Runella limosa sp. nov., isolated from activated sludge

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A Gram-negative bacterium, designated strain EMB111^T, was isolated from activated sludge performing enhanced biological phosphorus removal in a sequencing batch reactor. Cells were long and rod-shaped. The isolate was strictly aerobic and non-motile. The strain grew optimally at 25–30 °C and pH 7.5–8.0. The predominant fatty acids of strain EMB111^T were iso-C_{15:0}, C_{16:1}ω5c, iso-C_{17:0} 3-OH, iso-C_{15:0} 3-OH, C_{16:0} 3-OH, C_{16:0} and summed feature 3 (C_{16:1}ω7c and/or iso-C_{15:0} 2-OH). The strain contained a large amount of phosphatidylglycerol and small amounts of two unknown phospholipids (PL1, PL2) as the polar lipids. The major isoprenoid quinone was menaquinone-7. The G + C content of the genomic DNA was 42.7 mol%. Phylogenetic analysis showed that strain EMB111^T formed a phyletic cluster with members of the genus *Runella* within the family *Flexibacteraceae* and was most closely related to *Runella slithyformis* ATCC 29530^T with a 16S rRNA gene sequence similarity of 94.8%. On the basis of chemotaxonomic data and molecular properties, strain EMB111^T represents a novel species within the genus *Runella*, for which the name *Runella limosa* sp. nov. is proposed. The type strain is EMB111^T (=KCTC 12615^T=DSM 17973^T).

The genus *Runella*, a member of the family *Flexibacteraceae*, phylum *Bacteroidetes* (formerly *Flexibacter–Bacteroides–Flavobacterium*), was first erected by Larkin & Williams (1978). At the time of writing, the genus comprised two recognized species, *Runella slithyformis* and *Runella zeae* (Larkin & Williams, 1978; Chelius *et al.*, 2002). *R. slithyformis* has an aquatic lifestyle; by contrast, *R. zeae* was isolated from the stems of surface-sterilized maize (*Zea mays*). This suggests that members of the genus *Runella* can be isolated from diverse environmental habitats. In this study, we describe a novel species of the genus *Runella* isolated from an activated sludge process performing enhanced biological phosphorus removal.

Strain EMB111^T was isolated from activated sludge performing enhanced biological phosphorus removal in a laboratory-scale sequencing batch reactor. Sodium acetate

was supplied as the sole carbon source, and the operation of the reactor was as described by Jeon *et al.* (2003). A sludge sample was serially diluted in 1% (w/v) saline solution, spread on R2A agar (Difco) and incubated at 20 °C for 5 days. Subcultivation was on R2A agar at 25 °C for 3 days. Gram staining was performed using the bioMérieux Gram stain kit according to the manufacturer's instructions. Cell morphology and motility were studied by using phase-contrast microscopy and transmission electron microscopy (JEM-1010; JEOL) as described by Jeon *et al.* (2005). Physiological characteristics of strain EMB111^T were examined by growing the isolate on R2A medium at different temperatures and pH values. R2A media with different pH values were prepared as described by 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, Tweens 20 and 80, aesculin, casein, starch, gelatin and urea was assessed on R2A agar after 5 days incubation according to the methods described by Lanyi (1987) and Gerhardt *et al.* (1994). Nitrate reduction was performed according to the method of Lanyi (1987) and

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain EMB111^T is DQ372985.

