***Staphylococcus fleurettii* sp. nov., isolated from goat's milk cheeses**

Christine Vernozzy-Rozand,¹ Christine Mazuy,¹ Hélène Meugnier,² Michèle Bes,² Yves Lasne,³ Franz Fiedler,⁴ Jérôme Etienne² and Jean Freney²

Author for correspondence: Jean Freney. Tel: +33 4 72 11 07 62. Fax: +33 4 72 11 07 64.
e-mail: jfreney@laennec.univ-lyon1.fr

¹ Unité de Microbiologie Alimentaire et Prévisionnelle, Ecole Nationale Vétérinaire de Lyon, 1 avenue Bourgelat, BP 83, 69280 Marcy l'Etoile, France

² EA 1655, Centre National des Toxémies à Staphylocoques, Faculté de Médecine Laennec, 69372 Lyon cedex 08, France

³ Laboratoire des Radioisotopes, Hôpital Edouard Herriot, Place d'Arsonval, 69437 Lyon cedex 03, France

⁴ Institut für Genetik und Mikrobiologie, Lehrstuhl für Mikrobiologie, Mariast. 1a, 80638 Munich, Germany

A new coagulase-negative and novobiocin-resistant species of the genus *Staphylococcus*, *Staphylococcus fleurettii*, isolated from raw-milk cheeses, is described. This species is differentiated from the other novobiocin-resistant staphylococci on the basis of ribotype and intergenic transcribed spacer patterns, DNA–DNA reassociation reactions, cell wall composition and phenotypic characteristics. *S. fleurettii* could be distinguished by its oxidase activity, by its ability to produce acid aerobically from D-trehalose, D-mannose, D-turanose and maltose and by its inability to produce acid from D-cellobiose. The type strain of *S. fleurettii* is CIP 106114^T (= DSM 13212^T).

Keywords: *Staphylococcus fleurettii* sp. nov., goat's milk cheeses

INTRODUCTION

A total of 29 species and 8 subspecies are already recognized in the genus *Staphylococcus*, as listed in the 9th edition of *Bergey's Manual of Determinative Bacteriology* (Holt *et al.*, 1994). In addition, seven new staphylococcal species have been validly published since 1992 (*Staphylococcus muscae*, *Staphylococcus pasteurii*, *Staphylococcus piscifermentans*, *Staphylococcus pulvereri*, *Staphylococcus vitulinus*, *Staphylococcus lutrae* and *Staphylococcus condimenti*). Four new subspecies have been recently described: *Staphylococcus sciuri* subsp. *carnaticus*, *S. sciuri* subsp. *rodentium*, *Staphylococcus hominis* subsp. *novobiocin-septicus* and *Staphylococcus carnosus* subsp. *utilis*.

Within the cluster of coagulase-negative staphylococci, 16 novobiocin-resistant species or subspecies (*Staphylococcus arlettae*, *Staphylococcus cohnii* subsp. *cohnii*, *S. cohnii* subsp. *urealyticum*, *Staphylococcus equorum*, *Staphylococcus gallinarum*, *Staphylococcus kloosii*, *Staphylococcus lentus*, *S. pulvereri*, *Staphylococcus saprophyticus* subsp. *saprophyticus*, *S. saprophyticus* subsp. *bovis*, *S. sciuri* subsp. *sciuri*, *S. sciuri* subsp. *carnaticus*, *S. sciuri* subsp. *rodentium*, *S.*

vitulinus, *Staphylococcus xylosus* and *S. hominis* subsp. *novobiocin-septicus*) have been established so far.

Staphylococcus species have been isolated from various mammals and birds, as well as from food of animal origin. It is likely that novel species will be discovered if additional host organisms and new environments are examined. As the presence of coagulase-negative staphylococci or their toxins in goat's milk cheese poses a possible health hazard, it was of interest to identify and study the enterotoxigenicity of coagulase-negative staphylococci isolated from milk and cheeses (Vernozzy-Rozand *et al.*, 1996b).

In this paper, a new taxonomic group of novobiocin-resistant staphylococci is described. These bacteria were isolated from goat's milk cheeses in a previous study (Vernozzy-Rozand *et al.*, 1996a). They were allocated on the basis of their physiological and biochemical properties, cell wall composition and genetic relatedness to the other oxidase-positive and novobiocin-resistant staphylococcal species.

