Reclassification of *Lactobacillus maltaromicus* (Miller *et al.* 1974) DSM 20342^T and DSM 20344 and *Carnobacterium piscicola* (Collins *et al.* 1987) DSM 20730^T and DSM 20722 as *Carnobacterium maltaromaticum* comb. nov.

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Phenotypic and genotypic characterizations of *Lactobacillus maltaromicus* strains DSM 20342^T and DSM 20344 provided evidence for the reclassification of this species in the genus *Carnobacterium*. Moreover, phenotypic and genotypic comparisons made between *L. maltaromicus* and *Carnobacterium piscicola* highlighted that these two species should be considered synonyms. For these reasons, the species *Carnobacterium maltaromaticum* comb. nov. (type strain DSM 20342^T = ATCC 27865^T = CCUG 30142^T = CIP 103135^T = JCM 1154^T = LMG 6903^T = NRRL B-14852^T) is proposed to accommodate *L. maltaromicus* and *C. piscicola*.

Modern taxonomic methods have led to numerous reclassifications and rearrangements of bacterial strains, species and genera. In this context, the genus *Carnobacterium* was created by the reclassification of atypical *Lactobacillus* species isolated from vacuum-packed meat and fish (Collins *et al.*, 1987). The genus *Carnobacterium* was created to group heterofermentative, rod-shaped lactic acid bacteria that produce L-lactic acid from glucose and are characterized by the presence of *meso*-diaminopimelic acid in their cell-wall composition. At the time of writing, the genus *Carnobacterium* comprised seven species, *Carnobacterium alterfunditum*, *Carnobacterium funditum* (Franzmann *et al.*, 1991), *Carnobacterium divergens* (Holzapfel & Gerber, 1983; Collins *et al.*, 1987), *Carnobacterium gallinarum*, *Carnobacterium mobile* (Collins *et al.*, 1987), *Carnobacterium inhibens* (Jöborn *et al.*, 1999) and *Carnobacterium piscicola* (Hiu *et al.*, 1984; Shaw & Harding, 1985). Collins *et al.* (1991) inferred the phylogenetic relationships among *Lactobacillus* species, *Carnobacterium* species and related lactic acid bacteria on the basis of 16S rDNA sequence data. In that study, Collins and colleagues suggested the revision of the taxonomic position of *Lactobacillus maltaromicus* due to the high 16S rDNA sequence similarity (100% sequence similarity for a comparison based on 1340 nt of 16S rDNA sequence) detected between this species and *C. piscicola*, formerly *Lactobacillus piscicola* (Hiu *et al.*, 1984). *L. maltaromicus* was

first described by Miller *et al.* (1974) as a new lactic acid bacteria isolated from milk and producing a malty-like flavour and aroma.

In this study, a phenotypic and genotypic comparison among *L. maltaromicus* strains DSM 20342^T and DSM 20344 (Miller *et al.*, 1974) and *C. piscicola* strains DSM 20730^T and DSM 20722 was carried out with the aim of clarifying the taxonomic position of these two species. Specifically, the aforementioned *Lactobacillus* and *Carnobacterium* species were compared by evaluating their carbohydrate fermentation patterns, by determining whether *meso*-diaminopimelic acid was present in their cell-wall composition, and by determining the nature of the enantiomeric form of the lactic acid produced by their metabolism. Moreover, all the strains were characterized genetically by restriction analysis of their amplified 16S rDNA, by amplification of the internal transcribed spacers between their 16S and 23S rDNA and by evaluation of their DNA–DNA relatedness.

L. maltaromicus strains DSM 20342^T and DSM 20344, *C. piscicola* strains DSM 20730^T and DSM 20722, and *C. divergens* DSM 20623^T and *C. gallinarum* DSM 4847^T were maintained routinely at 4 °C after growth at 30 °C for 12 or 24 h in TSBY medium (30 g trypticase soy broth l⁻¹, 3 g yeast extract l⁻¹, pH 7). For long-term maintenance, stock cultures were stored in 20% (v/v) glycerol/80% (v/v) TSBY medium at –80 °C.

