***Streptococcus garvieae* sp. nov. and *Streptococcus plantarum* sp. nov.**

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Biochemical and chemotaxonomical studies were performed on some streptococci from frozen peas and bovine mastitis in an attempt to clarify their taxonomy. The results of the present and earlier studies indicate the pea and mastitis isolates represent two new species of the genus *Streptococcus*. The isolates from frozen peas are named *Streptococcus plantarum* sp. nov. and those from mastitis, *Streptococcus garvieae* sp. nov. The type strains are NCDO 1869 and NCDO 2155, respectively.

INTRODUCTION

At present only three species of streptococci are recognized which grow at 10 °C but not at 45 °C: *Streptococcus lactis*, *S. cremoris* and *S. raffinolactis* (Deibel & Seeley, 1974; Garvie, 1978; Skerman *et al.*, 1980). All three species contain the serological group N antigen. There have been reports of other streptococci which behave similarly under these temperature conditions but which cannot be allocated to any of these three species (Cavett *et al.*, 1965; Garvie *et al.*, 1981). Cavett *et al.* (1965) reported the isolation of some arginine-negative, group N streptococci from frozen peas, which apparently resembled *S. cremoris*. Subsequent studies have confirmed that these strains possess the group N antigen (Cavett & Garvie, 1967), although they were unrelated to other group N streptococci on the basis of DNA–DNA homologies (Garvie *et al.*, 1981). Similarly, Garvie *et al.* (1981) described the characteristics of some streptococci from bovine mastitis which biochemically and physiologically resembled *S. lactis*. Despite the phenotypic resemblance of these mastitic streptococci and *S. lactis*, these taxa were shown to be genetically quite distinct (Garvie *et al.*, 1981).

In the present paper additional biochemical and chemotaxonomical studies have been performed on the mastitic and pea streptococci in an attempt to further clarify their taxonomy.

METHODS

Cultures and cultivation. The test strains are listed in Tables 1 and 2. Cells for cell wall and lipid analyses were grown in yeast glucose phosphate broth (Garvie, 1978) at 30 °C for 3 d. Cultures were checked for purity, harvested by centrifugation, washed with distilled water and freeze-dried.

Cell wall peptidoglycan. The peptidoglycan type of two strains (NCDO 1869 and NCDO 2155) was determined. Purified walls were prepared from about 500 mg of dry cells as described by Schleifer & Kandler (1972). The amino acid composition of the wall hydrolysates was determined by paper chromatography and an automatic amino acid analyser (Schleifer & Kandler, 1972).

Fatty acid analyses. Dry cells (approximately 50 mg) were degraded by acid methanolysis and fatty acid methyl esters examined by GLC as described previously (Farrow *et al.*, 1983).

Isoprenoid quinone analyses. Isoprenoid quinones were extracted from dry cells (approximately 200 mg) and purified as described previously (Collins *et al.*, 1977). Mass spectra of the quinones were recorded on an AEI MS9 instrument using a direct insertion probe, an ionizing voltage of 70 eV and a temperature of 200 °C. Quantitative determination of the quinones was by HPLC as described by Collins *et al.* (1982).

Table 1. *Percentage fatty acid composition of test strains*

Abbreviations for fatty acids are illustrated by the following examples: C_{18:0}, octadecanoic acid; C_{18:1}, octadecenoic acid; Δ C₁₉, *cis*-11,12-methylenoctadecanoic acid; *iso*-C_{16:0}, 14-methylpentadecanoic acid. Components constituting >10% are indicated in bold type.

