Ideonella sakaiensis sp. nov., isolated from a microbial consortium that degrades poly(ethylene terephthalate)

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A Gram-stain-negative, aerobic, non-spore-forming, rod-shaped bacterium, designed strain 201-F6^T, was isolated from a microbial consortium that degrades poly(ethylene terephthalate) (PET) collected in Sakai city, Japan, and was characterized on the basis of a polyphasic taxonomic study. The cells were motile with a polar flagellum. The strain contained cytochrome oxidase and catalase. It grew within the pH range 5.5–9.0 (optimally at pH 7–7.5) and at 15–42 °C (optimally at 30–37 °C). The major isoprenoid quinone was ubiquinone with eight isoprene units (Q-8). $C_{16:0}$, $C_{17:0}$ cyclo, $C_{18:1}\omega7c$ and $C_{12:0}$ 2-OH were the predominant cellular fatty acids. The major polar lipids were phosphatidylethanolamine, *lyso*-phosphatidylethanolamine, phosphatidylglycerol and diphosphatidylglycerol. The G+C content of genomic DNA was 70.4 mol%. Phylogenetic analysis using the 16S rRNA gene sequences showed that strain 201-F6^T was affiliated to the genus *Ideonella*, and was closely related to *Ideonella dechloratans* LMG 28178T (97.7 %) and *Ideonella azotifigens* JCM 15503^T (96.6 %). Strain 201-F6^T could be clearly distinguished from the related species of the genus *Ideonella* by its physiological and biochemical characteristics as well as by its phylogenetic position and DNA–DNA relatedness. Therefore, the strain represents a novel species of the genus *Ideonella*, for which the name *Ideonella sakaiensis* sp. nov. (type strain 201-F6^T=NBRC 110686^T=TISTR 2288^T) is proposed.

The genus *Ideonella* belongs to the family *Comamonadaceae* of the class *Betaproteobacteria*, as proposed by Malmqvist *et al.*, (1994), with the type species *Ideonella dechloratans*, a chlorate-respiring bacterium. In 2009, *Ideonella azotifigens* strain 1a22^T, isolated from grass rhizosphere soil, was proposed to represent a second species (Noar & Buckley, 2009). Strains belonging to the genus *Ideonella* are Gram-stain-negative, aerobic and mesophilic, straight or slightly curved, asporogenous rods. They are motile by two or several polar or subpolar flagella. Colonies are circular, smooth and nonpigmented. These bacteria are positive for catalase and oxidase. The major cellular fatty acids are $C_{18:1}$ $\omega7c$ and $C_{16:0}$. The DNA G+C content ranges from 67.4 to 68.1 mol%. The members of the genus *Ideonella* are chemo-organotrophs that utilize organic acids, amino acids and carbohydrates as sole carbon sources. Lipolytic and proteolytic activities are positive. Recently, we isolated a bacterium designated 201-F6^T that degrades and assimilates poly(ethylene terephthalate) (PET) (Yoshida *et al.*, 2016). In this study, strain 201-F6^T was assessed on the basis of phenotypic and chemotaxonomic analyses and by DNA–DNA relatedness studies. Accordingly, we describe a novel bacterium, *Ideonella sakaiensis* sp. nov., evaluated by means of a polyphasic taxonomic study.

We screened for microorganisms that were able to utilize PET film as the major carbon source for growth by using PET-debris-contaminated environmental samples such as sediment, soil, waste water and sludge as targets. We succeeded in isolating a microbial consortium (named No. 46)

Abbreviation: PET, poly(ethylene terephthalate).

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain 201-F6^T is LC002525.

One supplementary table and two supplementary figures are available with the online Supplementary Material.

