DNA Base Composition, DNA–DNA Homology and Long-chain Fatty Acid Studies on *Streptococcus thermophilus* and *Streptococcus salivarius*

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DNA base composition, DNA–DNA homology and long-chain fatty acid studies were performed on *Streptococcus thermophilus* and *Streptococcus salivarius*. These species possess similar mol % G + C values (about 37 to 41), long-chain fatty acid profiles and belong to a single DNA homology group. On the basis of the present and earlier studies it is proposed that *Streptococcus thermophilus* (Orla-Jensen) be reclassified as *Streptococcus salivarius* subsp. *thermophilus* comb. nov.

INTRODUCTION

The species *Streptococcus thermophilus* was described and named by Orla-Jensen (1919) during his studies on the bacteriology of lactic acid bacteria occurring in milk and dairy products. The taxonomic interrelationships of *S. thermophilus* have, however, always been controversial (see Jones, 1978). Sherman (1937) classified *S. thermophilus* in his 'viridans' division of the streptococci which encompassed *S. bovis*, *S. equinus* and *S. salivarius*. London & Kline (1973), on the basis of FDP aldolase studies, grouped *S. thermophilus* with *S. lactis* and *S. cremoris*. The DNA–DNA homology studies of Ottogalli *et al.* (1979) however showed no close relationship between *S. thermophilus* and streptococci of serological group N. Indeed, DNA–rRNA hybridization studies (Garvie & Farrow, 1981; Kilpper-Bälz *et al.*, 1982) have shown that *S. thermophilus* is unrelated to *S. lactis* and *S. cremoris* at the generic level, but indicate that it is related at the generic level to *S. bovis*, *S. equinus* and *S. salivarius*. Furthermore, Kilpper-Bälz *et al.* (1982) showed high DNA–DNA homology (about 75 to 97%) between four strains of *S. thermophilus* and *S. salivarius*. The recent numerical phenetic study of Bridge & Sneath (1983) however showed no such close relationship between these species. Indeed, in the latter study *S. thermophilus* and *S. salivarius* were recovered as two quite distinct phena.

In the present study, mol % G + C, DNA–DNA hybridization and long-chain fatty acid studies have been performed on a large number of *S. thermophilus* and *S. salivarius* strains in an attempt to clarify their taxonomic interrelationships.

METHODS

Strains. The test strains shown in Table 1 were obtained from the National Collection of Dairy Organisms (NCDO), Shinfield, Reading, UK.

DNA preparation and base composition determination. Preparation of DNA followed a modification (Farrow *et al.*, 1983) of the method of Garvie (1976). The DNA base composition was estimated by thermal denaturation in standard saline citrate as described by Garvie (1978), using DNA from *Leuconostoc mesenteroides* NCDO 768 and *Escherichia coli* K12 NCDO 1984 as standards.

DNA–DNA hybridizations. These were performed under optimum conditions using the membrane filter technique described previously (Garvie *et al.*, 1981).

Analysis of fatty acids. Strains were grown in YGPB (Garvie, 1978) at 30°C for 2 d. Broth cultures were checked for purity on blood agar plates before harvesting by centrifugation. Cells were washed with distilled water and freeze-dried. Dry cells (about 50 mg) were examined using the acid methanolysis and thin-layer chromatography (TLC) procedure described by Minnikin *et al.* (1975). Fatty acid methyl esters were purified on Kieselgel 60

plastic-backed sheets (Merck 5735; 10 × 10 cm) using petroleum ether (b.p. 60–80°C)/diethyl ether (85:15, v/v) as developing solvent. A Carlo Erba (Swindon, UK) Fractovap series 4160 flame-ionization gas chromatograph was used for the analysis of fatty acid methyl esters. Analyses were performed on a glass 25 m OV-1 (Carlo Erba) wall-coated open tubular (WCOT) column, operated isothermally at 175 °C using helium as carrier gas (1.5 ml min⁻¹). The identity of individual esters was established by comparison of the retention times with those of standard straight-chain saturated and monounsaturated esters. The presence of unsaturated esters was confirmed by hydrogenation using H₂ with Pd on charcoal as catalyst. Cyclopropane-ring containing esters were not affected by this treatment.