The carbohydrate fermentation patterns obtained using the

API CH50 system (bioMérieux), with incubation at 30 °C for 12–24 h, were very similar for the *L. maltaromicus* and *C. piscicola* strains. The strains were able to ferment glycerol, ribose, galactose, D-glucose, D-fructose, D-mannose, methyl α -D-mannopyranoside, N-acetylglucosamine, amygdalin, arbutin, aesculin, salicin, cellobiose, maltose, sucrose, trehalose and β -gentibiose. A weak positive reaction was detected for methyl α -D-glucopyranoside. Lactose was fermented by the *L. maltaromicus* strains, while a weak positive reaction was observed for the *C. piscicola* strains. Starch and mannitol were fermented by the *C. piscicola* strains, while a weak positive reaction was detected for the *L. maltaromicus* strains. Neither species fermented erythritol, D-/L-arabinose, D-/L-xylose, adonitol, methyl β -D-xylopyranoside, L-sorbose, rhamnose, dulcitol, inositol, sorbitol, melibiose, inulin, melezitose, D-raffinose, xylitol, D-turanose, D-lyxose, D-tagatose, D-/L-fucose, D-/L-arabitol, gluconate, 2-ketogluconate nor 5-ketogluconate.

All *Lactobacillus* and *Carnobacterium* strains tested produced the L(+) enantiomeric form of lactic acid, as determined by using the D-L lactic acid kit (Roche). Moreover, the presence of meso-diaminopimelic acid was detected in the cell wall of all the strains tested using the method of Hancock (1994). Genotypic characterizations, based on a randomly amplified polymorphic DNA (RAPD) fingerprinting analysis, a 16S–23S rDNA intergenic spacer analysis and a restriction analysis of amplified 16S rDNA, were carried out for *L. maltaromicus* strains DSM 20342^T and DSM 20344 and for *C. piscicola* DSM 20730^T in comparison with the two closest phylogenetic neighbours of these species, *C. divergens* DSM 20623^T and *C. gallinarum* DSM 4847^T (Collins *et al.*, 1991). DNA extraction, PCR and restriction protocols were performed as described previously (Mora *et al.*, 1998, 2000); RAPD fingerprinting analysis was carried out with primer OPI-02mod (5'-GCTCGGAGGAGAGG-3').

The amplified 16S–23S rDNA intergenic spacer analysis showed an identical electrophoretic profile for the *L. maltaromicus* and *C. piscicola* strains (Fig. 1), which was characterized by a main fragment of 480 bp and secondary fragments ranging in size from 510 to 700 bp. *C. divergens* DSM 20623^T and *C. gallinarum* DSM 4847^T were easily distinguished from the *L. maltaromicus* and *C. piscicola* strains due to the presence of main amplification fragments of 310 and 420 bp, respectively, in their electrophoretic profiles. Likewise, the *L. maltaromicus* and *C. piscicola* strains showed identical *Hae*III/*Hinf*I restriction profiles for their amplified 16S rDNA (Fig. 2), while distinct patterns were obtained for *C. divergens* DSM 20623^T and *C. gallinarum* DSM 4847^T. The high level of similarity of the ribosomal locus detected for the *L. maltaromicus* and *C. piscicola* strains was also confirmed by RAPD fingerprinting analysis, as shown in Fig. 3.

The high genetic similarity detected among *L. maltaromicus* and *C. piscicola* strains substantiated the results obtained by the phenotypic characterization and suggests a revision of

Fig. 1. 16S–23S rDNA intergenic spacer amplification patterns of *L. maltaromicus*, *C. piscicola*, *C. gallinarum* and *C. divergens*. Lanes: 1, *L. maltaromicus* DSM 20342^T; 2, *L. maltaromicus* DSM 20344; 3, *C. piscicola* DSM 20730^T; 4, *C. gallinarum* DSM 4847^T; 5, *C. divergens* DSM 20623^T; M, 100 bp ladder (MBI Fermentans).

the taxonomic position of *L. maltaromicus* and *C. piscicola*. In this context, due to the high genotypic and phenotypic similarity between *L. maltaromicus* strains DSM 20342^T and DSM 20344, and between *C. piscicola* strains DSM 20730^T and DSM 20722, an evaluation of the DNA–DNA relatedness of these strains was carried using *C. piscicola*

Fig. 2. *Hae*III/*Hinf*I restriction analysis of amplified 16S rDNA of *L. maltaromicus*, *C. piscicola*, *C. gallinarum* and *C. divergens*. Lanes: 1, *L. maltaromicus* DSM 20342^T; 2, *C. piscicola* DSM 20730^T; 3, *C. piscicola* DSM 20722; 4, *C. gallinarum* DSM 4847^T; 5, *C. divergens* DSM 20623^T; M, GeneRuler 100 bp ladder plus (MBI Fermentans); M', GeneRuler 100 bp DNA ladder (MBI Fermentans).

