Deoxyribonucleic Acid Homology Among *Lactobacillus* Species of the Subgenus *Streptobacterium* Orla-Jensen

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The deoxyribonucleic acid (DNA) relationship among *Lactobacillus* species of the subgenus *Streptobacterium* Orla-Jensen was assessed by means of DNA/DNA hybridization experiments. High genetic homologies were found among *Lactobacillus casei* subsp. *casei*, *L. casei* subsp. *alactosus*, *L. casei* subsp. *pseudoplanarum*, and *L. casei* subsp. *tolerans*; however, there was only a low level of homology between the DNA of *L. casei* subsp. *rhamnosus* and the DNA of other subspecies of *L. casei*. The DNAs of *Lactobacterium zeae* ATCC 15820 and *L. casei* subsp. *casei* ATCC 393 were nearly completely homologous. Although the latter strain has been proposed as the neotype for *L. casei*, it is not related to any of the other strains within the subspecies of *L. casei*. *Lactobacillus plantarum* and strains identified as belonging to *L. plantarum* subsp. *rudensis* and *L. plantarum* subsp. *arabinosus* formed a fairly homogeneous and easily recognized group. The DNA of *Lactobacillus pentosus* was unrelated to that of any known streptobacteria, and we therefore advocate the recognition of this organism as an independent species. There is a high degree of homology between *Lactobacillus sake* and *Lactobacillus homohiochi*; the name *L. sake* has priority, and *L. homohiochi* is very probably a synonym; for the same reason, *Lactobacillus yamanashiensis* has priority over its later subjective synonym, *Lactobacillus mali*. The general DNA/DNA homology relationships among *Lactobacillus casei*, *L. plantarum*, *L. coryniformis*, *L. curvatus*, *L. xylosus*, *L. alimentarius*, *L. farciminis*, and *Sporolactobacillus inulinus* justify their status as separate species.

In a previous deoxyribonucleic acid (DNA)-DNA hybridization study on thermophilic lactobacilli (6), a close genetic relationship between *Lactobacillus helveticus* and *Lactobacillus jugurti* and also between *Lactobacillus lactis* and *Lactobacillus bulgaricus* was demonstrated. Their close phenotypic similarities were confirmed by genetic data.

Orla-Jensen (25) originally divided the subgenus *Streptobacterium* into only two species: *Lactobacillus casei* was described as a "typical cheese bacterium" and *Lactobacillus plantarum* was described as a "typical vegetable bacterium." Others have proposed *Lactobacillus coryniformis* (1), *Lactobacillus curvatus* (1), *Lactobacillus sake* (15), *Lactobacillus homohiochi* (17), *Lactobacillus xylosus* (16), *Lactobacillus mali* (5), *Lactobacillus yamanashiensis* (24), *Lactobacillus farciminis* (29), *Lactobacillus alimentarius* (29), and *Sporolactobacillus inulinus* (18) as members of the subgenus *Streptobacterium*.

Few DNA-DNA hybridization studies have been made on streptobacteria. Miller et al. (21) showed that there was a low homology (50%) between *L. casei* subsp. *casei* and *L. casei*

subsp. *rhamnosus*, and that *L. casei* and *L. plantarum* are completely unrelated. Johnson (14) found high genetic similarity (77 to 100%) between *L. casei* subsp. *casei* and *L. casei* subsp. *alactosus*, whereas *L. casei* subsp. *casei* and *L. casei* subsp. *rhamnosus* were poorly homologous (33 to 37%). Mills and Lessel (22), in agreement with our presently reported results, found a close genetic similarity (82%) between *L. casei* ATCC 393, the neotype strain of *L. casei* subsp. *casei*, and *Lactobacterium zeae* ATCC 15820.

In this work, a large number of known and proposed *Streptobacterium* species was investigated by DNA-DNA hybridization duplex formation to determine if taxa considered species by phenotypic characters may also be considered as genotypic species.

MATERIALS AND METHODS

Bacterial strains. The *Lactobacillus* strains used in this work are listed in Table 1. Most of them were received from foreign collections. The authenticity of all strains was checked by comparing their main physiological characteristics with original descriptions, if available.

