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Staphylococcus condimenti sp. nov., from soy sauce mash, and *Staphylococcus carnosus* (Schleifer and Fischer 1982) subsp. *utilis* subsp. nov.

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Based on the sequence data of 23S rRNA of *Staphylococcus carnosus*, *Staphylococcus piscifermentans*, *Staphylococcus aureus* and *Staphylococcus epidermidis*, species-specific probes were constructed. Their application revealed a heterogeneity within 18 strains previously identified as *S. carnosus*. Strains of this group were selected, and their 23S rRNA sequence was determined. It was revealed that the strains of *S. carnosus* can be placed in at least three sub-groups. This grouping was supported by physiological data and DNA–DNA similarity studies. Based on these results, we propose the new species *Staphylococcus condimenti* sp. nov. The type strain is *S. condimenti* F-2^T (= DSM 11674^T). The phylogenetic position of the new species within the radiation of other staphylococcal strains is reflected by a 16S rRNA-based tree. Furthermore, it is proposed to designate the new subspecies of *Staphylococcus carnosus* Schleifer and Fischer 1982, *Staphylococcus carnosus* subsp. *utilis* subsp. nov. The type strain of *S. carnosus* subsp. *utilis* is SK 11^T (= DSM 11676^T).

Keywords: *Staphylococcus condimenti* sp. nov., *Staphylococcus carnosus* subsp. *utilis* subsp. nov., soy mash

INTRODUCTION

Strains of the species *Staphylococcus carnosus* were originally isolated from fermenting sausages (17). It has been shown that they exert positive effects on the formation of flavour and the reddening reaction and therefore strains of this species are used as common components in starter cultures for the production of fermented sausage and cured ham (3). In more recent studies (20, 21), it was revealed that strains of *S. carnosus* participate in the fermentation of fish and soy sauces in Asia. A closely related group of strains isolated from fermenting fish sauce was characterized and described as the new species *Staphylococcus piscifermentans* (21). In this communication, we propose an emended classification within the species *S. carnosus* based on DNA–DNA similarity studies as well as 16S and 23S rDNA sequence analysis in combination with physiological data. Moreover, 23S rDNA-targeted oligonucleotide probes were constructed for the rapid identification of these species and other food-relevant staphylococci such as *Staphylococcus aureus* and *Staphylococcus epidermidis*.

The EMBL accession numbers for the 16S and 23S rRNA sequences reported in this paper are Y15754 and Y15751 (*S. piscifermentans* SK 03^T) and Y15750 and Y15755 (*S. condimenti*), and Y15752 for the partial 23S rRNA sequence (*S. epidermidis* DSM 20044^T).

METHODS

Organisms and growth conditions. The bacterial strains investigated and the strains used to construct and evaluate the specificity of the oligonucleotide probes are those compiled in Table 1. Staphylococci, *Kocuria varians* and *Escherichia coli* were grown at 37 °C on Standard I medium (Merck). Lactic acid bacteria were grown anaerobically at 30 °C on MRS medium (2), with the exception of *Tetragenococcus halophilus* DSM 20339^T and strains of *Carnobacterium* sp. These strains were cultivated on MRS medium containing 6·5% NaCl and CASO-yeast medium (Merck), respectively.

Physiological characterization. Physiological characteristics were determined with the aid of the ID 32 STAPH system