METHODS

Bacterial strains. Six strains [81, 90, 91, 125, 193 and 241^T (= CIP 106114^T = DSM 13212^T)] were isolated from goat's

Table 1. DNA relatedness between *Staphylococcus fleurettii* and other species in the genus *Staphylococcus*

Abbreviations: ATCC, American Type Culture Collection, Manassas, VA, USA; CCM, Czech Collection of Microorganisms, Masaryk University, Brno, Czech Republic; CIP, Collection of Bacterial Strains of Institut Pasteur, Paris, France; DSM, DSMZ –Deutsche Sammlung von Mikroorganismen und Zellkulturen, Braunschweig, Germany; JCM, Japan Collection of Microorganisms, RIKEN, Wako, Japan.

Species and subspecies	Strain	Relative binding with labelled DNA from CIP 106114 ^T (%) at 55 °C*
<i>S. fleurettii</i>	81	86
	90	96.6
	91	100
	125	87
	193	100
	241 ^T (CIP 106114 ^T)	100
<i>S. vitulinus</i>	ATCC 51145 ^T	44 (20)
<i>S. intermedius</i>	CCM 5739 ^T	37
<i>S. caseolyticus</i>	ATCC 13548 ^T	35
<i>S. cohnii</i> subsp. <i>urealyticum</i>	ATCC 49330 ^T	32
<i>S. sciuri</i> subsp. <i>rodentium</i>	ATCC 700061 ^T	32 (12)
<i>S. sciuri</i> subsp. <i>sciuri</i>	ATCC 29062 ^T	32 (10)
<i>S. sciuri</i> subsp. <i>carnaticus</i>	ATCC 700058 ^T	31 (10)
<i>S. lentus</i>	ATCC 29070 ^T	29
<i>S. chromogenes</i>	CCM 3387 ^T	24
<i>S. simulans</i>	CCM 2705 ^T	24
<i>S. capitis</i> subsp. <i>capitis</i>	CCM 2734 ^T	23
<i>S. hyicus</i>	CCM 2368 ^T	21
<i>S. aureus</i>	CCM 885 ^T	20
<i>S. carnosus</i>	DSM 20501 ^T	20
<i>S. equorum</i>	DSM 20674 ^T	18
<i>S. gallinarum</i>	CCM 3572 ^T	17
<i>S. schleiferi</i> subsp. <i>schleiferi</i>	ATCC 43808 ^T	17
<i>S. felis</i>	ATCC 49168 ^T	15
<i>S. hominis</i>	DSM 20328 ^T	15
<i>S. xylosus</i>	CCM 2738 ^T	15
<i>S. arlettae</i>	DSM 20671 ^T	14
<i>S. epidermidis</i>	CCM 2124 ^T	14
<i>S. saprophyticus</i>	CCM 883 ^T	14
<i>S. schleiferi</i> subsp. <i>coagulans</i>	JCM 7470 ^T	14
<i>S. capitis</i> subsp. <i>ureolyticus</i>	ATCC 49326 ^T	13
<i>S. lugdunensis</i>	ATCC 43809 ^T	12
<i>S. pasteurii</i>	ATCC 51129 ^T	12
<i>S. auricularis</i>	ATCC 33753 ^T	11
<i>S. kloosii</i>	DSM 20676 ^T	11
<i>S. piscifermentans</i>	JCM 6057 ^T	11
<i>S. warneri</i>	CCM 2790 ^T	11
<i>S. cohnii</i> subsp. <i>cohnii</i>	CCM 2736 ^T	10
<i>S. haemolyticus</i>	CCM 2737 ^T	10
<i>S. caprae</i>	CCM 3573 ^T	9
<i>S. muscae</i>	CCM 4175 ^T	9
<i>S. delphini</i>	DSM 20771 ^T	8

* Values in parentheses were determined at 70 °C.

milk cheeses made in the Poitou–Charentes region (in France) during a period of 6 months. These strains were not epidemiologically related. The isolation medium was nutrient broth (Oxoid) supplemented with 7% (w/v) NaCl and blood agar base no. 2 (Oxoid) mixed with 5% (v/v) defibrinated ovine blood. The latter was also used for the propagation of all of the isolates.

