Comparison of the *Sma*I-Digested Chromosomes of *Staphylococcus epidermidis* and the Closely Related Species *Staphylococcus capitis* and *Staphylococcus caprae*

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Pulsed-field gel electrophoresis was used to examine the chromosomal polymorphisms existing within and between four closely related members of the *Staphylococcus epidermidis* species group, *S. epidermidis*, *Staphylococcus caprae*, *Staphylococcus capitis* subsp. *capitis*, and *S. capitis* subsp. *ureolyticus*. *Sma*I was chosen as the restriction endonuclease for this study because it generated only a few well-separated chromosomal fragments. Each of the species and subspecies showed distinct *Sma*I digest patterns. The strains examined in this study were collected over a 20-year period from various geographical locations. The results indicate that DNA fragment patterns are unique to each species and subspecies and represent a reasonably stable component in the chromosome structure. *S. caprae* and *S. capitis* demonstrated considerable conservation in chromosome structure as indicated by the large numbers of conserved *Sma*I digest fragments. The polymorphisms found within each species appear to be linked to the species' character variability. The genome size of each *Staphylococcus* strain was extrapolated from the *Sma*I digest fragment pattern obtained by pulsed-field gel electrophoresis. The average genome size for *S. epidermidis* is $2,364 \pm 119$ kb; for *S. caprae* strains from humans it is $2,600 \pm 157$ kb and for *S. caprae* strains from goats it is $2,493 \pm 15$ kb; for *S. capitis* subsp. *capitis* it is $2,456 \pm 71$ kb; and for *S. capitis* subsp. *ureolyticus* it is $2,276 \pm 90$ kb.

The genus *Staphylococcus* has been organized into 32 species comprising at least six species groups (5, 15, 23, 24). Phylogenetic relationships have been suggested by DNA-DNA hybridization and supportive phenotypic tests (15). Recently, pulsed-field gel electrophoresis (PFGE) has been used to examine

number of different polymorphisms found in each species is linked to that species' genetic variability. For staphylococci, several different restriction endonucleases have been examined by us and others (18, 21) for the purpose of obtaining a small number of well-spaced chromosome fragments to provide a

FIG. 1. Dendrogram of the phylogenetic relationships among *S. epidermidis*, *S. caprae*, *S. capitis* subsp. *capitis*, and *S. capitis* subsp. *ureolyticus* based on optimal DNA-DNA reassociation conditions.

chromosomal polymorphisms existing within and between bacterial species (2, 18, 21). Chromosomal polymorphisms relate to differences in the numbers and sizes of specific fragments of genomic DNA that are generated by restriction enzymes. The

readable DNA profile. The restriction endonuclease *Sma*I was selected because this enzyme generates only 17 to 28 chromosome fragments, most of which are widely spaced.

In this study, *Sma*I digestion and PFGE were used to examine the chromosomal polymorphisms of four closely related members of the *Staphylococcus epidermidis* species group (Fig. 1), *S. epidermidis*, *Staphylococcus caprae*, *Staphylococcus capitis* subsp. *capitis*, and *S. capitis* subsp. *ureolyticus* (15), to estimate the extent of variability found within each

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TABLE 1. List of *Staphylococcus* strains and their isolation dates, sources, and locations

