Reclassification of *Lactobacillus casei* subsp. *casei* ATCC 393 and *Lactobacillus rhamnosus* ATCC 15820 as *Lactobacillus zeae* nom. rev., Designation of ATCC 334 as the Neotype of *L. casei* subsp. *casei*, and Rejection of the Name *Lactobacillus paracasei*

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The type strain of *Lactobacillus casei* subsp. *casei* (ATCC 393) exhibits low levels of DNA homology with other strains of *L. casei* subsp. *casei* (8 to 46%) and strains of *Lactobacillus paracasei* (30 to 50%), but exhibits a level of DNA similarity of 80% with *Lactobacillus rhamnosus* ATCC 15820, the original type strain of “*Lactobacterium zeae*” Kuznetsov 1959. Strains ATCC 393^T (T = type strain) and ATCC 15820^T are members of one protein profile cluster that is separate from the other *Lactobacillus* spp. The randomly amplified polymorphic DNA PCR profile of strain ATCC 393^T is also different from the profiles obtained for the other species. *L. casei* ATCC 334^T is genetically closely related to *L. casei* subsp. *casei* strains (71 to 97%) and *L. paracasei* strains (71 to 91%), is a member of the same protein profile cluster as these organisms, and shares several DNA amplicons with *L. paracasei* strains. On the basis of these results, we propose that *L. casei* subsp. *casei* ATCC 393^T and *L. rhamnosus* ATCC 15820 should be reclassified as members of *Lactobacillus zeae* nom. rev. (type strain, ATCC 15820), that strain ATCC 334 should be designated the neotype strain of *L. casei* subsp. *casei*, and that the name *L. paracasei* should be rejected.

In a previous paper (4), we proposed that *Lactobacillus casei* subsp. *casei* ATCC 334 should be designated the neotype strain of *Lactobacillus casei*. However, our request for an opinion on this effect was rejected by the Judicial Commission of the International Committee on Systematic Bacteriology (15) on the basis of the fact that the requirements of Rules 18e and 18g of the *International Code of Nomenclature of Bacteria* (10) had not been clearly and convincingly met. However, the true basis for the rejection was a misunderstanding of the wording, because the previously published facts do not support the opinion that “awarding an exception to the Rules” (15) is necessary. The facts are summarized below.

In 1966 the Subcommittee on Lactobacilli and Closely Related Organisms listed strain ATCC 393 (*Streptobacterium casei* No. 7 of Orla-Jensen) as the type strain of *Lactobacillus casei* Holland (7). There is, however, no evidence that strain ATCC 393^T (T = type strain) is one of the original strains described by Von Freudenreich (14). Hansen and Lessel (8) found some similarity between strain ATCC 393 and *Bacillus* sp. strain α , one of Von Freudenreich's strains, and designated strain ATCC 393 the neotype strain of *Lactobacillus casei*. Subsequently, Mills and Lessel (11) studied the DNA-DNA relatedness of *Lactobacillus casei* and “*Lactobacterium zeae*” Kuznetsov 1959 and showed that the type strains of *Lactobacillus casei* (ATCC 393) and “*Lactobacterium zeae*” (ATCC 15820; later listed as a *Lactobacillus casei* subsp. *rhamnosus* strain in the American Type Culture Collection *Catalogue of Strains* [1]) are closely related (level of relatedness, 82%).

These authors concluded that “*Lactobacterium zeae*” Kuznetsov 1959 is a subjective synonym of *Lactobacillus casei*.