Fig. 3. RAPD fingerprinting analysis of *L. maltaromicus*, *C. piscicola*, *C. gallinarum* and *C. divergens*. Lanes: 1, *L. maltaromicus* DSM 20342^T; 2, *L. maltaromicus* DSM 20344; 3, *C. piscicola* DSM 20730^T; 4, *C. gallinarum* DSM 4847^T; 5, *C. divergens* DSM 20623^T; M, GeneRuler 100 bp DNA ladder plus (MBI Fermentans); M', GeneRuler 1 kb ladder (MBI Fermentans).

DSM 20730^T, *C. divergens* DSM 20623^T, *C. gallinarum* DSM 4847^T and *L. maltaromicus* DSM 20342^T as reference strains. DNA–DNA reassociation experiments were carried out as described previously (Mora *et al.*, 1998) using the optical renaturation rate method and a model Response spectrophotometer (Gilford systems, CIBA-Corning Diagnostics). The results obtained suggested that *C. piscicola* DSM 20730^T and *L. maltaromicus* DSM 20342^T belong to the same species, showing 85 % DNA–DNA relatedness. DNA–DNA relatedness values of 26 and 29 % were obtained between *L. maltaromicus* DSM 20342^T and *C. gallinarum* DSM 4847^T, and between *L. maltaromicus* and *C. divergens* DSM 20623^T, respectively.

In conclusion, the genotypic and phenotypic comparisons carried out among *L. maltaromicus* and *C. piscicola* strains provide evidence for the reclassification of *L. maltaromicus* DSM 20342^T and DSM 20344 in the genus *Carnobacterium*. Moreover, the results of DNA–DNA reassociation analyses highlight that *L. maltaromicus* and *C. piscicola* belong to the same species. On the basis of the results presented here, we propose the reclassification of *L. maltaromicus* DSM 20342^T and DSM 20344 (Miller *et al.*, 1974) and *C. piscicola* DSM 20730^T and DSM 20722 (Hiu *et al.*, 1984; Collins *et al.*, 1987) as *Carnobacterium maltaromaticum*.

Description of *Carnobacterium maltaromaticum* comb. nov.

Basonym: *Lactobacillus maltaromicus* Miller *et al.* 1974; *Carnobacterium piscicola* Collins *et al.* 1987.

Carnobacterium maltaromaticum (malt.a.ro.mat.ic'um. N.L. neut. n. *maltum* -i malt; L. adj. *aromaticus* -a -um aromatic, fragrant; N.L. neut. adj. *maltaromaticum* possessing a malt-like aroma).

The description includes data compiled by Hiu *et al.* (1984) and Collins *et al.* (1987), and those generated in this study. Asporogenous, Gram-positive rods of varying length, which occur singly or in chains. Cells are non-motile, and catalase- and oxidase-negative. Facultatively anaerobic. L(+) -Lactic acid, ethanol and acetate are produced heterofermentatively. Gas production is weak and frequently undetectable. Growth occurs in MRS, TSBY and brain–heart infusion media. Growth occurs at 4 and 15 °C, but not at 45 °C; optimum growth occurs between 28 and 32 °C. Arginine and aesculin are hydrolysed. Nitrate is not reduced to nitrite. Acid is produced from glycerol, ribose, galactose, D-glucose, D-fructose, D-mannose, lactose, methyl α -D-mannopyranoside, methyl α -D-glucopyranoside, N-acetylglucosamine, amygdalin, arbutin, aesculin, salicin, cellobiose, maltose, sucrose, trehalose, β -gentiobiose, mannitol and starch. The peptidoglycan is of the *meso*-diaminopimelic acid direct type. Major cellular fatty acids are straight-chain saturated and monounsaturated acids, with tetradecanoic, hexadecanoic and 9- and 10-octadecenoic acids predominating. G+C content of the DNA ranges from 33.7 to 36.4 mol%.

The type strain of *Carnobacterium maltaromaticum* is DSM 20342^T (=ATCC 27865^T=CCUG 30142^T=CIP 103135^T=JCM 1154^T=LMG 6903^T=NRRL B-14852^T).

Note added in proof

Since this article was accepted for publication, *Carnobacterium viridans* has been described (Holley *et al.*, 2002).

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