Staphylococcus hyicus (Sompolinsky 1953) comb. nov. and *Staphylococcus hyicus* subsp. *chromogenes* subsp. nov.

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Although the name *Staphylococcus hyicus* has appeared in the literature, it has never been validly published. Supportive evidence is given for the transfer of *Micrococcus hyicus* Sompolinsky to the genus *Staphylococcus*, and the name *Staphylococcus hyicus* (Sompolinsky) comb. nov. is proposed. A description of this organism is given on the basis of a taxonomic analysis of 168 strains of this species isolated from swine with infectious epidermitis, from the skin and nares of healthy pigs and poultry, and from cows with mastitis. One hundred thirty-two strains which produced unpigmented colonies and a heat-stable nuclease were placed in the type subspecies *S. hyicus* subsp. *hyicus* subsp. nov. Thirty-six pigmented strains were placed in the subspecies *S. hyicus* subsp. *chromogenes* subsp. nov. Strain NCTC 10350 (= CCM 2368 = ATCC 11249 = D. Sompolinsky no. 1) is here designated the type strain of *S. hyicus* and of *S. hyicus* subsp. *hyicus*, and strain NCTC 10530 (= A. C. Baird Parker CBCC 1462) is designated the type strain of *S. hyicus* subsp. *chromogenes*. A scheme for the differentiation of *S. hyicus* from certain other *Staphylococcus* species is included.

In 1950, Sompolinsky described a gram-positive coccus isolated from pigs with exudative epidermitis, and he succeeded in reproducing the disease by inoculating cultures of this organism into apparently healthy pigs (25). He named his strains *Micrococcus hyicus* and gave a description of their most prominent characters (26). After a later redefinition of the genera *Staphylococcus* and *Micrococcus* (10), it was realized that these bacteria were not members of the genus *Micrococcus*. Baird-Parker (3) examined one strain of *M. hyicus* and found that its characteristics were identical to those of his *Staphylococcus* subgroup III. However, he did not make a formal proposal of a new combination, and he continued to use the name *M. hyicus* in his paper (3). The name *Staphylococcus hyicus* came into general use in veterinary publications but apparently was never formally proposed. The 1974 edition of *Bergey's Manual* (4) lists *M. hyicus* as belonging to *S. epidermidis* biotype 2. One of the objectives of the present paper is to effect the valid publication of the name *Staphylococcus hyicus*.

The above-mentioned situation was further complicated by the irregular, often negative, but sometimes delayed positive, results which may be obtained with *M. hyicus* strains in tube coagulase tests with different kinds of plasma (1, 9, 27, 28). Furthermore, the strains produce a heat-resistant nuclease, a characteristic gener-

ally accepted as a unique feature of *S. aureus* (4, 19). Strains isolated from bovine mastitis and poultry skin which have been referred to as coagulase-positive members of *S. epidermidis* subgroup 2 (5) and *S. aureus* avian biotype 2 (21), respectively, were found to be indistinguishable from *M. hyicus* strains from pigs (9). The poultry and swine strains occur frequently as skin inhabitants (8, 9, 21).

These findings, coupled with the fact that a new and more restrictive description has now been given to *S. epidermidis* (23), led us to reexamine the taxonomic position of *M. hyicus*.

MATERIALS AND METHODS

Bacterial strains. The designations, origins, and references containing the first descriptions of the strains studied are listed in Table 1. With few exceptions, the strains were obtained from different farms. The type strains of various *Staphylococcus* species obtained from the Czechoslovak Collection of Microorganisms (CCM), J. E. Purkyne University, Brno, were included in the test series for comparative purposes.

Methods. Cell and colony characteristics; the production of catalase, clumping factor, heat-stable and heat-labile nucleases, hyaluronidase, and phosphatase; hemolysis; hippurate hydrolysis; nitrate reduction; hydrolysis of polysorbates and gelatin; anaerobic growth in thioglycolate medium; and final pH in a fluid glucose medium were determined as described previously (9).

Twenty strains from different sources were examined for motility by the hanging-drop method.

The following tests were also carried out as previously described (11): oxidase production; benzidine test; coagulase activity on rabbit plasma; growth on Simmons citrate medium; production of arginine dihydrolase, caseinase, hydrogen sulfide, indole, phenylalanine deaminase, and fibrinolysin; hydrolysis of esculin; reduction of tellurite; and susceptibility to phages used for typing staphylococci from human and bovine sources.

