Runella defluvii sp. nov., isolated from a domestic wastewater treatment plant

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A long, Gram-negative, rod-shaped bacterium, designated strain EMB13T, was isolated from a wastewater treatment plant in Korea. The isolate was strictly aerobic and non-motile. The strain grew optimally at 30–35 °C and pH 7.5–8.0, and the predominant fatty acids were iso-C15:0, summed feature 3 (C16:1ω7c and/or iso-C15:0 2-OH), C16:1ω5c and iso-C17:0 3-0H. The strain contained a large amount of phosphatidylethanolamine and small amounts of phosphatidylcholine and an unknown phospholipid as the polar lipids. The G+C content of the genomic DNA was 40.1 mol% and the major isoprenoid quinone was menaquinone-7. Phylogenetic analysis based on 16S rRNA gene sequences showed that strain EMB13T belonged to the genus Runella and was most closely related to Runella limosa EMB111T, with a 16S rRNA gene sequence similarity of 97.1%. DNA–DNA relatedness between strain EMB13T and R. limosa EMB111T was approximately 25%. On the basis of phenotypic, chemotaxonomic and molecular data, it is clear that strain EMB13T represents a novel species within the genus Runella, for which the name Runella defluvii sp. nov. is proposed. The type strain is EMB13T (=KCTC 12614T = DSM 17978T).

The genus Runella, a member of the family ‘Flexibacteraceae’, was first proposed by Larkin & Williams (1978). Currently, the genus contains three species, Runella slithyformis (Larkin & Williams, 1978), Runella zeae (Chelius et al., 2002) and Runella limosa (Ryu et al., 2006). R. zeae was isolated from the stems of surface-sterilized Zea mays; in contrast, R. slithyformis and R. limosa were isolated from aquatic habitats, suggesting that aquatic environments may be important habitats for the genus Runella. Activated sludge processes have been used to remove organic compounds as well as nutrients from wastewater, and insight into the bacterial communities is a prerequisite for understanding activated sludge processes. Therefore, efforts have been made in our laboratory to isolate and characterize members of the bacterial community in activated sludge (Lu et al., 2006; Park et al., 2006, 2007). In this study, we describe another novel species belonging to the genus Runella isolated from an activated sludge process treating domestic sewage.

Strain EMB13T was isolated from activated sludge of a domestic wastewater treatment plant in Pohang, Korea. A sludge sample was serially diluted with 1% (w/v) saline solution, spread on R2A agar (Difco) and incubated at 20 °C for 5 days. Subcultivation was done on R2A agar at 30 °C for 3 days.

Gram staining was performed using the bioMérieux Gram stain kit according to the instructions of the manufacturer. Cell morphology and motility were studied using phase-contrast microscopy and transmission electron microscopy (JEM-1010; JEOL) as described by Jeon et al. (2005). Physiological characteristics of strain EMB13T were examined by growing the isolate on R2A agar at different temperatures (5–50 °C at 5 °C intervals) and in R2A broth adjusted at different pH values (pH 5.0–10.0 at 0.5 pH unit intervals) (Gomori, 1955). Oxidase activity was tested by oxidation of 1% (w/v) tetramethyl-p-phenylenediamine (Merck) and catalase activity was evaluated by production of oxygen bubbles in 3% (v/v) aqueous hydrogen peroxide solution. Hydrolysis of tyrosine, Tween80 and 20, aesculin, casein, starch, gelatin and urea was assessed on R2A agar after 5 days incubation according to methods described previously (Lanyi, 1987; Smibert & Krieg, 1994). Nitrate reduction was assessed according to the method of Lanyi (1987) and acid production from carbohydrates was

*These authors contributed equally to this work.

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain EMB13T is DQ372980.

