Comparative Study of the Total Cellular Fatty Acids of *Staphylococcus* Species of Human Origin†

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The cellular fatty acid compositions of 100 strains representing 10 different species of *Staphylococcus* were analyzed by gas-liquid chromatography. Results indicated that the fatty acid compositions of the various species were qualitatively similar with the exception of that of *S. warneri*, which contained a C₂₂ fatty acid component. The species differed quantitatively with respect to relative percentages of fatty acid components. The major fatty acids were C_{15Br}, C_{17Br}, C₁₆, C₁₈, and C₂₀. *S. warneri* and *S. saprophyticus* can be differentiated in most cases from all of the other species studied by the presence of significantly higher percentages of C₂₀ and C₁₆ fatty acids, respectively. Other species or groups of related species could also be separated on the basis of combinations of fatty acid components. These findings corroborate the newly proposed scheme of classification of the staphylococci, but the particular usefulness of gas-liquid chromatography of fatty acids for the identification of clinically significant, coagulase-negative staphylococci from humans is primarily limited to *S. saprophyticus* and possibly *S. haemolyticus*. However, application of the method may be useful in clarifying further the phenetic relationships of certain species and may have some ecological value.

The identification and differentiation of bacteria by gas-liquid chromatography (GLC) was proposed by Abel et al. (1). GLC analysis of metabolic products (3, 4, 22, 24) and structural components, particularly fatty acids (2, 5, 17, 23, 25) and hydrocarbons (21, 29), has been recognized as a potential tool for delineating bacterial genera and species.

Recent studies on the natural populations of cutaneous bacteria from human and other animal sources have resulted in amended descriptions of *S. epidermidis* and *S. saprophyticus* and the characterization of eight new coagulase-negative species (11, 13, 28). Although the cellular fatty acids and lipids of *S. aureus* and *S. epidermidis* have been investigated (8, 14-16, 30, 31), they have not yet been studied in *S. saprophyticus* and the newly proposed coagulase-negative species. (Whether strains used in the investigations cited here represent *S. epidermidis sensu stricto* or other coagulase-negative species is unknown.) The present study was initiated to compare the cellular fatty acid compositions of all known human *Staphylococcus* species to provide additional information on their phenetic relationships and to provide the diagnostic laboratory possessing GLC capability with additional criteria for identification.

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MATERIALS AND METHODS

Bacterial strains. The strains used in this study are listed in Table 1.

Media and growth conditions. The organisms were grown in 125-ml Erlenmeyer flasks containing 50 ml of Trypticase soy broth with dextrose (BBL). Cultures were prepared by inoculating the above-mentioned medium with approximately 10⁸ colony-forming units from an 18-h-old agar slant culture and then incubating on a rotary shaker (200 rpm) at 34°C for 24 h. Cells were harvested by centrifugation and washed twice with 67 mM Sorenson phosphate buffer (pH 7.0).

Preparation of fatty acid methyl esters. Cellular fatty acids were converted to methyl esters directly from wet-cell preparations by transesterification as described by Dunlap and Perry (6). Washed-cell pellets were suspended in a solution of 3 ml of 14% boron trifluoride-methanol and 3 ml of benzene. Suspensions were transferred to Teflon-lined, screw-capped tubes and incubated overnight in a water bath at 60°C. The benzene layer, separated from the emulsion by the addition of 4 ml of distilled water and subsequent centrifugation, was removed and brought to dryness under nitrogen. The fatty acid methyl esters were suspended in hexane and analyzed by GLC.

