Ottowia pentelensis sp. nov., a floc-forming betaproteobacterium isolated from an activated sludge system treating coke plant effluent

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A Gram-negative-staining, short-rod-shaped, floc-forming bacterium, designated strain RB3-7T, was isolated from a laboratory-scale activated sludge system treating coke plant effluent. Comparative analysis of the 16S rRNA gene sequence demonstrated that the novel isolate was distantly related (<95.8% similarity) to Ottowia thiooxydans K11T within the family Comamonadaceae. Strain RB3-7T was catalase- and oxidase-positive and non-motile. The predominant fatty acids were C16:0, cyclo C17:0, C18:1ω7c and C16:1ω9c, and the major respiratory quinone was Q-8. The G+C content of the genomic DNA of strain RB3-7T was 68.5 mol%. On the basis of phenotypic, chemotaxonomic and molecular data, strain RB3-7T is considered to represent a novel species of the genus Ottowia, for which the name Ottowia pentelensis sp. nov. is proposed. The type strain is RB3-7T (=DSM 21699T=NCAIM B 02336T).

During investigation of the microbial composition of a laboratory-scale activated sludge system removing thiocyanate and phenols from coke plant effluent (Felföldi laboratory-scale, 1999), isolates were retrieved showing low pairwise similarity values of partial 16S rRNA gene sequences to members of the family Comamonadaceae (Wen et al., 1999). Strains of this ‘Ottowia-related’ operational taxonomic unit tended to form flocs in liquid cultures (Felföldi et al., 2010). In this paper, the detailed taxonomic characterization of one isolate from this operational taxonomic unit, RB3-7T, is described, and this strain is proposed to represent a novel species of the genus Ottowia (Spring et al., 2004).

Strain RB3-7T was isolated from a laboratory-scale activated sludge system treating coke plant effluent by using the standard dilution plating technique on modified TC medium (Katayama et al., 1992; Felföldi et al., 2010) containing potassium thiocyanate (500 mg L−1), at Eötvös Loránd University (Budapest, Hungary) in 2007. The strain was maintained on trypticase soy agar (TSA; Merck) for direct observation of single colonies. Cell morphology and motility were studied with native preparations and with Gram staining according to Claus (1992). Poly-β-hydroxybutyrate granules were stained with Nile Blue A (Ostle & Holt, 1982). The presence of flagella was examined as described by Heimbrook et al. (1989). Oxidase activity was studied according to the method of Tarrand & Gröschel (1982), and catalase production was demonstrated with the method of Cowan & Steel (1974). Growth at pH 4–13 (intervals of 1 pH unit) was determined by using R2A broth and growth at 4, 8, 20, 28, 30, 37 and 45 °C was determined by using R2A broth and TSA media. Denitrification was studied in an anaerobic chamber (Forma Scientific) according to the guidelines of Smibert & Krieg (1994). Utilization of thiosulfate was tested in SFW-pyruvate medium supplemented with 10 mM thiosulfate, as described by Spring et al. (2004). Autotrophic growth with H2 was tested according to Malik & Schlegel (1981). Carbon source utilization and other metabolic tests were performed with API 50 CH and API 20 NE (bioMérieux) systems in accordance with the manufacturer’s instructions and as described in Kämpfer et al. (1991).

The cell-wall diamino acid of strain RB3-7T was determined from whole-cell hydrolysates as described by Hasegawa et al. (1983). Isoprenoid quinones were extracted according to the method of Collins et al. (1977), and the profile was analysed by HPLC (HP 9001) (Groth et al., 1997) after the cells had been cultivated in liquid Rich
medium (Yamada & Komagata, 1972). For fatty acid analysis, the age of cells was standardized in trypticase soy broth (TSB) medium (Merck), reaching an OD<sub>575</sub> of 1.425 ± 0.5% after 20 h of growth. Cellular fatty acids were extracted with the method of Stead et al. (1992) and were analysed by GC (Kämpfer & Kroppenstedt, 1996). Polar lipids were determined according to the method described by Tindall (1990a, b) from cells grown in TSB and were identified by two-dimensional TLC. Chloroform/methanol/water (65:25:4, by volume) was used in the first direction, followed by chloroform/acetic acid/methanol/water (80:15:12:4, by volume) in the second direction. The following spray reagents were used for detection: 5% ethanolic molybdophosphoric acid (for total lipids), molybdenum blue (for phospholipids), ninhydrin (for amino lipids) and α-naphthol-sulfuric acid (for sugar-containing lipids, which were absent).