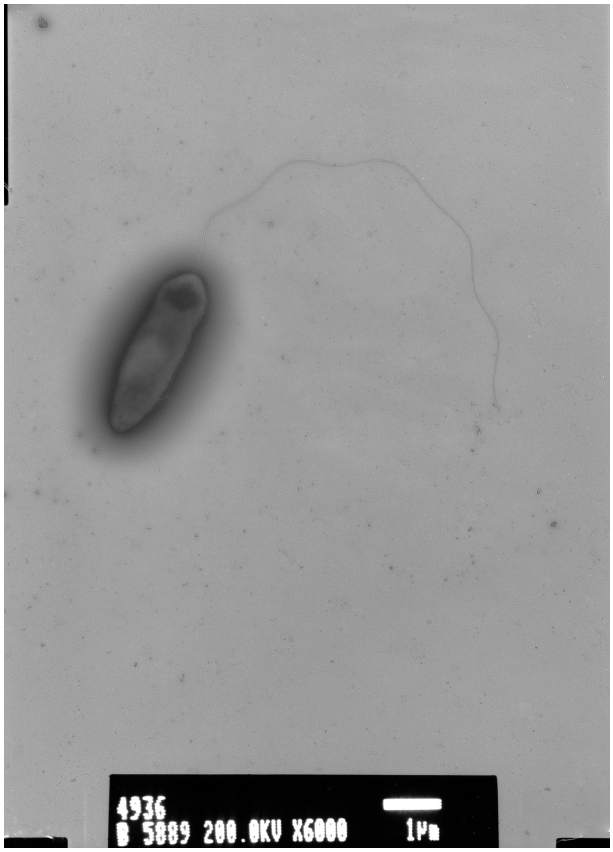

Fig. 1. Transmission electron micrograph of a cell of strain 201-F6^T grown in NBRC no. 802 broth at 30 °C for 24 h. Bar, 1 μm.

that degraded and grew on PET from the samples collected at a PET-bottle recycling site in Sakai city, Japan. Strain 201-F6^T was isolated from the consortium No. 46 as a bacterium that was mainly involved in the degradation of PET. Cells grown on NBRC medium no. 802 (Wako Pure Chemical Industries, Ltd.) agar plates [composed of (l^{-1}) 10 g polypeptone, 2 g yeast extract, 1 g $MgSO_4 . 7H_2O$ and 15 g agar; pH 7.0] at 30 °C for 48 h were examined for their morphological and cultural characteristics, including cell shape, colony appearance and pigmentation. The presence of flagella of cells cultivated in NBRC no. 802 broth at 30 °C was assessed by using (JEOL Ltd. Tokyo, Japan. JEM-2000EX) transmission electron microscopy. Catalase and oxidase activities, the methyl red/Voges–Proskauer reactions, and hydrolysis of casein, aesculin, DNA and starch were determined as described by Barrow & Feltham (1993). Growth was tested by using NBRC no. 802 broth based on 50 mM acetate buffer (pH 4.0–5.5), phosphate buffer (pH 6.0–8.0) or Tris buffer (pH 9.0) (Sorokin, 2005). Furthermore, the growth capacity on NBRC no. 802 medium with 3 or 5 % (w/v) NaCl added was evaluated. The photosynthetic characteristics of the strain cultivated under anaerobic conditions with or without lighting were determined as described by Willems *et al.* (1991). The lighting was provided by a 100 W filament lamp placed at 50 cm away from the samples. Biochemical characteristics were investigated using API 20NE strips (bioMérieux), in accordance with the manufacturer's directions. Additional enzymic activities of the strain incubated at 30 °C for 48 h were tested by using API ZYM kits (bioMérieux). PET-hydrolysis was investigated using YSV medium containing a low-crystallinity PET thin film (T_g: 77 °C, T_m: 255 °C, crystallinity: 1.9 %) as the main carbon source. The YSV medium contained 0.1 g yeast extract, 0.2 g $NaHCO_3$, 1 g $(NH_4)_2SO_4$, 0.1 g $CaCO_3$, 10 mg $FeSO_4 \cdot 7H_2O$, 1 mg $CuSO_4 \cdot 5H_2O$, 1 mg $MnSO_4 \cdot 7H_2O$, 1 mg $ZnSO_4 \cdot 7H_2O$, 10 mg $MgSO_4 \cdot 7H_2O$, 2.5 mg thiamine hydrochloride, 0.05 mg biotin and 0.5 mg vitamin B_{12} in one litre of 10 mM phosphate buffer, pH 7.0, as described by Yoshida *et al.* (2016). The quinone component was analysed as described by Komagata & Suzuki (1987). Fatty acid methyl esters were prepared according to the method described by the manufacturer's instuction of the Sherlock Microbial Identification System (MIDI) and analysed using gas chromatography and a HP computer with the MIDI database (Sasser, 1990; Kämpfer & Kroppenstedt, 1996). Polar lipids were extracted and determined by two-dimensional TLC following the procedure of Minnikin *et al.* (1984).

