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Identification of Staphylococci with a Self-Educating System Using Fatty Acid Analysis and Biochemical Tests

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We characterized all of the 35 aerobic taxa of the genus *Staphylococcus* by using an objective, self-learning system combining both whole-cell fatty acid (FA) analysis and the results of 35 biochemical tests. Isolates were compared with the type strain for each taxon to generate an FA profile library and a biochemical table of test responses. Isolates were accepted into the system if they had a similarity index of ≥0.6 for a taxon within the FA profile library and if they were identified as the same taxon by a computer program using a probability matrix constructed from the biochemical data. These stringent criteria led to acceptance of 1,117 strains assigned to legitimate taxa. Additional FA groups were assembled from selected strains that did not meet the inclusion criteria based on the type strains and were added to the system as separate entries. Currently, 1,512 isolates have been accepted into the system. This approach has resulted in a comprehensive table of biochemical test results and an FA profile library, which together provide a practical system for valid identifications.

The genus *Staphylococcus* has undergone extensive revision since 1975, when Schleifer and Kloos (29, 45) published the results of their investigation of staphylococci from human skin. There has since been a steady addition of species and subspecies that have been isolated from human, veterinary, and environmental sources (26, 27), and there are now 33 species and 8 subspecies that have been validly described. The newer species include some, such as *Staphylococcus lugdunensis* (16, 46), which may be primary pathogens of humans and animals and should be routinely identified in clinical laboratories. Furthermore, other coagulase-positive species, such as *S. intermedius* and *S. schleiferi* subsp. *coagulans*, which may be mistaken for *S. aureus*, have also been implicated in human infections (47, 48, 52).

The advent of nucleic acid methodologies has resulted in the recognition of new taxa which may not be identified by conventional methods (6), and older species descriptions have been changed by the division of a species into two or more subspecies, as in the cases of *S. cohnii* (31) and *S. capitis* (3). Commercial identification systems often do not include newer species in their databases, and a significant proportion of isolates are not identifiable, presumably because they represent hitherto undescribed taxa.

Fatty acid (FA) analysis has been used for the identification of a wide range of bacteria, and the topic has been reviewed by Welch (55). In the present study an objective, self-learning system that combined FA analysis with a standardized biochemical procedure was used to characterize the aerobic staphylococci. All of the known aerobic taxa were represented, and strains from diverse locales and habitats were included. This approach has resulted in a library of FA profiles and a complete table of biochemical data which can be used in combination to identify these important bacteria.

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MATERIALS AND METHODS

Bacterial strains. The sources of the type strains, reference strains, and other strains that were used to establish additional taxa defined by FA profiles are listed in Table 1. Other isolates were obtained from commercial culture collections, the collections of other researchers, clinical specimens, and a variety of animal and environmental sources in the field. The anaerobes *S. saccharolyticus* and *S. aureus* subsp. *anaerobius* were excluded because their growth requirements are not compatible with our methods.

Biochemical tests. All of the strains were subjected to 35 biochemical tests, as shown in Table 2. The test mixtures were incubated for 48 h at 35°C except as noted below.

The modified oxidase test (14) was performed with cultures cultivated for 24 h on horse blood agar. The tube coagulase test was performed with 0.5 ml of rabbit plasma (0803-46; Difco, Inc., Detroit, Mich.) and 0.1 ml of an overnight heart infusion broth culture. The mixture was incubated for 4 h at 35°C and then for 20 h at room temperature (18 to 24°C). Growth on medium containing 6.5% sodium chloride and resistance to 0.02% furazolidone (2) were both determined on P agar medium (30). The production of beta-glucosidase (56) was determined after incubation for 24 h.

Pyroglutamate aminopeptidase activity (22) was tested in 0.5-ml amounts of Todd-Hewitt broth (11736; BBL) containing 0.01% L-pyroglutamic acid β-naphthylamide (P-5891; Sigma), as previously described (27).

Arginine hydrolysis and ornithine decarboxylase activity were tested in a medium containing 1.5 g of thiotone E peptone (12302; BBL), 0.76 g of beef extract (0126-01-8; Difco), 0.2 g of yeast extract (0127-01-7; Difco), 0.03 g of glucose, 6 ml of 1% phenol red solution, and 1.2% of the appropriate L-amino acid in 300 ml of distilled water. The pH was adjusted to 6.5, and the medium was dispensed in 1-ml amounts prior to sterilization. The medium was inoculated to give a heavy suspension and was overlaid with mineral oil. A deep red color was interpreted as a positive result, and an orange or red-orange color was interpreted as negative. This medium is more sensitive than Moeller medium, which accounts for positive arginine reactions found with some species, e.g., *S. saprophyticus*, which are usually described as negative (27).

Acid production from carbohydrates was tested in phenol red broth base (11506; BBL) in which the indicator concentration was doubled to 0.036 g/liter. Carbohydrates were added to give a final concentration of 1.2%, and the pH was adjusted to 7.4. The medium (0.5-ml amounts) was inoculated with approximately 0.1 ml of a heavy suspension of growth that was harvested from an overnight blood agar plate and suspended in 0.85% saline. Reactions were interpreted as positive (yellow), weak (yellow-orange), or negative (red-orange or red).

The remaining biochemical tests were performed as previously described (46). For data analysis, weak reactions were recorded as positive results.

Cellular FA analysis. The strains of staphylococci were subcultured on blood agar at least twice at 24-h intervals in order to obtain organisms in a reproducible physiological state before analysis. The bacteria were then grown on Trypticase soy agar (11043; BBL) containing 5% sheep blood incubated at 35°C for 24 (± 1) h. The methyl esters of their FAs were extracted and analyzed as described by Miller and Berger (37). A Hewlett-Packard HP5890 series II gas chromatograph fitted with a flame ionization detector was used. Chromatograph control, peak

TABLE 1. Staphylococcal type and reference strains

Taxon	Strain designation[a]	Source[b]
S. arlettae	ATCC 43957^T	ATCC
S. aureus subsp. *aureus*	ATCC 12600^T	ATCC
S. aureus subsp. *aureus* FA group B	UHL 5066	UHL
S. auricularis	ATCC 33753^T	ATCC
S. capitis subsp. *capitis*	ATCC 27840^T	ATCC
S. capitis subsp. *ureolyticus*	ATCC 49326^T	ATCC
S. capitis subsp. *ureolyticus* FA group b	UHL 2312	UHL
S. caprae	ATCC 35538^T	ATCC
S. carnosus	DSM 20501^T	LWA
S. carnosus FA group B	JCM 6065	LWA
S. caseolyticus	ATCC 13548^T	ATCC
S. caseolyticus FA group B	OVI-153	RF
S. chromogenes	ATCC 43764^T	ATCC
S. cohnii subsp. *cohnii*	ATCC 29974^T	ATCC
S. cohnii subsp. *urealyticum*	ATCC 49330^T	ATCC
S. delphini	ATCC 49171^T	LWA
S. epidermidis	ATCC 14990^T	ATCC
S. epidermidis FA group b	ATCC 35983	ATCC
S. epidermidis FA group c	ATCC 35984	ATCC
S. epidermidis FA group d	UHL 5252	UHL
S. epidermidis FA group e	UHL 5933	UHL
S. epidermidis FA group f	UHL 5978	UHL
S. epidermidis FA group g	UHL 2771	UHL
S. equorum	ATCC 43958^T	ATCC
S. equorum FA group b	UHL H87	UHL
S. felis	ATCC 49168^T	ATCC
S. felis FA group b	UHL 7741	UHL
S. gallinarum	ATCC 35539^T	ATCC
S. haemolyticus	ATCC 29970^T	ATCC
S. haemolyticus FA group b	UHL 3099	UHL
S. hominis	ATCC 27844^T	ATCC
S. hominis FA group b	ATCC 27847	ATCC
S. hominis FA group c	UHL 4309	UHL
S. hominis FA group d	UHL 3144	UHL
S. hominis FA group E	UHL 5403	UHL
S. hominis FA group f	UHL 3573	UHL
S. hominis FA group g	UHL 5821	UHL
S. hyicus	ATCC 11249^T	ATCC
S. hyicus FA group b	Ab5A	CL
S. intermedius	ATCC 29663^T	ATCC
S. intermedius FA group B	ATCC 49051	ATCC
S. kloosii	ATCC 43959^T	ATCC
S. kloosii FA group b	DCC 2869	DCC
S. lentus	ATCC 29070^T	ATCC
S. lugdunensis	ATCC 43809^T	ATCC
S. lugdunensis FA group b	UHL 4827	UHL
S. muscae	ATCC 49910^T	VH
S. pasteuri	ATCC 51129^T	NES
S. piscifermentans	JCM 6057^T	LWA
S. piscifermentans FA group b	JCM 6063	LWA
S. pulvereri	NT 215^T	PCM
S. saprophyticus	ATCC 15305^T	ATCC
S. schleiferi subsp. *coagulans*	ATCC 49545^T	ATCC
S. schleiferi subsp. *schleiferi*	ATCC 43808^T	ATCC
S. schleiferi subsp. *schleiferi* FA group b	UHL 1834	UHL
S. schleiferi subsp. *schleiferi* FA group c	UHL 4667	UHL
S. sciuri	ATCC 29062^T	ATCC
S. sciuri FA group b	791	EB
S. simulans	ATCC 27848^T	ATCC
S. vitulus	ATCC 51145^T	ATCC
S. warneri	ATCC 27836^T	ATCC
S. warneri FA group b	UHL 3975	UHL
S. warneri FA group c	UHL 5417	UHL
S. xylosus	ATCC 29971^T	ATCC
S. xylosus FA group b	ATCC 29967	ATCC
Unnamed group 1	B19199	LWA
Unnamed group 2	W18338	LWA
Unnamed group 3	F58366	LWA
Unnamed group 4	TNA 1078	LWA