Assignment	C _{12:0}	C _{14:0}	C _{15:0}	C _{16:0}	C _{16:1}	<i>iso</i> - C _{16:0}	C _{17:0}	C _{18:0}	C _{18:1} ω 7	C _{18:1} ω 9	Δ C ₁₉
<i>Streptococcus cremoris</i>											
NCDO 508	—	3.0	—	29.0	1.5	—	—	6.5	31.5	1.5	27.0
NCDO 607	—	3.5	—	33.0	2.5	—	—	5.0	27.0	1.5	27.5
NCDO 1986	—	3.5	0.5	33.0	2.5	—	—	7.5	26.5	2.0	24.5
<i>S. garvieae</i>											
NCDO 2155	—	4.5	—	38.0	1.5	—	3.5	14.5	25.5	1.5	11.0
NCDO 2156	—	3.5	0.5	36.0	1.5	—	2.5	15.0	27.5	2.5	11.0
NCDO 2157	—	3.5	0.5	36.5	1.0	—	2.0	13.5	26.5	2.0	14.5
<i>S. lactis</i>											
NCDO 505	—	7.0	—	35.5	2.5	—	—	6.5	23.5	2.0	23.0
NCDO 604	—	3.5	—	28.5	3.5	—	—	3.5	31.0	2.0	28.0
<i>S. plantarum</i>											
NCDO 1869	—	8.0	0.5	45.0	2.5	—	—	5.0	21.0	3.5	14.5
NCDO 1870	0.5	8.0	—	46.5	3.0	—	—	5.0	17.0	4.5	15.5
NCDO 1871	0.5	6.5	0.5	46.0	3.0	—	—	6.0	18.0	5.5	14.0
<i>S. raffinolactis</i>											
NCDO 617	—	7.5	1.0	30.5	2.5	—	—	5.0	50.0	3.5	—
NCDO 619	—	8.0	0.5	33.5	2.0	—	1.0	4.5	48.0	2.5	—
NCDO 2126	1.0	14.5	1.0	39.0	2.0	3.0	—	4.0	32.5	3.0	—

Biochemical tests. The tests were performed using the API 20STREP and API 50CH systems (API Products, Basingstoke, U.K.), according to the manufacturer's instructions. Tests were incubated at 37 °C and readings were made at 4, 24 and 48 h.

RESULTS AND DISCUSSION

The purified peptidoglycans of NCDO 1869 and NCDO 2155 contained lysine as the dibasic amino acid. Quantitative determinations revealed the presence of group A peptidoglycan types: Lys-serine₁-alanine₁ in NCDO 1869, and Lys-alanine₁-glycine₁-alanine₁ in NCDO 2155. The presence of two different cell wall peptidoglycan types in the pea and mastitis strains reinforces the distinctiveness of these taxa. Further, the peptidoglycan types of these taxa are quite different from those of other group N streptococci. The peptidoglycan type of *S. lactis* and *S. cremoris* is Lys-D-aspartic acid (Schleifer & Kandler, 1972), whereas that of *S. raffinolactis* is Lys-threonine-alanine (O. Kandler, unpublished).

The long-chain fatty acid composition of the test strains is shown in Table 1. *Streptococcus cremoris*, *S. lactis*, the pea and mastitis strains possessed closely related fatty acid profiles consisting of predominantly straight-chain saturated, monounsaturated and cyclopropane-ring acids. The major fatty acids in all four taxa consisted of hexadecanoic (C_{16:0}), octadecenoic (C_{18:1}) and *cis*-11, 12-methylenoctadecanoic (Δ C_{19:0}) acids (Table 1). *Streptococcus raffinolactis* also possessed major amounts of C_{16:0} and C_{18:1}. However, *S. raffinolactis* differed from the other taxa examined in lacking cyclopropane-ring acids (*cis*-11,12-methylenoctadecanoic acid).

Neither menaquinones (vitamin K₂) nor ubiquinones (coenzyme Q) were detected in the neutral lipid fractions of the three pea isolates (NCDO 1869 to 1871). In contrast, the mastitis streptococci (NCDO 2155, 2156, 2157, 2159) possessed low levels of a quinone which co-chromatographed with vitamin K₁. On mass spectral analysis the quinones produced a base peak at *m/e* 225, and a second intense peak at *m/e* 187, in accordance with published data for menaquinones (Collins & Jones, 1981). Peaks corresponding to molecular ions (M⁺) were also observed at *m/e* 648, 716, **784** and 852 (major component in bold) attributable to unsaturated menaquinones with 7, 8, 9 and 10 isoprene units, respectively. Quantification by HPLC indicated MK-9 constituted the major component (about 56 to 62%) together with substantial

Table 2. *Differential biochemical properties of S. garvieae, S. plantarum, S. cremoris, S. lactis and S. raffinolactis*

