Jeongeupia chitinilytica sp. nov., a chitinolytic bacterium isolated from soil

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A novel bacterium, designated strain JchiT, was isolated from soil in Taiwan and characterized using a polyphasic approach. Cells of strain JchiT were aerobic, Gram-stain-negative, motile and rod-shaped. They contained poly-β-hydroxybutyrate granules and formed dark-yellow colonies. Growth occurred at 20–37 °C (optimum between 25 and 30 °C), at pH 6.0–8.0 (optimum between pH 7.0 and pH 8.0) and with 0–2 % NaCl (optimum between 0 and 1 %). Phylogenetic analyses based on 16S rRNA gene sequences indicated that strain JchiT belonged to the genus Jeongeupia and that its closest neighbour was Jeongeupia naejangsanensis BIO-TAS4-2T (98.0 % sequence similarity). The major fatty acids (>10 %) of strain JchiT were summed feature 3 (comprising C16:1ω7c and/or C16:1ω6c), C16:0 and C18:1ω7c. The major cellular hydroxy fatty acid was C12:0 3-OH. The isoprenoid quinone was Q-8 and the genomic DNA G+C content was 66.1 mol%. The polar lipid profile consisted of a mixture of phosphatidylethanolamine, phosphatidylglycerol, diphosphatidylglycerol, phosphatidylserine and two unidentified phospholipids. The DNA–DNA relatedness value between strain JchiT and J. naejangsanensis BIO-TAS4-2T was about 41.0 %. On the basis of the genotypic and phenotypic data, strain JchiT represents a novel species in the genus Jeongeupia, for which the name Jeongeupia chitinilytica sp. nov. is proposed. The type strain is JchiT (=BCRC 80367T =KCTC 23701T).

The genus Jeongeupia, first proposed by Yoon et al. (2010), belongs to the family Neisseriaceae of the order Neisseriales in the class Betaproteobacteria (Garrity et al., 2005). The genus Jeongeupia presently comprises only a single species: Jeongeupia naejangsanensis, which was isolated from forest soil collected on Naejang Mountain in South Korea (Yoon et al., 2010). In the present study, a polyphasic approach was used to clarify the taxonomic position of a novel Jeongeupia-like bacterium, which was designated strain JchiT.

During the characterization of micro-organisms from a soil sample collected from Kaoshiung County in Taiwan, at a sampling site (22° 43’ 33.7” N 120° 18’ 42.9” E) where the soil was pH 7.5, and the air temperature was 25 °C, several dark-yellow-coloured bacterial colonies were isolated on trypticase soy agar (TSA; Difco). Strains showing similar colony morphology were segregated and a representative strain, designated JchiT, was selected for detailed study. Strain JchiT was maintained on TSA at 25 °C. It was preserved at –80 °C, in trypticase soy broth (TSB; Difco) with 20 % (v/v) glycerol, and also by lyophilization. Jeongeupia naejangsanensis BIO-TAS4-2T was used as reference strain in the phenotypic and genotypic tests.

Cell morphology was investigated by phase-contrast microscopy (DM 2000, Leica), using cells that had been grown on TSA at 25 °C for 6 h (lag growth phase), 18 h (exponential phase) or 36 h (stationary phase). Motility was tested by the hanging-drop method (Murray et al., 1994). The Gram Stain Set S kit (BD) and the Ryu non-staining KOH method (Powers, 1995) were used for Gram staining. Accumulation of poly-β-hydroxybutyrate granules was observed under light microscopy after staining the cells with Sudan black. Capsule formation was studied using the Hiss staining method (Murray et al., 1994). Colony morphology was observed on TSA, using a stereoscopic microscope (SMZ 800, Nikon).