RESULTS AND DISCUSSION

The mol % G + C contents of the test strains are shown in Table 1. *Streptococcus salivarius* and *S. thermophilus* strains possessed mol % G + C ranges of 39.5 to 41.0 and 37.2 to 39.8, respectively. These data are in good agreement with the previous reports of about 38 to 42 mol % G + C within these species (Deibel & Seeley, 1974; Kilpper-Bälz *et al.*, 1982).

The results of DNA–DNA hybridization experiments are shown in Table 2. *Streptococcus salivarius* and *S. thermophilus* strains formed a single homology group, 61 to 100% related to the type strain of *S. salivarius* NCDO 1779, and 67 to 97% related to the type strain of *S. thermophilus* NCDO 573 under optimum hybridization conditions. The type strains of the two species were approximately 70% related to each other (Table 2). Strains of *S. salivarius* which lack the group K antigen were not significantly different from the group K-containing strains in their homology values with either label. The type strains of *S. mitis*, *S. mutans*, *S. oralis*, *S. sanguis* and '*S. milleri*' exhibited <43% homology to *S. salivarius* NCDO 1779 and <51% homology to *S. thermophilus* NCDO 573 (see Table 2). Kilpper-Bälz *et al.* (1982) recently reported high DNA homology (about 75 to 97%) between two strains of *S. salivarius* and two strains of *S. thermophilus*. The high degree of overall DNA base sequence similarity between *S. salivarius* and *S. thermophilus* in the present study substantiates the findings of Kilpper-Bälz *et al.* (1982) and indicates that these taxa represent a single genetical species.

Whole-organism methanolysates of the test strains showed the presence upon TLC of single spots corresponding to non-hydroxylated long-chain fatty acid methyl esters. The non-hydroxylated fatty acids were composed of predominantly straight-chain saturated and monounsaturated acids. Cyclopropane-ring acids were either absent, or present in only trace amounts (Table 3). *Streptococcus salivarius* and *S. thermophilus* possessed similar fatty acid profiles with hexadecanoic (C_{16:0}), octadecenoic (C_{18:1}) and eicosenoic (C_{20:1}) acids constituting the major components. It is worth noting that the presence of substantial levels of oleic acid (C_{18:1}, ω9) in many of the strains were unexpected (Table 3). Members of the genus *Streptococcus* generally synthesize *cis*-vaccenic acid (C_{18:1}, ω7) (Collins *et al.*, 1983; Farrow *et al.*, 1983; Teixeira *et al.*, 1983). *Streptococcus mitis*, *S. oralis* and *S. sanguis* differed from *S. salivarius* and *S. thermophilus* in lacking substantial levels (about 12 to 17%) of C_{20:1} fatty acids (Table 3). The presence of closely related fatty acid profiles within *S. salivarius* and *S. thermophilus* further supports the close relatedness between these taxa as shown by DNA–DNA homology studies (Kilpper-Bälz *et al.*, 1982; Table 2).

Although *S. salivarius* and *S. thermophilus* are physiologically and biochemically very distinct, and have in the past been considered to be well-defined species (see Jones, 1978; Bridge & Sneath, 1983), the present work and the earlier study of Kilpper-Bälz *et al.* (1982) show that they belong to a single DNA homology group. The apparently large number of phenetic differences between *S. salivarius* and *S. thermophilus* despite the high degree of overall DNA base sequence similarity is difficult to explain. It is possible that the two taxa share a large proportion of 'redundant' DNA or that the biochemical/physiological differences between the taxa may be due to their differing environments (i.e. phenetic divergence due to environmental pressure). Taking 70% homology under optimum hybridization conditions as the borderline for species differentiation, *S. salivarius* and *S. thermophilus* nevertheless represent a single genetical species. It is not however suggested that *S. salivarius* and *S. thermophilus* be reduced to synonymy. The separate identity of *S. thermophilus* should be retained since it is phenetically quite distinct from *S. salivarius*. We therefore formally propose that *S. thermophilus* (Orla-Jensen, 1919) be

