**Lactobacillus panisapium** sp. nov., from honeybee *Apis cerana* bee bread

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**Abstract**

A novel facultatively anaerobic, Gram-stain-positive, non-motile, non-spore-forming, catalase-negative bacterium of the genus *Lactobacillus*, designated strain Bb 2-3ᵀ, was isolated from bee bread of *Apis cerana* collected from a hive in Kunming, China. The strain was regular rod-shaped. Optimal growth occurred at 37°C, pH 6.5 with 5.0 g l⁻¹ NaCl. The predominant fatty acids were C₁₈:1ω9c, C₁₆:0 and C₁₉:0 iso. Respiratory quinones were not detected. Seven glycolipids, three lipids, phosphatidylglycerol and diphosphatidylglycerol were detected. The peptidoglycan type A4 was detected. Strain Bb 2-3ᵀ was closely related to *Lactobacillus bombicola* DSM 28793ᵀ, *Lactobacillus apis* LMG 26964ᵀ and *Lactobacillus helsingborgensis* DSM 26265ᵀ, with 97.8, 97.6 and 97.0% 16S rRNA gene sequence similarity, respectively. A comparison of two housekeeping genes, *pfoA* and *pheS*, revealed that strain Bb 2-3ᵀ was well separated from the reference strains of species of the genus *Lactobacillus*. The average nucleotide identity between strain Bb 2-3ᵀ and the type strains of closely related species was lower than the 95–96% threshold value for delineation of genomic prokaryotic species. The G+C content of the genomic DNA of strain Bb 2-3ᵀ was 37.4 mol%. On the basis of phenotypic, chemotaxonomic and phylogenetic analyses, strain Bb 2-3ᵀ is proposed to represent a novel species of the genus *Lactobacillus*, for which we propose the name *Lactobacillus panisapium* sp. nov. The type strain is Bb 2-3ᵀ (=DSM 102188ᵀ=ACC 19955ᵀ).

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In recent years, metagenomic analyses and experimental investigations have shown that members of the genus *Lactobacillus* are widely distributed in the gut of honey bees and have positive effects on bee health [1–4]. At the time of writing, there are more than 200 species and subspecies with validly published names (data from EBI datasets, https://www.ezbiocloud.net/search?tn=Lactobacillus), but only 11 species (*Lactobacillus apinorum*, *Lactobacillus melifer*, *Lactobacillus mellis*, *Lactobacillus melliventris*, *Lactobaclulus kimbladii*, *Lactobacillus helsingborgensis*, *Lactobacillus bombi*, *Lactobacillus bombicola*, *Lactobacillus apis*, *Lactobacillus kulaebergensis* and *Lactobacillus vespuale*) have been isolated from the honey stomach or bee gut [5–9]. Previous studies have revealed that *Lactobacillus* exists in bee bread and plays a key role in its production [10, 11]. In the present study, we characterized a novel species of the genus *Lactobacillus* isolated from the bee bread of *Apis cerana* by using polyphasic and genomics approaches.

During an investigation and comparison of microbial diversity in bee stomach, gut and bread, a novel strain designated Bb 2-3ᵀ was isolated from bee bread of *A. cerana* collected from a hive in Kunming, Yunnan province, China. For isolation, the bee bread was mashed and resuspended in sterilized 0.9% NaCl under anaerobic conditions, followed by serial dilution in anaerobic culture tubes containing sterilized de Man Rogosa and Sharpe (MRS) broth (Thermo Scientific Oxoid) supplemented with 0.5 g l⁻¹ L-cysteine, 1.0 mg l⁻¹ resazurin and 15 g l⁻¹ agar. The MRS medium was prepared and dispensed under a gaseous atmosphere of 100% nitrogen. Strains were isolated by Hungate roll-tube technique [12, 13]. After incubation for 48 h at 37°C, a single white, round colony was obtained, designated as strain Bb 2-3ᵀ, and subjected to taxonomic analysis based on phenotypic and phylogenetic studies.