However, this conclusion was not supported by the results of a more comprehensive DNA-DNA reassociation study (3), which included *Lactobacillus casei* ATCC 393^T, “*Lactobacterium zeae*” ATCC 15820^T, and representatives of all subspecies of *Lactobacillus casei*, including *Lactobacillus casei* subsp. *casei*, *Lactobacillus casei* subsp. *alactosus*, *Lactobacillus casei* subsp. *rhamnosus*, *Lactobacillus casei* subsp. *tolerans*, *Lactobacillus casei* subsp. *pseudoplatanturum*, and *Lactobacillus casei* subsp. *fusiformis*. In their study Dellaglio et al. (3) obtained evidence that the level of DNA-DNA relatedness of strains ATCC 393^T and ATCC 15820^T is in fact more than 80%, but that these strains are only moderately related (levels of relatedness 8 to 46%) to all of the *Lactobacillus casei* subspecies (including *Lactobacillus casei* subsp. *rhamnosus*). On the other hand, high levels of DNA homology were found among and between *Lactobacillus casei* subsp. *casei* (the majority of strains), *Lactobacillus casei* subsp. *alactosus* Mills and Lessel 1973, and *Lactobacillus casei* subsp. *pseudoplatanturum* Abo-El-naga and Kandler 1965 strains, which is consistent with the genotype of *Lactobacillus casei*, and among *Lactobacillus casei* subsp. *rhamnosus* strains, indicating that these strains have a separate genotype (later designated *Lactobacillus rhamnosus* Collins, Phillips, and Zannoni 1989). *Lactobacillus rhamnosus* is only moderately related to strains ATCC 393^T and ATCC 15820^T and the *Lactobacillus casei* genotype. Thus, it is clear that “*Lactobacterium zeae*” is not a synonym of *Lactobacillus casei* and that the neotype strain of *Lactobacillus casei* subsp. *casei* (ATCC 393) is genotypically closely related to neither *Lactobacillus casei* nor the subspecies of *Lactobacillus casei* and therefore is not representative of the species. Another strain of *Lactobacillus casei* should be designated the neotype

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TABLE 1. Levels of genotypic relatedness among *Lactobacillus zeae* nom. rev., *Lactobacillus casei*, *Lactobacillus paracasei*, and *Lactobacillus rhamnosus* strains

Strain	% DNA homology with DNA from ^a :		Original name on Approved Lists of Bacterial Names ^b
	<i>Lactobacillus casei</i> ATCC 393 ^T	<i>Lactobacillus casei</i> ATCC 334 ^T	
<i>Lactobacillus paracasei</i> NCFB 2713 (= ATCC 1983 = ATCC 27216 = DSM 20020)	50	85	<i>Lactobacillus casei</i> subsp. <i>alactosus</i> (former type strain)
<i>Lactobacillus paracasei</i> NCFB 151 ^T (= ATCC 25302 ^T = NCDO 141 ^T = DSM 5622 ^T) ^c	22	95	<i>Lactobacillus casei</i> subsp. <i>casei</i>
<i>Lactobacillus paracasei</i> DSM 20006 ^c	35	79	<i>Lactobacillus casei</i> subsp. <i>alactosus</i> (formerly " <i>Lactobacillus casei</i> subsp. <i>fusiformis</i> ")
<i>Lactobacillus casei</i> ATCC 334 ^{Tc}	43	100	<i>Lactobacillus casei</i> subsp. <i>casei</i>
<i>Lactobacillus paracasei</i> NCFB 2743 (= ATCC 25598 = DSM 20008) ^c	33	86	<i>Lactobacillus casei</i> subsp. <i>pseudoplatanturum</i> (former type strain)
<i>Lactobacillus rhamnosus</i> STPS I ^c	ND ^d	ND	
<i>Lactobacillus paracasei</i> DSM 20207 ^c (= NCFB 1977)	40	85	<i>Lactobacillus casei</i> subsp. <i>pseudoplatanturum</i>
<i>Lactobacillus</i> sp. strain LHS ^c	ND	ND	
<i>Lactobacillus rhamnosus</i> ATCC 15820 ^T (= DSM 20178 ^T = NCIB 9537 ^T)	80	42	" <i>Lactobacterium zeae</i> " (type strain)
<i>Lactobacillus paracasei</i> ATCC 25180	47	83	<i>Lactobacillus casei</i> subsp. <i>alactosus</i>
<i>Lactobacillus casei</i> ATCC 393 ^T (= NCDO 161 ^T = DSM 20011 ^T) ^c	100	36	<i>Lactobacillus casei</i> subsp. <i>casei</i>
<i>Lactobacillus rhamnosus</i> NCFB 243 ^T (= ATCC 7469 ^T = DSM 20021 ^T = NCIB 6375 ^T) ^c	45	47	<i>Lactobacillus casei</i> subsp. <i>rhamnosus</i>

^a Data from references 3 and 4.^b See reference 13.^c Strain used in RAPD-PCR analyses.^d ND, not determined.

strain, as suggested by Dellaglio et al. (3). Surprisingly, these important facts have not been taken into account previously, as strain ATCC 393 was retained as the type strain of *Lactobacillus casei* (Orla-Jensen) Hansen and Lessel 1971 on the Approved Lists of Bacterial Names (13).

