Staphylococcus auricularis sp. nov.: an Inhabitant of the Human External Ear†

WESLEY E. KLOOS^{1*} AND KARL H. SCHLEIFER²

Department of Genetics, North Carolina State University, Raleigh, North Carolina 27650,¹ and Lehrstuhl für Mikrobiologie, Technische Universität München, D-8000 Munich 2, Federal Republic of Germany²

A new species, *Staphylococcus auricularis*, was isolated from the human external ear (external auditory meatus) and is described here. This species can be distinguished from other staphylococci primarily on the basis of growth rate and colony morphology on agar, carbohydrate reaction pattern, cell wall composition, and deoxyribonucleic acid sequence relationships. The type strain of this species is ATCC 33753.

Before the introduction of sound, molecular criteria for distinguishing staphylococci from micrococci, several skin microbiologists reported that micrococci were the most prevalent bacteria of healthy, external human ears (16). Upon reinvestigation of the microflora of human ears, we have found that the predominant gram-positive, catalase-positive cocci inhabiting this specific site belong to the new species *Staphylococcus auricularis* (see below) and to *Staphylococcus capitis* (7). Of a total of 25 individuals sampled, 23 (92%) demonstrated populations of *S. auricularis* in the external auditory meatus. In 22 of the 23 individuals, *S. auricularis* was the major *Staphylococcus* species present in this habitat. *Staphylococcus epidermidis*, *Staphylococcus warneri*, *Staphylococcus hominis*, and *Micrococcus kristinae* were isolated only occasionally.

In this paper we describe the characteristics of *S. auricularis*, including the criteria establishing it as a new species.

MATERIALS AND METHODS

Bacterial strains. Staphylococci were isolated by procedures described previously (8, 12). A total of 20 strains from 16 different people were selected for this study (Table 1). Each of four of the persons carried two different strains of this species in one ear. The strains of the other species included in this study are listed in Table 2. All inocula were prepared by aerobic growth of cells on a medium containing peptone, yeast extract, glucose, and NaCl (P broth or agar) (12). Unless noted otherwise, the incubation temperature was 34°C.

Character determinations. The procedures used for determining deoxyribonucleic acid (DNA) base composition, colony morphology and pigment, motility, aerobic and anaerobic growth in thioglycolate, salt tolerance, growth temperature, growth on Tween 80,

catalase and benzidine activities, acetylmethylcarbinol production, nitrate reduction, coagulase, hemolysis, deoxyribonuclease, alkaline phosphatase, carbohydrate reactions, and susceptibility to various antibiotics, lysozyme, and lysostaphin were similar to the procedures described previously by Kloos and co-workers (10-13, 20) for cutaneous micrococci and staphylococci. The preparation of cell walls and the determination of the peptidoglycan type were performed as described by Schleifer and Kandler (18). Cell wall teichoic acids were determined by the procedures of Schleifer and co-workers (17, 21). The oxidase test was performed as described by Faller and Schleifer (3). The class of fructose-1,6-biphosphate aldolase was determined by the method of Götz et al. (4). The configuration of the lactic acid produced from glucose was determined by the method of Schleifer and Kocur (21). DNA was isolated and purified by the procedures of Brenner and co-workers (1), as modified by Kloos and Wolfsohl (13). DNA-DNA reassociation reactions and the separation of single-stranded, unreacted DNA from double-stranded, hybridized DNA on hydroxyapatite were performed by the procedures described by Brenner and co-workers (1).

RESULTS AND DISCUSSION

Description of *Staphylococcus auricularis* sp. nov. *Staphylococcus auricularis* (au. ri. cu. la'ris. NL. adj. *auricularis* pertaining to the ear). The following description of *S. auricularis* is based on a total of 20 strains.