Extraction and purification of genomic DNA, PCR amplification, and sequencing of the 16S rRNA gene were performed.

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**Keywords:** bee bread; *Lactobacillus*; *Lactobacillus panisapium*; *Apis cerana*.

**Abbreviations:** ANI, average nucleotide identity; DDH, DNA–DNA hybridization; GGDC, Genome-Genome Distance Calculator.

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The GenBank/EMBL/DDBJ accession numbers for the 16S rRNA gene and *pheS* gene sequences of strain Bb 2-3ᵀ are KX447147 and KY622000, respectively. The NCBI accession numbers for the draft genome sequences of strain Bb 2-3ᵀ, *Lactobacillus bombicola* DSM 28793ᵀ and *Lactobacillus apis* LMG 26964ᵀ are KPNN000000000, KPNG000000000 and KPDP000000000, respectively. One supplementary table and six supplementary figures are available with the online version of this article.
as described previously [14]. All obtained sequences were submitted to NCBI for initial alignment with highly similar sequences using BLASTn, and 16S rRNA gene sequences of the closely related organisms were retrieved from NCBI (https://www.ncbi.nlm.nih.gov/) and EzTaxon (http://www.ezbiocloud.net/) databases. Housekeeping genes rpoA (encoding DNA-directed RNA polymerase subunit alpha) and pheS (encoding phenylalanyl-tRNA synthetase subunit alpha) were also used for phylogenetic analysis. rpoA and pheS gene sequences were retrieved from the genome of strain Bb 2-3^T. The sequence of pheS was confirmed by PCR amplification according to De Bruyne and Snaauwaert [7, 15, 16]. Phylogenetic trees were reconstructed with the software package MEGA version 5.0 using the neighbour-joining and maximum-likelihood methods [17, 18]. The robustness of the topology of the phylogenetic trees was evaluated by bootstrap analysis based on 1000 replications. Phylogenetic analysis based on 16S rRNA gene sequences using the neighbour-joining method revealed that strain Bb 2-3^T represented a separated lineage within the genus Lactobacillus, together with *L. bombicola* (Fig. 1). Pairwise comparisons of 16S rRNA gene sequences indicated that strain Bb 2-3^T was most closely related to *L. bombicola* DSM 28793^T (97.8 % 16S rRNA gene sequence similarity), followed by *L. apis* DSM 26964^T (97.6 %) and *L. helsingborgensis* DSM 26265^T (97.0 %). All other type strains shared less than 97.0 % 16S rRNA gene sequence similarities. Phylogenetic analysis of rpoA gene sequences showed that strain Bb 2-3^T was closest to *L. apis* DSM 26964^T with 91.1 % sequence similarity, followed by *L. helsingborgensis* DSM 26265^T (86.6 %) and *L. bombicola* DSM 28793^T (84.4 %) (Fig. S1, available in the online version of this article). A pheS-based phylogenetic tree reconstructed using the neighbour-joining method indicated that the nearest phylogenetic neighbours to strain Bb 2-3^T were *L. apis* DSM 26964^T and *L. bombicola* DSM 28793^T, both sharing 80.1 % sequence similarity (Fig. S2). The phylogenetic position was also confirmed by trees generated using the maximum-likelihood algorithm method (Fig. S3).

