–DNA hybridization (Kloos & Schleifer, 1986; Freney *et al.*, 1988). Although several schemes (Kloos & Schleifer, 1986; Schleifer *et al.*, 1984; Devriese *et al.*, 1985; Hajek *et al.*, 1986; Freney *et al.*, 1988) have been recommended for the identification of staphylococcal species, many strains cannot be identified by conventional methods. Improved methods are needed to identify species, subspecies and biotypes of the genus *Staphylococcus*.

Staphylococci can cause severe infections. The three coagulase- and thermonuclease-producing species *Staphylococcus aureus*, *S. intermedius* and *S. hyicus* often cause disease in previously healthy hosts. The so-called coagulase negative staphylococci (CNS), with the exception of *S. saprophyticus*, are opportunistic pathogens which cause infections in humans mainly after implantation of foreign bodies or in immunocompromised patients; *S. saprophyticus* is often involved in urinary tract infections (Kloos & Schleifer, 1986; Eykyn, 1988). The increased incidence of nosocomial infections caused by *S. aureus* and CNS have

† Present address: Centre National d'Etudes Vétérinaires et Alimentaires, Laboratoire Central d'Hygiène Alimentaire, 43 rue de Dantzig, 75015 Paris, France.

Abbreviation: CNS, coagulase negative staphylococci.

stimulated interest in the use of epidemiological markers to fingerprint and compare clinical strains (Collins *et al.*, 1984; Mickelsen *et al.*, 1985; Parisi *et al.*, 1986).

Recently, patterns of restriction fragments hybridizing with labelled rRNA from *Escherichia coli* have been used to fingerprint bacterial strains (Grimont & Grimont, 1986; Irino *et al.*, 1988). Probes which can be used to visualize rRNA gene restriction patterns are cloned rRNA genes (Yogev *et al.*, 1988; Bercovier *et al.*, 1986; Gottlieb & Rudner, 1985; Saunders *et al.*, 1988), 5'-end labelled rRNA (Grimont & Grimont, 1986; Stull *et al.*, 1988), or cDNA (Pitcher *et al.*, 1987). Since rRNA sequences have been remarkably conserved during evolution, a single probe can be used to visualize patterns in taxonomically distant bacteria. These rRNA gene restriction patterns can be species-, subspecies- or type-specific depending on the degree of intra-species genomic heterogeneity (Grimont & Grimont, 1986; Irino *et al.*, 1988).

The purpose of this study was to evaluate the potential usefulness of the rRNA gene restriction pattern as a taxonomic tool and a molecular epidemiological marker for staphylococcal species of human origin or those species difficult to distinguish from human-associated species.

METHODS

Bacterial strains. The 110 epidemiologically unrelated staphylococcal strains studied were isolated from human (52 clinical isolates), animal (54 isolates), hospital environment (2 isolates) and unknown sources (2 isolates). The origins of the strains are listed in Table 1. Twelve species are represented and the type strain of each species is included.

E. coli strains used were HVC45 (Dagert & Ehrlich, 1979) harbouring plasmid pBR322 (Sutcliffe, 1978), and SF8 harbouring plasmid pBA2 (Iglesias *et al.*, 1983). Plasmid pBA2 contains a 2.3 kb *Bacillus subtilis* DNA fragment encoding 16S rRNA inserted in the *Hind*III site of pBR322.

Species identification of staphylococci. (a) *Identification by the French National Reference Centre for Staphylococci* (FNRCs). The strains were Gram-positive and catalase-positive cocci. Staphylococci were differentiated from micrococci by use of the following tests (De Buyser *et al.*, 1987): production of acid from glucose under anaerobic conditions; production of acid from glycerol under aerobic conditions; sensitivity to lysostaphin, nitrofurantoin, vibriostatic agent O/129 and bacitracin.