The pH range for growth was determined by measuring the optical densities (at 600 nm) of cultures in nutrient broth (BD Difco). Prior to sterilization, the pH of the nutrient broth was adjusted to pH 4.0–9.0 (at intervals of 1.0 pH unit) using appropriate biological buffers (Breznak & Costilow, 1994): citrate/Na2HPO4 (pH 4.0–5.0), phosphate

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain JchiT is JF809864.

Two supplementary figures are available with the online version of this paper.
Bacterial strains were examined for a broad range of phenotypic properties. Activities of catalase, oxidase, DNase, urease and lipase (corn oil), and hydrolysis of starch, casein, gelatin and Tweens 20, 40, 60 and 80 were determined using standard methods (Smibert & Krieg, 1994). Hydrolysis of carboxymethylcellulose was tested as described by Bowman (2000), using TSA as the basal medium. Chitin hydrolysis was assessed on chitinase-detection agar (Wen et al., 2002) and visualized by the formation of clear zones around the colonies. A culture of the novel strain was mixed with phosphate buffer containing 0.5 % (w/v) colloidal chitin and incubated for 12 h at 30 °C before any hydrolysed chitin in the suspension was analysed by mass spectrometry. Mass spectra were recorded by using a quadrupole time-of-flight (Q-TOF) mass spectrometer (Micromass). The quadrupole mass analyser was scanned over a mass-to-charge ratio range of 100–1500 atomic mass units (m/z) with a scan step of 2 s and inter-scan of 0.1 s/step. Additional biochemical tests were performed using API ZYM and API 20NE kits (bioMérieux). Carbon source utilization was evaluated using GN2 MicroPlates (Biolog). All commercial phenotypic tests were performed according to the manufacturers’ recommendations.

Sensitivity of strain JchiT to antibiotics was tested by the disc diffusion method after spreading cell suspensions (0.5 McFarland) on TSA plates. The 8-mm-wide discs (Oxoid) contained the following antibiotics: ampicillin (10 μg), chloramphenicol (30 μg), gentamicin (10 μg), kanamycin (30 μg), nalidixic acid (30 μg), novobiocin (30 μg), rifampicin (5 μg), penicillin G (10 μg), streptomycin (10 μg), sulfamethoxazole (23.75 μg) plus trimethoprim (1.25 μg), or tetracycline (30 μg). The effect of antibiotics on cell growth was assessed after 2 days at 25 °C. The strain was considered susceptible, intermediate and resistant when the diameter of the inhibition zone measured >13 mm, 10–12 mm and <10 mm, respectively, as described by Nokhal & Schlegel (1983).

Genomic DNA was isolated by using a bacterial genomic DNA purification kit (DP02-150, GeneMark Technology) and the 16S rRNA gene sequence was analysed as described by Chen et al. (2001). Primers FD1 (5′-AGAGTTTGATCC-TGGCTCAG-3′) and RD1 (5′-AAGGAGGTGATCCAGCC-3′) were used for amplification of bacterial 16S rRNA genes by PCR. These primers correspond to nucleotide positions 8–27 and 1525–1541 of the Escherichia coli 16S rRNA gene, respectively, and can be used to amplify the almost-complete 16S rRNA gene sequence. The PCR product was purified, and direct sequencing was performed in a DNA sequencer (ABI Prism 3730, Applied Biosystems), using the primers FD1, RD1, 520F and 800R (Weisburg et al., 1991; Anzai et al., 1997). The almost-complete 16S rRNA gene sequence (1422 nt) of strain JchiT was compared with 16S rRNA gene sequences available from the EzTaxon server (Kim et al., 2012), the Ribosomal Database Project (Maidak et al., 2001) and the GenBank database (http://blast.ncbi.nlm.nih.gov/Blast.cgi). The sequence data were analysed, using the BioEdit software package (Hall, 1999) and version 5 of the MEGA package (Tamura et al., 2011), after multiple alignments within CLUSTAL_X (Thompson et al., 1997). The resulting multiple sequence alignment was corrected manually and gaps at the 5′ and 3′ ends of the alignment were omitted in further analyses. Evolutionary distances (corrected according to Kimura’s two-parameter model; Kimura, 1983) were calculated before clustering was performed with the neighbour-joining method (Saitou & Nei, 1987). Maximum-likelihood (Felsenstein, 1981) and maximum-parsimony (Kluge & Farris, 1969) trees were also constructed, by using the treeing algorithms contained in the PHYLIP software package (Felsenstein, 1993). The topology of each tree was evaluated by bootstrap analysis, with 1000 replications.

