A study of small-colony, β -haemolytic, Lancefield group C streptococci within the anginosus group: description of *Streptococcus constellatus* subsp. *pharyngis* subsp. nov., associated with the human throat and pharyngitis

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β -Haemolytic, Lancefield group C streptococci within the anginosus-species group were shown by genetic and phenotypic criteria to be heterogeneous and to constitute two distinct taxa related at subspecies level to *Streptococcus constellatus* and *Streptococcus anginosus*, respectively. The first group, referred to here as DNA group 1, comprised six strains with 86–100% intragroup overall genomic DNA relatedness; five of the strains were originally isolated from the human throat and one was from an abdominal mass. They shared 61–77% DNA relatedness (ΔT_m values = 1.2–1.5 °C) with reference strains of *S. constellatus* and were clearly differentiated from *S. constellatus* (now named *Streptococcus constellatus* subsp. *constellatus*) by the ability to produce β -N-acetylgalactosaminidase, β -N-acetylglucosaminidase, β -D-fucosidase, β -D-galactosidase and β -D-glucosidase. The name *S. constellatus* subsp. *pharyngis* is proposed for these strains on the grounds that they are genetically and phenotypically distinct and exhibit a predilection for the human throat, being isolated also from cases of pharyngitis. The DNA G+C content is 35–37 mol%. The type strain is MM9889a^T (= NCTC 13122^T). The second group (DNA group 2) was formed by five β -haemolytic, Lancefield group C strains originally isolated from various human infections. DNA group 2 strains (81–100% intragroup DNA relatedness) shared 60–72% DNA relatedness (ΔT_m values = 2.1–4.1 °C) with *S. anginosus* strains NCTC 10713^T and MAS 283 but were not clearly differentiated phenotypically from *S. anginosus*, showed no clear pattern of clinical association, and therefore are not formally proposed as a new subspecies here.

Keywords: human throat, pharyngitis, *Streptococcus constellatus* subsp. *pharyngis* subsp. nov., *Streptococcus constellatus* subsp. *constellatus*

INTRODUCTION

Within the genus *Streptococcus*, the 'anginosus' group includes three relatively closely related species, *Streptococcus anginosus*, *Streptococcus constellatus* and *Streptococcus intermedius* as shown by DNA–DNA hybridization studies and 16S rRNA gene sequence analysis (Whiley & Beighton, 1991; Kawamura *et al.*, 1995). Collectively, these form part of the flora of the mouth and the female urogenital tract, are isolated from the gastrointestinal tract, and

are frequently associated with purulent infections in humans (Whiley *et al.*, 1992; Jacobs *et al.*, 1995). Evidence of further taxonomic heterogeneity within the group has been supported by phenotypic and genotypic criteria that include biochemical and cultural characteristics (Winstanley *et al.*, 1992; Bergman *et al.*, 1995), long-chain fatty acid composition (Cookson *et al.*, 1989), pyrolysis mass spectra (Winstanley *et al.*, 1992), DNA hybridization (Whiley & Hardie, 1989; Whiley *et al.*, 1997), and rRNA-based studies (Doitt *et al.*, 1994; Bergman *et al.*, 1995;

Table 1. DNA relatedness (%) among strains ΔT_m values (°C) are shown in parentheses.

Unlabelled DNA	Labelled DNA						
	MM9889a	I751s	MAS 624	NMH3	NCTC 10713 ^T	NCDO 2226 ^T	NCDO 2227 ^T
DNA group 1							
I751s	97 (1.5)	100	51	41	51	61	46
M5823	96 (1.0)	100	51 (7.5)	50	47	62	46
MM4055	98 (0.0)	86		46		61	49
I1299	94	89		43			
MM9889a	100	89	49 (6.25)		58	74 (1.5)	50
MM6375	100 (1.5)	100		50		63	
<i>S. constellatus</i>							
NCDO 2226 ^T	74 (1.2)	77	50 (7.4)	45	58 (7.9)	100	56 (8.0)
NCTC 10714	72 (1.2)		51	39	44	85	
DNA group 2							
MAS624	58		100	91	72 (2.2)		
NMH3		55		100	69		
MAS158	57 (4.5)		88 (0.0)	86	63	48	44
W558		56	90 (0.1)	81	67		
MM5068	57 (5.0)		100 (0.0)	100	67 (2.1)	49	42
<i>S. anginosus</i>							
NCTC 10713 ^T	50 (6.4)	55 (6.5)	67 (4.1)	60	100	44	36
MAS283	50	49		62	65	43	
<i>S. intermedius</i>							
NCDO 2227 ^T	55 (2.6)	48	41 (6.0)	42	43 (9.4)	49	100