Isolates were assigned to known staphylococcal species according to identification schemes (Kloos & Schleifer, 1986; Schleifer *et al.*, 1984; Devriese *et al.*, 1985; Hajek *et al.*, 1988; Frenay *et al.*, 1988), by use of the following tests: colony diameter and pigment; sensitivity to novobiocin (5 µg per disk); nitrate reduction; proteolytic activity on casein; production of urease, haemolysins, DNAase, heat-stable DNAase, clumping factor, free coagulase (rabbit plasma), alkaline phosphatase, acetoin, arginine dihydrolase, ornithine decarboxylase; production of acid under aerobic conditions from maltose, sucrose, D-trehalose, D-mannitol, D-cellobiose, lactose, D-fructose, D-melezitose, D-xylose, L-arabinose, D-raffinose, D-melibiose, D-mannose, D-turanose, D-ribose; production of acid from D-mannitol under anaerobic conditions. Staphylococcal species were also differentiated by use of the API Staph gallery (API System). *S. cohnii* subspecies 1 and 2 were differentiated by use of the Staph Ident gallery (API System). A seroinhibition test, with antibodies raised against *S. aureus* heat-stable DNAase (Staphynuclease kit, Biomérieux), was used to distinguish *S. aureus* nuclease from other staphylococcal nucleases.

(b) *Identification by API Research Laboratory (APIRL)*. Sixty-eight strains were also identified by APIRL, using the ATB 32 Staph gallery (API System).

Plasmid isolation and labelling. Plasmids pBR322 and pBA2 were used as probes in the hybridization experiments. Plasmids were isolated and purified in a caesium chloride/ethidium bromide gradient according to Maniatis *et al.* (1982). They were labelled with [α -³²P]dCTP (Amersham) using the Multiprime DNA labelling system (Amersham) according to the manufacturer's instructions. The specific activity of the probes was approx. 10⁸ c.p.m. (µg DNA)⁻¹.

Isolation and purification of staphylococcal DNA. Staphylococcal strains were grown in 25 ml Brain Heart Infusion (Difco) for 18 h at 37 °C, then harvested and washed in 10 mM-Tris, pH 8.00, 1 mM-EDTA (TE buffer) and resuspended in TE buffer. They were treated with lysostaphin (Sigma) at a final concentration of 2 units ml⁻¹ for 30 min at 37 °C. Lysis was checked on samples treated with 1% (w/v) SDS. If lysis was incomplete, cells were further incubated at 37 °C with lysostaphin at a final concentration of 12 units ml⁻¹. Lysates were then treated with SDS and proteinase K (Merck) at final concentrations of 1% (w/v) and 10 units ml⁻¹ respectively, for 30 min at 37 °C. Potassium acetate was added at a final concentration of 1 M and the mixtures were incubated for 30 min at 4 °C. Proteins were denatured and removed by sequential extraction with equal volumes of phenol, phenol/chloroform/isoamyl alcohol (25:24:1, by vol.) and chloroform/isoamyl alcohol (24:1, v/v). 2-Propanol (0.6 vol.) was added to the aqueous layer. Precipitated DNA was recovered by centrifugation, washed in 70% (v/v) ethanol, dried and resuspended in TE buffer, treated with RNAase (Calbiochem, Behring Diagnostics) at a final concentration of 3 units ml⁻¹ for 2 h at 37 °C, and kept at -20 °C until use.