The 36 type strains of the genus *Staphylococcus* obtained from the collections given in Table 1 were used for DNA–DNA hybridizations.

All of the strains were maintained as frozen suspensions in glycerol broth at -25°C and also in a freeze-dried state.

Phenotypic characteristics. The following properties were determined by using methods previously described in detail (Hájek, 1976; Hájek *et al.*, 1992): cell and colony morphology and pigmentation on P agar (Freney *et al.*, 1999); motility; anaerobic growth in thioglycolate medium; production of catalase and oxidase; hydrolysis of aesculin; production of coagulase in rabbit plasma; production of clumping factor, urease, arginine dihydrolase, alkaline phosphatase, heat-labile and heat-stable nucleases, acetoin, β -galactosidase, ornithine decarboxylase and acid from carbohydrates (arabinose, cellobiose, fructose, fucose, galactose, glucose, glycerol, lactose, maltose, mannose, melezitose, raffinose, salicine, sucrose, trehalose, turanose, xylitol) under aerobic conditions; nitrate reduction; and tolerance to NaCl. In addition, the production of arginine arylamidase, pyrrolidonyl arylamidase, β -glucuronidase and *N*-acetylglucosaminidase were examined with the ID 32 Staph strip (bioMérieux) (Brun *et al.*, 1990).

Antibiotic susceptibilities. Susceptibility testing with novobiocin, bacitracin, vibriostatic compound O/129, lysozyme and lysostaphin was performed as described previously (Freney *et al.*, 1988; Hájek, 1976). The MICs of 22 different antibiotics were determined by the dilution micromethod with Mueller–Hinton broth (Oxoid). The reference strains *Escherichia coli* CCM 3988, *Staphylococcus aureus* CCM 3953, *S. saprophyticus* CCM 883 and *Kocuria (Micrococcus) kristinae* CCM 2690 were used as controls.

Enterotoxin production and detection. The detection and production of enterotoxins (i.e. SEA, SEB, SEC₁, SEC₂, SEC₃, SED and SEE) were performed according to methods described previously (Vernozy-Rozand *et al.*, 1996b).

Cell wall analysis. Cells were disintegrated by shaking with glass beads and the cell walls were purified with 4% SDS by using the procedure of Glauner *et al.* (1988). For total amino acid analysis, SDS-purified cell walls were hydrolysed with 4 M HCl for 16 h at 100°C . The levels of amino acids were determined with an amino acid analyser (LC 6001; Biotronik). The peptidoglycan structure was deduced from the molar ratios of glutamic acid to glycine, alanine, serine, lysine, glucosamine and muramic acid, respectively (Schleifer & Kandler, 1972). Phosphorus in cell walls was determined subsequent to acid hydrolyses, according to the method of Ames (1966). The composition of teichoic acids was analysed in hydrofluoric acid (HF) hydrolysates (60%, w/v, HF) of cell walls (Anderson *et al.*, 1977) or of extracted and purified teichoic acids in the case of strains 193 and CIP 106114^T. Extraction of teichoic acids from cell walls and purification were achieved using the methods of Kaya *et al.* (1985). The analyses of polyols and alditols as peracetyl-derivatives were performed by GLC. The details of the procedures have been described elsewhere (Webster *et al.*, 1994).

DNA–DNA hybridization. Published methods for the extraction (Heath *et al.*, 1986) and purification of DNA (Brenner *et al.*, 1982) were used. DNA was labelled *in vitro* by nick translation with tritium-labelled nucleotides (Amersham). The S1 nuclease method (Crosa *et al.*, 1973) was used according to the trichloroacetic procedure described in detail elsewhere (Grimont *et al.*, 1980); the reassociation temperature was 55°C . The hybridization experiments were carried out with labelled DNA from strain CIP 106114^T as a probe. The temperature (T_m) at which 50% of the re-associated DNA became hydrolysable by the S1 nuclease was determined (Crosa *et al.*, 1973). The difference (ΔT_m) between the T_m of the homologous and heterologous reactions is an estimate of the divergence between the two DNAs (Brenner, 1978); this procedure was done twice.

DNA base composition. The G+C content of the DNA (mol%) was obtained after hydrolysis with nuclease P1 (Sigma) (Gherke *et al.*, 1984) and after reverse-phase chromatography on a Merck–Clevenot chromatograph using the HPLC method as described elsewhere (Peyret *et al.*, 1989). *Legionella gratiana* strain ATCC 49413, with a G+C content of 38.0 mol%, was included as a standard.