Table 1. Strains and sources of micro-organisms

T = Type strain. The following strains were used for the evaluation of the probes: *Carnobacterium divergens* (DSM 20623^T), *Carnobacterium piscicola* (DSM 20730^T), *Enterococcus faecalis* (DSM 20478^T), *Escherichia coli* LTH 1288, *Kocuria varians* (DSM 20033^T), *Lactobacillus curvatus* LTH 1432, *Lactobacillus pentosus* (DSM 20314^T), *Lactobacillus plantarum* (DSM 20174^T), *Lactobacillus sakei* LTH 677, *Leuconostoc carnosum* (DSM 5576^T), *Pediococcus acidilactici* (DSM 20284^T), *Pediococcus pentosaceus* (DSM 20336^T), *Staphylococcus arlettae* (DSM 20672^T), *Staphylococcus auricularis* (DSM 20609^T), *Staphylococcus capitis* (DSM 20326^T), *Staphylococcus caprae* (DSM 20608^T), *Staphylococcus caseolyticus* (DSM 20597^T), *Staphylococcus chromogenes* (DSM 20454^T), *Staphylococcus cohnii* (DSM 20260^T), *Staphylococcus equorum* (DSM 20674^T), *Staphylococcus gallinarum* (DSM 20610^T), *Staphylococcus haemolyticus* (DSM 20263^T), *Staphylococcus hominis* (DSM 20328^T), *Staphylococcus hyicus* (DSM 20459^T), *Staphylococcus intermedius* (DSM 20373^T), *Staphylococcus kloosii* (DSM 20676^T), *Staphylococcus lentus* (DSM 20352^T), *Staphylococcus saprophyticus* (DSM 20229^T), *Staphylococcus simulans* (DSM 20322^T), *Staphylococcus warneri* (DSM 20316^T), *Staphylococcus xylosus* (DSM 20266^T), *Tetragenococcus halophilus* (DSM 20339^T). DSM, DSMZ–Deutsche Sammlung von Mikroorganismen und Zellkulturen; LTH, strain collection of the Institut für Lebensmitteltechnologie, Universität Hohenheim, Germany.

Species	Strain	Source
S. carnosus	SK 361^T (DSM 20501^T)	Schleifer & Fischer (17)
	833 (LTH 3840)	Montel *et al*. (15)
	836 (LTH 3841)	
	F-2^T (LTH 3734^T)	Tanasupawat *et al*. (20)
	F-8 (LTH 3735)	
	SK 06 (LTH 3723)	
	SK 07 (LTH 3724)	
	SK 08 (LTH 3728)	
	SK 09 (LTH 3726)	
	SK 10 (LTH 3727)	
	SK 11^T (LTH 3728^T)	
	SK 12 (LTH 3729)	
	SK 13 (LTH 3730)	
	LTH 18	Meat starter strains
	LTH 53	
	LTH 175	
	LTH 1574	
	LTH 2102	
S. piscifermentans	(10 strains)	Tanasupawat *et al*. (21)
S. aureus	(10 strains)	Type strain and strains from various foods
S. epidermidis	(8 strains)	Type strain and strains of various origin

(bioMérieux). Acid production from additional carbohydrates (D-galactose, D-melezitose, D-sorbitol) and glycerol was studied as described by Kloos *et al*. (8). Catalase activity was detected according to Knauf *et al*. (9). The sensitivity to lysozyme (400 μg ml^{-1} and 1·6 mg ml^{-1}) and lysostaphin (200 μg ml^{-1}) was determined on agar plates (8). Tolerance to NaCl was examined by incubating for 24 h on P-agar (8) containing NaCl at concentrations of 0·5, 5, 10 and 15%. Relation to temperature was determined on P-agar at 8, 15, 25, 34, 42 and 45 °C. The effect of pH on growth was investigated under aerobic conditions in P-medium containing 20 mM ammonium citrate adjusted to an initial pH of 5·0.

Isolation of genomic DNA. Cells of a 5 ml overnight culture were harvested by centrifugation, washed in 2 ml 5 × TE buffer (16) and resuspended in 100 μl 5 × TE buffer containing 100 μg RNase ml^{-1}. Staphylococci were lysed by adding 50 μl 10 mM Tris/HCl (pH 7·6) containing 5 mg lysozyme ml^{-1} (Serva) and 0·5 mg lysostaphin ml^{-1} (Sigma). All other organisms were lysed by the addition of 50 μl of 10 mM Tris/HCl (pH 7·6) containing 10 mg lysozyme ml^{-1}. Cell suspensions were incubated at 37 °C until they became viscous. Thereafter, 150 μl 2% SDS and 50 μl proteinase K (18 mg ml^{-1}; Boehringer Mannheim) were added. The mixtures were incubated for 30 min at 55 °C. The DNA was purified by repeated phenol–chloroform extractions, precipitated with ethanol and dissolved in 100 μl of 1 × TE buffer.

rDNA sequencing. Overlapping stretches of 23S rDNA were amplified with *Pwo* polymerase (Boehringer Mannheim) in a GeneAmp 2400 PCR system (Perkin-Elmer). PCR frag-

ments were cloned using the pCR-Script Amp SK(+) cloning kit (Stratagene). Plasmid DNA was isolated from *E. coli* with the aid of Qiagen-tip 100 columns (Qiagen). Sequencing was performed using an AutoRead sequencing kit (Pharmacia) as recommended by the supplier. Sequences were read by an ALF DNA sequencer (Pharmacia) and analysed with DNASIS (Hitachi Europe). 16S rDNA was amplified *in vitro* and sequenced directly as described previously (18).