Whiley *et al.*, 1997). In a recent investigation undertaken in our laboratory, 21 strains identified as *S. anginosus* were shown to exhibit sufficient heterogeneity to warrant division at both species and subspecies level, although the numbers of strains belonging to these taxa were too few to support any formal taxonomic proposals (Whiley *et al.*, 1997). Of particular interest in that study were four β -haemolytic, Lancefield group C strains that formed two groups. The first group included two strains (I751s and M5823) with the following properties: they shared 100 and 60% DNA relatedness under optimum (60 °C) and stringent (75 °C) hybridization conditions, respectively; they possessed a 380 bp 16S–23S rRNA (rRNA) intergenic spacer; they produced unique ribotype patterns from *Hind*III genomic digests; they were unusual in being able to produce β -galactosidase; and they shared less than 50% overall DNA relatedness with other anginosus-group strains tested under stringent hybridization conditions. The strains comprising the second group (strains NMH3 and W558) were characterized by a 600 bp intergenic spacer, β -galactosidase activity, the production of hyaluronidase, and shared 87% interstrain DNA relatedness under stringent hybridization conditions. The latter data indicated that the second group might warrant subspecies status (Whiley *et al.*, 1997).

In view of several reports on the possible clinical significance of Lancefield group C strains that resembled *S. anginosus* (Lawrence *et al.*, 1985; Fox *et al.*, 1993; Vance, 1992; Willcox *et al.*, 1993; Willcox, 1995), together with the fact that too few strains have been sufficiently characterized to allow any formal taxonomic proposals (Whiley *et al.*, 1997), this study was undertaken with the purpose of clarifying the taxonomic position of these streptococci. On that basis, more extensive DNA reassociation studies and phenotypic characterization were applied to an increased number of strains.

METHODS

Bacterial strains and growth conditions. The following reference strains were used: the *S. anginosus* type strain, NCTC 10713^T (ATCC 33397^T, DSM 20536^T); the *S. intermedius* type strain, NCDO 2227^T (NCTC 11324^T, ATCC 27225^T); the *S. constellatus* type strain, NCDO 2226^T (NCTC 11325^T, ATCC 27823^T); and the *S. constellatus* reference strain, NCTC 10714. Clinical strains used were as follows: strains I751s and I1299 (from throats) and M5823 (from an abdominal mass) were originally obtained from T. Winstanley, Royal Hallamshire Hospital, Sheffield, UK; strain W558 (from an arm-burn swab) and strains MM4055,

MM9889a, MM6375 and MM5068 (from the throats of subjects with pharyngitis) were obtained from the Department of Medical Microbiology, St Bartholomew's and the Royal London School of Medicine and Dentistry, London, UK; and strains MAS 158, MAS 624 and MAS 283 (from abdominal infections) were obtained from Dr Jan Jacobs, Department of Medical Microbiology, University Hospital of Maastricht, Maastricht, The Netherlands. Most of the strains were received as β -haemolytic, Lancefield group C (or Lancefield group G in the case of strains NCTC 10713^T and MM5068) *S. anginosus*. There were some exceptions to this: strain MAS 283 belonged to Lancefield group C but was non-haemolytic; and the type strains NCDO 2226^T (*S. constellatus*) and NCDO 2227^T (*S. intermedius*) were both serologically ungroupable against Lancefield grouping antisera and were non-haemolytic.

Strains were maintained in the laboratory on Columbia blood-agar plates (Gibco-BRL, Life Technologies) containing 5% (v/v) defibrinated horse blood (TCS Microbiological), incubated at 37 °C in an atmosphere containing, by volume, 20 % hydrogen, 10% CO₂ and 70% nitrogen. Cells for large-scale preparation of DNA for hybridization experiments and DNA minipreps for PCR were obtained by growing strains in brain-heart infusion broth (Oxoid Unipath) as described previously (Whiley *et al.*, 1997).

DNA reassociation and ΔT_m determinations. DNA was prepared from approximately 3 g (wet wt) of cells according to the method originally described by Garvie (1976). DNA purities and concentrations were determined spectrophotometrically at 230, 260, 280 and 300 nm. Radioactive DNA was obtained by simultaneous labelling with both [1',2',5-³H]dCTP and [1',2'-³H]dGTP by nick translation using a commercial kit according to the manufacturer's instructions (Amersham International).