Since the 16S rRNA gene sequence similarity between strain Bb 2-3^T and *L. bombicola* DSM 28793^T, *L. apis* DSM 26964^T or *L. helsingborgensis* DSM 26265^T exceeded 97 %, genomic relatedness between strain Bb 2-3^T and its three phylogenetically closest relatives was determined on the basis of genome sequences. For the comparison of genome relatedness, the genome sequences of strain Bb 2-3^T, *L. bombicola* DSM 28793^T and *L. apis* DSM 26964^T were recently sequenced by Majorbio (Shanghai, PR China) using the Illumina MiSeq sequencing platform. All the obtained draft genomes were submitted to the GenBank database. Genomic data for *L. helsingborgensis* DSM 26265^T was obtained from the GenBank database. The level of pairwise genome-based similarity was evaluated based on both the average nucleotide identity (ANI) value determined by using ChunLab’s online ANI calculator (http://www.ezbiocloud.net/tools/ani) described by Yoon et al. [19], and the genome-to-genome distance calculator (GGDC) software version 2.1 (http://ggdc.dsmz.de/distcalc2.php) and Formula 2 was used as recommended for the calculation of DDH (DNA–DNA hybridization) for incomplete genomes. The ANI values of strain Bb 2-3^T and its related species *L. bombicola* DSM 28793^T, *L. apis* DSM 26964^T and *L. helsingborgensis* DSM 26265^T were 76.0, 77.0 and 75.7 %, respectively, which were much lower than the cut-off value of 95–96 %. The digital DDH values between

![](image.png)

**Fig. 1.** Neighbour-joining phylogenetic tree based on 16S rRNA gene sequences showing the relationship between strain Bb 2-3^T and its phylogenetically close relatives. Bootstrap values based on 1000 replications are listed as percentages at the branching points. Bar, 0.01 substitutions per site.
strain Bb 2-3<sup>T</sup> and <i>L. bombicola</i> DSM 28793<sup>T</sup>, <i>L. apis</i> LMG 26964<sup>T</sup> and <i>L. helsingborgensis</i> DSM 26265<sup>T</sup> were 19.9, 20.4 and 18.6%, respectively, which were lower than the cut-off point of 70% for the delineation of a novel species. Furthermore, the genomic G+C content was calculated directly from the respective genome sequence and was determined to be 37.4, 34.6, 36.5 and 36.3 mol% for strain Bb 2-3<sup>T</sup>, <i>L. bombicola</i> DSM 28793<sup>T</sup>, <i>L. apis</i> LMG 26964<sup>T</sup> and <i>L. helsingborgensis</i> DSM 26265<sup>T</sup>, respectively (Table 1). Thus, the phylogenetic and genomic results supported the view that strain Bb 2-3<sup>T</sup> represents a novel species of the genus <i>Lactobacillus</i>.

Phenotypic characteristics were determined for strain Bb 2-3<sup>T</sup> and the reference type strains <i>L. bombicola</i> DSM 28793<sup>T</sup>, <i>L. apis</i> LMG 26964<sup>T</sup> and <i>L. helsingborgensis</i> DSM 26265<sup>T</sup>. Cell morphology of a 24-hour-old Bb 2-3<sup>T</sup> culture incubated in MRS medium at 37 °C was examined using a scanning electron microscope (JSM-7500F; JEOL) and transmission electron microscope (JEM-1230; JEOL). Motility was tested using the hanging-drop technique [20]. Gram staining was performed according to Claus et al. [21]. Spore-forming ability was examined by phase-contrast microscope (Nikon 80i). Catalase activity, oxidase activity and fermentation type were determined as previously described [7]. Ammonia production from arginine and the Voges–Proskauer test were carried out according to Lopes et al. [22]. Hydrolysis of aesculin was tested according to the method described in Tjandraatmadja et al. [23]. Hydrolysis of starch was

| Table 1. Phenotypic comparison of strain Bb 2-3<sup>T</sup> with its phylogenetically closest relatives |
|---------------------------------------------------------------|
| Strains: 1, Bb 2-3<sup>T</sup>; 2, <i>L. bombicola</i> DSM 28793<sup>T</sup>; 3, <i>L. apis</i> LMG 26964<sup>T</sup>; 4, <i>L. helsingborgensis</i> DSM 26265<sup>T</sup>. | +, Positive; —, negative; L, lipid; GL, glycolipid; PL, phospholipid; PG, phosphatidylglycerol; PGL, phosphoglycolipid; DPG, diphasphatidylglycerol. All data are from this study except where indicated. |