Cells were gram-positive cocci, 0.8 to 1.2 µm in diameter, nonmotile, and nonsporeforming and occurred predominantly in tetrads and pairs. Colonies on P agar were convex, opaque, circular, slightly glistening, white, and very small, ranging from 1.4 to 2.8 mm in diameter (after incubation for 3 days at 34°C and for 2 days at 25°C). Small colony size was also observed on several commercially prepared agar media (e.g., nutrient, tryptic soy, and brain heart infusion agars). The colony texture of 90% of the strains was smooth; 10% of strains had colonies with a granular texture and ornate, wrinkled, or rough

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TABLE 1. Characteristics that varied among 20 strains of *S. auricularis*

Strain	Colony surface ^a	Colony diam (mm)	Growth on 15% NaCl agar	Growth at 45°C	Nitrate reduction	Deoxyribo-nuclease	Acid produced aerobically from:					Novobiocin MIC (μg/ml) ^b	Peptidoglycan (mol/mol of glutamic acid)	
							Fructose	Trehalose	Maltose	Sucrose	Mannose		Lactose or galactose	Serine
ATCC 33753 ^T	S	2.0	± ^c	±	±	—	+	+	+	±	—	0.1	0.5	4.4
ATCC 33750	S	2.3	+	±	+	±	+	+	±	—	—	0.8	0.6	4.5
ATCC 33751	S	2.8	+	±	—	±	—	+	±	—	—	0.4	0.2	4.7
ATCC 33752	S	2.5	+	±	±	±	+	+	—	—	—	0.4	0.1	4.8
ATCC 33754	OW	2.7	±	+	—	—	+	+	+	+	±	0.4	0.2	4.6
LK 811M	S	2.4	+	+	±	±	+	+	+	—	—	0.8	0.1	4.6
LK 812M	S	2.3	+	±	±	±	±	±	—	—	—	0.2	0	4.7
DB 811M	S	2.3	+	±	±	±	+	+	—	—	—	0.4	0.1	4.7
HK 811M	S	1.8	±	±	—	—	+	+	—	—	—	0.2	0	4.8
BO 4	R	2.5	±	+	—	—	+	+	+	+	+	0.4	0.3	4.5
BO 2	S	2.8	±	+	—	±	+	±	—	—	—	0.4	0.3	4.6
MLK 2	S	2.0	±	±	—	±	+	+	±	—	—	0.4	0.1	4.6
JM 2	S	1.4	±	±	—	—	—	+	±	—	—	0.4	0	4.8
MM 2	S	2.2	±	±	—	±	+	±	—	—	—	0.4	0.2	4.7
BB 1	S	1.6	±	±	—	±	+	—	—	—	—	0.8	0.3	4.5
MA 2	S	2.2	±	±	+	±	+	+	+	—	—	0.4	0.2	4.6
JP 2	S	2.8	±	+	—	—	+	—	—	—	—	0.1	0	4.7
JS 1	S	2.4	±	—	—	±	+	—	—	—	—	0.4	0.5	4.3
JS 2	S	1.5	±	—	±	—	+	+	—	—	—	0.4	0	4.7
TK 2	S	2.0	±	+	—	±	+	—	—	—	—	0.2	ND ^d	ND

^a S, Smooth; OW, ornate wrinkled; R, rough.^b MIC, Minimal inhibitory concentration.^c +, Positive reaction; ±, weak reaction; —, negative reaction.^d ND, Not determined.

TABLE 2. DNA hybridization of staphylococci with [methyl-³H]thymidine-labeled DNA from *S. auricularis* ATCC 33753^T

