Characterization of *Staphylococcus sciuri* sp. nov. and Its Subspecies¹

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Several strains previously classified as group III staphylococci, by the scheme of Schleifer and Kocur, and numerous strains isolated from animal and human skin that appeared to be related to group III strains were subjected to a taxonomic study. As a result of this study, all group III and related strains were placed in the newly proposed species *Staphylococcus sciuri*. This species can be differentiated from all other staphylococci on the basis of colony morphology, cell wall peptidoglycan, acid production from cellobiose and usually from fucose under aerobic conditions, and a combination of other characteristics. Thirty-five strains that produced large colonies, usually moderate to light anaerobic growth in thioglycolate, and acid from galactose, sucrose, glycerol, and often from melezitose were placed in the type subspecies, *S. sciuri* subsp. *sciuri*. Nine strains that produced very small, unpigmented colonies, usually no detectable anaerobic growth in thioglycolate, and acid from sucrose and often from galactose, glycerol, lactose, and raffinose were placed in the subspecies *S. sciuri* subsp. *lentus*. The type strains of these subspecies are ATCC 29062 and ATCC 29070, respectively. A group of three strains that produced relatively small, unpigmented colonies, moderate anaerobic growth in thioglycolate, and acid from glycerol but failed to produce acid from sucrose, melezitose, raffinose and usually galactose may also deserve subspecies status. A summary of the character variation found in *S. sciuri* and in other novobiocin-resistant species and a simplified scheme for distinguishing *S. sciuri* and its subspecies are included in this paper.

In 1973, Schleifer and Kocur (13) recognized a group of nine strains of staphylococci that contained a unique cell-wall peptidoglycan type, L-Lys-L-Ala-Gly₄, and formed L-lactic acid from glucose under anaerobic conditions. According to their classification scheme, based on peptidoglycan type, teichoic acid type, configuration of lactic acid, and pH values of a glucose medium after anaerobic incubation, these strains were placed in *Staphylococcus* groups IIIA, B, and C. More recent studies by Kocur and Boháček (7) have determined that the guanine plus cytosine (G+C) contents of the deoxyribonucleic acids (DNA) of several of the strains in group IIIA are in the range of 29.8 to 33.3 mol%. One of the strains (CCM 2614; I3) in group IIIC was previously designated as *Staphylococcus lactis* by Davison and co-workers (1-3); however, since the name *S. lactis* is a later objective synonym of *Micrococcus varians* Migula 1900 (8), it cannot be appropriately applied to staphylococci. To date, staphylococci in group III have remained in an uncertain taxonomic status.

In independent studies characterizing staphy-

lococci from animal and human skin, we have isolated certain strains that could not be identified with currently recognized species of *Staphylococcus*, namely, *S. aureus*, *S. simulans*, *S. xylosus*, *S. cohnii*, *S. saprophyticus*, *S. haemolyticus*, *S. warneri*, *S. hominis*, *S. epidermidis*, and *S. capitis* (4, 12). Interestingly, a large number of these strains isolated from animal skin and several isolated from human skin had the same peptidoglycan type and produced the same lactic acid isomer as members of group III.

Since numerous strains of group III staphylococci are now available for analysis, we proceeded, in the presently reported study, to determine the taxonomic status of this unique group.

MATERIALS AND METHODS

Bacterial strains. Representative strains of *Staphylococcus* group III (13) used in this study included CCM 2611 (J896) obtained from L. Janíková, State Veterinary Institute, Prague, CSSR, and isolated from milk, CCM 2614 (I3) isolated from the uropygeal gland of a chicken and CCM 2615 (A4) obtained from A. R. Archibald, Newcastle, England; and CCM 2598 (K2), CCM 2599 (K6), K14, K15, K17, K18, K19, K20, and K21 obtained from M. Roguinsky, Nouzilly, France, and isolated from goat udders.

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A total of 35 strains were isolated from the healthy skins of Eastern gray squirrels (*Sciurus carolinensis*), Southern flying squirrels (*Glaucomys volans*), opossums (*Didelphis virginiana*), a raccoon (*Procyon lotor*), a dog (*Canis familiaris*), sheep (*Ovis aries*), and humans (Table 1). Three strains were also isolated from marsh grass (*Spartina alterniflora*) and beach sand frequented by animals.

For comparative purposes, 64 strains of the presumed related species, *Staphylococcus xylosus*, and 20 strains of *S. cohnii* were isolated from the above-mentioned animals and were characterized.

Procedures for isolating staphylococci. The sampling techniques and the isolation medium used were described previously (4, 6, 12).

Culture conditions. Culture conditions were similar to those described previously for the study of cutaneous micrococci (6).

Character determinations. Procedures for determining DNA base composition, colony morphology and pigment, cell morphology, motility, aerobic and anaerobic growth in thioglycolate, salt tolerance, growth temperature, catalase and benzidine activities, acetylmethylcarbinol production, nitrate reduction, coagulase, hemolysis, deoxyribonuclease (DNase), phosphatase, and bacteriolytic activity, carbohydrate reactions, and susceptibility to various antibiotics, lysozyme, and lysostaphin were similar to those described previously for cutaneous micrococci and staphylococci (5, 6, 12).

Procedures for determining gelatinase and lecithinase (egg yolk reaction) activity, hydrolysis of Tween 80, and lipase activity on tributyrin and triolein agars were described previously (14). Casein hydrolysis was determined with the medium of Martley et al. (9). Elastase activity was determined by the method of Varadi and Saqueton (15).

Procedures for making cell wall hydrolysates and determining peptidoglycan type, teichoic acid, configuration of lactic acid, and the anaerobic fermentation of glucose have also been described previously (10, 11, 13, 15). The amount of free glycerol was enzymatically determined in acid hydrolysates (2 N HCl, 100 C, 3 h) of extracted teichoic acids. The amount of glycerol mono- and diphosphates was not determined by this method. The different peptidoglycan types are abbreviated according to the system of Schleifer and Kandler (11).