	<i>S. garvieae</i> (NCDO 2155, 2156, 2157, 2159; from bovine mastitis)	<i>S. plantarum</i> (NCDO 1869, 1870, 1871; from frozen peas)	<i>S. cremoris</i> (NCDO 607, 508, 764, 924, 1217, 1986)	<i>S. lactis</i> (NCDO 712, 604, 505, 507, 509)	<i>S. raffinolactis</i> (NCDO 617, 618, 619, 2112, 2114, 2136)
<i>Acid from:*</i>					
L-Arabinose	0	0	0	0	33
Amygdalin	100	100 (weak)	0	20	33
Arbutin	100	100	0	80	83
Cellobiose	100	100	67	80	100
Galactose	100	0	100	100	100
β -Gentiobiose	100	67	17	80	67
Gluconate	50 (weak)	0	0	0	0
Inulin	0	0	0	20	50
Lactose	100	0	100	100	100
Maltose	75	100	0	100	100
D-Mannitol	75	100	0	20	67
Melibiose	0	0	0	0	100
Melizitose	0	100	0	0	50
α -Methyl-D-glucoside	0	0	0	0	17
Raffinose	0	0	0	0	100
Ribose	100	0	0	100	17
Salicin	100	100	17	80	100
L-Sorbose	0	0	0	0	17
Sorbitol	0	100	0	0	0
Sucrose	50	100	0	20	100
D-Tagatose	50	0	0	0	0
Trehalose	100	100	17	100	100
D-Turanose	0	67	0	0	50
D-Xylose	0	0	0	0	83
Starch	0	0	0	40	100
<i>Hydrolysis of:†</i>					
Aesculin	100	100	50	100	100
Hippurate	0	0	100	100	0
<i>Production of:‡</i>					
Arginine dehydrolase	100	0	0	100	17
α -Galactosidase	0	33	0	0	100
β -Glucuronidase	100	0	0	0	0
Pyrrolidonylarylamidase	100	0	0	0	0

* Acid from carbohydrates was determined using the API 50CH system; readings were made at 24 h. All strains produced acid from glucose, fructose, mannose and *N*-acetylglucosamine; acid was not produced from D-arabinose, arabinol, adonitol, 2-keto-gluconate, 5-keto-gluconate, dulcitol, erythritol, D-fucose, L-fucose, glycerol, glycogen, inositol, D-lyxose, α -methyl-D-mannoside, β -methylxyloside, rhamnose, L-xylose and xylitol.

† Hydrolysis of aesculin and hippurate was determined using the API 20STREP system; readings for hippurate and aesculin hydrolysis were made at 4 h and 24 h, respectively.

‡ Enzyme activity was determined using the API 20 STREP system; readings were made at 4 h. All strains were leucine arylamidase-positive and β -galactosidase-negative.

amounts of MK-8 (about 25 to 32%). Minor amounts of MK-7 (11 to 12%) and MK-10 (2 to 3%) were also detected. The detection of MK-9 in the mastitis isolates serves to distinguish these from the pea strains and *S. raffinolactis* which lack respiratory menaquinones. Major amounts of MK-9 have, however, been previously reported in *S. cremoris* and *S. lactis* (Collins & Jones, 1979).

The results of the biochemical tests are presented in Table 2. The pea and mastitis strains were readily distinguished from each other, and from *S. cremoris*, *S. lactis* and *S. raffinolactis*. In the study of Garvie *et al.* (1981), *S. lactis* and the mastitis isolates were found to be phenotypically indistinguishable. These taxa however can be readily distinguished using the API 50CH and 20STREP systems (Table 2).

Table 3. *Differential characteristics of S. garvieae, S. plantarum, S. cremoris, S. lactis and S. raffinolactis*

	<i>S. garvieae</i>	<i>S. plantarum</i>	<i>S. cremoris</i>	<i>S. lactis</i>	<i>S. raffinolactis</i>
Cell wall peptidoglycan type	Lys-Ala ₁ -Gly ₁ -Ala ₁	Lys-Ser ₁ -Ala ₁	Lys-D-Asp ₁	Lys-D-Asp ₁	Lys-Thr ₁ -Ala ₁
Major menaquinone	MK-9	—	MK-9	MK-9	—
Major fatty acids	C _{16:0} , C _{18:1} , Δ C _{19:0}	C _{16:0} , C _{18:1} , Δ C _{19:0}	C _{16:0} , C _{18:1} , Δ C _{19:0}	C _{16:0} , C _{18:1} , Δ C _{19:0}	C _{16:0} , C _{18:1}
G + C (mol %)	38.3–38.7	36.9–38.1	34.8–35.6	34.4–36.3	40.0–42.6

In the earlier study of Garvie *et al.* (1981) the mastitis and pea isolates were shown to be genetically quite distinct from each other, and from other group N streptococci. The results of the present study confirm this distinctiveness. The mastitis and pea strains can be distinguished from *S. raffinolactis* in possessing high levels of *cis*-11,12-methylenoctadecanoic acid, whilst they differ from *S. cremoris*, *S. lactis* and each other in peptidoglycan composition (see Table 3). The mastitis and pea isolates are also biochemically quite distinct (Table 2). Thus on the basis of the present and earlier studies (Cavett & Garvie, 1967; Garvie *et al.*, 1981) we consider the pea and mastitis strains constitute two new species. The isolates from frozen peas are named *Streptococcus plantarum* sp. nov. and those from mastitis, *Streptococcus garvieae* sp. nov.

