**Bacillus wuyishanensis** sp. nov., isolated from rhizosphere soil of a medical plant, *Prunella vulgaris*

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A Gram-staining-positive, rod-shaped, endospore-forming, aerobic bacterium (FJAT-17212T) was isolated from the rhizosphere soil of a medical plant, *Prunella vulgaris* (common selfheal), on the Wuyishan mountain of China. Isolate FJAT-17212T grew at 10–50 °C (optimum 30 °C), pH 5–11 (optimum pH 7) and with 0–6 % (w/v) NaCl (optimum 2 %). Phylogenetic analyses based on 16S rRNA gene sequences showed that isolate FJAT-17212T was a member of the genus *Bacillus* and was most closely related to *Bacillus galactosidilyticus* DSM 15595T (97.3 %). DNA–DNA relatedness between isolate FJAT-17212T and *B. galactosidilyticus* DSM 15595T was low (35.2 % ± 2.3). The diagnostic diamino acid of the peptidoglycan of isolate FJAT-17212T was meso-diaminopimelic acid and the predominant isoprenoid quinone was MK-7 (80.8 %). The major cellular fatty acids were iso-C₁₅ : 0 (35.7 %), anteiso-C₁₅ : 0 (29.8 %), iso-C₁₄ : 0 (9.9 %) and iso-C₁₆ : 0 (9.9 %) and the DNA G + C content was 39.8 mol%. Phenotypic, chemotaxonomic and genotypic properties clearly indicated that isolate FJAT-17212T represents a novel species within the genus *Bacillus*, for which the name *Bacillus wuyishanensis* sp. nov. is proposed. The type strain is FJAT-17212T (=DSM 27848T=CGMCC 1.12709T).

Species of the genus *Bacillus* accommodate rod-shaped, aerobic or facultatively anaerobic, Gram-stain-positive, endospore-forming bacteria, which exhibit a wide range of physiological abilities that enable them to live in every natural environment, such as soil, hot springs, water, marine sediments or airborne dust (Berkeley, 2002). Species of the genus *Bacillus* in the rhizosphere and rhizoplane of plants have attracted a great deal of attention (Singh et al., 2014) and increasing numbers of novel species have been isolated, such as *Bacillus rhizosphaerae*, an novel diazotrophic bacterium isolated from sugar cane rhizosphere soil (Madhaiyan et al., 2011), *Bacillus methylotrophicus*, a methanol-utilizing, plant-growth-promoting bacterium isolated from rice rhizosphere soil (Madhaiyan et al., 2010) and *Bacillus koreensis*, a spore-forming bacterium, isolated from the rhizosphere of willow roots in Korea (Lim et al., 2006). It is of great significance that species of the genus *Bacillus* could have a role to further define the diversity, ecology and biocontrol activities in the promotion of plant growth and the suppression of soil-borne pathogens (Ait Kaki et al., 2013; Lee et al., 2014). In this study, a novel isolate designated strain FJAT-17212T was described with its phylogenetic and phenotypic characteristics for classification.

