Novosphingobium fluoreni sp. nov., isolated from rice seeds

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A yellow-pigmented, Gram-stain-negative, rod-shaped, non-spore-forming bacterium designated strain HLJ-RS18T, which could degrade fluorene, was isolated from rice seeds collected from Heilongjiang Province, China. Similarities of full-length of 16S rRNA gene sequences between strain HLJ-RS18T and the type strains of the genus Novosphingobium with validly published names ranged from 93.8 to 97.1 %. Phylogenetic analysis with maximum-likelihood and neighbour-joining methods revealed that strain HLJ-RS18T belonged to genus Novosphingobium and strain HLJ-RS18T formed a distinct clade to Novosphingobium chloroacetimidivorans BUT-14T (96.9 % similarity based on 16S rRNA gene). DNA–DNA hybridization of HLJ-RS18T and BUT-14T showed a low relatedness value of 22.4 ± 0.9 %, which indicated that strain HLJ-RS18T represents a novel species of the genus Novosphingobium. The genomic DNA G+C content of strain HLJ-RS18T was 62 mol%. Ubiquinone Q-10 was the major respiratory quinone. Spermidine was the predominant polyamine. Polar lipids consisted mainly of aminophospholipid, phosphatidylglycerol, phosphatidylyethanolamine, phospholipid and sphingoglycolipid. The predominant fatty acid composition of HLJ-RS18T were summed 8 (C18 : 1ω7c and/or C18 : 1ω6c, 61.5 %), C16 : 0 (14.2 %), summed 3 (C16 : 1ω7c and/or C16 : 1ω6c, 13.5 %) and C14 : 0 2-OH (6.8 %). Phylogenetic analysis, DNA–DNA hybridization, chemotaxonomic data and phenotypic characteristics support the conclusion that HLJ-RS18T represents a novel species within the genus Novosphingobium. Therefore, we propose the species Novosphingobium fluoreni sp. nov. with HLJ-RS18T (=DSM 27568T =ACCC19180T) as the type strain.

The genus Novosphingobium, which was dissected from the genus Sphingomonas, was proposed by Takeuchi et al. (2001) based on phylogenetic and chemotaxonomic analysis. At the time of writing this manuscript, the genus Novosphingobium had 30 recognized species (Parte, 2014; Huo et al., 2015) Members of the genus Novosphingobium play a significant role in the degradation of polycyclic aromatic hydrocarbons (PAH) and related compounds. PAHs are ubiquitous pollutants consisting of two or more fused aromatic rings and are primarily from petrogenic, pyrogenic and biogenic sources (See et al., 2009). Novosphingobium pentaromativorans US6-1T can degrade two- to five-ring PAHs (Sohn et al., 2004). Novosphingobium indicum H25T can degrade several classes of aromatic hydrocarbons, including biphenyls, PAHs, dibenzofurans and dibenzothiophenes (Yuan et al., 2009). Novosphingobium naphthalenivorans can metabolize naphthalene and co-metabolize dibenzofuran in the presence of naphthalene (Suzuki & Hiraishi, 2007). Novosphingobium aromaticivorans can degrade a broad range of aromatic compounds, including xylene, toluene and naphthalene (Fredrickson et al., 1991). We isolated a rod-shaped, yellow-pigmented bacterial strain HLJ-RS18T from rice seeds collected from Heilongjiang Province, China. This strain metabolized fluorene. In the present study, we describe the phenotypic, chemotypic and genotypic properties of strain HLJ-RS18T to determine its taxonomic position.

Strain HLJ-RS18T was isolated from rice seeds collected from Heilongjiang Province, China. For this study, 100 rice seeds were suspended in 40 ml of PBS containing Tween-20 (PBST) (per litre: 8.0 g NaCl, 1.15 g Na2HPO4, 0.2 g KH2PO4, 0.2 g KCl, 0.5 g Tween-20, pH 7.4). After the
solution was shaken with a model 25 Controlled Environment Incubator Shaker (New Brunswick Scientific Co., Inc., Edison, NJ) at 120 r.p.m. for 30 min, rice seeds were removed by filtration with gauze. After centrifugation at 10 000 g for 10 min, the pellet was resuspended in 1 ml of PBST. The mixture was serially diluted and plated on LB with 100 µg cycloheximide ml⁻¹ and incubated at 28 ºC for 3 days. Since HLJ-RS18T grew well on LB agar and in LB broth at 28 ºC, routine cultivation was done on LB agar and LB broth at 28 ºC and all morphological, physiological and biochemical characteristics of HLJ-RS18T were investigated under these conditions.