[a] A superscript T following the strain designation indicates a type strain.

[b] ATCC, American Type Culture Collection, Bethesda, Md.; CL, C. Lämmler, Giessen, Germany; DCC, Delaware Culture Collection, Newark; EB, E. Bucher, Munich, Germany; LWA, Leona W. Ayers, Columbus, Ohio; NES, N. El Solh, Institut Pasteur, Paris, France; PCM, Polish Collection of Microorganisms, Wroclaw, Poland; RF, R. de la Fuente, Madrid, Spain; UHL, clinical isolates (except UHL H87 [from a horse] and UHL 7741 [from a cat]), University Hospital, London, Ontario, Canada; VH, V. Hájek, Olomouc, Czechoslavakia.

naming, and data handling were performed with the MIS software (Microbial Identification, Inc., Newark, Del.).

Development of biochemical table and FA library. Initially, a table containing biochemical test data (as in Table 2) was constructed solely from the results of type strains. Variability in the test results for a type strain was addressed by repeating the biochemical tests at least four times and expressing the data as the percentages of positive responses. The biochemical table served as a probability matrix for a computer program, RefID (4), which identifies unknown isolates by probability calculations as described by Lapage et al. (34).

The type strains were also subjected to at least six FA analyses, and a library entry for each taxon was constructed by using the MIS library generation software.

Further development of the biochemical table and the FA library was done in a heuristic fashion as shown in Fig. 1. The investigation was performed in numerous repetitive phases. New strains that met the inclusion criteria were added to the biochemical table and FA library with each iteration of the identification system. For acceptance of a strain, the FA comparison required a similarity index value of at least 0.6 with the profile of a taxon within the library, and the same taxon was required to be the most likely identification when the biochemical results were analyzed with the RefID program.

At a later stage, additional FA groups were created for reference strains and other representative strains whose similarity index values were less than 0.6 with the type strain profiles and therefore did not meet the inclusion criteria for these groups. FA groups with uncertain affinities are referred to as unnamed groups.

The ability to differentiate each of the taxa by biochemical tests was assessed with the RefID program. For each taxon in turn, a hypothetical organism, the centrostrain, was created from the probability matrix by determining the most common test result for each biochemical test. The test results for this centrostrain were then used as if it were an unknown, and its identity was calculated. The identification score (relative probability) for the centrostrain decreases as the similarity with another taxon increases. An identification score of less than 98% for the centrostrain was considered unacceptable for differentiating the taxon represented by the centrostrain from the other taxa, since a score of below 98% indicates that there is no test that discriminates the taxon represented by the centrostrain from the next most similar taxon.

RESULTS

The biochemical and FA data for the aerobic members of the genus *Staphylococcus* were accumulated by a heuristic procedure that began with the type strains of the genus. After 25 iterations of the procedure, 1,117 isolates met the inclusion criteria for acceptance into the system. The biochemical and FA characteristics of these isolates are listed by species name in Tables 2 and 3, respectively.

Our stringent acceptance criteria excluded a significant proportion of isolates. Among the excluded isolates were reference strains and other strains that were indistinguishable, in terms of their biotypes, from strains that had been accepted. Thus, a species may contain more than the one FA group represented by the type strain. Therefore, FA library entries and biochemical data were assembled for additional FA groups, and the identification procedure was continued.

Some of the additional FA groups of a species contained isolates that were identical or very similar biochemically to isolates in the type strain group. Therefore, we could not differentiate between some of the FA groups of a species by biochemical tests. For this reason the biochemical data for two