Table 1. Strain details and DNA base composition

Strain designation	NCDO no.	Comments	Mol % G + C
<i>Streptococcus salivarius</i>	1779	Type strain, group K, NCTC 8618	41.0
	2107	Cow's teeth	40.1
	2685	Kiel 45807, group K (K. H. Schleifer, Munich, FRG)	39.7
	2686	Kiel 21237 (K. H. Schleifer, Munich, FRG)	ND
	2700	M 36 (J. Hardie, London, UK)	39.8
	2701	A 385, group K (J. Hardie, London, UK)	39.5
<i>Streptococcus thermophilus</i>	573	Type strain, NCIB 8510, ATCC 19258	39.6
	271	Cheese curd	38.9
	822	German yogurt	39.7
	1409		39.3
	1469	Bulgarian yogurt	37.2
	1611		39.3
	1968	Bulgarian cheese, ferments lactose only	39.7
	2549	Yogurt starter	39.7
	2635	Gruyère cheese starter	39.2
	2687	NCDO 489, DSM 20479 (K. H. Schleifer, Munich, FRG)	39.8
	2688	Kiel 68323 (K. H. Schleifer, Munich, FRG)	39.2
<i>Streptococcus sanguis</i>	2064	Type strain NCTC 7863, ATCC 10556	43.4
<i>Streptococcus mitis</i>	2495	Type strain NCTC 3165	39.5
<i>Streptococcus mutans</i>	2062	Type strain NCTC 10449	37.0
<i>Streptococcus oralis</i>	2680	Type strain NCTC 11427	41.3
' <i>Streptococcus milleri</i> '	2077	NCTC 10708	39.0
<i>Streptococcus</i> sp.	1786	' <i>Streptococcus salivarius</i> '	40.4
	2104	Cow's teeth	40.6
	2105	Cow's teeth	39.2

ND, Not determined.

Table 2. DNA homologies of *S. salivarius* and *S. thermophilus* with other streptococci using optimal hybridization conditions

Strain	NCDO no.	Percentage DNA homology* using ³ H-labelled DNA from:	
		<i>S. salivarius</i> 1779	<i>S. thermophilus</i> 573
<i>S. salivarius</i>	1779	100	74
	2107	86	91
	2685	92	91
	2686	96	67
	2700	71	72
	2701	100	86
<i>S. thermophilus</i>	573	67	100
	271	74	97
	822	66	81
	1409	64	87
	1469	78	96
	1611	68	97
	1968	71	95
	2549	61	73
	2635	73	91
	2687	76	97
<i>S. mitis</i>	2688	64	86
	2495	43	46
	2062	29	47
	2680	35	51
	2064	34	36
	2077	35	31
	1786	49	38
	2104	30	35
<i>Streptococcus</i> sp.	2105	31	38

* Values > 60% are shown in bold print.

