**Rhodanobacter fulvus sp. nov., a \( \beta \)-galactosidase-producing gammaproteobacterium**

Wan-Taek Im, Sung Taik Lee, and Akira Yokota

Department of Biological Sciences, Korea Advanced Institute of Science and Technology, 373–1 Guseong-dong, Yuseong-gu, Daejeon 305–701, Republic of Korea

Institute of Molecular and Cellular Biosciences, The University of Tokyo, Bunkyo-ku, Tokyo 113–0032, Japan

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A taxonomic study was carried out on a bacterial strain designated as Jip2\(^T\) isolated from a soil sample mixed with rotten rice straw. It was a Gram-negative, aerobic, motile, and rod-shaped bacterium. It grew well on nutrient agar medium and utilized a fairly narrow spectrum of carbon source. The G+C content of the genomic DNA was 65.3 mol%. The major ubiquinone was Q-8. The major fatty acids were branched fatty acids, especially large amounts of iso C\(_{15:0}\) and iso C\(_{17:1}\) \( \omega 9c \) were detected in the cells grown on TSA agar for 24 h. Comparative 16S rDNA study showed a clear affiliation of this bacterium to the genus *Rhodanobacter*. The 16S rDNA sequence of strain Jip2\(^T\) showed 96.4% sequence similarity to that of *Rhodanobacter lindaniclasticus* RP5575\(^T\). On the basis of phenotypic characteristics and 16S rDNA sequence analysis, strain Jip2\(^T\) is clearly distinct from *Rhodanobacter lindaniclasticus*. We propose the name *Rhodanobacter fulvus* sp. nov. for strain Jip2\(^T\) (=IAM 15025\(^T\)=KCTC 12098\(^T\)).

**Key Words**—bacterial taxonomy; \( \gamma \)-Proteobacteria; *Rhodanobacter fulvus*; 16S rRNA sequence

Introduction

Many humans are intolerant to lactose in their diet because of the inability to digest a significant amount. This phenomenon is called lactose intolerance. As a result, many humans are unable to consume unfermented dairy products comfortably. One technological approach to solve this problem is to use \( \beta \)-galactosidase in the processing of dairy foods. Due to the possible benefits arising from the isolation of \( \beta \)-galactosidase-producing bacteria, we targeted lactose-utilizing bacteria from a number of sources such as human feces, fermented dairy products, and environmental soils and wastewater sludges. During the course of the isolation of \( \beta \)-galactosidase-producing strains, we isolated a strain designated as Jip2\(^T\) from soil mixed with rotten rice straw, which was found to be closely related to *Rhodanobacter lindaniclasticus* RP5575\(^T\) based on a 16S rDNA sequence study with a similarity value of 96.4%.

The aim of this study is to determine the taxonomic position of strain Jip2\(^T\) using phenotypic characteristics and 16S rDNA sequence analysis. The results provide evidence that strain Jip2\(^T\) represents a new bacterial species.

Materials and Methods

Isolation of bacterial strain and media. Strain Jip2\(^T\) was isolated on \( O \)-nitrophenyl-\( \beta \)-d-galactopyranoside medium (ONPG). This defined medium was made to mix an ONPG solution [ONPG 1.5 g in 250 ml sodium
phosphate buffer (0.01 M, pH 7.5) with peptone water [7.5 g peptone and 3.75 g NaCl in 750 ml of water]. The strain was isolated from soil of a composed paddy field in Daejeon, Korea. In ONPG agar, lactose-utilizing bacteria could be easily detected because of the creation of a yellow zone. After strain Jip2T was isolated, it was conserved by transferring it on nutrient agar every month and deposited in the Korean Collection for Type Cultures as KCTC 12098T (=IAM 15025T).

**Morphological and phenotypic characteristics.** Motility was determined with an optical microscope using the hanging drop technique. Morphology was determined using a JEOL Transmission Electron Microscope (JEOL, Tokyo, Japan) with 40,000 magnification. Growth at a different temperature was observed in nutrient agar broth at 10, 25, 30, 37 and 42°C. The growth experiment was performed using a cap tube containing 3 ml nutrient broth at pH 4.0–10.0 and temperature at 25°C. Growth was estimated by monitoring the OD600. Carbon-source utilization and some enzyme activities were tested by using the API 20NE, API 20E and API ID32 gallery methods (bioMérieux, Marcy l’Etoile, France). Catalase activity was determined by bubble production in 3% (v/v) H2O2 and oxidase activity was determined using 1% (w/v) tetramethyl p-phenylenediamine.