Minimal inhibitory concentrations (MICs) of various antibiotics, lysostaphin (Schwarz/Mann; lot no. AZ-1975; specific activity, 237 U/mg), lysozyme from egg white (Sigma Chemical Co., grade I), and NaCl were determined by the agar dilution technique (29), using overnight broth cultures diluted 100-fold as inocula and tryptone soy agar (Oxoid Ltd.) as the test medium. This medium was substituted by Wellcotest medium (Wellcome Research Laboratories) when testing for susceptibility to sulfamethoxazole and trimethoprim. Penicillinase activity was determined by the agar diffusion test (30).

Acid production from sugars and sugar alcohols was determined on peptone-free ASS medium (24) modified to contain 0.5% yeast extract (Difco Laboratories). Negative cultures were retested in phenol red broth (Difco). The tests were observed for 3 days on ASS plate medium and for 5 days on phenol red broth. All sugars were used at a 1% concentration, and any evidence of pH decline was recorded as a positive reaction. Acetoin production was determined by the method of Davis and Hoyle (6). Lipolytic activity was determined on tributyrin agar (Oxoid) after 2 days of incubation at 37°C. Urease production was tested for on Christensen urea agar (Oxoid) with incubation for 14 days.

Serological determination of teichoic acid and protein A was performed by an agar precipitation technique previously described (9). In addition to the reference systems used by Devriese and Oeding (9), the strains were tested against a specific system consisting of strain VA308 and the homologous rabbit antiserum. Cell wall peptidoglycan type and configuration of lactic acid were determined as described by Schleifer and Kocur (22).

The guanine-plus-cytosine content of the deoxyribonucleic acid (DNA) of strain CBCC 1462 was determined by the method of Meyer and Schleifer (20). The DNA-DNA hybridization technique with nitrocellulose filters was used to determine genetic relatedness. Nitrocellulose filters (Sartorius, diameter, 50 mm; pore size, 0.2 μ m) were loaded by the procedure of Gillespie and Spiegelmann (12) with unlabeled, alkali-denatured DNA. Small disks (diameter, 5 mm) were punched from the larger filters and were preincubated by the procedure of Denhardt (7). [3 H]thymidine-labeled DNA was sheared with a French press at 20,000 lb/in² and dissolved in reassociation buffer (0.6 M NaCl-0.06 M trisodium citrate [pH 7.0] containing 20% formamide) to a final concentration of 0.4 μ g/200 μ l and heat denatured. Two hundred microliters of labeled DNA was added to the preincubated filters, and the reaction was allowed to proceed for 30 h at 60°C. Between 50 and 60% of the added labeled DNA

bound to the filter in the homologous reaction. This was considered to represent 100% homology, and all heterologous reactions were normalized accordingly. DNA homology values, therefore, refer to the relative binding capacity expressed as the ratio (given as a percentage) of the amount of labeled DNA which reassociated with filter-bound DNA of various reference strains (heterologous reaction) to the amount of labeled DNA which reassociated with the filter-bound DNA from the same strain from which the labeled DNA was obtained (homologous reaction). Allowing DNA-DNA hybridization to proceed at 25°C below the melting point of the DNA in a particular buffer is considered to represent the optimal reassociation condition. Higher reaction specificity can be obtained by decreasing the temperature span between the DNA melting point and the temperature of reassociation so that the formation of stable hybrids between the labeled DNA and the filter-bound DNA is restricted to more closely matching base sequences. By employing formamide and using an incubation temperature of 60°C, we achieved restrictive reassociation conditions about 18°C below the melting point of the DNA. A detailed description of the DNA hybridization procedure used in this study is given by Meyer and Schleifer (Arch. Microbiol., in press).

RESULTS

The strains under investigation clustered into two groups. One hundred and thirty-two strains, which were very similar to strain NCTC 10350, one of the strains originally isolated and named *M. hyicus* by Sompolinsky (26), were placed in group A (Table 1). Thirty-six other strains isolated from cows with subclinical mastitis and from the intact skin of pigs (Table 1) were placed in group B. Their characters were similar to those of strain NCTC 10530 originally isolated by Baird-Parker (2) from the skin of a pig and subsequently placed in *Staphylococcus epidermidis* biotype 2 (4).

Characterization of group A strains. Cells of group A strains were nonsporeforming, nonmotile, gram-positive cocci, 0.6 to 1.3 μ m in diameter (predominantly 0.8 to 1.0 μ m), arranged in pairs, tetrads, and small groups; occasionally they occurred singly.