Table 3. *Percentage fatty acid composition of the test strains*

Assignment*... Equivalent chain length†	C _{12:0}	C _{13:0}	C _{14:1}	C _{14:0}	C _{16:1}	C _{16:0}	C _{17:0}	C _{18:1}			C _{18:0}	C _{19Δ}	C _{20:1}	C _{20:0}
								ω9	ω7	ω7				
<i>Streptococcus mitis</i> NCDO 2495	0.9	0.4	0.6	5.5	1.4	3.0	33.6	0.5	5.5	29.3	14.2	—	—	—
<i>Streptococcus oralis</i> NCDO 2680	0.6	0.3	0.2	4.0	1.6	3.3	33.0	0.6	8.5	29.0	18.9	—	—	—
<i>Streptococcus sanguis</i> NCDO 2064	0.7	0.3	0.2	4.3	2.5	7.6	28.5	0.1	7.6	40.9	7.3	—	—	—
<i>Streptococcus salivarius</i> NCDO 1779	—	0.3	—	2.0	3.9	1.9	27.2	—	11.9	30.7	8.5	—	11.4	0.3
NCDO 2685	—	—	—	0.6	4.5	1.2	10.2	—	10.2	55.4	2.3	—	12.4	0.5
NCDO 2686	—	1.2	—	2.7	1.2	1.0	37.2	—	10.0	22.3	12.2	—	11.7	0.5
<i>Streptococcus thermophilus</i> NCDO 573	—	—	—	2.0	2.0	1.4	9.6	—	60.3	7.8	tr	—	16.0	0.9
NCDO 1968	—	0.5	—	2.7	1.8	1.0	10.3	—	60.3	7.8	tr	1.0	12.3	0.4
NCDO 2635	—	—	—	1.9	3.0	1.0	11.9	—	54.2	14.4	tr	1.0	11.9	0.3
NCDO 2688	—	6.6	—	1.7	4.0	1.6	29.1	—	9.2	27.5	5.9	tr	13.8	0.6
NCDO 2687	0.2	3.6	0.3	1.7	3.8	1.4	23.4	0.6	10.9	27.5	8.2	—	17.0	1.4
NCDO 2549	—	2.2	—	1.9	4.0	4.0	10.5	0.2	40.3	18.5	1.5	3.2	13.0	0.6

tr, trace amounts only.

* Abbreviations for fatty acids are illustrated by the following examples: C_{16:0}, hexadecanoic acid; C_{16:1}, hexadecenoic acid; C_{19Δ}, for 19 carbon cyclopropane ring acid (position of ring not determined).† According to Miwa *et al.* (1960).

reclassified as *S. salivarius* subsp. *thermophilus* comb. nov., thereby automatically creating the subspecies *S. salivarius* subsp. *salivarius*.

Description of Streptococcus salivarius subsp. thermophilus comb. nov.

The following is based on the description of *S. thermophilus* in the 8th edition of *Bergey's Manual of Determinative Bacteriology* (Deibel & Seeley, 1974) and the studies of Schleifer & Kandler (1972), Kilpper-Bälz *et al.* (1982), Bridge & Sneath (1983) and the present work.

Spherical or ovoid cells (about 0.8 to 1.0 µm diam.) in pairs to long chains. Most strains produce a weak α-reaction on blood agar. Catalase negative. Grows at 45 °C; many strains grow at 50 °C. No growth at 15 °C. Heat tolerance marked, survives heating at 65 °C for 30 min. Does not grow at pH 9.6 or 0.1% methylene blue. Most strains do not grow in 2% (w/v) NaCl; no growth in 3% NaCl. Nutritionally exacting, requiring B-vitamins and some amino acids. Facultatively anaerobic. Chemo-organotroph: metabolism fermentative. Acid produced from fructose, glucose, lactose, mannose and sucrose. Acid not produced from *N*-acetylglucosamine, adonitol, amygdalin, arabinose, cellobiose, dulcitol, erythritol, gluconate, glycerol, glycogen, inulin, maltose, mannitol, methyl D-glucoside, methyl D-mannoside, methyl D-xyloside, rhamnose, salicin, sorbitol, trehalose and xylose. Variable results may be obtained for arbutin, galactose, melezitose, melibiose, raffinose and ribose. Aesculin, arginine, casein, gelatin and hippurate are not hydrolysed. Starch hydrolysis is variable. DNAase and urease are not produced. Voges-Proskauer positive. Acid phosphatase, alkaline phosphatase, α-galactosidase, cysteine arylamidase, β-glucuronidase, α-glucosidase, β-glucosidase, α-fucosidase, pyrrolidonyl-arylamidase, *N*-acetyl-β-glucosaminidase and valine arylamidase negative. β-Galactosidase and leucine arylamidase positive. Group A type peptidoglycan based upon lysine (type: Lysine-alanine_n). No group-specific antigen demonstrated. Menaquinones are absent. Major long-chain fatty acids are C_{16:0}, C_{18:1} and C_{20:1}. The guanine plus cytosine content of DNA ranges from 37.2 to 40.3 as determined by melting temperature (*T_m*).

Source: milk and milk products. Type strain: NCDO 573.

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