TABLE 1. *List of strains studied*

Strains	Source	Former designations and comments ^a
<i>L. casei</i> subsp. <i>casei</i> (Orla-Jensen) Hansen and Lessel (12) ATCC 393	Cheese	<i>Streptobacterium casei</i> Orla-Jensen (25); neotype for <i>L. casei</i> subsp. <i>casei</i> (Hansen [11])
NIRD C15	Cheese	Same as ATCC 393 (see above); received from M. E. Sharpe
ATCC 334	Emmenthal cheese	J. M. Sherman
NIRD C46		Serological group B (M. E. Sharpe, personal communication); received from M. E. Sharpe
NIRD C2	Grana cheese	Serological group C (M. E. Sharpe, personal communication); received from M. E. Sharpe
IMPC CP34		Serological group B; from our collection
NIRD A148		Not grouped serologically (M. E. Sharpe, personal communication); received from M. E. Sharpe
IMPC MFP1	Grana cheese	Serological group B; from our collection
ATCC 27092b	Human feces	A. Murata
ATCC 4646	Dental caries	<i>L. acidophilus</i> (Hadley et al. [10])
ATCC 11578	Oral cavity	Tilden and Svec (32)
ATCC 11582	Oral cavity	Tilden and Svec (32); lactose-negative
ATCC 15008	Mouth	F. J. Orland (26)
NCIB 4113	Grana cheese	<i>L. helveticus</i> ; formerly ATCC 335
NCIB 4114		<i>L. licopersici</i> ; formerly ATCC 4223
NCIB 6106		<i>L. plantarum</i> ; A. Cunningham
IMPC MFP3		Serological group B; from our collection
<i>L. casei</i> subsp. <i>alactosus</i> Rogosa et al. (28) ATCC 27216	Saliva of child	Rogosa et al. (28); type strain of <i>L. casei</i> subsp. <i>alactosus</i> (Mills and Lessel [22])
ATCC 25180		N. B. Williams
NCIB 8001		<i>Lactobacillus</i> sp.; formerly ATCC 10746
<i>L. casei</i> subsp. <i>rhamnosus</i> Rogosa et al. (28) ATCC 7469		Previously <i>L. casei</i> and later <i>L. helveticus</i> (F. M. Strong); type strain of <i>L. casei</i> subsp. <i>rhamnosus</i> (Hansen [11])
ATCC 7469a		Derived from ATCC 7469 (see above)
NIRD YOG3		Same as ATCC 7469 (see above); serological group C (M. E. Sharpe, personal communication); received from M. E. Sharpe
ATCC 12116		Same as ATCC 7469 (see above)
NCIB 6375		Same as ATCC 7469 (see above)
NIRD A10		Serological group C (M. E. Sharpe, personal communication); received from M. E. Sharpe
IMPC MFP2	Milk	Serological group C; from our collection
ATCC 11982		<i>L. helveticus</i> (Research Association of British Floor Millers)
ATCC 10863	Oral	<i>L. delbrueckii</i> ; production of bacterial koji and soy sauce (Prescot and Dunn [27])
ATCC 14957		<i>L. casei</i> ; P. A. Hansen; standard strain for production of FI serum (Orland [26])
ATCC 8530		<i>L. acidophilus</i> ; C. P. Hegarty; candidate for discard by ATCC
ATCC 9595		<i>L. casei</i> (Merck & Co., Inc.)
ATCC 4961		<i>L. acidophilus</i> ; L. F. Rettger; candidate for discard by ATCC
ATCC 11981		<i>L. helveticus</i> ; A. M. Bradford; <i>L. casei</i> subsp. <i>rhamnosus</i> according to Sharpe and Wheeler (31)

TABLE 1—Continued

Strains	Source	Former designations and comments ^a
NIRD BR26 ATCC 13075 ATCC 14435 ATCC 15820	Swiss cheese Grass silage Corn-steep liquor	Received from M. E. Sharpe R. G. Benedict; candidate for discard by ATCC <i>L. casei</i> ; Langston and Bouma (20); motile Type strain of <i>Lactobacterium zeae</i> Kuznetsov (19); Syn. of <i>L. casei</i> (22)
NCIB 9537 <i>L. casei</i> subsp. <i>pseudoplatanturum</i> Abo-Elnaga and Kandler (1) DSM 20008	Corn-steep liquor	Same as ATCC 15820 (see above)
DSM 20207	Milking machine	Abo-Elnaga and Kandler (1); received from N. Weiss Abo-Elnaga and Kandler (1); received from N. Weiss
<i>L. casei</i> subsp. <i>fusiformis</i> Eschenbecher (8) DSM 20006 DSM 20013	Beer Beer	Eschenbecher (8); received from N. Weiss Eschenbecher (8); received from N. Weiss
<i>L. casei</i> subsp. <i>tolerans</i> Abo-Elnaga and Kandler (1) DSM 20012	Pasteurized milk	Abo-Elnaga and Kandler (1); received from N. Weiss
<i>L. plantarum</i> Bergey et al. (3) ATCC 14917	Pickled cabbage	<i>Streptobacterium plantarum</i> Orla-Jensen (25); type strain of <i>L. plantarum</i> (Hansen [11]) <i>L. arabinosus</i> (E. McCoy)
ATCC 8014 NCIB 6376 NIRD P5 IMPC TL7 NIRD P19 ATCC 14431 ATCC 10012	Corn silage Corn silage Milk Grass silage	Same as ATCC 8014 (see above) Received from M. E. Sharpe From our collection Received from M. E. Sharpe Langston and Bouma (20); catalase positive <i>Streptobacterium plantarum</i> (R. Kuhn); raffinose negative
IMPC TL8 NIRD SL7 NIRD AR5 NIRD P3 IMPC HP10 ATCC 4008 ATCC 11580	Silage Silage	From our collection Received from M. E. Sharpe Received from M. E. Sharpe Received from M. E. Sharpe From our collection <i>L. acidophilus-aerogenes</i> (C. S. Pederson)
ATCC 10241	Oral cavity	Tilden et al. (32); candidate for discard by ATCC
ATCC 8008 ATCC 21028	Sauerkraut Butter	M. Stephenson; type serum for <i>L. plantarum</i> ; M. E. Sharpe (30) Orla-Jensen (25) <i>L. delbrueckii</i> (Kyowa Ferm. Ind. Co. Ltd. KY 3648); patent strain
NIRD AB41 NCIB 1406	Dental caries	Received from M. E. Sharpe <i>L. acidophilus-odontolyticus</i> (J. J. McIntosh); formerly ATCC 11974
IMPC PC8 NIRD P2 ATCC 10776 ATCC 8041 NCIB 8026 ATCC 8292 NIRD P31 IMPC 2-18	Maize silage Fermenting olives Cecal feces of turkeys	From our collection Received from M. E. Sharpe E. B. Fred; candidate for discard by ATCC <i>L. pentosus</i> (E. E. Snell) Same as ATCC 8041 (see above) R. H. Vaughn; candidate for discard by ATCC Received from M. E. Sharpe Harrison and Hansen (13); from our collection