Labelled DNA was prepared from the type strains of *S. anginosus* (NCTC 10713^T), *S. constellatus* (NCDO 2226^T) and *S. intermedius* (NCDO 2227^T), and from β -haemolytic, Lancefield group C strains MM9889a, I751s, MAS 624 and NMH3. DNA relatedness was determined using the S1 nuclease-trichloroacetic acid method (Grimont, 1988): briefly, approximately 10 ng labelled and 75 μ g unlabelled DNA were heat-denatured in 0.042 M NaCl, the final NaCl concentration was adjusted to 0.42 M (final volume, 0.5 ml), and hybridization was allowed to proceed at 60 °C for 18 h. The T_m value (i.e. the temperature at which 50% of the reassociated DNA became hydrolysable by S1 nuclease) was determined for those strains from which radioactive reference DNA had been prepared; the ΔT_m value (i.e. the difference between the T_m value of the homologous reaction and that of a heterologous reaction) was used to estimate the level of divergence between two strains (Brenner, 1978).

DNA base composition determinations. G + C compositions were determined in 0.15 M NaCl and 0.015 M trisodium citrate (standard saline citrate) by thermal melting (Marmur & Doty, 1962) as previously described (Whiley & Hardie, 1989).

PCR amplification of the 16S–23S rRNA intergenic spacer regions. Cell growth, treatment, lysis and PCR amplification were performed as previously described (Whiley *et al.*, 1995).

Phenotypic tests. All strains were tested for preformed enzyme activities (β -N-acetylgalactosaminidase, β -N-acetylglucosaminidase, α -arabinosidase, α -L-fucosidase, β -D-fucosidase, α -D-galactosidase, β -D-galactosidase, α -D-glu-

cosidase, β -D-glucosidase, β -D-glucuronidase and sialidase), for acid production from carbohydrate substrates (amygdalin, arbutin, inulin, β -N-acetylglucosamine, lactose, mannitol, melibiose, sorbitol and raffinose), for the production of acetoin (Voges–Proskauer test) and for the hydrolysis of arginine and aesculin, as described previously (Beighton *et al.*, 1991; Whiley & Beighton, 1991). Tests for the activities of preformed enzymes on 4MU-linked fluorogenic substrates were carried out using cells suspended in 50 mM TES buffer (pH 7.5) to an optical density of 0.4 at 620 nm and were scored by visual assessment as either positive (including weak activities above that of the negative controls) or negative. Hyaluronidase activity was detected using the method of Smith & Willet (1968). Lancefield serological group reactions were detected with a commercial kit (Oxoid Unipath). Haemolytic reactions were determined on layered Columbia blood-agar plates (Gibco-BRL, Life Technologies) containing 5% (v/v) defibrinated horse blood (TCS Microbiological), incubated anaerobically at 37 °C for 24 and 48 h.

Those strains that had been received together with accompanying biochemical or cultural data were, nevertheless, re-examined in all the above tests at the commencement of this study and the results obtained reported here.

RESULTS

DNA reassociation and ΔT_m determinations

The results of these experiments are shown in Table 1. All of the β -haemolytic, Lancefield group C strains fell into one of two DNA reassociation groups (referred to as DNA groups 1 and 2 for the purposes of this communication), each showing $\geq 81\%$ intragroup overall genomic sequence relatedness and ΔT_m values of ≤ 5 °C. Intergroup DNA relatedness values for these two DNA groups were 41–58% and ΔT_m values were 4.5–7.5 °C. DNA group 1 included β -haemolytic, Lancefield group C strains I751s, M5823, MM4055, I1299, MM9889a and MM6375 while DNA group 2 comprised Lancefield group C strains NMH3, MAS 624, MAS 158 and W558 together with Lancefield group G strain MM5068.

DNA group 1 also exhibited a weaker, although still relatively close, relationship with *S. constellatus* strains NCDO 2226^T and NCTC 10714, with overall reassociation values of 61–77% and ΔT_m values of 1.2–1.5 °C. DNA group 2 shared 60–72% DNA relatedness and showed ΔT_m values of 2.1–4.1 °C with *S. anginosus* strains NCTC 10713^T and MAS 283. Reassociation levels with *S. intermedius* type strain NCDO 2227^T were 46–55% ($\Delta T_m = 2.6$ °C) and 41–44% ($\Delta T_m = 6.0$ °C) for DNA groups 1 and 2, respectively.

16S rRNA intergenic spacer-size determinations

All strains in DNA group 1 produced a 380 bp PCR product, as did both of the *S. constellatus* strains tested (NCDO 2226^T and NCTC 10714). The DNA group 2 strains all produced a 600 bp product, *S. anginosus* strains NCTC 10713^T and MAS 283 gave a 450 bp

product, and the type strain of *S. intermedius*, NCDO 2227^T, gave a 330 bp PCR product.