A transmission electron micrograph of cells of strain EMB13T and API 50CH utilization results for strain EMB13T are available as supplementary material with the online version of this paper.
tested as described by Leifson (1963). Additional enzyme activities and biochemical features were determined using API ZYM, API 20E and API 50CH kits as recommended by the manufacturer (bioMérieux) except that kits were incubated at 30 °C. Strain EMB13T on R2A agar formed slightly raised, circular, salmon-coloured colonies when grown at 30 °C for 3 days. Growth was observed at temperatures between 15 and 40 °C, with an optimum growth temperature of 30–35 °C. The strain grew at pH 6.0–9.5, with an optimum of pH 7.5–8.0. Cells of the isolate were non-motile rods (0.5–0.9 μm wide and 2.2–6.0 μm long) without flagella (Supplementary Fig. S1 available in IJSEM Online). Cells of strain EMB13T were Gram-negative, oxidase-negative and catalase-positive and did not reduce nitrate to nitrite. The isolate was negative for production of indole, H2S and acetoin and citrate utilization (API 20E). Anaerobic growth was not observed under anaerobic conditions over a 7 day incubation at 30 °C on R2A agar.

Analysis of fatty acid methyl esters was performed according to the instructions of the Microbial Identification System (MIDI; Microbial ID, Inc.). The DNA G+C content of strain EMB13T was determined using an HPLC fitted with a reversed-phase column (GROM-SIL 100 ODS-2FE; GROM) according to the method of Tamaoka & Komagata (1984). Analyses of polar lipids and isoprenoid quinones were carried out using the methods described by Komagata & Suzuki (1987). The major respiratory lipoquinone of strain EMB13T was menaquinone-7 (MK-7). The isolate contained iso-C15 : 0 (29.0 %), summed feature 3 (C16 : 1ω7c and/or iso-C15 : 0 2-OH; 20.2 %), C16 : 1ω9c (10.8 %) and iso-C17 : 0 3-OH (9.2 %) as the major fatty acids, which is similar to the profiles of other Runella species (Table 1). The G+C content of the genomic DNA was 40.1 mol%. The polar lipid profile of strain EMB13T was dominated by a large amount of phosphatidylethanolamine, and small amounts of phosphatidylglycerol and an unknown phospholipid (PL1) were also present, which shows that the isolate can be clearly differentiated from R. limosa EMB111T (Table 1).

Typical phenotypic characteristics of strain EMB13T are summarized and compared with those of the type strains of closely related taxa in Table 1 and Supplementary Table S1. Some properties, such as the fatty acid composition, major