GLC. The GLC analysis of the fatty acid methyl esters was done on a Varian gas chromatograph, series 2400, equipped with dual hydrogen-flame detectors. The chromatographic analysis was carried out in a copper column (1.8 m by 0.3 cm) containing 15% diethylene glycol succinate on 60/70-mesh Gas-Chrom Q. Operating conditions were as follows: column tem-

TABLE 1. *Bacterial strains of human origin used in this study*

Species	Strain no.	Source or reference	Species	Strain no.	Source or reference
<i>Staphylococcus simulans</i>	ATCC 27848 ^a	11	<i>S. haemolyticus</i>	DSM 20263 ^a	28
	ATCC 27849	11		DSM 20264	28
	ATCC 27850	11		DSM 20265	28
	ATCC 27851	11		KES 23	28
	CE 13	11		KL 109	28
	MAW 222	11		AS 22	28
	AW 232	11		DM 293	28
	DW 196	11		HM 26	28
	KL 299	11		MK 67	28
	KH 12	11		DM 70	28
<i>S. cohnii</i>	DSM 20260 ^a	28	<i>S. hominis</i>	ATCC 27844 ^a	11
	DSM 20261	28		ATCC 27845	11
	DSM 20262	28		ATCC 27846	11
	JRM 24	28		ATCC 27847	11
	LK 478	28		WK 227	11
	CK 27	28		DGS 13	11
	KH 201	28		SM 67	11
	BB 3	28		JL 248	11
	DM 272	28		AW 178	11
	MK 247	28		TW 52	11
<i>S. xylosus</i>	DSM 20266 ^a	28	<i>S. epidermidis</i>	JRM 1	28
	DMS 20267	28		MCS 21	28
	DM 37	28		MK 214	28
	AW 124	28		AW 269	28
	GH 30	28		DM 206	28
	TW 41	28		KL 280	28
	AW 244	28		PM 340	28
	SE 4	28		RK 13	28
	KH 168	28		RM 351	28
	LK 236	28		WK 5	28
<i>S. saprophyticus</i>	SE 11	28	<i>S. capitis</i>	ATCC 27840 ^a	11
	AW 163	28		ATCC 27841	11
	DM 115	28		ATCC 27842	11
	KL 150	28		ATCC 27843	11
	RM 454	28		GS 14	11
	GH 196	28		KH 220	11
	KL 20	28		RK 23	11
	TW 111	28		PM 378	11
	JRM 27	28		CK 17	11
	SM 115	28		KES 14	11
<i>S. warneri</i>	ATCC 27836 ^a	11	<i>S. aureus</i>	DBM 34	W. E. Kloos
	ATCC 27837	11		DM 171	W. E. Kloos
	ATCC 27838	11		PM 26	W. E. Kloos
	ATCC 27839	11		335	W. H. Sperber
	SM 251	11		R 126	W. H. Sperber
	KH 208	11		S 36	W. H. Sperber
	SM 244	11		CDC 41734-74 ^b	G. A. Hancock
	JL 286	11		75:556 ^b	G. A. Hancock
	KL 105	11		75:560 ^b	G. A. Hancock
	TW 98	11		SAH	R. M. Cody

^a Type strain.^b Clinical isolates from the Center for Disease Control, Atlanta, Ga.

perature, 170°C; injector and detector temperature, 190°C; and argon flow rate, 20 ml/min.

Fatty acid methyl esters were tentatively identified by comparisons of retention time to standard fatty

acid methyl esters (Applied Science Laboratories). Confirmation of unsaturated fatty acids was accomplished by hydrogenation of methyl esters with hydrogen-platinum oxide and examination for possible peak

shifts (8). Quantitation of fatty acids was determined as previously described (6).

Mass spectrometry. Confirmation of fatty acid components was accomplished by analyzing fatty acid methyl esters of selected species with a Hewlett Packard HP5982A GLC-mass spectrometer and a HP5934A data system. The chromatographic analysis was carried out in a 500-foot by 0.02-inch (inner diameter; about 151 m by 0.05 cm) stainless-steel column coated with OV-17. The GLC oven temperature was programmed from 150 to 290°C, and the source temperature was 200°C. The ionizing voltage of the mass spectrometer was 70 eV. GLC mass spectrometry of fatty acid methyl esters was performed by J. D. Weete (Auburn University, Auburn, Ala.).

