**Enterococcus camelliae** sp. nov., isolated from fermented tea leaves in Thailand

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A Gram-positive and catalase-negative coccus that formed chains, strain FP15-1T, isolated from fermented tea leaves ('miang'), was studied systematically. The strain was facultatively anaerobic and produced L-lactic acid from glucose. Demethylmenaquine (DMK-7) was the major menaquinone. Straight-chain unsaturated fatty acids C16:1 and C18:1 were the dominant components. The DNA G+C content was 37.8 mol%. On the basis of 16S rRNA and RNA polymerase \( \alpha \) subunit \((\text{rpoA})\) gene sequence analysis, strain FP15-1T was closely related to *Enterococcus italicus* KCTC 5373\(^T\), with 99.2 and 93.8% similarity, respectively. The strain could be clearly distinguished from *E. italicus* ATCC 5373\(^T\) by low DNA–DNA relatedness (≤33.8%) and phenotypic characteristics. Therefore, this strain represent a novel species of the genus *Enterococcus*, for which the name *Enterococcus camelliae* sp. nov. is proposed. The type strain is FP15-1\(^T\) (=KCTC 13133\(^T\) =NBRC 101868\(^T\) =NRIC 0105\(^T\) =TISTR 932\(^T\) =PCU 277\(^T\)).

The enterococci comprise an important group of lactic acid bacteria found ubiquitously in the environment, the gastrointestinal tract, traditional fermented foods and dairy products. The classification of the genus *Enterococcus* has undergone considerable changes as a consequence of the increasing number of species and also improvements in methods to discriminate between species (Baele et al., 2000; Merquior et al., 1994; Naser et al., 2005). At the time of writing, 33 *Enterococcus* species names have been validly published (Euzéby, 1997; last full update 1 February 2007). Four species groups, the *Enterococcus faecium*, *E. avium*, *E. gallinarum* and *E. cecorum* groups, and other ungrouped species, including *Enterococcus faecalis*, *E. saccharolyticus*, *E. sulfureus* and *E. dispar*, were revealed by comparative 16S rRNA gene sequence analysis (Hardie & Whiley, 1997). Recently, *Enterococcus saccharominimus*, represented by isolates from contaminated pasteurized cow’s milk (Vancanneyt et al., 2004), was reclassified as a later synonym of *Enterococcus italicus*, isolated from artisanal Italian cheeses (Fortina et al., 2004), by comparison of partial sequences for three housekeeping genes, phenylalanyl-tRNA synthase \( \zeta \) subunit \((\text{pheS})\), RNA polymerase \( \alpha \) subunit \((\text{rpoA})\) and the \( \zeta \) subunit of ATP synthase \((\text{atpA})\), and as confirmed by DNA–DNA hybridization (Naser et al., 2006). In the present paper, we describe a novel *Enterococcus* strain from fermented tea leaves ('miang') based on phenotypic and chemotaxonomic characteristics, DNA–DNA relatedness and 16S rRNA and *rpoA* gene sequence analysis.

Samples of fermented tea leaves were collected from Chiangmai province, in the northern part of Thailand. Coci in chains were isolated from the samples using GYP-CaCO\(_3\) agar (Tanasupawat et al., 1992). GYP-sodium acetate-mineral salt broth (Tanasupawat et al., 1992) (pH 7.2) was used for working cultures. All tests were performed by incubating the cultures at 30 °C. Cell shape, size and arrangement and colony appearance were examined using cells grown on GYP agar for 3 days. Gram staining was done as described by Hucker & Conn (1923). Spore formation was examined in the Gram-stained specimen. Results of the oxidation/fermentation test and motility were examined in soft agar (Whittenbury, 1963). Catalase activity,

Abbreviation: DMK, demethylmenaquine.

The GenBank/EMBL/DDBJ accession numbers for the 16S rRNA and *rpoA* gene sequences of strain FP15-1\(^T\) are respectively EF154454 and EF197993.