TABLE 2. Biochemical data

Taxon	No. of strains	% of positive reactions[a]																																									
		Oxidase (modified)	Coagulase (tube)	Glucose fermentation	Urea hydrolysis	Arginine hydrolysis	Ornithine decarboxylase	Glucose acid	Lactose acid	Sucrose acid	D-Mannitol acid	Salicin acid	Sorbitol acid	L-Arabinose acid	D-Raffinose acid	Maltose acid	D-Xylose acid	D-Trehalose acid	D-Cellobiose acid	D-Fructose acid	D-Mannose acid	D-Galactose acid	Xylitol acid	D-Melezitose acid	Esculin hydrolysis	Nitrate reduction	PYR	Phosphatase	6.5% NaCl growth	Novobiocin resistance	Gelatinase	DNase	Tween 80 lipase	Furazolidone resistance	Acetoin	Beta-glucosidase
S. arlettae	2	0	0	100	0	0	0	100	100	100	100	0	0	100	0	100	100	100	0	100	0	0	0	17	100	0	0	100	100	100	100	17	0	0	0	0
S. aureus subsp. *aureus*	130	0	99	100	80	100	0	100	79	100	94	2	0	0	0	100	0	96	1	100	100	86	0	7	52	98	0	100	100	1	78	99	83	0	95	32
S. aureus subsp. *aureus* group B	3	0	100	100	100	100	0	100	100	100	100	0	0	0	0	100	0	100	0	100	100	100	0	0	0	100	0	100	100	0	0	100	0	0	100	0
S. aureus subsp. *aureus* total	133	0	99	100	80	100	0	100	80	100	94	2	0	0	0	100	0	96	1	100	100	86	0	7	51	98	0	100	100	1	77	99	81	0	95	31
S. auricularis	2	0	0	10	0	100	0	100	0	50	0	0	0	0	0	100	0	100	0	100	0	0	0	0	0	80	100	0	10	0	0	0	0	0	0	0
S. capitis subsp. *capitis*	39	0	0	97	0	100	10	100	0	71	97	0	0	0	0	3	0	0	0	100	97	0	0	0	0	92	0	0	100	0	10	60	3	0	92	0
S. capitis subsp. *ureolyticus*	34	0	0	100	76	100	0	100	91	97	97	0	0	0	0	100	0	0	0	100	97	73	0	0	0	97	0	3	100	0	100	100	0	0	97	0
S. capitis subsp. *ureolyticus* group b	2	0	0	100	100	100	0	100	100	100	100	0	0	0	0	100	0	0	0	100	100	0	0	0	0	100	0	0	100	0	100	100	0	0	100	0
S. capitis subsp. *ureolyticus* total	36	0	0	100	78	100	0	100	92	97	97	0	0	0	0	100	0	0	0	100	97	69	0	0	0	97	0	3	100	0	100	100	0	0	97	0
S. caprae	38	0	0	100	92	100	0	100	50	95	95	0	0	0	0	89	0	97	0	100	95	71	0	0	0	79	100	92	100	0	74	100	37	0	87	0
S. carnosus	9	0	0	100	0	100	0	100	78	0	100	0	89	0	0	0	0	11	0	100	100	0	0	0	0	100	56	100	100	0	0	0	0	0	11	0
S. carnosus group B	10	0	0	100	0	100	0	100	0	0	0	20	0	0	0	0	0	100	0	100	20	30	0	0	20	80	50	20	100	0	0	20	0	0	0	20
S. carnosus total	19	0	0	100	0	100	0	100	37	0	47	11	42	0	0	0	0	58	0	100	58	16	0	0	11	89	53	58	100	0	0	11	0	0	5	11
S. caseolyticus	2	90	0	70	0	0	0	100	100	0	0	0	0	0	0	100	0	100	70	100	0	70	0	0	0	100	100	0	100	50	100	0	0	0	100	0
S. caseolyticus group B	2	100	0	100	0	0	0	100	0	100	0	0	0	0	0	100	0	100	0	100	100	0	0	0	0	100	100	0	100	50	100	0	0	0	100	0
S. caseolyticus total	4	95	0	85	0	0	0	100	50	50	0	0	0	0	0	100	0	100	35	100	50	35	0	0	0	100	100	0	100	50	100	0	0	0	100	0
S. chromogenes	5	0	0	100	80	100	0	100	84	100	0	0	0	0	0	20	0	96	0	100	100	40	0	0	0	100	60	100	100	0	100	100	0	0	0	0
S. cohnii subsp. *cohnii*	7	0	0	100	0	0	0	100	0	21	100	0	50	0	0	100	0	100	0	100	43	14	21	0	0	0	0	71	100	100	0	0	71	0	71	0
S. cohnii subsp. *urealyticum*	32	0	0	100	91	29	0	100	97	17	97	47	6	0	3	97	0	100	0	100	100	16	47	0	47	3	69	53	100	100	75	0	59	0	31	46
S. delphini	2	0	100	100	100	100	0	100	100	100	90	0	0	0	0	100	0	0	0	100	100	100	0	0	0	100	100	100	100	0	100	90	100	0	0	38
S. epidermidis	155	0	0	100	98	94	1	100	99	100	2	0	1	0	1	99	0	3	0	100	98	94	0	88	1	99	0	92	100	3	87	1	85	0	100	0
S. epidermidis group b	6	0	0	100	100	83	0	100	100	100	0	0	17	0	0	100	0	0	0	100	83	83	0	100	0	100	0	100	100	0	83	0	100	0	100	0
S. epidermidis group c	39	0	0	100	92	95	0	100	100	100	3	0	3	0	0	100	0	3	0	100	97	100	0	97	0	95	0	31	100	0	54	0	64	0	100	0
S. epidermidis group d	3	0	0	100	100	100	0	100	100	100	0	0	0	0	0	100	0	0	0	100	100	100	0	100	0	67	0	100	100	0	100	0	67	0	100	0
S. epidermidis group e	9	0	0	100	100	78	0	100	89	100	0	0	22	0	0	100	0	0	0	100	100	89	0	89	0	100	0	100	100	0	67	0	78	0	100	0
S. epidermidis group f	2	0	0	100	100	100	0	100	50	100	0	0	0	0	0	100	0	0	0	100	100	100	0	100	0	100	0	100	100	0	100	0	100	0	100	0
S. epidermidis group g	3	0	0	100	100	100	0	100	100	100	0	0	0	0	0	100	0	0	0	100	100	100	0	0	0	100	0	100	100	0	100	0	33	0	100	0
S. epidermidis total	217	0	0	100	97	94	0	100	98	100	2	0	3	0	0	100	0	2	0	100	98	95	0	89	0	98	0	82	100	2	81	0	80	0	100	0
S. equorum	48	0	0	94	100	56	0	100	92	98	94	94	23	94	0	100	96	88	48	100	100	50	0	8	94	100	46	50	100	98	71	0	0	0	10	99
S. equorum group b	21	0	0	100	100	52	0	100	100	100	100	100	24	86	0	100	100	100	62	100	100	19	0	0	24	100	14	76	100	100	71	0	0	0	5	100
S. equorum total	69	0	0	96	100	55	0	100	94	99	96	96	23	91	0	100	97	91	52	100	100	41	0	6	72	100	36	58	100	99	71	0	0	0	9	99
S. felis	21	0	0	100	100	100	0	100	100	43	5	0	0	0	0	0	0	100	0	100	100	100	0	0	0	100	100	100	100	0	0	48	95	0	0	0
S. felis group b	20	0	0	100	100	100	0	100	100	100	95	0	0	0	0	0	0	100	0	100	100	100	0	0	0	100	100	100	100	0	0	29	100	0	0	0
S. felis total	41	0	0	100	100	100	0	100	100	71	49	0	0	0	0	0	0	100	0	100	100	100	0	0	0	100	100	100	100	0	0	39	98	0	0	0
S. gallinarum	2	0	0	100	100	100	0	100	50	100	100	100	100	100	100	100	100	100	50	100	100	100	0	0	0	100	100	50	100	100	100	83	17	0	0	50	100
S. haemolyticus	167	0	0	100	5	98	0	100	89	100	73	0	2	0	0	100	0	99	0	94	1	87	0	30	1	98	97	0	100	0	1	7	8	0	81	26
S. haemolyticus group b	14	0	0	100	0	100	0	100	100	100	50	0	0	0	0	100	0	79	0	93	0	100	0	43	0	100	100	0	100	0	0	21	0	0	100	40
S. haemolyticus total	181	0	0	100	4	98	0	100	90	100	71	0	2	0	0	100	0	98	0	94	1	88	0	31	1	98	97	0	100	0	1	8	7	0	82	27
S. hominis	5	0	0	100	100	0	0	100	20	100	0	0	0	0	0	100	0	100	0	100	0	20	0	45	20	100	0	0	100	0	0	0	65	0	5	0
S. hominis group b	2	0	0	100	100	0	0	100	63	100	0	0	0	0	0	100	0	100	0	100	0	63	0	50	0	100	0	0	100	0	0	0	50	0	13	0
S. hominis group c	13	0	0	100	92	0	0	100	62	100	38	0	0	0	0	100	0	15	0	100	0	38	0	85	0	92	8	0	100	77	0	8	0	0	15	0
S. hominis group d	48	0	0	100	77	4	0	100	85	100	29	0	0	0	0	100	0	65	2	92	0	83	2	92	0	98	10	0	100	2	0	0	46	0	38	0
S. hominis group E	2	0	0	100	100	0	0	100	100	100	0	0	0	0	0	100	0	0	0	100	0	100	0	100	0	100	0	0	100	100	0	0	0	0	100	50
S. hominis group f	5	0	0	100	60	20	0	100	80	100	20	0	0	0	0	100	0	80	0	100	0	80	0	80	0	100	20	0	100	0	0	0	25	0	40	0
S. hominis group g	10	0	0	100	100	10	0	100	50	100	0	0	0	0	0	100	0	80	0	100	0	60	0	60	0	100	0	0	100	0	0	0	30	0	50	0
S. hominis total	85	0	0	100	84	5	0	100	73	100	24	0	0	0	0	100	0	61	1	95	0	70	1	83	1	98	8	0	100	15	0	1	36	0	35	0
S. hyicus	46	0	72	100	2	100	0	100	83	98	0	0	0	0	0	0	0	80	0	100	100	80	0	0	0	100	0	98	100	0	100	100	100	0	0	8
S. hyicus group b	10	0	80	100	0	100	0	100	100	100	0	0	0	0	0	0	0	100	0	100	100	90	0	0	0	100	0	100	100	0	100	100	100	0	0	0
S. hyicus total	56	0	74	100	2	100	0	100	86	98	0	0	0	0	0	0	0	84	0	100	100	82	0	0	0	100	0	98	100	0	100	100	100	0	0	5
S. intermedius	1	0	100	100	100	20	0	100	100	100	100	0	0	0	0	100	0	100	0	100	100	0	0	0	0	100	100	100	100	0	100	100	100	0	0	0
S. intermedius group B	85	0	94	100	100	100	0	100	100	100	12	0	0	0	0	100	0	100	0	100	100	100	0	0	0	100	99	100	100	0	100	100	98	0	1	4