Fluorene degradation by strain HLJ-RS18T was studied. Strain HLJ-RS18T was cultured in LB medium to the exponential phase of growth. An aliquot of 3 ml of the HLJ-RS18T culture was centrifuged at 7000 g for 1 min and the pellet was then washed six times with mineral medium (MM; per litre: 8.8 g Na₂HPO₄·2H₂O, 3.0 g KH₂PO₄, 1.0 g KH₂PO₄, 0.5 g NaCl, 1.0 ml of 1 M MgSO₄ and 2.5 ml of a trace element solution (per litre: 23 mg MnCl₂·2H₂O, 30 mg MnCl₄·H₂O, 31 mg H₃BO₃, 36 mg CoCl₂·6H₂O, 10 mg CuCl₂·2H₂O, 20 mg NiCl₂·6H₂O, 30 mg Na₂MoO₄·2H₂O and 50 mg ZnCl₂), pH 7) (Seo et al., 2007). The washed cells were resuspended in 1 ml of MM. An aliquot of 25 µl of 10 mg fluorene µl⁻¹ (>98% purity, Sigma Aldrich) stock solution in acetone was placed in a culture tube, followed by evaporation of acetone with 99.99% nitrogen gas to complete dryness. An aliquot of 5 ml of MM was then added, followed by addition of 100 µl of the cell suspension. After 0, 1, 2, 3 and 6 days of incubation, fluorene was extracted with 5 ml of ethyl acetate three times. The combined extracts were concentrated with a rotary evaporator to near dryness. One millilitre of acetonitrile was used to dissolve the residues. Fluorene in the extract was measured with a Shimadzu LC-10AS HPLC as described by Gao et al. (2013). The dead cells of HLJ-RS18T, which were boiled for 5–8 min, were used as a negative control. Experiments performed in triplicate unless otherwise stated. Biodegradation rate constants (k) and half-lives (t₁/₂) were calculated. Strain HLJ-RS18T could degrade 60% of fluorene within 6 days (Fig. S1, available in the online supplementary material). The degradation followed a pseudo first-order kinetic reaction. The degradation rate constant and half-life of fluorene were 0.13 day⁻¹ and 2.8 days, respectively, under the cultured conditions.

Gram-staining was performed as described by Gerhardt et al. (1994). Motility was checked on the motility agar medium (Tittsler & Sandholzer, 1936). Morphological characteristics were observed under a scanning electron microscope (S3400N; Hitachi). Flagellation was observed under a transmission electron microscope (JEM-1230) at 80 kV after double staining the cells harvested from 48 h culture with uranyl acetate and lead citrate. Growth in LB broth at 28 ºC at different pH values (pH 1.0 to 9.0, adjusted with 1.0 M HCl or 1.0 M NaOH) and salt concentrations (0–3%, w/v, NaCl) was studied as described by Jones et al. (1979). Phenotypic characterization was conducted in comparison to Novosphingobium barchaimii LL02T, Novosphingobium lindaniclasticum LE124T and Novosphingobium soli CC-TPE-1T using API 20NE gallery by the Identification Service at the Leibniz-Institut DSMZ, including arginine dihydrolase, catalase-activity, aesculin hydrolysis, β-galactosidase, gelatin hydrolysis, indole production, nitrate reduction, oxidase activity and urease-activity. Further substrate assimilation tests were performed as described by Stanier et al. (1966).

Strain HLJ-RS18T was a yellow-pigmented, non-spore-forming and rod-shaped bacterium. Colonies of HLJ-RS18T were approximately 1 mm in diameter after 3 days, circular, domed and smooth with entire margins. Cell size was 1–1.5 µm long and 0.5–0.7 µm wide. HLJ-RS18T cells were motile with a single polar flagellum observed at polar position (Fig. S2). HLJ-RS18T could grow under pH 4.4–7.6 and the optimum pH was 5.5. It could tolerate NaCl at concentrations from 0 to 2.1% (w/v) NaCl and the optimum was 1% (w/v) NaCl. By Gram staining we observed the cells of strain HLJ-RS18T were slightly curved rods with rounded ends and were Gram-stain-negative. Catalase and oxidase activities of HLJ-RS18T were both positive. The catalase activity of N. soli CC-TPE-1T determined in the present study was positive (Table 1), but was negative as reported by Kämpfer et al. (2011). Arginine dihydrolase activity, gelatin hydrolysis, glucose fermentation, indole production, nitrate reduction and urease were all negative in the type strains investigated. The nitrate reduction of N. soli CC-TPE-1T was negative in the present study, which is consistent with Saxena et al. (2013) while a positive reaction was reported by Niharika et al. (2013). The assimilation results of HLJ-RS18T were