Chemicals. Egg-white lysozyme (muramidase), triolein, elastin, and phenolphthalein monophosphate were purchased from Sigma Chemical Co., St. Louis, Mo.; lysostaphin was from Schwarz/Mann, Orangeburg, N.Y.; α -naphthylamine was from Matheson Coleman and Bell, Cincinnati, Ohio; 4-aminoantipyrine was from K & K Laboratories, Inc., Plainview, N.Y.; penicillin G and tetracycline hydrochloride were purchased from Calbiochem, Los Angeles, Calif. Erythromycin base was kindly supplied by Abbott Laboratories, North Chicago, Ill., and novobiocin was kindly supplied by the Upjohn Co., Kalamazoo, Mich.

RESULTS AND DISCUSSION

Characterization of *S. sciuri* sp. nov. and its subspecies. Staphylococci examined in this

study that were novobiocin resistant, produced acid from D-(+)-cellobiose and usually from D-(+)-fucose, produced only the L-lactic acid isomer from glucose, and contained cell wall peptidoglycan of the type L-Lys-L-Ala-Gly₄ were clearly different from previously described species (4, 12). These staphylococci are considered here as members of a new species, *Staphylococcus sciuri* (sci'ur-i. L. mas.n. *Sciurus* generic name of a squirrel on whose skin this species is commonly found in large populations; L. gen.n. *sciuri* of the squirrel). Strains that produced large distinctive colonies, failed to produce acid from raffinose and usually α -lactose, but often produced acid from D-(+)-melezitose, and were usually pigmented (Table 1) were placed in *S. sciuri* subsp. *sciuri*. The K series of strains isolated from goats that produced slow-growing, small colonies, usually produced acid from raffinose and α -lactose but no acid from D-(+)-melezitose, and were unpigmented were placed in *S. sciuri* subsp. *lentus* (len'tus. L. adj. *lentus* slow; pertaining to slow growth). Strains CCM 2611, CCM 2614, and CCM 2615 appear to be related to both subspecies but differ from them in several distinctive characteristics and combinations of characteristics and, therefore, may belong to a different subspecies. Further studies are needed to resolve this matter.

At present, we have chosen to divide the species *S. sciuri* into at least two subspecies rather than to make proposals for separate species. This choice is somewhat arbitrary, but it is based primarily on the unique characteristics that are shared by these staphylococci. However, as staphylococci from more animal species are studied, we could conceivably find that what appear at present to be unique characters may actually be rather common in nature. To date, most of our knowledge about staphylococci has come from the analysis of human and certain domestic-animal strains. Also, in this study we have been reluctant to assign *S. sciuri* subsp. *lentus* a higher taxonomic rank because of the limited source and number of strains available for analysis. As additional sources and strains of this subspecies are studied, we should be better able to evaluate the difference in character parameters observed between this organism and *S. sciuri* subsp. *sciuri*. Future DNA hybridization studies may also be helpful in evaluating the genetic relationship of these organisms.

The following description of *S. sciuri* is based on a total of 44 strains (35 strains of *S. sciuri* subsp. *sciuri* and 9 strains of *S. sciuri* subsp. *lentus*), unless otherwise noted.

Cells were gram-positive cocci, 0.7 to 1.2 μ m in diameter, nonmotile, and nonsporeforming, and occurred singly, in pairs, and tetrads.

TABLE 1. Sources of *Staphylococcus sciuri* subsp. *sciuri* strains studied

Strain	Host animal (skin) or other habitat	Animal no.	Collection location	Collection date
SC 120	Eastern gray squirrel (<i>Sciurus carolinensis</i>)	11	Sunbury, N.C., woodland	8/72
ATCC 29062, SC 114		13		
SC 231		23	Raleigh, N.C., residential	9/72
SC 255		24		
SC 226		25		
SC 251	Southern flying squirrel (<i>Glaucomys volans</i>)	26	Sunbury, N.C., swamp	12/72
SC 407, -409		38		
SC 414		39		
SC 446, -474		43		
GV 234		19	Raleigh, N.C., residential	9/72
GV 212	Opossum (<i>Didelphis virginiana</i>)	20		
ATCC 29061		21	Sunbury, N.C., swamp	8/72
DM 24, -81		4		
ATCC 29059, DM 30, -71, -88, -91		5		
DM 25	Raccoon (<i>Procyon lotor</i>)	10	Sunbury, N.C., woodland	12/72
DM 393		35		
PL 37		8	Sunbury, N.C., swamp	
CF 3	Dog (<i>Canis familiaris</i>)	1	Raleigh, N.C., residential	11/71
OA 526		2		
ATCC 29060, GH 10	Sheep (<i>Ovis aries</i>)		Raleigh, N.C., Univ. farm	3/75
	Human (<i>Homo sapiens</i>)		Raleigh, N.C.	7/71
KH 30	Beach sand		New Brunswick, N.J.	11/71
DW 144				10/71
SK 24				10/72
OW 2				
HA 10T				
	Marsh grass (<i>Spartina alterniflora</i>)		Cape Hatteras, N.C.	
HA 12	Beach sand			

Usually 40 to 70% of the cells of *S. sciuri* subsp. *lentus* occurred in tetrad arrangements, which may be the reason why Roguinsky (8, 13; personal communication) called these organisms "*Gaffkya tetragena*-like."

The colonies of the two subspecies were remarkably different. Colonies of *S. sciuri* subsp. *sciuri* on a P agar medium (6) were large (7.0 to 11.0 mm in diameter), circular, smooth, glistening, opaque, raised, and, with age, developed an elevated center. The colony edge of 86% of the strains was slightly to moderately undulate. Colonies usually developed numerous pinpoint depressions near the edge within 5 to 7 days. Eighty percent of the strains produced colonies that were gray-white with a yellowish or cream-colored tint toward the center, 14%

produced yellowish or gray-yellow colonies (see color photograph of strain GH 9 in reference 4), and 6% produced gray-white (unpigmented) colonies. Pigment was considerably intensified during growth at low temperatures (15 to 20 °C). Colonies of *S. sciuri* subsp. *lentus* were very small (2.2 to 3.0 mm in diameter), circular, smooth, glistening to wet in appearance, opaque, and convex. The colony edge of 67% of the strains was slightly undulate. All strains produced gray-white to white colonies.