Colonies were circular, entire, 3 to 5 mm in diameter, low convex, and opaque with a glistening surface. Pigments were not produced. In liquid media, uniform turbidity with a deposit was seen. The strains did not grow or grew weakly in nutrient broth (Oxoid) at 45°C and showed delayed growth in static nutrient broth cultures incubated at 22°C.

Tests in which all strains reacted uniformly positively or negatively are listed in Table 2, which contains results obtained with strains belonging to both groups described in this paper. All group A strains were also positive in one

TABLE 1. *Strains investigated*

Laboratory no.	Origins			Refer- ence(s)
	Animal species	Country	Source ^a	
Group A strains				
VA302, VA303, VA305-VA308, VA310, VA317, VA319, VA323, VA325-VA330, VA332, VA333, VA341-VA344, VA346-VA351	Swine (lesions)	Belgium	1	8, 9
VA353-VA356, VA358-VA362, VA501-VA505, VA507-VA511, VA513-VA539, VA541-VA551	Swine (healthy)	Belgium	1	8, 9
SH1, SH3-SH9	Swine (lesions)	Czechoslovakia	2	
2869c/70, 5170/73	Swine (lesions)	Germany	3	1
H310, H343	Swine (lesions)	England	4	15
NCTC 10350 = CCM 2368	Swine (lesions)	Denmark	5	26
A4, A252, A273, A313, A332, A355, A387, 201-1	Cows (lesions)	United States	6	5
VII26, VII27, VII28, VII52, VII53, VII63, VII76, VII96, VII99, VII100, VII108, VII111	Poultry (healthy)	Belgium	1	9
K13, K19, K20, K24, K46, K57, K127, K129, K130	Poultry (healthy)	Rwanda	7	9
J12, J13, J14, J16, J17	Poultry (healthy)	Japan	8	9, 21
Group B strains				
CBCC 1462 = NCTC 10530		England	9	2
VA102, VA103, VA104, VA110, VA111, VA401, VA404, VA406, VA608, VA647, VA650, VA652, VA654, VA656, VA666, VA669, VA672	Swine (healthy)	Belgium	1	
Bo4, Bo14, Bo18, Bo43, Bo49, Bo52, Bo59, Bo64, Bo66, Bo81, Bo83, Bo90, Bo91, Bo93, Bo98, Bo129, Bo153, Bo157	Cows (lesions)	Belgium	1	

^a 1, L. A. Devriese, Gent, Belgium; 2, V. Hájek, Olomouc, C.B.S.R.; 3, G. Amtsberg, Hannover, B.R.D.; 4, D. Hunter, Leeds, England; 5, National Collection of Type Cultures, London, England and Czechoslovak Collection of Microorganisms, Purkyně University, Brno, C.S.S.R.; 6, R. W. Brown, Ames, Iowa; 7, L. Van Damme, Butare, Rwanda; 8, G. Sato, Sapporo, Japan; 9, A. C. Baird-Parker, Sharnbrook, Bedford, England.

TABLE 2. *Characteristics of S. hyicus: tests for which all strains reacted uniformly*

Positive reaction	Negative reaction
Anaerobic growth ^a	Motility
Benzidine test	Growth on medium with 15% NaCl
Catalase test	Growth on Simmons citrate agar
Clearing on milk agar	Oxidase production
Gelatinase production	Clumping-factor production
Nitrate reduction beyond nitrite	Hemolysin α , β , or δ
Phosphatase production	Hydrogen sulfide production
Acid from:	Indole production
Sucrose	Phenylalanine-deaminase production
Mannose	Tellurite reduction
	Acetylmethylcarbinol production
	Hydrolysis of esculin
	Acid from: Adonitol
	Amygdaline
	L-(+)-Arabinose
	Arabitol
	Cellobiose
	Dulcitol
	L-(-)-Fucose
	β -Gentiobiose
	Lyxose
	Melibiose
	Melezitose
	Raffinose
	L-(+)-Rhamnose
	Salicin
	Sorbitol
	Sorbose
	Tagatose
	Xylitol
	Xylose

^a Tested in thioglycolate broth as described by Evans and Kloos (11).

further test: the heat-stable deoxyribonuclease reaction. In some tests, a great majority of the group A strains reacted uniformly, but a few exceptions were noted (Table 3). In a few other tests, the strains reacted differently depending upon the nature and geographic origin of the host species (Table 4).