Source of unlabeled DNA		% Relative binding ^a to labeled <i>S. auricularis</i> ATCC 33753 ^T DNA at:		Source of unlabeled DNA		% Relative binding ^a to labeled <i>S. auricularis</i> ATCC 33753 ^T DNA at:	
Taxon	Strain	55°C (optimal conditions)	70°C (stringent conditions)	Taxon	Strain	55°C (optimal conditions)	70°C (stringent conditions)
<i>S. auricularis</i>	ATCC 33753 ^T	100	100	<i>S. saprophyticus</i>	CCM 883 ^T	34	9
	ATCC 33750	97	97	<i>S. cohnii</i>	ATCC 29974 ^T	42	10
	ATCC 33751	93	96	subsp. 1			
	ATCC 33752	100	99	<i>S. cohnii</i>	PAY 12J3	39	7
	ATCC 33754	96	91	subsp. 2			
	LK 811M	99	99	<i>S. cohnii</i>	AU 5033	35	6
	LK 812M	98	97	subsp. 3			
	DB 811M	97	97	<i>S. xylosus</i>	ATCC 29971 ^T	32	7
	HK 811M	98	98	<i>Staphylococcus</i> sp. ("3")	DM 130	39	10
	BO 4	91	90				
<i>S. epidermidis</i>	ATCC 14990 ^T	33	7				
<i>S. capitis</i>	ATCC 27840 ^T	34	10	<i>S. simulans</i>	ATCC 27848 ^T	36	9
<i>S. hominis</i>	ATCC 27844 ^T	35	5	<i>S. carnosus</i>	CH	31	7
<i>S. warneri</i>	ATCC 27836 ^T	35	8	<i>S. intermedius</i>	CFDD	26	8
subsp. 1							
<i>S. warneri</i>	PA 13 C11	38	12	<i>S. hyicus</i>	NCTC 10350 ^T	25	8
subsp. 2				subsp. <i>hyicus</i>			
<i>S. haemolyticus</i> subsp. 1	ATCC 29970 ^T	33	8	<i>S. sciuri</i>	ATCC 29062 ^T	24	7
				subsp. <i>sciuri</i>			
<i>S. haemolyticus</i> subsp. 2	LED 324	35	9	<i>S. sciuri</i>	ATCC 29070 ^T	28	8
				subsp. <i>lentus</i>			
<i>S. "simians"</i>	GOB 18F3	41	13	<i>S. caseolyticus</i>	ATCC 29750	13	8
<i>S. saccharolyticus</i>	ATCC 14953 ^T	35	15	<i>B. subtilis</i>	ATCC 6051 ^T	10	0
<i>S. aureus</i>	ATCC 12600 ^T	34	10				

^a Relative binding data represent the means of duplicate reassociation reactions. The sample standard deviation had a mean of 2%. In the homologous reaction, labeled DNA reassociated with unlabeled DNA at a level of $97 \pm 3\%$. The percentage of radioactivity bound in the heterologous reaction was normalized to the percentage of radioactivity bound in the homologous reaction. Selected reciprocal reassociation reactions, in which the labeled DNA was from other species (e.g., *S. epidermidis* ATCC 14990^T, *S. hominis* ATCC 27836^T, *S. capitis* ATCC 27840^T, *S. cohnii* ATCC 29974^T, *S. sciuri* subsp. *lentus* ATCC 29070^T) and the unlabeled DNA was from *S. auricularis* ATCC 33753^T, produced results similar to the data shown above ($\pm 3\%$).

^b T, Type strain.

surface. The colony edges were entire to slightly undulate and usually became crimped after 4 to 5 days of incubation. Young colonies (1 to 3 days old) exhibited an unusually high profile for staphylococci, which was rather similar to the profiles of certain micrococci (e.g., *M. kristinae* and *Micrococcus sedentarius* [12]).

Growth was weak to moderate in the aerobic portion of a semisolid thioglycolate medium and very weak to weak in the anaerobic portion, suggesting a slight facultative anaerobic capability. All strains produced only L-lactic acid from glucose under anaerobic conditions.

All strains grew well at NaCl concentrations up to 10%. A total of 70% of the strains grew poorly at an NaCl concentration of 15%; 30% of the strains grew moderately at this high salt

concentration. The optimal growth temperature range was 30 to 40°C. No strain grew at 15°C, and 90% of the strains grew weakly to moderately at 45°C.

All strains had a moderate to strong catalase activity, were positive for the benzidine test, and were oxidase negative. Like most staphylococci, all strains contained class I fructose-1,6-biophosphate aldolase. All failed to produce coagulases or to exhibit clear hemolysin activity, although upon prolonged incubation (more than 3 days) some partial hemolysis or dark "greening" of the surrounding bovine blood agar medium became evident. A total of 50% of the strains failed to produce acetylmethylcarbinol, whereas the remaining 50% produced only low levels of this compound. No strain produced

alkaline phosphatase activity; 35% of the strains reduced nitrates to nitrites slowly, and 70% demonstrated weak deoxyribonuclease activity. Growth was not stimulated by the addition of Tween 80 to P agar at concentrations ranging from 0.2 to 3%.