### Table 1. Differential phenotypic characteristics of strain EMB13T and selected related species

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
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</thead>
<tbody>
<tr>
<td><strong>Morphology</strong></td>
<td></td>
<td></td>
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<tr>
<td>Colony colour</td>
<td>Rods</td>
<td>Filamentous</td>
<td>Curved rods</td>
<td>Bent rods</td>
<td>Rods</td>
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<tr>
<td><strong>Oxidase</strong></td>
<td>–</td>
<td>–</td>
<td>+</td>
<td>+</td>
<td>+</td>
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<tr>
<td><strong>Catalase</strong></td>
<td>+</td>
<td>+</td>
<td>W</td>
<td>+</td>
<td>+</td>
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<tr>
<td><strong>Starch hydrolysis</strong></td>
<td>–</td>
<td>–</td>
<td>W</td>
<td>–</td>
<td>–</td>
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<tr>
<td><strong>Acid produced from glucose</strong></td>
<td>–</td>
<td>+</td>
<td>–</td>
<td>+</td>
<td>+</td>
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<td><strong>Sole carbon sources for growth</strong></td>
<td></td>
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<tr>
<td><strong>Glycerol</strong></td>
<td>+</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>w</td>
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<tr>
<td><strong>D-Argitol</strong></td>
<td>–</td>
<td>–</td>
<td>W</td>
<td>–</td>
<td>+</td>
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<tr>
<td><strong>L-Argitol</strong></td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>W</td>
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<tr>
<td><strong>Dulcitol</strong></td>
<td>–</td>
<td>–</td>
<td>W</td>
<td>W</td>
<td>W</td>
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<tr>
<td><strong>Inositol</strong></td>
<td>–</td>
<td>–</td>
<td>W</td>
<td>W</td>
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<tr>
<td><strong>Mannitol</strong></td>
<td>–</td>
<td>–</td>
<td>W</td>
<td>–</td>
<td>+</td>
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<td><strong>Sorbitol</strong></td>
<td>–</td>
<td>–</td>
<td>W</td>
<td>W</td>
<td>W</td>
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<tr>
<td><strong>Glycogen</strong></td>
<td>W</td>
<td>W</td>
<td>+</td>
<td>+</td>
<td>–</td>
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<tr>
<td><strong>Sorbose</strong></td>
<td>W</td>
<td>W</td>
<td>W</td>
<td>W</td>
<td>+</td>
</tr>
<tr>
<td><strong>5-Ketogluconate</strong></td>
<td>W</td>
<td>+</td>
<td>–</td>
<td>W</td>
<td>+</td>
</tr>
<tr>
<td><strong>Methyl β-xylulose</strong></td>
<td>+</td>
<td>–</td>
<td>+</td>
<td>+</td>
<td>+</td>
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<tr>
<td><strong>Maltose</strong></td>
<td>–</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
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<tr>
<td><strong>Trehalose</strong></td>
<td>–</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
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<tr>
<td><strong>Major cellular fatty acids</strong></td>
<td>iso-C15 : 0, C16 : 1ω5c, SF3</td>
<td>iso-C15 : 0, C16 : 1ω5c, SF3</td>
<td>NA</td>
<td>C16 : 1ω5c, iso-C15 : 0, SF3</td>
<td>iso-C15 : 0, C16 : 1ω5c, C16 : 1ω7c</td>
</tr>
<tr>
<td><strong>Major polar lipid</strong></td>
<td>PE</td>
<td>PG</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td><strong>DNA G+C content (mol%)</strong></td>
<td>40.1</td>
<td>44.5</td>
<td>49</td>
<td>49</td>
<td>48</td>
</tr>
</tbody>
</table>

*Results for R. limosa EMB111T were obtained in this study.

†SF3, Summed feature 3, containing C16 : 1ω7c and/or iso-C15 : 0 2-OH.

§PE, Phosphatidylethanolamine; PG, phosphatidylglycerol.
lipoquinone and nitrate reduction, are in accordance with those of members of the genus Runella, whereas some others such as DNA G+C content and major polar lipids allow the differentiation of strain EMB13T from closely related species.

Sequencing of the 16S rRNA gene of strain EMB13\textsuperscript{T} was carried out as described previously (Lane, 1991). The PCR product was cloned using a TOPO cloning kit (Invitrogen) and sequenced. The resulting 16S rRNA gene sequence (1427 nucleotides) was compared with available 16S rRNA gene sequences from GenBank using the BLAST program (http://www.ncbi.nlm.nih.gov/BLAST/) to determine an approximate phylogenetic affiliation, and gene sequences were aligned with those of closely related species by using the CLUSTAL W software (Thompson et al., 1994). Phylogenetic trees were constructed by using three different algorithms, neighbour-joining, maximum-likelihood and maximum-parsimony algorithms; these methods are available in PHYLIP software, version 3.6 (Felsenstein, 2002). Sequence similarity values between the novel strain and related organisms were computed using the FASTA3 program in EBI (http://www.ebi.ac.uk/fasta33/nucleotide.html). A bootstrap analysis was performed according to the Kimura two-parameter model (Kimura, 1980) of the neighbour-joining method in the PHYLIP package. DNA–DNA hybridization was carried out to evaluate the genomic DNA relatedness between strain EMB13\textsuperscript{T} and \textit{R. limosa} EMB111\textsuperscript{T} using the fluorometric microplate method (Ezaki \textit{et al}, 1989). Fluorometric data recorded after 30 min incubation were used for the calculation of a DNA–DNA hybridization value; the DNA relatedness value is the mean of three values.