TABLE 1—Continued

Strains	Source	Former designations and comments ^a
<i>L. plantarum</i> subsp. <i>rudensis</i> (Davis and Mattick) Breed and Pederson (J. Bacteriol. 36:667) ATCC 13649	Cheddar cheese	Breed and Pederson (J. Bacteriol. 36:667); candi- date for discard by ATCC
<i>L. coryniformis</i> subsp. <i>corynifor-</i> <i>mis</i> Abo-Elnaga and Kandler (1) ATCC 25602	Silage	Abo-Elnaga and Kandler (1); suggested reference strain for <i>L. coryniformis</i> subsp. <i>coryniformis</i>
DSM 20001	Silage	Abo-Elnaga and Kandler (1); received from N. Weiss
DSM 20007	Silage	Abo-Elnaga and Kandler (1); received from N. Weiss
<i>L. coryniformis</i> subsp. <i>torquens</i> Abo-Elnaga and Kandler (1) ATCC 25600	Cow dung	Abo-Elnaga and Kandler (1); suggested reference strain for <i>L. coryniformis</i> subsp. <i>torquens</i>
DSM 20004	Cow barn	Abo-Elnaga and Kandler (1); received from N. Weiss
DSM 20005	Cow dung	Abo-Elnaga and Kandler (1); received from N. Weiss
<i>L. sake</i> Katagiri et al. (15) ATCC 15521 K 15521 ATCC 15578	"Moto" starter of sake Moto starter of sake Moto starter of sake	Suggested type strain of <i>L. sake</i> by Kitahara Same as ATCC 15521; received from Kitahara S. Fukui
<i>L. homohiochi</i> Kitahara et al. (17) ATCC 15434	Spoiled sake	T. Kaneko
<i>L. xylosus</i> Kitahara (16) ATCC 15577	"Anchu" mash	Suggested as type strain of <i>L. xylosus</i> by Kitahara
<i>L. mali</i> Carr and Davies (5) ATCC 27053	Apple juice	Carr and Davies (5); suggested reference strain for <i>L. mali</i> ; catalase-positive
J 123	Apple juice	Carr and Davies (5); same as ATCC 27054; catalase positive; received from Carr
J 26	Apple juice	Carr and Davies (5); same as ATCC 27055; catalase positive; received from Carr
<i>L. yamanashiensis</i> Nonomura et al. (24) N 239	Japanese wine	Nonomura et al. (24); suggested reference strain for <i>L. yamanashiensis</i> ; motile; diaminopimelic acid positive
<i>L. alimentarius</i> Reuter (29) DSM 20249	Meat products	Reuter (29); received from N. Weiss
<i>L. farciminis</i> Reuter (29) DSM 20180 DSM 20184	Meat products Meat products	Reuter (29); received from N. Weiss Reuter (29); received from N. Weiss
<i>S. inulinus</i> Kitahara and Suzuki (18) K 15538	Chicken feed	Kitahara and Suzuki (18); type strain of <i>S.</i> <i>inulinus</i> ; received from K. Kitahara
ATCC 15538	Chicken feed	Same as K 15538 (see above)
NCIB 9743	Chicken feed	Same as K 15538 (see above)

^aThis information was obtained from culture collection catalogues or from the pertinent literature. Abbreviations and symbols: NIRD, National Institute for Research in Dairying, Reading, England; NCIB, National Collection of Industrial Bacteria, Aberdeen, Scotland; IMPC, Istituto di Microbiologia, Facoltà di Agraria, Piacenza, Italy; and J. G. Carr, University of Bristol, Bristol, England. For other abbreviations, see footnotes to Table 3.

Cultures of the strains were maintained on MRS agar (7) and propagated in MRS broth, except for *L. homohiochi* ATCC 15434, which was grown on MRS medium supplemented with mevalonic acid (0.05 g/liter).

The medium employed for labeling DNA was MRS broth containing added [¹⁴C]uracil and [¹⁴C]adenine (each 50 γ Ci/liter). DNA preparations were purified and sheared. These conditions and those for DNA immobilization on membrane filters (Sartorius SM 11306) and DNA reassociation at 60 C (25 C below thermal denaturation *T*) have been described previously (6).

RESULTS AND DISCUSSION

The DNA homology values obtained are reported in Tables 2 and 3.

(i) ***L. casei* subsp. *casei* (Orla-Jensen) Hansen and Lessel (12).** The DNAs of 16 strains received as *L. casei* subsp. *casei* were hybridized with reference DNA from *L. casei* subsp. *casei* ATCC 393, *L. casei* subsp. *casei* ATCC 334, *L. casei* subsp. *alactosus* ATCC 27216, *L. casei* subsp. *rhamnosus* ATCC 7469, and *L. plantarum* ATCC 14917.