A transmission electron micrograph showing the general morphology of negatively stained cells of strain EMB111^T is available as a supplementary figure in IJSEM Online.

acid production from carbohydrates was tested as described by Leifson (1963). Additional enzyme activities and biochemical characteristics were determined by using the API ZYM and API 20E kits as recommended by the manufacturer (bioMérieux). Strain EMB111^T on R2A agar formed slightly raised, circular, salmon-pink colonies when grown at 25 °C for 3 days. Growth was observed at temperatures between 15 and 40 °C, with an optimum growth temperature range of 25–30 °C. The strain grew at pH 6.0–9.0, with an optimum pH range of 7.5–8.0. Cells of the isolate were non-motile, long rods (0.7–0.9 µm wide and 4.0–10.0 µm long) that lacked flagella (see Supplementary Fig. S1 in IJSEM Online). Cells of strain EMB111^T 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 for utilization of citrate (API 20E). Growth was not observed under anaerobic conditions over a 7-day incubation period 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.). Analyses of polar lipids and isoprenoid quinones were carried out using the methods described by Komagata & Suzuki (1987). The DNA G + C content of strain EMB111^T was determined using an HPLC system fitted with a reversed-phase column (GROM-SIL 100 ODS-2FE; GROM) according to the method of Tamaoka & Komagata (1984). The major respiratory lipoquinone of strain EMB111^T was menaquinone-7 (MK-7). The cellular fatty acids of the isolate included iso-C_{15:0} (29.5 %), summed feature 3 (C_{16:1}ω7c and/or iso-C_{15:0} 2-OH; 19.5 %), C_{16:1}ω5c (12.9 %), iso-C_{17:0} 3-OH (9.2 %), iso-C_{15:0} 3-OH (6.7 %), C_{16:0} 3-OH (5.7 %), C_{16:0} (5.5 %), C_{15:0} (2.5 %), iso-C_{15:1}G (2.4 %), iso-C_{13:0} (1.8 %), anteiso-C_{15:0} (1.7 %), iso-C_{16:0} 3-OH (0.8 %), iso-C_{16:0}

(0.8 %), C_{14:0} (0.8 %), iso-C_{17:0} (0.3 %) and iso-C_{14:0} (0.2 %). The strain contained a large amount of phosphatidylglycerol (PG) and small amounts of two unknown phospholipids (PL1, PL2). The G + C content of the genomic DNA of strain EMB111^T was 42.7 mol%. The phenotypic characteristics of strain EMB111^T are summarized and compared with those of the type strains of closely related taxa in Table 1.

Sequencing and assembling of the 16S rRNA gene were carried out as described by DeLong (1992). The resultant 16S rRNA gene sequence of strain EMB111^T was compared with available sequences from GenBank using the BLAST program (<http://www.ncbi.nlm.nih.gov/blast/>) to determine an approximate phylogenetic affiliation, and the gene sequence was aligned with those of closely related strains using CLUSTAL W software (Thompson *et al.*, 1994). Phylogenetic trees were constructed using three different methods, the neighbour-joining, maximum-likelihood and maximum-parsimony algorithms, which are available in the PHYLIP software, version 3.6 (Felsenstein, 2002). Sequence similarity values were computed by using Similarity Matrix version 1.1 (Ribosomal Database Project II; <http://35.8.164.52/html/>; Cole *et al.*, 2003). A bootstrap analysis was performed according to the algorithm of the Kimura two-parameter model (Kimura, 1980) of the neighbour-joining method using the PHYLIP package. Phylogenetic analysis using a nearly complete 16S rRNA gene sequence (1428 nt) of strain EMB111^T indicated that the isolate formed a phyletic cluster with members of the genus *Runella* with 100 % bootstrap support (Fig. 1). The strain was most closely related to *R. slithyformis* ATCC 29530^T with a 16S rRNA gene sequence similarity of 94.8 %. The topologies of phylogenetic trees built using the maximum-likelihood and maximum-parsimony algorithms also supported the notion that the isolate belongs to the genus *Runella* (data not

Table 1. Differential characteristics of strain EMB111^T and type strains of selected related species

Strains: 1, strain EMB111^T; 2, *R. slithyformis* ATCC 29530^T (data from Chelius & Triplett, 2000); 3, *R. zeae* NS12^T (Chelius *et al.*, 2002); 4, *Dyadobacter fermentans* NS114^T (Chelius & Triplett, 2000; Reddy & Garcia-Pichel, 2005); 5, *Flectobacillus major* ATCC 29496^T (Chelius & Triplett, 2000). Symbols: +, positive; –, negative; (+), slightly positive; NA, no data available.