Growth of *S. sciuri* subsp. *sciuri* occurred in the aerobic portion and 91% of the strains produced growth in the anaerobic portion of a semi-solid thioglycolate medium. Twenty percent of the strains produced uniformly moderate to dense anaerobic growth, 67% produced a gra-

dient of light to moderate to light or undetectable anaerobic growth down the tube, and one strain (3%) produced a gradient of anaerobic growth plus individual discrete colonies. Ten percent of the strains failed to show detectable anaerobic growth. Growth of *S. sciuri* subsp. *lentus* occurred in the aerobic portion, but only one strain (11%) produced detectable growth in the anaerobic portion of the thioglycolate medium. This strain produced a gradient of light to undetectable anaerobic growth down the tube.

Twenty-seven selected strains, including members of both subspecies, demonstrated weak fermentation of glucose by lowering the pH of a yeast extract-glucose broth from 6.8 to 5.7 to 6.0 after anaerobic incubation. All of these strains produced low amounts of L-lactic acid from glucose.

All strains grew well at NaCl concentrations up to 10%, although strains of *S. sciuri* subsp. *lentus* grew very slowly and required 72 to 96 h of incubation for comparative evaluation. Strains usually grew poorly at an NaCl concentration of 15%. The optimal growth temperature range was 25 to 35 C. All strains of *S. sciuri* subsp. *sciuri* grew moderately well at 15 C, whereas strains of *S. sciuri* subsp. *lentus* grew poorly or failed to grow at this temperature. Sixty-three percent of *S. sciuri* subsp. *sciuri* strains grew poorly and 26% failed to grow at 45 C. All *S. sciuri* subsp. *lentus* strains failed to grow at 45 C.

All strains had moderate catalase activity and were positive for the benzidine test. All failed to demonstrate coagulase or hemolysin activity. All strains reduced nitrates and demonstrated weak to moderate phosphatase activity. Ninety-one percent of the strains failed to produce acetylmethylcarbinol, and the few remaining strains produced only low amounts of this compound. Ninety-eight percent of the strains had weak to moderate DNase activity, and 68% failed to demonstrate bacteriolytic activity.

Thirty-one selected strains of *S. sciuri* subsp. *sciuri* demonstrated moderate to strong caseinolytic and gelatinase activities and failed to demonstrate lipolytic activity on triolein, tributyrin, or lecithin or elastase activity. Ninety-four percent of the strains failed to hydrolyze Tween 80. All strains of *S. sciuri* subsp. *lentus* demonstrated moderate caseinolytic and gelatinase activities.

By comparison with other staphylococci, *S. sciuri* produced acid from a very wide range of carbohydrates, which included some not previously reported to be attacked by staphylococci (4, 12). All strains of this species produced acid,

aerobically, from D-(+)-glucose, β -D-(-)-fructose, D-(-)-ribose, D-(+)-cellobiose, and D-mannitol, and 68% produced small to moderate amounts of acid from maltose. In addition, all strains of *S. sciuri* subsp. *sciuri* produced acid from D-(+)-galactase, D-(+)-fucose, sucrose, and glycerol, whereas all strains of *S. sciuri* subsp. *lentus* produced acid from D-(+)-mannose, sucrose, D-(+)-trehalose, salicin, and β -gentiobiose. Ninety-one percent of *S. sciuri* subsp. *sciuri* strains produced acid from D-(+)-trehalose and β -gentiobiose, 77% produced acid from D-sorbitol, 71% produced acid from salicin, 49% produced acid from D-(+)-melezitose, 37% produced acid from L-(+)-arabinose, 31% produced small amounts of acid from D-(+)-mannose, and 23% produced acid from L-(+)-rhamnose. Sixty-seven to 78% of *S. sciuri* subsp. *lentus* strains produced acid from L-(+)-arabinose, D-(+)-galactose, α -lactose, L-(+)-rhamnose, and D-sorbitol, and small to moderate amounts of acid from D-(+)-fucose and glycerol. Eighty-nine percent of the strains of this subspecies uniquely produced acid from raffinose. One strain (3%) of *S. sciuri* subsp. *sciuri* produced acid from raffinose within 24 h, but this was neutralized by 72 h. Six percent of *S. sciuri* subsp. *sciuri* strains produced a small amount of acid and 33% of *S. sciuri* subsp. *lentus* strains produced small to moderate amounts of acid from D-(+)-xylose. All strains failed to produce acid from xylitol, D-arabitol, dulcitol, meso-erythritol, D-erythrose, tagatose, L-(-)-fucose, D-lyxose, or L-sorbose, and only one strain (11%) of *S. sciuri* subsp. *lentus* produced small amounts of acid from D-(+)-melibiose.

All strains were resistant to lysozyme. Twenty-seven percent of *S. sciuri* subsp. *sciuri* strains were susceptible (minimal inhibitory concentration [MIC] 25 to 50 μ g/ml) and 73% were slightly resistant (MIC 100 to 400 μ g/ml) to lysostaphin. On the other hand, all strains of *S. sciuri* subsp. *lentus* were very susceptible (MIC 12.5 to 25.0 μ g/ml) to lysostaphin. All strains were slightly resistant (MIC 1.6 to 12.5 μ g/ml) to novobiocin. All strains of *S. sciuri* subsp. *sciuri* were slightly resistant (MIC 0.4 to 6.2 μ g/ml) to penicillin G, whereas all strains of *S. sciuri* subsp. *lentus* were susceptible (MIC 0.05 to 0.2 μ g/ml) to this antibiotic. Eighty-six percent of the strains were susceptible (MIC 0.4 to 0.8 μ g/ml) to erythromycin.