Sequence data analysis. The rRNA sequence data were added to alignments of deposited complete primary structures of 16S and 23S rRNA. Phylogenetic analyses were performed by applying maximum-parsimony and maximum-likelihood approaches on data sets varying with respect to the selection of reference sequences as well as sequence positions. The corresponding tools of the ARB program package (11) were used for alignment, selection of positions according to variability, calculation of similarities as well as tree reconstruction, evaluation and drawing.

Design of species-specific probes. A comparative analysis of aligned 23S rDNA sequences of different staphylococci species revealed regions that were used as target sites for species-specific oligonucleotide probes. To optimize the specificity of the probes, target sites were chosen with respect to the most destabilizing effect of mismatches on the oligonucleotide–DNA hybrid (7, 13).

Probe hybridizations. For the application of species-specific oligonucleotide probes, 5 μg DNA from each strain was denatured in 0·2 M NaOH/2 × SSC (16) at 37 °C for 5 min and transferred to a nylon membrane (Qiabrane; Qiagen) using a dot-blot apparatus (Schleicher & Schuell). Subsequently, the DNA was immobilized by incubating at 80 °C for 1 h. Oligonucleotides were labelled with the 3′-digoxigenin (DIG) oligolabelling kit (Boehringer Mannheim). Hybridizations were carried out as described by the supplier. The membranes were washed twice for 10 min in 2 × SSC/0·1 % SDS. The temperatures used for hybridization and washing are shown in Table 3. Detection of hybrids was performed using a DIG luminescent detection kit (Boehringer Mannheim). For further hybridizations, oligonucleotides and antibodies were removed by washing the membranes twice in 0·2 M NaOH/0·5 % SDS at 37 °C for 10 min. Subsequently, the membranes were washed five times in 2 × SSC to avoid carry-over of NaOH.

DNA–DNA hybridization. DNA similarity was determined using radioactively labelled genomic DNA of strain F-2^T as a probe. DNA (5 μg) from selected type strains was transferred to and immobilized on a nylon membrane as described above. To determine the accessibility of DNA, hybridization with the radioactively labelled 16S rDNA bacterial probe EUB338 (1) was carried out as described previously (6). Radioactive-labelling of probe EUB338 was done with the aid of Ready-To-Go T4 polynucleotide kinase (Pharmacia) and Redivue [γ-^{32}P]ATP (Amersham). Genomic DNA of strain F-2^T was labelled with a Ready-To-Go DNA-labelling kit and Redivue [α-^{32}P]dATP. Subsequent hybridization was performed at 68 °C for 24 h. The membrane was washed twice at 70 °C for 15 min in 2 × SSC/0·1 % SDS and once in 0·1 × SSC/0·5 % SDS to ensure stringent conditions (23). Autoradiograms were digitized with a flatbed scanner (Hewlett-Packard). The DNA similarities were calculated using WinCam software (Cybertech), taking into consideration the accessibility of immobilized DNA for hybridization (10).

RESULTS

Design and application of oligonucleotide probes

To construct species-specific probes, the complete 16S and 23S rRNA sequences of strain *S. piscifermentans* SK 03^T and parts of the 23S rRNA sequence of *S. epidermidis* (DSM 20044^T) were determined by sequencing of *in vitro* amplified or cloned rDNA. The 16S and 23S rRNA sequences of *S. carnosus* and *S. aureus* have been described previously (14). The comparison of variable regions of these 23S rRNA sequences permitted the construction of specific oligonucleotide probes. The application revealed that probes Stpis2, Staur and Stepi were specific for *S. piscifermentans*, *S. aureus* and *S. epidermidis*, respectively, while probe Stcar2 hybridized with the DNA of only a few strains of *S. carnosus*. Therefore, 16S and 23S rRNA sequences of strain F-2^T as well as parts of the 23S rRNA sequences of strains F-8, SK 06 and SK 12 were analysed, and the results were used to construct probes F2son2 and Stcar4. By applying probes Stcar2, Stcar4 and F2son2, the strains formerly classified as *S. carnosus* could be allotted to groups F, A and B (Table 2). The specificities of all probes were evaluated with DNA isolated from the staphylococci and type strains of species relevant in fermenting high-protein food substrates. The sequences of all probes and the temperatures used for hybridization and washing are given in Table 3.