Phylogenetic analysis based on 16S rRNA gene sequences indicated that strain EMB13\textsuperscript{T} formed a phylogenetic lineage with \textit{R. limosa} EMB111\textsuperscript{T} with 100 % bootstrap value (Fig. 1). The overall topologies of the maximum-likelihood and maximum-parsimony trees were essentially the same as that of the neighbour-joining tree (data not shown). Comparative 16S rRNA gene sequence analysis showed that strain EMB13\textsuperscript{T} was most closely related to \textit{R. limosa} EMB111\textsuperscript{T}, \textit{R. zeae} NS12\textsuperscript{T} and \textit{R. slithyformis} ATCC 29530\textsuperscript{T}, with sequence similarities of 97.1, 93.9 and 93.6 %, respectively. The DNA–DNA relatedness between strain EMB13\textsuperscript{T} and \textit{R. limosa} EMB111\textsuperscript{T} was about 25 %, which is clearly below the 70 % threshold generally accepted for species delineation (Stackebrandt \textit{et al}, 2002). The physiological, biochemical and phylogenetic properties of strain EMB13\textsuperscript{T} suggest that the isolate should be considered as representing an additional novel species belonging to the genus \textit{Runella}, for which the name \textit{Runella defluvii} sp. nov. is proposed.

**Description of \textit{Runella defluvii} sp. nov.**

\textit{Runella defluvii} (de.flu’v’i. L. gen. n. defluvii of sewage).

Colonies are slightly raised, circular and salmon pink in colour on R2A agar. Cells are Gram-negative, non-motile rods, 0.5–0.9 \textmu m wide and 2.2–6.0 \textmu m long at 30 °C on R2A agar. Growth occurs at 15–40 °C (optimum, 30–35 °C) and at pH 6.0–9.5 (optimum, pH 7.5–8.0). Nitrate is not reduced to nitrite. Catalase-positive and oxidase-negative. No anaerobic growth after 7 days at 30 °C on R2A agar. Tyrosine, Tween 80 and ascin are hydrolysed, but casein, Tween 20, starch, gelatin and urea are not hydrolysed. Acid is produced from raffinose, \textit{myo}-inositol, lactose, L-arabinose, D-galactose, D-mannose, D-mannitol and melibiose, but not from D-glucose, D-fructose, arbutin or salicin. Indole, H\textsubscript{2}S and acetoin are not produced and citrate is not utilized (API 20E). Alkaline phosphatase, trypsin, \textit{a}-chymotrypsin, \textit{N}-acetyl-\textit{b}-glucosaminidase and naphthol-AS-BI-phosphohydrolase are produced, but tryptophan deaminase, esterase (C4), lipase (C14) and \textit{b}-glucuronidase are not produced. Weak activities are observed for esterase lipase (C8), leucine arylamidase, valine arylamidase, cystine arylamidase, acid phosphatase, \textit{a}-galactosidase, \textit{b}-galactosidase, \textit{b}-glucosidase, \textit{a}-mannosidase and \textit{a}-fucosidase (API ZYM). Utilizes glycerol, methyl \textit{b}-xylloside, methyl \textit{a}-D-mannoside and ascin as sole carbon sources but not erythritol, D- or L-arabinose, D-xylitol, adonitol, galactose, D-glucose, D-fructose, mannose, dulcitol, inositol, mannnitol, sorbitol, \textit{N}-acetylglucosamine, salicin, cellobiose, maltose, lactose, melibiose, sucrose, trehalose, inulin, melezitose, D-raffinose, D-turanose, D- or L-arabitol, gluconate or 2-ketogluconate. Weakly utilizes ribose, L-xylitol, sorbose, rhamnose, methyl \textit{a}-D-glucoside, amygdalin, arbutin, starch, glycogen, xylitol, \textit{b}-gentiobiose, D-lyxose, D-tagatose, D- and L-fucose and 5-ketogluconate (API 50CH). Contains a large amount of phosphatidylethanolamine and small amounts of phosphatidylcholine and an unknown phospholipid as polar lipids. The major isoprenoid quinone is menaquinone-7. The cellular fatty acids are
iso-C15:0 (29.0 %), summed feature 3 (C16:1ω7c and/or
iso-C15:0 2-OH; 20.2 %), C16:0ω5c (10.8 %), iso-C17:0 3-
OH (9.2 %), iso-C15:0 3-OH (7.4 %), C15:0 (6.5 %), iso-
C15:1 G (3.4 %), C16:0 3-OH (3.3 %), iso-C13:0 (2.2 %),
C15:1ω6c (1.8 %), C16:0 (1.4 %), C17:1ω6c (0.9 %), C14:0
(0.8 %), anteiso-C15:0 (0.6 %) and unknown ECL 14.959
(2.7 %). The G+C content of the DNA is 40.1 mol%
(HPLC).