Table 1. Origin and rRNA gene restriction patterns of 110 staphylococcal strains

Taxon and strain designation	Origin* or reference	Source	HindIII rRNA gene restriction pattern	EcoRI rRNA gene restriction pattern
<i>S. aureus</i>				
ATCC 12600†	ATCC	Man	H ₁	E ₁
RN 450	Novick (1967)	Unknown	H ₁	E ₁
BM 3250‡	El Solh <i>et al.</i> (1984)	Man	H ₁	E ₁
BM 3254‡	El Solh <i>et al.</i> (1984)	Man	H ₁	E ₁
BM 3121‡	El Solh <i>et al.</i> (1981)	Man	H ₁	E ₁
ATCC 6538P	ATCC	Unknown	H ₁	E ₂
BM 3247‡	El Solh <i>et al.</i> (1984)	Man	H ₂	E ₃
CH 1	De Buyser <i>et al.</i> (1987)	Goat	H ₃	E ₄
CH 96	De Buyser <i>et al.</i> (1987)	Goat	H ₃	E ₄
CH 102	De Buyser <i>et al.</i> (1987)	Goat	H ₃	E ₄
CH 54	De Buyser <i>et al.</i> (1987)	Goat	H ₃	E ₄
CH 31	De Buyser <i>et al.</i> (1987)	Goat	H ₄	E ₄
CH 73	De Buyser <i>et al.</i> (1987)	Goat	H ₄	E ₄
CH 49	De Buyser <i>et al.</i> (1987)	Goat	H ₅	E ₄
CH 74	De Buyser <i>et al.</i> (1987)	Goat	H ₆	E ₄
64 048	Pillet & Orta (1973)	Poultry	H ₇	E ₅
64 043	Pillet & Orta (1973)	Poultry	H ₇	E ₆
BM 3319	El Solh <i>et al.</i> (1984)	Man	H ₇	E ₇
86 315	FNRCS	Man	H ₇	E ₈
BM 3251	El Solh <i>et al.</i> (1984)	Man	H ₇	E ₈
86 002	FNRCS	Man	H ₇	E ₉
BM 3248	El Solh <i>et al.</i> (1984)	Man	H ₈	E ₁₀
85 282	FNRCS	Man	H ₈	E ₁₀
85 522	FNRCS	Man	H ₈	E ₁₁
BM 3152‡	El Solh <i>et al.</i> (1981)	Man	H ₉	E ₁₂
<i>S. intermedius</i>				
subsp. pigeon				
ATCC 29663†	ATCC	Pigeon	H ₁₀	E ₁₃
subsp. carnivora				
81 508	FNRCS	Dog	H ₁₁	E ₁₄
61 221	I. Live	Dog	H ₁₂	E ₁₅
74 337	FNRCS	Dog	H ₁₂	E ₁₅
75 619	FNRCS	Dog	H ₁₂	E ₁₅
83 743	FNRCS	Man	H ₁₂	E ₁₅
<i>S. hyicus</i>				
ATCC 11249†	L. A. Devriese	Pig	H ₁₃	E ₁₆
VA 503	L. A. Devriese	Pig	H ₁₄	E ₁₆
BOD 28	L. A. Devriese	Cow	H ₁₄	E ₁₆
E 80	FNRCS	Cow	H ₁₅	E ₁₇
PA 81	L. A. Devriese	Horse	H ₁₆	E ₁₈
VII 96	L. A. Devriese	Poultry	H ₁₇	E ₁₉
<i>S. chromogenes</i>				
NCTC 10530†	L. A. Devriese	Pig	H ₁₈	E ₂₀
VA 608	L. A. Devriese	Pig	H ₁₉	E ₂₀
BO 64	L. A. Devriese	Cow	H ₁₉	E ₂₀
BO 153	L. A. Devriese	Cow	H ₁₉	E ₂₀
CH 22	De Buyser <i>et al.</i> (1987)	Goat	H ₁₉	E ₂₀
CH 62	De Buyser <i>et al.</i> (1987)	Goat	H ₁₉	E ₂₀
<i>S. epidermidis</i>				
ATCC 14990†	W. E. Kloos	Man	H ₂₀	E ₂₁
85 711	FNRCS	Man	H ₂₀	E ₂₁
86 166	FNRCS	Man	H ₂₀	E ₂₁
86 060	FNRCS	Man	H ₂₁	E ₂₁
52 260	Pillet & Orta (1970)	Man	H ₂₂	E ₂₁
75 864	FNRCS	Man	H ₂₂	E ₂₁
84 331	FNRCS	Man	H ₂₂	E ₂₁
CH 8	De Buyser <i>et al.</i> (1987)	Goat	H ₂₂	E ₂₁
CH 93	De Buyser <i>et al.</i> (1987)	Goat	H ₂₂	E ₂₁
CH 106	De Buyser <i>et al.</i> (1987)	Goat	H ₂₂	E ₂₁

Table 1. (continued)