Phenotypic characterization

All strains were positive in the Voges–Proskauer test and did not produce α -D-arabinosidase, α -L-fucosidase, α -D-galactosidase or β -D-glucuronidase. All strains produced acid from β -D-N-acetylglucosamine, and both hydrolysed arginine and aesculin. Acid was not produced from inulin, mannitol, raffinose, sorbitol or melibiose. The variable reactions for the strains studied, including typical test results for *S. anginosus*, *S. constellatus* and *S. intermedius* obtained using the test methods employed here, are shown in Table 2.

DISCUSSION

The results of the DNA relatedness studies demonstrate a close relationship between DNA group 1 and *S. constellatus*, and between DNA group 2 and *S. anginosus*. We believe that the 61–77% range in DNA relatedness and the ΔT_m values of 1.2–1.5 °C obtained between DNA group 1 strains and *S. constellatus*, when considered along with the high intra-DNA group 1 levels (86–100%) and lower ΔT_m values (0.0–1.5 °C), allows for the division of *S. constellatus* into two subspecies, according to currently accepted guidelines (Wayne *et al.*, 1987; Grimont, 1988). The DNA reassociation data in this case are supported by clear discrimination of DNA group 1 strains from ‘typical’ *S. constellatus* strains on the basis of phenotypic testing; we feel that the proposal to recognize these strains as a distinct subspecies is further justified on clinical grounds by the fact that they exhibit an association with human throats and pharyngitis in particular.

The situation with DNA group 2 strains is different. Although these form a discrete sub-group, having a close relationship to *S. anginosus* (on the basis of DNA reassociation), and are characterized by a distinctive 600 bp 16S–23S rRNA intergenic spacer, as previously described (Whiley *et al.*, 1995), their unequivocal differentiation by biochemical testing is not possible at present. On the one hand, these strains have β -D-galactosidase activity (in contrast to ‘typical’ *S. anginosus* strains) but on the other hand, it has been demonstrated that the results of this test can be strongly influenced by the incubation conditions employed (Ahmet *et al.*, 1995). At best, only a single phenotypic test difference has been found between these and other *S. anginosus* strains (under our experimental conditions) and no distinct pattern of association with particular clinical infections or anatomical sites has been observed so far; consequently, there are insufficient grounds for proposing subspecies status for DNA group 2 strains at present. On the basis of the DNA reassociation data reported here from reciprocal experiments, DNA groups 1 and 2 are

clearly less closely related to *S. intermedius*. This observation supports our previously reported conclusions regarding the DNA relatedness of these taxa (Whiley *et al.*, 1997). The surprisingly low ΔT_m value of 2.6 °C between DNA group 1 strains MM9889a and NCDO 2227^T, though still significantly greater than the 0.0–1.5 °C range obtained between DNA group 1 strains, requires confirmation in future taxonomic studies on these streptococci. The doubt cast by us previously on the suitability of strain NCTC 10713^T as the type strain of *S. anginosus* is reinforced here by the low reassociation level (65%) obtained with a typical *S. anginosus* strain (MAS 283). This observation further supports our decision, here, not to make limited taxonomic proposals for *S. anginosus*; this species clearly needs to be the subject of a larger study involving many more strains. Virtually all of the results obtained here confirm or extend those obtained in our previous study (Whiley *et al.*, 1997): in the latter case, DNA reassociation experiments performed under optimum (60 °C) and stringent (75 °C) conditions gave 100 and 60% homology, respectively, between strains I751s and M5823 (both were within homology group 1 in the present study) and 87% homology between strains W558 and NMH3 (both were within DNA group 2 in the present study). We have now determined (by agarose gel electrophoresis) that the low reassociation level (16%) previously obtained between strain I751s and *S. constellatus* type strain NCDO 2226^T was the result of degradation of the DNA preparation that was being used at the time; this problem led to the erroneous conclusion in that paper that strains I751s and M5823 were representatives of a new species (Whiley *et al.*, 1997). In contrast, the subspecies-level relationship between DNA group 1 strains and strain NCDO 2226^T revealed in the present study is also supported by the result obtained here between *S. constellatus* reference strain NCTC 10714 and another DNA group 1 strain, MM9889a (72% DNA relatedness, $\Delta T_m = 1.2$ °C) and also by the presence of a 380 bp 16S–23S rRNA intergenic spacer within DNA group 1 strains and *S. constellatus*, as shown previously (Whiley *et al.*, 1995). Furthermore, the low DNA reassociation levels [22% (optimum) and 20% (stringent)] obtained previously between *S. anginosus* type strain NCTC 10713^T and strain NCDO 2226^T when inadvertently using the degraded DNA from strain NCDO 2226^T, are in contrast to the significantly higher values consistently obtained between these two strains in both the present study and on other previous occasions (Whiley & Hardie, 1989).