Thirty-eight selected strains were found to produce predominantly L-lactic acid from glucose under anaerobic conditions. The pH in peptone-yeast extract-glucose broth (10) was lowered from 6.8–7.1 to 4.8–5.3 after 5 days of anaerobic incubation.

The strains were uniformly resistant to lysozyme (MIC, >800 µg/ml). They were susceptible to novobiocin (MIC, 0.05 to 0.1 µg/ml; with one exception: strain VA346), lysostaphin (MIC, 12.5 to 25 µg/ml), cloxacillin (MIC, 0.1 to 0.2 µg/ml),

cephaloridine (MIC, 0.025 to 0.1 µg/ml), bacitracin (MIC, 0.01 to 0.8 U/ml), neomycin (MIC, 0.1 to 0.4 µg/ml; with two exceptions), rifamycin (MIC, 0.006 to 0.025 µg/ml), furazolidone (MIC, 0.8 to 1.6 µg/ml), trimethoprim (MIC, 0.8 to 1.6 µg/ml), sulfamethoxazole (MIC, 25 to 50 µg/ml), and penicillin G (MIC, 0.025 to 0.05 µg/ml). Some strains, however, possessed penicillinase activity, and their penicillin G MIC levels were not regularly reproducible. Levels of susceptibility to erythromycin, lincomycin, streptomycin, sulfamethoxazole, tetracycline hydrochloride, and chloramphenicol were bimodally distributed. This was probably caused by resistance determinants. The polymyxin B sulfate MICs were 100 to 200 µg/ml.

None of the strains was susceptible to phages of the human and bovine *S. aureus* typing sets.

TABLE 3. Characteristics of *S. hyicus* subsp. *hyicus* (group A): tests in which there were strain differences independent of the origin of the strains

Characteristic	No. of strains positive/total no. of strains	Reaction of type strain (NCTC 10350)	Reference numbers of strains that gave the less common result
Hydrolysis of hippurate	130/132	+	VA341, VA348
Hydrolysis of arginine	131/132	+	A4
Hydrolysis of Tweens	130/132	+	VA323, K130
Production of hyaluronidase	128/132	+	VII26, VII27, VII76, VA548
Acid from ribose	131/132	+	A4
Acid from glycerol	130/132	–	NCTC 10530, VA350
Acid from fructose	130/132	+	A4, VII99

TABLE 4. Characters of *S. hyicus* subsp. *hyicus* (group A) which vary according to the nature and geographic localization of the host species

Characteristic	No. positive strains/no. strains tested				
	Pigs	Poultry			Cows
	Europe	Europe	Africa	Japan	America
Tube coagulase (rabbit plasma)					
Clotting after 1 h	3/98	0/12	0/9	1/5	0/8
3 h	3/98	0/12	4/9	4/5	0/8
24 h	12/98 ^a	2/12 ^a	5/9 ^a	5/5	8/8
Trehalose reaction	97/98 ^b	9/12 ^b	9/9	5/5	2/8 ^b
Lactose/galactose reactions	97/98 ^c	8/12 ^c	4/9 ^c	5/5	8/8
Urease	97/98 ^d	12/12	3/9 ^d	5/5	8/8
Fibrinolysin production	54/98 ^e	0/12	0/9	0/5	0/8
Polysaccharide C in cell wall	25/95 ^f	7/12 ^f	8/8	4/4	6/8 ^f

^a The following strains were positive after 24 h in rabbit plasma: K13, K19, K20, K127, K129, VII28, VII111, H310, VA305, VA306, VA307, VA350, VA359, VA510, VA521, VA528, VA541, VA542, VA547.

^b The following strains were negative: VA519, A252, A273, A313, A332, A355, A387, VII28, VII108, VII111.

^c The following were negative: K13, K19, K20, K127, K129, VII52, VII96, VII99, VII101, VA354.

^d The following were negative: K13, K19, K20, K57, K127, K129, NCTC 10350.

^e The following strains were positive: NCTC 10350, H310, H343, SH1, SH3–SH9, VA306, VA307, VA310, VA326–VA329, VA333, 2869c/70, 5170/73, VA341, VA344, VA348, VA349, VA351, VA353, VA354, VA356, VA361, VA501, VA502, VA599–VA511, VA514, VA515, VA518–VA520, VA522–VA525, VA529, VA530, VA532, VA533, VA535, VA538, VA541, VA542, VA551.