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**Table 1. Phenotypic characteristics of strain HLJ-RS18T and type strains of closely related species of the genus Novosphingobium**

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>1</th>
<th>2</th>
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<th>4</th>
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<tbody>
<tr>
<td>Aesculin hydrolysis</td>
<td>−</td>
<td>+</td>
<td>−</td>
<td></td>
</tr>
<tr>
<td>β-Galactosidase</td>
<td>−</td>
<td>−</td>
<td>+</td>
<td>−</td>
</tr>
<tr>
<td>Assimilation of</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>L-Arabinitol</td>
<td>−</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Maltose</td>
<td>−</td>
<td>+</td>
<td>−</td>
<td></td>
</tr>
<tr>
<td>L-Rhamnose</td>
<td>−</td>
<td>−</td>
<td>+</td>
<td>−</td>
</tr>
<tr>
<td>L-Alanine</td>
<td>+</td>
<td>+</td>
<td>w</td>
<td>w</td>
</tr>
<tr>
<td>DL-Lactate</td>
<td>+</td>
<td>+</td>
<td>−</td>
<td></td>
</tr>
<tr>
<td>L-Histidine</td>
<td>−</td>
<td>−</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>Na-Propionate</td>
<td>+</td>
<td>+</td>
<td>−</td>
<td>−</td>
</tr>
</tbody>
</table>

Strains: 1, HLJ-RS18T; 2, N. barchaimii LL02T; 3, N. lindaniclasticum LE124T; 4, N. soli CC-TPE-1T. All strains were positive for catalase, oxidase and assimilation of D-glucose, L-proline and DL-malate. All strains were negative for nitrate reduction, gelatin hydrolysis, indole production, arginine dihydrolase, urease, glucose fermentation and assimilation of sucrose, D-mannose, N-acetylglucosamine, L-serine, and D-sorbitol. All data were obtained in the present study. +, Positive; −, negative; w: weakly positive.
positive for utilization of D-glucose, L-proline, DL-malate and negative for mannose, sucrose and N-acetylglucosamine. Strain HLJ-RS18<sup>T</sup> was clearly distinguished from other type strains of the genus Novosphingobium by several features (Table 1).

For determination of fatty acid composition, cells of HLJ-RS18<sup>T</sup>, N. barchaimii LL02<sup>T</sup>, N. lindaniclasticum LE124<sup>T</sup> and N. soli CC-TPE-1<sup>T</sup> were harvested from LB agar after incubation at 28 °C for 3 days. All the strains were revived on tryptic soy agar for 48 h. The growth phase of the type strains was standardized by observing growth equally in all four quadrants of all cultures. Fatty acid methyl esters were extracted from 40 mg cells (Miller, 1982; Kuykendall et al., 1988) and analysed on an Agilent 6890N gas chromatograph fitted with a DB5 capillary column (0.2 mm x 25 m) and a flame-ionization detector using Sherlock Microbial Identification System (MIDI) and identification of the fatty acid using the Aerobe (TSBA6, version 6.10) database at the Identification Service at the Leibniz-Institut DSMZ. The predominant fatty acids were summed feature 8 (C<sub>18:1ω7c</sub> and/or C<sub>18:1ω6c</sub>, 61.5 %), C<sub>16:0</sub> (14.2 %), summed 3 (C<sub>16:1ω7c</sub> and/or C<sub>16:1ω6c</sub>, 13.5 %) and hydroxyl fatty acid C<sub>14:0 2-OH</sub> (6.8 %) (Table 2). The fatty acid profile determined in the present study agreed with that published in the literature (Ka¨mpfer et al., 2011; Saxena et al., 2013; Niharika et al., 2013). The fatty acid profile obtained for HLJ-RS18<sup>T</sup> was characteristic for species of the genus Novosphingobium (Table 2).