Strain FJAT-17212T was isolated from samples of rhizosphere soils of *Prunella vulgaris* (latitude 27° 44’ 4.27” N, longitude 117° 38’ 8.11” E and altitude 1147 m) on Wuyishan, a mountain in Fujian Province, China. The sample was suspended in sterilized water, serially diluted, spread on nutrient agar (NA) and incubated at 30 °C for 48 h (Atlas, 1993). Pure cultures were obtained by several successive single colony isolations. The isolate was stored both on NA slants at 4 °C and as suspensions in Luria–Bertani (LB) broth with 20 % (v/v) glycerol at −80 °C. The reference strains *Bacillus galactosidilyticus* DSM 15595T, *Bacillus panacisoli* JCM 19226T and *Bacillus ruris* DSM 17057T were obtained from the culture collections indicated and used as controls in the phenotypic tests.
Phenotypic tests for isolate FJAT-17212^T and the reference strains B. galactosidilyticus DSM 15595^T, B. panacisoli JCM 19226^T and B. ruris DSM 17057^T were performed as described by Logan et al. (2009). Cell morphology was observed by light microscopy (DMI3000B, Leica). Gram staining and the KOH lysis test were carried out according to the methods described by Smitbt & Krieg (1994) and Gregersen (1978). Endospores were examined according to the Schaeffer-Fulton staining method (Murray et al., 1994). Motility was examined on motility agar (Chen et al., 2007). Catalase activity was determined by investigating bubble production with 3 % (v/v) H_2O_2, and oxidase activity was determined using 1 % (v/v) tetramethyl p-phenylenediamine (Chen et al., 2007). Cell growth under anaerobic conditions was determined in a CO_2 incubator on anaerobically prepared maintenance media. Physiological characteristics, such as Voges-Proskauer test, hydrolysis of gelatin, activities of arginine dihydrolase, lysine decarboxylase, ornithine decarboxylase, tryptophan deaminase and urease, citrate utilization, ONPG, and H_2S and indole production, were performed using API 20E strips (bioMérieux). Nitrate reduction, and hydrolysis of casein and starch were determined as described by Cowan & Steel (1965) and Smitbt & Krieg (1994). Tests for carbon source utilization and acid production were performed using the API 50CHB system (bioMérieux). Growth at different temperatures (5–50 °C, in increments of 5 °C), pH (5.0–10.0, in increments of 1 pH unit) and NaCl concentrations (0–10 %, w/v, in increments of 2 % NaCl) was tested in nutrient broth (NB). All tests were repeated in triplicate (Atlas, 1993).

For phylogenetic analysis, chromosomal DNA was extracted and purified according to standard methods (Hopwood et al., 1985). The 16S rRNA gene was amplified by PCR with universal primers (Lane et al., 1985). Amplification was carried out with a DNA thermal cycler (C1000, Bio-Rad) according to the programme described by Liu et al. (2014). PCR products were purified and sequenced by Shanghai Biosune (Shanghai, PR China) with an Applied Biosystems automatic sequencer (ABI 3730). Pairwise sequence similarities were calculated using a global alignment algorithm implemented in the EzTaxon-e database (http://eztaxon-e.ezbiocloud.net/; Kim et al., 2012). After multiple alignments of data by CLUSTAL X (Thompson et al., 1997), phylogenetic trees were reconstructed using the neighbour-joining (NJ) (Saitou & Nei, 1987), maximum-parsimony (MP) (Fitch, 1971) and maximum-likelihood (ML) (Felsenstein, 1981) methods implemented with MEGA version 6 (Tamura et al., 2013). Evolutionary distances were computed according to the Jukes-Cantor model (Jukes & Cantor, 1969). The reliability of each branch was evaluated by bootstrap analysis based on 1000 replications (Felsenstein, 1985).

DNA G+C content was determined using the thermal denaturation method described by Marron & Doty (1962) using Escherichia coli K-12 DNA as a calibration standard. For analysis of DNA–DNA relatedness, levels of DNA–DNA hybridization were determined using a modification of the optical renaturation method described by De Ley et al. (1970) and Huß et al. (1983), using a UV/VIS spectrometer equipped with a temperature programmer controller (Lambda 35, Perkin-Elmer). DNAs were sheared by sonication (SCIENTZ) at 40 W for three periods of 5 s. The renaturation was performed in 2 × saline sodium citrate buffer at 67.3 °C. Three replicate hybridizations were carried out.

For measurement of chemotaxonomic characteristics, the isoprenoid quinone system was analysed as described by Collins et al. (1977) using reverse-phase HPLC (Groth et al. 1996). The cell-wall peptidoglycan was isolated after disruption of the cells by shaking with glass beads and subsequent total hydrolysis (4 M HCl, 100 °C, 16 h). The amino acids and peptides in the hydrolysate were analysed by two-dimensional ascending TLC on cellulose plates using previously described solvent systems (Schleifer, 1985). For determination of cellular fatty acids, strains were harvested after cultivation on trypticase soy agar (TSA) BD at 30 °C for 48 h. The cellular fatty acids in the cell walls were extracted and analysed according to the standard protocol of the Microbial Identification System (MIDI) by using GC (model 7890, Agilent) (Sassier, 1990).

