

The status of the species *Lactobacillus casei* (Orla-Jensen 1916) Hansen and Lessel 1971 and *Lactobacillus paracasei* Collins *et al.* 1989. Request for an Opinion

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On the basis of considerable published evidence, it is concluded that the species *Lactobacillus casei* is not correctly represented by the strain actually designated as the type strain ATCC 393. It is proposed that the Judicial Commission consider: (1) that ATCC 393^T is scientifically unsuitable as the type strain of *Lactobacillus casei* and should be reclassified as *Lactobacillus zeae*; (2) that *Lactobacillus casei* ATCC 334 and *Lactobacillus paracasei* strains are members of the same taxon and therefore can be united within the name *Lactobacillus casei* (Rules 42 and 23a), the name *Lactobacillus paracasei* being rejected; and (3) designating ATCC 334 as the neotype strain for the species *Lactobacillus casei*.

Keywords: *Lactobacillus casei*, *Lactobacillus paracasei*, Request for an Opinion, Bacteriological Code

A previous Request for an Opinion on the nomenclature of the *Lactobacillus casei* species group (Dellaglio *et al.*, 1991) was rejected by the Judicial Commission because ‘... there is not enough basis for concern about confusion leading to serious consequences in industrial microbiology to justify awarding an exception to the Rules’ (Wayne, 1994). However, in recent years we have assisted with a series of publications dealing with new *Lactobacillus* species in which the reference strain or, in some cases the type strain, of the species *Lactobacillus casei* was alternatively ATCC 334 (Yoon *et al.*, 2000; Cai *et al.*, 1999; Morlon-Guyot *et al.*, 1998) or ATCC 393 (Edwards *et al.*, 2000; Kato *et al.*, 2000; Swezey *et al.*, 2000; Tanasupawat *et al.*, 2000). Moreover, either ATCC 393 (= LMG 6904) (Morlon-Guyot *et al.*, 1998) or LMG 17315 (= ATCC 15820) (Cai *et al.*, 1999) has been used as the type strain for the species *Lactobacillus zeae*.

The precise individuation of a type strain for analysis has importance from both scientific and industrial points of view. In fact, taxonomic and phylogenetic relationships between species are often delineated entirely on the basis of the type strains, which therefore have to be representative. This also has practical implications, as these *Lactobacillus* species are often isolated from foods and are largely employed in the food industry.

A chronological summary of the scientific evidence concerning the 25-year-old taxonomic problem of *Lactobacillus casei* is reported here for ease of comprehension.

(1) The emended description of *Lactobacillus casei* (Orla-Jensen 1916) comb. nov. by Hansen & Lessel (1971) was based on the neotype strain ATCC 393. No original strains representing *Lactobacillus casei* in live form were available other than strain ATCC 393, which was believed to be a subculture of strain no. 7 from the Orla-Jensen strain collection, and it was believed that it represented a typical *Lactobacillus casei*. However, this assumption was made on the basis of phenotypic traits only.

(2) DNA–DNA hybridization experiments performed in two independent studies (Mills & Lessel, 1973; Dellaglio *et al.*, 1975) showed that *Lactobacillus casei* subsp. *casei* ATCC 393^T was genotypically strongly related to *Lactobacillus casei* subsp. *rhamnosus* ATCC 15820, the former type strain of ‘*Lactobacterium zeae*’ (Kuznetsov, 1959). Moreover, it was not related to any of the other strains within the subspecies of *Lactobacillus casei* (homology values were between 43 and 50%). Therefore, shortly after its designation, strain ATCC 393^T was shown to be unsuitable as the neotype strain of *Lactobacillus casei* species.

(3) The true status of '*Lactobacterium zeae*' (actually *Lactobacillus zeae*; Dicks *et al.*, 1996) was overlooked and, accordingly, neglected during the preparation of the Approved List of Bacterial Names in 1980.

(4) DNA homology studies performed by Collins *et al.* (1989) revealed that the majority of strains designated as *Lactobacillus casei* (divided into different subspecies) exhibited high levels of DNA relatedness with each other (61–100%) but were unrelated (10–20%) to *Lactobacillus casei* subsp. *casei* ATCC 393^T. In the light of those experiments, these workers proposed to create the species *Lactobacillus paracasei* for strains unrelated to *Lactobacillus casei* ATCC 393^T. However, Collins *et al.* omitted to test the relatedness between ATCC 393^T and *Lactobacillus casei* subsp. *rhamnosus* ATCC 15820.

(5) Numerical analysis of total soluble cell protein and DNA–DNA hybridization experiments performed by Dellaglio *et al.* (1991) corresponded to the results of Dellaglio *et al.* (1975) and Collins *et al.* (1989), but the request for ATCC 334 to be designated, in place of ATCC 393, as the neotype strain of *L. casei* (together with the rejection of the name *Lactobacillus paracasei*) was denied (Wayne, 1994).

(6) The randomly amplified polymorphic DNA PCR fingerprints obtained by Dicks *et al.* (1996) strongly confirmed the results obtained previously (Dellaglio *et al.*, 1975, 1991; Collins *et al.*, 1989). In this paper, the species *Lactobacillus zeae* was validly proposed but this epithet referred only to strain ATCC 15820.