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TABLE 2—*Continued*

Taxon	No. of strains	% of positive reactions[a]																																				
		Oxidase (modified)	Coagulase (tube)	Glucose fermentation	Urea hydrolysis	Arginine hydrolysis	Ornithine decarboxylase	Glucose acid	Lactose acid	Sucrose acid	D-Mannitol acid	Salicin acid	Sorbitol acid	L-Arabinose acid	D-Raffinose acid	Maltose acid	D-Xylose acid	D-Trehalose acid	D-Cellobiose acid	D-Fructose acid	D-Mannose acid	D-Galactose acid	Xylitol acid	D-Melezitose acid	Esculin hydrolysis	Nitrate reduction	PYR	Phosphatase	6.5% NaCl growth	Novobiocin resistance	Gelatinase	DNase	Tween 80 lipase	Furazolidone resistance	Acetoin	Beta-glucosidase
S. intermedius total	86	0	94	100	100	99	0	100	100	100	13	0	0	0	0	100	0	100	0	100	100	99	0	0	0	100	99	100	100	0	100	100	98	0	1	4
S. kloosii	2	0	0	100	100	30	0	100	100	90	100	0	0	100	0	100	0	100	0	100	0	20	0	0	50	0	100	100	100	100	0	0	0	0	30	0
S. kloosii group b	2	0	0	100	100	50	0	100	100	50	100	0	0	50	0	100	0	100	0	100	0	0	0	0	50	0	100	100	100	100	0	0	0	0	50	0
S. kloosii total	4	0	0	100	100	40	0	100	100	70	100	0	0	75	0	100	0	100	0	100	0	10	0	0	50	0	100	100	100	100	0	0	0	0	40	0
S. lentus	12	100	0	100	25	0	0	100	92	100	100	100	58	100	92	100	90	100	100	100	100	100	0	17	100	100	19	92	100	100	83	0	0	0	2	75
S. lugdunensis	105	0	0	100	56	0	100	100	72	100	1	0	0	0	0	100	0	99	0	100	100	72	0	7	0	98	99	2	100	0	15	96	0	0	99	43
S. lugdunensis group b	4	0	0	100	50	0	100	100	100	100	0	0	0	0	0	100	0	100	0	100	100	100	0	0	0	100	100	0	100	0	25	75	0	0	100	0
S. lugdunensis total	109	0	0	100	56	0	100	100	73	100	1	0	0	0	0	100	0	99	0	100	100	73	0	6	0	98	99	2	100	0	16	95	0	0	99	43
S. muscae	2	0	0	100	0	0	0	100	0	100	0	0	0	0	0	0	100	100	0	100	0	0	0	0	0	100	0	100	100	0	0	100	100	0	0	67
S. pasteuri	2	0	0	100	100	100	0	100	0	100	100	0	0	0	0	100	0	100	0	100	50	100	0	88	0	100	0	0	100	0	88	0	50	0	100	100
S. piscifermentans	6	0	0	100	100	100	0	100	100	100	0	100	0	0	0	67	0	100	0	100	21	100	0	100	100	100	46	96	100	0	0	67	100	0	0	100
S. piscifermentans group b	2	0	0	100	100	100	0	100	100	100	0	100	0	0	0	100	0	100	0	100	0	50	0	50	100	100	50	100	100	0	0	100	100	0	50	100
S. piscifermentans total	8	0	0	100	100	100	0	100	100	100	0	100	0	0	0	75	0	100	0	100	16	88	0	88	100	100	47	97	100	0	0	75	100	0	13	100
S. pulvereri	5	100	0	100	0	0	0	100	0	100	100	0	0	0	0	0	100	100	0	100	100	0	0	0	20	100	0	0	100	100	100	0	0	0	0	0
S. saprophyticus	73	0	0	100	100	71	0	100	86	100	88	0	0	0	0	100	0	99	0	100	0	8	12	1	0	4	15	14	100	100	73	1	0	0	99	6
S. schleiferi subsp. *coagulans*	4	0	100	100	100	100	0	100	81	0	25	0	0	0	0	0	0	0	0	100	100	100	0	0	0	100	100	100	100	0	100	100	100	25	100	0
S. schleiferi subsp. *schleiferi*	8	0	0	100	0	100	0	100	13	0	0	0	0	0	0	0	0	88	0	100	100	100	0	3	0	100	100	100	100	0	100	100	48	0	100	0
S. schleiferi subsp. *schleiferi* group b	17	0	0	100	0	100	0	100	0	0	0	0	0	0	0	0	0	88	0	100	100	100	0	0	0	94	100	100	100	0	82	100	88	0	100	14
S. schleiferi subsp. *schleiferi* group c	6	0	33	100	0	100	0	100	0	0	0	0	0	0	0	0	0	100	0	100	100	100	0	0	0	100	100	100	100	0	100	100	83	0	100	0
S. schleiferi subsp. *schleiferi* total	31	0	6	100	0	100	0	100	3	0	0	0	0	0	0	0	0	90	0	100	100	100	0	1	0	97	100	100	100	0	90	100	77	0	100	8
S. sciuri	27	100	0	100	0	0	0	100	81	100	100	100	96	67	0	100	0	100	100	100	100	52	0	22	100	100	4	100	100	100	100	59	0	0	0	100
S. sciuri group b	2	100	0	100	0	0	0	100	50	100	100	100	100	100	0	100	0	100	100	100	100	100	0	100	100	100	0	100	100	100	100	100	0	0	0	100
S. sciuri total	29	100	0	100	0	0	0	100	79	100	100	100	97	69	0	100	0	100	100	100	100	55	0	28	100	100	3	100	100	100	100	62	0	0	0	100
S. simulans	33	0	0	100	94	100	0	100	97	100	79	0	0	0	0	9	0	97	0	100	24	58	0	0	0	100	97	9	100	0	18	27	97	0	15	0
S. vitulus	13	100	0	100	0	0	0	100	0	100	100	31	52	0	0	23	65	94	69	100	100	0	0	0	77	100	0	4	100	100	100	0	0	0	0	25
S. warneri	76	0	0	100	92	95	4	100	8	100	64	0	0	0	0	100	0	100	0	97	7	11	0	9	0	43	1	1	100	0	63	0	89	0	91	58
S. warneri group b	4	0	0	100	100	100	0	100	0	100	0	0	0	0	0	100	0	100	0	100	0	0	0	0	0	100	0	0	100	0	75	0	100	0	75	50
S. warneri group c	5	0	0	100	80	80	0	100	20	100	60	0	0	0	0	100	0	100	0	100	0	20	0	20	0	80	0	0	100	0	40	0	100	0	100	75
S. warneri total	85	0	0	100	92	94	4	100	8	100	61	0	0	0	0	100	0	100	0	98	6	11	0	9	0	48	1	1	100	0	62	0	91	0	91	59
S. xylosus	2	0	0	100	100	38	0	100	100	100	100	0	0	50	0	100	100	100	0	100	100	0	50	0	0	100	88	100	100	100	100	0	0	0	50	0
S. xylosus group b	31	0	0	100	100	65	0	100	100	100	100	0	19	87	0	100	100	97	0	100	100	19	14	0	7	97	83	100	100	100	65	3	0	0	30	13
S. xylosus total	33	0	0	100	100	63	0	100	100	100	100	0	18	85	0	100	100	97	0	100	100	18	16	0	7	97	83	100	100	100	67	3	0	0	32	13
Unnamed group 1	2	0	0	100	13	0	0	100	0	100	0	0	0	0	0	0	0	0	0	88	0	0	0	0	0	100	25	88	100	0	0	0	0	0	25	0
Unnamed group 2	5	0	0	100	100	100	0	100	0	92	0	0	0	0	0	100	0	0	0	100	100	40	0	24	0	80	0	100	100	0	100	0	80	0	100	0
Unnamed group 3	3	0	0	100	0	100	0	100	0	100	67	0	0	0	0	100	0	100	0	100	0	0	0	0	0	100	100	0	100	0	0	33	0	0	100	17
Unnamed group 4	3	0	0	100	100	92	0	100	67	100	100	100	100	100	67	100	100	100	0	100	100	8	0	0	100	100	42	100	100	100	100	33	0	0	58	100

[a] Multiple examinations of reference strains were reduced to a single data set prior to inclusion in the database. PYR, pyroglutamate aminopeptidase.