RESULTS AND DISCUSSION

The percentages of the total cellular fatty acids for *Staphylococcus* species isolated from human skin are presented in Table 2. The fatty acid compositions of the staphylococci were qualitatively similar, with the exception of that of *S. warneri*, which contained C₂₂ fatty acid. All species had fatty acids which were in the range of C₁₂ to C₂₁ and which consisted of iso and anteiso methyl-branched structures and occasional normal structures for odd-numbered carbon chains and iso and normal structures for even-numbered chains. We were unable to completely resolve C_{15Br}, C_{17Br}, C_{19Br}, and C_{21Br} into iso and anteiso components for the conditions described. However, mass spectra analysis of selected strains confirmed that iso and anteiso forms were present for these chain lengths. The predominant isomers of C_{15Br} and C_{17Br} were the anteiso derivatives. Similar results have been reported for *S. aureus* (31) and *S. epidermidis* (14). The major fatty acids were C_{15Br}, C_{17Br}, C_{19Br}, C₁₆, C₁₈, and C₂₀. No unsaturated fatty acids were detected.

The results indicated that *S. warneri* and *S. saprophyticus* could be separated in most cases from all other species by their higher percentages of C₂₀ and C₁₆ fatty acids, respectively (Fig. 1). Only one strain of *S. capitis* (namely GS14) contained 47.3% C₂₀, a value which overlapped with that obtained with *S. warneri*, and one strain of *S. warneri* (namely KL105) contained 43.9% C₂₀, a value which overlapped with that obtained with *S. capitis*. Two unusual strains of *S. saprophyticus* (namely JRM27 and GH196) contained 6.9 and 8.7% C₁₆, respectively, values that were low enough to overlap with those of *S. xylosus* and *S. simulans*. Other species or groups of related species could also be separated on the basis of combinations of fatty acid constituents.

S. aureus strains isolated from healthy human skin and clinical infections represented a fairly homogeneous group. The fatty acid compositions obtained in this study were similar to those previously reported, with C_{15Br} as the major fatty

acid (8, 29, 31). Considerable amounts of C_{17Br}, C₁₈, and C₂₀ fatty acids were also detected. It is particularly interesting that *S. aureus* and *S. haemolyticus* are similar in fatty acid composition, with the exception of the C_{19Br} fraction.

S. simulans, a relatively uncommon species which shares several phenotypic characteristics with *S. aureus*, also contained a C_{15Br} fraction as its predominant fatty acid and a relatively large percentage of C₁₈ and C₂₀ fatty acids. This species differs from *S. aureus* in that it contains a significantly higher percentage of C₁₆ and usually a lower percentage of C_{17Br} fatty acid (Fig. 1).

S. warneri, *S. hominis*, and *S. haemolyticus* comprise a group of related coagulase-negative species that may occasionally be confused with one another on the basis of simple character analysis (12). Most errors, when they occur, are between *S. warneri* and the other species. Relative rates of migration of esterase enzymes using gel electrophoresis (33) are quite useful in differentiating *S. hominis* from *S. warneri* and *S. haemolyticus* but have limited use in separating *S. warneri* from *S. haemolyticus*. In the present study, we have shown that fatty acid composition clearly distinguishes *S. warneri* from *S. hominis* and *S. haemolyticus*. The normal C₂₀ fraction and C_{15Br}, C_{17Br}, and C_{19Br} fractions can be used to separate *S. warneri* from these species (Fig. 1). In addition, a C₂₂ fraction was detected only in *S. warneri*. *S. haemolyticus* and *S. hominis* yielded rather similar fatty acid patterns but possessed minor differences in the C₁₈ and C₂₀ fractions.

S. capitis can be readily distinguished from *S. hominis* and *S. haemolyticus* by their levels of C_{15Br}, C_{17Br}, C_{19Br}, and C₂₀ fatty acids. Moreover, the unique percentages of C_{15Br} and C₁₈ fractions, taken together, can be utilized to separate *S. capitis* from all other species. The fatty acid composition of this species parallels that of *S. warneri* with the exception of the C₁₈ and C₂₀ fractions (Fig. 1).