Description of Streptococcus plantarum sp. nov. (*L. gen. pl. noun plantarum of plants*)

Colonies on blood agar or nutrient agar are circular, smooth and entire. Non-pigmented. Non-haemolytic. Spheres or ovoid cells elongated in the direction of the chain; mostly in pairs or short chains. Gram-positive, non-motile. Facultatively anaerobic. Catalase negative. Growth at 10 °C but not at 40 °C. Grows in 4% (w/v) NaCl. Chemo-organotroph: metabolism fermentative. Acid produced from amygdalin, arbutin, cellobiose, dextrin, fructose, glucose, maltose, mannose, D-mannitol, melizitose, *N*-acetylglucosamine, salicin, sucrose and trehalose. Acid not produced from adonitol, D-arabinose, L-arabinose, D-arabitol, L-arabitol, 2-ketogluconate, 5-ketogluconate, dulcitol, erythritol, D-fucose, L-fucose, galactose, gluconate, glycogen, glycerol, inositol, inulin, lactose, D-lyxose, melibiose, α -methyl-D-glucoside, α -methyl-D-mannoside, β -methylxyloside, raffinose, ribose, rhamnose, L-sorbose, D-tagatose, D-xylose, L-xylose and xylitol. Variable results may be obtained for β -gentiobiose and D-turanose. Aesculin hydrolysed. Starch, hippurate and gelatin not hydrolysed. Leucine arylamidase positive. β -Galactosidase, β -glucuronidase, arginine dehydrolase and pyrrolidonylaryl-amidase negative. Some strains α -galactosidase positive. Contains Lancefield group N antigen.

The peptidoglycan type of NCDO 1869 is Lys-serine₁-alanine₁. Menaquinones are absent. Major non-hydroxylated long-chain fatty acids are hexadecanoic, octadecanoic (*w*7) and *cis*-11,12-methylenoctadecanoic acid (Δ C_{19:0}). The guanine plus cytosine content of DNA ranges from 36.9 to 38.1 mol% as determined by melting temperature (*T_m*). The type strain is NCDO 1869. Source: frozen peas.

Description of Streptococcus garvieae sp. nov. (*garvieae* of Garvie; named for E. I. Garvie, a British microbiologist)

Colonies on blood agar or nutrient agar are circular, smooth and entire. Non-pigmented. Non-haemolytic. Ovoid cells elongated in the direction of the chain; mostly in pairs or short chains. Gram-positive, non-motile. Facultatively anaerobic. Catalase negative. Growth at 10 °C and 40 °C; no growth at 45 °C. Grows in 4% (w/v) NaCl. Chemoorganotroph: metabolism fermentative. Acid produced from galactose, glucose, fructose, cellobiose, amygdalin, arbutin, mannose, ribose, trehalose, salicin, β -gentiobiose and *N*-acetylglucosamine. Acid not produced from D-arabinose, L-arabinose, D-arabitol, L-arabitol, adonitol, 2-ketogluconate, 5-ketogluconate, dulcitol, erythritol, D-fucose, L-fucose, glycogen, inulin, inositol, melibiose, melezitose, β -methylxyloside, α -methyl-D-glucoside, α -methyl-D-mannoside, D-lyxose, raffinose, rhamnose, L-sorbose, sorbitol, D-turanose, xylitol, D-xylose and L-xylose. Variable results may be obtained for

maltose, D-mannitol, sucrose and D-tagatose. Aesculin hydrolysed. Starch and hippurate hydrolysis negative. β -Glucuronidase, arginine dehydrolase, leucine arylamidase and pyrrolidonylarylamidase positive. α -Galactosidase and β -galactosidase negative.

The peptidoglycan type of NCDO 2155 is Lys-ala₁-gly₁-ala₁. Low levels of menaquinones produced with MK-9 predominating. Major non-hydroxylated long-chain fatty acids are hexadecanoic, octadecenoic (ω 7) and *cis*-11,12-methylenoctadecanoic acid (Δ C_{19:0}). The guanine plus cytosine content of DNA ranges from 38.3 to 38.7 mol% as determined by melting temperature (T_m). The type strain is NCDO 2155. Source: bovine mastitis.

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