FA groups were merged whenever the identification scores (relative probabilities) became less than 98% for their centrostrain biotypes. Merged FA groups were assigned a letter of the alphabet in lowercase; FA groups that did not merge were assigned uppercase letters. The FA groups included 395 additional isolates, making a total of 1,512. Their biochemical and FA characteristics are listed in Tables 2 and 3, respectively.

DISCUSSION

In addition to conventional biochemical tests, various other methods for identifying staphylococci have been used, including the determination of bacteriolytic-activity patterns (53), sodium dodecyl sulfate-polyacrylamide gel electrophoresis of proteins (39), and several kinds of chromosomal analysis (7, 8, 17, 18, 51, 54). Commercial identification systems using biochemical and enzyme tests are also available (26–28, 40), and their limitations and reliabilities have been discussed in a recent review by Kloos and Bannerman (27).

FA analysis has been used for the identification of a wide range of microorganisms, including bacteria, yeasts, and filamentous fungi (55). Durham and Kloos (12) determined the FA compositions of 100 staphylococci representing 10 species and found that the FA compositions of all strains were qualitatively similar but quantitatively different. Eerola and Lehtonen (13) performed FA analysis on strains of various bacteria, including three species of staphylococci. They found that the occurrences and amounts of the various fatty acids were generally constant for members of the same species and that repeated analysis of the same strain produced essentially identical results. Kotilainen et al. (32) extended that work to the analysis of seven species: *S. epidermidis*, *S. warneri*, *S. hominis*,

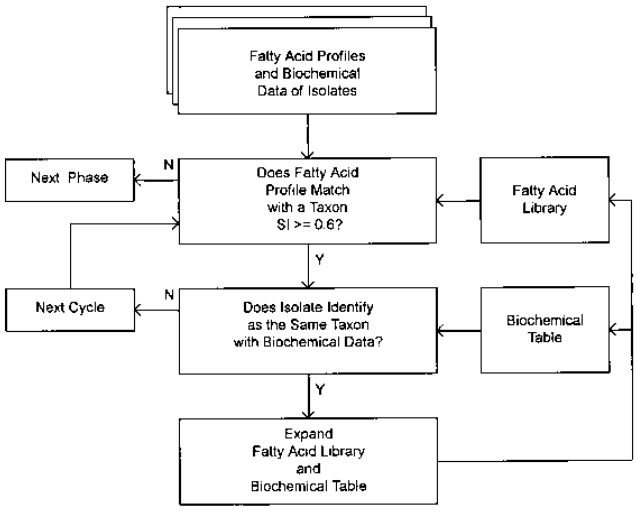

FIG. 1. Flow diagram for the development of the FA profile library and biochemical table. SI, similarity index; N, no; Y, yes.

S. haemolyticus, *S. capitis*, *S. lugdunensis*, and *S. simulans*. When the data were subjected to cluster analysis, they found that isolates belonging to the same species formed separate clusters, but the distinctions were not absolute; some strains were incongruently positioned in various clusters, and some strains identified as *S. epidermidis* and *S. warneri* remained outside all clusters.

Identification schemes in general suffer from the limitation that the reliability of the identification depends on the range of taxa represented in the database. Incorrect identifications can result if some taxa are omitted, and this is a particular weakness in probabilistic programs, where a high percent probability figure may be completely misleading if the database is restricted. For this reason, we felt it imperative to include all of the aerobic taxa in our system.

The FA analysis method produces a similarity index which is a measure of how much an unknown profile resembles "average" profiles stored in the FA library. A perfect match gives a similarity index of 1.0. We chose a threshold value of 0.6 or greater for acceptance, which is considered a good match for an individual taxon (36). However, the profile of an unknown isolate may match the profiles of two or more taxa at a high level of similarity, in which case FA analysis cannot be relied upon alone (as with strains of *S. capitis* and *S. caprae*). An advantage of our system is that mistakes are less likely to occur, since isolates are identified by combining conventional biochemical tests with FA analysis.

The heuristic, or self-educating, approach which we developed is an objective procedure that is based only on the characteristics of the type strains and the stringency of the acceptance criteria. Preconceptions regarding the key features of a taxon are not needed.

Characteristics of selected species. The type strain group of *S. aureus* subsp. *aureus* contains human, animal, and environmental strains; the FA group B form of the subspecies contains three human strains only. One strain belonging to the type strain group was resistant to novobiocin, and occasional strains were negative in the tube coagulase test.

S. auricularis grows slowly, which makes FA analysis problematic and not reproducible. Only one strain (ATCC 33750) met the acceptance criteria; six other strains were biochemically similar to *S. auricularis*, but they had FA similarity indices of less than 0.6. Some carbohydrate reactions were weak, as was the glucose fermentation test. The species is characterized by high proportions (about 20%) of the $C_{20:0}$ fatty acid.

The reactions of the two subspecies of *S. capitis* agreed well with published data (3, 29). DNase reactions were almost always weak. Occasional maltose-positive strains of *S. capitis* subsp. *capitis* may be difficult to distinguish from urease-negative strains of *S. capitis* subsp. *ureolyticus*. These latter strains can be distinguished from *S. capitis* subsp. *capitis* in that they produce acid from lactose, maltose, and galactose, and they produce gelatinase. Rare ornithine decarboxylase-positive strains of *S. capitis* subsp. *capitis* may be mistaken for *S. lugdunensis*, but they are easily separated by other tests, and *S. lugdunensis* is usually yellow pigmented and beta-hemolytic on blood agar.

S. caprae was described on the basis of 10 strains isolated from goat's milk (11), but strains have since been isolated from human clinical samples (25, 51), and these show some divergence with respect to *Sma*I-digested-DNA electrophoresis patterns (17). Vandenesch et al. (51) found eight *Eco*RI ribotypes among 26 strains from humans and goats, and they distinguished human from goat ribotypes on the basis of a constant four-band pattern in the human isolates. Our strains include 4 goat strains and 34 human strains, many from bone and joint infections. Contrary to the original description, in our tests the type strain produced acid from sucrose, mannitol, and fructose and gave a negative result in the test for acetoin. Our type strain results agree (except for acetoin) with those found by Vandenesch et al. (51), as do the results for the human strains examined, but goat strains were more likely to give negative maltose reactions. All strains gave a strongly positive DNase reaction, but the positive acetoin reactions were weak. FA profiles are very similar for *S. caprae*, for both subspecies of *S. capitis*, and, to a lesser extent, for *S. haemolyticus* and *S. warneri*. For this reason, FA analysis should not be used alone to identify *S. caprae.*

There are considerable biochemical differences between the *S. carnosus* type strain group and *S. carnosus* FA group B. The type strain group includes four commercial starter cultures for sausage production and three strains of unknown origin. All group B strains came from fermented fish in Japan. In both cases our results agree closely with those published previously (41, 49).