| Characteristic | 1 | 2 | 3 | 4 |
|---------------|---|---|---|---|
| Isolation source* | Bee bread of A. cerana | Bumble bee gut | Stomach of A. mellifera | Stomach of A. mellifera |
| Morphology† | Rods (0.3–0.45×1.5–4.1 µm) | Rods (0.5–1×3.0 µm) | Rods (0.5–1×2.1–6.3 µm) | Rods (0.5–0.8×2.0–7.0 µm) |
| Temperature for growth (°C, optimum) | 15–55 (37) | 15–55 (37) | 15–50 (37) | 15–55 (40) |
| pH for growth (optimum) | 3.5–9.0 (6.5) | 4.5–8.5 (6.5) | 4.0–9.0 (6.5) | 4.0–8.5 (6.5) |
| NaCl tolerance (% w/v; optimum) | 0–8.5 (0.5) | 0–4.0 (1.0) | 0–6.0 (0.5) | 0–5.0 (0.5) |
| Major cellular fatty acids | C<sub>16:0</sub>, C<sub>18:1</sub>ω9c, C<sub>16:0</sub>iso | C<sub>18:1</sub>ω9c, C<sub>16:0</sub>ω9c | C<sub>18:1</sub>ω9c, C<sub>16:0</sub> | C<sub>18:1</sub>ω9c, C<sub>16:0</sub> |
| Polar lipids | L, GL, PG, DPG | L, GL, PG, DPG | L, GL, PG, DPG | L, GL, PG, DPG |
| G+C content (mol%) | 37.4 | 34.6 | 36.5 | 36.3 |
| Substrates | | | | |
| D-Galactose | + | — | — | + |
| D-Glycerate | — | — | + | + |
| D-Inositol | — | — | + | + |
| L-Lactose | — | — | + | + |
| D-Mannitol | — | — | + | + |
| Melibiose | + | — | — | — |
| D-Ribose | + | — | — | — |
| D-Sorbitol | — | — | + | + |
| Sucrose | — | — | + | + |
| Trehalose | — | — | + | + |
| D-Xylose | — | — | + | + |
| (+)-Maltose | — | — | — | — |
| (+)-Raffinose | — | — | — | — |
| L-Arabinose | + | — | — | + |
| L-Rhamnose | + | — | — | + |
| L-Sorbose | — | — | — | + |
| Sodium gluconate | — | — | — | + |
| Main fermentation products (mM)‡ | | | | |
| Lactic acid | 87.0 | 61.7 | 26.0 | 10.8 |
| Acetic acid | 13.5 | 0.0 | 39.6 | 0.0 |
| Propionic acid | 14.1 | 0.0 | 0.0 | 0.0 |
| Butyric acid | 8.8 | 11.1 | 0.0 | 0.0 |

*Data from [5, 7, 8].

†Main fermentation products are produced from glucose.
determined based on the formation of clear zones around colonies after applying the suitable staining solutions described previously [24]. H₂S production was investigated using triple sugar iron agar (BD Difco) supplemented with 2.0 % NaCl. The production of D- and L-lactic acid was analysed using a D-lactic acid/L-lactic acid enzyme kit (Magazyme) according to the manufacturer’s instructions. The growth behaviour in the presence of oxygen was determined in a sealed serum bottle containing liquid MRS medium prepared under anaerobic conditions. Nitrogen (0.4, 1.0, 2 or 4 ml) was removed from the 20 ml headspace and the same volume of filter-sterilized oxygen (99.9%) was added before inoculation to provide 2, 5, 10 or 20 % oxygen. All cultures were incubated without shaking and growth rates were measured.