DNA similarity

Quantitative DNA–DNA reassociation studies were performed with DNA of the strains of group F and the type strains of *S. carnosus* and *S. piscifermentans*. As shown in Table 4, strain F-2^T exhibited a high level of DNA relatedness with strain F-8, but only low levels with *S. carnosus* SK 361^T (58 %) and *S. piscifermentans* SK 03^T (51 %).

Phylogenetic implications

It was calculated that the 16S as well as the 23S rRNA sequences of strain F-2^T, *S. piscifermentans* SK 03^T and the type strain of *S. carnosus* (14) share more than 99·9 and 98·9 % overall sequence similarity, respectively. For the small-subunit rRNAs, only one to three base changes were found. On the other hand, 13–29 base changes were detected in the 23S rRNA of these strains, confirming the higher overall fraction of variable residues within large-subunit rRNA sequences (12, 14). A 16S rRNA-based phylogenetic tree reflecting the position of the strains within the radiation of other staphylococcal strains is shown in Fig. 1.

Cultural and morphological properties

To support the grouping depicted in Table 2, the strains were studied in more detail. All strains grew well on P-agar between 15 and 42 °C. None of the

Table 2. Specificity of oligonucleotide probes F2son2, Stcar2, Stcar4, Stpis2, Staur and Stepi

Species	Strain	Reaction with probe*:					
		F2son2	Stcar2	Stcar4	Stpis2	Staur	Stepi
Group F	F-2^T(LTH 3734)	+	−	−	−	−	−
	F-8 (LTH 3735)	+	−	−	−	−	−
Group A	SK 361^T† (DSM 20501)	−	+	−	−	−	−
	SK 07 (LTH 3724)	−	+	−	−	−	−
	SK 13 (LTH 3730)	−	+	−	−	−	−
	833 (LTH 3840)	−	+	−	−	−	−
	836 (LTH 3841)	−	+	−	−	−	−
	LTH 18	ND	+	−	ND	ND	ND
	LTH 53	ND	+	−	ND	ND	ND
	LTH 175	ND	+	−	ND	ND	ND
	LTH 1574	ND	+	−	ND	ND	ND
	LTH 2102	ND	+	−	ND	ND	ND
Group B	SK 06 (LTH 3723)†	−	−	+	−	−	−
	SK 08 (LTH 3728)	−	−	+	−	−	−
	SK 09 (LTH 3726)	−	−	+	−	−	−
	SK 10 (LTH 3727)	−	−	+	−	−	−
	SK 11^T (LTH 3728)	−	−	+	−	−	−
	SK 12 (LTH 3729)†	−	−	+	−	−	−
S. piscifermentans	(10 strains)	−	−	−	+	−	−
S. aureus	(10 strains)	−	−	−	−	+	−
S. epidermidis	(8 strains)	−	−	−	−	−	+

ND, Not determined.

* No hybrids were obtained with DNA of other strains listed in Table 1.

† Strain used for construction of specific probe.

Table 3. Description of specific probes for the strains of groups F, A and B as well as of *S. piscifermentans*, *S. aureus* and *S. epidermidis*

Target organism	Probe	Sequence (5′–3′)	Temperature (°C) used for:	
			Hybridization	Washing
Group F	F2son2	CGCCATTCTCAAGGT	43	45
Group A	Stcar2	ACCTTGAGAATAGCG	43	46
Group B	Stcar4	ACCTTGTGAATAGCG	43	46
S. piscifermentans	Stpis2	CGCCATTCATAAGGT	43	45
S. aureus	Staur	AGCCTTAACGAGTACCGG	50	53
S. epidermidis	Stepi	CGGCACTCATAAGGCTG	50	52
Bacteria	EUB338*	GCTGCCTCCCGTAGGAGT	43	45

* Targeted against 16S rRNA (1).

strains grew at 8 °C. After 48 h incubation, colonies (0·5–2 mm diameter) appeared circular, smooth, slightly raised and cream-coloured or orange (strains SK 07 and SK 13). All strains tolerated concentrations of NaCl up to 15%.