Twenty-seven selected strains, including members of both subspecies, contained peptidoglycan of the type L-Lys-L-Ala-Gly₄. In some strains of *S. sciuri* subsp. *sciuri*, small amounts of glycine are replaced by L-serine. Their cell wall teichoic acid contained glycerol and glucosamine, with the exception of one strain (DM

TABLE 2. *Variable characters of Staphylococcus sciuri*

Strain	G+C content (mol%)	Colony diam (mm)	Colony pigment ^a	Anaerobic thioglycolate growth ^b	Anaerobic fermentation of glucose (pH)	Growth on 15% NaCl agar	Growth at 45 C	Acetyl-methyl-carbinol	Phosphatase	Deoxy-ribonuclease	Bacteriolytic activity
<i>S. sciuri</i> subsp. <i>sciuri</i>											
ATCC 29062	36.5	9.5	yt	±	5.8	± ^c	±	-	+	+	-
SC 114		9.0	yt	±	5.8	±	-	-	+	+	+
SC 120		7.2	yt	±	5.8	±	±	-	+	+	+
SC 226		8.5	yt	±	5.7	±	±	-	+	+	+
SC 231		8.0	yt	±	5.9	±	±	-	±	+	-
SC 251		8.5	yt	±	5.9	±	±	-	±	+	-
SC 255		7.2	yt	±	6.0	±	±	-	±	+	+
SC 407		7.8	yt	±	6.0	-	±	-	±	+	+
SC 409		8.5	yt	±	5.9	+	±	-	±	+	-
SC 414		8.5	yt	-	5.9	-	±	-	±	+	-
SC 446		7.8	yt	-	6.0	±	±	±	+	±	+
SC 474		8.5	yt	±	5.9	±	±	-	+	+	+
ATCC 29061	36.0	10.0	yt	± ^C	5.9	±	±	-	+	+	-
GV 212		11.0	yt	±		±	±	-	+	+	-
GV 234		9.0	yt	±	5.7	±	±	-	+	±	+
ATCC 29059	35.6	7.5	yt	±	5.9	±	±	-	+	±	-
DM 24		8.2	yt	±		+	-	-	+	+	-
DM 25		7.1	yt	±		±, -	-	±	+	+	-
DM 30		7.0	yt	+		-	-	-	+	-	-
DM 71		7.8	yt	-	6.0	±	±	-	±	±	±
DM 81		8.5	gw	+		±	±	-	±	+	-
DM 88		9.5	gy	±		±	±	-	±	+	-
DM 91		9.5	gy	±		-	±	-	±	+	-
DM 393		7.2	yt	±	5.8	±	±	-	±	±	-
PL 37		11.0	yt	-	5.7	±	±	-	±	±	+
CF 3		7.5	gy	±		±	±	±	+	±	+
OA 526		7.5	yt	±		±	±	±	+	±	+
ATCC 29060	34.5	8.0	yt	±	5.2	±	±	-	+	±	+
GH 10		9.0	gy	±	5.1	+	+	-	+	+	+
KH 30		7.0	gy	±		+	±	-	+	+	-
DW 144		8.5	yt	+		±	±	-	±	±	-
SK 24		9.0	gw	±		±	±	-	+	+	-
OW 2		8.5	yt	±		±	±	-	+	+	-
HA 10T		7.5	yt	+		±	±	-	+	+	-
HA 12		8.0	yt	+		+	±	-	+	+	-

[illegible]

TABLE 2—Continued

Strain	Acid (aerobically) from													Lyso-staphin MIC (μg/ml)	Peptidoglycan (mol/mol of glutamic acid)			Teichoic acid components		
	Maltose	Lactose	Xylose	Arabinose	Galactose	Mannose	Trehalose	Melezitose	Salicin	Rhamnose	Sorbitol	Gentiobiose	Fucose		Raffinose	Glycerol	Serine	Glycine	Alanine	Glycerol (free, nmol/mg cell wall)
DM 71	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	0.1	4.0	3.1	0 ^c	0.7
DM 81	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+					
DM 88	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+					
DM 91	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+					
DM 393	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	0	4.1	3.2	57	0.6
PL 37	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	0	4.2	3.1	71	0.4
CF 3	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+					
OA 526	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+					
ATCC 29060	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	0	4.1	3.1		
GH 10	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	0	4.0	3.2		
KH 30	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+					
DW 144	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+					
SK 24	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+					
OW 2	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+					
HA 10T	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+					
HA 12	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+					
<i>S. sciuri</i> subsp. <i>lentus</i>	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	0	3.7	2.6	80	1.2
ATCC 29070	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+					
K 2	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+					
K 6	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+					
K 14	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	0	3.9	2.7	62	0.8
K 15	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	0	4.0	2.9	75	1.1
K 17	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	0	3.8	2.8	82	0.5
K 18	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	0	4.1	3.0	65	1.1
K 19	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	0	3.9	2.9	57	0.5
K 20	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	0	4.0	3.0	65	0.8
Strains of uncertain relationship	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+					
CCM 2611	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	0	4.0	3.0		0.7
CCM 2614	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	0	4.2	3.1	9	
CCM 2615	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	0	4.1	3.2	23	0.7

^a Symbols for colony pigment: gy, gray-yellow; yt, gray-white with yellowish or cream tint toward center; gw, gray-white; w, white; w/gw, predominantly white with gray-white sectors; gw/w, predominantly gray-white with white sectors.

^b Symbols for anaerobic growth: +, uniformly moderate or dense growth; $\pm$, gradient of growth from light to moderate to light or no growth down the tube; $\pm$ C, gradient of growth plus individual colonies; -, no growth.

^c Reactions: ++, strong positive (carbohydrates only); +, positive; $\pm$, weak; (-), weak within 24 h, but negative by 72 h (raffinose only); -, negative.

^d Teichoic acid contains glucose instead of glucosamine.

^e Teichoic acid contains ribitol instead of glycerol.

^f DNA base composition determined by Kocur and Boháček (7).