The DNA base composition was determined from bacterial cells disrupted with the use of a French press. After purification of the DNA on hydroxyapatite according to the procedure of Cashion et al. (1977), the DNA was degraded into nucleosides by using P1 nuclease and bovine intestinal mucosa alkaline phosphatase, as described by Mesbah et al. (1989). The nucleosides were separated by reversed-phase HPLC (Shimadzu LC 20A) according to the methods described by Tamaoka & Komagata (1984). The G+C content of the DNA was calculated from the ratio of deoxyguanosine to thymidine.

The 16S rRNA gene of strain RB3-7<sup>T</sup> was amplified and sequenced by using primers 27F, 357F, 519R, 803F and 1492R as described by Sipos et al. (2007) and Felöffdi et al. (2010). Alignment and comparative analysis of this sequence with those taxa listed in Fig. 1 (accession numbers given in parentheses) was carried out with MEGA 4 software (Tamura et al., 2007).

Results of the physiological and chemotaxonomic investigations of strain RB3-7<sup>T</sup> are given in the species description below and in Table 1. Photomicrographs illustrating cell morphology and floc-forming ability of strain RB3-7<sup>T</sup> are available as Supplementary Fig. S1 in IJSEM Online, and the polar lipid pattern after separation by two-dimensional TLC is shown in Supplementary Fig. S2. The lipid extract of Ottowia thiooxydans K11<sup>T</sup> was yellow and a series of yellow pigments were visible by eye on the polar lipid plates, but they did not stain with the molybdophosphoric

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**Fig. 1.** Phylogenetic position of strain RB3-7<sup>T</sup> and related species within the family Comamonadaceae. The phylogenetic tree was based on a CLUSTAL W alignment of almost-complete 16S rRNA gene sequences (1330 unambiguous nucleotide positions) and was reconstructed with the neighbour-joining (NJ) method, supplemented with bootstrap values for the minimum-evolution (ME) and maximum-parsimony (MP) methods (only bootstrap values >50% are shown, order: NJ/ME/MP). GenBank accession numbers are given in parentheses. The sequence of Escherichia coli (L10328) was used as outgroup (not shown). Bar, 0.02 substitutions per nucleotide position.
The two strains are oxidase- and catalase-positive, lack flagella, are negative for autotrophic growth with H₂ and are able to oxidize thiosulphate to sulphate. Data are from the present study, except where indicated. The two type strains were investigated under the same conditions. The fatty acid composition was determined from cells grown in TSB. For fatty acids, values are given as percentages of the total fatty acids and only values ≥1.0 % are shown. +, Positive; −, negative; W, weak reaction.