16S rRNA gene sequence-based maximum-likelihood and maximum-parsimony trees are available as supplementary material with the online version of this paper.
hydrolysis of gelatin, aesculin, arginine and starch, nitrate reduction, production of gas from glucose, gluconate and citrate and acid formation from carbohydrates were tested as reported by Tanasupawat et al. (1992). Additional biochemical characters were recorded after 2 days incubation in API 50 CH galleries. Hydrolysis of horse blood was assessed as described by Barrow & Feltham (1993). Growth on Slanetz–Bartley agar (Oxoid) and on kanamycin aesculin azide agar (Merck) was tested. The reaction in litmus milk was investigated after incubating cultures for 3, 7 and 14 days. Effects of temperature (10–45°C) was investigated after incubation for 2 days in API 50 CH galleries. Hydrolysis of gelatin, aesculin, arginine and starch, nitrate reduction, production of gas from glucose, gluconate and citrate and acid formation from carbohydrates were tested as reported by Tanasupawat et al. (1992). Additional biochemical characters were recorded after 2 days incubation in API 50 CH galleries. Hydrolysis of horse blood was assessed as described by Barrow & Feltham (1993). Growth on Slanetz–Bartley agar (Oxoid) and on kanamycin aesculin azide agar (Merck) was tested. The reaction in litmus milk was investigated after incubating cultures for 3, 7 and 14 days. Effects of temperature (10–45°C) was investigated after incubation for 2 days in API 50 CH galleries. Hydrolysis of gelatin, aesculin, arginine and starch, nitrate reduction, production of gas from glucose, gluconate and citrate and acid formation from carbohydrates were tested as reported by Tanasupawat et al. (1992). Additional biochemical characters were recorded after 2 days incubation in API 50 CH galleries. Hydrolysis of horse blood was assessed as described by Barrow & Feltham (1993). Growth on Slanetz–Bartley agar (Oxoid) and on kanamycin aesculin azide agar (Merck) was tested. The reaction in litmus milk was investigated after incubating cultures for 3, 7 and 14 days. Effects of temperature (10–45°C) was investigated after incubation for 2 days in API 50 CH galleries. Hydrolysis of gelatin, aesculin, arginine and starch, nitrate reduction, production of gas from glucose, gluconate and citrate and acid formation from carbohydrates were tested as reported by Tanasupawat et al. (1992). Additional biochemical characters were recorded after 2 days incubation in API 50 CH galleries. Hydrolysis of horse blood was assessed as described by Barrow & Feltham (1993). Growth on Slanetz–Bartley agar (Oxoid) and on kanamycin aesculin azide agar (Merck) was tested. The reaction in litmus milk was investigated after incubating cultures for 3, 7 and 14 days. Effects of temperature (10–45°C) was investigated after incubation for 2 days in API 50 CH galleries. Hydrolysis of gelatin, aesculin, arginine and starch, nitrate reduction, production of gas from glucose, gluconate and citrate and acid formation from carbohydrates were tested as reported by Tanasupawat et al. (1992). Additional biochemical characters were recorded after 2 days incubation in API 50 CH galleries. Hydrolysis of horse blood was assessed as described by Barrow & Feltham (1993). Growth on Slanetz–Bartley agar (Oxoid) and on kanamycin aesculin azide agar (Merck) was tested. The reaction in litmus milk was investigated after incubating cultures for 3, 7 and 14 days. Effects of temperature (10–45°C) was investigated after incubation for 2 days in API 50 CH galleries. Hydrolysis of gelatin, aesculin, arginine and starch, nitrate reduction, production of gas from glucose, gluconate and citrate and acid formation from carbohydrates were tested as reported by Tana...
KCTC 5373T (21.6 %), with 33.8 % relatedness in the reciprocal reaction. In addition, strain FP15-1T could be differentiated from closely related Enterococcus species by growth in 6.5 % NaCl and at 10°C, acid production and DNA G+C content (Table 1). Therefore strain FP15-1T should be classified in the genus Enterococcus as the type strain of a novel species, Enterococcus camelliae sp. nov.

**Description of Enterococcus camelliae sp. nov.**

*Enterococcus camelliae* [ca.mel’li.ae. N.L. gen. n. camelliae of Camellia, referring to the isolation of the type strain from fermented tea leaves (*Camellia sinensis*)].

Cells are Gram-positive, facultatively anaerobic, non-motile, non-spore-forming, spherical or ovoid, 0.5–1 μm in diameter and arranged in pairs or in chains. Colonies on GYP agar plates are circular, raised or low-convex with entire margins, and non-pigmented. Small, red colonies appear on Slanetz–Bartley agar. No growth is observed on kanamycin aesculin azide agar. Positive for hydrolysis of aesculin but weak reactions for starch hydrolysis and blood haemolysis. Negative for catalase, hydrolysis of arginine and gelatin, reduction of nitrate and production of gas from glucose, gluconate and citrate. Utilizes glucose fermentatively. No acidification, coagulation, reduction or liquefaction in litmus milk. Grows at pH 5.0–9.6, at 15–45°C and in 2–6 % NaCl. Acid is produced from D-glucose, D-fructose, D-cellobiose, aesculin, D-mannose, maltose, mannotol, N-acetylglucosamine, trehalose, sucrose and salicin, but not from glycerol, erythritol, D- or L-arabinose, D-ribose, D- or L-xylitol, adonitol, methyl β-D-xlyoside, D-galactose, D-sorbitose, rhamnose, dulcitol, inositol, methyl α-D-mannoside, methyl β-D-glucoside, D-amylodextrin, butuin, lactose, inulin, D-melibiose, D-melezitose, D-sorbitol, raffinose, starch, glycogen, xylitol, β-gentiobiose, D-turanose, D-lyxose, D-tagatose, D- or L-fucose, D- or L-arabitol, gluconate or 2- or 5-ketogluconate. Riboflavin, niacin and calcium pantothenate are required for growth. DMK-7 is the major menaquinone. C16 : 1 is the dominant component of the fatty acid profile. The DNA G+C content of the type strain is 37.8 mol%.

The type strain, FP15-1T (=KCTC 13133T =NBRC 101868T =NRIC 0105T =TISTR 932T =PCU 277T), was isolated from fermented tea leaves (‘miang’) produced in Thailand.

**Table 1. Differential characteristics of FP15-1T and related Enterococcus species**

<table>
<thead>
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<th>Characteristic</th>
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<td>Growth in 6.5 % NaCl</td>
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<td>–</td>
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<td>Growth at 10°C</td>
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<td>Acid production from:</td>
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<td>DNA G+C content (mol%)</td>
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**References**