^f The following strains were positive: VA302, VA310, VA323, VA327, VA328, VA332, VA333, VA341, VA342, VA348, VA350, VA351, VA354, VA355, VA358, VA530, VA535, VA536, VA537, VA539, VA546, VA547, VA549, VA551, SH4.

^g The following strains were negative: VII52, VII63, VII96, VII99, VII100, A4, 201-1.

All of the strains except five (2869c, H310, H343, K24, J14) were examined serologically with respect to their type of cell wall teichoic acid. All produced a precipitation line showing a reaction of identity with the VA308 reference line. The teichoic acid responsible for this line, provisionally designated A1 (9), has recently been identified as an α -*N*-acetylglucosaminyl glycerol teichoic acid (Osland, Grov, and Oeding, submitted for publication). Fifty strains contained β -*N*-acetylglucosaminyl glycerol teichoic acid (polysaccharide C) as well (Table 4). Strains VA310, VA333, A4, J12, and VII96 lacked L-serine in their cell wall peptidoglycan. The other strains examined (SH1, SH3-SH9, VA302, VA303, VA305-VA308, VA317, VA319, VA323, VA325-VA330, VA332, VA355-VA357, VA501-VA503, 2869c/70, 5170/73, NCTC 10350) contained only trace amounts of serine (0.1 to 0.3 mol of serine per mol of glutamic acid).

Characterization of group B strains. The cell morphology and characteristics of the group B strains were similar to those of the group A strains, except that the group B strains produced a yellow, orange, or creamy pigment.

In addition to the characteristics listed in Table 2, the following reactions were uniformly negative with the group B strains: tube coagulase with rabbit plasma, precipitation on media containing Tween 80 or Tween 85, and production of hyaluronidase and fibrinolysin. In the deoxyribonuclease plate tests, zones of clearing, after addition of HCl, were seen which were always smaller than the zones produced by group A strains. In the heat-stable-deoxyribonuclease test, most strains produced small reaction zones which were difficult to read. All group B strains produced zones of clearing on tributyrin agar which were narrower than the zones seen with group A strains.

All group B strains were positive in the hippurate, arginine dihydrolase, and urease tests. All produced acid from glycerol, ribose, fructose, trehalose, lactose, and galactose. Twenty-one strains (58%) were mannitol positive, 18 (50%) produced acid from maltose, and 16 (44%) produced acid from turanose. The reactions with mannitol, maltose, and turanose were only weakly positive and were most readily demonstrable in phenol red broth. When the fluid medium of Evans et al. (10) was used with mannitol replacing glucose, the pH was lowered from 6.8 to 4.7-6.5. In similar tests with maltose, pH values ranging from 5.1 to 6.7 were obtained. Other positive results are listed in Table 2.

The susceptibility levels of the group B strains to antimicrobial agents were similar to those described for group A strains, except that

slightly lower MICs were obtained with tetracycline hydrochloride and trimethoprim. The trimethoprim MICs were 0.1 to 0.4 μ g/ml with group B and 0.8 to 1.6 μ g/ml with group A strains. The tetracycline hydrochloride susceptibility levels were 0.1 to 0.2 μ g/ml for group B and 0.4 to 0.8 μ g/ml for group A strains. The tetracycline-resistant group B strains had MICs of 6.3 to 12.5 μ g/ml, whereas the tetracycline-resistant group A strains were inhibited at 25 to 100 μ g/ml.

The strains were not lysed by the phages used for typing human and bovine *S. aureus* strains.

All except two of the strains (Bo64, Bo83) were examined serologically with respect to their type of cell wall teichoic acid. All except one (VA404) produced the A1 precipitation line. Seventeen strains (CBCC1462, VA103, VA104, VA110, VA11, VA401, VA608, VA647, VA650, VA656, VA669, Bo14, Bo18, Bo81, Bo93, Bo129, Bo153) contained β -*N*-acetylglucosaminyl glycerol teichoic acid as well. Strain VA404 contained exclusively β -*N*-acetylglucosaminyl glycerol teichoic acid. The strains VA406 and Bo18 lacked L-serine in their cell wall peptidoglycan. The other strains studied (CBCC 1462, VA650, Bo4, Bo43, and Bo59) contained only trace amounts of serine (0.1 to 0.3 mol/mol of glutamic acid). Fifteen selected strains produced predominantly L-lactic acid from glucose under anaerobic growth conditions. The guanine-plus-cytosine content of the DNA of strain NCTC 10530 was 33.9 mol%.

