Three novel strains isolated from brewery environments are described. These strains were Gram-positive, facultatively anaerobic, heterofermentative rods that did not exhibit catalase activity. Phylogenetic analysis based on 16S rRNA gene sequence similarity showed that these strains belong to the genus Lactobacillus and are most closely related to Lactobacillus collinoides (approximately 99% similarity). The novel strains could be differentiated from L. collinoides on the basis of DNA–DNA relatedness, differences in beer-spoilage ability and the inability to utilize D-fructose. These isolates represent a novel species, for which the name Lactobacillus paracollinoides sp. nov. is proposed. The type strain is LA2T (DSM 15502T = JCM 11969T).

Although the majority of bacteria are incapable of growing in beer, a limited number of species of lactobacilli exhibit strong beer-spoilage ability (Back, 1981). Lactobacillus brevis is known to be the most prevalent beer-spoilage species (Back et al., 1988; Back, 1994a). Three brewery isolates, LA2T, LA3 and LA4, that possess strong beer-spoilage ability have been reported previously (Funahashi et al., 1998). Since these three strains showed identical ribotypes and morphological features, the isolates were indistinguishable at the strain level. Coupled with the fact that these three strains were isolated from one brewery, they may well be considered to be identical. The representative strain, LA2T, did not show sufficient DNA–DNA relatedness to be classified as any of the validly published Lactobacillus species, although it was most closely related to Lactobacillus collinoides JCM 1123T on the basis of 16S rRNA gene sequence comparisons (Funahashi et al., 1998). L. collinoides strains are not generally considered to be beer-spoilage bacteria (Back, 1994b; Carr & Davies, 1972, 1974), but LA2T was able to grow in beer.

Recently, strains LA7 and LA8 have been isolated from different breweries in Japan. These strains also exhibited strong beer-spoilage ability. 16S rRNA gene sequence analysis indicated that these strains are potentially related to L. collinoides or Lactobacillus sp. LA2T at the species level. These findings led us to characterize these novel beer-spoilage strains and to investigate their taxonomic relationship with L. collinoides and Lactobacillus sp. LA2T. Based on these results, a novel species, Lactobacillus paracollinoides sp. nov., is described.

Lactobacillus strains used in this study were grown in MRS broth (Merck) at 25 °C under anaerobic conditions. Carbohydrate fermentation profiles were determined using the API 50CH system (bioMérieux). API tests were performed in accordance with the manufacturer's instructions. Each strain was examined for morphological features, motility and Gram staining by microscopy. Hydrogen peroxide (3%) was used to test for catalase activity. Gas production from glucose was examined using Durham tubes. An F-kit di-lactic acid (Boehringer Mannheim) was used to determine production of D- and L-lactic acids. Beer-spoilage ability was determined by inoculating degassed commercial beers (pH 4.2) with each strain at 3 × 10⁵ cells ml⁻¹. The inoculated beers were incubated anaerobically at 25 °C and examined regularly for visible growth for up to 90 days (Suzuki et al., 2002).

The ribotype of each strain was obtained using a Riboprinter (Qualicon) in accordance with the manufacturer's instructions, with EcoRI as a restriction enzyme (Bruce et al., 1995; Hubner et al., 1995; Olsen et al., 1991). G+C contents were determined by HPLC as described by Mesbah et al. (1989). Experimental procedures for 16S rRNA gene analysis were described previously (Funahashi et al., 1998). Sequences were edited with the DNAsis PRO software package (Hitachi Software Engineering). The CLUSTAL W algorithm (Thompson et al., 1994) provided in DNAsis PRO was used to align sequences and to construct a neighbour-joining tree with 1000 bootstrap iterations. A DNA–DNA hybridization study was carried out as described by De Ley et al. (1970) with some modifications (Escara & Hutton, 1980; Huß et al., 1983). A model 2600

**Correspondence**
Koji Suzuki
koji.suzuki@asahibeer.co.jp

**International Journal of Systematic and Evolutionary Microbiology** (2004), 54, 115–117
DOI 10.1099/ijs.0.02722-0

**Lactobacillus paracollinoides** sp. nov., isolated from brewery environments

Koji Suzuki,¹ Wataru Funahashi,² Masahiro Koyanagi¹ and Hiroshi Yamashita¹

Analytical Technology Laboratory¹ and Brewing Research & Development Laboratory², Asahi Breweries Ltd, 1-2 Midori 1-chome, Moriya-shi, Ibaraki, 302-0106, Japan