Taxon and strain designation	Origin* or reference	Source	HindIII rRNA gene restriction pattern	EcoRI rRNA gene restriction pattern
<i>S. epidermidis</i> —contd				
CH 109	De Buyser <i>et al.</i> (1987)	Goat	H ₂₂	E ₂₁
CH 133	De Buyser <i>et al.</i> (1987)	Goat	H ₂₂	E ₂₁
CH 145	De Buyser <i>et al.</i> (1987)	Goat	H ₂₂	E ₂₁
CH 58	De Buyser <i>et al.</i> (1987)	Goat	H ₂₃	E ₂₁
<i>S. warneri</i>				
ATCC 27836†	W. E. Kloos	Man	H ₂₄	E ₂₂
MCS 4	W. E. Kloos	Man	H ₂₄	E ₂₂
75 594	FNRCs	Man	H ₂₄	E ₂₂
76 066	FNRCs	Man	H ₂₄	E ₂₂
CH 251	De Buyser <i>et al.</i> (1987)	Goat	H ₂₄	E ₂₂
CH 108	De Buyser <i>et al.</i> (1987)	Goat	H ₂₄	E ₂₂
CH 98	De Buyser <i>et al.</i> (1987)	Goat	H ₂₄	E ₂₂
CH 225	De Buyser <i>et al.</i> (1987)	Goat	H ₂₄	E ₂₂
CH 17	De Buyser <i>et al.</i> (1987)	Goat	H ₂₅	E ₂₂
CH 30	De Buyser <i>et al.</i> (1987)	Goat	H ₂₅	E ₂₂
CH 60	De Buyser <i>et al.</i> (1987)	Goat	H ₂₅	E ₂₂
<i>Unidentified</i> §				
CH 199	De Buyser <i>et al.</i> (1987)	Goat	H ₂₆	E ₂₃
83 796	FNRCs	Man	H ₂₆	E ₂₃
<i>S. hominis</i> 1				
ATCC 27844†	W. E. Kloos	Man	H ₂₇	E ₂₄
SM 67	W. E. Kloos	Man	H ₂₇	E ₂₄
75 611	FNRCs	Man	H ₂₇	E ₂₄
78 354	FNRCs	Man	H ₂₇	E ₂₄
86 116	FNRCs	Man	H ₂₇	E ₂₄
<i>S. hominis</i> 2				
CH 181	De Buyser <i>et al.</i> (1987)	Goat	H ₂₈	E ₂₅
<i>S. lugdunensis</i>				
ATCC 43809†	J. Freney	Man	H ₂₉	E ₂₆
CRS 307	J. Freney	Man	H ₃₀	E ₂₇
CH 97	De Buyser <i>et al.</i> (1987)	Goat	H ₃₀	E ₂₇
CRSN 850412	J. Freney	Man	H ₃₁	E ₂₇
83 091	FNRCs	Man	H ₃₁	E ₂₇
86 428	FNRCs	Man	H ₃₁	E ₂₇
CH 80	De Buyser <i>et al.</i> (1987)	Goat	H ₃₁	E ₂₇
84 373	FNRCs	Man	H ₃₁	E ₂₈
<i>S. haemolyticus</i>				
ATCC 29970†	W. E. Kloos	Man	H ₃₂	E ₂₉
KES 23	W. E. Kloos	Man	H ₃₂	E ₂₉
75 581	FNRCs	Man	H ₃₂	E ₂₉
77 028	FNRCs	Man	H ₃₂	E ₂₉
85 474	FNRCs	Man	H ₃₃	E ₂₉
85 333	FNRCs	Man	H ₃₄	E ₃₀
<i>S. saprophyticus</i>				
ATCC 15305†	W. E. Kloos	Man	H ₃₅	E ₃₁
KL 20	W. E. Kloos	Man	H ₃₅	E ₃₁
75 168	FNRCs	Man	H ₃₅	E ₃₁
75 751	FNRCs	Man	H ₃₅	E ₃₁
<i>S. xylosus</i>				
ATCC 29971†	W. E. Kloos	Man	H ₃₆	E ₃₂
CH 167	De Buyser <i>et al.</i> (1987)	Goat	H ₃₆	E ₃₂
CH 61	De Buyser <i>et al.</i> (1987)	Goat	H ₃₇	E ₃₃
CH 232	De Buyser <i>et al.</i> (1987)	Goat	H ₃₇	E ₃₃
77 020	FNRCs	Horse	H ₃₇	E ₃₃
78 037	FNRCs	Horse	H ₃₈	E ₃₄
CH 65	De Buyser <i>et al.</i> (1987)	Goat	H ₃₈	E ₃₅
CH 100	De Buyser <i>et al.</i> (1987)	Goat	H ₃₉	E ₃₆

Table 1. (continued)