Characteristic	1	2	3	4	5
Morphology	Long rods	Curved rods	Bent rods	Rods	Curved rods
Colony colour	Salmon pink	Salmon pink	Salmon pink	Yellow	Pink
Optimum temperature (°C)	25–30	20–30	28	28	20–30
Oxidase	–	+	+	+	+
Catalase	+	(+)	+	+	(+)
Gelatin hydrolysis	–	–	–	–	(+)
Starch hydrolysis	–	(+)	–	–	+
Major cellular fatty acids	iso-C _{15:0} , C _{16:1} ω5c, summed feature 3*	NA	C _{16:1} ω5c, iso-C _{15:0} , summed feature 3*	iso-C _{15:0} , C _{16:1} ω5c, C _{16:1} ω7c	NA
DNA G + C content (mol%)	42.7	49	49	48	40

*Summed feature 3 comprises C_{16:1}ω7c and/or iso-C_{15:0} 2-OH.

Fig. 1. Neighbour-joining tree based on 16S rRNA gene sequences showing the phylogenetic relationships of strain EMB111^T and related taxa. Bootstrap values are shown as percentages of 1000 replicates; only values >50% are shown. *Escherichia coli* ATCC 11775^T was used as an outgroup. Bar, 0.1 changes per nucleotide position.

shown). Therefore, the physiological, biochemical and phylogenetic properties of strain EMB111^T suggest that it represents a novel species of the genus *Runella* within the family *Flexibacteraceae*, for which the name *Runella limosa* sp. nov. is proposed.

Description of *Runella limosa* sp. nov.

Runella limosa (li.mo'sa. L. fem. adj. *limosa* muddy, pertaining to sludge, the natural habitat of the species).

Colonies are slightly raised, circular and salmon-pink in colour on R2A agar. Growth occurs optimally at 25–30 °C and pH 7.5–8.0. Cells are Gram-negative, non-motile, long rods, 0.7–0.9 µm wide and 4.0–10.0 µm long when grown at 25 °C on R2A agar. Nitrate is not reduced to nitrite. Catalase-positive and oxidase-negative. Tyrosine, Tween 80 and aesculin are hydrolysed. Casein, Tween 20, starch, gelatin and urea are not hydrolysed. Acids are produced from D-glucose, D-raffinose, *myo*-inositol, D-lactose, D-mannitol and melibiose, but not from sorbitol, sucrose, rhamnose, amygdalin, D-fructose, D-galactose, D-mannose, L-arabinose, arbutin or salicin. Produces alkaline phosphatase, α -chymotrypsin, *N*-acetyl- β -glucosaminidase and naphthol-AS-BI-phosphohydrolase, but not esterase (C4), esterase lipase (C8), lipase (C14) or cystine arylamidase. Weak enzymic activities are observed for leucine arylamidase, valine arylamidase, trypsin, β -galactosidase, α -glucosidase, β -glucosidase, acid phosphatase, α -galactosidase, β -glucuronidase, α -mannosidase and α -fucosidase. The strain contains a large amount of phosphatidylglycerol and small amounts of two unknown phospholipids (PL1, PL2). The major isoprenoid quinone is MK-7. The major fatty acids are iso-C_{15:0}, C_{16:1}ω5c, iso-C_{17:0} 3-OH, iso-C_{15:0} 3-OH, C_{16:0} 3-OH, C_{16:0} and summed feature 3

(C_{16:1}ω7c and/or iso-C_{15:0} 2-OH). The DNA G + C content is 42.7 mol% (HPLC).

The type strain, EMB111^T (=KCTC 12615^T=DSM 17973^T), was isolated from sludge performing enhanced biological phosphorus removal.

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