S. caseolyticus (9, 44) differs from other staphylococci in several respects, notably the positive oxidase and benzidine reactions, the occurrence of a class II Fructose-1,6-diphosphate aldolase, and its possession of two types of cytochrome *c*. However, the results of DNA-rRNA hybridization tests support its inclusion in the genus (44). Our type strain group contains only the type strain and one other strain of unknown origin. Group B contains two of the strains described by De La Fuente et al. (9). Four other lamb strains received from R. De La Fuente gave biochemical reactions similar to those of group B strains. Although their FA profiles were similar to those of accepted strains, they did not meet the acceptance criteria. The FA profile of *S. caseolyticus* is quite different from that of other staphylococci, including the oxidase-positive *S. sciuri* species group. In particular, the extremely low levels of 15:0 anteiso, the presence of large amounts of 16:0, and the absence of 15:0 iso, 17:0 iso, and 17:0 anteiso FAs set *S. caseolyticus* apart from all other staphylococcal species.

Most of the biochemical characteristics that we list for *S. cohnii* agree with published data (31, 45). However, a clear distinction between *S. cohnii* subsp. *cohnii* and *S. saprophyticus* on the basis of acid production from sucrose is not supported by our data. A combination of tests (urea hydrolysis, gelati-

TABLE 3. FA library entries[a]

Taxon	% FA																																		
	No. of strains	11:0 iso	12:0 iso	12:0	13:0 iso	13:0 anteiso	14:0 iso	14:0	15:0 iso	15:0 anteiso	15:0	16:1 iso E	Unknown 15.549	16:0 iso	Unknown 15.665	16:1 A	16:0	17:1 iso E	17:0 iso	17:0 anteiso	17:0	18:3 cis 6,12,14	18:0 iso	18:1 cis 9	18:0	19:0 iso	19:0 anteiso	19:0	20:0 iso	Unknown 19.735	20:1 cis 11	20:0	Sum 5	Sum 6
S. arlettae	2						1.1	0.4	11.8	45.9				1.4			3.2		8.7	13.7			0.6	1.0	5.6	1.9	2.0					2.6		
S. aureus subsp. *aureus*	130						1.5		6.3	42.7				2.2			2.0		4.3	14.4	0.5		1.6	1.6	7.5	1.8	4.0	1.4		0.7		5.5		1.0
S. aureus subsp. *aureus* group B	3						0.4		4.3	45.3				1.0			1.5		4.0	22.1			0.7	0.9	6.3	1.8	6.2	0.4			0.7	3.9		0.4
S. auricularis	2						2.8		6.2	37.8							1.0		1.0	1.8			0.9		4.6	7.7	8.9	1.1	3.0		0.8	20.8		1.0
S. capitis subsp. *capitis*	39								7.0	40.0							1.5		8.7	20.2				1.3	6.9	4.2	6.7					2.5		0.6
S. capitis subsp. *ureolyticus*	34								8.0	37.6							1.4		8.7	15.7				1.4	8.5	5.3	6.5				0.7	3.9		0.6
S. capitis subsp. *ureolyticus* group b	2						1.0		13.0	30.2				0.8			1.7		10.6	8.6			0.9	1.8	9.6	8.6	4.4	1.2			0.5	6.2		0.8
S. caprae	38								5.6	41.0							1.4		7.8	21.0				1.6	6.9	3.7	6.9				0.6	2.0		0.8
S. carnosus	9						0.9		6.9	56.8				1.3			3.9		3.0	16.2				1.3	6.4		1.2					1.9		
S. carnosus group B	10								4.3	53.9							4.7		2.5	20.2					7.9		2.1					3.2		
S. caseolyticus	2						16.1	13.2		1.0		1.5	2.4	1.6		21.7	9.8					0.7		28.9	2.1						1.2			
S. caseolyticus group B	2			1.0			3.5	24.7					3.5			15.6	11.5					2.5		29.1	5.3						1.6	0.8		0.9
S. chromogenes	5						0.5		17.0	39.6				1.2			1.6		6.7	16.1			0.3	1.1	3.6	3.4	3.9			0.3		3.0		1.1
S. cohnii subsp. *cohnii*	7						0.7		10.4	49.4				1.8			2.6		5.2	15.9				1.1	4.3	2.0	1.9					4.0		
S. cohnii subsp. *urealyticum*	32						1.0		18.6	44.1				2.3			2.1		8.7	11.4			0.5	0.8	3.5	2.8	1.1					2.7		
S. delphini	2			0.5				1.0	43.5	22.7				1.0			3.6		9.0	5.6				1.7	5.3	0.8						3.9		0.5
S. epidermidis	155						1.3		8.4	34.3				1.3			2.2		7.2	11.9			0.9	2.1	11.8	4.0	4.0	1.2		1.0	1.3	6.2		0.7
S. epidermidis group b	6						1.5		6.9	34.6				1.2			1.5		5.2	10.8			1.6	1.5	9.3	5.4	7.2	0.9	0.4	0.7	2.4	8.0		
S. epidermidis group c	39						1.5		6.1	36.6				1.5			1.9		5.7	14.3			1.5	1.6	10.7	3.5	5.5	1.0		0.6	1.2	6.5		
S. epidermidis group d	3						0.7		7.5	35.7							3.2		6.5	11.9				3.4	13.3	3.9	4.1			0.8	1.7	6.3		
S. epidermidis group e	9						1.5		9.1	36.0				0.7			2.3		6.7	10.9				1.7	12.5	4.1	3.9	0.8		1.4		7.9		
S. epidermidis group f	2						1.6		8.0	31.4				1.0			1.9		4.8	8.1			1.1	2.1	12.7	5.1	4.6	1.6		1.0	3.6	11.4		
S. epidermidis group g	3			1.2			1.2	0.7	8.0	33.9							4.7		3.6	5.7				2.3	22.1	0.8	1.2			1.3		11.3		1.2
S. equorum	48			1.4			1.2	0.9	13.8	62.5				0.6			2.9		2.5	4.9				1.8	2.6	0.9						2.3		0.8
S. equorum group b	21			0.8			1.0	0.5	11.1	55.3				1.3			2.7		4.5	8.5				1.9	3.0	2.6	1.3				0.6	3.3		1.0
S. felis	21							0.7	25.7	30.5				0.9			3.3		7.2	10.7				1.8	5.9	1.9	2.1	0.4		1.5	1.5	4.3		1.2
S. felis group b	20			0.5				0.9	30.3	27.0				0.8			3.9		7.5	8.3				2.1	6.3	1.8	1.5	0.5		1.5	1.5	4.0		1.3
S. gallinarum	2			1.0			0.9		13.7	39.5				0.8			2.7		15.4	10.3			0.4	1.2	4.7	4.7	2.4				0.4	1.7		
S. haemolyticus	167								5.6	42.7				0.5			2.1		5.6	23.1				1.4	9.0	1.8	4.0					2.3		0.4
S. haemolyticus group b	14						1.6	0.5	4.0	39.9				1.0			2.5		3.2	17.3	0.5		0.7	1.2	13.9	1.2	3.8	1.2				6.4		0.5
S. hominis	5						0.4		6.1	41.1							2.1		5.6	15.6				2.3	10.3	3.4	6.3	0.8		0.8		3.8		0.8
S. hominis group b	2								5.6	40.4							1.5		4.8	12.8				2.4	9.2	6.5	10.4	0.7			1.0	4.9		
S. hominis group c	13			0.6			1.6		10.9	32.9							2.7		6.2	6.9				3.0	14.3	4.4	3.1	2.2		1.3		7.0		1.4
S. hominis group d	48						0.9		9.0	35.5							2.0		7.1	9.3			0.8	2.5	11.1	6.8	5.8	1.3			1.0	5.9		
S. hominis group E	2						1.6		11.5	32.7							2.8		6.6	7.3				2.5	15.6	3.7	2.7	1.8		1.7		8.0		1.6
S. hominis group f	5								7.2	37.6							1.5		6.4	11.5			1.0	1.5	7.9	8.4	9.9	0.7		0.8		4.1		
S. hominis group g	10						0.6		7.2	38.8							2.5		6.1	12.3				2.6	12.5	4.0	5.4	0.8		0.9		5.0		1.1
S. hyicus	46			0.4			0.9	0.7	38.7	27.8				1.9			2.9		8.6	5.8				1.2	3.9	1.7	0.7				0.5	2.9		0.8
S. hyicus group b	10			0.6			2.3	0.9	36.3	22.6				2.8			4.1		7.0	3.2				1.3	6.4	1.7	0.4	0.5		0.3	0.6	7.2		1.2
S. intermedius	1			0.4			0.4		43.2	19.3				1.6			2.1		13.0	5.7				1.6	3.6	2.2	0.8			0.5	0.9	3.5		0.8
S. intermedius group B	85								46.8	14.1	0.4			1.1			2.2		13.4	5.3	0.5			2.0	3.8	2.0	0.5	0.6		1.1	1.2	3.1		1.2
S. kloosii	2						1.1		10.8	39.6				1.3			2.8		11.2	13.0			0.7	0.6	6.7	3.7	3.1					4.7		
S. kloosii group b	2						0.8		8.6	45.0				1.1			2.4		10.0	15.6			0.6	1.0	5.1	2.9	3.2					3.2		
S. lentus	11			0.5			0.6	0.4	31.8	35.2				0.9	1.2	0.5	2.2	1.6	7.9	10.7				1.1	1.5	0.8	0.9					1.0	0.7	0.5
S. lugdunensis	105						1.0		3.7	46.6				1.5			2.5		2.5	22.6	0.6		0.6	1.1	7.0	0.8	4.5	1.0				2.8		
S. lugdunensis group b	4						1.8	0.6	2.8	44.8	0.4			1.9			3.4		1.5	17.2	1.3		0.7	1.2	10.2	0.4	3.5	2.4				4.9		
S. muscae	2			1.5				1.6	48.5	20.4							6.2		6.0	3.6				3.0	5.7	0.7						1.3		1.2
S. pasteuri	2						1.7		4.7	45.2				2.3			1.9		6.7	19.5			1.4	1.0	5.4	1.4	3.7	0.7			0.8	2.7		0.6
S. piscifermentans	6						1.1		12.7	43.8				1.4			3.3		7.4	16.2	0.6			1.0	6.8	1.1	1.5					1.7		0.5
S. piscifermentans group b	2						1.2		13.0	44.3				1.6			4.0		7.0	14.1	0.6			1.7	7.5	1.0	1.2					1.7		0.9
S. pulvereri	5			2.6	1.1	1.0			37.7	38.1						1.8	0.9	4.8	1.9	3.1				2.3	1.0	0.5							2.6	0.5
S. saprophyticus	73								12.6	51.7				1.0			2.4		6.8	13.8				1.0	3.1	2.4	1.2					3.5		
S. schleiferi subsp. *coagulans*	4			0.5					42.6	18.5				0.8			2.1		13.7	9.4				1.1	3.5	2.9	1.1			0.4		2.6		0.7
S. schleiferi subsp. *schleiferi*	8								47.7	11.7				0.9			2.2		17.1	5.9				1.5	4.5	3.2	0.6					2.8		0.6