Three novel strains isolated from brewery environments are described. These strains were Gram-positive, facultatively anaerobic, heterofermentative rods that did not exhibit catalase activity. Phylogenetic analysis based on 16S rRNA gene sequence similarity showed that these strains belong to the genus Lactobacillus and are most closely related to Lactobacillus collinoides (approximately 99% similarity). The novel strains could be differentiated from L. collinoides on the basis of DNA–DNA relatedness, differences in beer-spoilage ability and the inability to utilize D-fructose. These isolates represent a novel species, for which the name Lactobacillus paracollinoides sp. nov. is proposed. The type strain is LA2T (DSM 15502T = JCM 11969T).

Although the majority of bacteria are incapable of growing in beer, a limited number of species of lactobacilli exhibit strong beer-spoilage ability (Back, 1981). Lactobacillus brevis is known to be the most prevalent beer-spoilage species (Back et al., 1988; Back, 1994a). Three brewery isolates, LA2T, LA3 and LA4, that possess strong beer-spoilage ability have been reported previously (Funahashi et al., 1998). Since these three strains showed identical ribotypes and morphological features, the isolates were indistinguishable at the strain level. Coupled with the fact that these three strains were isolated from one brewery, they may well be considered to be identical. The representative strain, LA2T, did not show sufficient DNA–DNA relatedness to be classified as any of the validly published Lactobacillus species, although it was most closely related to Lactobacillus collinoides JCM 1123T on the basis of 16S rRNA gene sequence comparisons (Funahashi et al., 1998). L. collinoides strains are not generally considered to be beer-spoilage bacteria (Back, 1994b; Carr & Davies, 1972, 1974), but LA2T was able to grow in beer.

Recently, strains LA7 and LA8 have been isolated from different breweries in Japan. These strains also exhibited strong beer-spoilage ability. 16S rRNA gene sequence analysis indicated that these strains are potentially related to L. collinoides or Lactobacillus sp. LA2T at the species level. These findings led us to characterize these novel beer-spoilage strains and to investigate their taxonomic relationship with L. collinoides and Lactobacillus sp. LA2T. Based on these results, a novel species, Lactobacillus paracollinoides sp. nov., is described.

Lactobacillus strains used in this study were grown in MRS broth (Merck) at 25 °C under anaerobic conditions. Carbohydrate fermentation profiles were determined using the API 50CH system (bioMérieux). API tests were performed in accordance with the manufacturer's instructions. Each strain was examined for morphological features, motility and Gram staining by microscopy. Hydrogen peroxide (3%) was used to test for catalase activity. Gas production from glucose was examined using Durham tubes. An F-kit di-lactic acid (Boehringer Mannheim) was used to determine production of D- and L-lactic acids. Beer-spoilage ability was determined by inoculating degassed commercial beers (pH 4.2) with each strain at 3 × 10⁵ cells ml⁻¹. The inoculated beers were incubated anaerobically at 25 °C and examined regularly for visible growth for up to 90 days (Suzuki et al., 2002).

The ribotype of each strain was obtained using a Riboprinter (Qualicon) in accordance with the manufacturer's instructions, with EcoRI as a restriction enzyme (Bruce et al., 1995; Hubner et al., 1995; Olsen et al., 1991). G+C contents were determined by HPLC as described by Mesbah et al. (1989). Experimental procedures for 16S rRNA gene analysis were described previously (Funahashi et al., 1998). Sequences were edited with the DNAsis PRO software package (Hitachi Software Engineering). The CLUSTAL W algorithm (Thompson et al., 1994) provided in DNAsis PRO was used to align sequences and to construct a neighbour-joining tree with 1000 bootstrap iterations. A DNA–DNA hybridization study was carried out as described by De Ley et al. (1970) with some modifications (Escara & Hutton, 1980; Huß et al., 1983). A model 2600
spectrophotometer equipped with a model 2527-R thermoprogrammer and plotter (Gilford Instrument Laboratories) was used for determining DNA–DNA relatedness. Renaturation rates were computed with the program TRANSFER.BAS (Jahnke, 1992).