(7) A comparative sequence analysis of the genes encoding 16S rRNA of *Lactobacillus casei*-related taxa has recently shown that *Lactobacillus casei* ATCC 334 and other strains of the species *Lactobacillus paracasei* had the same 16S rDNA sequences. In contrast, ATCC 393^T had its own sequence, which differs from that of *Lactobacillus zeae* ATCC 15820^T and from that of the *Lactobacillus casei* ATCC 334/*Lactobacillus paracasei* group. Moreover, the difference between ATCC 393^T and the *Lactobacillus casei* ATCC 334/*Lactobacillus paracasei* group is greater (twofold) than that between ATCC 393^T and *Lactobacillus zeae* (Mori *et al.*, 1997). Their findings support the request for the taxonomic position of the *Lactobacillus casei* group proposed by Dellaglio *et al.* (1991).

(8) An RFLP analysis and a chromotyping analysis performed on type, reference, clinical, health-food and stock isolates of *Lactobacillus* species with *Bcl*I and *Dra*I revealed a different pattern for *Lactobacillus casei* ATCC 393 and *Lactobacillus casei* ATCC 334. In contrast, ATCC 334 and the *Lactobacillus paracasei* strains (Zhong *et al.*, 1998) shared a similar profile.

(9) In 1999, Tynkkynen *et al.* demonstrated that the exact identification of *Lactobacillus casei* group strains by phenotypic traits was not reliable at the species level. Moreover, randomly amplified polymorphic DNA PCRs, ribotyping, PFGEs, and species-specific PCRs produced results in accordance with the classification

of the *Lactobacillus casei* group suggested by Dellaglio *et al.* (1991) and Dicks *et al.* (1996).

(10) A further analysis of the genes encoding 23S–5S rRNA intergenic spacer regions, together with an rRNA RFLP, showed that *Lactobacillus casei* ATCC 393^T has spacers 197 and 117 bp in length while those of the *Lactobacillus casei*-related strains are 198 and 122 bp long. Moreover, the sequence of the long intergenic spacer region of strain ATCC 393^T has only about 80.2% similarity with the spacers of the other strains of *Lactobacillus paracasei* and 72.4% for the short intergenic spacer region. Finally, the *Bam*HI and *Pst*I ribotypes of strain ATCC 393^T were distinct from the others in the group, whereas strain ATCC 334 shared high levels of similarity with them (Chen *et al.*, 2000). Their results support the request for the taxonomic position of the *Lactobacillus casei* group proposed by Dellaglio *et al.* (1991).

(11) A comparative analysis performed on partial sequences of the *recA* gene strongly support the view that ATCC 393 and *Lactobacillus zeae* ATCC 15820^T share a great degree of similarity but are clearly distinct from *Lactobacillus casei* ATCC 334 and *Lactobacillus paracasei* strains. Moreover, ATCC 334 and the *Lactobacillus paracasei* group are genetically very closely related and belong to the same taxon (Felis *et al.*, 2001).

On the basis of the data reported, we conclude that ATCC 393 is not, and never has been, a typical *Lactobacillus casei* strain. We are referring this matter to the Judicial Commission (Rule 18g of the Bacteriological Code; Lapage *et al.*, 1992) and propose that the Judicial Commission consider the following course of action.

(1) Strain ATCC 393, as the neotype of the species *Lactobacillus casei* (Orla-Jensen 1916) Hansen and Lessel 1971, should be recognized as scientifically unsuitable (Rule 18g) since it does not genetically represent the species *Lactobacillus casei*. Furthermore, recent genotypic evidence (Mori *et al.*, 1997; Tynkkynen *et al.*, 1999; Chen *et al.*, 2000; Felis *et al.*, 2001) strongly supports previous indications (Mills & Lessel, 1973; Dellaglio *et al.*, 1975, 1991; Dicks *et al.*, 1996) that strains ATCC 393 and ATCC 15820 are members of the same taxon. Even though the valid status of the species *Lactobacillus zeae* was recognized only in 1996 (Dicks *et al.*, 1996), the misidentified strain ATCC 393 is closely related to the type strain of this species. We ask the Judicial Commission to pronounce on the classification of strain ATCC 393. On the basis of the evidence reported, we propose that it could be allotted to the species *Lactobacillus zeae* (ex '*Lactobacterium zeae*' Kuznetsov 1959) (Dicks *et al.*, 1996).

(2) The strains of the *Lactobacillus casei* and *Lactobacillus paracasei* species form a single taxon, and therefore we ask the Judicial Commission to unite them in a single species with the name *Lactobacillus*

casei, the oldest legitimate name (Rules 42 and 23a), and to reject the name *Lactobacillus paracasei* (Collins *et al.*, 1989). We also ask the Judicial Commission to maintain the previous subspecies of *Lactobacillus casei*, i.e. *Lactobacillus casei* subsp. *casei* and *Lactobacillus casei* subsp. *tolerans*.

(3) On the basis that the species *Lactobacillus casei* has no type strain (see Request 1), we propose strain ATCC 334 as the neotype, since it has been already proposed (Dellaglio *et al.*, 1991; Dicks *et al.*, 1996), supported (Mori *et al.*, 1997; Tynkkynen *et al.*, 1999; Chen *et al.*, 2000; Felis *et al.*, 2001) and used as a reference strain (Yoon *et al.*, 2000; Cai *et al.*, 1999; Morlon-Guyot *et al.*, 1998) (Rule 19). Furthermore, strain LMG 9191^T may be retrieved as the type strain of the subspecies *Lactobacillus casei* subsp. *tolerans*.

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