**Chemotaxonomic characteristics.** Ubiquinones were analyzed as described previously (Komagata and Suzuki, 1987). Cellular fatty acids were analyzed for cells grown on a trypticase soy agar (TSA) (Difco Lab., Detroit, MI) for 24 h. The cellular fatty acids were saponified, methylated, and extracted according to the protocol of the MIDI/Hewlett Packard Microbial Identification System. Fatty acid methylster extracts were analyzed by using the API 20NE, MO, USA) was used as the calibration reference. The API 20NE, API 20E and API ID32 gallery methods (bioMérieux, Marcy l’Etoile, France). Catalase activity was determined by bubble production in 3% (v/v) H2O2 and oxidase activity was determined using 1% (w/v) tetramethyl p-phenylenediamine.

**DNA extraction.** Chromosomal DNA was extracted as described previously (Ausubel et al., 1995). Colonies grown on an agar plate were collected in a 15 ml conical tube and washed with distilled water, and suspended in 1.8 ml of 1/10 TE buffer. The suspension was supplemented with 210 μl SDS (10%, w/v) and 30 μl proteinase K (10 mg ml⁻¹), and then incubated at 37°C for 1 h. To degrade the RNA, 30 μl of Ribonuclease A and T₁ solution (10 mg and 2,000 units) ml⁻¹ was added and incubated for 30 min at room temperature. Three hundred sixty microliters of 5 m NaCl and 270 μl of cetyltrimethylammonium bromide (CTAB)/NaCl solution was added, and incubated at 65°C for 10 min. An equal volume of phenol/chloroform/isoamyl alcohol (25:24:1, v/v/v) was added to the reaction mixture and centrifuged at 5,000 rpm for 10 min. The experiment was performed twice, then an equal volume of chloroform/isoamyl alcohol (24:1, v/v) was added to the supernatant, centrifuged at 11,000 × g for 10 min. The DNA in the supernatant was precipitated by adding 0.6 volume of isopropanol, collected by centrifugation, and washed with 70% ethanol. The obtained DNA was suspended in 200 μl of distilled water.

**Determination of G+C content.** DNA base composition was determined using the HPLC method. DNA was enzymatically degraded into nucleotides as described by Mesbah et al. (1989). The nucleotide mixture obtained was then separated by HPLC using a Cosmosil 5C18R column thermostatted at 40°C. The solvent was 0.2 M NH₄H₂PO₄ with 2.5% acetonitrile. Unmethylated lamda phage DNA (Sigma, St. Louis, MO, USA) was used as the calibration reference.

**PCR amplification and 16S rRNA gene sequencing.** The 16S rDNA was amplified from the chromosomal DNA of strain Jip2T by using a universal eubacterial primer set, 9F (5’-GAGTTTGATCCTGCTGCTG-3’) and 1512R (5’-ACCG(H)TACCTTGTTACGACTT) as described by William et al. (1991). After purifying the PCR product with a GFX™ PCR DNA and Gel Band Purification Kit (Amersham Bioscience, Piscataway, NJ, USA), the resulting PCR product was sequenced with an ABI Prism BigDye Terminator cycle sequencing ready reaction kit (Applied Biosystems, Foster City, CA, USA) and an automatic DNA sequencer (model 310; Applied Biosystems). The primers used for sequencing were 9F [5’-GAGTTTGATCCTGCTGCTG-3’; positions 9-27 (Escherichia coli 16S rRNA numbering)], 341F [5’-CTTACGGAGGCAGCAGCAGCAG-3’; positions 341-357], 519F [5’-CAGCACCCTGCTATATT-3’; positions 519-536], 907F [5’-AAACTCAAAK-3’; positions 907-926], 536R [5’-GAATTGACGG-3’; positions 1110-1111], 1512R [5’-ACGGHTACCTTGTTACGACTT-3’; positions 1512-1492].

**Phylogenetic analysis.** The 16S rDNA full sequences were edited by combining 16S rDNA partial sequences using the BioEdit program (Hall, 1999).
The 16S rDNA sequences of related taxa were obtained from the GenBank. Those obtained sequences including 16S rDNA sequence of strain Jip2T, and related taxa were analyzed. For the multiple alignments, Clustal X program (Thompson et al., 1997) was used. Gaps were edited with the BioEdit program. The evolutionary distance sequences were computed based on the no-gap option and using the Kimura two-parameter model (Kimura, 1980). A phylogenetic tree was constructed by using the neighbor-joining method (Saitou and Nei, 1987) in the MEGA 2 Program (Kumar et al., 2001). The bootstrap neighbor-joining method was used to obtain the confidence level of neighbor-joining analysis with a 1,000 bootstrap data set (Felsenstein, 1985).