Growth experiments to determine the pH, temperature, and NaCl concentration ranges were performed in triplicate using anaerobic culture tubes with 5 ml anaerobic MRS medium supplemented with 0.5 g l⁻¹ L-cysteine and 1.0 mg l⁻¹ resazurin. The growth rates were measured to evaluate the growth characteristics. The pH range was tested between pH 2.5 and pH 10.0 (at intervals of 0.5 pH units). The sterile anaerobic buffers Na₂HPO₄-citric acid (for pH 2.5, 3.0, 3.5, 4.0, 4.5, 5.0 and 5.5), MES (pH 6.0 and 6.5), PIPES (pH 7.0 and 7.5), Tris (pH 8.0 and 8.5), and CHES (pH 9.0, 9.5 and 10.0) were used at a final concentration of 0.02 M to maintain the pH. The temperature range for growth was tested at 10–55 °C at intervals of 5 °C, and the NaCl concentration range was 0–90.0 g l⁻¹ (w/v) at intervals of 5.0 g l⁻¹. The ability to utilize organic substrates as carbon sources was determined in peptone-yeast extract (PY) medium containing (l⁻¹): 0.5 g peptone, 1.0 g yeast extract, 0.5 g trypticase, 4.0 ml salt solution II, 0.5 g L-cysteine and 1.0 mg resazurin. The salt solution II contained the following (g l⁻¹): 0.2 CaCl₂·2H₂O, 0.48 MgSO₄·7H₂O, 1.0 K₂HPO₄, 1.0 KH₂PO₄, 10.0 NaHCO₃ and 2.0 NaCl. Cellulose, D-fructose, D-galactose, D-glucose, D-glycogen, D-inositol, lactose, D-mannitol, melibiose, D-ribose, D-sorbitol, sucrose, trehalose, D-xylene, (+)-maltose, (+)-d-mannose, (+)-raffinose, L-arabinose, L-rhamnose, L-sorbose and sodium gluconate were added to tubes of PY medium as filter-sterilized solution, at a final concentration of 20.0 g l⁻¹. Growth was determined using the method as described by Ma et al. [25]. Liquid fermentation products were analysed by liquid chromatography (Agilent 1200). CO₂ was analysed by gas chromatography (Agilent 7820A) [25].

The phenotypic characteristics of strain Bb 2-3T are presented in the species description, Table 1 and Figs S4 and S5. A scanning electron micrograph and transmission electron micrograph of strain Bb 2-3T showed cells were regular-shaped rods with a size of 0.3–0.45×1.5–4.1 μm (Fig. S4). The differential characteristics between strain Bb 2-3T and its closest relatives obtained from the above experiments are listed in Table 1. The key difference between strain Bb 2-3T and its three closest phylogenetic neighbours was the carbohydrate fermentation pattern. Strain Bb 2-3T did not utilize D-glycogen, D-inositol, lactose, D-mannitol, D-sorbitol, trehalose, D-xylene, (+)-maltose, D-sorbose or sodium gluconate. However, strain Bb 2-3T could produce up to 87.0 mM lactic acid when grown on glucose, which was much higher than that of L. bombicola DSM 28793T (61.7 mM), L. apis LMG 26964T (26.0 mM) and L. helsingborgensis DSM 26265T (10.8 mM).

The profiles of cellular fatty acids, respiratory quinones, polar lipids and peptidoglycan structure of strain Bb 2-3T, L. bombicola DSM 28793T, L. apis LMG 26964T and L. helsingborgensis DSM 26265T were determined by the Identification Service of the Deutsche Sammlung von Mikroorganismen und Zellkulturen (Braunschweig, Germany) using methods described previously [24, 26–31]. Cultures used for measurement of chemotaxonomic characteristics were harvested after cultivation on MRS medium at 37 °C for 18 h.

The cellular fatty acid profile of strain Bb 2-3T comprised C₁₈:ω9c (54.8 %), C₁₆:0 (16.2 %) and C₁₉:0 iso (10.5 %) as the major fatty acids (>10.0 %). The fatty acid profile of the strain was clearly qualitatively and quantitatively different from those of closely related species (Table 2). The polar lipid profile of strain Bb 2-3T comprised three lipids, seven glycolipids, phosphatidylglycerol and diphosphatidylglycerol. Lipids, glycolipids, phosphatidylglycerol and diphosphatidylglycerol were widely distributed in strain Bb 2-3T and its three closest relatives, however, the absence of

