Ideonella azotifigens sp. nov., an aerobic diazotroph of the *Betaproteobacteria* isolated from grass rhizosphere soil, and emended description of the genus *Ideonella*

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Strain 1a22^T, a nitrogen-fixing bacterium, was isolated from soil associated with the rhizosphere of a perennial grass growing in a fallow agricultural field in Ithaca, New York, USA. Analysis of the 16S rRNA gene sequence placed the strain in the *Rubrivivax–Roseateles–Leptothrix–Azohydromonas–Aquincola–Ideonella* branch of the *Betaproteobacteria* and the closest characterized relative was the type strain of *Ideonella dechloratans* (97.7 % 16S rRNA sequence similarity). Cells of strain 1a22^T were Gram-negative, motile, straight rods, which formed polyhydroxybutyrate-like granules and were positive for oxidase and weakly positive for catalase. Cells were chemo-organotrophic, unable to grow by reduction of chlorate or nitrate and grew exclusively through aerobic respiration. Growth with mannitol on N-free solid media caused the strain to produce copious amounts of slime. The G+C content of the genomic DNA was 67.4 mol%. The major cellular fatty acids were $C_{16:1}$ *cis*-9 and $C_{16:0}$ and cells contained significant amounts of the hydroxy fatty acids $C_{10:0}$ 3-OH, $C_{12:0}$ 2-OH and $C_{12:0}$ 3-OH. Based on DNA–DNA hybridization studies, 16S rRNA gene sequence analysis, fatty acid analysis, and morphological and physiological characteristics, strain 1a22^T represents a novel species in the genus *Ideonella*, for which the name *Ideonella azotifigens* sp. nov. is proposed. The type strain of *Ideonella azotifigens* is 1a22^T (=JCM 15503^T=DSM 21438^T).

The diversity of diazotrophs detected in the environment through recovery of *nifH* gene sequences vastly exceeds that of cultivated isolates such that nearly half of described *nifH* clusters do not contain even a single cultivated isolate (Zehr *et al.*, 2003). A novel approach for isolating diazotrophs was evaluated using plate-wash PCR (PW-PCR) screening of isolates for *nifH* as an alternative to selection on N-free media. PW-PCR has been used previously to isolate novel strains of '*Verrucomicrobia*' and '*Acidobacteria*' by screening with 16S rRNA gene primers specific for these groups (Stevenson *et al.*, 2004). An isolate obtained using this approach, strain 1a22^T, was identified as a novel diazotroph whose closest characterized relative was *Ideonella dechloratans*, a betaproteobacterium belonging to the *Rubrivivax–Roseateles–Leptothrix–Azohydromonas–Aquincola–Ideonella* branch of the *Burkholderiales*.

I. dechloratans is remarkable among related species for its ability to use chlorate as an electron acceptor for anaerobic respiration (Malmqvist *et al.*, 1994). The genera most closely related to *Ideonella* are *Aquincola* and *Rubrivivax*, species of which can be distinguished phenotypically from those of *Ideonella* by their respective abilities to use *tertiary* alkyl moieties such as 2-hydroxyisobutyric acid as a source of carbon and energy (Lechner *et al.*, 2007) and to grow phototrophically (Ramana *et al.*, 2006). The *Rubrivivax–Roseateles–Leptothrix–Azohydromonas–Aquincola–Ideonella* branch probably represents a recent phylogenetic radiation (Lechner *et al.*, 2007), as has been suggested for other lineages of aerobic proteobacteria (Stackebrandt, 2006). DNA–DNA hybridization values between strains of *Aquincola*, *Ideonella*, *Leptothrix* and *Rubrivivax* can be as high as 43–58 %, with that between the type strains of *I. dechloratans* and *Aquincola tertiaricarbonis* being 58 % (Lechner *et al.*, 2007). Nitrogen fixation has been documented in species of *Azohydromonas* and *Rubrivivax*, but has not been investigated previously in characterized strains of *Ideonella* or *Aquincola*. Based on phenotypic and molecular characterization, strain 1a22^T was determined to represent a novel species of the genus *Ideonella*.

In the current study, PW-PCR screening for *nifH* was used following partial enrichment in N-free media containing

Abbreviation: PW-PCR, plate-wash PCR.

The GenBank/EMBL/DDBJ accession number for the 16S rRNA and *nifH* gene sequences of *Ideonella azotifigens* strain 1a22^T are EU542576 and EU542577, respectively.