Intergenic transcribed spacer 16S–23S PCR. The 16S–23S rDNA intergenic PCR analysis was performed using the method described by Mendoza *et al.* (1998).

Ribotyping. Ribotyping was performed as described previously (Regnault *et al.*, 1997).

RESULTS AND DISCUSSION

The six strains investigated are Gram-positive, spherical cocci that occur singly, in pairs, in short chains and in irregular clusters and that are non-motile and non-spore-forming. All of the strains are able to grow in the presence of 10% NaCl and grow both aerobically and anaerobically in the semi-solid Brewer thioglycolate medium. They produce catalase and are resistant to bacitracin, vibriostatic agent O/129 and lysozyme but are sensitive to lysostaphin. The mean base composition in all of the strains reached 31 mol% G+C. The characteristics mentioned above permit us to include the strains in the genus *Staphylococcus*.

All of the strains are coagulase-negative in rabbit plasma, heat-stable nuclease- and clumping factor-negative and novobiocin-resistant. The DNA–DNA hybridization studies have shown that the six strains are genetically closely related at the species level. However, they are not related to any of the other staphylococcal species.

Description of *Staphylococcus fleurettii* sp. nov.

Staphylococcus fleurettii (fleu.rett'i.i. L. gen. n. in honour of the French microbiologist Jean Fleurette for his contribution to the taxonomy of staphylococci).

The description of *S. fleurettii* is based on the results of a study of six strains.

Physiological and biochemical characteristics

The organism has Gram-positive spherical cells, 0.8 to 1.4 μm in diameter, that occur singly, in short chains and in irregular clumps. The organism is non-motile

Table 2. Variable characteristics of *S. fleurettii* strains

Characteristic	No. positive strains/no. strains tested (%)
Colony pigment	1/6 (16)
Acetoin production	2/6 (33)
Alkaline phosphatase	2/6 (33)
Aesculin hydrolysis	5/6 (83)
Acid produced aerobically from:	
L-Arabinose	4/6 (67)
D-Xylose	4/6 (67)

and non-spore-forming. Colonies are less than 3 mm in diameter when the organisms are grown aerobically on P agar at 37 °C. Colonies on P agar are usually raised, with ulcerated, irregular centres, opaque and often have sectorized or irregular edges; five of the six strains studied produce unpigmented colonies and one strain produces cream pigmentation. Colonies grown on tryptic soy agar are much larger (8–12 mm in diameter) and not as irregular as colonies grown on P agar.

All of the strains are negative for staphylocoagulase, thermonuclease, pyrrolidonyl arylamidase, ornithine decarboxylase, urease, arginine dihydrolase, arginine arylamidase, β -glucuronidase and β -galactosidase activities. All strains are negative for aerobic production of acid from the following substrates: D-raffinose, D-galactose, L-fucose, α -lactose, D-melezitose, D-cellobiose, salicine and D-xylitol.

All of the strains are positive for catalase and nitrate reductase activities, modified oxidase activity and aerobic production of acid from the following substrates, D-fructose, maltose, D-mannose, D-trehalose, sucrose and turanose. The variable characteristics of *S. fleurettii* are listed in Table 2.

Enterotoxin production and detection

The six strains of *S. fleurettii* do not produce the seven different enterotoxins that were investigated.

Antibiotic susceptibilities

In disk diffusion tests, resistance to novobiocin, bacitracin and vibriostatic compound O/129 is noted. In contrast, all of the strains are sensitive to lyso-staphin. All strains are resistant to 1000 μ g lysozyme ml⁻¹.

As shown by the following MIC values (mg l⁻¹), all of the strains are susceptible to penicillin G (0.06–0.1), gentamicin (0.06–0.1), kanamycin (0.5), tobramycin (0.06), neomycin (0.06), minocycline (0.06), chloramphenicol (4.0), erythromycin (0.06–0.5), lincomycin

Table 3. Composition of peptidoglycans determined from acid hydrolysates of SDS-purified cell walls

Values are in mol (mol lysine)⁻¹. *S. aureus* was used as a control. The composition of Mur, Glu and Lys was equimolar for all strains.

