INTERNATIONAL JOURNAL OF SYSTEMATIC BACTERIOLOGY, Apr. 1982, p. 153–156 Vol. 32, No. 2
0020-7713/82/020153-04$02.00/0

Description of a New Species of the Genus *Staphylococcus*: *Staphylococcus carnosus*

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From dry sausage, a new species, *Staphylococcus carnosus*, has been isolated and described. The new species is established primarily on the basis of its deoxyribonucleic acid-deoxyribonucleic acid hybridization relationships, its biochemical reactions, and its cell wall composition. The type strain of this species is DSM 20501.

It has been known for a long time that micrococci take part in the fermentation of dry sausage (11–13). More recent studies have shown that many of these micrococci were incorrectly identified and are in fact staphylococci (4, 14). One group of these staphylococci was tentatively identified as belonging to *Staphylococcus simulans* because they closely resembled the type strain (ATCC 27848) of *S. simulans* (4). The present communication describes this group of "*S. simulans*" strains as a new species of *Staphylococcus*, namely, *S. carnosus*.

MATERIALS AND METHODS

Strains identified as members of "*S. simulans*" were isolated from dry sausage and from a starter culture used for the production of dry sausage (Fa. Müller, Giessen, F. R. G.) by plating samples on plate-count agar or on Schleifer and Krämer's (17) selective medium for staphylococci. The other strains included in this study are listed in Table 2. All organisms, with the exception of *Lactobacillus gasseri* DSM 20243, were cultivated aerobically in peptone-yeast extract-glucose-NaCl broth (7). The strain of *L. gasseri* was grown under microaerophilic conditions in MRS medium (1). The incubation temperature was 32°C. Carbohydrate and physiological reactions were determined by methods previously described (7, 16). The preparation of cell walls and the determination of the peptidoglycan type were performed as described by Schleifer and Kandler (15). Cell wall teichoic acids were extracted with 60% hydrofluoric acid. Their chemical compositions were determined by hydrolysis with 2 N HCl at 100°C for 3 h and subsequent gas chromatography of the *N,O*-trifluoracetylated products. The oxidase test was carried out as described by Faller and Schleifer (3). The class of fructose-1,6-biphosphate-aldolase was determined by the method of Götz et al. (5). The cytochrome pattern was elucidated as previously reported (2). The guanine-plus-cytosine (G+C) content of the deoxyribonucleic acid (DNA) was determined by the thermal denaturation method of Marmur and Doty (9). Radioactive labeling of DNA and the DNA-DNA hybridization experiments were also carried out as previously described (10, 18).

RESULTS AND DISCUSSION

The description of the new species, based on 14 strains from dry sausages and 1 strain (no. 61) from a starter culture for dry sausages, is as follows.

Description of *Staphylococcus carnosus* sp. nov. (car.nó sus. L. adj. *carnosus* pertaining to flesh).

Cocci, 0.5 to 1.5 μm in diameter, occurring predominantly in pairs and singly. Gram positive. Nonmotile. Nonsporeforming. Colonies were slightly raised, circular, smooth, slightly glistening, and usually gray-white and ranged from about 1 to 3 mm in diameter.

All strains grew facultatively anaerobically and produced, under anaerobic growth conditions, equal amounts of D- and L-lactate from glucose. The pH of a peptone-yeast extract-glucose broth was lowered from 6.8 to 4.2 after 2 days of growth under anaerobic conditions.

All strains grew well at 15 and 45°C and at NaCl concentrations up to 15%. All strains reduced nitrate and produced acetoin. All failed to produce coagulase and to exhibit hemolysin activity. All were negative for the oxidase test and resistant to 50 μg of lysozyme per ml. Their growth was inhibited by 200 μg of lysostaphin or 1.6 μg of novobiocin per ml.

All strains produced acid aerobically from glucose, fructose, mannose, mannitol, and glycerol. All failed to produce acid from xylose, sucrose, maltose, xylitol, rhamnose, turanose, raffinose, fucose, and melibiose.