Taxon and strain designation	Origin* or reference	Source	HindIII rRNA gene restriction pattern	EcoRI rRNA gene restriction pattern
<i>S. cohnii</i>				
subsp. 1				
ATCC 29974†	W. E. Kloos	Man	H ₄₀	E ₃₇
87 100	FNRCS	Dust	H ₄₀	E ₃₈
CH 227	De Buyser <i>et al.</i> (1987)	Goat	H ₄₁	E ₃₉
87 106	FNRCS	Man	H ₄₂	E ₄₀
subsp. 2				
MK 247	W. E. Kloos	Man	H ₄₃	E ₄₁
79 006	FNRCS	Dust	H ₄₃	E ₄₂
78 073	FNRCS	Man	H ₄₃	E ₄₃
CH 20	De Buyser <i>et al.</i> (1987)	Goat	H ₄₄	E ₄₄

* ATCC, American Type Culture Collection, Rockville, Md, USA; NCTC, National Collection of Type Cultures, London UK; FNRCS, National Reference Centre for Staphylococci, Paris, France; I. Live, Philadelphia, Pa., USA; L. A. Devriese, Gent, Belgium; W. E. Kloos, Raleigh, NC, USA; J. Freney, Lyon, France.

† Type strain.

‡ Methicillin-resistant *S. aureus* strains isolated in French hospitals. All the other *S. aureus* strains studied were methicillin-susceptible.

§ The two unidentified strains could correspond either to *S. warneri* or to *S. hominis* according to their biochemical characteristics. Their rRNA gene restriction patterns were intermediate between those observed with *S. warneri* and with *S. hominis*.

|| *S. hominis* 1 and *S. hominis* 2 were differentiated according to the ATB 32 Staph gallery (API System) by use of three biochemical characteristics: production of alkaline phosphatase (1% of *S. hominis* 1 strains and 94% of *S. hominis* 2 strains are positive), production of arginine dihydrolase (5% of *S. hominis* 1 strains and 73% of *S. hominis* 2 strains are positive), and production of acid from mannose (1% of *S. hominis* 1 strains and 75% of *S. hominis* 2 strains are positive).

Restriction endonuclease digestion and agarose gel electrophoresis of DNA. DNA (2 to 5 µg) was cleaved with restriction endonuclease HindIII (Amersham) or EcoRI (Genofit), according to the manufacturer's instructions. Restriction DNA fragments were separated electrophoretically in 0.8% (w/v) agarose (Sigma) gel in Tris/borate buffer as described by Maniatis *et al.* (1982).

Bg/II and HindIII-EcoRI fragments of bacteriophage λ DNA (Biolabs) were used as size markers. The sizes of these fragments were the same as those published by Maniatis *et al.* (1982).

Southern blotting and hybridization. After electrophoresis, agarose gels were sequentially soaked in 0.25 M-HCl for 5 min, 1.5 M-NaCl, 0.5 M-NaOH for 30 min and 0.5 M-Tris/HCl (pH 7.2), 1.5 M-NaCl, 1 mM-EDTA for 30 min. Southern blotting (Southern, 1975) was carried out on Hybond-N nylon membrane (Amersham). The blotted DNA was fixed by UV irradiation at 305 nm for 5 min. The prehybridization, hybridization and washing steps were performed as previously described (Grimont & Grimont, 1986). The blots were exposed to Fuji RX films at -70 °C with intensifying screens for 24 h to several days.

RESULTS

Biochemical identification of staphylococcal species

Identification showed discrepancies in 12 (17.6%) out of 68 strains identified at both FNRCS and APIRL. Strain 64048 identified with *S. aureus* (FNRCS) or *S. chromogenes* (APIRL). This strain showed delayed production of free coagulase and was clumping-factor-positive, acetoin-negative and heat-stable-DNAase-positive. Activity of this latter enzyme was inhibited by antiserum raised against *S. aureus* heat-stable DNAase. Strains CH133 and CH145, which showed delayed aerobic acid production from sucrose, identified with *S. epidermidis* (FNRCS) or was unidentified (APIRL). Three strains (CH98, CH225 and 83796) identified with *S. hominis* (FNRCS) or *S. warneri* (APIRL), whereas strain 85474 identified with *S. hominis* (FNRCS) or *S. haemolyticus* (APIRL). Five strains (CH108, CH17, CH60, CH199 and CH181)

Fig. 1. Autoradiogram of *Hind*III digests of DNA from 14 staphylococcal strains, separated by electrophoresis and probed with 32 P-labelled plasmid pBA2. Lanes A to C, *S. haemolyticus* strains ATCC 29970, 85474, 85333; lanes D to H, *S. warneri* strains CH251, CH30, CH60, CH108, CH98; Lane I, *S. hominis* 1 strain ATCC 27844; lane J, *S. hominis* 2 strain CH181; lanes K to M, *S. lugdunensis* strains 86428, CH80, CH97; lane N, unidentified strain CH199.

identified with either *S. warneri* or *S. hominis* without possible distinction between the species (FNCRS); however, they identified with either *S. warneri* (CH108, CH17, CH60, CH199) or *S. hominis* 2 (CH181) at APIRL.