The results of the 16S rRNA gene sequence analysis indicated that strain JchiT belonged to the family Neisseriaceae of the order Neisseriales in the class Betaproteobacteria. Strain JchiT formed a distinct subline within the genus Jeongeupia in the neighbour-joining tree (Fig. 1). The overall topologies of the maximum-likelihood and maximum-parsimony trees were similar. Sequence similarity calculations (over 1400 nt) indicated that strain JchiT was most closely related to Jeongeupia naejangsanensis BIO-TAS4-2T (98.0 % 16S rRNA gene sequence similarity). The novel strain showed lower sequence similarities (<95.0 %) to representative members of all of the other genera shown in Fig. 1.

DNA–DNA hybridization experiments were carried out at 54 °C with photobiotin-labelled probes, using the method of Ezaki et al. (1989). The level of DNA–DNA relatedness recorded between strain JchiT and J. naejangsanensis BIO-TAS4-2T, 41.0 ± 1.3 %, fell well below the 70 % cut-off point recommended for the assignment of strains to the same genomic species (Wayne et al., 1987). Strain JchiT therefore appeared to represent a novel species that belonged in the genus Jeongeupia.

The fatty acid profiles of strain JchiT and J. naejangsanensis BIO-TAS4-2T were determined using cells grown on TSA at 25 °C for 2 days. The physiological age of the different bacterial cultures at the time of harvest was standardized by the choice of sector from a quadrant streak on each of the R2A agar plates (Sasser, 1990). The two strains investigated in this study exhibited very similar growth rates on R2A agar. The fatty acid methyl esters were prepared, separated and identified by following the standard protocol of version 6.0 of the Sherlock Microbial Identification
System (MIDI; Sasser, 1990) and using the RTSBA6.00 database. The major fatty acids (>10%) of both strain JchiT and J. naejangsanensis BIO-TAS4-2T were summed feature 3 (C16:1ω7c and/or C16:1ω6c), C16:0 and C18:1ω7c (Table 1). The major cellular hydroxy fatty acid of both strains was C12:0 3-OH.

Isoprenoid quinones were extracted and purified according to the method of Collins (1985) and were analysed by HPLC. The only detected respiratory quinone of strain JchiT was Q-8. All other current members of the class Betaproteobacteria, including J. naejangsanensis BIO-TAS4-2T (Yoon et al., 2010), have Q-8 as their predominant respiratory quinone. The genomic DNA G+C content of strain JchiT, determined by the HPLC-based method of Mesbah et al. (1989), was 66.1±1.0 mol%.

Polar lipids were extracted and analysed by two-dimensional TLC as described by Embley & Wait (1994). Molybdophosphoric acid was used for the detection of the total polar lipids, ninhydrin for amino lipids, the Zinzadze reagent for phospholipids, and the α-naphthol reagent for glycolipids. Strain JchiT exhibited a complex polar lipid profile consisting of phosphatidylethanolamine (PE), phosphatidylglycerol (PG), diphosphatidylglycerol (DPG), phosphatidylserine (PS) and two uncharacterized phospholipids (PL1 and PL2) (Fig. S1, available in IJSEM Online). The corresponding profile of J. naejangsanensis BIO-TAS4-2T was very similar and also contained PE, PG, DPG, PS, PL1 and PL2.

Mass spectrometry revealed that, when the novel strain was offered chitin as a substrate, a monosaccharide (N-acetyl-D-glucosamine) was produced (Fig. S2).