Physiological characterization

The study of physiological characteristics supported the genotypic grouping of the strains. As shown in Table 5, strains of groups F, A and B exhibited

Table 4. DNA similarities of strains F-2^T, F-8, *S. carnosus* SK 361^T and *S. piscifermentans* SK 03^T using ^{32}P-labelled DNA of strain F-2^T as a probe

Strain	EUB338 (relative signal)	F-2^T probe (relative signal)	Calculated DNA homology (%)
F-2^T	100	100	100
F-8	99	97	98
SK 361^T	102	59	58
SK 03^T	83	42	51

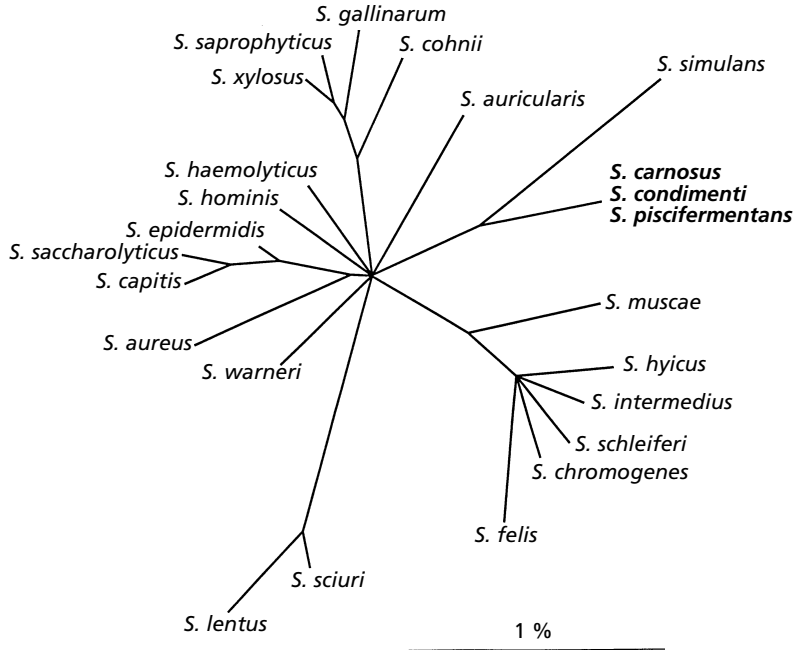

Fig. 1. 16S rRNA-based tree reflecting the phylogenetic relationships of staphylococci. The tree is based on a maximum-likelihood tree and a data set containing all available almost complete 16S rRNA sequences from staphylococci and selected reference organisms from other phylogenetic groups. For the calculations, only those alignment positions sharing identical residues in at least 50% of all staphylococcal sequences were used. The tree topology was corrected according to the results of distance matrix as well as maximum-parsimony analyses. Multifurcations indicate that a common branching order was not supported by applying alternative treeing methods. The bar indicates 1% estimated sequence divergence.

catalase and arginine dihydrolase activity but no β-glucuronidase activity. They reduced nitrate to nitrite (except strain SK 09), and nitrite was also reduced as described previously (5). Acid was produced from glucose, fructose, *N*-acetylglucosamine and glycerol. All strains were susceptible to novobiocin. None of these strains produced acid from raffinose, ribose, cellobiose, arabinose and turanose. Strains assigned to group A exhibited phosphatase activity and produced acid from D-mannose and D-sorbitol. None of these characteristics was present in the strains of group B. Strains F-2^T and F-8 were allotted to group F and exhibited urease as well as high lipolytic activity.

DISCUSSION

Staphylococci in food, such as *S. carnosus* and *S. piscifermentans*, may exert desirable effects as components of a fermentation flora, whereas other species, for example *S. aureus* and *S. epidermidis*, are known as food poisoning organisms or potential pathogens. To develop a system for their identification and differentiation, oligonucleotide probes were designed by the comparative analysis of the large subunit rRNA primary structures of the type strains. The evaluation of the specificity of these probes revealed a genetic heterogeneity of the *S. carnosus* strains studied. Further sequence analyses of 23S rRNA genes led to the design of probes that, in combination with the type strain-specific probe, allowed the detection of all strains formerly classified as *S. carnosus*. The strains could be divided into three sub-groups (F, A and B).