Only strain NIRD C15 shared a complete DNA relationship to *L. casei* subsp. *casei* ATCC 393. However, this is not surprising because NIRD C15 is the same as ATCC 393 (M. E. Sharpe, personal communication).

All other *L. casei* strains gave homology levels of 8 to 46% with strain ATCC 393. Ten strains of *L. casei* gave homology values of 71 to 85% with reference DNA from *L. casei* subsp. *casei* ATCC 334 and nearly similar values of 75% to 99% with reference DNA from *L. casei* subsp. *alactosus* ATCC 27216.

Although ATCC strains 11582 and 15008 are listed in the ATCC catalogue (2) as *L. casei* (Orla-Jensen) Hansen and Lessel, i.e., presumably *L. casei* subsp. *casei*, these strains ferment rhamnose and have the other phenotypic characteristics of *L. casei* subsp. *rhamnosus*. It is, therefore, not surprising that the DNA of these strains and that of the reference strain of *L. casei* subsp. *rhamnosus* ATCC 7469 are related (79 and 95% for ATCC strains 11582 and 15008, respectively). Rhamnose fermentation occurred also in strains NIRD C2 and ATCC 11578, where there was high homology with reference DNA from *L. casei* subsp. *casei* ATCC 334 and *L. casei* subsp. *alactosus* ATCC 27216. This is more difficult to explain because here phenotype and genotypy do not appear to be correlated. NCIB 6106 is listed in the NCIB catalogue (23) as *L. casei* (Orla-Jensen) Holland; however, it was originally deposited as *L. plantarum*, and its present characteristics, including melibiose and raffinose fermentations, conform to those of *L. plantarum* and not to those

of *L. casei*. The high level of homology (76%) between NCIB 6106 and *L. plantarum* reference DNA is therefore to be expected.

(ii) ***L. zeae* Kuznetsov [syn. *L. casei* (Orla-Jensen) Hansen and Lessel (12)].** We received Kuznetsov's strain of *L. zeae* from the ATCC (ATCC 15820) as *L. casei* subsp. *rhamnosus* and from NCIB (NCIB 9537) as *L. zeae*. Our results confirm those obtained by Mills and Lessel (22): ATCC 15820 and NCIB 9537 were 80 and 84% homologous, respectively, to the reference strain *L. casei* subsp. *casei* ATCC 393.

These strains were considerably less related to all other reference DNAs used.

L. casei subsp. *casei* ATCC 393 has been recently designated as the neotype strain of *L. casei* (12). However, our results indicated that only *L. zeae* ATCC 15820 (= NCIB 9537) is genetically homologous to this strain. Evidently the choice of the type strain for the species *L. casei* should be reconsidered.

(iii) ***L. casei* subsp. *alactosus* Rogosa et al. (28).** The three lactose-negative strains of *L. casei* subsp. *alactosus* studied (ATCC 27216, ATCC 25180, and NCIB 8001) competed to nearly the same high levels (from 78 to 91%) with both *L. casei* subsp. *alactosus* ATCC 27216 (lactose negative) and *L. casei* subsp. *casei* ATCC 334 (lactose positive). Moreover, reciprocal hybridization of the reference DNAs from the latter two strains give similar high homology levels.

(iv) ***L. casei* subsp. *rhamnosus* Rogosa et al. (28).** Thirteen of the 17 strains received as *L. casei* subsp. *rhamnosus* formed a rather homologous group and displayed a close genetic relationship (80 to 106%), with *L. casei* subsp. *rhamnosus* ATCC 7469 used as reference. The same strains showed lower levels of similarity to *L. casei* subsp. *casei* ATCC 393, *L. casei* subsp. *casei* ATCC 334, *L. casei* subsp. *alactosus* ATCC 27216, and *L. plantarum* ATCC 14917. Two strains (NIRD BR26 and ATCC 13075) were not related to the reference strain *L. casei* subsp. *rhamnosus* ATCC 7469, but they were homologous to *L. casei* subsp. *casei* ATCC 334 and *L. casei* subsp. *alactosus* ATCC 27216.

We were not able to detect any fermentation of rhamnose by these two strains. (However, rhamnose fermentation by these strains was rechecked by C. Mills and was found to be positive. Additional experiments are needed to resolve this matter.)

Only one strain (ATCC 14435), received as *L. casei* subsp. *rhamnosus* and isolated by Langston and Bouma (20), remained unassigned even though its DNA was tested in all our reference systems.

Our results strongly indicate that *L. casei*

subsp. *rhamnosus* should not be considered as a subspecies but should be elevated to the rank of species.

(v) ***L. casei* subsp. *fusiformis* Eschenbecher (8).** The two strains (DSM 20006 and DSM 20013) received as *L. casei* subsp. *fusiformis* were hybridized with almost all of our homologous systems, and one of them, DSM 20006, was found to be homologous with both *L. casei* subsp. *casei* ATCC 334 and *L. casei* subsp. *alactosus* ATCC 27216, and the other, DSM 20013, showed a high degree of homology (80%) with *L. casei* subsp. *rhamnosus* ATCC 7469 reference DNA.

The fermentation patterns of these two strains are very different: DSM 20006 ferments maltose, melezitose, and mannitol and does not ferment rhamnose, lactose, or melibiose; DSM 20013 has the opposite reactions. Therefore, the recognition of this subspecies is seriously questioned.