| Fatty acid | 1 | 2 | 3 | 4 |
|-----------|---|---|---|---|
| Saturated |   |   |   |   |
| C₁₄:0     | 1.3 | 0.8 | 0.6 | 0.9 |
| C₁₆:0     | 16.2 | 15.2 | 13.0 | 18.6 |
| C₁₈:0     | 5.1 | 3.3 | 5.5 | 6.0 |
| Unsaturated |   |   |   |   |
| C₁₉:1ω9c | 54.8 | 69.0 | 64.6 | 63.0 |
| C₁₉:1ω7c | 4.9 | 3.9 | 5.4 | – |
| Branched-chain |   |   |   |   |
| C₁₉:1 iso 1 | 1.5 | – | 1.2 | 0.6 |
| C₁₉:0 iso | 10.5 | 2.1 | 7.0 | – |
| Summed features* |   |   |   |   |
| 3 | 1.0 | 0.7 | 1.0 | 1.1 |
| 7 | 2.5 | 4.0 | 1.0 | 4.4 |
| 8 | 4.9 | 3.9 | 5.4 | 4.8 |

*Summed features represent groups of two or three fatty acids that could not be separated by GLC with the MIDI system. Summed feature 3 comprised C₁₆:1ω7c/C₁₆:1ω6c; summed feature 7 comprised T₁₉:0 cyclo ω10c/C₁₉:1ω6c; summed feature 8 comprised C₁₈:1ω7c/C₁₈:1ω6c.
phospholipid and phosphoglycolipid could distinguish strain Bb 2-3\textsuperscript{T} from its closest relatives within the genus Lactobacillus (Fig. S6). Strain Bb 2-3\textsuperscript{T} displayed the peptidoglycan type A\textalpha \textbeta L-lys–D-Asp (type A11.31 according to http://www.peptidoglycan-types.info/) in the cell wall, which is identical with that of L. bombicola DSM 28793\textsuperscript{T}, L. apis LMG 26964\textsuperscript{T} and L. helsingborgensis DSM 26265\textsuperscript{T}. The molar ratio of the amino acids in the peptidoglycan hydrolysate of strain Bb 2-3\textsuperscript{T} was as follows: 1.5 Ala : 0.8 Asp : 1.0 Glu : 0.7 Lys (Table S1).

Therefore, based on phylogenetic, physiological and chemotaxonomic analyses, strain Bb 2-3\textsuperscript{T} represents a novel species of the genus Lactobacillus, for which the name Lactobacillus panisapium sp. nov. is proposed.

**DESCRIPTION OF LACTOBACILLUS PANISAPIUM SP. NOV.**

Lactobacillus panisapium (pa.nis.a’pi.um. L. masc. n. panis bread; L. fem. n apis bee; N.L. gen. n. panisapium of bee bread).

Cells growing in liquid medium (MRS) under anaerobic conditions are Gram-stain-positive, non-motile, non-spore-forming, long rods with rounded ends, occurring singly, 0.3–0.45 μm wide and 1.5–4.1 μm long, heterofermentative and facultatively anaerobic. The best growth is observed under anaerobic conditions, weaker growth occurs in the presence of oxygen. Colonies on MRS agar under anaerobic conditions after 48 h are round, white and semi-transparent, with a diameter of 2–3 mm. Growth occurs at 15–55 °C (optimum 37 °C), pH 3.5–9.0 (optimum 6.5), and NaCl concentration of 0–85.0 g L\textsuperscript{-1} (optimum 5.0 g L\textsuperscript{-1}). Doubling time under the optimal conditions is 0.46 h. Utilizes cellobiose, D-fructose, D-galactose, D-glucose, melibiose, D-ribose, sucrose, (+)-D-mannose, (+)-raf-finose, L-aráabinose and L-rhamnose. Negative results in tests for starch hydrolysis, ammonia production from arginine, catalase, Voges–Proskauer reaction and H\textsubscript{2}S production, but positive for asaccharolysis.