Species, subspecies, and strains	Host or source	Location ^a	Isolation date
<i>S. epidermidis</i>			
MAW 815	Human	Raleigh, N.C.	1981
MAW 815 (91)	Human	Boone, N.C.	1991
MAW 8111	Human	Raleigh, N.C.	1981
MAW 8114	Human	Raleigh, N.C.	1981
CJ 879	Human	Fayetteville, N.C.	1987
CJ 877	Human	Fayetteville, N.C.	1987
CJ 8726	Human	Fayetteville, N.C.	1987
CB 877-4	Human	Fayetteville, N.C.	1987
CB 877-6	Human	Fayetteville, N.C.	1987
CB 8712	Human	Fayetteville, N.C.	1987
MR 8718	Human	Fayetteville, N.C.	1987
MR 8719	Human	Fayetteville, N.C.	1987
MR 8711	Human	Fayetteville, N.C.	1987
<i>S. caprae</i>			
142.22	Goat milk	Nouzilly, France	1980s
141.15	Goat milk	Nouzilly, France	1980s
143.15	Goat milk	Nouzilly, France	1980s
2122	Human	Oklahoma City, Okla.	1991
H2132	Human	Columbus, Ohio	1990s
UHL 2455	Human	London, Ontario, Canada	1990s
G15752	Human	Columbus, Ohio	1990s
T11022	Human	Columbus, Ohio	1990s
G7344	Human	Columbus, Ohio	1990s
3051	Human	Chicago, Ill.	1991
B3300	Human	Columbus, Ohio	1990s
M8756	Human	Columbus, Ohio	1990s
106	Human	Vancouver, British Columbia, Canada	1990s
<i>S. capitis</i> subsp. <i>capitis</i>			
WK 811	Human	Raleigh, N.C.	1981
CJ 872	Human	Fayetteville, N.C.	1987
JLJ 853	Human	Raleigh, N.C.	1985
EK 11	Human	North Branch Station, N.J.	1973
MAW 913	Human	Boone, N.C.	1991
MAW 911	Human	Boone, N.C.	1991
CJ 874	Human	Fayetteville, N.C.	1987
LK 811	Human	Raleigh, N.C.	1981
HK 811	Human	Raleigh, N.C.	1981
CB 871	Human	Fayetteville, N.C.	1987
CB 872	Human	Fayetteville, N.C.	1987
CB 873	Human	Fayetteville, N.C.	1987
MR 875	Human	Fayetteville, N.C.	1987
<i>S. capitis</i> subsp. <i>ureolyticus</i>			
MAW 8436	Human	Raleigh, N.C.	1984
CB 8735	Human	Fayetteville, N.C.	1987
CMM 8536	Human	Raleigh, N.C.	1985
DLB 8531	Human	Raleigh, N.C.	1985
NRC 8535	Human	Raleigh, N.C.	1985
CMM 8539	Human	Raleigh, N.C.	1985
AW 8127	Human	Raleigh, N.C.	1981
NRC 8533	Human	Raleigh, N.C.	1985
3239	Human	Raleigh, N.C.	1990
3069	Human	Raleigh, N.C.	1990
1115	Human	Raleigh, N.C.	1989
Hartford-1	Human	Hartford, Conn.	1985
DEM 8530	Human	Raleigh, N.C.	1985

^a Location refers to the host's place of residence or the original location of the source.

MATERIALS AND METHODS

member and to extrapolate their genome sizes. In this study, the use of PFGE as an epidemiological tool for identifying strains within each of the above-mentioned species and sub-species was also assessed.

Bacterial strains. The *Staphylococcus* strains used in this study are listed in Table 1. All strains of *S. epidermidis* and *S.*

FIG. 2. PFGE of *Sma*I-digested, large genomic fragments of *S. epidermidis*, *S. caprae*, *S. capitis* subsp. *capitis*, and *S. capitis* subsp. *ureolyticus* strains.

capitis were isolated from humans. Ten strains of *S. caprae* were isolated from humans, and three strains of this species were isolated from goats. Species and subspecies identification was done according to the conventional methods of Kloos and coworkers (11, 13, 15) and on the basis of original character descriptions (3, 6, 14, 22). The following strains were verified by DNA-DNA hybridization (4, 17): *S. epidermidis* MAW 815 and MAW 8114; *S. caprae* 141.15, 2122, 3051, H2132, G15752, UHL 2455, and T11022 (hybridization kindly performed by T. L. Bannerman in our laboratory); *S. capitis* subsp. *capitis* WK811 and EK11; and *S. capitis* subsp. *ureolyticus* MAW 8436, CMM 8536, NRC 8533, DLB 8531, NRC 8535, and DEM 8530 (3). Individual strains were resolved on the basis of colony morphology, enzyme activities, carbohydrate reactions, anti-biograms, plasmid composition, and plasmid restriction enzyme analysis (13, 15, 17, 19, 20).