All strains produced acid aerobically from D-(+)-glucose and glycerol; 90% of the strains produced acid from β -D-(-)-fructose and D-(+)-trehalose, 70% produced acid from maltose, and 40% produced acid from sucrose. A total of 10% of the strains produced acid from D-(+)-mannose, α -lactose, and D-(+)-galactose, and 5% produced weak acid from D-(-)-ribose; 35% of the strains produced very weak acid from D-(+)-turanose. All failed to produce acid from β -gentiobiose, D-sorbitol, L-(-)-sorbose, salicin, D-(+)-raffinose, L-(+)-rhamnose, xylicol, D-(+)-xylose, L-(+)-arabinose, D-(+)-fucose, *meso*-inositol, D-mannitol, D-(+)-melezitose, and D-(+)-cellobiose.

When we used the API STAPH-IDENT system (Analytab Products, Plainview, N.Y.) with 10 miniaturized biochemical tests (14), all *S. auricularis* strains were negative for alkaline phosphatase, urease, β -glucosidase, and β -glucuronidase activities and failed to produce acid aerobically from D-mannitol and salicin. A total of 80% of the strains produced acid from D-(+)-trehalose, and 10% produced acid from D-(+)-mannose; 60% utilized arginine, and 25% exhibited weak to moderate β -galactosidase activity.

All strains were resistant to 50 μ g of lysozyme per ml and were susceptible to 100 μ g of lysostaphin per ml and 1.6 μ g of novobiocin per ml. The minimum inhibitory concentration for novobiocin was from 0.1 to 0.8 μ g/ml. Agar disk diffusion tests indicated that all strains were susceptible to penicillin G, methicillin, erythromycin, tetracycline, chloramphenicol, streptomycin, kanamycin, gentamicin, lincomycin, neomycin, vancomycin, and bacitracin (9, 11).

All strains contained cell wall peptidoglycan of the basic type Lys-Gly₅; however, 15% of the strains had peptidoglycans that had significant amounts of glycine (0.5 to 0.6 mol/mol of glutamic acid) replaced by serine. All strains possessed an atypical cell wall teichoic acid, namely, poly(N-acetylglucosamine 1-phosphate) (15, 19). The guanine-plus-cytosine contents of the DNAs of the two strains tested (ATCC 33753^T [type strain] and ATCC 33754) were 38 and 39 mol%, respectively. The variable characteristics of the strains are listed in Table 1.

DNA-DNA reassociation reactions performed under optimal and stringent conditions indicated that the 10 strains of *S. auricularis* examined were very closely related ($\geq 90\%$ relative DNA binding or homology) to one another at the species level (Table 2). When *S. auricularis* and

other *Staphylococcus* species were compared, the DNA homology values were much lower (5 to 15% under stringent reassociation conditions and, with the one exception of *Staphylococcus caseolyticus* strain ATCC 29750, 24 to 41% under optimal reassociation conditions) and were as expected for separate species. These values were significantly higher than those between *S. auricularis* and a member of another gram-positive genus, *Bacillus subtilis*.

S. auricularis can be distinguished from all other staphylococci primarily on the basis of its slow growth and resulting colonial morphology, carbohydrate reaction pattern, cell wall composition, and DNA sequence relationships. There are also some significant differences between this species and some other species in the parameters of cell arrangement, anaerobic growth pattern in thioglycolate, various enzyme activities, and susceptibility to novobiocin (2, 5, 6, 9, 11, 17, 20, 21). Simple characters useful in separating *S. auricularis* from other *Staphylococcus* species are shown in Table 3.

Strain ATCC 33753 (originally designated WK 811M) is the type strain of *S. auricularis*. A description of this strain follows.

Cells: spheres 1.0 to 1.2 μ m in diameter, occurring predominantly in tetrads and pairs. Nonmotile and nonsporeforming. Gram positive.