The type strain is EMB13 T (=KCTC 12614 T =DSM
17976 T), isolated from activated sludge of a domestic
wastewater treatment plant.

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References

sp. nov., a novel Gram-negative bacterium isolated from
surface-sterilized Zea mays stems. Int J Syst Evol Microbiol 50,
751–758.

nov., a novel Gram-negative bacterium from the stems of surface-

deoxyribonucleic acid-deoxyribonucleic acid hybridization in micro-
dilution wells as an alternative to membrane filter hybridization in
which radioisotopes are used to determine genetic relatedness among

Felsenstein, J. (2002). PHYLIP (phylogeny inference package) version
3.6a. Distributed by the author. Department of Genomics,
University of Washington, Seattle, USA.

Methods Enzymol 1, 138–146.

(2005). Reclassification of Bacillus haloalkaliphilus Fritzte 1996 as
Alkalibacillus haloalkaliphilus gen. nov., comb. nov. and the descrip-
tion of Alkalibacillus salitarius sp. nov., a novel halophilic bacterium
isolated from a salt lake in China. Int J Syst Evol Microbiol 55,
1891–1896.

of base substitutions through comparative studies of nucleotide


Techniques in Bacterial Systematics, pp. 115–175. Edited by


sp. nov., a curved, nonflexible, pink bacterium. Int J Syst Bacteriol 28,
32–36.

Leifson, E. (1963). Determination of carbohydrate metabolism of

Analysis of microbial communities using culture-dependent and
culture-independent approaches in an anaerobic/aerobic SBR reactor.

Park, M., Lu, S., Ryu, S. H., Chung, B. S., Park, W., Kim, C. J. & Jeon,
C. O. (2006). Flavobacterium croceum sp. nov., isolated from activated

nov., from biological soil crusts in the Colorado Plateau, USA, and an
emended description of the genus Dyadobacter Chelius and Triplett

Ryu, S. H., Nguyen, M. T., Park, W., Kim, C.-J. & Jeon, C. O.
(2007). Flavobacterium defluvii sp. nov., isolated from activated

Methods for General and Molecular Bacteriology, pp. 607–654. Edited
Washington, DC: American Society for Microbiology.

Stackebrandt, E., Frederiksen, W., Garrity, G. M., Grimont, P. A. D.,
Kämpfer, P., Maiden, M. C., Nesme, X., Rossello-Mora, R., Swings, J.,
and other authors (2002). Report of the ad hoc committee for the re-
evaluation of the species definition in bacteriology. Int J Syst Evol Microbiol 52,
1043–1047.

composition by reversed-phase high-performance liquid chromato-

Thompson, J. D., Higgins, D. G. & Gibson, T. J. (1994). CLUSTAL W:
improving the sensitivity of progressive multiple sequence alignment
through sequence weighting, position-specific gap penalties and