Results and Discussion

Morphological and physiological characteristics

Strain Jip2T is an aerobic, Gram-negative, motile and rod-shaped organism of 0.6 μm in diameter and 2.4 μm long, and has one long flagellum on one side and two short flagella on the opposite side (Fig. 1). Colonies formed on nutrient agar (Difco) are yellow and flat with clean edges. The stain gave positive results for catalase and oxidase. This strain was strictly aerobic and did not reduce nitrite or nitrate. This strain grew at 10–37°C but not at 42°C. The optimum growth temperature was 25°C. The optimum pH was in a range of pH 6.0–7.0. As shown in Table 1, strain Jip2T showed positive results for α-glucosidase and β-galactosidase. This strain could grow on a fairly narrow range of carbon source: D-glucose, mannose, salicin, d-melibiose, d-sucrose, N-acetyl-glucosamine and maltose as a sole carbon source. However, it did not utilize acetate, adipate, L-alanine, L-arabinose, caprate, citrate, L-fucose, gluconate, glycerogen, L-histidine, 3-hydroxybenzoate, 4-hydroxybenzoate, 3-hydroxybutyrate, inositol, itaconate, lactate, malate, malonate, mannitol, phenylacetate, L-proline, propionate, rhamnose, d-ribose, L-serine, d-sorbitol, suberate, or valerate. In the API 20E test, strain Jip2T showed positive results for only α-glucosidase. It showed negative results for the following: urease, arginine dihydrolase, lysine decarboxylase, ornithine decarboxylase, gelatin hydrolysis, citrate utilization, H2S production, indole production, Voges-Proskauer test, tryptophane deaminase, and acid production from glucose, mannitol, inositol, sorbitol, rhamnose, sucrose, melibiose, amygdaline, and arabinose.

Chemotaxonomic characteristics

The major ubiquinone of this strain was Q-8. The fatty acid profile of strain Jip2T is shown in Table 2.

![An electron micrograph of the strain Jip2T.](image)
The major fatty acids of strain Jip2\(^T\) were branched fatty acids, with an especially large amount for iso C\(_{15:0}\) and iso C\(_{17:1}\)\(\omega9c\). The G+C content of the DNA of strain Jip2\(^T\) was 65.3 mol%.

**Phylogenetic analysis**

A nearly complete 16S rDNA sequence (nucleotide positions 9–1482, *E. coli* numbering) of strain Jip2\(^T\) was determined. Comparison of the Jip2\(^T\) 16S rDNA sequence with the GenBank database using the BLASTN program (Altschul et al., 1990) and RDP (Ribosomal Data Project) (Maidak et al., 1997) revealed that the most closely related species was *Rhodanobacter lindanolicasticus* RP5557\(^T\) (AF039167) with 96.4% 16S rDNA sequence identity between positions 32 and 1482 (*E. coli* numbering).

It has been suggested that in bacterial strains with less than 97% 16S rDNA sequence identity, that the DNA-DNA hybridization level is less than 70% (Stackebrandt and Goebel, 1994), which defines genomic species (Wayne et al., 1987). Thus, based on the 16S rDNA sequence analysis, a new taxon could be detected. The 16S rDNA sequence relatedness between the isolate and *Fulvimonas soli* LMG 19981\(^T\) (Mergaert et al., 2002) was 95.3%, and that between the isolate and *Frateuria aurantia* LMG 1558\(^T\) (Swings et al., 1980) was 95.2%. Other members of the \(\gamma\)-Proteobacteria including members of *Xanthomonas*, *Stenotrophomonas* and *Xylella*, showed less than 90% sequence similarity.

The phylogenetic tree based on \(K_{\text{unc}}\) values shown in Fig. 2 indicates that strain Jip2\(^T\) is clustered with *Rhodanobacter lindanolicasticus* RP5557\(^T\) (bootstrap value 98%) and distinctively different from related genera. Physiological and chemotaxonomic studies shown in Table 1 indicate that strain Jip2\(^T\) has characteristics distinct from those of related taxa.