DNA preparation for PFGE analysis. DNA was prepared according to a modification of the procedures of Goering and Winters (8). *Staphylococcus* cultures were grown on the surface of a P agar medium (14, 16) for 18 h at 35°C as a source of inoculum. The overnight culture was transferred to 10 ml of Trypticase soy broth (approximately 10^7 CFU/ml) and then shaken (200 rpm) for 18 h at 35°C. A portion of the overnight culture was diluted with Trypticase soy broth in a 3-ml cuvette until its optical density at 540 nm was between 0.7 and 0.8. The

same fraction of cells from the original overnight culture was then centrifuged in a Sorvall RC2-B centrifuge at 8,000 rpm for 10 min, and the cell pellet was washed once and resuspended in 5 ml of 0.1 M Tris-0.15 M NaCl-0.1 M EDTA (TEN) buffer, pH 7.5. The cells were centrifuged again at 8,000 rpm for 10 min and then resuspended in 2 ml of 6 mM Tris·Cl-1 M NaCl-100 mM EDTA buffer containing 0.5% Brij 58, 0.2% deoxycholate, and 0.5% sodium lauroyl sarcosine (EC lysis buffer), pH 7.5. One milliliter of the cell suspension was transferred to a sterile 1.5-ml microcentrifuge tube, to which was then added 50 µl of lysostaphin solution (1 mg/ml in 20 mM sodium acetate, pH 4.5) (recombinant lysostaphin [Ambicin L]; Applied Microbiology, Inc., New York, N.Y.). The tube was immediately vortexed, and then 1 ml of cell suspension was removed and placed into 1 ml of warm (55°C) 2% low-gelling-temperature agarose (SeaPlaque; FMC, Rockland, Maine) prepared with EC lysis buffer. The agarose-cell suspension was immediately vortexed and transferred into a well of a 10-well sample plug mold (Bio-Rad, Hercules, Calif.). The agarose plug was cooled for 10 min at room temperature. The cooled agarose plug was removed from the mold with the aid of a small spatula and then transferred to 5 ml of EC lysis buffer and incubated for approximately 1 to 2 h at 37°C, until the plug was cleared (complete lysis). Following lysis, the EC lysis buffer was carefully decanted and replaced with 5 ml of 10

FIG. 3. PFGE of *Sma*I-digested, medium-size (upper panels) and small (lower panels) genomic fragments of *S. epidermidis*, *S. caprae*, *S. capitis* subsp. *capitis*, and *S. capitis* subsp. *ureolyticus*. Strains of *S. caprae*, *S. capitis* subsp. *capitis*, and *S. capitis* subsp. *ureolyticus* for which data are shown demonstrated different DNA fragment patterns. Those for which data are not included demonstrated the same fragment pattern as one of the strains for which data are shown did.

mM Tris · Cl–1 mM EDTA (TE) buffer, pH 7.6. The agarose plug in TE buffer was incubated at 55°C for 1 h. The TE buffer was then decanted and replaced with 5 ml of fresh TE buffer. The agarose plugs were stored at 4°C for up to 1 month.

***Sma*I digestion.** Digestion of total DNA by the restriction endonuclease *Sma*I was performed in a 1.5-ml microcentrifuge tube by using a 125- μ l assay and 20 U of enzyme according to the instructions of the manufacturer. The assay tube was vortexed and briefly centrifuged. A section (2 by 4 by 1.5 mm) of the agarose plug prepared as described above was added to the assay tube and then incubated with shaking (150 rpm) for 2 h at 25°C. After digestion, the agarose plug was removed from the assay tube and carefully placed into 1 well of a 15-well 1% agarose (SeaKem GTG; FMC) gel slab (12.5 by 14 by 0.9 cm) prepared with 0.5 $\times$ Tris-borate-EDTA (TBE) buffer as previously described (8). The agarose plug was positioned

against the forward wall of the well with the aid of a small spatula. Then the plug was sealed in the well with 0.8% low-gelling-temperature agarose prepared with 0.5 $\times$ TBE buffer.

PFGE procedures and analysis. *Sma*I digest fragments were separated in the agarose gel slab by using a Bio-Rad CHEF-DR II pulsed-field electrophoresis unit. Electrophoresis was performed in 0.5 $\times$ TBE buffer maintained at 14°C. To identify the DNA fragments, which had a large range of sizes, three separate gels were run, each under different conditions. For the largest DNA fragments (≥ 100 kb), a gel was run with a ramped pulse of 15 to 55 s at 200 V for 22 h. To identify the medium-size DNA fragments (50 to 100 kb), a second gel was prepared and run with a ramped pulse of 1 to 10 s at 200 V for 20 h. To identify the smallest DNA fragments (4 to 50 kb), a third gel was prepared and run with a ramped pulse of 1 to 12