Continued on following page

TABLE 3—*Continued*

Taxon	% FA																																
	No. of strains	11:0 iso	12:0 iso	12:0	13:0 iso	13:0 anteiso	14:0 iso	14:0	15:0 iso	15:0 anteiso	15:0	16:1 iso E	Unknown 15.549	16:0 iso	Unknown 15.665	16:1 A	16:0	17:1 iso E	17:0 iso	17:0 anteiso	17:0	18:3 cis 6,12,14	18:0 iso	18:1 cis 9	18:0	19:0 iso	19:0 anteiso	19:0	20:0 iso	Unknown 19.735	20:1 cis 11	20:0	Sum 5	Sum 6
S. schleiferi subsp. *schleiferi* group b	17								40.2	15.5			0.4			2.3		15.5	8.4				1.9	5.0	3.3	1.0	0.6			0.9	3.1		0.6
S. schleiferi subsp. *schleiferi* group c	6								38.6	19.8			0.5			3.4		11.4	8.3				2.2	6.4	1.7	0.6			0.5	0.8	3.8		1.2
S. sciuri	27				0.7		0.9	0.6	43.5	28.1			0.9	1.1	0.4	2.4		9.1	6.8				0.8	1.6	0.7	0.5					0.9	0.5	
S. sciuri group b	2				1.1		1.3	0.8	47.5	28.4	0.6		0.6		1.3	2.1	1.1	5.9	3.5				1.1	1.7	0.5						1.0	1.2	
S. simulans	33						1.2	0.5	11.3	51.6			1.0			4.6		4.5	11.4	0.7			1.9	6.3	0.6	0.9					1.5		1.2
S. vitulus	13				2.5	1.1	1.0		37.1	37.9	0.6			0.8	1.6	0.8	4.7	2.2	3.3				1.7	0.9	0.6							2.4	
S. warneri	76						0.8		5.2	43.0			1.1			2.1		7.3	18.5			0.8	1.6	7.1	2.3	4.7	0.5			0.6	3.1		0.8
S. warneri group b	4						0.8		3.4	42.8						0.8		1.8	6.7			0.9	0.6	4.3	6.0	17.5	0.8	1.2		1.9	10.1		
S. warneri group c	5						3.5		4.6	41.8			3.3			2.8		4.3	12.9	0.7		2.0	1.1	9.8	1.1	2.3	1.7				6.8		0.9
S. xylosus	2				1.0		0.9		21.1	48.2			1.2			2.8		7.8	6.4				1.1	3.8	2.1						2.3		
S. xylosus group b	31				0.5		0.5		17.3	54.3			0.9			2.5		6.5	8.6				1.3	3.2	1.5	0.5					1.6		
Unnamed group 1	2								13.1	32.6			0.8			1.1		9.9	29.6				0.7	2.8	3.1	3.9					2.4		
Unnamed group 2	5								9.3	28.4			2.1			2.9		5.3	25.3				2.5	10.7	2.1	6.2			0.6	1.3	2.7		
Unnamed group 3	3						1.4		8.2	35.8			1.2			2.5		6.0	10.9	0.7		0.6	1.2	16.4	2.4	2.5	1.6				7.6		0.4
Unnamed group 4	3	0.3	0.4	0.4	18.8	10.7	1.8	1.8	11.1	23.9			1.5			2.6		9.0	6.5			0.5	0.7	3.5	2.6	1.3					1.4		0.4

[a] Unknown fatty acids are listed with their equivalent chain lengths. Summed feature 5 (sum 5) is a combination of 17:1 iso I and 17:1 anteiso B fatty acids, and summed feature 6 (sum 6) is a combination of 18:2 cis 9, 12 and 18:0 anteiso fatty acids. The absence of an entry indicates that the fatty acid was not included in the library.

nase, and acid production from lactose, sucrose, and mannose) is required to distinguish between these two taxa. *S. cohnii* subsp. *urealyticum* is more prevalent in human clinical samples than *S. cohnii* subsp. *cohnii*, although neither is encountered frequently. All of our strains of *S. cohnii* subsp. *urealyticum* came from human samples. Urease-negative strains are otherwise typical.

Most of our strains of *S. epidermidis* were members of the type strain group. Generally, the lack of acid production from trehalose is a useful marker for this species, but trehalose-positive strains occur occasionally. Susceptibility to desferrioxamine (35) is also very useful for recognizing *S. epidermidis*. This test was not included at the beginning of the study, so it is not listed in Table 2. However, in our experience nearly all strains of *S. epidermidis* and *S. hominis* are susceptible, while strains of other species are resistant. We encountered one strain each of *S. epidermidis* and *S. hominis* that were resistant and one strain each of *S. warneri* and *S. cohnii* subsp. *cohnii* which were susceptible. In addition, two of six strains of *S. piscifermentans* were susceptible (5).