The main fermentation products from glucose are D-/L-lactic acid, acetic acid, propionic acid, butyric acid and CO\textsubscript{2}. The major fatty acids are C\textsubscript{18:1}ω9c, C\textsubscript{16:0} and C\textsubscript{19:0} iso. Respiratory quinones are not detected. The polar lipid profile is composed of lipids, glycolipids, phosphatidylglycerol and diphosphatidylglycerol. The determined peptidoglycan structure is type A\textalpha\textbeta L-Lys–D-Asp.

The type strain is Bb 2-3\textsuperscript{T} (=DSM 102188\textsuperscript{T}=ACCC 19955\textsuperscript{T}), isolated from bee bread of Apis cerana collected from a hive in Kunming, China. The genomic DNA G+C content of the type strain is 37.4 mol%.

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**Conflicts of interest**

The authors declare that there are no conflicts of interest.

**References**

1. Kwong WK, Moran NA. Gut microbial communities of social bees. Nat Rev Microbiol 2016;14:374–384.
2. Alberoni D, Gaggia F, Baffoni L, di Gioia D. Beneficial microorganisms for honey bees: problems and progresses. Appl Microbiol Biotechnol 2016;100:9469–9482.
3. Vásquez A, Forsgren E, Fries I, Paxton RJ, Flaberg E et al. Symbions as major modulators of insect health: lactic acid bacteria and honeybees. PLoS One 2012;7:e33188.
4. Rangberg A, Mathiesen G, Amdam GV, Diep DB. The paraphysiological potential of Lactobacillus kunkeei in the honey bee Apis mellifera. Benef Microbes 2015;6:513–523.
5. Olofsson TC, Alsterfjord M, Nilson B, Butler E, Vásquez A. Lactobacillus apinorum sp. nov., Lactobacillus mellifer sp. nov., Lactoba-cillus melilis sp. nov., Lactobacillus melliventris sp. nov., Lactoba-cillus kimbladii sp. nov., Lactobacillus helsingborgensis sp. nov. and Lactobacillus kullabergensis sp. nov. isolated from the honey stomach of the honeybee Apis mellifera. Int J Syst Evol Microbiol 2014;64:3109–3119.
6. Killer J, Votavová A, Valterová I, Loomis M, Ristić BI, Votava A. Lactic acid bacteria isolated from the bumblebee gut: Convivina intestini gen. nov., sp. nov., Lactobacillus bombicola sp. nov., and Weissella bombi sp. nov. Antonie van Leeuwenhoek 2015;107:1337–1349.
7. Killer J, Dubná S, Sedláček I, Švec P. Lactobacillus apis sp. nov., from the stomach of honeybees (Apis mellifera), having an in vitro inhibitory effect on the causative agents of American and European foulbrood. Int J Syst Evol Microbiol 2014;64:2611–2617.
8. Praet J, Meeus I, Cnockaert M, Houf K, Smagghe G. Novel lactic acid bacteria isolated from the digestive tract of laboratory-raised bumblebee queens (Bombus terrestris). Int J Syst Evol Microbiol 2014;64:3109–3117.
9. Hoang VA, Kim YJ, Nguyen NL, Kim SK, Yang DC. Lactobacillus vespuiae sp. nov., isolated from gut of a queen wasp (Vespula vulgaris). Int J Syst Evol Microbiol 2015;65:3326–3332.
10. Wang Cong HY, Ma Shichun DY, Jun GUO, Zhengyun W. Lactobacillus melliventris sp. nov., isolated from the stomach of honeybee Apis mellifera, having an in vitro inhibitory effect on the causative agents of American and European foulbrood. Int J Syst Evol Microbiol 2014;64:152–157.
11. Wang Cong HY, Ma Shichun DY, Jun GUO, Zhengyun W. Optimization of the fermentation conditions of bee pollen with Lactobacillus sp. strain 2-3. Science and Technology of Food Industry 2016;37:119–123.
12. Vásquez A, Olofsson TC. The lactic acid bacteria involved in the production of bee pollen and bee bread. J Apic Res 2009;48:189–195.