α-cellulose as sole carbon source. A 100 mg sample of soil obtained from grass rhizosphere (from a 30-year-old, well-drained fallow agricultural field on the Cornell campus, Ithaca, New York, USA) was used to inoculate 10 ml N-free dNfb broth [containing (l^{-1}): 0.5 g K_2HPO_4, 0.2 g $MgSO_4.7H_2O$, 0.1 g NaCl, 0.02 g $CaCl_2.2H_2O$, 2 ml minor element solution (containing, l^{-1}: 0.4 g $CuSO_4.5H_2O$, 0.12 g $ZnSO_4.7H_2O$, 1.4 g H_2BO_3, 1 g $Na_2MoO_4.2H_2O$ and 1.5 g $MnSO_4.H_2O$), 4 ml 1.64 % Fe.EDTA and 1 ml vitamin solution (containing, l^{-1}, 100 mg biotin and 200 mg pyridoxol.HCl), pH adjusted to 6.8 with KOH; adapted from Dobereiner *et al.* 1976)] containing 10 g α-cellulose l^{-1} and tubes were incubated (shaking at 200 r.p.m., 30 °C) for 1 month, by which time copious growth was observed. The enrichment was used to inoculate fresh media (1 : 100) and incubation continued for another month. This incomplete enrichment was followed by direct isolation of colonies on dilute nutrient broth agar [DNBA: 0.01 % (w/v) nutrient broth (Difco), 15 g agar l^{-1}]. Colonies formed on DNBA were arrayed onto fresh DNBA plates and screened by PW-PCR with primers that target the *nifH* gene [primer B1-112F (5′-GGCTGCGATCCCAAGGCTGA-3′) (Bürgmann *et al.*, 2004); primer CDHP 723R (5′-GATGTTCGCGCGGCA-CGAADTRNATSA-3′) (Steward *et al.*, 2004)]. PW-PCR was conducted as described previously (Stevenson *et al.*, 2004) with the exception that reactions were conducted in a volume of 50 µl as follows: 50 ng template DNA, 0.5 U AmpliTaq DNA polymerase with 1 × supplied reaction buffer (Applied Biosystems), 2.5 mM $MgCl_2$, 0.05 % BSA, 200 µM dNTPs and 0.25 µM of each primer. Tubes were held 5 min at 95.0 °C and reactions were performed for 40 cycles consisting of: 30 s at 95 °C, 30 s at 60 °C and 45 s at 72 °C. A total of 2 % of colonies screened in this way were positive for the presence of *nifH* and strain 1a22^T was selected for additional analyses.

Genomic DNA for use in genomic comparisons was obtained by using a standard phenol/chloroform extraction protocol (Sambrook & Russell, 2001). A nearly complete 16S rRNA gene sequence (1396 bp) was determined as described previously (Eden *et al.*, 1991). Partial *nifH* sequences were obtained for both strain 1a22^T (432 bp) and the type strain of *I. dechloratans* (535 bp; GenBank accession no. EU542578) using the primers and conditions described above for PW-PCR. Sequence assembly and alignment were performed in ARB (Strunk & Ludwig, 1997) and phylogenetic analysis was carried out with PHYLIP 3.64 (Felsenstein, 2005). DNA distance calculations were made using the F84 substitution model (Felsenstein & Churchill, 1996; Kishino & Hasegawa, 1989) implemented by DNADIST and, following translation, protein distance calculations were made using the amino acid substitution of Jones *et al.* (1992) implemented by PROTDIST (Jones *et al.*, 1992). Phylogenetic trees were constructed using maximum-likelihood and parsimony methods for either protein or DNA as appropriate with 100 bootstrap replications performed with jumbling of sequence input order.

Phylogenetic trees consistently demonstrated that strain 1a22^T formed a monophyletic group with the type strain of *I. dechloratans* and related strains of *Ideonella* that are as yet not formally characterized (Fig. 1). These analyses show that, although *Ideonella* and *Aquincola* are closely related genera, strain 1a22^T consistently associated with *I. dechloratans* ATCC 51718^T to the exclusion of *Aquincola tertiaricarbonis* L108 (Fig. 1). Phylogenetic analysis of the *nifH* gene placed strain 1a22^T with related genera in the *Burkholderiales* (Fig. 2) and identified the *nifH* gene found in the type strain of *I. dechloratans* as being most similar to that of strain 1a22^T (94.1 % nucleotide and 98.4 % amino acid similarities). Protein encoding genes from strains of a given species generally have a mean nucleotide similarily that is greater than 93–95 % (Konstantinidis & Tiedje, 2005). The next most similar *nifH* sequences were from the type strains of *Pelomonas saccharophila* (89.2 % nucleotide and 97.3 % amino acid similarities) and *Azohydromonas lata* (89.2 % nucleotide and 93.3 % amino acid similarities), which are both closely related to *Ideonella* based on 16S rRNA gene sequence analysis. It is interesting to note that while *Rubrivivax gelatinosus* is closely related to *Ideonella* based on 16S rRNA sequence similarity, its *nifH* gene is most similar to those found in the genus *Azospirillum* of the *Alphaproteobacteria.*