Variable characters of the strains are listed in Table 1. The peptidoglycan types of the strains were studied, and all were determined to be L-Lys-Gly_{5-6}. This type of peptidoglycan was previously found in *S. aureus*, *S. cohnii*, *S. simulans*, and *S. xylosus* (6, 15). The G+C contents of the DNAs of five strains were determined and were found to vary between 35 and 36 mol% (Table 1).

DNA-DNA hybridization studies indicated

TABLE 1. Variable characters of 15 strains of *S. carnosus*

Strain no.	G+C content of DNA (mol %)	Acid (aerobically) from:			Anaerobic fermentation of glucose (pH) in peptone-yeast extract-glucose broth
		Galactose	Lactose	Trehalose	
51	ND[a]	+	−	−	4.3
61	36	+	−	+	4.4
91	35	−	+	+	4.5
151	ND	−	−	+	4.5
191	ND	−	−	+	4.3
201	ND	−	−	+	4.2
211	ND	−	−	+	4.4
221	ND	−	−	+	4.5
271	ND	+	+	+	4.7
301	36	−	−	−	4.8
311	35	+	+	+	4.7
341	ND	−	−	−	4.7
351	ND	−	−	−	4.6
361 (= DSM 20501)	36	+	−	+	4.6
841	ND	−	+	−	4.3

[a] Not determined.

that the strains of *S. carnosus* are closely related to one another at the species level (Table 2). The DNA homology values with other staphylococci, with the exception of *S. simulans*, were rather low (10 to 20%) but were significantly higher than the values between the staphylococci and an unrelated gram-positive bacterium of low G+C content, namely, *Lactobacillus gasseri* DSM 20243 (Table 2). The DNA homology values (32 to 39%) between the type strain of *S. simulans*, ATCC 27848, and the strains of *S. carnosus* are higher than those between ATCC 27848 and the other staphylococci. This indicates that *S. carnosus* and *S. simulans* are closely related to each other but not closely enough to justify their union in one species. In

TABLE 2. DNA-DNA hybridization relationships among *S. simulans* ATCC 27848, *S. carnosus* DSM 20501, and various other strains of staphylococci[a]

Source of filter-bound DNA	% relative binding of labeled DNA from	
	S. simulans ATCC 27848	*S. carnosus* DSM 20501 (no. 361)
Staphylococcus simulans ATCC 27848[b]	100	32
S. xylosus DSM 20266 (= ATCC 29971)[b]	15	17
S. saprophyticus CCM 883 (= ATCC 15305)[b]	13	12
S. cohnii DSM 20260 (= ATCC 29974)[b]	13	13
S. haemolyticus ATCC 20263[b]	16	17
S. hominis ATCC 27844[b]	18	18
S. epidermidis ATCC 14990[b]	20	20
S. capitis ATCC 27840[b]	16	15
S. warneri ATCC 27836[b]	10	12
S. sciuri ATCC 29062[b]	11	10
S. intermedius CCM 5739 (= ATCC 29663)[b]	13	14
S. aureus ATCC 12600[b]	17	14
S. carnosus no. 61	39	105
S. carnosus no. 301	37	103
S. carnosus no. 311	38	101
S. carnosus DSM 20501[c] (no. 361)	38	100
L. gasseri DSM 20243[d]	4	4

[a] The percentage of radioactivity bound in the heterologous reaction was normalized to the percentage of radioactivity bound in the homologous reaction and expressed as a homology value. Optimal reassociation conditions were employed corresponding to approximately 25°C below the thermal melting point of the DNA (0.45 M NaCl plus 0.045 M sodium citrate adjusted to 10% formamide, 58°C).

[b] Type strain (19).

[c] Type strain (designated in this paper).

[d] Type strain (8).