(vi) ***L. casei* subsp. *pseudoplantarum* Abo-Elnaga and Kandler (1).** The only two strains studied (DSM 20008 and DSM 20207) showed high genetic homologies (86 and 85%, respectively) with *L. casei* subsp. *casei* ATCC 334 and (98 and 93%, respectively) with *L. casei* subsp. *alactosus* ATCC 27216 as reference strains.

Their fermentation patterns were in accordance with the report of Abo-Elnaga and Kandler (1); in particular, these strains did not ferment rhamnose.

These two strains displayed some levels of DNA homology with *L. plantarum* used as reference. It is interesting to note that, although these strains undoubtedly belong to *L. casei*, they are somewhat more related genetically to *L. plantarum* ATCC 14917 than all other strains of *L. casei* (30 and 38%, respectively [Table 2]).

(vii) ***L. casei* subsp. *tolerans* Abo-Elnaga and Kandler (1).** *L. casei* subsp. *tolerans* (DSM 20012), according to the description of Abo-Elnaga and Kandler (1), ferments only lactose and galactose. Low DNA-DNA homology values were obtained with all reference DNAs except in the case of *L. casei* subsp. *casei* ATCC 334 and *L. casei* subsp. *alactosus* ATCC 27216, where the homology values were 71 and 82%, respectively.

(viii) ***L. plantarum* Bergey et al. (3).** The DNAs of 20 of the 28 strains received as *L. plantarum* had similarity values ranging from 76 to 101% to the reference DNA of the type strain of *L. plantarum* ATCC 14917.

The other eight strains behaved quite differently. *L. plantarum* strains ATCC 8292 and NIRD P31 were only 57 and 65% homologous, respectively, to the reference ATCC 14917, but were completely homologous to strain ATCC

8041 (*L. pentosus*) which was used as additional reference in the experiments with this species. *L. plantarum* NCIB 8026 is simply a deposit of strain ATCC 8041 in the NCIB. As expected, there was complete homology between the two DNA preparations.

ATCC 10776, NIRD P2, IMPC PC8, and ATCC 8008 were received as *L. plantarum*, but their DNAs were distinctly unrelated to *L. plantarum* and *L. pentosus* reference DNAs; furthermore, they were not related to ATCC 10776 used as additional reference DNA. Therefore, these strains were not assigned to any species.

In the interspecies hybridization tests, the DNAs of the *L. plantarum* strains showed little or no similarity with the *L. casei* DNAs used as reference, namely *L. casei* ATCC 393, *L. casei* ATCC 334, *L. casei* subsp. *alactosus* ATCC 27216, and *L. casei* subsp. *rhamnosus* ATCC 7469.

Our results showed conclusively that the majority of the strains received as belonging to *L. plantarum* form a fairly homogeneous group whose DNA matched almost completely with the DNA of the type strain of this species, ATCC 14917.

However, three strains of *L. plantarum* which fermented xylose at variance with all other strains of this species behaved quite differently; their DNA annealed completely to the filter-bound DNA obtained from ATCC 8041, formerly considered a representative of the species *L. pentosus* established by Fred et al. (9).

The lack of competition observed with the DNAs of these strains in all other homologous systems we used strongly suggests that this species might be reconsidered to be a valid specific taxon.

Apparently the same does not hold for *L. arabinosus* (9), because two strains, ATCC 8014 and NCIB 6376, which were originally identified as belonging to *L. arabinosus*, were completely homologous to *L. plantarum* ATCC 14917. This supports the widely accepted view, based on phenotypic characteristics, that these strains belong to *L. plantarum*.

(ix) ***L. coryniformis* Abo-Elnaga and Kandler (1).** Three strains (ATCC 25602, DSM 20001, and DSM 20007) were received as *L. coryniformis* subsp. *coryniformis* and three (ATCC 25600, DSM 20004, and DSM 20005) as *L. coryniformis* subsp. *torquens*. Their DNAs competed to very high levels of homology (87 to 98%) with reference DNA from *L. coryniformis* subsp. *coryniformis* ATCC 25602. There was little or no homology with the DNAs of any of the other reference systems. The fermentation patterns of these strains, including the inability