DNA of strain 201-F6^T was extracted from the cells grown on NBRC no. 802 agar for 18–24 h and purified by the method of Saito & Miura (1963). DNA base composition was determined by reversed-phase HPLC (Tamaoka & Komagata, 1984). DNA–DNA hybridization was conducted in multidilution-well plates as reported by Ezaki *et al.* (1989). 16S rRNA gene sequencing was carried out by using the primers 8-27F (5′-AGAGTTTGATCCTGGCTCAG-3′) and 1525R (5′-AAAGGAGGTGATCCAGCC-3′). The PCR product was purified and sequenced as described previously (Tanasupawat *et al.*, 2004). BLAST analysis for 16S rRNA gene sequences was done on the EzTaxon-e database (Kim *et al.*, 2012). The sequence was multiply aligned with the selected sequences of type strains obtained from GenBank/EMBL/DDBJ databases by using Clustal W version 1.81 (Thompson *et al.*, 1997). A phylogenetic tree was reconstructed using MEGA5.0 software (Tamura *et al.*, 2011), based on the neighbour-joining (Saitou & Nei, 1987), maximum-parsimony (Felsenstein, 1983) and maximum-likelihood (Felsenstein, 1981) methods. The *nifH* gene was amplified using the primers B1-112F (5′- GGCTGCGA TCCCAAGGCTGA-3′) (Bürgmann *et al.*, 2004) and CDHP 723R (5′-GATGTTCGCGCGGCACGAADTRNATSA-3′) (Steward *et al.*, 2004). The amplification was performed at 95 °C for 5 min, followed by 40 cycles at 95 °C for 30 s, 60 °C for 30 s and at 72 °C for 45 s (Noar & Buckley, 2009).

Cells of strain 201-F6^{T}were Gram-stain-negative, aerobic, non-spore forming rods (0.6–0.8×1.2–1.5 μm) and motile with a polar flagellum (Fig. 1). Colonies were 0.5–1.0 mm in diameter, circular, raised and translucent with entire margin and non-pigmented or cream after incubation at 30 °C on NBRC no. 802 agar medium for 2 days. Cytochrome oxidase and catalase were positive. Growth was