DISCUSSION

The two groups of bacteria examined in this study differ from *Micrococcus* species in the following characters: they have a low guanine-plus-cytosine content in their DNA; they have a teichoic acid in their cell walls and a peptidoglycan different from that of micrococci; they are susceptible to lysostaphin; they have a low-convex colony profile; they grow anaerobically in thioglycolate medium; and they produce acid from glycerol.

One of the group A strains, viz. NCTC 10350 (= Sompolinsky strain 1), is one of the strains on which Sompolinsky based his original description of *Micrococcus hyicus* (26). This strain possesses the characters given in the original description of the species, and it is here designated the type strain of *M. hyicus*. Because the type strain of *M. hyicus*, together with the other group A and B strains, is here regarded as belonging to the genus *Staphylococcus* as a distinct species, the name of this species is *Staphylococcus hyicus* (Sompolinsky) comb. nov.

A description of this species follows: Non-

sporeforming, gram-positive cocci, predominantly 0.8 to 1.0 μm in diameter, arranged in pairs, tetrads, and small groups; occasionally cells occur singly. Colonies are circular, entire, 3 to 5 mm in diameter, low convex, and opaque with a glistening surface. Broth cultures are uniformly turbid with a deposit. Physiological characteristics common to all of the known strains of this species are listed in Table 2. Nearly all of the strains hydrolyze hippurate and arginine and produce acid from ribose, glycerol, and fructose. The strains produce predominantly L-lactic acid from glucose under anaerobic conditions. They are resistant to lysozyme and susceptible to lysostaphin and the commonly used antibiotics active against gram-positive bacteria. Their cell walls contain α -N-acetylglucosaminyl glycerol teichoic acid and lack L-serine or contain only trace amounts of this substance. The strains are not susceptible to the phages used for typing *S. aureus* strains from human and bovine sources.

Type strain: Strain D. Sompolsky 1 (= ATCC 11249 = CCM 2368 = NCTC 10350); from the skin of a pig affected with exudative epidermitis in Seeland, Denmark.

A DNA-DNA hybridization study of *S. hyicus* strains which differ in several characteristics from each other (Table 4; see Table 6) revealed that the strains could be subdivided into at least two groups, each composed of strains with homology values above 90% (strain VA 306 with NCTC 10350 and strain CBCC 1462 with Bo4 in Table 5). However, when members from these different groups were compared, lower hybridization rates ranging from 30 to 50% were de-

tected. Such low homology values indicate that these two groups are related to each other at a subspecies level. Group A and group B strains differ from each other in several easily recognizable characters (Table 6). Therefore, subspecies status is proposed here for these two groups. Group A contains the type strain of the species, and therefore the name of this subspecies is *S. hyicus* subsp. *hyicus*. For the other subspecies (group B), the name *S. hyicus* subsp. *chromogenes* subsp. nov. is proposed (chro.mó.ge.nes. Gr. n. *chroma* color; Gr. v. *gennaio* to produce; M.L. adj. *chromogenes* producing color). The characteristics which differentiate *S. hyicus* subsp. *hyicus* from *S. hyicus* subsp. *chromogenes* are given in Table 6.

The following characteristics are considered essential for membership in *S. hyicus* subsp. *hyicus*: presence of a heat-stable nuclease and absence of clumping-factor activity and hemolysins; presence of α -N-acetylglucosaminyl glycerol teichoic acid in the cell wall; ability to produce acid from mannose and sucrose but not from maltose, mannitol, xylose, arabinose, or cellobiose; reduction of nitrates; and absence of pigment production. For membership in *S. hyicus* subsp. *chromogenes*, the following features are essential: weak or absent heat-stable nuclease; absence of clumping-factor activity and hemolysins; presence of α -N-acetylglucosaminyl glycerol teichoic acid in the cell wall; ability to reduce nitrates and to produce acid from mannose, trehalose, and sucrose; and absence of activity on xylose, arabinose, and cellobiose. These characteristics, together with the diagnostic reactions which distinguish the two *S. hyicus* sub-