Agar colonies: circular, entire, convex, smooth with slight glossy surface, opaque, white, and 1.8 to 2.2 mm in diameter (after 5 days). Older colonies develop a crimped edge.

Catalase and benzidine tests are positive. Oxidase is negative.

Chemoorganotroph; metabolism is respiratory.

Slightly facultatively anaerobic.

Temperature relationships: no growth at 15°C and poor growth at 45°C; optimum growth temperature, 34 to 37°C.

Growth on agar containing NaCl: good growth with 10% NaCl, poor growth with 15%.

Coagulases are not produced. Nitrates are reduced slowly and weakly; phosphatase and acetylmethylcarbinol are not produced. Deoxyribonuclease activity is very weak. Hemolysins are not detected.

Acid is produced aerobically from glucose, glycerol, fructose, maltose, and trehalose and weakly from sucrose and turanose. No acid is produced from gentiobiose, sorbitol, sorbose, salicin, raffinose, rhamnose, xylose, xylicol, arabinose, fucose, inositol, mannitol, mannose, lactose, melezitose, cellobiose, galactose, or ribose.

Antibiotic susceptibilities: susceptible to lysostaphin (100 μ g/ml) and novobiocin (≥ 0.1 μ g/ml); susceptible to penicillin G, methicillin,

TABLE 3. Simple characters useful in separating *S. auricularis* from various human coagulase-negative staphylococci^a

Species	Colony size ^b	Pigment	Aerobic growth	Alkaline phosphatase	Hemolysis	Novobiocin resistance ^c	Acid produced aerobically from:					Urease ^d
							Maltose	Trehalose	Mannitol	Sucrose	Xylose or arabinose or both	
<i>S. auricularis</i>	VS	— ^e	± or —	—	—	—	+ or —	+ or ±	—	— or +	—	—
<i>S. epidermidis</i>	S or M	—	+	+	— or ±	—	+	—	—	+	—	+
<i>S. hominis</i>	S or M	+ or —	— or ±	—	— or ±	—	+	+ or —	— or +	+	—	+
<i>S. haemolyticus</i>	M or L	— or +	± or +	—	— or ±	—	+	+	+ or —	+	—	—
<i>S. warneri</i>	S or M	+ or —	+	—	— or ±	—	+ or ±	+	+ or —	+	—	+
<i>S. capitis</i>	S	—	+ or ±	—	— or ±	—	—	—	+	+ or —	—	—
<i>S. saprophyticus</i>	M or L	+ or —	+ or ±	—	—	+	+	+ or —	+ or —	+	—	+
<i>S. cohnii</i>	M or L	—	± or +	— or ±	— or ±	+	± or +	+	+ or ±	+	—	— or ±
<i>S. xylosus</i>	M or L	+ or —	+ or ±	+ or —	— or ±	+	+ or ±	+	+ or ±	+	+	+
<i>S. simulans</i>	L	—	+	± or —	± or —	—	— or ±	+ or —	+ or ±	+	—	+

^a Data taken from references 7, 9, and 10.
^b Colony sizes (diameters) were determined after incubation on P agar (12) at 34 to 35°C for 3 days and at room temperature for an additional 2 days. L, Large (≥7 mm); M, medium (5 to 6 mm); S, small (2 to 4 mm); VS, very small and slow growing (1 to 2 mm).
^c Minimal inhibitory concentration, ≥3.1 µg/ml.
^d Character originally determined to be useful by the API STAPH and API STAPH-IDENT staphylococcal systems.
^e +, Positive reaction; ±, weak reaction; —, negative reaction.

erythromycin, chloramphenicol, streptomycin, kanamycin, tetracycline, gentamicin, neomycin, lincomycin, vancomycin, and bacitracin.

Fructose-1,6-biphosphate aldolase: class I.

Peptidoglycan type: L-Lys-Gly_{4,4}, Ser_{0,5}.

Cell wall teichoic acid composition: poly(*N*-acetylglucosamine 1-phosphate). Atypical.

Guanine-plus-cytosine content of the DNA: 38.8 mol%.

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