71), which contained ribitol instead of glycerol, and one strain (SC 225) which contained glucose instead of glucosamine. Most of the strains of *S. sciuri* subsp. *sciuri* produced low concentrations of free glycerol in the acid hydrolysates of cell walls; this finding might suggest the presence of an atypical teichoic acid (1, 2).

The G+C content of the DNA, as determined in six strains, was 35.3 ± 0.4 mol%.

S. sciuri can be distinguished from all other staphylococci primarily on the basis of its distinctive colonial morphology, carbohydrate reaction pattern (including the unique production of acid from fucose and cellobiose), cell wall peptidoglycan, and a combination of characteristics including novobiocin resistance, L-lactic acid configuration, lack of acetylmethylcarbinol production, weak to moderate phosphatase and DNase activities, nitrate reduction, moderate to strong caseinolytic and gelatinase activities, and lack of lipolytic activity. There are also some significant differences between *S. sciuri* and certain other species in the parameters of growth at extreme temperatures and different NaCl concentrations, cell arrangement, growth in a thioglycolate medium, and coagulase, hemolysin, and bacteriolytic activities. Several of the above-mentioned properties appear to overlap with those of *S. xylosus*, suggesting a relatively close relationship between these species. Some variable characters and the G+C contents of the DNAs of representative strains of *S. sciuri* are given in Table 2.

Description of the type strain of *S. sciuri* subsp. *sciuri*. Strain ATCC 29062 (originally designated SC 116), isolated from the skin of an Eastern gray squirrel, is the type strain of *S. sciuri* subsp. *sciuri*, the type subspecies of *S. sciuri*. A description of this strain follows.

Cells: Spheres, 0.8 to 1.2 μ m in diameter, occurring in pairs, singly, and in tetrads, in order of frequency. Nonmotile and nonspore-forming. Gram positive.

Agar colonies: Circular, 9.0 to 9.5 mm in diameter, moderately undulate edge, raised with slightly elevated center, smooth with glistening surface. Opaque, gray-white with cream tint toward center.

Catalase and benzidine tests are positive.

Chemoorganotroph; metabolism is respiratory.

Facultatively anaerobic.

Growth at extreme temperatures: Moderate growth at 15 C, poor growth at 45 C.

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

Coagulases are not produced.

Phosphatase activity is moderate.

Nitrates are reduced.

Hemolysins: No hemolysis of bovine blood.

Acetylmethylcarbinol is not produced.

DNase activity is moderate.

Proteolytic activity: Caseinolysis is strong, gelatinase activity is moderate, and there is no elastase activity.

Lipolytic activity: No lipolysis of triolein, tributyrin, or Tween 80; no lecithinase activity.

Acid produced aerobically from D-(+)-glucose, β -D(-)-fructose, D-(+)-galactose, D-(+)-fucose, D(-)-ribose, sucrose, D-(+)-cellobiose, D-mannitol, glycerol, L-(+)-arabinose, D-(+)-trehalose, D-(+)-melezitose, salicin, and D-sorbitol; small amounts of acid are produced from β -gentiobiose and maltose. No acid from α -lactose, D-(+)-xylose, D-(+)-mannose, L-(+)-rhamnose, D-(+)-turanose, xylitol, *meso*-inositol, adonitol, dulcitol, D-arabitol, *meso*-erythritol, D-erythrose, raffinose, D-(+)-melibiose, tagatose, D-lyxose, or L-sorbose.

Antibiotic, lysostaphin, and lysozyme susceptibilities: Slightly resistant to penicillin G (MIC 1.6 μ g/ml), novobiocin (MIC 12.5 μ g/ml), streptomycin (MIC 25 μ g/ml), and tetracycline (MIC 6.2 μ g/ml); susceptible to erythromycin (MIC 0.8 μ g/ml); slightly resistant to lysostaphin (MIC 400 μ g/ml); resistant to lysozyme.

Cell wall peptidoglycan: L-Lys-L-Ala-Gly₄.

Cell wall teichoic acid: Glycerol and glucosamine.

Lactic acid configuration: L-lactic acid.

G+C content of DNA: 36.5 mol%.

Description of the type strain of *S. sciuri* subsp. *lentus*. Strain ATCC 29070 (originally designated K21), isolated from the udder of a goat, is the type strain of *S. sciuri* subsp. *lentus*. A description of this strain follows.

Cells: Spheres, 0.9 to 1.2 μ m in diameter, occurring in tetrads and occasionally in pairs. Nonmotile and nonsporeforming. Gram positive.

Agar colonies: Circular, 2.8 to 3.0 mm in diameter, entire, convex, smooth, and glistening wet surface. Opaque white.

Catalase and benzidine tests are positive.

Chemoorganotroph; metabolism is respiratory.

Aerobic with very slight facultative anaerobic capability.

Growth at extreme temperatures: No growth at 15 C or 45 C.

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

Coagulases are not produced.

Phosphatase activity is weak.

Nitrates are reduced.

Hemolysins: No hemolysis of bovine blood.

Acetylmethylcarbinol is not produced.

DNase activity is moderate.

Proteolytic activity: Caseinolytic and gelatinase activities are moderate.

Acid produced aerobically from D-(+)-glucose, β -D(-)-fructose, D-(+)-galactose, D(-)-ribose, sucrose, D-(+)-cellobiose, D-mannitol, α -lactose, glycerol, D-(+)-trehalose, salicin, D-sorbitol, β -gentiobiose, and raffinose; a small amount of acid is produced from L-(+)-arabinose, D-(+)-mannose, and D(-)-fucose. No acid from maltose, D-(+)-xylose, D-(+)-turanose, D-(+)-melezitose, xylitol, L-sorbose, D-arabitol, D-(+)-melibiose, L-(+)-rhamnose, *meso*-inositol, adonitol, dulcitol, D-arabitol, *meso*-erythritol, D-erythrose, tagatose, D-lyxose, or L-sorbose.