TABLE 5. DNA-DNA homology values of *S. hyicus* strains and other strains

DNA _a ^a from:	DNA _b ^b from:								
	A4	VA 306	NCTC 10350	CBCC 1462	Bo4	H11	ATCC 12600	ATCC 14990	AM63
<i>Staphylococcus hyicus</i> subsp. <i>hyicus</i>									
A4	100	48	49	35	30	17	15	14	8
VA306	48	100	93	32	32	10	10	10	2
NCTC 10350	52	95	100	45	40	19	14	17	3
<i>S. hyicus</i> subsp. <i>chromogenes</i>									
CBCC 1462	47	55	52	100	95	17	19	19	3
Bo4	33	40	36	92	100	17	9	13	3
<i>S. intermedius</i>									
H11	10	11	12	11	10	100	7	7	2
<i>S. aureus</i>									
ATCC 12600	12	11	10	10	11	10	100	16	6
<i>S. epidermidis</i>									
ATCC 14990	15	10	14	19	9	12	16	100	6
<i>Lactobacillus acidophilus</i>									
AM63	9	9	6	7	8	8	8	8	100

^a DNA_a, Labeled DNA in solution.

^b DNA_b, DNA immobilized on filter.

species from the most phenotypically related other *Staphylococcus* species (Table 7), can be used for the identification of *S. hyicus*. None of the other presently known *Staphylococcus* species (13, 16, 17, 23) possess the combination of characteristics described here for *S. hyicus*.

S. hyicus subsp. *hyicus* occurs commonly on the skin of poultry and pigs (8, 21) and is more rarely isolated from cows with mastitis (5). Bovine and poultry strains do not cause a visible effect when inoculated in colostrum-deprived pigs, whereas porcine strains are highly pathogenic (9). The poultry strains are probably not pathogenic for chickens (21).

S. hyicus subsp. *chromogenes* can be isolated from the skin of pigs and mastitic milk of cows, but its importance is unknown.

Description of the type strain of *S. hyicus* subsp. *hyicus*. (Strain D. Sompolinsky 1 = ATCC 11249 = CCM 2368 = NCTC 10350).

Cells are gram-positive spheres, 0.6 to 1.3 μ m in diameter, occurring in pairs, tetrads, and ir-

regular groups; occasionally they occur singly. Endospores are not produced. Nonmotile.

Agar colonies: Circular, entire, 3 to 5 mm in diameter, low convex, opaque with a glistening surface. No pigment.

Broth cultures: Uniformly turbid with a deposit.

The biochemical characters of this strain are listed in Tables 2 and 8.

Antibiotic susceptibilities: Resistant to lysozyme. Susceptible to novobiocin, lysostaphin, penicillins, bacitracin, neomycin, streptomycin, rifamycin, furazolidone, erythromycin, lincomycin, tetracycline, chloramphenicol, sulfamethoxazole, and trimethoprim.

Not susceptible to phages of the international *S. aureus* typing set or to those used for typing bovine *S. aureus* strains.

Cell wall composition: Peptidoglycan is of the L-Lys-Gly₅₋₆ type (22); trace amounts of glycan can be replaced by L-serine. Teichoic acid: α -N-acetylglucosaminyl glycerol teichoic acid.

Guanine-plus-cytosine content of the DNA: 32.9 mol% (18).

Pathogenic to pigs.

Description of the type strain of *S. hyicus* subsp. *chromogenes*. The strain originally isolated in 1963 by Baird-Parker from the skin of a pig and designated CBCC 1462 (= NCTC 10530) is the type strain of *S. hyicus* subsp. *chromogenes*. The characteristics of this strain are as follows.

Cells: Gram-positive spheres, 0.7 to 1.3 μ m in diameter, occurring in pairs, tetrads, and irregular groups; occasionally they occur singly. Non-sporeforming. Nonmotile.

Agar colonies: Circular, entire, 3 to 5 mm in diameter, low convex, and opaque with a glistening surface. Orange pigment.

TABLE 6. Characters useful in differentiating the subspecies of *S. hyicus*^a

Character	<i>S. hyicus</i> subsp. <i>hyicus</i> (group A)	<i>S. hyicus</i> subsp. <i>chromogenes</i> (group B)
Heat-stable nuclease	+	Slight or -
Hyaluronidase production	+	-
Pigment production	-	+
Tween 80 and 85 splitting	+	-
Acid from mannitol	-	D
Acid from maltose	-	D
Acid from turanose	-	D
Coagulase (rabbit plasma) production	D	-
Production of fibrinolysin	D	-

^a Reactions: +, >90% positive; -, >90% negative; D, 11 to 89% positive.

