Microbiology, Vol. 71, No. 5, 2002, pp. 614–616. Translated from Mikrobiologiya, Vol. 71, No. 5, 2002, pp. 713–716.

SHORT COMMUNICATIONS

Confirmation of the Systematic Position of *Streptococcus salivarius*

A. M. Lysenko*, S. G. Botina, and V. V. Sukhodolets****

**Institute of Microbiology, Russian Academy of Sciences, pr. 60-letiya Oktyabrya 7, k. 2, Moscow, 117811 Russia*

***State Scientific Research Institute of Genetics and Selection of Industrial Microorganisms, Pervyi Dorozhnyi proezd 1, Moscow, 113545 Russia*

Received January 24, 2002; in final form, April 22, 2002

Although the initial classification of bacteria of the genus *Streptococcus*, which was based on immunological tests and the analysis of some physiological and biochemical characteristics, appeared to be convenient for the identification and differentiation of multiple streptococci, it was disapproved by many authors as artificial [1, 2]. The use of more perfect taxonomic criteria, such as the DNA–DNA and RNA–DNA hybridization levels and 16S RNA sequencing data [3, 4], allowed the genus *Streptococcus* to be divided into three allied genera, *Streptococcus* sensu stricto, *Lactococcus*, and *Enterococcus*, and the group of anaerobic streptococci.

However, the intrageneric taxonomy of true streptococci still has a number of weak points. In particular, the species *Streptococcus salivarius* and *Streptococcus thermophilus* have not so far been reliably differentiated. The initial classification of these bacteria as separate species was questioned after the DNA–DNA hybridization analysis of some *S. salivarius* and *S. thermophilus* strains had indicated a rather high homology level (more than 80%) of their DNA nucleotide sequences [5, 6], since it is known that such a high DNA homology level is typical of strains belonging to one species [7]. Moreover, based on the genetic relatedness of some *S. salivarius* and *S. thermophilus* strains and the similarity of their fatty acid composition, Farrow and Collins [8] proposed to reclassify the species *S. thermophilus* into *Streptococcus salivarius* subsp. *thermophilus.* It should be noted that further investigations of the two species showed that their DNA–DNA hybridization levels are within 60% [4, 9], i.e., do not exceed the value typical of different species.

The use of the improved DNA–DNA hybridization technique with stricter DNA reassociation conditions allowed the revival of *S. thermophilus* as a separate species [10]. The taxonomic status of both species was recognized by placing them in *The List of Approved Bacterial Names* published in 1995. Nevertheless, the earlier data on the genetic relatedness of *S. salivarius* and *S. thermophilus* [8] have not so far received convincing explanation. We cannot exclude the possibility of methodological errors in the experiments of Farrow and Collins. Furthermore, the number of the strains used for analysis (as a rule, no more than 3–4) was insufficient to adequately compare the two strains. At the same time, the large number of the collection strains of *S. thermophilus* available to us offers considerable scope for the appropriate investigation of the intraspecific genetic relations of these strains.

Earlier, the investigation of 19 strains of thermophilic streptococci, widely used as starters in the domestic dairy industry, allowed us to divide them into five distinct groups differing in DNA homology levels [11]. A further investigation of 39 new strains obtained from different geographical regions confirmed the heterogeneity of *S. thermophilus* in the degree of DNA–DNA hybridization [12]. These data laid the basis for the investigation of the taxonomic position of the allied species *S. salivarius.*

The aim of the present study was to prove the independent taxonomic status of *S. salivarius* and *S. thermophilus* based on the DNA reassociation and membrane techniques.

The subjects of this study were thermophilic streptococcal strains used as dairy starters or isolated from dairy products produced in various regions of the Commonwealth of Independent States. Of the 58 strains of different origin which were studied earlier [11, 12], we chose six reference strains representing six DNA homology groups, namely, strain B3371 from group I, strain 32 from group II, strain 5 from group III, strain 722 from group IV, strain 6kb from group V, and strain T-48 from group VI. The other reference strains were the type strains *S. salivarius* ATCC 7073 and *S. thermophilus* ATCC 19258, *Lactococcus lactis* subsp. *lactis* AC021 (a derivative of ATCC 11454), *L. lactis* subsp. *cremoris* B4461, *Enterococcus faecium* ATCC 8043, and *E. faecalis* M74.