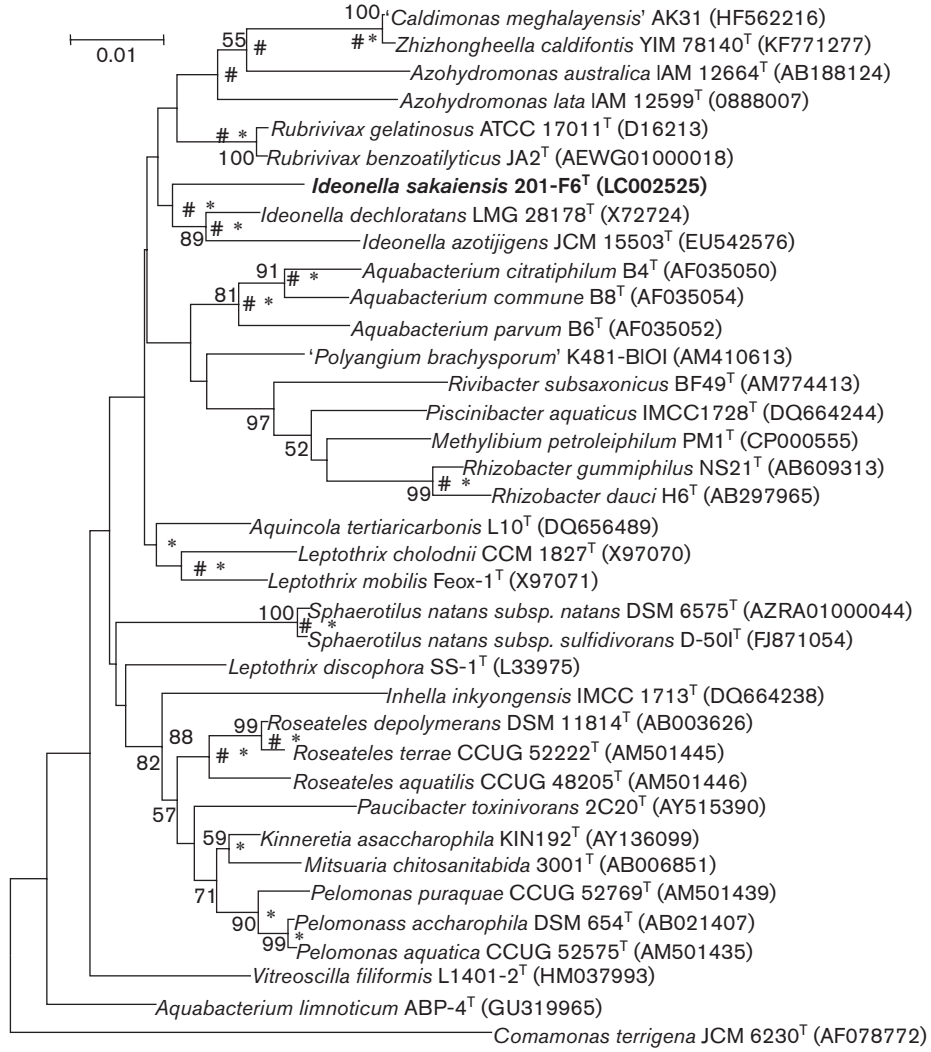

Fig. 2. Neighbour-joining tree based on an almost complete 16S rRNA gene sequence (1493 nt) showing the relationships among strain 201-F6^T and related type strains. *Comamonas terrigena* JCM 6230^T was used as an outgroup. Asterisks (*) and hashes (#) indicate that the corresponding nodes were also found in trees generated with the maximum-likelihood and maximum-parsimony algorithms, respectively. The numbers at branch nodes indicate bootstrap percentages derived from 1000 replications; only values >50 % are shown. Bar, 0.01 substitutions per single nucleotide position.

observed within the pH range 5.5–9 (optimally at pH 7–7.5) and at 15–42 °C (optimally at 30–37 °C), but not at 45 °C. Growth did not occur with 3 % (w/v) NaCl. The strain showed positive results for the assimilation of *N*-acetylglucosamine, maltose and potassium gluconate, and activity of leucine arylamidase, the same as *I. dechloratans* LMG 28178^T (=CCUG 30898^T) and *I. azotifigens* JCM 15503^T (=1a22^T=DSM 21438^T) but did not show capacities for either nitrate reduction or D-glucose assimilation, which was in contrast to the results for *I. dechloratans* LMG 28178^T (Malmqvist *et al.*, 1994). Strain 201-F6^T did not grow under anaerobic conditions with or without lighting. Under aerobic conditions, lighting did not affect the growth rate of strain 201-F6^T. Furthermore, strain 201-F6^T did not have any visual pigment that is usually observed in photosynthetic bacteria. Strain 201-F6^T contained ubiquinone with eight (Q-8) isoprene units as a major quinone (97.5 %) and a small amount of Q-9 (2.5 %). Q-8 as a major ubiquinone is the same as strain *I. dechloratans* LMG 28178^T (Table 1), as reported by Lechner *et al.* (2007). The DNA G+C content was 70.4 mol%. The predominant fatty acids were $C_{16:0}$ (37.0 %), $C_{17:0}$ cyclo (13.7 %), $C_{18:1}\omega7c$ (12.5 %) and $C_{12:0}$ 2-OH (5.9 %). Strain 201-F6^T, *I. dechloratans* LMG 28178^T and *I. azotifigens* JCM 15503^T showed resemblance to each other only with regard to the presence of $C_{16:0:0}$, $C_{18:1:1\omega7c}$, $C_{12:0:0}$ 2-OH and summed feature 3 fatty acids as major fatty acids, whereas distinct differences could be

Table 1. Differential characteristics of strain 201-F6^T and related species of the genus *Ideonella*

Strains: 1, 201-F6^T; 2, *I. dechloratans* LMG 28178^T; 3, *I. azotifigens* JCM 15503^T. All phenotypic characteristics were determined in this study. +, Positive; W, weakly positive; −, negative.