Strain	GlcN	Ala	Gly
<i>S. fleurettii</i> 193	1	3.6	4
<i>S. fleurettii</i> CIP 106114 ^T	1	3.3	4
<i>S. aureus</i> H	1.3	2.3	4.7

Fig. 1. Schematic representation of ribotype patterns for *S. fleurettii* and type strains of *S. vitulinus* and *S. sciuri* species and subspecies.

(0.06–0.5), clindamycin (0.06–0.1), pristinamycin (0.25–10), rifampicin (0.1), teicoplanin (0.5–20), vancomycin (0.5–1.0) and tetracycline (0.25).

Two strains (90 and CIP 106114^T) are resistant to oxacillin and chloramphenicol.

Cell wall analysis

The composition of peptidoglycans is given in Table 3. The molar ratios of the amino acids and amino sugars occurring in the peptidoglycans of strains 193 and CIP 106114^T are based on lysine. The peptidoglycans tested appear to have tetraglycine interpeptide bridges that also contain alanine.

Lysostaphin is able to hydrolyse the cell walls, whereas

Fig. 2. Intergenic transcribed spacer-PCR of *S. fleurettii* strains and type strains of *S. vitulinus* and *S. sciuri* species and subspecies. Lanes: M, 100 bp DNA ladder; 1–6, *S. fleurettii* strains 81, 90, 91, 125, 193 and CIP 106114^T; 7, *S. vitulinus* ATCC 51145^T; 8, *S. sciuri* subsp. *carnaticus* ATCC 700058^T; 9, *S. sciuri* subsp. *rodentium* ATCC 700061^T; 10, *S. sciuri* subsp. *sciuri* ATCC 29062^T.

lysozyme has only weak hydrolysis activity, suggesting acetylation of muramic acid (as is the case for *S. aureus* peptidoglycan).

The composition of the cell walls of strains 193 and CIP 106114^T was 0.7 and 0.9 µmol phosphorus (mg cell wall)^{−1}. Glycerol and glucose were found both in cell walls and in isolated teichoic acids. As a product obtained by HF-hydrolysis of cell walls, isolated teichoic acid α-glucosyl-glycerol was recognized by GLC (Fiedler *et al.*, 1981). This indicated that glucose is linked α-glycosidically to glycerol phosphate units of poly-(glycerolphosphate) teichoic acids.

Lipoteichoic acids were found and their structure corresponds to that described for staphylococci (Ruhland & Fiedler, 1990).

DNA–DNA hybridization

The DNA relationships among the six *S. fleurettii* strains and between strain CIP 106114^T and other *Staphylococcus* species are shown in Table 1. In DNA–DNA hybridization reactions performed under optimal conditions (55 °C), *S. fleurettii* strains exhibited high levels of DNA similarity (86–100%). However, the level of DNA relatedness between the new species and the *Staphylococcus* species were significantly low (8–37%). Under stringent conditions (70 °C), the levels of DNA similarity were 20% for *S. vitulinus*, 10% for *S. sciuri* subsp. *sciuri*, 10% for *S. sciuri* subsp. *carnaticus* and 12% for *S. sciuri* subsp. *rodentium*. The ΔT_m between strain CIP 106114^T and *S. vitulinus* was greater than 5 °C (5.6 °C).

DNA base composition

The G + C content of the DNA of all strains tested varied over a narrow range between 30.9 and 32 mol %.

Ribotyping

The ribotypes determined for the six isolates were entered into our database, which contains information on staphylococcal strains (32 species and 4 subspecies). The six *S. fleurettii* strains shared very close rDNA restriction profiles and a core of 10–18 common hybridizing bands (Fig. 1). A similar pattern was observed for three *S. fleurettii* strains (81, 193 and 241^T). The three other *S. fleurettii* strains presented unique patterns. The ribotypes of the six *S. fleurettii*

Table 4. Characteristics that differentiate *S. fleurettii* from the other novobiocin-resistant, oxidase-positive *Staphylococcus* species

+, 90% or more of the strains are positive; +/−, 90% or more of strains are weakly positive; −, 0–10% of strains are positive; d, 11–89% of the strains are positive. Parentheses indicate that a response is delayed.