The biochemical test results reveal that the majority of the DNA group 1 strains produce hyaluronidase, with the exception of strains I751s and M5823 (which have remained negative for this characteristic in repeated testing). The initial impression gained during our previous study when only these two strains were available for examination, namely, that this taxon was characteristically hyaluronidase-negative, has not been confirmed here; this vindicates the decision made then

Table 2. Biochemical characteristics of strains

Abbreviations refer to the production of the following: Haem, haemolysis on blood agar; Hyal, hyaluronidase; β -D-fuc, β -D-fucosidase; β -NA gal, β -N-acetyl-galactosaminidase; Sial, sialidase (neuraminidase); β -NA glu, β -N-acetylglucosaminidase; α -D-glu, α -D-acetylglucosaminidase; β -D-glu, β -D-glucosidase; β -D-gal, β -D-galactosidase; Amyg, acid from amygdalin; Arb, acid from arbutin; Lac, acid from lactose. Typical species reactions are taken from Whiley & Beighton (1991). NG, non-groupable against Lancefield grouping antisera; NH, non-haemolytic.

Strain	Lancefield grp	Haem	Hyal	β -D-fuc	β -NA gal	Sial	β -NA glu	α -D-glu	β -D-glu	β -D-gal	Amyg	Arb	Lac
DNA group 1													
I751s	C	β	—	+	+	—	—	+	+	+	+	+	+
M5823	C	β	—	+	+	—	+	+	+	+	+	+	+
MM4055	C	β	+	+	+	—	+	+	+	+	+	+	+
I1299	C	β	+	+	+	—	+	+	+	+	+	+	+
MM9889a	C	β	+	+	+	—	+	+	+	+	+	+	+
MM6375	C	β	+	+	+	—	+	+	+	+	+	+	+
<i>S. constellatus</i>													
NCDO 2226 ^T	NG	NH	+	—	—	—	—	+	—	—	—	—	+
NCTC 10714	F	β	+	—	—	—	—	+	—	—	—	—	—
Typical species reactions	F/NG (C, G, A)	β , NH	+	—	—	—	—	+	—	—	±	+	±
DNA group 2													
NMH3	C	β	+	—	—	—	—	—	+	+	+	+	+
MAS 624	C	β	+	—	—	—	+	—	+	+	+	+	+
MAS158	C	β	+	—	—	—	—	—	+	+	+	—	+
W558	C	β	+	—	—	—	—	—	+	+	+	—	+
MM5068	G	β	+	—	—	—	+	—	+	+	+	+	+
<i>S. anginosus</i>													
NCTC 10713 ^T	G	β	—	—	—	—	—	—	+	—	+	+	+
MAS 283	C	NH	—	—	—	—	—	—	+	—	+	+	+
Typical species reactions	F/NG (C, G, A)	NH, β	—	—	—	—	—	±	+	—	+	+	+
<i>S. intermedius</i>													
NCDO 2227 ^T	NG	NH	+	+	+	+	+	+	+	+	+	+	+
Typical species reactions	NG/F(C)	NH, β	+	+	+	+	+	+	±	+	±	+	+

not to make any formal taxonomic proposals until further strains had been studied.

We are aware that the present proposal to further divide *S. constellatus* into two subspecies may be considered by some to be unfounded in the belief that the anginosus group should be considered as a single species (Vandamme *et al.*, 1998). We have previously acknowledged that members of this species group are relatively closely related when compared to many examples within the oral streptococci, but we cannot agree with the proposal that these should be reduced to single-species status. The levels of relatedness obtained by us with the S1 nuclease/trichloroacetic acid approach in these experiments demonstrate unequivocally that the type strains of *S. anginosus* (NCTC 10713^T), *S. constellatus* (NCDO 2226^T) and *S. intermedius* (NCDO 2227^T) represent separate species. We have observed this both when examining the group previously under optimum and stringent hybridization conditions

(Whiley & Hardie, 1989; Whiley *et al.*, 1997) and in the present study of ΔT_m values. Similar conclusions have also been reached by other workers using the filter method for DNA hybridization (Kilpper-Bälz *et al.*, 1984). The DNA homology data, together with the phenotypic differentiation of *S. anginosus*, *S. constellatus* and *S. intermedius* (Whiley & Beighton, 1991; Ahmet *et al.*, 1995; Jacobs *et al.*, 1995) and the strong clinical association patterns for these taxa (Whiley *et al.*, 1992; Jacobs *et al.*, 1995), refute the idea that these constitute a single species. The evidence reported in this paper supports the further subdivision of *S. constellatus* into two subspecies.