### Table 1. Differential characteristics between strain RB3-7ᵀ and *Ottowia thiooxydans* K11ᵀ

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>RB3-7ᵀ</th>
<th><em>Ottowia thiooxydans</em> K11ᵀ</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cell shape</td>
<td>Short rods</td>
<td>Rods</td>
</tr>
<tr>
<td>Denitrification</td>
<td>−</td>
<td>+</td>
</tr>
<tr>
<td>Utilization of:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fumarate</td>
<td>−</td>
<td>+</td>
</tr>
<tr>
<td>Gluconate</td>
<td>W</td>
<td>−</td>
</tr>
<tr>
<td>DL-Lactate</td>
<td>−</td>
<td>+</td>
</tr>
<tr>
<td>Fatty acids</td>
<td></td>
<td></td>
</tr>
<tr>
<td>C₁₂:₀</td>
<td>32.8</td>
<td>23.4 (19.9*)</td>
</tr>
<tr>
<td>cyclo C₁₇:₀</td>
<td>20.8</td>
<td>11.3 (3.5)</td>
</tr>
<tr>
<td>C₁₈:₁₀₇c</td>
<td>18.1</td>
<td>24.0 (20.4)</td>
</tr>
<tr>
<td>C₁₆:₁₀₇c</td>
<td>14.1</td>
<td>41.4 (42.7)</td>
</tr>
<tr>
<td>C₁₂:₀ 3-OH</td>
<td>4.8</td>
<td>2.6 (2.8)</td>
</tr>
<tr>
<td>cyclo C₁₉:₀₈c</td>
<td>3.6</td>
<td>− (−)</td>
</tr>
<tr>
<td>C₁₂:₀</td>
<td>3.5</td>
<td>3.0 (2.8)</td>
</tr>
<tr>
<td>C₁₂:₀ 2-OH</td>
<td>−</td>
<td>2.2 (2.1)</td>
</tr>
<tr>
<td>C₁₆:₁₀₅c</td>
<td>−</td>
<td>− (2.6)</td>
</tr>
<tr>
<td>C₁₄:₀</td>
<td>−</td>
<td>− (2.1)</td>
</tr>
<tr>
<td>DNA G + C content</td>
<td>68.5</td>
<td>59†</td>
</tr>
</tbody>
</table>

*Results of fatty acid analysis for *O. thiooxydans* K11ᵀ taken from Spring *et al.* (2004) are shown in parentheses.

†Data from Spring *et al.* (2004).

acid stain. In contrast, lipid extracts of strain RB3-7ᵀ did not contain these pigments in detectable amounts.

The 16S rRNA gene sequence of strain RB3-7ᵀ retrieved was a continuous stretch of 1451 nt, and the closest relatives of this strain, based on pairwise similarity values to the relevant type strains (*Chun et al.*, 2007), were *Ottowia thiooxydans* K11ᵀ (95.8 %), *Hydrogenophaga caeni* DSM 17962ᵀ (95.3 %), *Acidovorax caeni* DSM 19327ᵀ (95.2 %), *Variovorax dokdonensis* KCTC 12544ᵀ (95.1 %), *Ramlibacter tatouinensis* DSM 14655ᵀ (95.1 %), *Variovorax soli* DSM 18216ᵀ (95.0 %), *Pseuacidovorax intermedius* CCUG 54492ᵀ (95.0 %) and *Hylenonella gracilis* ATCC 19624ᵀ (95.0 %). All other members of the family *Comamonadaceae* showed lower sequence similarity values (<95 %) to strain RB3-7ᵀ. Phylogenetic analysis of the 16S rRNA gene sequences with the neighbour-joining, minimum-evolution and maximum-parsimony methods gave trees with almost identical topologies, and confirmed the separate position of strain RB3-7ᵀ in the family *Comamonadaceae*, class Betaproteobacteria (Fig. 1). *Ottowia thiooxydans*, the single recognized species of the genus *Ottowia*, clustered with the novel strain with high bootstrap values in the 16S rRNA gene-based phylogenetic tree (Fig. 1), suggesting that strain RB3-7ᵀ belongs to the same genus.

The fatty acid profile of strain RB3-7ᵀ was dominated by C₁₆:₀, cyclo C₁₇:₀, C₁₈:₁₀₇c and C₁₆:₁₀₇c, C₁₀:₀ 3-OH, cyclo C₁₉:₀₈c and C₁₂:₀ were present as minor components. Although most of the detected fatty acids were present in *O. thiooxydans* K11ᵀ, significant differences were detected in their ratios (Table 1). In contrast to *O. thiooxydans* K11ᵀ (Spring *et al.*, 2004), the novel strain was not capable of denitrification, was unable to utilize fumarate or DL-lactate as carbon source, had a higher maximal growth temperature (37 versus 29 °C), showed no stimulatory growth on thiosulfate and had a significantly different genomic DNA G+C content (68.5 mol% for strain RB3-7ᵀ versus 59 mol% for *O. thiooxydans* K11ᵀ); based on these observations strain RB3-7ᵀ represents a novel species. Other physiological properties (e.g. absence of flagella, restricted substrate spectrum, ability to oxidize thiosulfate to sulfate) and polar lipid patterns were similar, supporting the placement of the two strains in the same genus.