<i>L. casei</i> subsp. <i>rhamnosus</i>	45	47	49	100	2	14	—	20	4	—	23	36	18	18	22	23
ATCC 7469	44	—	51	102	10	—	—	—	—	—	—	—	—	—	—	—
ATCC 7469-a	42	42	50	96	9	7	12	15	—	—	—	—	—	—	—	—
NIRD YOG3	49	—	27	100	2	—	—	—	—	—	—	—	—	—	—	—
ATCC 12116	52	41	—	96	11	—	—	—	—	—	—	—	—	—	—	—
NCIB 6375	47	—	49	93	17	—	—	—	—	—	—	—	—	—	—	—
NIRD A10	48	32	25	98	7	—	—	—	—	—	—	—	—	—	—	—
IMPC MFP2	43	43	51	106	1	—	—	—	—	—	—	—	—	—	—	—
ATCC 11982	47	44	27	105	12	—	—	—	—	—	—	—	—	—	—	—
ATCC 10863	39	44	28	86	0	10	15	5	10	—	9	25	10	—	8	—
ATCC 14957	40	42	38	97	0	—	—	—	—	—	—	—	—	—	—	—
ATCC 8530	40	—	—	98	9	—	—	—	—	—	—	—	—	—	—	—
ATCC 9595	37	45	32	99	18	—	—	—	—	—	—	—	—	—	—	—
ATCC 4961	33	39	29	105	12	—	—	—	—	—	—	—	—	—	—	—
ATCC 11981	42	41	49	79	3	—	—	—	—	—	—	—	—	—	—	—
<i>L. casei</i> subsp. <i>casei</i>	46	—	24	95	5	—	—	—	—	—	—	—	—	—	—	—
ATCC 11582	7	5	15	80	2	—	—	19	8	—	10	—	1	—	—	—
ATCC 15008	8	10	12	9	100	44	60	15	—	12	14	15	12	19	18	22
<i>L. casei</i> subsp. <i>fusiformis</i>	0	—	16	22	95	31	53	14	—	—	—	—	—	—	—	—
<i>L. plantarum</i>	5	—	16	13	86	33	—	—	—	—	—	—	—	—	—	—
ATCC 14917	2	—	8	1	99	39	—	—	—	—	—	—	—	—	—	—
NCIB 6376	6	4	—	3	89	—	—	—	—	—	—	—	—	—	—	—
NIRD P5	—	—	18	25	81	—	—	—	—	—	—	—	—	—	—	—
IMPC TL7	19	—	0	2	84	21	52	—	—	—	—	—	—	—	—	—
NIRD P19	0	1	4	20	82	38	57	—	—	—	—	—	—	—	—	—
ATCC 14431	26	—	2	—	92	35	—	—	—	—	—	—	—	—	—	—
ATCC 10012	4	—	0	—	90	—	—	—	—	—	—	—	—	—	—	—
ATCC 4008	13	—	4	—	89	—	—	—	—	—	—	—	—	—	—	—
IMPC TL8	14	—	4	—	89	—	—	—	—	—	—	—	—	—	—	—
NIRD P3	15	—	0	—	101	29	56	—	—	—	—	—	—	—	—	—
NIRD SL7	19	—	6	—	100	31	54	—	—	—	—	—	—	—	—	—
NIRD AR5	35	—	10	—	94	33	—	—	—	—	—	—	—	—	—	—
IMPC HP10	28	0	0	—	84	—	—	—	—	—	—	—	—	—	—	—
ATCC 11580	15	—	—	—	95	37	—	—	—	—	—	—	—	—	—	—
ATCC 10241	15	20	—	—	94	26	—	—	—	—	—	—	—	—	—	—
ATCC 21028	16	—	—	—	88	46	—	—	—	—	—	—	—	—	—	—
NIRD AB41	10	—	—	—	76	51	—	—	—	—	—	—	—	—	—	—
NCIB 1406	—	—	—	—	92	—	—	—	—	—	—	—	—	—	—	—
IMPC 2-18	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—

TABLE 2—Continued

Strains	<i>L. casei</i> subsp. <i>casei</i> ATCC 393	<i>L. casei</i> subsp. <i>casei</i> ATCC 334	<i>L. casei</i> subsp. <i>alactosus</i> ATCC 27216	<i>L. casei</i> subsp. <i>rhamnosus</i> ATCC 7469	<i>L. plantarum</i> ATCC 14917	<i>L. pentosus</i> ATCC 8041	<i>L. plantarum</i> ATCC 10776	<i>L. coryniformis</i> subsp. <i>coryniformis</i> ATCC 25602	<i>L. curvatus</i> DSM 20010	<i>L. sake</i> ATCC 15521	<i>L. homohiochi</i> ATCC 15434	<i>L. xylo-sus</i> ATCC 15577	<i>L. mali</i> ATCC 27053	<i>L. yamamotoi</i> ATCC N239	<i>L. farci-minis</i> DSM 20180	<i>S. inulinus</i> K 15538
<i>L. plantarum</i> subsp. <i>rudensis</i> ATCC 13649	13	0	0	—	84	31	—	—	—	—	—	—	—	—	—	—
<i>L. casei</i> subsp. <i>casei</i> NCIB 6106	8	15	—	6	91	45	—	—	—	—	—	—	—	—	—	—
IMPC MF07	18	10	35	31	99	20	—	19	—	—	—	—	0	—	—	—
<i>L. pentosus</i> ATCC 8041	16	3	8	9	40	100	42	0	7	—	—	—	13	31	13	—
<i>L. plantarum</i> NCIB 8026	9	—	—	5	46	106	48	0	—	—	—	—	—	—	—	—
ATCC 8292	21	1	10	—	57	101	50	—	—	—	—	—	—	—	—	—
NIRD P31	42	—	0	—	65	102	—	—	—	—	—	—	—	—	—	—
ATCC 10776	6	3	7	0	57	41	100	—	—	—	—	—	—	—	—	—
NIRD P2	3	0	0	0	64	38	70	—	—	—	—	—	—	—	—	—
IMPC PC8	9	—	1	0	66	41	67	—	—	—	—	—	—	—	—	—
ATCC 8008	27	0	—	—	62	31	57	—	—	—	—	—	—	—	—	—
<i>L. coryniformis</i> subsp. <i>coryniformis</i> ATCC 25602	11	10	4	14	16	2	—	100	20	—	—	17	6	25	25	7
DSM 20001	10	2	—	2	10	—	—	92	—	—	—	—	—	—	—	—
DSM 20007	8	—	—	—	18	—	—	87	26	—	—	—	—	—	—	—
<i>L. coryniformis</i> subsp. <i>torquens</i> ATCC 25600	0	7	—	11	29	—	—	98	10	—	—	—	—	—	—	—
DSM 20004	13	7	—	15	13	—	—	88	—	—	—	—	—	—	—	—
DSM 20005	7	3	—	3	16	—	—	94	—	—	—	—	—	—	—	—
<i>L. curvatus</i> DSM 20010	15	3	7	0	21	11	—	30	100	—	—	31	6	—	0	—
DSM 20019	4	21	—	—	20	8	—	22	108	—	—	—	—	—	—	—
<i>L. sake</i> ATCC 15521	7	14	12	10	11	18	—	19	47	100	89	10	0	18	—	—