Further evidence which supports the conclusion that strain ATCC 393 is not a *Lactobacillus casei* strain was presented by Collins et al. (1a). However, the type strain of "*Lactobacterium zeae*" (ATCC 15820) was not included in the study of these authors, and the level of genetic relatedness between this spe-

FIG. 1. Simplified dendrogram showing the clustering of *Lactobacillus zeae* nom. rev., *Lactobacillus casei*, and *Lactobacillus rhamnosus* obtained when we performed a numerical analysis of total soluble cell protein patterns. Based on the dendrogram published previously by Dellaglio et al. (4).

cies and the type strain of *Lactobacillus casei* (ATCC 393) was not determined. Because of this, the true reason for the poor definition of the species *Lactobacillus casei* was not recognized. Collins et al. (1a) retained ATCC 393 as the type strain of *Lactobacillus casei* and reclassified *Lactobacillus casei* subsp. *casei* (which included the majority of the strains studied, including ATCC 334^T), *Lactobacillus casei* subsp. *alactosus*, *Lactobacillus casei* subsp. *pseudopiantarum*, and *Lactobacillus casei* subsp. *tolerans* strains as members of *Lactobacillus paracasei*.

In this paper we describe the DNA polymorphisms of representative strains having the different genotypes and present additional evidence that strain ATCC 393 should be excluded from the species *Lactobacillus casei* (12) Hansen and Lessel 1971. We also show that ATCC 334 is the most representative strain of *Lactobacillus casei* subsp. *casei*, *Lactobacillus casei* subsp. *alactosus*, and *Lactobacillus casei* subsp. *pseudopiantarum* and thus the most appropriate choice to be the neotype strain of *Lactobacillus casei*. These findings imply that the name *Lactobacillus paracasei* Collins, Phillips, and Zanoni 1989 should be rejected.

The strains included in this study are listed in Table 1. A simplified dendrogram based on the protein profile groups identified by Dellaglio et al. (4) is shown in Fig. 1. The randomly amplified polymorphic DNA PCR (RAPD-PCR) fingerprints of the type strains of *Lactobacillus casei* (ATCC 393), *Lactobacillus paracasei* (NCFB 151), and *Lactobacillus rhamnosus* (NCFB 243) and representative strains of subspecies included on the Approved Lists of Bacterial Names (13) are shown in Fig. 2. Strain STPS I was classified as a *Lactobacillus rhamnosus* strain (Table 1) on the basis of the results of physiological and biochemical tests (data not shown). Strain LHS was isolated from South African fortified wines. The RAPD-PCR analyses were performed as described by Du Plessis and Dicks (5). Two single primers, primers OPL-01 (GGCATGA CCT) and OPL-05 (ACGCAGGCAC) were used in this analysis.

Three genetic clusters were identified on the basis of RAPD-PCR data (Fig. 2), confirming the results obtained in DNA-DNA hybridization experiments (1a, 3) and a numerical analysis of total soluble cell protein patterns (4). The three clusters contained the type strains of *Lactobacillus casei* (ATCC 393), *Lactobacillus paracasei* (NCFB 151), and *Lactobacillus rhamnosus* (NCFB 243). The status of *Lactobacillus rhamnosus* as a separate species, as proposed by Collins et al. (1a) was clearly supported by our data.

The RAPD-PCR fingerprints of *Lactobacillus casei* subsp. *casei* ATCC 393^T are different from the RAPD-PCR fingerprints obtained for other *Lactobacillus casei* subsp. *casei* strains (Fig. 2), indicating that strain ATCC 393^T belongs to a separate genetic cluster. *Lactobacillus casei* subsp. *casei* ATCC 393^T exhibited low DNA levels of homology (8 to 46%) with other *Lactobacillus casei* subsp. *casei* strains, but a high level of DNA homology (80%) with "*Lactobacterium zeae*" ATCC 15820^T (3). Furthermore, *Lactobacillus casei* subsp. *casei* ATCC 393^T and *Lactobacillus rhamnosus* ATCC 15820^T belong to one protein profile cluster that is separate from the cluster containing other *Lactobacillus* spp. (Fig. 1). Thus, it is clear that strain ATCC 393^T, as described by Hansen and Lessel (8), is not a typical *Lactobacillus casei* strain but is a typical "*Lactobacterium zeae*" strain, as described by Kuznetsov (9). According to Rule 18g of the *International Code of Nomenclature of Bacteria*, replacement of a neotype strain can be requested if the strain has become unsuitable. On the basis of DNA-DNA hybridization results, the results of a numerical analysis of total soluble cell protein profiles, and the results of RAPD-PCR analyses, we propose that *Lactobacillus casei*

FIG. 2. Agarose gel electrophoresis of PCR amplification products. (A) Primer OPL-1. (B) Primer OPL-5.

subsp. *casei* ATCC 393^T and *Lactobacillus rhamnosus* ATCC 15820 should be reclassified as *Lactobacillus zeae* (ex Kuznetsov 1959) nom. rev. strains (type strain, ATCC 15820). L-(+)-Rhamnose is fermented by *Lactobacillus zeae* ATCC 15820^T, but not by strain ATCC 393^T, and thus this characteristic is not a distinctive trait of the species.