Respiratory quinones and polar lipids were extracted from 100 mg of freeze-dried cell material according to procedures reported (Tindall, 1990a, b; Bligh & Dyer, 1959). Polyamines were extracted according to the procedures reported by Busse & Auling (1988). Respiratory quinones were separated into their different classes by TLC on silica gel (Macherey-Nagel Art. No. 805 023), using hexane/tert-butylmethylether (9: 1, v/v) as solvent. Further analysis was carried out on a LDC HPLC (Thermo Separation Products) fitted with a reverse phase column (Macherey-Nagel, 2 mm x 125 mm, 3 μm, RP18) using methanol/heptane (9: 1, v/v) as the mobile phase. Respiratory lipoquinones were detected at 269 nm. The respiratory quinone observed was ubiquinone Q-10 (100 %). Polar lipids were separated by two dimensional silica gel TLC (Macherey-Nagel Art. No. 818 135) through first dimension in chloroform/methanol/water (65: 25: 4, by vol.) and the second in chloroform/methanol/acetic acid/water (80: 12: 15: 4, by vol.). Total lipids and specific functional groups were detected by dodecanoylbutyrophosphoric acid (total lipids), Zinzadze reagent (phosphate), ninhydrin (free amino groups), periodate-Schiff (α-glycols), Dragendorff (quaternary nitrogen) and α-naphthol-sulphuric acid (glycolipids) as reported by Tindall et al. (2007). The polar lipids detected were mainly of sphingoglycolipid, phospholipid, phosphatidylethanolamine, aminophospholipid and phosphatidyglycerol (Fig. S3). Polyamine was detected by HPLC interfaced with a reversed-phase column (250 × 4 mm, hypersil octyldecyl silane, 5 μm). A linear 40 to 85 % acetonitrile-water gradient was applied for 35 min and then a linear 85 to 100 % acetonitrile-water gradient applied for 15 min at 40 °C was followed as described by Altenburger et al. (1997). The predominant polyamine was spermidine.

DNA base compositions were determined by the Institute of Microbiology, Chinese Academy of Sciences (Beijing, China) according to the method of thermal denaturation (Marmur & Doty, 1962) and DNA from Escherichia coli K-12 (CGMCC 1.365) used as the standard for calibration of the Tm value. The genomic DNA G+C content of HLJ-RS18<sup>T</sup> was 62 mol%, which agreed with that described for the genus Novosphingobium (62–67 %; Takeuchi et al., 2001).

Genomic DNA was extracted with UltraClean Microbial DNA Isolation kit (MO BIO laboratories, Inc.) from 1.8 ml bacterial culture grown in LB medium at 28 °C for 48 h. Universal primers 27F (5′-AGAGTTTGATCMTGGCTCAG-3′) and 1492R (5′-GGTTACCTTGTAGACCTT-3′) (Lane, 1991) were used to amplify the 16S rRNA gene. PCR products (about 1.5 kb) were purified with a PCR-purification kit (Qiagen) and then 50 ng PCR products were ligated with 50 ng pGEM-T Easy Vector (Promega) according to the manufacturer’s instruction. The ligation product (2 μl) was then transformed into 100 μl JM109 High

Table 2. Cellular fatty acid profile of strain HLJ-RS18<sup>T</sup> and the type strains of closely related species of the genus Novosphingobium