K 15521	—	24	—	22	5	—	—	24	27	97	96	24	6	—	—	—
ATCC 15578	20	13	—	10	—	10	—	27	—	62	58	20	7	—	—	—
<i>L. homohiochi</i>	15	8	—	15	10	10	—	12	37	82	100	16	26	21	—	28
ATCC 15434	14	7	—	40	10	9	—	21	24	4	10	100	14	12	—	24
<i>L. xyloso</i>																
ATCC 15577																
<i>L. mali</i>																
ATCC 27053	5	7	20	8	11	14	—	10	3	—	—	—	100	84	10	31
J 123	2	11	24	4	18	—	—	15	—	—	—	—	86	95	—	—
J 26	4	4	23	3	9	—	—	9	—	—	—	—	96	82	—	—
<i>L. yamanashiensis</i>																
N 239	17	16	16	10	16	—	—	5	0	—	26	—	78	100	27	30
<i>L. farciminis</i>																
DSM 20180	24	29	17	15	18	—	—	23	7	—	—	—	9	19	100	—
DSM 20184	15	23	15	20	26	—	—	14	4	—	—	—	—	—	88	—
<i>S. inulinus</i>																
K 15538	18	12	20	18	12	6	—	13	11	10	13	22	20	23	16	100
ATCC 15538																
NCIB 9743	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	91
<i>L. alimentarius</i>	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	94
DSM 20249	24	14	25	12	16	21	—	27	14	11	18	16	14	29	22	26
<i>L. casei</i> subsp. <i>rhamnosus</i>																
ATCC 14435	10	11	11	27	19	14	7	31	3	41	27	24	30	19	17	24

^a —, Not tested.

TABLE 3. General survey of DNA homology relationships between type, neotype, or other reference strains of the known species of the subgenus *Streptobacterium*

Competitor strains	Reference strains														
	ATCC ^a 393	ATCC 334	ATCC 27216	ATCC 7469	ATCC 14917	ATCC 8041	ATCC 25602	DSM ^b 20010	ATCC 15521	ATCC 15434	ATCC 15577	ATCC 27053	N ^c 239	DSM 20180	K ^d 15538
<i>Lactobacillus casei</i> subsp. <i>casei</i> ATCC 393	100	36	38	38	7	21	17	— ^e	10	16	5	11	16	20	25
<i>L. casei</i> subsp. <i>casei</i> ATCC 334	43	100	90	46	6	12	19	8	18	—	8	3	25	35	—
<i>L. casei</i> subsp. <i>alactosus</i> ATCC 27216	50	85	100	43	10	—	10	—	16	—	—	10	14	34	26
<i>L. casei</i> subsp. <i>rhamnosus</i> ATCC 7469	45	47	49	100	2	14	20	4	—	23	36	18	18	22	23
<i>L. plantarum</i> ATCC 14917	8	10	12	9	100	44	15	—	12	14	15	12	19	18	22
<i>L. pentosus</i> ATCC 8041	16	3	8	9	40	100	0	7	—	—	—	13	31	13	—
<i>L. coryniformis</i> subsp. <i>coryniformis</i> ATCC 25602	11	10	4	14	16	2	100	20	—	—	17	6	25	25	7
<i>L. curvatus</i> DSM 20010	15	3	7	0	21	11	30	100	—	—	31	6	—	0	—
<i>L. sake</i> ATCC 15521	7	14	12	10	11	18	19	47	100	89	10	0	18	—	—
<i>L. homioichi</i> ATCC 15434	15	8	—	15	10	10	12	37	82	100	16	26	21	—	28
<i>L. xylosum</i> ATCC 15577	14	7	—	40	10	9	21	24	4	10	100	14	12	—	24
<i>L. mali</i> ATCC 27053	5	7	20	8	11	14	10	3	—	—	—	100	84	10	31
<i>L. yamanashiensis</i> N 239	17	16	16	10	16	—	5	0	—	—	—	78	100	27	30
<i>L. farciminis</i> DSM 20180	24	29	17	15	18	—	23	7	—	—	—	9	19	100	—
<i>Sporolactobacillus inulinus</i> K 15538	18	12	20	18	12	6	13	11	10	13	22	20	23	16	100

^a ATCC, American Type Culture Collection, Rockville, Md.^b DSM, Deutsche Sammlung für Mikroorganismen, Munich, Germany.^c N, H. Nonomura, Yamanashi University, Kofu, Japan.^d K, K. Kitahara, University of Tokyo, Tokyo, Japan.^e —, Not tested.

of *L. coryniformis* subsp. *coryniformis* to ferment lactose, agree with the original description given by Abo-Elnaga and Kandler (1).