Furthermore, it is clear to us from the results of this and our previous study that more taxa exist within the anginosus group than are currently recognized. It remains to be determined whether these taxa are distinct species and subspecies or constitute nodes within one large continuum of types; such clarification

will require future examination of many more strains and intermediate/atypical isolates, and lies beyond the scope of this paper.

Description of *Streptococcus constellatus* Prevot 1924 emend. Holdman and Moore 1974, emend. Whiley and Beighton 1991

The description of *Streptococcus constellatus* is the same as that given by Whiley & Beighton (1991) except that it is amended to include some strains producing β -*N*-acetylgalactosaminidase, β -*N*-acetylglucosaminidase, β -fucosidase, β -galactosidase and β -glucosidase. *S. constellatus* was described by Whiley & Beighton (1991) as including very few strains producing β -galactosidase or β -glucosidase and no strains able to produce β -*N*-acetylglucosaminidase or β -fucosidase. The proportion of strains producing β -*N*-acetylgalactosaminidase was not included in the previous description.

Description of *Streptococcus constellatus* subsp. *constellatus* subsp. nov.

The description of *Streptococcus constellatus* subsp. *constellatus* is based on the previously reported characteristics of 50 clinical and reference strains of *Streptococcus constellatus* (emend.) (Whiley & Beighton, 1991). This subspecies is the type subspecies of *S. constellatus* and contains the type strain according to Rules 40a and 40b of the Bacteriological Code (Lapage *et al.*, 1992).

Small (0.5–1.0 μ m in diameter), Gram-positive, non-spore, non-motile cocci in short chains. Catalase-negative. Colonies on blood agar are 0.5–2.0 mm in diameter, white or translucent, and convex with entire margins; some strains produce colonies that are 0.5–1.0 mm in diameter, white and matte. Growth is reduced under aerobic conditions and is frequently enhanced by the addition of CO₂. Some strains require anaerobic conditions for growth. Acetoin is produced. Arginine and aesculin are hydrolysed, but urea is not. Hippurate is not split. Alkaline phosphatase and leucine arylamidase are produced. Most strains produce α -glucosidase but very few produce β -galactosidase and β -glucosidase. β -*N*-acetylglucosaminidase, β -D-fucosidase, α -galactosidase, β -glucuronidase, pyrrolidonylarylamidase and sialidase are not produced. Acid is produced from glucose and frequently from salicin and trehalose. Lactose fermentation and amygdalin fermentation are variable characteristics of this species. A few strains produce acid from cellobiose, melibiose and raffinose. Arabinose, glycerol, inulin and sorbitol are not fermented. Few strains produce hydrogen peroxide. Most strains produce hyaluronidase. Strains are frequently β -haemolytic and belong to Lancefield group F or are non-haemolytic (α and γ) and serologically ungroupable. A few strains react with Lancefield group A, C and G antisera. The habitats are oral cavities and upper

respiratory tracts. Strains are isolated from human purulent infections including appendicitis. The DNA G+C content is 37–38 mol%.

Description of the type strain. The type strain of *Streptococcus constellatus* subsp. *constellatus* is NCDO 2226^T (= NCTC 11325^T = ATCC 27823^T), which was the type strain of *S. constellatus* (Whiley & Beighton, 1991). It has all of the uniform characteristics of the subspecies described above, is serologically upgroupable against Lancefield grouping antisera, is non-haemolytic, produces hyaluronidase and α -glucosidase, and does not produce β -*N*-acetylglucosaminidase, β -D-fucosidase, α -galactosidase, β -galactosidase, β -glucosidase, β -glucuronidase, pyrrolidonylarylamidase or sialidase. Lactose is fermented but amygdalin is not.

Description of *Streptococcus constellatus* subsp. *pharyngis* subsp. nov.

Streptococcus constellatus subsp. *pharyngis* (pha.ryn'gis. Gr. n. *pharynx* throat; Gr. gen. n. *pharyngis* of the throat).