Antibiotic, lysostaphin, and lysozyme susceptibilities: Slightly resistant to novobiocin (MIC 1.6 μ g/ml); susceptible to penicillin G (MIC 0.2 μ g/ml), streptomycin (MIC 0.8 μ g/ml), tetracycline (MIC 0.4 μ g/ml), and erythromycin (MIC 1.6 μ g/ml); susceptible to lysostaphin (MIC 25 μ g/ml); resistant to lysozyme.

Cell wall peptidoglycan: L-Lys-L-Ala-Gly₄.

Cell wall teichoic acid: Glycerol and glucosamine.

Lactic acid configuration: L-lactic acid.

G+C content of DNA: 34.2 mol%.

Summary of character variation in *S. sciuri* and other novobiocin-resistant species. Summary data of the morphological, physiological, biochemical, and antibiotic characters found in *S. sciuri* and other presumably related novobiocin-resistant *Staphylococcus* species, namely *S. xylosus*, *S. cohnii*, and *S. saprophyticus*, are given in the following tables: colony morphology and pigment and cell arrangements, Table 3; anaerobic growth in thioglycolate, growth on 10% and 15% NaCl agar, and growth at 15 and 45 C, Table 4; hemolysin activity, acetylmethylcarbinol production, nitrate reduction, phosphatase, DNase, and bacteriolytic activities, Table 5; carbohydrate reactions, Tables 6, 7 and 8; lysostaphin and antibiotic susceptibilities, Table 9; proteolytic and lipolytic activities, Table 10. The summary data on strains of *S. xylosus*, *S. cohnii*, and *S. saprophyticus* isolated from human skin have been published previously (4, 12). They are used here for making comparisons with similar species isolated from animal skin and with *S. sciuri*, a species isolated predominantly from animals.

As can be seen from the data on various characters, *S. xylosus* and *S. sciuri* strains isolated from animals had character parameters similar to strains isolated from humans. However, some question arises as to the original

TABLE 3. Morphological characters of novobiocin-resistant *Staphylococcus* species

Novobiocin-resistant species	Source	Total no. of strains studied	Colony diam ^a (mm)	Colony pigment ^b						Colony profile ^b			Colony edge ^b		Cell arrangement ^b				
				Yellow-orange	Yellowish	Gray or gray-white	White with yellowish tint	White with yellowish tint	Gray to gray-white	White	Raised	Low convex	Convex to slight umbonate	Entire	Undulate to crenate	Tetrads, <10% pairs and singles	Tetrads, 10-50% pairs and singles	10-<50% tetrads, pairs and singles	Pairs and singles, <10% tetrads
<i>S. sciuri</i> subsp. <i>lentus</i> <i>S. sciuri</i> subsp. <i>sciuri</i>	Animals	9	2.8 ± 0.1		10	87			33	67	100 ^c		100	33	67	33	56	11	37
	Animals	30	8.4 ± 0.2		40	40			20	3	100			3	97 ^d		3	60	37
	Humans	5	8.4 ± 0.4		40	40						100			40	60		60	40
<i>S. xylosus</i>	Animals	64	7.3 ± 0.2	25	17	26	3		20	9	56	22	22	52	48			29	71
	Humans	98	7.0 ± 0.1	24	16	35	5		12	8	71	17	12	68	32			12	88
<i>S. cohnii</i>	Animals	20	7.6 ± 0.1	10			15		35	40		25	75	100				15	85
	Humans	42	5.9 ± 0.2			5	21		21	53	7	10	83	95	5			7	93
<i>S. saprophyticus</i>	Humans	83	6.5 ± 0.1	6	10	26	18		11	29	20	80		98	2			7	93

^a $\bar{x}$ and s_x .

^b Numbers indicate percentages of strains possessing the character indicated.

^c Colony profile of *S. sciuri* is raised and with age develops an elevated center. Most strains produce colonies that develop pinpoint depressions near periphery within 5 to 7 days.

^d Colony edge of most *S. sciuri* strains is slightly to moderately undulate.

TABLE 4. *Physiological characters of novobiocin-resistant Staphylococcus species*

Novobiocin-resistant species	Source	Total no. of strains studied	Anaerobic growth in thioglycolate semisolid medium ^a					Growth on: ^a				Growth at: ^a			
			Uniformly moderate or dense	Gradient: moderate to light	Scattered colonies >50	Scattered colonies <50	None	10% NaCl agar		15% NaCl agar		15 C		45 C	
								+	±	+	±	+	±	+	±
<i>S. sciuri</i> subsp. <i>lentus</i>	Animals	9	11		3		89	89	11						100
<i>S. sciuri</i> subsp. <i>sciuri</i>	Animals	30	20	67			10	100				33	67	7	66
	Humans	5	20	80				100						40	40
<i>S. xylosus</i>	Animals	64	67	10	14	3	6	ND ^c				100		ND	20
	Humans	98	70	8	14	4	4	100				ND		19	62
<i>S. cohnii</i>	Animals	20	50	45	5	7	5	ND				ND		ND	7
	Humans	42	24	47	17	1	1	100						76	17
<i>S. saprophyticus</i>	Humans	83	59	30	9	1	1	100						38	26

^a Numbers indicate percentages of strains possessing the character indicated.^b Growth: +, good; ±, weak; -, none.^c ND, Character not determined.

TABLE 5. Biochemical characters of novobiocin-resistant *Staphylococcus* species

Novobiocin-resistant species	Source	Total no. of strains studied	Hemolysis ^a		Acetyl methyl-carbinol production ^a			Nitrate reduction ^a		Phosphatase ^a		Deoxyribonuclease ^a		Bacteriolytic activity ^a	
			+	±	+	±	-	+	±	+	±	+	±	+	±
<i>S. sciuri</i> subsp. <i>lentus</i>	Animals	9						100				56	44		
<i>S. sciuri</i> subsp. <i>sciuri</i>	Animals	30						100		78	22	67	30	7	30
	Humans	5						100		80	20	60	40	40	60
<i>S. xylosus</i>	Animals	64	2	6	ND ^c			88	6	62	26	6	12	ND	82
	Humans	98	5	10	85	22		83	17	70	10	20	21	79	14
<i>S. cohnii</i>	Animals	20	20	25	55	ND		5	95	5	10	85	5	20	75
	Humans	42	14	31	55	41		3	94	17	83	21	79	14	62
<i>S. saprophyticus</i>	Humans	83	3	3	97	77		5	92	2	14	84	21	79	100