<table>
<thead>
<tr>
<th>Fatty Acids</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
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<tbody>
<tr>
<td>Saturated fatty acids</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C&lt;sub&gt;14:0&lt;/sub&gt;</td>
<td>0.9</td>
<td>1.1</td>
<td>1.6</td>
<td>0.5</td>
</tr>
<tr>
<td>C&lt;sub&gt;16:0&lt;/sub&gt;</td>
<td>14.2</td>
<td>4.4</td>
<td>10.2</td>
<td>7.1</td>
</tr>
<tr>
<td>C&lt;sub&gt;18:0&lt;/sub&gt;</td>
<td>0.3</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>Unsaturated fatty acids</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C&lt;sub&gt;16:1ω5c&lt;/sub&gt;</td>
<td>1.3</td>
<td>1.4</td>
<td>3.2</td>
<td>2.2</td>
</tr>
<tr>
<td>C&lt;sub&gt;17:1ω6c&lt;/sub&gt;</td>
<td>0.6</td>
<td>0.9</td>
<td>3.3</td>
<td>5.7</td>
</tr>
<tr>
<td>C&lt;sub&gt;18:1ω7c&lt;/sub&gt;</td>
<td>ND</td>
<td>ND</td>
<td>0.5</td>
<td>0.9</td>
</tr>
<tr>
<td>C&lt;sub&gt;18:1ω5c&lt;/sub&gt;</td>
<td>0.9</td>
<td>2.1</td>
<td>0.9</td>
<td>0.9</td>
</tr>
<tr>
<td>11-methyl C&lt;sub&gt;18:1ω7c&lt;/sub&gt;</td>
<td>ND</td>
<td>1.3</td>
<td>3.1</td>
<td>3.4</td>
</tr>
<tr>
<td>Summed feature 3*</td>
<td>13.5</td>
<td>17.9</td>
<td>12.7</td>
<td>7.1</td>
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<tr>
<td>Summed feature 8*</td>
<td>61.5</td>
<td>58.6</td>
<td>54.9</td>
<td>62.4</td>
</tr>
<tr>
<td>Hydroxy fatty acids</td>
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<td></td>
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<tr>
<td>C&lt;sub&gt;14:0 2-OH&lt;/sub&gt;</td>
<td>6.8</td>
<td>8.7</td>
<td>6.9</td>
<td>7.7</td>
</tr>
<tr>
<td>C&lt;sub&gt;15:0 2-OH&lt;/sub&gt;</td>
<td>ND</td>
<td>ND</td>
<td>0.7</td>
<td>0.8</td>
</tr>
<tr>
<td>C&lt;sub&gt;16:0 2-OH&lt;/sub&gt;</td>
<td>ND</td>
<td>1.8</td>
<td>1.8</td>
<td>0.8</td>
</tr>
<tr>
<td>C&lt;sub&gt;16:1 2-OH&lt;/sub&gt;</td>
<td>ND</td>
<td>0.5</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>iso-C&lt;sub&gt;16:0 3-OH&lt;/sub&gt;</td>
<td>ND</td>
<td>1.2</td>
<td>ND</td>
<td>ND</td>
</tr>
</tbody>
</table>
Efficiency Competent Cells using the heat-shock method. Plasmid extracts from positive clone (200–300 ng) were used for sequencing using 357F, 533F, 902R, SP6 and T7 primers (Advanced Studies in Genomics, Proteomics and Bioinformatics, University of Hawaii at Manoa). A continuous stretch of 1450 bp was obtained. The 16S rRNA gene sequences of other type strains of the genus *Novosphingobium* were retrieved from NCBI (http://www.ncbi.nlm.nih.gov) and RDP (http://rdp.cme.msu.edu/) databases. Similarity was calculated on the basis of pairwise comparison with DNASTAR (Burland, 2000). Multiple alignment of the sequences performed using CLUSTAL W and phylogenetic trees were reconstructed by maximum-likelihood method with Kimura two-parameter model (Kimura, 1980) and neighbour-joining method with Kimura two-parameter model, using MEGA 6.06 (Tamura et al., 2013).

A comparison of the 16S rRNA gene sequences of strain HLJ-RS18<sup>T</sup> and the type strains of all recognized species of the genus *Novosphingobium* showed that strain HLJ-RS18<sup>T</sup> was most closely related to *N. barchaimii* (LL02<sup>T</sup>, 97.1%), *Novosphingobium chloroacetimidivorans* (BUT-14<sup>T</sup>, 96.9%), *N. stygium* IFO16085<sup>T</sup> (AB025013), *N. fuchskuhlense* FNE08-7<sup>T</sup> (JN695619), *N. taihuense* W-51<sup>T</sup> (AJ416411), *N. pentaromativorans* US6-1<sup>T</sup> (AF502400), *N. acidiphilum* FSW06-204<sup>T</sup> (EU336977), *N. aromaticivorans* DSM 12444<sup>T</sup> (CP000248), *N. capsulatum* Gifu 11526<sup>T</sup> (D16147), *N. chloroacetimidivorans* BUT-14<sup>T</sup> (KF676669), *N. soli* CC-TPE-1<sup>T</sup> (FJ425737), *N. capsulatum* Gifu 11526<sup>T</sup> (D16147), *N. soli* CC-TPE-1<sup>T</sup> (FJ425737).