The description below is based on the characteristics of the six strains included in this study. Small (0.5–1.0 μ m in diameter), Gram-positive, non-spore-forming, non-motile cocci in short chains. Catalase-negative. Colonies on blood agar are 0.5–2.0 mm in diameter, white or translucent, and convex with entire margins. Growth is reduced under aerobic conditions and is enhanced by the addition of CO₂. Acetoin is produced. Arginine and aesculin are hydrolysed. Urea and hippurate are not hydrolysed. Alkaline phosphatase is produced. Strains produce β -*N*-acetylgalactosaminidase, β -D-fucosidase, β -galactosidase, α -glucosidase and β -glucosidase. The majority of strains produce β -*N*-acetylglucosaminidase. Strains do not produce α -galactosidase, β -glucuronidase, pyrrolidonylarylamidase or sialidase. Acid is produced from amygdalin, arbutin, lactose, glucose or β -D-*N*-acetylglucosamine. Acid is not produced from inulin, mannitol, melibiose, raffinose or sorbitol. The majority of strains produce hyaluronidase. Strains are β -haemolytic and belong to Lancefield group C. Strains were isolated from infections of the human throat (pharyngitis) and clinical specimens (strains M5823 was from an abdominal mass). The DNA G+C content is 35–37 mol%.

Description of the type strain. The type strain of *Streptococcus constellatus* subsp. *pharyngis* is MM9889a^T (= NCTC 13122^T), which was isolated from the human throat in a subject with pharyngitis. It has all of the uniform characteristics described above and produces β -*N*-acetylglucosaminidase and hyaluronidase. The DNA G+C content is 35 mol%.

Distinguishing characteristics. The phenotypic characteristics that are useful for distinguishing *S. constellatus* subsp. *pharyngis* from *S. constellatus* subsp. *constellatus*, and from the closely related species *S.*

anginosus and *S. intermedius*, are summarized in Table 2. *S. constellatus* subsp. *pharyngis* can be distinguished from *S. constellatus* subsp. *constellatus* by the ability of the former to produce β -N-acetylgalactosaminidase, β -N-acetylglucosaminidase, β -D-fucosidase, β -D-galactosidase and β -D-glucosidase. It can be differentiated from *S. intermedius* by an inability to produce sialidase and by the possession of a 380 bp 16S–23S rRNA intergenic spacer, and from *S. anginosus* by the production of β -N-acetylgalactosaminidase, β -N-acetylglucosaminidase, β -D-fucosidase, β -D-galactosidase, α -D-glucosidase, β -D-glucosidase and hyaluronidase.