Strains LA2\(^T\), LA7 and LA8 were Gram-positive rods that exhibited no catalase activity. When grown on MRS agar, their colonies were small and non-pigmented. Growth was observed at 15 °C but not at 45 °C in MRS broth. All strains produced predominantly D-lactic acid from glucose with a smaller amount of L-lactic acid; the proportion of D-lactic acid ranged between 66-5 and 79-1 %, depending on the strain. Gas production was observed for each strain. Compared with L. collinoides JCM 1123\(^T\), the major difference in carbohydrate utilization profiles was the inability of the brewery isolates to ferment D-fructose. The ability to utilize L-arabinose was variable. Except for these differences, carbohydrate utilization by the isolates was identical to that observed in L. collinoides JCM 1123\(^T\). The DNA \(G + C\) content of strain LA2\(^T\) was 44-8 mol\%, which is within the range for the genus Lactobacillus (32–53 mol\%) (Kandler & Weiss, 1986).

The beer-spoilage ability of the strains was compared with that of three L. collinoides strains, JCM 1123\(^T\), ATCC 27610 and ATCC 27611. Strains LA2\(^T\), LA7 and LA8 exhibited strong beer-spoilage ability and formed visual turbidity in beer within 7 days. This degree of beer-spoilage ability is comparable with that of the most serious beer-spoilage bacterium, Lactobacillus brevis (Suzuki et al., 2002). In contrast, none of the three L. collinoides strains tested in this study was able to grow in beer, even after 90 days of observation. Thus, strains LA2\(^T\), LA7 and LA8 are distinguishable from L. collinoides in terms of beer-spoilage ability.

The 16S rRNA gene sequences of LA7 and LA8 showed approximately 99% similarity to those of L. collinoides JCM 1123\(^T\) (DDJB accession number AB005893) and Lactobacillus sp. LA2\(^T\) (E16651), suggesting that these four strains are closely related. DNA–DNA relatedness data showed that LA7 and LA8 showed 86-8 and 70-7 % relatedness, respectively, to LA2\(^T\). In contrast, DNA similarity between the three brewery isolates and L. collinoides JCM 1123\(^T\) was relatively low (46-8–57-6 %). These results, together with differences in beer-spoilage ability and the ability to utilize D-fructose, show that the three brewery isolates are most likely to be related at the species level and should be regarded as a species distinct from L. collinoides. Ribotyping of LA7 and LA8 yielded ribopatterns that were distinct from that of LA2\(^T\), indicating these three brewery isolates are distinguishable at the strain level. A phylogenetic tree showing the relationship of LA2\(^T\) with other Lactobacillus species is shown in Fig. 1.

Taken collectively, these results allowed us to assign the brewery isolates described in the present study to a novel species, for which the name Lactobacillus paracollinoides sp. nov. is proposed.

**Description of Lactobacillus paracollinoides sp. nov.**

Lactobacillus paracollinoides (pa.ra.col.li.no’i.des. Gr. pref. para beside; N.L. masc. adj. collinoides hill-shaped, referring to the colony form of Lactobacillus collinoides; N.L. masc. adj. paracollinoides beside collinoides, referring to the close relationship to L. collinoides).

Cells are Gram-positive, non-motile, non-spore-forming rods, occurring singly or in short chains. Facultatively anaerobic, catalase-negative and heterofermentative. All strains so far isolated grow at 15 °C, but not at 45 °C. A predominant amount of D-lactic acid, with a smaller amount of L-lactic acid, is produced from glucose. Acid is produced from ribose, D-xylose, D-glucose, maltose and melibiose. Acid production from L-arabinose is variable. No acid is produced from glycerol, erythritol, D-arabinose, L-xylose, adonitol, methyl \(\beta\)-xiloside, D-fructose, D-mannose, D-sorbose, rhamnose, dulcitol, inositol, mannotol, sorbitol, methyl \(\alpha\)-D-mannoside, methyl \(\alpha\)-D-glucosamine, myagdalin, arbutin, salicin, cellobiose, lactose, sucrose, trehalose, inulin, melezitose, D-raffinose, starch, glycogen, xylitol, \(\beta\)-gentiobiose, D-turanose, D-lyxose, D-tagatose, D- or L-fucose, D- or L-arabitol, glucosate or 2- or 5-ketogluconate.

The DNA G + C content of the type strain, strain LA2\(^T\) (= DSM 15502\(^T\) = JCM 11969\(^T\)), is 44-8 mol\%. Isolated from brewery environments. All the strains presently isolated exhibit strong beer-spoilage ability.


References