Isolates of *S. equorum* were first obtained from the skin of healthy horses (43). Our 68 strains include two of the original strains (DSM 20674 [ATCC 43958] and PA 219), two from German fish meal, three from healthy rabbits, one from an environmental sample, and one from a human clinical sample, and we collected the remaining 59 strains from healthy local horses. *S. equorum* is difficult to distinguish biochemically from *S. xylosus*, and our set of 35 tests did not always accomplish this. Colonial morphology is a useful indicator, for *S. equorum* forms small (<1-mm) colonies on blood agar plates incubated at 35°C for 24 h, whereas *S. xylosus* forms much larger colonies which are often yellow pigmented. On P agar at 35°C, colonies of *S. equorum* are very small, are irregular in shape, and hardly increase in size with prolonged incubation. Colonies of *S. xylosus* grow well on P agar. In addition, *S. equorum* is beta-glucosidase positive and gives a weak phosphatase reaction by the method described here, whereas *S. xylosus* is usually beta-glucosidase negative and gives a strong phosphatase reaction. Other quantitative differences are shown in Table 2. The two species were readily differentiated by FA analysis.

Of nine strains of *S. gallinarum* that we examined, only one strain, P57 of Devriese et al. (11), was accepted into the type strain group. The others gave similar biochemical reactions but did not fulfill the FA similarity criterion, even though the species was the first choice.

In general, strains of *S. haemolyticus* were readily identified by FA analysis and showed high similarity indices, but there was some ambiguity with *S. capitis* subsp. *capitis* and with *S. caprae*. These species are easily differentiated by biochemical tests. The biochemical resemblance between *S. haemolyticus* and unnamed group 3 is discussed below.

The FA results for *S. hominis* are variable, because of the low growth rate of the organisms. Similarity indices tend to be low, and few strains gave the value of 0.6 required for inclusion in the type strain group. The creation of six FA groups allowed the inclusion of many more strains. The biochemical results are generally similar for all of the groups, but the existence of novobiocin-resistant strains may easily cause confusion with other species.

Our two subgroups of *S. hyicus* each contain strains from cattle and pigs, and the two groups show similar biochemical reactions which correspond to published data (10, 33). All trehalose-negative strains came from cattle, but not all of the bovine strains were trehalose negative. There is some heterogeneity within this species as indicated by DNA-DNA hybridization studies, possibly representing the inclusion of unnamed subspecies (10, 38), and FA analysis tends to support this.

There appears to be both genotypic and phenotypic varia-

tion between strains of *S. intermedius* isolated from different animal hosts. De Buyser et al. (8) found that the type strain (a pigeon isolate) showed a ribotype different from that of strains isolated from carnivores, and biochemical differences have also been noted by Hájek et al. (19–21). In our system, the type strain gave results for acid production from mannitol and galactose different from those for our other strains. For this reason, no strains were accepted into the type strain group. Contrary to the original description, we found that the type strain produced acid from maltose as well as from mannitol. The majority of our strains of *S. intermedius* were isolated from dogs; a few came from other animals and from animal bite wounds of humans. Our strains appear to belong to biotype 1 or 3, as described by Hàjek et al. (20, 21), although only a minority were mannitol positive. The biochemical distinction between *S. intermedius* biotype 2 and *S. schleiferi* subsp. *coagulans* remains problematic.

S. lugdunensis, first described in 1988 (16), has been shown to be a human pathogen capable of causing serious disease (23, 46), and it should be routinely identified in clinical laboratories. The species is well identified by FA analysis and appears to be homogeneous.

All of our strains of *S. piscifermentans* came from fermented fish or shrimp. The biochemical results are similar for the two FA groups that we established, and generally the results agree with those published previously (50). The DNase reaction was weak.

The biochemical results for *S. schleiferi* agree closely with those published previously (16, 24). As noted above, biochemical differentiation between *S. schleiferi* subsp. *coagulans* and *S. intermedius* biotype 2 is difficult, but FA analysis usually separates these two groups. Occasional strains of *S. schleiferi* subsp. *schleiferi* produce a pseudo-coagulase phenomenon (52), as is shown in our FA group c. The strong DNase reaction may cause confusion with other species, particularly *S. aureus*. Grattard et al. (18), using *Hin*dIII endonuclease, divided 31 strains of *S. schleiferi* subsp. *schleiferi* into six ribotypes. There was limited correlation between the ribotypes and our FA groups. When examined by the same method, two of our strains (one of FA group b and one of FA group c), established new ribotypes (15).

The members of the *S. sciuri* species group are *S. sciuri*, *S. lentus*, *S. pulvereri*, and *S. vitulus* (26, 42, 54, 57). They possessed characteristic FA profiles that contained small amounts of 16:1 A (present otherwise only in *S. caseolyticus*) and 17:1 iso E (present only in this group) acids. Very small amounts of 20:0, if any, were present; this FA is characteristic for the other species of the genus.

The type strain group of *S. sciuri* contains strains from human, environmental, and animal (horse, cow, rat, and rabbit) sources. The two strains in FA group b were from barley straw. Almost all strains of *S. lentus* gave a strong positive phosphatase reaction.

Webster et al. (54) described 11 strains of *S. vitulus* obtained from animals and meat products. Our strains of *S. vitulus* were all isolated from healthy horses.

The five strains of *S. pulvereri* described by Zakrzewska-Czerwinska et al. (57) fulfilled our acceptance criteria for *S. vitulus*, but because *S. pulvereri* was described as a species, we show the data for the two groups separately. The biochemical and FA data are similar, and it is likely that they are synonomous. *S. vitulus* was not included in the DNA-DNA hybridization experiments done with *S. pulvereri*, and further work is required to clarify the relationship.

Discrimination between *S. pasteuri* and *S. warneri* is difficult and currently requires the use of nucleic acid techniques (6). We found that some strains which were identified as *S. pasteuri* by FA analysis were identified biochemically as *S. warneri*. Also, three of the five *S. warneri* FA group c strains were described by Chesneau et al. (6) as *S. pasteuri* (BM 10425, BM 10427, and BM 9363). It is likely that some of the other strains included as *S. warneri* are actually *S. pasteuri*. The one strain which was accepted as *S. pasteuri* came from an orangutan.

The type strain group of *S. xylosus* contains only one other strain isolated from salami in Germany. FA group b strains came from a variety of animal and environmental sources. We have never isolated *S. xylosus* from a human clinical sample. Both groups show similar biochemical profiles, but FA analysis suggests some heterogeneity within this species. It is interesting that De Buyser et al. (8) described the existence of two varieties within this species on the basis of an examination of rRNA gene restriction patterns. As indicated above, *S. xylosus* may be confused with *S. equorum* biochemically.

We have recognized four unnamed FA groups that require further study. (i) Unnamed group 1 consists of two strains of human origin which have distinct biochemical and FA patterns. These correspond to those noted recently by Ayers and Solomon (1). (ii) Unnamed group 2 consists of four strains of human origin. Their biochemical pattern is similar to that of *S. epidermidis*, but the FA profile revealed a much larger amount of 17:0 anteiso and a smaller amount of 20:0 FAs than in *S. epidermidis*. (iii) Unnamed group 3 consists of two strains of human origin and one of bovine origin. The biochemical pattern resembles that of *S. haemolyticus*, but acid production from lactose and from galactose is consistently negative. The FA profile is closer to those of *S. epidermidis* and *S. hominis*. (iv) The three strains in unnamed group 4 exhibit biochemical results which are similar to those for *S. gallinarum*. Two strains were from bovine sources, and the other was a contaminant in a culture of *S. gallinarum* sent to us. Their FA profile was quite different from that of *S. gallinarum* and indeed was different from those of all other staphylococci, with major differences in the contents of 13:0 iso, 13:0 anteiso, 17:0 iso, and 17:0 anteiso fatty acids. The DNase reaction is weak or negative, and colonies on P agar after 5 days are irregular in outline, 6 to 9 mm in diameter, flat, matt, and pale yellow.

Our examination of the aerobic species of *Staphylococcus* represents a new attempt to standardize the identification of these important bacteria. The combination of FA analysis and biochemical testing overcomes most of the identification errors that would result from using either method alone. The methodology is useful for reference laboratories and adaptable for use in the clinical laboratory. The heuristic system we have described is an objective approach for characterizing recognized species of bacteria, and it also serves well as a research tool for distinguishing undescribed taxa.

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