Table 1. Cellular fatty acid contents (%) of strain JchiT and Jeongeupia naejangsanensis BIO-TAS4-2T

<table>
<thead>
<tr>
<th>Fatty acid</th>
<th>Strain JchiT</th>
<th>J. naejangsanensis BIO-TAS4-2T</th>
</tr>
</thead>
<tbody>
<tr>
<td>C12:0</td>
<td>1.9</td>
<td>1.8</td>
</tr>
<tr>
<td>C14:0</td>
<td>7.6</td>
<td>6.3</td>
</tr>
<tr>
<td>C16:0</td>
<td>25.5</td>
<td>23.4</td>
</tr>
<tr>
<td>C17:0</td>
<td>0.5</td>
<td>0.6</td>
</tr>
<tr>
<td>C18:0</td>
<td>1.7</td>
<td>1.8</td>
</tr>
<tr>
<td>C18:0 3-OH</td>
<td>5.4</td>
<td>5.5</td>
</tr>
<tr>
<td>C17:0 cyclo</td>
<td>8.2</td>
<td>7.0</td>
</tr>
<tr>
<td>C14:105c</td>
<td>0.3</td>
<td>0.3</td>
</tr>
<tr>
<td>C15:106c</td>
<td>0.4</td>
<td>0.5</td>
</tr>
<tr>
<td>C18:107c</td>
<td>12.6</td>
<td>12.2*</td>
</tr>
<tr>
<td>C18:2o6,9c</td>
<td>0.6</td>
<td>0.5</td>
</tr>
<tr>
<td>Summed feature 3†</td>
<td>29.0</td>
<td>31.5</td>
</tr>
</tbody>
</table>

*Not detected by Yoon et al. (2010).
† Summed features are groups of two or three fatty acids that cannot be separated by GLC using the MIDI system. Summed feature 3 contained C16:1ω7c and/or C16:1ω6c.
clearly differentiated from *J. naejangsanensis* BIO-TAS4-2<sup>T</sup> by its lower optimal NaCl concentration for growth, by its growth at relatively low pH values, by its inability to hydrolyse cellulose, by its apparent lack of esterase lipase (C8) and N-acetyl-β-glucosaminidase activities, by its detectable α-glucosidase activity, by its ability to assimilate maltose and to utilize Tween 80, maltose, citric acid, D-gluconic acid, D-glucosaminic acid, β-hydroxybutyric acid and succinamic acid as sole carbon sources, and by its inability to utilize glycogen and L-serine as sole carbon sources.

The characteristics of strain Jchi<sup>T</sup> are consistent with the description of the genus *Jeongeupia* (Yoon et al., 2010). On the basis of the data obtained from the 16S rRNA gene sequence comparisons, strain Jchi<sup>T</sup> occupies a distinct position within the genus *Jeongeupia*. The phylogenetic insight is supported by the novel strain’s unique combination of chemotaxonomic and biochemical characteristics. It is clear from the fatty acid and polar lipid analyses that strain Jchi<sup>T</sup> belongs in the genus *Jeongeupia*. However, strain Jchi<sup>T</sup> could be differentiated from *J. naejangsanensis* BIO-TAS4-2<sup>T</sup> by a combination of physiological and biochemical characteristics, and therefore represents a novel species in the genus *Jeongeupia*, for which name *Jeongeupia chitinilytica* sp. nov., is proposed.

**Description of Jeongeupia chitinilytica* sp. nov.**

*Jeongeupia chitinilytica* [chi.ti.ni.ly’ti.ca. N.L. n. chitinum, chitin; N.L. adj. lyticus -a -um (from Gr. adj. lutikos -e -on), able to loose, able to dissolve; N.L. fem. adj. chitinilytica dissolving chitin].