Based on the data presented in this study, strain RB3-7ᵀ is considered to represent a novel species of the genus *Ottowia*, for which the name *Ottowia pentelensis* sp. nov. is proposed.

### Description of *Ottowia pentelensis* sp. nov.

*Ottowia pentelensis* (pen.te.len’sis. N.L. fem. adj. pentelensis of or belonging to Pentele, referring to the traditional local name of Dunaujváros from where the inoculating sludge of the laboratory-scale activated sludge system was collected, from where the type strain was isolated).

Growth occurs on TSA after 2–5 days at 28 °C. Colonies on TSA medium are beige, circular, smooth and slightly raised with an entire margin, and 0.2–1.0 mm in diameter. Cells are non-motile short rods (0.5–0.7 × 1.0–1.7 μm). Gram-negative-staining and oxidase- and catalase-positive. Aerobic and mesophilic. Growth occurs at 4–37 °C, but not at 45 °C (optimum, 20–28 °C), and at pH 5–12 (optimum, pH 6–8). Flocs are formed in R2A liquid cultures. Poly-β-hydroxybutyrate granules are present inside cells grown on TSA. Negative for reduction of nitrate to nitrite, for autotrophic growth with H₂ and for denitrification. Thiosulfate can be utilized as co-substrate for aerobic growth and is oxidized to sulfate. Aesculin is not hydrolysed. Negative for indole production, gelatin hydrolysis, D-glucose fermentation, and for arginine dihydrolase, β-galactosidase and urease activity. Positive for the following carbon substrate utilization tests: potassium gluconate (weak), adipic acid (weak), malic acid (weak), trisodium citrate and phenylacetic acid. Negative for D-glucose, L-arabinose, D-mannose, D-mannitol, N-acetylglucosamine, maltose, capric acid, fumarate, DL-lactate and malonate. None of the carbon sources in the
API 50 CH test system are fermented. The major isoprenoid quinone is Q-8. Major cellular fatty acids are C16:0, cyclo C17:0 and C18:1ω7c. Polar lipids are dominated by phosphatidylethanolamine, phosphatidylglycerol, phosphatidylinositolmonomethylethanolamine and diphosphatidylglycerol. The cell wall contains meso-diaminopimelic acid.

The type strain, RB3-7T (=DSM 21699=NCIMB 02336T), was isolated from the activated sludge of a laboratory-scale system treating coke plant effluent. The G + C content of the genomic DNA of the type strain is 68.5 mol%.

**Emended description of the genus Ottowia Spring et al. 2004**

The description of the genus Ottowia is as given by Spring et al. (2004), with the following amendments. Major cellular fatty acids are C16:0, C18:1ω7c and C16:1ω7c, while cyclo C17:0, C10:0 3-OH, cyclo C19:0ω9c, C12:0, C12:0 2-OH, C16:1ω5c and C14:0 may present in various amounts. Polar lipids are dominated by phosphatidylethanolamine, phosphatidylglycerol, phosphatidylmonomethylethanolamine and diphosphatidylglycerol. The G + C content of the genomic DNA is 59–68.5 mol%.

**Acknowledgements**

This work was supported by the Hungarian Economic Competitiveness Operational Program (GVOP-3.2.2-2004-0019/3.0). We thank Katalin Barkacs for her advice and help with chemical measurements, to Gergely Scheirich, Gabrielle Pötter, Judit András and Agnes Simka for their technical assistance, and to Gábor Cech for correction of the species etymology. T.F. was supported by a scholarship from the Ministry of Education and Culture, Hungary (DFÖ 0051/2009).

**References**