The strains of group F were originally found in soy sauce mash and described as the new species '*Tetracoccus soyae*' by Ueno & Omata (22). However, this species was never validated. In more recent studies, these strains have been identified as *S. carnosus* (20), mainly because of their DNA–DNA similarity level of 69–72%. However, the strains exhibited certain specific physiological characteristics, and the results of our DNA–DNA hybridization studies (51–58% similarity) are also indicative of their separation from the species *S. carnosus*. On the other hand, the overall similarities of rRNA sequences of strains F-2^T, *S. piscifermentans* SK 03^T and the corresponding sequences of strain *S. carnosus* SK 361^T were rather

Table 5. Differential characteristics of the strains of groups F, A and B and *S. piscifermentans* SK 03^T

T = Type strain; ND, not determined; w, weak reaction.

Characteristic	Group F		Group A										Group B						SK 03^T
	F-2^T	F-8	SK 361^T	SK 07	SK 13	833	836	LTH 18	LTH 53	LTH 175	LTH 1574	LTH 2102	SK 06	SK 08	SK 09	SK 10	SK 11^T	SK 12	
Urease	+	+	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	+
Phosphatase	+	+	w	+	+	+	+	+	+	+	+	+	−	−	−	−	−	−	w
Lipolytic activity	+	+	−	−	−	ND	ND	−	−	−	−	−	−	−	−	−	−	−	+
Aesculin hydrolysis	−	−	−	w	w	−	−	−	−	−	−	−	−	−	−	−	−	−	+
Acid from:																			
D-Galactose	+	w	+	−	+	−	−	−	−	−	−	−	−	−	−	−	−	−	+
D-Mannose	+	+	+	+	+	+	+	+	+	+	+	+	−	−	−	−	−	−	−
Lactose	+	w	+	−	−	+	+	+	+	+	+	+	−	−	−	−	−	−	+
Maltose	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	+
Sucrose	w	w	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	+
Turanose	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	+
Trehalose	+	+	+	+	+	−	−	−	−	−	−	−	−	+	+	+	+	+	+
D-Melezitose	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	+
Mannitol	+	+	+	−	−	+	+	+	+	+	+	+	−	−	−	−	−	−	−
D-Sorbitol	+	+	+	+	+	+	+	+	+	+	+	+	−	−	−	−	−	−	−
Growth at 45 °C	+	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	+
Growth at pH 5	+	w	−	w	−	+	+	+	w	+	+	+	w	w	+	+	+	+	w
Growth on 15% NaCl	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Susceptibility to (μg ml^{-1}):																			
Lysozyme (400)	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
Lysozyme (1600)	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
Lysostaphin (200)	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+

high (> 98·9%). The comparative rRNA sequence analysis at this level of phylogenetic relatedness can only provide differentiating information and is not sufficient to be used for species definition. It is accepted (19) that the superior method for phylogenetic investigation at or below the species level is the quantitative hybridization analysis of genomic DNA. The quantitative DNA–DNA reassociation studies confirmed a close relationship of the F group strains (98% similarity), which were clearly separated from the type strains of *S. carnosus* and *S. piscifermentans*. Taking into account the recommended threshold value of 70% genomic DNA relatedness as a limit for species definition (23), together with the characteristic epigenetic properties, e.g. urease and lipolytic activity (Table 5), the strains of group F represent a new species. We propose that the strains of this group should be placed in a new *Staphylococcus* species, *Staphylococcus condimenti*.

The evaluation of the probe specificities also revealed a heterogeneity among the remaining strains of *S. carnosus*. According to their high levels of DNA–DNA similarity (> 73%) reported by Tanasupawat *et al.* (20), these strains have to be grouped in one single species. There exist characteristic differences among these strains in their physiological properties as well as in their reaction with specific 23S rRNA-directed probes. Based on these results, we propose to place the strains of group B in a new subspecies of *Staphylococcus carnosus* Schleifer and Fischer 1982, *Staphylococcus carnosus* subsp. *utilis*. In accordance with Rule 40 of the International Code of Nomenclature of Bacteria, the strains of group A are placed in the subspecies *Staphylococcus carnosus* subsp. *carnosus*. This grouping is consistent with the results obtained in studies of the potential of strains of *S. carnosus* to form biogenic amines and to exhibit haemolytic activities (4). It was observed that the strains of group B were virtually free of that potential, whereas in strains of group A, these activities were frequently present.