The overall RAPD-PCR profiles obtained for *Lactobacillus casei* subsp. *casei* ATCC 334^T, representative strains of the original genospecies *Lactobacillus casei* included in 1980 on the Approved Lists of Bacterial Names, and *Lactobacillus paracasei* Collins, Phillips, and Zanoni 1989 were similar (Fig. 2), indicating that these organisms belong to one genetic cluster. The majority of the typical strains of *Lactobacillus casei* previously placed in several subspecies, including *Lactobacillus casei*

subsp. *casei*, *Lactobacillus casei* subsp. *alactosus*, *Lactobacillus casei* subsp. *tolerans* (not included in this study), and *Lactobacillus casei* subsp. *pseudoplatantarum*, belong to the same genospecies as *Lactobacillus casei* ATCC 334^T. This important fact was ignored by Collins et al. (1a), who placed *Lactobacillus casei* subsp. *casei* (the majority of the strains studied), *Lactobacillus casei* subsp. *alactosus*, *Lactobacillus casei* subsp. *tolerans*, and *Lactobacillus casei* subsp. *pseudoplatantarum* in the species *Lactobacillus paracasei* because the neotype strain of *Lactobacillus casei* subsp. *casei* (ATCC 393) belonged to a separate genospecies. Collins et al. (1a) did not consider the possibility that an appropriate type strain should be selected for the species *Lactobacillus casei*, as proposed by Dellaglio et al. (3). Our results clearly show that strain ATCC 334^T is the most representative strain of the genospecies *Lactobacillus casei* and *Lactobacillus paracasei*. Furthermore, strain ATCC 334^T has been included as a prominent and suitable reference strain in previous taxonomic studies (2, 3, 6). According to Rule 18e, the neotype strain of a species can be replaced by an original strain which was discovered subsequently. Rule 19 also applies; i.e., a reference strain which has no standing in nomenclature may be made a neotype strain. On the basis of these rules, we propose that strain ATCC 334 should be designated the neotype strain of *Lactobacillus casei* (Orla-Jensen 1916) Hansen and Lessel 1971 and that the name *Lactobacillus paracasei* Collins, Phillips, and Zanoni 1989 should be rejected.

Description of *Lactobacillus zeae* nom. rev. Surface colonies on MRS agar are smooth, glistening, white, and 1 to 2 mm in diameter. Cells are rod shaped (ca. 0.5 to 0.6 by 1.2 to 2.4 µm) and occur singly or in chains, depending on the growth conditions. Gram positive. Nonmotile. Spores are not formed. Grows at 10 and 45°C. Facultatively heterofermentative. Produces mainly L-(+)-lactic acid and a minor amount of D-(-)-lactic acid. Acid is produced from amygdalin, cellobiose, fructose, galactose, gluconate, glucose, lactose, maltose, mannitol, mannose, melezitose, ribose, salicin, sucrose, tagatose, trehalose, and turanose. Acid is not produced from arabinose, dextrin, inositol, inulin, lactate, melibiose, raffinose, sorbitol (only strain ATCC 393^T), sorbose, starch, and xylose. Strain ATCC 15820^T ferments rhamnose, whereas strain ATCC 393^T does not. Gas is produced from gluconate. Catalase, benzidine, urease, and indole tests are negative. Nitrate is not reduced to nitrite, and ammonia is not produced from arginine. Esculin is hydrolyzed. The L-(+)-lactic acid dehydrogenase polyacrylamide gel electrophoretic pattern is identical to the pattern obtained for *Lactobacillus casei* and *Lactobacillus rhamnosus*. The murein type is Lys-D-Asp. The G+C content of the DNA

is 48 to 49 mol%. The type strain is strain ATCC 15820. Isolated from corn steep liquor.

Description of *Lactobacillus casei* neotype strain ATCC 334. Neotype strain ATCC 334 of *Lactobacillus casei* has been described by Dellaglio et al. (4).

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