International Journal of Systematic and Evolutionary Microbiology (2000), **50**, 247–249 Printed in Great Britain

NOTE

DNA–DNA reassociation studies of *Streptococcus constellatus* with unusual 16S rRNA sequences

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DNA–DNA reassociation studies were performed on previously described 'CI strains', which form an unusual 16S rRNA population within the 'anginosus' group of *Streptococcus*. The CI strains displayed reassociation values of >70% with the *Streptococcus constellatus* NCDO 2226^T strain, with T_m values <1 °C, indicating phylogenetic species identity.

Keywords: *Streptococcus constellatus*, *Streptococcus intermedius*, anginosus group of *Streptococcus*

Streptococcus constellatus, *Streptococcus intermedius* and *Streptococcus anginosus* are the three species currently included within the 'anginosus group' of viridans streptococci (Kawamura *et al*., 1995). The 'anginosus group' of streptococci, formerly known as the '*Streptococcus milleri* group', is associated with purulent infections at various sites of the human body (Gossling, 1988).

Recently, we described an unusual rRNA population of 'anginosus group' strains. In a line blot assay, these strains reacted with oligonucleotide probes homologous to the 213–231 bp region of the 16S rRNA gene from both the type strains *S. intermedius* ATCC 27335^T and *S. constellatus* ATCC 27823^T. For this reason, these dually reactive strains were referred to as CI strains. The CI strains phenotypically resembled *S. constellatus* and accounted for 42/136 (31%) of the randomly selected *S. constellatus* strains (Jacobs *et al*., 1996). Similar strains have been found with comparable frequency by Limia *et al*. (1998). According to our findings, the CI strains were homogeneous at the 16S rRNA level. They uniformly hybridized with an oligonucleotide probe directed to the 213–231 bp region of a sequenced CI strain. Comparison of the partial 16S rRNA gene sequences (487 bp) of the three 'anginosus group' type strains and the CI strains revealed that the CI strains were most closely related to *S. constellatus*. They displayed 16S rRNA sequence similarities of 98·1 and 97·7% with the ATCC type strains *S. constellatus* ATCC 27823^T and *S. intermedius* ATCC 27335^T, respectively (Jacobs *et al*., 1996). At such high levels of 16S rRNA sequence similarity, DNA–DNA reassociation studies are needed to measure the degree of relatedness (Stackebrandt & Goebel, 1994). In order to clarify the taxonomic status of the CI strains, we performed DNA–DNA reassociation studies on a selection of these strains.

Seven CI strains and the type strains *S. intermedius* NCDO 2227^T (=NCTC 11324^T, ATCC 27335^T), *S. constellatus* NCDO 2226^T (=NCTC 11325^T, ATCC 27823^T) and *S. anginosus* NCTC 10713^T (=ATCC 33397^T) were selected. The CI strains and their sources are shown in Table 1. Strains were grown in 2 l Streptococcus Sugar Base broth as previously described (Whiley *et al*., 1997). DNA was isolated and purified from approximately 3 g (wet weight) cells by the method of Garvie (1976). DNA purity was monitored by measuring the A_{260} and A_{280} and by agarose gel electrophoresis. Native DNA of strains CI 1 and NCDO 2226^T was labelled *in vitro* with deoxy(1′,2′,5′-^{3}H)cytidine 5′-triphosphate and deoxy(1′,2′-^{3}H)guanosine 5′-triphosphate by nick translation using a commercial kit (Amersham International).

DNA–DNA reassociations were done using the S1 nuclease/trichloroacetic acid procedure (Crosa *et al*.,

Table 1. Results of DNA–DNA reassociation studies on representative CI strains and the species reference strains of the 'anginosus group' of *Streptococcus*

Strain and origin	Reassociation (%) with:	
	Strain CI 1 (ΔT_m)	*S. constellatus* NCDO 2226^T
CI 1, pleural empyema	100	75
CI 19, oral cavity	81	ND
CI 22, abdominal wound	75	ND
CI 57, subcutaneous abscess	78	ND
CI 65, abdominal wound	75	ND
CI 76, urine	81 (0·5 °C)	ND
CI 295, abdominal cavity	75	ND
S. constellatus NCDO 2226^T	76 (0·2 °C)	100
S. anginosus NCTC 10713^T	42	44
S. intermedius NCDO 2227^T	52	49

ND, Not done.

1973) as slightly modified by Grimont *et al.* (1978). Reactions were performed in duplicate. Approximately 10 ng [^{3}H]DNA and 75 μg unlabelled DNA were heat-denatured in 0·042 M NaCl. The NaCl concentration was subsequently adjusted to 0·42 M and reassociation reactions were allowed to proceed at 60 °C for 16 h before S1 nuclease treatment (nuclease S1; Boehringer Mannheim). For two strains, the thermal stability of the hybrids (ΔT_m) was determined in which the T_m (temperature at which 50 % of the reassociated DNA became hydrolysable by S1 nuclease) was determined for strain CI 1. The ΔT_m values (the difference between the T_m of the homologous reaction and that of a heterologous reaction) were used to estimate the level of divergence between strains (Brenner, 1978; Grimont, 1988).

The levels of DNA–DNA reassociation obtained under relaxed conditions and the ΔT_m values are shown in Table 1. From these data, it is clear that the CI strains form a homogeneous group but do not constitute a separate species. The CI strains and the *S. constellatus* type strain NCDO 2226^T are related at the species level, as they fulfil both criteria of phylogenetic species definition: DNA–DNA reassociation values of >70 % and ΔT_m values <5 °C (Wayne *et al.*, 1987). The DNA–DNA relatedness with the *S. intermedius* NCDO 2227^T strain (the nearest relative of the CI strains on the basis of partial 16S rRNA sequence similarity) was 52 %, which is consistent with separate species.

The present results are in line with the poor biochemical delimitation of the CI strains versus the *S. constellatus* strains as defined by 16S rRNA hybridization (Jacobs *et al.*, 1996). At this moment, we are investigating an extended series of CI strains in order to trace specific clinical characteristics such as anatomical distribution or relation with abscesses at defined body sites. For the time being, however, both the phenotypic characteristics of the CI strains and the results of the present DNA–DNA reassociation studies do not warrant consideration of this 16S rRNA population as a distinct subspecies.

The existence of an unusual rRNA population within a single species of the 'anginosus group' of *Streptococcus* has been reported before. Based on a phenotypic characteristic (i.e. a gliding type of motility on certain types of chocolate agar) and a different pattern for the 16S rRNA sequences, Bergman *et al.* (1995) recognized a group of strains closely related to the *S. anginosus* species. Extensive genotypic and phenotypic characterizations of these 'motile' strains, however, did not support consideration of these strains as a distinct subspecies (Whiley *et al.*, 1997). In view of the biochemical heterogeneity of the 'anginosus group' of streptococci (Whiley *et al.*, 1997), it can be expected that more ribosomal subpopulations will be recognized when further rRNA typing studies are performed.

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