The strains were grown using the M21 medium [12]. DNA was isolated by the method of Marmur [13] with modifications described earlier [11]. DNA reassociation rates were measured by the method of De Ley [14]. Radiolabeled samples of the *S. salivarius* DNA were obtained by nick-translation according to the Amersham protocol. The subsequent DNA–DNA hybridiza-

0026-2617/02/7105-0614$27.00

Genomic characteristics of *Streptococcus salivarius* with respect to thermophilic streptococci, lactococci, and enterococci

Strain	G+C content, mol %	DNA–DNA hybridization (%) with		
		S. salivarius ATCC7073 (optical reassociation)	*S. salivarius* ATCC7073 (membrane technique)	*S. thermophilus* ATCC19258 (optical reassociation)
Streptococcus salivarius ATCC7073	41.0	100	100	
Streptococcus thermophilus ATCC19258	39.6	45	48	100
S. thermophilus B3371 (I)*	38.3	33	31	54
S. thermophilus 32 (II)	39.3	25	21	35
S. thermophilus 5(III)	40.2	31	34	43
S. thermophilus 722 (IV)	38.4	33	37	47
S. thermophilus 6kb (V)	38.4	34	39	43
S. thermophilus T-48 (VI)	39.4	44	51	65
Lactococcus lactis subsp. *lactis* ACO21	37.1	25	15	22
L. lactis subsp. *cremoris* B4461	36.5	23	14	26
Enterococcus faecium ATCC8043	39.0	17	8	15
E. faecalis M74	39.2	17	5	12

* Parenthesized are DNA homology groups.

tion was carried out by the membrane technique [15]. The table presents data on the DNA–DNA hybridization of *S. salivarius* with the type strain *S. thermophilus* and the members of the six DNA homology groups of thermophilic streptococci, as well as with some lacto- and enterococci. As was shown earlier, thermophilic streptococci exhibit close values of the G+C content of DNA (from 38 to 40 mol %) and DNA homology (80 to 90%) within the particular DNA homology groups, whereas the degree of DNA homology between the members of different groups varied from 20 to 60% [10, 11]. The degree of the DNA homology of these members with the type strain *S. thermophilus* ATCC 19258 varied from 30 to 70%, the members of only one homology group (VI) showing fairly high DNA–DNA reassociation values (55–70%) with *S. thermophilus* ATCC 19258. The existence of six homology groups sufficiently distant from the type *S. thermophilus* species in the DNA–DNA hybridization levels suggested that some members of these groups might be genetically related to the type *S. salivarius* strain. However, the data presented in the table, which were obtained by the two independent methods, indicate that the level of DNA homology between *S. salivarius* and thermophilic streptococci is relatively low (typically, no more than 40%). According to the results reported by De Ley [14], the DNA–DNA hybridization values obtained from DNA renaturation rates and by the membrane technique agree well in the range of DNA homology from 30 to 100%. At lower DNA homology values, especially within 0–20%, the membrane technique provides a better resolution. This inference is confirmed by comparing data on the genetic relatedness between *S. salivarius* and members of the genera *Lactococcus* and *Enterococcus* (see table).

It was of particular interest to determine the DNA homology of the type strains *S. salivarius* and *S. thermophilus* and to compare the results obtained with the relevant data of other authors [4, 8, 9]. As can be seen from the table, the DNA–DNA hybridization level of these strains is within 45–48%, which is close to the DNA homology values shown of thermophilic streptococci from group VI. These results agree with those obtained by other authors, who reported DNA–DNA hybridization levels of about 60% [4, 9] and from 25 to 40% [10].

In view of this, the high DNA–DNA reassociation values reported by Farrow and Collins [8] for some strains of *S. salivarius* and *S. thermophilis* deserve a closer consideration. The membrane technique used by these authors gave hybridization values for some strains of these species exceeding 90%. At the same time, the DNA–DNA hybridization value for the type strains of these species was less than 70%. The authors also compared these species with the type strains of other streptococci, namely, *S. mutans, S. sanguis, S. mitis*, and *S. oralis*, and found that their DNA homology is from 30 to 50%. It should be noted that investigations with the use of S1 nuclease for detecting hybrid duplexes [9] gave lower DNA homology values for the two species (from 5 to 15%). We cannot exclude the possibility of a methodological error in the measurements by Ferrow and Collins, which may be associated with the improper choice of the conditions of molecular hybridization.

There is further evidence indicating that *S. salivarius* and *S. thermophilus* are different species. First, thermophilic streptococci are mainly found in dairy products, whereas *S. salivarius* occur only in the human and mammalian oral cavities [4]. According to numerical taxonomic data, the known strains of *S. salivarius* form a distinct and homogeneous cluster relative to other species of oral streptococci and, which is no less important, to representatives of *S. thermophilus* [2]. Second, approximately half of the investigated *S. salivarius* strains belong to serological group K, while none of the known *S. thermophilus* strains was found to belong to this serological group [4]. It is also noteworthy that various *S. salivarius* strains contain two types of murein, Lys-Ala2–32 (also found in *S. thermophilus*) and Lys-Thr-Gly [4]. However, *S. salivarius* strains with different types of murein form one genetic group.

Thus, the presented data on the degree of DNA homology of *S. salivarius* and *S. thermophilus* and the known differences in their physiological and biochemical characteristics confirm the taxonomic status of *S. salivarius* and *S. thermophilus* as separate species.

ACKNOWLEDGMENT

This work was supported, in part, by grant no. 00-04-48147 from the Russian Foundation for Basic Research.

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