Characteristic	1	2	3
Temperature for growth (°C)	15–42	12–42	4–35
pH for growth	5.5–9	5–9	5.5–8
Nitrate reduction	−	+	−
Gelatin hydrolysis	−	+	+
PET hydrolysis	+	−	−
Assimilation of:			
D-Glucose	−	+	+
L-Arabinose	−	+	+
D-Mannose	−	+	−
D-Mannitol	−	+	+
Capric acid	−	+	−
Adipic acid	+	+	−
Malic acid	W	+	+
Trisodium citrate	W	+	−
Phenylacetic acid	−	+	−
Enzyme activities (API ZYM)			
Esterase lipase (C8)	+	W	W
Valine arylamidase	−	+	+
Cystine arylamidase	−	−	W
α-Chymotrypsin	−	−	W
Acid phosphatase	W	W	+
α-Glucosidase	+	W	+
Ubiquinone (%)			
Q-8	97.5	99.9	99
Q-9	2.5	0.1	1
DNA G+C content (mol%)	70.4	68.1	67.4
Isolation source	Microbial consortium No. 46*	Sewage water†	Soil‡

*Data from Yoshida *et al.* (2016).
†Data from Malmqvist *et al.* (1994).
‡Data from Noar & Buckley (2009).

observed in the presence and absence of $C_{10:0}$ 3-OH, $C_{12:0}$ 3-OH, $C_{14:0}$ 2-OH, iso-$C_{16:0}$, $C_{17:0}$ cyclo, $C_{19:0}$ cyclo ω8*c*, $C_{17:1}$ω8*c*, $C_{18:1}$ω9*c*, unknown 12.484, unknown 13.957, unknown 14502, summed feature 1 and summed feature 2 fatty acids as shown in Table 2. Phosphatidylethanolamine, *lyso*-phosphatidylethanolamine, phosphatidylglycerol and diphosphatidylglycerol, together with two unidentified phospholipids, two unidentified lipids, an unknown glycolipid and an unknown aminophospholipid were detected as polar lipids (Fig. S1, available in the online Supplementary Material). This was almost the same as in strain *I. dechloratans* LMG 28178^T, which contained phosphatidylethanolamine, *lyso*-phosphatidylethanolamine, phosphatidylglycerol, diphosphatidylglycerol, two unknown phospholipids and four unknown lipids (Fig. S2).

A comparison of the 16S rRNA gene sequence of strain 201-F6^T (1493 nt) with those of other members of the family *Comamonadaceae* placed this strain in a monophyletic cluster consisting of members of the genus *Ideonella*, based on the neighbour-joining, maximum-parsimony and maximum-likelihood methods (Fig. 2). Strain 201-F6^T exhibited 97.7 % similarity to *I. dechloratans* LMG 28178^T and 96.6 % to *I. azotifigens* JCM 15503^T. The DNA–DNA hybridization results indicated that strain 201-F6^T showed low DNA–DNA relatedness to *I. dechloratans* LMG 28178^T and *I. azotifigens* JCM 15503^T, with 21.6±1.5 % and 26.7 ±4.7 % relatedness, respectively, whereas strain *I. dechloratans* LMG 28178^T exhibited reciprocally low DNA–DNA relatedness to strain 201-F6^T (15.2±1.4 %) (Table S1). The DNA–DNA relatedness of strain 201-F6^T with its closest phylogenetic neighbours was well below the 70 % cut-off point recommended for the assignment of the strains to the same genomic species (Wayne *et al.*, 1987). On the basis of the above DNA–DNA relatedness data, strain 201-F6^T warrants identification as a representative of a separate species of the genus *Ideonella.* In addition, the *nifH* gene of this strain was not detected, in contrast to*I. dechloratans* LMG 28178^T and *I. azotifigens* JCM 15503^T as reported previously (Noar & Buckley, 2009). On the basis of its physiological, biochemical and phylogenetic data, including DNA–DNA relatedness, strain 201-F6^T was identified as a representative of a novel species in the genus *Ideonella*, for which the name *Ideonella sakaiensis* is proposed.

Description of *Ideonella sakaiensis* sp. nov.