Cells of isolate FJAT-17212^T were Gram-staining-positive, moderately halophilic and facultatively alkaliphilic straight rods (Fig. S1a, available in the online Supplementary Material), with endospores (Fig. S1b) and terminal flagella observed (Fig. S1c). The isolate grew at salt concentrations in the range 0–6 % (w/v) NaCl (optimum 2 %). Growth was observed at 10–50 °C (optimum 30 °C) and pH 5–10 (optimum pH 7.0). The strain was oxidase-negative and catalase-positive and did not reduce nitrate to nitrite. The phenotypic properties that differentiate strain FJAT-17212^T from its closest phylogenetic neighbours are given in Table 1.

An almost-complete 16S rRNA gene sequence (1440 bp) of the isolate was determined. Phylogenetic analysis using the neighbour-joining algorithm revealed that FJAT-17212^T represented a separate lineage (Fig. 1). The phylogenetic position was also confirmed by trees generated using the maximum-parsimony (Fig. S2) and maximum-likelihood algorithms (Fig. S3). The results of phylogenetic analysis revealed that isolate FJAT-17212^T belongs to the genus Bacillus. Comparison of the sequences from isolate FJAT-17212^T and the three reference strains indicated that the novel isolate exhibited 16S rRNA gene sequence similarities of 97.3, 96.9 and 96.6 % with B. galactosidilyticus DSM 15595^T, B. panacisoli JCM 19226^T and B. ruris DSM 17057^T, respectively.

The DNA G+C content of isolate FJAT-17212^T was calculated to be 39.8 mol%, which is within the range of 35.6–44.8 % described for the genus Bacillus (Logan & De Vos, 2009). The isolate showed 35.2 % ± 2.3 sd DNA–DNA relatedness to the closest reference strain, B. galactosidilyticus DSM 15595^T, which is lower than the cut-off point (70 %).
for the delineation of novel species (Wayne, 1988; Stackebrandt & Goebel, 1994). These results support the view that isolate FJAT-17212\textsuperscript{T} represents a novel species in the genus Bacillus.

Isolate FJAT-17212\textsuperscript{T} contained MK-7 (80.8 %) as the major menaquinone, with MK-6 (1.8 %) and MK-8 (14.9 %) present as minor constituents. Analysis of the cell-wall peptidoglycan showed that the isolate contained meso-diaminopimelic acid as the diagnostic diamino acid, as is typical of the large majority of members of the genus Bacillus (Priest et al., 1988). The cellular fatty acid profile of the isolate comprised iso-C\textsubscript{15}:0, anteiso-C\textsubscript{15}:0, iso-C\textsubscript{14}:0, iso-C\textsubscript{16}:0 and anteiso-C\textsubscript{17}:0 as the major fatty acids (>4 %). The fatty acid profile of the isolate is clearly qualitatively and quantitatively different from the closely related type strain B. galactosidilyticus DSM 15595\textsuperscript{T} (Table 2). These iso- and anteiso-branched fatty acids of the 14- and 17-carbon series are typical of those observed in profiles of type strains of the genus Bacillus, for which the name Bacillus wuyishanensis sp. nov. is proposed.

### Description of Bacillus wuyishanensis sp. nov.

Bacillus *wuyishanensis* (wu.yi.shan.en'si.s. N.L. masc. adj. wuyishanensis belonging to Wuyishan, a mountain located in Wuyishan town in Fujian province, China, where a rhizosphere soil sample of a medical plant, *Prunella vulgaris*, was collected for isolation of the organism).