**Fig. 1.** Maximum-likelihood tree based on 16S rRNA gene sequence showing the phylogenetic position of strain HLJ-RS18<sup>T</sup> among type strains of the genus *Novosphingobium*. GenBank accession numbers are given in parentheses. A discrete Gamma distribution and invariable (G + I) was used to model the rate variation among sites. There were a total of 1307 positions in the final dataset. Numbers at branch nodes refer to bootstrap values >70% (1000 resamplings). Nodes supported by high bootstrap value in Neighbour-joining tree were marked with asterisk *.

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Described for *Novosphingobium acidiphilum* E. coli between position 1256 and 1278 (2009) and *Novosphingobium fluoreni* BUT-14T (Figs 1 and S4). Other members of the genus 1215A and 1218C (Takeuchi et al., 2007) was not present in the 16S rRNA gene sequence of *Cells of HLJ-RS18T are Gram-stain-negative, strictly aerobic, motile with a polar flagellum, short rod-shaped, yellow pigment, 1.0–1.5 μm long and 0.5–0.7 μm wide. It grows at pH 4.4–7.6 and with 0–2.1% (w/v) NaCl. Oxidase and catalase activities are positive. Aesculin hydrolysis, nitrate reduction, gelatin hydrolysis, indole production, arginine dihydrolase, urease, β-galactosidase and glucose fermentation are negative. Utilization test results with D-glucose, L-proline, L-alanine, DL-malate, DL-lactate, Na-propionate are positive and L-arabinose, D-mannose, maltose, L-rhamnose, sucrose, N-acetylglucosamine, L-histidine, L-serine and D-sorbitol are negative. Sphingoglycolipid is present. Spermidine is the major polyamine component. The isoprenoid quinone is ubiquinone Q-10. The major polar lipids are aminophospholipid, phosphatidylglycerol, phosphatidylethanolamine, phospholipid and sphingoglycolipid. The fatty acids summed feature 8 (C₁₈:1ω9c and/or C₁₈:1ω6c), C₁₆:0 and summed feature 3 (C₁₆:1ω7c and/or C₁₆:1ω6c), in addition to a few hydroxy fatty acids C₁₆:1ω7OH are produced. The signature nucleotides HLJ-RS18T are consistent with the genus *Novosphingobium*: 52C, 134G, 359G, 593U, 987G, 990U, 1215A and 1218C (Takeuchi et al., 2001).

The type strain is HLJ-RS18T (=DSM 27568T = ACCCC19180T), with the capability of degrading fluorene, was isolated from rice seeds collected from Heilongjiang Province, China. The genomic DNA G+C content is 62 mol%.

**Acknowledgements**

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**References**


**Description of *Novosphingobium fluoreni* sp. nov.**

*Novosphingobium fluoreni* fluoreni (flu.o.re'ni. N.L. neut. n. fluor-enum fluoreni; N.L. gen. neut. n. fluoreni of fluorene).

Cells of HLJ-RS18T are Gram-stain-negative, strictly aerobic, motile with a polar flagellum, short rod-shaped, yellow pigment, 1.0–1.5 μm long and 0.5–0.7 μm wide. It grows at pH 4.4–7.6 and with 0–2.1% (w/v) NaCl. Oxidase and catalase activities are positive. Aesculin hydrolysis, nitrate reduction, gelatin hydrolysis, indole production, arginine dihydrolase, urease, β-galactosidase and glucose fermentation are negative. Utilization test results with D-glucose, L-proline, L-alanine, DL-malate, DL-lactate, Na-propionate are positive and L-arabinose, D-mannose, maltose, L-rhamnose, sucrose, N-acetylglucosamine, L-histidine, L-serine and D-sorbitol are negative. Sphingoglycolipid is present. Spermidine is the major polyamine component. The isoprenoid quinone is ubiquinone Q-10. The major polar lipids are aminophospholipid, phosphatidylglycerol, phosphatidylethanolamine, phospholipid and sphingoglycolipid. The fatty acids summed feature 8 (C₁₈:1ω9c and/or C₁₈:1ω6c), C₁₆:0 and summed feature 3 (C₁₆:1ω7c and/or C₁₆:1ω6c), in addition to a few hydroxy fatty acids C₁₆:1ω7OH are produced. The signature nucleotides HLJ-RS18T are consistent with the genus *Novosphingobium*: 52C, 134G, 359G, 593U, 987G, 990U, 1215A and 1218C (Takeuchi et al., 2001).

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**References**