^a Numbers indicate percentages of strains giving the reaction indicated.^b Reactions: +, positive; ±, weak; -, negative.^c ND, Character not determined.TABLE 6. Carbohydrate reactions of novobiocin-resistant *Staphylococcus* species: monosaccharides under aerobic conditions

Novobiocin-resistant species	Source	Total no. of strains studied	β-D-(-)-Fructose ^a		D-(+)-Galactose ^a			D-(+)-Mannose ^a			D-(+)-Fucose ^a		L-(-)-Rhamnose ^a		D-(-)-Xylose ^a		L-(+)-Arabinose ^a		D-(-)-Ribose ^a	
			++	+	+	±	-	++	+	±	+	±	+	±	+	±	++	+	++	+
<i>S. sciuri</i> subsp. <i>lentus</i>	Animals	9	100					67	11	22			33	67	11	22	67	11	33	100
<i>S. sciuri</i> subsp. <i>sciuri</i>	Animals	30	80	20				13	30	57	100		23	77		3	97	27	7	66
	Humans	5	80	20				40	60	100			20	80		20	80	40	20	100
<i>S. xylosus</i>	Animals	64	79	21			8	96	4				ND ^c	80		70	10	20	83	5
	Humans	98	95	5			30	10	95	5			100	18		86	10	4	69	8
<i>S. cohnii</i>	Animals	20	95	5			50	30	20	95			100	2			100	100	100	5
	Humans	42	100				10	90	51	24			100	2			100	100	100	100
<i>S. saprophyticus</i>	Humans	83	92	5	3		8	92		100			100				100	100	100	5

^a Numbers indicate percentages of strains giving reaction indicated.^b Reactions: ++, strong acid; +, moderate acid; ±, weak acid; -, negative.^c ND, Character not determined.

TABLE 7. Carbohydrate reactions of novobiocin-resistant *Staphylococcus* species: disaccharides under aerobic conditions

Novobiocin-resistant species	Source	Total no. of strains studied	Maltose ^a		α -Lactose ^a		Sucrose ^a		D-(+)-Trehalose ^a		D-(+)-Turannose ^a		D-(+)-Cellobiose ^a		β -Gentiobiose ^a	
			++, + ^b	±	++, +	±	++, +	±	++, +	±	+	±	+	±	++, +	±
<i>S. sciuri</i> subsp. <i>lentus</i>	Animals	9	56	11	33	78	22	100	89	11			100		100	
<i>S. sciuri</i> subsp. <i>sciuri</i>	Animals	30	7	63	30	3	10	87	83	7	10	7	93	100	57	33
	Humans	5	20	60	20			100	80	20			100		60	40
<i>S. xylosus</i>	Animals	64	87	6	7	64	5	31	90	3	7	24	52	100	37	6
	Humans	98	85	12	3	66	12	22	96	4	36	54	10	2	28	14
<i>S. cohnii</i>	Animals	20	100			75	5	20	5	100	5	95	95	100	100	100
	Humans	42	28	53	19	31	69	12	88	100	5	95	95	100	100	100
<i>S. saprophyticus</i>	Humans	83	98	2		85	15	100	94	6	92	8		100		100

^a Numbers indicate percentages of strains giving reaction indicated.^b Reactions: ++, strong acid; +, moderate acid; ±, weak acid; -, negative.TABLE 8. Carbohydrate reactions of novobiocin-resistant *Staphylococcus* species: trisaccharides and sugar alcohols under aerobic conditions

Novobiocin-resistant species	Source	Total no. of strains studied	Trisaccharides ^a				Sugar alcohols ^a											
			D-(+)-Melezitose		Raffinose		D-Mannitol		Xylitol		Salicin	D-Sorbitol		meso-Inositol				
			++ +, +	±	-	+, ±	-	++ +	±	+	-	++ +, +	++ +, +	-	++ +, +	±	-	
<i>S. sciuri</i> subsp. <i>lentus</i>	Animals	9																
<i>S. sciuri</i> subsp. <i>sciuri</i>	Animals	30	43	7	100	89	11	100						100	100	78	22	100
	Humans	5	40		50	(3) ^c	97	100						100	70	73	27	3
<i>S. xylosus</i>	Animals	64			60	100	100	100						100	80	20	100	100
	Humans	98			100	100	100	90	10	12	3	85	ND ^d	3	97	3	97	7
<i>S. cohnii</i>	Animals	20			100	100	100	89	6	5	18	82	3	97	11	89	3	3
	Humans	42			100	100	100	100		40	10	50		100	100	5	100	5
<i>S. saprophyticus</i>	Humans	83			100	100	100	62	26	12	17	21	62	100	100	2	98	100
					100		100	95	1	4	60	20		100	100		100	100

^a Numbers indicate percentages of strains giving reaction indicated.^b Reactions: ++, strong acid; +, moderate acid; ±, weak acid; -, negative.^c Strain produced small amount of acid within 24 h; acid became neutral by 72 h.^d ND, Character not determined.