(x) *L. curvatus* Abo-Elnaga and Kandler (1). The two strains (DSM 20010 and DSM 20019) received as *L. curvatus* were genetically identical and very distantly related, if at all, to other reference strains. Therefore, our results confirm the validity at the genetic level of the species established by Abo-Elnaga and Kandler (1), namely *L. coryniformis* and *L. curvatus*.

(xi) *L. sake* Katagiri et al. (15). ATCC 15521, suggested as the type strain of *L. sake*, was used as DNA filter reference; *L. sake* ATCC 15578 was only 62% homologous to the reference.

Strain K 15521, received directly from Kitahara, proved to be identical to strain ATCC 15521. The DNA homology was low with all other reference systems used.

The fermentation patterns of the two ATCC strains were different; ATCC 15521 fermented galactose, cellobiose, lactose, maltose, melibiose, sucrose, and trehalose while ATCC 15578 fermented galactose, cellobiose, lactose, maltose, sucrose, trehalose, melezitose, salicin, and mannitol.

(xii) *L. homohiochi* Kitahara et al. (17). Only one strain (ATCC 15434) of this species was studied in our competition experiments; it displayed a high level of similarity (89%) only with *L. sake* ATCC 15521. Furthermore, reverse competition between the two strains gave the same high level of homology. The phenotypic characters of ATCC 15434 agree with those in the original description of *L. homohiochi* (17).

Our results concerning the species *L. sake* and *L. homohiochi*, although revealing homology levels that suggest the identity of these two species, should probably not be taken as definitive because they were based on a limited number of strains. It was clearly demonstrated that the strains of these two species studied are unrelated to any of the species of streptobacteria studied.

(xiii) *L. xylosus* Kitahara (16). The only strain of *L. xylosus* tested, ATCC 15577, displayed some level of homology only when tested with DNA from *L. casei* subsp. *rhamnosus* ATCC 7469 and showed almost zero homology with all other reference DNAs used. The phenotypic traits of ATCC 15577, the suggested type strain, agreed with the description of *L. xylosus* given by Kitahara (16). It is of interest to note that the DNA of ATCC 15577 is not related to the DNA of *L. pentosus* ATCC 8041, although both species are characterized by their behavior toward xylose.

(xiv) *L. mali* Carr and Davies (5). ATCC

27053 was used as reference for *L. mali*. Two additional strains of this species, J123 (= ATCC 27054) and J26 (= ATCC 27055), received directly from Carr, proved to be genetically identical to the selected reference.

The distinctive characteristics given by Carr and Davies (5) for this species, i.e., production of catalase and sugar fermentation patterns, were confirmed.

(xv) *L. yamanashiensis* Nonomura et al. (24). Nonomura's strain N239 displayed a high level of homology only with the reference *L. mali* ATCC 27053. Conversely, in DNA competition experiments, the three strains of *L. mali* showed similarity ranging from 82 to 95% when tested in the *L. yamanashiensis* reference system.

Some of the characters given by Nonomura et al. (24), e.g., motility, absence of catalase, and inability to ferment melibiose, raffinose, or arabinose, were checked in strain N239 and were found to correspond to those given in the original description of *L. yamanashiensis*. The close relationship between *L. mali* ATCC 27053, *L. mali* ATCC 27055, and N239 was indicated by the G+C contents of their DNAs: ATCC 27053 = 32.5 ± 0.15 mol%; ATCC 27055 (J26) = 32.2 ± 0.12 mol%; and N239 = 32.9 ± 0.19 mol%.

Although our results suggested a very close relationship between *L. mali* and *L. yamanashiensis*, one must bear in mind that only very few strains of both species were studied. The difference in the phenotypic characters of these organisms (J. G. Carr, personal communication) suggests that a larger number of strains must be studied to obtain conclusive results.

(xvi) *L. farciminis* Reuter (29). *L. farciminis* DSM 20180 was used as reference. Strain DSM 20184 displayed 88% homology to the reference, suggesting a high degree of genetic relationship. Insignificant reassociation was found with all other competitors used.

The relevant physiological characters of this strain as given by Reuter (29) were confirmed by our tests.

(xvii) *L. alimentarius* Reuter (29). *L. alimentarius* DSM 20249 was genetically unrelated to all of our competition systems.

The phenotypic traits of this strain checked by us agree with those given in Reuter's (29) original description of this species.

Reuter's two species appear to be valid, but additional data are required to confirm this.

(xviii) *S. inulinus* Kitahara and Suzuki (18). *S. inulinus* K 15538, received directly from Kitahara, was used as reference strain. As expected, *S. inulinus* ATCC 15538 and NCIB

9743, subcultures from the same strain deposited by Kitahara in both collections, were highly homologous (91 and 84%, respectively).

The same fermentation patterns, absence of catalase, formation of spores, and motility, were found in ATCC 15538 and NCIB 9743 in accordance with the description of *S. inulinus* by Kitahara and Suzuki (18).

Although we did not test all of our DNA competitors against the *S. inulinus* reference strain (K 15538), our data are sufficient to permit the conclusion that this species is genetically distinct from the other species considered in this investigation.

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