13. Hungate RE. Chapter IV A roll tube method for cultivation of strict anaerobes. In: Norris JR and Ribbons DW (editors). Methods in Microbiology. Millbrae, CA, USA: Academic Press; 1969. pp. 117–132.
14. Hungate RE, Mau J. The roll-tube method for cultivation of strict anaerobes. Bulletin from the Ecological Research Committee 1973; 3:123–126.
15. Huang Y, Sun Y, Ma S, Chen L, Zhang H et al. Isolation and characterization of Keratinibaculum paraltunense gen. nov., sp. nov., a novel thermophilic, anaerobic bacterium with keratinolytic activity. FEMS Microbiol Lett 2013;334;56–63.
16. Snuauwerta I, Papalexandratou Z, de Vuyst L, Vandamme P. Characterization of strains of Weissella faibalis sp. nov. and Fructobacillus tropaeoli from spontaneous cocoa bean fermentations. Int J Syst Evol Microbiol 2013;63:1709–1716.
17. Tamura K, Peterson D, Peterson N, Stecher G, Nei M et al. MEGA5: molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. Mol Biol Evol 2011;28:2731–2739.
18. Tamura K, Nei M, Kumar S. Prospects for inferring very large phylogenies by using the neighbor-joining method. Proc Natl Acad Sci USA 2004;101:11030–11035.
19. Yoon SH, Ha SM, Lim J, Kwon S, Chun J. A large-scale evaluation of algorithms to calculate average nucleotide identity. Antonie van Leeuwenhoek 2017;110:1281–1286.
20. Harrigan WF. Laboratory methods in food microbiology, 3rd ed. San Diego, CA: Academic Press; 1998.
21. Claus D. A standardized Gram staining procedure. World J Microbiol Biotechnol 1992;8:451–452.
22. Lopes MF, Pereira CI, Rodrigues FM, Martins MP, Mimoso MC et al. Registered designation of origin areas of fermented food products defined by microbial phenotypes and artificial neural networks. Appl Environ Microbiol 1999;65:4484–4489.
23. Tjandraatmadja M, Norton BW, Rae ICM. A numerical taxonomic study of lactic acid bacteria from tropical silages. J Appl Bacteriol 1990;68:543–553.
24. Tindall BJ, Sikorski J, Smibert RA, Krieg NR. Phenotypic characterization and the principles of comparative systematics. In: Beveridge JTB, Marzluf GA, Schmidt TM and Snyder LR (eds). Methods for General and Molecular Microbiology. Washington, DC: ASM Press; 2007. pp. 330–393.
25. Ma S, Huang Y, Wang C, Fan H, Dai L et al. Defluviitalea raffinosden sp. nov., a thermophilic, anaerobic, saccharolytic bacterium isolated from an anaerobic batch digester treating animal manure and rice straw. Int J Syst Evol Microbiol 2017;67:1607–1612.
26. Kämpfer P, Kroppenstedt RM. Numerical analysis of fatty acid patterns of coryneform bacteria and related taxa. Can J Microbiol 1996;42:989–1005.
27. Kuykendall LD, Roy MA, O’Neill JJ, Devine TE. Fatty acids, antibiotic resistance, and deoxyribonucleic acid homology groups of Bradyrhizobium japonicum. Int J Syst Bacteriol 1988;38:358–361.
28. Miller LT. Single derivatization method for routine analysis of bacterial whole-cell fatty acid methyl esters, including hydroxy acids. J Clin Microbiol 1982;16:584–586.
29. Tindall BJ. Lipid composition of Halobacterium lacusprofundi. FEMS Microbiol Lett 1990;66:199–202.
30. Tindall BJ. A comparative study of the lipid composition of Halo bacterium saccharovorum from various sources. Syst Appl Microbiol 1990;13:128–130.
31. Schumann P. Peptidoglycan structure. Methods Microbiol 2011;38:101–129.

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