S. epidermidis possessed fatty acid patterns qualitatively similar to those previously reported (8, 14). However, lower levels of C_{15Br} and higher levels of C₂₀ fatty acids were observed in this study. *S. epidermidis* strains contained C₂₀ (33.0%), C_{15Br} (27.6%), and C₁₈ (15.4%) as the principal fatty acids. It is interesting to note that the fatty acid compositions of *S. epidermidis* and *S. hominis* are nearly identical. These organisms are the most prevalent and persistent *Staphylococcus* species isolated from humans, and they produce large populations in warm, moist habitats of the skin (10).

S. saprophyticus, *S. xylosus*, and *S. cohnii* comprise a group of related species that are distinguished from other staphylococci by their

TABLE 2. Mean percentages and standard deviations of cellular fatty acid components of *Staphylococcus* species

<i>Staphylococcus</i> species	C ₁₂	C _{14 iso}	C ₁₄	C _{15 Br} ^a	C _{16 iso}	C ₁₆	C _{17 Br} ^a	C _{18 iso}	C ₁₈	C _{19 Br} ^a	C ₁₉	C ₂₀	C _{21 Br}	C ₂₂
<i>S. aureus</i>	0.7 ± 0.2 ^b	1.8 ± 0.7	0.2 ± 0.05	34.8 ± 3.7	1.5 ± 0.06	3.1 ± 0.5	10.2 ± 3.1	0.6 ± 0.3	19.5 ± 2.5	1.9 ± 0.8 (2.5 ± 0.9)	0.9 ± 0.2	21.7 ± 4.6	0.6 ± 0.1	ND ^c
<i>S. simulans</i>	1.2 ± 0.4	2.3 ± 0.6	1.0 ± 0.4	37.2 ± 6.9	0.6 ± 0.2	7.9 ± 1.3	4.8 ± 2.8	0.3 ± 0.1	23.1 ± 3.5	1.1 ± 0.4	0.3 ± 0.2	20.2 ± 5.1	ND	ND
<i>S. epidermidis</i>	0.8 ± 0.4	0.7 ± 0.2	0.3 ± 0.3	27.6 ± 4.2	0.3 ± 0.2	2.0 ± 1.1	6.8 ± 2.5	0.3 ± 0.2	15.4 ± 2.7	7.2 ± 2.9 (5.1 ± 3.7)	0.1 ± 0.2	33.0 ± 7.6	0.4 ± 0.5	ND
<i>S. capitis</i>	2.9 ± 1.1	0.8 ± 0.3	1.5 ± 0.9	20.0 ± 4.2	0.3 ± 0.1	4.7 ± 2.4	2.7 ± 1.5	0.4 ± 0.3	23.2 ± 4.0	2.4 ± 1.5	0.5 ± 0.2	39.9 ± 7.1	0.5 ± 0.6	ND
<i>S. warneri</i>	0.6 ± 0.2	1.1 ± 0.4	4.1 ± 1.6	18.7 ± 4.8	0.2 ± 0.1	5.1 ± 1.9	1.5 ± 0.4	0.2 ± 0.9	14.7 ± 2.2	0.9 ± 0.3 (0.8 ± 0.3)	ND	48.3 ± 2.1	0.5 ± 0.3	3.2 ± 1.6
<i>S. haemolyticus</i>	0.3 ± 0.3	0.9 ± 0.4	0.2 ± 0.09	33.6 ± 2.5	0.8 ± 0.3	2.8 ± 0.9	10.7 ± 2.3	0.4 ± 0.2	23.0 ± 3.7	4.7 ± 1.6 (4.0 ± 1.8)	0.7 ± 0.4	17.6 ± 1.5	0.3 ± 0.2	ND
<i>S. hominis</i>	0.8 ± 0.8	0.5 ± 0.2	0.2 ± 0.2	32.4 ± 3.2	0.2 ± 0.08	1.5 ± 0.8	7.3 ± 3.1	0.3 ± 0.1	16.6 ± 3.6	7.2 ± 2.8 (8.1 ± 3.0)	ND	24.2 ± 5.8	0.7 ± 0.8	ND
<i>S. saprophyticus</i>	1.7 ± 0.6	2.0 ± 1.0	6.8 ± 5.0	27.5 ± 7.6	0.4 ± 0.3	12.1 ± 2.8	4.0 ± 2.7	0.3 ± 0.3	15.6 ± 4.0	2.2 ± 1.2	0.4 ± 0.2	26.8 ± 5.1	0.2 ± 0.3	ND
<i>S. cohnii</i>	0.5 ± 0.4	1.2 ± 0.9	1.3 ± 0.8	42.5 ± 6.0	1.2 ± 0.9	5.6 ± 1.5	11.4 ± 3.5	0.5 ± 0.2	14.5 ± 3.2	3.3 ± 0.9	0.3 ± 0.2	17.4 ± 3.0	0.3 ± 0.2	ND
<i>S. xylosus</i>	2.3 ± 0.7	2.2 ± 0.7	3.2 ± 1.5	36.4 ± 5.9	0.8 ± 0.4	8.0 ± 1.4	8.8 ± 2.6	0.4 ± 0.2	17.3 ± 2.7	3.3 ± 1.0	0.4 ± 0.1	16.9 ± 3.5	0.07 ± 0.2	ND