Cells are Gram-staining-positive, aerobic, moderately halophilic, facultatively alkaliphilic, straight, motile rods (0.4–0.6 \times 1.3–5.0 \textmu m), with rounded ends and one flagellum at the end, and occurred singly or in pairs. Oval endospores are located subterminally and give rise to swollen sporangia. Colonies on NA are cream–yellow, flat, opaque, smooth, have circular margins and are 2–9 mm in diameter. Growth occurs at salinities of 0–6% (w/v) NaCl (optimum 2%), pH 5.0–10 (optimum pH 7.0) and 10–50 °C (optimum 30 °C). Cells are catalase-positive and oxidase-negative. Nitrate is not reduced to nitrite. H\textsubscript{2}S and indole are not produced. Reactions for hydrolysis of gelatin and arginine dihydrolase, \(\beta\)-galactosidase, urease, Voges–Proskauser, citrate utilization, lysine decarboxylase, ornithine decarboxylase and tryptophan deaminase are negative. Acids are produced from L-arabinose, ribose, D-xylose, galactose, glucose, melezitose, amygdalin, aesculin, salicin, cellobiose, lactose, sucrose, trehalose, raffinose and N-acetyl-D-glucosamine, but not from arbutin, fructose, maltose, melibiose, methyl \(\beta\)-D-xyloside, rhamnose, methyl \(\alpha\)-D-glucoside, methyl \(\alpha\)-D-mannoside, inulin, starch, gentiobiose, glyceral, erythrol, D-arabinose, L-xylose, adonitol, sorbose, dulcitol, inositol, mannitol, sorbitol, glycerogen, xylitol, D-lyxose, D-tagatose, turanose,
L-fucose, D-fucose, D-arabitol, L-arabitol, gluconate, 2-keto-D-gluconate or 5-keto-D-gluconate. Acid is produced weakly from mannose. The cell-wall peptidoglycan contains meso-diaminopimelic acid and the major isoprenoid quinone is MK-7. The major fatty acids are iso-C15:0, anteiso-C15:0, iso-C14:0 and iso-C16:0.

The type strain, FJAT-17212T (\(5\) DSM 27848\(5\) \(5\) CGMCC 1.12709\(5\)) was isolated from the rhizosphere of \(Prunella\) \(vulgaris\) (common selfheal) on the Wuyishan mountain of Fujian Province in China. The DNA G+C content of the type strain is 39.8 mol%.

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**Table 2.** Fatty acid compositions of strain FJAT-17212\(T\) and related species of the genus Bacillus

<table>
<thead>
<tr>
<th>Fatty acid (%)</th>
<th>1</th>
<th>2</th>
</tr>
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<tbody>
<tr>
<td>iso-C(_{15}):0</td>
<td>35.7</td>
<td>31.9</td>
</tr>
<tr>
<td>anteiso-C(_{15}):0</td>
<td>29.8</td>
<td>17.5</td>
</tr>
<tr>
<td>iso-C(_{16}):0</td>
<td>9.9</td>
<td>5.8</td>
</tr>
<tr>
<td>iso-C(_{14}):0</td>
<td>9.9</td>
<td>4.2</td>
</tr>
<tr>
<td>anteiso-C(_{17}):0</td>
<td>4.7</td>
<td>3.8</td>
</tr>
<tr>
<td>C(_{16}):0(\alpha)(7)c alcohol</td>
<td>1.7</td>
<td>0.5</td>
</tr>
<tr>
<td>C(_{16}):0(\alpha)(11)c</td>
<td>1.5</td>
<td>2.1</td>
</tr>
<tr>
<td>C(_{16}):0</td>
<td>1.5</td>
<td>21.5</td>
</tr>
<tr>
<td>C(_{14}):0</td>
<td>1.3</td>
<td>4.0</td>
</tr>
<tr>
<td>iso-C(_{17}):0</td>
<td>0.8</td>
<td>4.0</td>
</tr>
<tr>
<td>iso-C(_{17}):0(\alpha)(10)c</td>
<td>0.4</td>
<td>0.4</td>
</tr>
<tr>
<td>C(_{10}):0</td>
<td>0.3</td>
<td>0.3</td>
</tr>
<tr>
<td>C(_{12}):0</td>
<td>0.2</td>
<td>0.2</td>
</tr>
</tbody>
</table>

L-fucose, D-fucose, D-arabitol, L-arabitol, gluconate, 2-keto-D-gluconate or 5-keto-D-gluconate. Acid is produced weakly from mannose. The cell-wall peptidoglycan contains meso-diaminopimelic acid and the major isoprenoid quinone is MK-7. The major fatty acids are iso-C\(_{15}\):0, anteiso-C\(_{15}\):0, iso-C\(_{14}\):0 and iso-C\(_{16}\):0. The type strain, FJAT-17212\(T\) (=DSM 27848\(T\)=CGMCC 1.12709\(T\)) was isolated from the rhizosphere of \(Prunella\) \(vulgaris\) (common selfheal) on the Wuyishan mountain of Fujian Province in China. The DNA G+C content of the type strain is 39.8 mol%.

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Bacillus wuyishanensis sp. nov.