Characteristic	<i>S. fleurettii</i>	<i>S. vitulinus</i>	<i>S. lentus</i>	<i>S. sciuri</i>
Colony size > 6 mm	−	−	−	+
Alkaline phosphatase activity	−	−	(+/−)	+
Aesculin hydrolysis	d	d	+	+
Acid produced aerobically from:				
D-Trehalose	+	d	+	+
D-Mannose	+	−	+	(d)
D-Turanose	+	−	(+/−)	(+/−)
D-Cellobiose	−	d	+	+
Maltose	+	−	d	(d)
Raffinose	−	−	+	−

strains were different from those of the *S. sciuri* and *S. vitulinus* type strains and are distinguishable by the presence of an *S. fleurettii*-specific core of 10 common hybridizing bands (Fig. 1).

Intergenic transcribed spacer 16S–23S PCR (ITS-PCR)

The *S. fleurettii* patterns shared a core of common fragments and were different from those of the *S. sciuri* and *S. vitulinus* type strains (Fig. 2).

Distinguishing characteristics

S. fleurettii can be phenotypically distinguished from the other novobiocin-resistant and oxidase-positive *Staphylococcus* species on the basis of the lack of acid production from cellobiose and raffinose and the acid production from trehalose, mannose, turanose and maltose (Table 4).

Description of the type strain

Type strain 241^T (= CIP 106114^T = DSM 13212^T), isolated from a cheese made from raw goat's milk, has the following characteristics. Colonies are 3 mm in diameter, unpigmented and raised with ulcerated centres. The type strain produces catalase and is resistant to bacitracin, vibriostatic agent O/129 and lysozyme but is sensitive to lysostaphin. The type strain is novobiocin-resistant and negative for staphylocoagulase, clumping factor, thermonuclease, alkaline phosphatase, pyrrolidonyl arylamidase, ornithine decarboxylase, urease, β -glucuronidase and β -galactosidase activities, arginine utilization and acetoin production. It is negative for aerobic production of acid from the following substrates: D-raffinose, D-galactose, L-fucose, α -lactose, D-melezitose, D-cellobiose, salicine and D-xylitol. The type strain is positive for modified oxidase activity, aesculin hydrolysis and aerobic production of acid from the following substrates, D-fructose, maltose, D-mannose, D-trehalose, L-arabinose and D-turanose. The G + C content of its DNA is 30.7 mol %.

ACKNOWLEDGEMENTS

A portion of this work was presented at the 98th General Meeting of the American Society for Microbiology (Abstract D9).

REFERENCES

- Ames, B. N. (1966). Assay of inorganic phosphate, total phosphate and phosphatases. *Methods Enzymol* **8**, 115–118.
- Anderson, A. J., Green, R. S. & Archibald, A. R. (1977). Specific determination of ribitol teichoic acid in whole bacteria and isolated walls of *Bacillus subtilis* W23. *Carbohydr Res* **57**, C7–C10.
- Brenner, D. J. (1978). Characterization and clinical identification

of *Enterobacteriaceae* by DNA hybridization. *Prog Clin Pathol* **7**, 71–117.

Brenner, D. J., McWorter, A. C., Leete Knutson, J. K. & Steigerwalt, A. G. (1982). *Escherichia vulneris*: a new species of *Enterobacteriaceae* associated with human wounds. *J Clin Microbiol* **15**, 1133–1140.

Brun, Y., Bes, M., Boeufgras, J. M. & 9 other authors (1990). International collaborative evaluation of the ATB 32 Staph gallery for identification of the *Staphylococcus* species. *Zentbl Bakteriol* **273**, 319–326.

Crosa, J. H., Brenner, D. J. & Falkow, S. (1973). Use of a single-strand specific nuclease for analysis of bacterial and plasmid deoxyribonucleic acid homo- and heteroduplexes. *J Bacteriol* **115**, 904–911.

Fiedler, F., Schaffler, M. J. & Stackebrandt, E. (1981). Biochemical and nucleic acid hybridisation studies on *Brevibacterium linens* and related strains. *Arch Microbiol* **129**, 85–93.

Freney, J., Brun, Y., Bes, M., Meugnier, H., Grimont, F., Grimont, P. A. D., Nervi, C. & Fleurette, J. (1988). *Staphylococcus lugdunensis* sp. nov. and *Staphylococcus schleiferi* sp. nov., two species from human clinical specimens. *Int J Syst Bacteriol* **38**, 168–172.