Although identified without discrepancy at both FNCRS and APIRL, three *S. aureus* strains and three *S. lugdunensis* strains were found to be atypical. The three *S. aureus* strains were atypical with respect to one or two characteristics that are usually shared by more than 90% of the *S. aureus* strains (Kloos & Schleifer, 1986). Strain 64043 produced no heat-stable DNAase. Strain CH96 failed to produce acid from mannitol under anaerobic conditions. Strain 86315 was acetoin-negative and free coagulase-negative. Heat-stable DNAase was produced by the latter two atypical strains. Enzyme activity was inhibited by antiserum raised against *S. aureus* heat-stable DNAase, which confirmed that these two strains belonged to *S. aureus* species. The three *S. lugdunensis* strains (CH97, 86428 and CH80) were clumping-factor-negative even when tested on the recommended medium, Columbia blood agar (Freney *et al.*, 1988).

rRNA gene restriction patterns

When the *Hind*III- or *Eco*RI-cleaved staphylococcal DNA was probed with pBR322, no restriction fragments hybridized with the probe. Hence, pBA2 was used as a probe for the visualization of rRNA genes without the need for separating the insert from the pBR322 vector.

The patterns of radiolabelled bands hybridizing with pBA2 were designated the rRNA gene restriction patterns. *Hind*III rRNA gene restriction patterns of 13 strains belonging to four species and one unidentified strain are shown in Fig. 1. A total of 44 distinct *Hind*III rRNA gene restriction patterns, designated H1 to H44, and 44 distinct *Eco*RI rRNA gene restriction patterns, designated E1 to E44, could be distinguished (Table 1). As shown in Figs 2 and 3, *Hind*III patterns consisted of 5 to 10 bands whereas *Eco*RI patterns consisted of 6 to 13 bands. Each species showed 1 to 9 *Hind*III patterns and 1 to 12 *Eco*RI patterns. Strains belonging to different species did not share the same pattern.

Among *S. aureus* strains, the goat isolates belonging to biotype *ovis*, CH1, CH96, CH102, CH54, CH31, CH73, CH49 and CH74 (De Buyser *et al.*, 1987), specifically showed a single *Eco*RI pattern, E4, and four *Hind*III patterns, H3, H4, H5 and H6, which were not shown by the other *S. aureus* strains. The five methicillin-resistant *S. aureus* strains studied showed three distinct pairs of *Hind*III- and *Eco*RI-patterns, H1-E1, H2-E3 and H9-E12. One of these pairs of patterns, H1-E1, was also shown by methicillin-susceptible *S. aureus* strains (Table 1).

Each species could be characterized by a core of common rRNA gene restriction fragments which constituted a species-specific pattern. However, when *S. cohnii* DNA was cleaved by *Hind*III, no species-specific pattern was observed but subspecies-specific patterns could be detected. Strains of *S. cohnii* subspecies 1 (Kloos & Schleifer, 1986) shared two *Hind*III fragments and this pair of fragments was not found in other patterns. Strains of *S. cohnii* subspecies 2 showed six common *Hind*III fragments. When considering the eight *S. xylosus* strains studied, only one and three common bands were shown among *Hind*III- and *Eco*RI-patterns, respectively. However, on the basis of their *Hind*III- and *Eco*RI-patterns, two taxa could be distinguished among these strains. The first taxon included five strains showing patterns H36, H37 (five common bands) and E32, E33 (eight common bands). The second one included three strains showing patterns H38, H39 (six common bands) and E34, E35, E36 (nine common bands). When considering *S. intermedius* strains, only two common bands were observed among *Eco*RI patterns, whereas eight common bands were observed within *S. intermedius* subsp. *carnivora* *Eco*RI patterns.