The mean G+C content of *Fulvimonas soli* is 71.7% which is 6.4% higher than that of strain Jip2\(^T\). This difference of G+C content is satisfactory to differentiate strain Jip2\(^T\) from *Fulvimonas soli* at the genus level. The next most closely related *Frateuria aurantia* showed distinctive phenotypic characteristics that were oxidase negative and production of acid from various

### Table 2. Cellular fatty acid composition of strain Jip2\(^T\).

<table>
<thead>
<tr>
<th>Fatty acid</th>
<th>Content (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>C(_{16:0})</td>
<td>6.27</td>
</tr>
<tr>
<td>iso C(_{11:0})</td>
<td>3.52</td>
</tr>
<tr>
<td>iso C(_{15:0})</td>
<td>21.25</td>
</tr>
<tr>
<td>iso C(_{15:1}) (\omega9c)</td>
<td>1.24</td>
</tr>
<tr>
<td>iso C(_{16:0})</td>
<td>2.24</td>
</tr>
<tr>
<td>iso C(_{17:0})</td>
<td>10.91</td>
</tr>
<tr>
<td>iso C(_{17:1}) (\omega9c)</td>
<td>24.58</td>
</tr>
<tr>
<td>anteiso C(_{15:0})</td>
<td>6.88</td>
</tr>
<tr>
<td>cyclo C(_{17:0})</td>
<td>2.03</td>
</tr>
<tr>
<td>iso C(_{11:0}) 3OH</td>
<td>4.35</td>
</tr>
<tr>
<td>iso C(_{13:0}) 3OH</td>
<td>2.95</td>
</tr>
<tr>
<td>Summed feature 4(^b)</td>
<td>5.89</td>
</tr>
<tr>
<td>Unknown fatty acid (11.798)</td>
<td>2.23</td>
</tr>
</tbody>
</table>

\(^a\) The double bond position indicated by a capital letter is unknown.

\(^b\) Summed features 4: C\(_{16:1}\) \(\omega7c/iso\) C\(_{15:0}\) 2OH.

![Fig. 2. Rooted phylogenetic tree based on the 16S rDNA sequences of strain Jip2\(^T\) and related bacteria in \(\gamma\)-Proteobacteria.](image)

This tree was made using the neighbor-joining method (Saitou and Nei, 1987) with a Kimura (1983) two-parameter distance matrix and pairwise deletion. Bootstrap values (expressed as percentages of 1,000 replications) greater than 70% are shown at the branch points. The bar represents 20 nucleotides substitution per 1,000 nucleotides.
Rhodanobacter lindaniclasticus RP5558T is also most closely related to this strain. However, strain Jip2T can be differentiated by its motility, its ability to grow on D-maltose, D-mannose, and N-acetyl-glucosamine but not caprate, citrate, or D-malate, with its ability to form β-D-galactosidase. Moreover, as monotype strain RP5558T of the species Rhodanobacter lindaniclasticus no longer exists, either with the authors or with the LMG Bacteria Collection, in which the strain was deposited as LMG 18385T (Mergaert et al., 2002). On the basis of morphological, physiological and chemotaxonomic characteristics, together with data from 16S rDNA sequence comparison described above, strain Jip2T should be placed into a single species, for which we propose the name, **Rhodanobacter fulvus** sp. nov.

**Descriptions**

**Description of Rhodanobacter fulvus** sp. nov.


Cells are Gram-negative and motile rods with size varying between 0.7 and 1.0 μm long. Colonies are circular, convex in form and yellow in pigment with a diameter of 0.2–0.5 mm with clean edges on nutrient agarplates after 3 days at 25°C. Spores are not observed. Catalase and oxidase are positive. Aerobic and chemoorganotrophic. No acid is produced from glucose. Optimum temperature for growth is 25°C. Growth is observed for D-glucose, maltose, D-melibiose, N-acetyl-glucosamine, salicin, and D-sucrose, but not for acetate, adipate, L-alanine, L-arabinose, caprate, citrate, L-fucose, gluconate, glycerogen, L-histidine, 3-hydroxybenzoate, 4-hydroxybenzoate, 3-hydroxybutyrate, inositol, itaconate, lactate, malate, malonate, mannitol, phenylacetate, L-proline, propionate, rhamnose, D-ribose, L-serine, D-sorbitol, suberate, or valerate. Does not hydrolyze gelatin. Does not reduce nitrate to nitrite. Indole production, arginine dihydrolase, urease, lysine decarboxylase, and tryptophan deaminase are negative. α-Glucosidase and β-galactosidase are positive. DNA G+C content is 65.3 mol%. Main composition of ubiquinone is Q-8. The major cellular fatty acids are iso C15:0 and iso C17:1ω9c.

The type strain is strain Jip2T (＝IAM 15025T＝KCTC 12098T).

The 16S rDNA sequence of strain Jip2T has been deposited under DDBJ accession number AB100608.

**References**