Ideonella sakaiensis (sa.kai.en′sis. N.L. fem. adj. *sakaiensis* pertaining to Sakai city in Japan where the first strain was isolated).

Cells are Gram-stain-negative, aerobic, non-spore-forming rods (0.6–0.8×1.2–1.5 μm), motile with a polar flagellum (Fig. 1). Colonies are 0.5–1.0 mm in diameter, circular, raised, translucent with entire margin and non-pigmented or cream after incubation at 30 °C on NBRC no. 802 agar medium for 2 days. Cytochrome oxidase and catalase are positive. Growth is observed within the pH range 5.5–9 (optimum pH 7–7.5) and at 15–42 °C (optimum 30–37 °C). No growth at 45 °C or with 3 % NaCl. In the API 20 NE tests, positive for assimilation of *N*-acetyl glucosamine, maltose, potassium gluconate and adipic acid; weakly positive for assimilation of malic acid and citrate; negative for nitrate reduction, denitrification, indole formation, glucose fermentation, β-galactosidase, hydrolysis of arginine, gelatin, urea and aesculin, and assimilation of L-arabinose, D-glucose, D-mannose, capric acid, D-mannitol and phenylacetic acid. In the API ZYM system, positive for alkaline phosphatase, esterase lipase (C8), leucine

Table 2. Cellular fatty acids composition of strain 201-F6^T and related type strains

Strains: 1, 201-F6^T; 2, *I. dechloratans* LMG 28178^T; 3, *I. azotifigens* JCM 15503^T. All values were determined in this study. Values are percentages of total fatty acids. –, Not detected.

Fatty acid	1	2	3
$C_{12:0}$	1.5	2.3	5.4
$C_{14:0}$	0.6	1.8	0.8
$C_{16:0}$	37.0	28.2	22.6
$C_{17:0}$	0.2	0.3	2.9
$C_{18:0}$	0.4	0.5	0.4
$C_{10:0}$ 3-OH	–	3.9	–
$C_{12:0}$ 2-OH	5.9	3.6	2.7
$C_{12:0}$ 3-OH	–	5.2	3.5
$C_{14:0}$ 2-OH	–	2.4	–
iso-$C_{16:0}$	–	1.1	–
$C_{17:0}$ cyclo	13.7	0.6	–
$C_{19:0}$ cyclo $\omega 8c$	2.7	–	–
$C_{14:1}\omega 5c$	–	0.2	–
$C_{17:1}\omega 8c$	–	–	5.4
$C_{18:1}\omega 7c$	12.5	14.9	0.7
$C_{18:1}\omega 9c$	–	–	35.7
Unknown 12.484	–	–	0.6
Unknown 13.957	1.2	–	–
Unknown 14.502	0.8	–	–
Summed feature 1*	–	–	0.5
Summed feature 2†	10.8	–	3.3
Summed feature 3‡	12.7	35.1	15.4

*Summed feature 1 contained $C_{13:0}$ 3-OH/iso-$C_{15:1}$ I/H.
†Summed feature 2 contained iso-$C_{16:1}$ I/$C_{14:0}$ 3-OH.
‡Summed feature 3, $C_{16:1}\omega 7c$/iso-$C_{15:0}$ 2-OH.

arylamidase and α-glucosidase; weak enzymic activities for acid phosphatase and naphthol-AS-BI-phosphohydrolase; negative for lipase (C14), valine arylamidase, *N*-acetyl-β-glucosaminidase, β-glucosidase, α-chymotrypsin, cystine arylamidase, esterase (C4), α-fucosidase, β-glucuronidase, α-mannosidase, α-galactosidase, β-galactosidase and trypsin activities. Q-8 is the predominant ubiquinone. The predominant fatty acids are $C_{16:0}$, $C_{17:0}$ cyclo, $C_{18:1}$ $\omega 7c$ and $C_{12:0}$ 2-OH. Major polar lipids are phosphatidylethanolamine, *lyso*-phosphatidylethanolamine, phosphatidylglycerol and diphosphatidylglycerol.

The type strain is 201-F6^T (=NBRC 110686^T=TISTR 2288^T). The G+C content of genomic DNA of the type strain is 70.4 mol%.

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