The characterization of taxon-specific cores of fragments among rRNA gene restriction patterns was found useful in assessing the identification of the six atypical strains and to identify nine out of the 12 strains for which discrepant results were obtained. According to rRNA gene restriction patterns, strains 64048, 64043, CH96 and 86315 identified with *S. aureus*; CH133 and CH145 with *S. epidermidis*; CH108, CH98, CH225, CH17 and CH60 with *S. warneri*; CH97, 86428 and CH80 with *S. lugdunensis*, and 85474 with *S. haemolyticus*. Strains CH199 and 83796, which were found biochemically to be either *S. warneri* or *S. hominis*, showed intermediate patterns (H26, E23) between those of these two species and could not be identified. Strain CH181, the only strain which identified with *S. hominis* 2 (a group detected using ATB 32 Staph gallery but not yet validated by molecular studies), showed different patterns (H28, E25) from that shown by the five strains identified with *S. hominis* 1 and the 11 identified with *S. warneri*.

DISCUSSION

The discrepancies in identification obtained at FNRCS and APIRL concerned 17.6% of the 68 staphylococcal strains studied in both laboratories. This indicates the insufficiency of present identification schemes based on phenotypic characteristics.

rRNA gene restriction patterns have been proposed as a taxonomic aid mainly for Gram-negative bacteria (Grimont & Grimont, 1986; Saunders *et al.*, 1988), and therefore it was interesting to evaluate the contribution of these patterns to the identification of Gram-positive bacteria such as staphylococci. Using this method, species- or subspecies-specific cores of restriction fragments have been observed. Thus, species usually considered as difficult to

Fig. 2. Schematic representation of the 44 *Hind*III rRNA gene restriction patterns (H1 to H44) detected among the 110 staphylococcal strains studied. Dotted lines indicate very weakly labelled bands.

Fig. 3. Schematic representation of the 44 *EcoRI* rRNA gene restriction patterns (E1 to E44) detected among the 110 staphylococcal strains studied. Dotted lines indicate very weakly labelled bands.

distinguish from each other using phenotypic characteristics, *S. aureus*, *S. intermedius*, *S. hyicus* and *S. chromogenes* on the one hand, *S. haemolyticus*, *S. warneri* and *S. hominis* on the other, could be easily differentiated. Moreover, two distinct taxa could be discerned among the *S. xylosus* strains studied on the basis of their rRNA gene restriction patterns, whereas the phenotypic characteristics did not enable differentiation of these taxa. *S. xylosus* strains should therefore be submitted to quantitative DNA-DNA hybridization in order to assess the validity of these two taxa. In addition, the use of rRNA gene restriction patterns enabled the identification of 15 out of 18 phenotypically atypical strains. The three remaining phenotypically atypical strains (CH199, 83796 and CH181) had unusual patterns and could belong either to a species not included in this study or to a new species or subspecies. They should also be submitted to quantitative DNA-DNA hybridization for further elucidation of their taxonomic position.

The present work concerned staphylococcal species frequently involved in human and animal infection and other species from which they need to be differentiated. rRNA gene restriction patterns of remaining staphylococcal species are now under study in our laboratory.

Genomic species (i.e. species defined by DNA relatedness) may show various degrees of heterogeneity. Homogeneous species often show one or a few rRNA gene restriction patterns (Grimont & Grimont, 1986) whereas heterogeneous species show many patterns (Irino *et al.*, 1988). Thus, in addition to taxonomic relevance, rRNA gene restriction patterns may have epidemiological relevance (Grimont & Grimont, 1986; Stull *et al.*, 1988; Irino *et al.*, 1988). In this study, a variety of patterns was observed within some species, depending on the restriction enzyme used to cleave DNA. Cleavage with *EcoRI* yielded 12 distinct patterns among the 25 *S. aureus* strains studied whereas only nine *HindIII* patterns were observed. In contrast, among the 14 *S. epidermidis* strains, the number of patterns was greater when *HindIII* was used (four distinct patterns) instead of *EcoRI* (single pattern). The variety of patterns observed among *S. aureus* strains, especially the methicillin-resistant ones, and the *S. epidermidis* strains, could be used to type these strains. However, such an epidemiological approach needs to be validated by study of many strains involved in different outbreaks.

In conclusion, the present work demonstrates the usefulness of rRNA gene restriction patterns in staphylococcal taxonomy. They may be used as a screening method to detect new taxa. The resulting banding patterns may also constitute additional epidemiological markers.

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