TABLE 9. *Lysostaphin and antibiotic susceptibilities of novobiocin-resistant Staphylococcus species*

Novobiocin-resistant species	Source	Total no. of strains studied	Lysostaphin at a level of susceptibility of: ^a				Penicillin G at a level of susceptibility of: ^b				Novobiocin at a level of susceptibility of:		Erythromycin at a level of susceptibility of:				Tetracycline at a level of susceptibility of:				Lincomycin at a level of susceptibility of: ^c								
			12.5-25 (+)	50 (+)	100 (+)	200-400 (+, ±)	0.012-0.025	(14-12)	0.050-0.1	(11-9)	0.2-0.4	(8-6)	0.8-6.2	(5-3)	1.6-3.1	6.2-12.5	0.4-1.6	(8-3)	3.2-25	(2-1)		100-400 (0)	0.4-0.8	(12-8)	1.6-6.2	(7-5)	50-200	(2-0)	(5-2)
<i>S. sciuri</i> subsp. <i>lentus</i>	Animals	9	100 ^d						78	22				100				78	22			100							ND ^e
<i>S. sciuri</i> subsp. <i>sciuri</i>	Animals	30	7	20	13	67				27	73			23	77			84	16			37	53		10			100	
<i>S. xylosus</i>	Humans	5			20	80				40	60			20	80			100				80	20						
	Animals	64								28	72			48	52			46	20		34	39	27	34	18			94	
<i>S. cohnii</i>	Humans	98	28	70	2		2	26	47	25	25			32	68			62	26		12	80	2	2	50			100	
	Animals	20	5	95						100				5	95			75	5		20	35	15	2	2			95	
<i>S. saprophyticus</i>	Humans	42		100			2		91	7	7			12	88			62	17		21	84	14	4	3			100	
	Humans	83		28	72				95	5	5			8	91			82	7		11	93	4					10	

^a Levels of susceptibility expressed as MIC (numbers) and spot inhibition (symbols in parentheses). MICs are expressed as micrograms per milliliter. Spot inhibition is expressed as: +, complete inhibition; ±, partial inhibition.

^b Levels of susceptibility expressed as MIC (numbers) and disk zone inhibition (numbers in parentheses). MICs are expressed as micrograms per milliliter (except not determined for lincomycin). Disk zone inhibition represents the width (in millimeters) of the zone of inhibition, measured from the edge of a 2-U penicillin G, 5-μg novobiocin, 2-μg erythromycin, 30-μg tetracycline, and 2-μg lincomycin sensitivity disk (Difco), respectively.

^c Lincomycin susceptibility was tested with only 20 strains of each species.

^d Data are given as percentages of strains.

^e ND, Character not determined.

TABLE 10. *Proteolytic and lipolytic activities of novobiocin-resistant Staphylococcus species*

Novobiocin-resistant species	Source	Total no. of strains studied	Proteolytic activity on: ^a				Lipolytic activity on: ^a				
			Casein		Gelatin		Triolein	Tributyrin	Lecithin	Tween 80	
			++, + ^b	±	+	++					
<i>S. sciuri</i> subsp. <i>lentus</i>	Animals	9	100								
<i>S. sciuri</i> subsp. <i>sciuri</i>	Animals	29	100				ND ^c	ND	ND	7	ND
	Humans	2	100				100	100	100	93	100
<i>S. xylosus</i>	Animals	42		2	84	40	60 ^d	43	100	29	71
	Humans	40	98	14	85	30	65	70	96	42	50
<i>S. cohnii</i>	Animals	13	8	15	100	100	5	69	2	85	8
	Humans	16	100				81	13	15	92	31
<i>S. saprophyticus</i>	Humans	23	6	13	87	19	35	65	8	69	57
			43	57	26	48	17	17	8	30	13

^a Numbers indicate percentages of strains giving reaction indicated.^b Lytic activity: ++, strong; +, moderate; ±, weak; -, negative.^c ND, Not determined.^d One-third of the strains of *S. xylosus* produced poor growth or failed to grow on the triolein-supplemented medium.TABLE 11. *Simplified scheme for distinguishing Staphylococcus sciuri and its subspecies from other novobiocin-resistant Staphylococcus species^a*

<i>Staphylococcus</i> species ^b	Colony diam (mm)	Colony pigment	Nitrate reduction	Acid (aerobically) from:									Peptidoglycan (mol/mol of glutamic acid)			
				D-(+)-Fructose	D-(-)-Ribose	α-Lactose	Sucrose	D-(-)-Cellobiose	D-(-)-Turannose	D-(-)-Meleziotose	Raffinose	Salicin	Proteolytic activity on casein	Serine	Glycine	Addi- tional alanine ^c
<i>S. sciuri</i> subsp. <i>lentus</i>	2-3	-	+	(±, +)	+	(+)	+	+	-	+	+	+	+	0	3.7-4.1	≈1.0
<i>S. sciuri</i> subsp. <i>sciuri</i>	7-10	+	+	+	(+)	(-)	+	+	-	+	+	(+)	+	0-0.4	3.6-4.2	≈1.0
<i>S. xylosus</i>	5-10	(+)	(+)	-	V	V	+	+	V	+	-	-	-	0-0.1	4.9-5.7	0
<i>S. cohnii</i>	5-8	(-)	-	-	-	V	-	+	-	-	-	-	-	0-0.2	4.9-6.0	0
<i>S. saprophyticus</i>	5-8	V	-	-	-	(+)	+	+	+	+	-	-	±, -	0.6-0.8	4.1-5.1	0

^a Characters were selected based on their ease of distinction and wide differences (≥70%) in their type frequencies between two or more species. A single listed symbol denotes a type frequency of 90 to 100%; parentheses around symbols denote a type frequency of 70 to 89%; two symbols are listed for a character when either type is in frequency below 70% but together equal 80 to 100%. Symbols for characters (unless noted otherwise): +, positive; ±, weak; -, negative; V, variable (+, ±, and -).^b Schemes for differentiating coagulase-positive and coagulase-negative species other than those listed here are given in references 4 and 5.^c Present in the interpeptide bridge.

source of *S. xylosus* and *S. sciuri* strains isolated from humans. Humans from whom these species were isolated had several pets or had frequent contact with farm animals, whose staphylococci may have contaminated their skin.

S. cohnii strains isolated from animals had several noteworthy differences in character parameters compared to strains isolated from humans, particularly with respect to colony size, certain carbohydrate reactions, lipolytic activity, and antibiotic susceptibilities. These differences might suggest a significant divergence between strains of *S. cohnii* living on animals and those living on humans.

A simplified scheme distinguishing *S. sciuri* and its subspecies from other novobiocin-resistant *Staphylococcus* species is shown in Table 11.

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REPRINT REQUESTS

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