^a C_{15 Br}, C_{17 Br}, and C_{19 Br} contained iso and anteiso components confirmed by mass spectra analysis of *S. warneri* MK 155, *S. haemolyticus* AS 22, and *S. hominis* WK 227. C_{19 Br} anteiso components, in parentheses, were resolved for *S. aureus*, *S. epidermidis*, *S. warneri*, *S. haemolyticus*, and *S. hominis*. Br, Branched.

^b Data represent the mean percentages and standard deviations of 10 strains for each species. Duplicate independent determinations for each strain varied from 1 to 25%.

^c ND, Not detected.

FIG. 1. Comparison of the relative percentages of the major fatty acid components of *Staphylococcus* species. Points and bars represent the means and the standard deviations of 10 strains for each species.

resistance to novobiocin, cell wall composition, low acid production from glucose under anaerobic conditions, high salt tolerance, and their lack of, or limited, organic nitrogen requirements (7, 11, 28). *S. saprophyticus* contained almost equivalent quantities of C_{15Br} and C₂₀ fatty acids. It was found to be different from *S. xylosum* and *S. cohnii* in the percentage of C₂₀ fatty acid. *S. xylosum* and *S. cohnii* are indistinguishable in their fatty acid patterns.

In light of the recent evidence that several coagulase-negative species, particularly *S. epidermidis*, *S. saprophyticus*, *S. haemolyticus*, and *S. hominis*, have been associated with human infections (18, 20, 26, 27), it was initially hoped that GLC analysis of the cellular fatty acids could be utilized for the clinical identification of these species. Results suggest that cellular fatty acid composition alone would not completely separate *S. hominis* and *S. epidermidis* from each other, although it would clearly separate *S. saprophyticus* from these and most other species. *S. haemolyticus*, in most instances, can be separated from *S. hominis* and *S. epidermidis* based on the percentage of C₂₀

fatty acid. In ecological studies, cellular fatty acid composition may be useful in separating *S. warneri* from other staphylococci. Recent studies have suggested that this species is only occasionally found on healthy human skin or in infections (10, 26), although its presumed subspecies are commonly found on the skins of primates other than man (W. E. Kloos, unpublished data). The occurrence of *S. warneri* in nonhuman primate infections has not yet been determined.

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REPRINT REQUESTS

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