Description of *Staphylococcus condimenti* sp. nov.

Staphylococcus condimenti (con.di.men′ti. L. n. *condimentum* spice; L. gen. n. *condimenti* of the spice).

Cells are Gram-positive cocci (diameter 1 μm), facultative anaerobic, non-motile, non-spore-forming and occur in pairs, short chains and small clusters. Colonies on P-agar are usually opaque, circular and slightly raised, with entire margins. After 48 h incubation, colonies are cream-coloured and 1–2 mm in diameter. They exhibit catalase, urease, arginine dihydrolase, nitrate reductase, nitrite reductase, β-galactosidase, phosphatase and lipolytic activity. Aesculin is not hydrolysed; no β-glucuronidase activity is detectable. Acid is produced from glucose, fructose, mannose, galactose, trehalose, mannitol, sorbitol and glycerol. Acid is not produced from raffinose, ribose, cellobiose, turanose and arabinose. Lecithinase and coagulase activity is not detectable. Strains grow at 15 and 42 °C and on P-agar containing 0·5–15% NaCl. Sensitive to lysostaphin (200 μg ml^{-1}) and resistant to lysozyme (400 and 1600 μg ml^{-1}). Variable characteristics are shown in Table 5. DNA G+C content is 35·2–36 mol%. MK-7 is the major menaquinone, and MK-6 or MK-6 and MK-8 are the minor mena-

quinones. Strains were isolated from soy sauce mash. Strain F-2^T (= DSM 11674^T) is the type strain, and its characteristics are those described above. Its G+C content is 35·2 mol %. MK-7 is the major menaquinone; MK-6 is the minor menaquinone. The sequences of the 16S and 23S rRNA have EMBL accession numbers Y15750 and Y15755. Strain F-8 (= DSM 11675) is a reference strain that exhibits different physiological characteristics. This strain does not grow at 45 °C, has a G+C content of 36 mol %, MK-7 as the major menaquinone and MK-6 and MK-8 as the minor menaquinones.

Description of *Staphylococcus carnosus* subsp. *utilis* subsp. nov.

Staphylococcus carnosus subsp. *utilis* (ut.ti′lis L. adj. *utilis* useful).

Cells are Gram-positive cocci (diameter 1 μm), facultative anaerobic, non-motile, non-spore-forming and occur predominantly in pairs and small clusters. Colonies on P-agar are usually opaque, circular and slightly raised, with entire margins. After 48 h incubation, colonies are cream-coloured and 0·5–1·5 mm in diameter. They exhibit catalase, arginine dihydrolase, nitrate reductase (except strain SK 09) and nitrite reductase activity. Aesculin is not hydrolysed; β-galactosidase, phosphatase and β-glucuronidase activity is not detectable. Acid is produced from glucose, fructose, trehalose, mannitol and glycerol. Acid is not produced from mannose, maltose, galactose, melezitose, lactose, mannitol, sorbitol, sucrose, raffinose, ribose, cellobiose, turanose and arabinose. Lecithinase and coagulase activity is not present. The strains grow at 15 and 42 °C and on P-agar containing 0·5–15% NaCl. They are sensitive to lysostaphin (200 μg ml^{-1}) and resistant to lysozyme (400 and 1600 μg ml^{-1}). Variable characteristics are shown in Table 5. The DNA G+C content is 34·8–37·1 mol %. They contain MK-7 as the major menaquinone and MK-6 or MK-6 and MK-8 as the minor menaquinones. Isolated from fermenting fish and shrimp sauces. Strain SK 11^T (= DSM 11676^T) is the type strain, and its characteristics are those described above. The G+C content is 34·8 %. MK-7 is the major menaquinone. Strain SK 09 (= DSM 11677) is a reference strain that exhibits different characteristics. This strain does not exhibit nitrate reductase activity. MK-7 is the major menaquinone; MK-6 and MK-8 are the minor menaquinones.

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