TABLE 7. Diagnostic features useful in distinguishing between the *S. hyicus* subspecies and related *Staphylococcus* species^a

Character	<i>S.</i> <i>hyicus</i> subsp. <i>hyicus</i>	<i>S. hyicus</i> subsp. <i>chromogenes</i>	<i>S.</i> <i>inter-</i> <i>medius</i>	<i>S.</i> <i>aureus</i>	<i>S. simulans</i>
Coagulase (rabbit plasma) production	D	-	+	+	-
Heat-stable nuclease production	+	Slight or -	+	+	-
Clumping-factor production	-	-	D	+	- ^b
Pigment production	-	+	-	+	Slight or -
Hemolysins α and/or β and/or δ	-	-	D	+	-
Hyaluronidase production	+	-	-	+	- ^b
Hydrolysis of Tween 80	+	-	+	D	+ ^b
Acid from mannitol	-	D	+	+	+
Phosphatase production	+	+	+	+	Weak or -

^a Based on references 13, 14, and 16. Reaction: +, >90% of strains positive; -, >90% of strains negative; D, 11 to 89% positive.

^b Based on unpublished results of tests with 2 strains only (CCM 2705 and CCM 2724).

TABLE 8. Biochemical characteristics of the type strains of *S. hyicus* subsp. *hyicus* and *S. hyicus* subsp. *chromogenes*

Characteristic	<i>S. hyicus</i> subsp. <i>hyicus</i> NCTC 10350	<i>S. hyicus</i> subsp. <i>chromogenes</i> NCTC 10530
Coagulase (rabbit plasma) production	—	—
Hemolysis on rabbit, cow, and sheep blood agar	—	—
Fibrinolysin production	+	—
Hippurate hydrolysis	+	+
Arginine dehydrolase	+	+
Hyaluronidase	+	—
Tweens 80 and 85 hydrolysis	+	—
Tributyrin hydrolysis	+	+
Heat-stable deoxyribonuclease production	+	Weak
Urease production	— ^a	+
L-Lactic acid from glucose anaerobically	+	+
Acid from: Ribose	+	+
Fructose	+	+
Lactose	+	+
Galactose	+	+
Trehalose	+	+
Glycerol	— ^a	+
Maltose	—	—
Mannitol	—	—
Turanose	—	—

^a Atypical character.

Broth cultures: Uniformly turbid with a deposit.

The biochemical characteristics of this strain are listed in Tables 2 and 8.

Antibiotic susceptibilities: Resistant to lysozyme. Susceptible to novobiocin, lysostaphin, penicillins, bacitracin, neomycin, streptomycin, rifamycin, furazolidone, erythromycin, lincomycin, tetracycline, chloramphenicol, sulfamethoxazole, and trimethoprim.

Not susceptible to the phages of the international *S. aureus* typing set or to those used for typing bovine *S. aureus* strains.

Cell wall composition: Peptidoglycan is of the L-Lys-Gly₅₋₆ type; trace amounts of glycan can be replaced by L-serine. Teichoic acid: α -N-acetylglucosaminyl glycerol teichoic acid and β -N-acetylglucosaminyl glycerol teichoic acid.

G+C content of the DNA: 33.9 mol%.

When *S. hyicus* strains were compared with reference strains of *S. aureus*, *S. intermedius*, or coagulase-negative organisms such as *S. epidermidis*, low DNA homology values (below 17%) were found (Table 5). Less than 8% homology resulted when *S. hyicus* was compared with a

phenotypically unrelated reference organism (*L. acidophilus*).

The DNA of group A strain A₄ is 30 to 52% homologous with the DNAs from other *S. hyicus* strains, an indication for another subspecies relationship. A₄ and the other strains isolated from cows in the United States all originated from one herd. Some of their phenotypic characters differed from those of the other *S. hyicus* subsp. *hyicus* strains, but none of the tests used in this study could clearly differentiate these strains (Table 4). Other *S. hyicus* strains from other animal hosts and other geographic regions also differed in several characteristics from each other (Table 4). It remains to be seen whether these host-adapted strains belong to different "biotypes" or formae speciales, analogous to the biotypes described in *S. aureus* (14) and *S. intermedius* (13, 14), or to different subspecies. Therefore, it is proposed that strain A₄ and similar strains from other origins should provisionally be placed in *S. hyicus* subsp. *hyicus*.

REPRINT REQUESTS

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