TABLE 2. Fragments conserved among species and subspecies

Fragment(s) ^a conserved among indicated percentage of strains									
<i>S. epidermidis</i>		<i>S. caprae</i> (goat), 100%	<i>S. caprae</i> (human)		<i>S. capitis</i> subsp. <i>capitis</i>			<i>S. capitis</i> subsp. <i>ureolyticus</i>	
100%	77%		100%	90%	100%	92%	77%	100%	92%
86, 41	215, 26, 19	388 ^b , 170, 120, 118, 76, 70, 52, 32 ^b , 28, 24 ^b , 22 ^b , 19, 4	214, 140, 130, 50, 32, 24, 22, 12	388, 224, 188	700, 229, 140, 89 ^c , 82 ^c , 64, 54 ^c , 35 ^c , 19 ^c , 16, 9, 7 ^c , 5 ^c	194	120, 102, 97	463, 240, 175, 148, 108, 89, 82, 76, 67, 54, 45, 35, 19, 7, 5	408

^a Fragments designated according to size in kilobases.^b Fragment shared by goat and human *S. caprae* strains^c Fragment shared by both subspecies of *S. capitis*.

s at 150 V for 20 h. The gels were stained with ethidium bromide (2 µg/ml) for 25 min and then destained in distilled water for 60 min to 24 h.

The genome size of each strain was estimated from the sum of the *Sma*I digest fragments appearing on gels following PFGE. The lambda ladder DNA size standard (Bio-Rad) was used as a reference for determining the sizes of the largest *Sma*I digest fragments. The sizes of the smaller *Sma*I digest fragments were determined with the Low Range PFG Marker (New England Biolabs) molecular size standard. The Soft Laser Densitometer (model SLR-2D/ID; Bio Med Instruments Inc., Fullerton, Calif.) was used to scan Polaroid negatives of each ethidium bromide-stained gel to analyze the light intensity of each band and estimate the numbers of fragments. Bands that were of greater intensity than surrounding ones were considered to represent more than one digest fragment.

RESULTS

The PFGE analysis of *S. epidermidis*, *S. caprae*, *S. capitis* subsp. *capitis*, and *S. capitis* subsp. *ureolyticus* DNA showed distinct species and subspecies *Sma*I digest fragment patterns. *S. epidermidis* showed the greatest diversity in fragment patterns of the three species (Fig. 2 and 3). A total of 17 to 22 fragments were detected; they ranged in size from 8 to 630 kb. MAW 815 and MAW 815 (91) represent the same strain and were collected from the forehead and anterior nares, respectively, of a patient receiving topical clindamycin therapy. MAW 815 was collected in 1981, whereas MAW 815 (91) was collected in 1991. These isolates represented the predominant *S. epidermidis* strain found on the patient. The fragment patterns of these two isolates were similar except for the positions of 3 of the 20 fragments. The genome sizes were the same.

S. epidermidis CB 877 isolates were obtained from a patient receiving topical clindamycin. CB 877-4 was collected from the anterior nares. CB 877-6 was collected 4 weeks later from the inguinal-perineal area. The strain to which these isolates belong was the predominant strain found on this patient. The isolates had identical *Sma*I digest fragment patterns, except for the position of a single unshared fragment. It was determined that CB 877-4 has a fragment of approximately 243 kb, whereas CB 877-6 has a fragment of approximately 293 kb, for a difference in size of 50 kb. The original identification of each MAW and CB isolate mentioned above was based on numerous phenotypic and plasmid characteristics (described in Materials and Methods) used for strain identification. Isolates belonging to the same strain had identical characteristics.

The DNA fragment pattern of *S. caprae* strains (Fig. 2 and 3) is more highly conserved than that of *S. epidermidis*. Table 2 lists the fragments conserved among species and subspecies as observed in this study. Approximately one-half of the *S. caprae*

fragments were conserved, whereas only about 20% of the *S. epidermidis* fragments were conserved. A total of 17 to 28 fragments were detected for *S. caprae*, which ranged in size from about 3 to 533 kb. The isolates were obtained from several different localities, demonstrating that the conserved DNA fragment pattern is not confined to one geographical area. Strains 143.22, 141.15, and 143.15 were collected from two different goat herds in Nouzilly, France. The remaining isolates were collected from human clinical specimens. The goat isolates had a fragment pattern different from that of the human isolates but shared fragments of 388, 32, 24, and 22 kb with human isolates (Table 2). Concurrently, Bannerman et al. (2) observed similar results with goat and human *S. caprae* isolates, and they observed a similar level of conservation of fragment patterns when either *Sma*I or *Eag*I digestion was used.