TABLE 3. DNA-DNA hybridization values obtained under stringent reassociation conditions[a]

Source of filter-bound DNA	% relative binding of labeled DNA from		
	S. simulans ATCC 27848	*S. carnosus* no. 301	*S. carnosus* DSM 20501 (= no. 361)
S. simulans ATCC 27848	100	22	20
S. saprophyticus CCM 883	10	10	10
S. epidermidis ATCC 14990	10	10	10
S. capitis ATCC 27840	10	10	10
S. carnosus no. 301	25	100	98
S. carnosus DSM 20501 (= no. 361)	28	100	100
L. gasseri DSM 20243	2	2	2

[a] These conditions occur at approximately 21°C below the thermal melting point of the DNA. For further explanations, see Table 2.

particular, DNA-DNA homology studies under stringent conditions (Table 3) clearly demonstrated that *S. simulans* and *S. carnosus* belong to separate species.

The main characters for the differentiation of *S. carnosus* from other staphylococci are listed in Table 4. *S. carnosus* can be distinguished from all other staphylococci primarily on the bases of its ability to produce acetoin, to reduce nitrate, and to grow on 15% NaCl and of its failure to produce acid from maltose and sucrose.

Strain DSM 20501 (originally designated no. 361) is the type of strain of *S. carnosus*. A description of this strain follows.

Spheres, about 1 μm in diameter, occurring predominantly in pairs and singly. Gram positive. Nonmotile and nonsporeforming. Colonies on peptone-yeast extract-glucose-NaCl-agar were circular, entire, about 1.0 to 2.5 mm in diameter, slightly convex, smooth with glossy surface, white, and opaque. Older colonies (≥6 days) showed a brownish pigment in their centers.

Chemoorganotrophic: Metabolism was respiratory and fermentative. Facultatively anaerobic.

Catalase and benzidine tests were positive. Coagulase negative. Bovine or sheep blood was not hemolyzed.

Temperature relationships: Growth occurred at 15 and 45°C.

Growth on NaCl-agar: Growth occurred with 10 and 15% NaCl. Nitrate was reduced. Acetoin was produced. Acid was produced aerobically

TABLE 4. Characters useful in the separation of *S. carnosus* from other staphylococci[a]

Character	*S. carnosus*	*S. aureus*	*S. simulans*	*S. xylosus*	*S. cohnii*	*S. saprophyticus*	*S. haemolyticus*	*S. warneri*	*S. hominis*	*S. epidermidis*	*S. capitis*
Coagulase	−[b]	+	−	−	−	−	−	−	−	−	−
Phosphatase	+	+	(±)	(+)	(−)	(−)	(−)	(−)	(−)	+	(−)
Hemolysis	−	(+)	±, −	(−)	(−, ±)	−	(+)	±, −	(−)	±, −	(−)
Growth on 15% NaCl	+	±, −	(±)	(+, ±)	±, +	±, +	(±, −)	(±, −)	−	−	±, −
Nitrate reduction	+	+	+	−	−	−	(+)	(−)	(+, ±)	(+, ±)	(+)
Acetoin production	+	+	(−)	±, −	+ , ±	(+)	+, ±	+	v	+	v
Acid (aerobically) from											
Maltose	−	+	−, ±	(+)	± , +	+	+	±, ±	+	+	−
Lactose	(−)	(+)	+	v	(−)	(+)	−, +	(−)	v	(+)	−
Sucrose	−	+	+	+	(−)	+	+	+	+	+	+
Resistance to novobiocin[c]	−	−	−	+	+	+	−	−	−	−	−

[a] With the exception of *S. carnosus*, the data are taken from Kloos and Schleifer (6).

[b] A single symbol denotes a 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 has a frequency below 70%, but together the frequencies equal 80 to 100%. +, Positive; ±, weak; −, negative; v, variable (+, ±, and −).

[c] Minimal inhibitory concentration ≥3.1 μg/ml.

from glucose, fructose, galactose, mannose, ribose, trehalose, mannitol, and glycerol. No acid from xylose, sucrose, maltose, lactose, xylitol, rhamnose, turanose, raffinose, fucose, or melibiose. Susceptible to lysostaphin (100 μg/ml) and novobiocin (1.6 μg/ml).

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

Peptidoglycan type: L-Lys-Gly_{5-6}.

Cell-wall teichoic acid composition: Glycerol, glucose, galactosamine, and traces of glucosamine were present.

G+C content of DNA: 36 mol%.

REPRINT REQUESTS

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