Freney, J., Kloos, W. E., Hajek, V., Webster, J. A., Bes, M., Brun, Y. & Vernozy-Rozand, C. (1999). Recommended minimal standards for description of new staphylococcal species. *Int J Syst Evol Microbiol* **49**, 489–502.

Gherke, C. W., McCune, R. A., Gama-Sosa, M. A., Ehrlich, M. & Kuo, K. C. (1984). Quantitative reversed-phase high-performance liquid chromatography of major and modified nucleosides in DNA. *J Chromatogr* **301**, 199–219.

Glauner, B., Holtje, J. V. & Schwarz, U. (1988). The composition of the murein of *Escherichia coli*. *J Biol Chem* **263**, 10088–10095.

Grimont, P. A. D., Popoff, M. Y., Grimont, F., Coynaud, C. & Lemelin, M. (1980). Reproducibility and correlation study of three deoxyribonucleic acid hybridization procedures. *Curr Microbiol* **4**, 325–330.

Hájek, V. (1976). *Staphylococcus intermedius*, a new species isolated from animals. *Int J Syst Bacteriol* **26**, 401–408.

Hájek, V., Ludwig, W., Schleifer, K. H., Springer, N., Zitzelsberger, W., Kroppenstedt, R. M. & Kocur, M. (1992). *Staphylococcus muscae*, a new species isolated from flies. *Int J Syst Bacteriol* **42**, 97–101.

Heath, L. S., Sloan, G. L. & Heath, H. E. (1986). A simple and generally applicable procedure for releasing DNA from bacterial cells. *Appl Environ Microbiol* **51**, 1138–1140.

Holt, J. G., Krieg, N. R., Sneath, P. H. A., Staley, J. T. & Williams, S. T. (editors) (1994). *Bergey's Manual of Determinative Bacteriology*, 9th edn. Baltimore: Williams & Wilkins.

Kaya, S., Araki, Y. & Ito, E. (1985). Characterization of a novel linkage unit between ribitol teichoic acid and peptidoglycan in *Listeria monocytogenes* cell walls. *Eur J Biochem* **146**, 517–522.

Mendoza, M., Meugnier, H., Bes, M., Etienne, J. & Freney, J. (1998). Identification of *Staphylococcus* species by 16S–23S rDNA intergenic spacer PCR analysis. *Int J Syst Bacteriol* **48**, 1049–1055.

Peyret, M., Freney, J., Meugnier, H. & Fleurette, J. (1989). Determination of G + C content of DNA using HPLC for the identification of staphylococci and micrococci. *Res Microbiol* **140**, 467–475.

Regnault, B., Grimont, F. & Grimont, P. A. D. (1997). Universal ribotyping method using a chemically labelled oligonucleotide probe mixture. *Res Microbiol* **148**, 649–659.

- Ruhland, G. J. & Fiedler, F. (1990). Occurrence and structure of lipoteichoic acids in the genus *Staphylococcus*. *Arch Microbiol* **154**, 375–379.
- Schleifer, K. H. & Kandler, O. (1972). Peptidoglycan types of bacterial cell walls and their taxonomic implications. *Bacteriol Rev* **36**, 407–477.
- Vernozy-Rozand, C., Mazuy, C., Perrin, G., Haond, F., Bes, M., Brun, Y. & Fleurette, J. (1996a). Identification of *Micrococcaceae* isolated from goat's milk and cheese in the Poitou-Charentes region. *Int J Food Microbiol* **30**, 373–378.
- Vernozy-Rozand, C., Mazuy, C., Prevost, C., Lapeyre, G., Bes, M., Brun, Y. & Fleurette, J. (1996b). Enterotoxin production by coagulase-negative staphylococci isolated from goat's milk and cheese. *Int J Food Microbiol* **30**, 271–280.
- Webster, J. A., Bannerman, T. L., Hubner, R. J., Ballard, D. N., Cole, E. M., Bruce, J. L., Fiedler, F., Schubert, K. & Kloos, W. E. (1994). Identification of the *Staphylococcus sciuri* species group with *EcoRI* fragments containing rRNA sequences and description of *Staphylococcus vitulinus* sp. nov. *Int J Syst Bacteriol* **44**, 454–460.