Cells are Gram-staining-negative, facultatively anaerobic, motile, non-spore-forming and rod-shaped. Poly-β-hydroxybutyrate accumulation is observed. After 24 h of incubation on TSA at 25 °C, the mean cell size is approximately 0.3–0.5 × 1.0–2.0 μm. Colonies are dark-yellow-pigmented, convex, round and smooth, with entire edges. After 48 h of incubation in TSA at 25 °C, colonies measure 1.0–2.0 mm in diameter. Growth occurs at 20–37 °C (optimum between 25 and 30 °C), at pH 6.0–8.0 (optimum between pH 7.0 and pH 8.0), and with 0–2 % (w/v) NaCl (optimum between 0 % and 1 %). Positive for oxidase and catalase activities and hydrolysis of chitin and Tweens 20 and 80. Negative for DNase and urease activities and hydrolysis of cellulose, casein, starch, corn oil and Tweens 40 and 60. In API 20NE tests, gives positive reactions for nitrate reduction, glucose acidification and the assimilation of glucose, mannose, N-acetyl-D-glucosamine, maltose, gluconate, capric acid, malic acid and citrate, but negative reactions for indole production, aesculin and gelatin hydrolysis, arginine dihydrolase, urease and β-galactosidase activities and the assimilation of arabinose, mannitol, adic acid and phenylacetic acid. Positive, in API ZYM tests, for esterase, leucine arylamidase (C4), acid phosphatase, β-glucosidase and N-acetyl-β-glucosaminidase activities but negative for alkaline phosphatase, esterase lipase (C8), lipase (C14), valine arylamidase, cystine arylamidase, trypsin, α-chymotrypsin, naphthol-AS-Bl-phosphohydrolase, α-galactosidase, β-galactosidase, β-glucuronidase, β-glucosidase, α-mannosidase and α-fucosidase activities. In GN2 MicroPlates, only the following compounds are utilized as sole carbon sources: dextrin, Tween 80, N-acetyl-D-galactosamine, N-acetyl-D-glucosamine, adonitol, D-fructose, D-glucose, maltose, D-mannose, D-psicose, xylitol, pyruvic acid methyl ester, succinamic acid, L-serine, a-glucosidase, a-glucosaminidase and a-glucuronidase activities. In GN2 MicroPlates, only the following compounds are utilized as sole carbon sources: dextrin, Tween 80, N-acetyl-D-galactosamine, N-acetyl-D-glucosamine, adonitol, D-fructose, D-glucose, maltose, D-mannose, D-psicose, xylitol, pyruvic acid methyl ester, succinamic acid, L-serine, α-glucosidase, α-glucosaminidase and α-glucuronidase activities.
ester, acetic acid, citric acid, d-gluconic acid, d-glucosaminic acid, β-hydroxybutyric acid, succinamic acid, l-alanine, l-allyl glycline, l-asparagine, l-aspartic acid, l-glutamic acid and l-proline. Sensitive to chloramphenicol, gentamicin, rifampicin, kanamycin, tetracycline, novobiocin, streptomycin, sulfamethoxazole plus trimethoprim and nalidixic acid, but resistant to penicillin G and ampicillin. The major fatty acids are summed feature 3 \((C_{16:0} 3\text{O7c} \text{and/or } C_{16:0} 3\text{O6c}, C_{16:0} \text{and/or } C_{18:1} 3\text{O7c})\). The major cellular hydroxy fatty acid is \(C_{12:0} 3\text{O-H}\). The respiratory quinone is Q-8. The polar lipid profile is complex, consisting of phosphatidylyethanolamine, phosphatidylglycerol, diphasphatidylglycerol, phosphatidylserine and two unidentified phospholipids.

The type strain, HL-12\(^T\) \((=\text{BCRC 80367}\quad =\text{KCTC 23701}\quad T\)\), was isolated from soil collected from Kaoshing County in Taiwan. The genomic DNA G+C content of the type strain is 66.1 mol%.

**References**