REFERENCES

- Ahmet, Z., Warren, M. & Houang, E. T. (1995). Species identification of members of the *Streptococcus milleri* group isolated from the vagina by ID 32 Strep System and differential phenotypic testing. *J Clin Microbiol* **33**, 1592–1595.
- Beighton, D., Hardie, J. M. & Whiley, R. A. (1991). A scheme for the identification of viridans streptococci. *J Med Microbiol* **35**, 367–372.
- Bergman, S., Selig, M. S., Collins, M. D., Farrow, J. A. E., Baron, E. J., Dickersin, G. R. & Ruoff, K. L. (1995). '*Streptococcus milleri*' strains displaying a gliding type of motility. *Int J Syst Bacteriol* **45**, 235–239.
- Brenner, D. J. (1978). Characterization and clinical identification of *Enterobacteriaceae* by DNA hybridization. *Prog Clin Pathol* **7**, 71–117.
- Cookson, B., Talsania, S., Chinn, S. & Phillips, I. (1989). A qualitative and quantitative study of the cellular fatty acids of '*Streptococcus milleri*' with capillary gas chromatography. *J Gen Microbiol* **135**, 831–838.
- Doitt, C., Grimont, F., Whiley, R. A., Regnault, B., Grimont, P. A. D., Hardie, J. M. & Bouvet, A. (1994). Ribotypes of the '*Streptococcus milleri*-group' allow discrimination between strains of *Streptococcus constellatus*, *Streptococcus intermedius* and *Streptococcus anginosus*. In *Pathogenic Streptococci Present and Future*, pp. 531–532. Edited by A. Totolian. St Petersburg, Russia: Lancer Publications.
- Fox, K., Turner, J. & Fox, A. (1993). Role of beta-hemolytic streptococci in pharyngitis: incidence and biochemical characteristics of *Streptococcus equisimilis* and *Streptococcus anginosus* in patients and healthy controls. *J Clin Microbiol* **31**, 804–807.
- Garvie, E. I. (1976). Hybridization between the deoxyribonucleic acid of some strains of hetero-fermentative lactic acid bacteria. *Int J Syst Bacteriol* **26**, 166–122.
- Grimont, P. A. D. (1988). Use of DNA reassociation in bacterial classification. *Can J Microbiol* **34**, 541–546.
- Jacobs, J. A., Pietersen, H. G., Stobberingh, E. E. & Soeters, P. B. (1995). *Streptococcus anginosus*, *Streptococcus constellatus* and *Streptococcus intermedius*. Clinical relevance, hemolytic and serologic characteristics. *Am J Clin Pathol* **104**, 547–553.
- Kawamura, Y., Hou, X.-G., Sultana, F., Miura, H. & Ezaki, T. (1995). Determination of 16S rRNA sequences of *Streptococcus mitis* and *Streptococcus gordonii* and phylogenetic relationships among members of the genus *Streptococcus*. *Int J Syst Bacteriol* **45**, 406–408.
- Kilpper-Bälz, R., Williams, B. L., Luttiken, R. & Schleifer, K. H. (1984). Relatedness of '*Streptococcus milleri*' with *Streptococcus anginosus* and *Streptococcus constellatus*. *Syst Appl Microbiol* **5**, 494–500.
- Lapage, S. P., Sneath, P. H. A., Lessel, E. F., Skerman, V. B. D., Seeliger, H. P. R. & Clark, W. A. (editors) (1992). *International Code of Nomenclature of Bacteria (1990 Revision). Bacteriological Code*. Washington, DC: American Society for Microbiology.
- Lawrence, J., Yuko, D. M. & Hadley, W. K. (1985). Incidence and characterization of beta-hemolytic *Streptococcus milleri* and differentiation from *S. pyogenes* (group A), *S. equisimilis* (group C), and large-colony group C streptococci. *J Clin Microbiol* **22**, 772–777.
- Marmur, J. & Doty, P. (1962). Determination of the base composition of deoxyribonucleic acid from its thermal denaturation temperature. *J Mol Biol* **5**, 109–118.
- Smith, R. F. & Willet, N. P. (1968). Rapid plate method for screening hyaluronidase- and chondroitin sulfatase-producing microorganisms. *Appl Microbiol* **16**, 1434–1436.
- Vance, D. W. (1992). Group C streptococci: '*Streptococcus equisimilis*' or *Streptococcus anginosus*? *Clin Infect Dis* **14**, 616.
- Vandamme, P., Torck, U., Falsen, E., Pot, B., Goossens, H. & Kersters, K. (1998). Whole-cell protein electrophoretic analysis of viridans streptococci: evidence for heterogeneity among *Streptococcus mitis* biovars. *Int J Syst Bacteriol* **48**, 117–125.
- Wayne, L. G., Brenner, D. J., Colwell, R. R. & 9 other authors (1987). International Committee on Systematic Bacteriology. Report of the ad hoc committee on reconciliation of approaches to bacterial systematics. *Int J Syst Bacteriol* **37**, 463–464.
- Whiley, R. A. & Beighton, D. (1991). Emended descriptions and recognition of *Streptococcus constellatus*, *Streptococcus intermedius* and *Streptococcus anginosus* as distinct species. *Int J Syst Bacteriol* **41**, 1–5.
- Whiley, R. A. & Hardie, J. M. (1989). DNA–DNA hybridization studies and phenotypic characterization of strains within the '*Streptococcus milleri* group'. *J Gen Microbiol* **135**, 2623–2633.
- Whiley, R. A., Beighton, D., Winstanley, T. G., Fraser, H. Y. & Hardie, J. M. (1992). *Streptococcus intermedius*, *Streptococcus constellatus*, and *Streptococcus anginosus* (the *Streptococcus milleri* group): association with different body sites and clinical infections. *J Clin Microbiol* **30**, 243–244.
- Whiley, R. A., Duke, B., Hardie, J. M. & Hall, L. M. C. (1995). Heterogeneity among 16S–23S rRNA intergenic spacers of species within the '*Streptococcus milleri* group'. *Microbiology* **141**, 1461–1467.
- Whiley, R. A., Hall, L. M. C., Hardie, J. M. & Beighton, D. (1997). Genotypic and phenotypic diversity within *Streptococcus anginosus*. *Int J Syst Bacteriol* **47**, 645–650.
- Willcox, M. D. P. (1995). Potential pathogenic properties of the '*Streptococcus milleri*' group in relation to the production of endocarditis and abscesses. *J Med Microbiol* **43**, 405–410.
- Willcox, M. D. P., Patrikakis, M., Loo, C. Y. & Knox, K. W. (1993). Albumin binding proteins on the surface of the *Streptococcus milleri* group and characterization of the albumin receptor of *Streptococcus intermedius*. *J Gen Microbiol* **139**, 2451–2458.
- Winstanley, T. G., Magee, J. T., Limb, D. I., Hindmarch, J. M., Spencer, R. C., Whiley, R. A., Beighton, D. & Hardie, J. M. (1992). A numerical taxonomic study of the '*Streptococcus milleri*' group based upon conventional phenotypic tests and pyrolysis mass spectrometry. *J Med Microbiol* **36**, 149–155.