The DNA fragment patterns of *S. capitis* subsp. *capitis* and *S. capitis* subsp. *ureolyticus* are both highly conserved, but they are quite different from each other (Fig. 2 and 3; Table 2). For *S. capitis* subsp. *capitis* 20 to 23 fragments were identified. They ranged in size from about 5 to 700 kb. Thirteen fragments were shared by all strains examined. These strains were obtained from a variety of geographical areas in the United States.

The DNA fragment pattern of *S. capitis* subsp. *ureolyticus* showed 18 to 19 fragments ranging in size from 5 to 463 kb. The fragment pattern of *S. capitis* subsp. *ureolyticus* is the most highly conserved of the patterns for the four taxa examined. With the exception of those of four strains (Fig. 2, lanes 1, 9, 10, and 13) all fragment patterns were identical. In the exceptional strains only one or two band differences were noted. *S. capitis* subsp. *ureolyticus* shared fragments of 89, 82, 54, 35, 19, 7, and 5 kb with *S. capitis* subsp. *capitis* (Table 2).

The average estimated genome size of each of the species and subspecies described above is shown in Table 3. The genome sizes of these species are somewhat smaller than that reported for *Staphylococcus aureus* (21). The genome sizes of

TABLE 3. Estimated genome size of *Staphylococcus* species and subspecies

Species and subspecies	Host	Genome size (kb)	
		Mean	Standard deviation
<i>S. aureus</i> ^a	Human	2,741	277
<i>S. epidermidis</i>	Human	2,364	119
<i>S. capitis</i> subsp. <i>capitis</i>	Human	2,456	71
<i>S. capitis</i> subsp. <i>ureolyticus</i>	Human	2,276	90
<i>S. caprae</i>	Human	2,600	157
<i>S. caprae</i>	Goat	2,493	15

^a Results reported by Prevost et al. (21).

various strains of *S. capitis* are more uniform than those of the other species examined in this study.

DISCUSSION

S. epidermidis is the most abundant and widely distributed *Staphylococcus* species on the human skin. It is found in greatest numbers in the moist anterior nares, axillae, inguinal-perineal area, and toe webs, but it also can be found on drier skin areas (10). The great adaptability of this species may account for the large amounts of chromosome fragment polymorphisms observed among isolates. In support of this principle, two predominant strains of *S. epidermidis*, MAW 815 and CB 877, exhibited some chromosome polymorphism within subpopulations isolated from different body sites and at different times. This species also demonstrates a wide variety of biotypes (9).

Strains of *S. caprae* gathered from the milk of goats showed some deviation from the human *S. caprae* strains with respect to their DNA fragment patterns. This divergence in pattern may reflect some evolutionary change within the species in the process of changing hosts. It is quite conceivable that humans and goats have come to share some of their bacteria during the course of a long association throughout the history of agriculture.

The two most closely related taxa in this study were *S. capitis* subsp. *capitis* and *S. capitis* subsp. *ureolyticus*, yet these subspecies showed very different DNA fragment patterns. These subspecies have approximately 79% DNA-DNA homology under stringent conditions and 89% homology under optimal conditions (3). When the DNA fragment patterns were analyzed, the relatively high degree of DNA homology was not apparent. The marked reduction in chromosome polymorphism compared with that of the related species *S. epidermidis* may be associated with the restricted habitat range of these subspecies and limited biotype variability (9). Chromosome fragment patterns are also highly conserved within the genus *Brucella* and especially in *Brucella melitensis* (1). *S. capitis* is primarily found on the head, though occasionally *S. capitis* subsp. *ureolyticus* may colonize other body sites on certain individuals following antibiotic therapy (12). These features may reflect less adaptability within the species.

It is apparent from our results that PFGE analysis can be used as an epidemiological tool for the identification of different *S. epidermidis* strains (7) but that this method would have limited use for monitoring strains or clones of *S. caprae* or *S. capitis*.

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