Strain 1a22^T was routinely grown aerobically at 30 °C in nutrient broth or on nutrient agar (Difco). Physiological tests for strain 1a22^T and *I. dechloratans* ATCC 51718^T (obtained from the American Type Culture Collection)

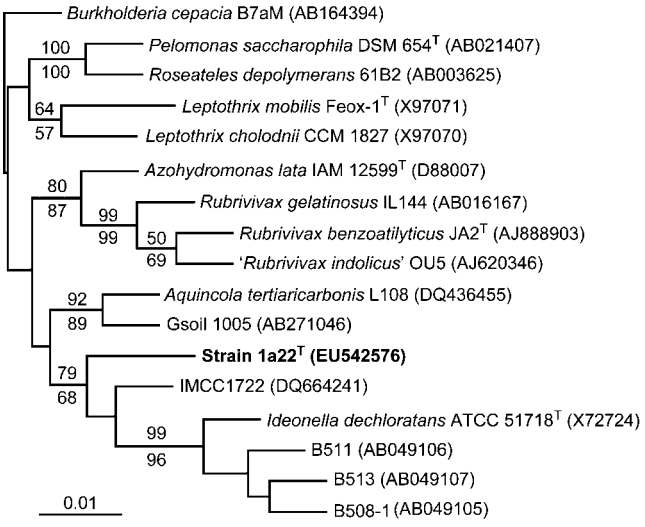

Fig. 1. Phylogenetic tree depicting relationships between 16S rRNA gene sequences from strain 1a22^T and closely related strains. The tree was generated by using maximum-likelihood analysis of 1321 nt positions. Numbers above and below the horizontal branches indicate bootstrap support generated from parsimony and maximum-likelihood analysis, respectively. Bootstrap values are omitted when both values were below 60. Bar, 0.01 changes per nucleotide.

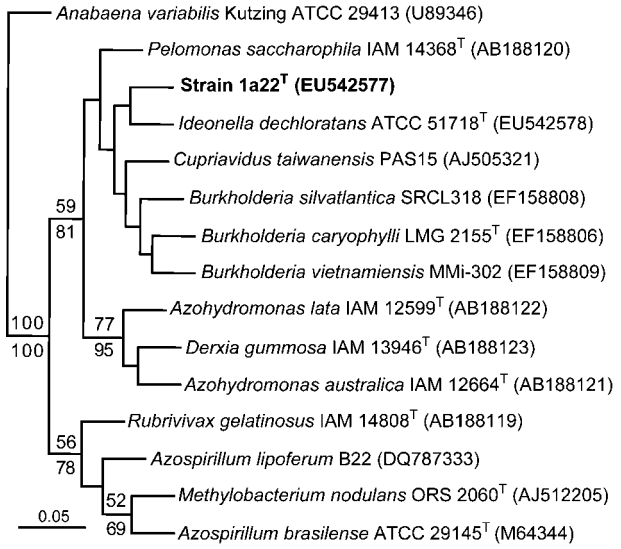

Fig. 2. Phylogenetic tree depicting relationships between *nifH* gene sequences from strain 1a22^T and closely related strains. The tree was generated by maximum-likelihood analysis of 100 amino acid positions. Numbers above and below the horizontal branches indicate bootstrap support generated from parsimony and maximum-likelihood analysis, respectively. Bootstrap values are omitted when both values were below 60. Bar, 0.05 changes per nucleotide.

were performed in dNfb liquid media supplemented with 0.25 g NH_4Cl l^{-1} and with 10 mM substrate unless otherwise noted. Growth was measured by changes in OD at 600 nm. Tests for cellular pigmentation, casein hydrolysis and lipase activity were performed as described by Tindall *et al.* (2007). Both strain 1a22^T and the type strain of *I. dechloratans* were also tested using API 20NE strips (bioMérieux), although growth-dependent tests on these strips gave inconsistent results for strain 1a22^T and were confirmed in dNfb. All tests were repeated a minimum of three times for both the type strain of *I. dechloratans* and strain 1a22^T unless otherwise noted. Results of genotypic and phenotypic tests are provided in the species description and in Table 1. The physiological and morphological characteristics of strain 1a22^T are generally consistent with those described for the genus *Ideonella* (Malmqvist *et al.*, 1994) with the exception of chlorate reduction, which is absent in strain 1a22^T.

Cellular fatty acid analysis was performed following growth on nutrient agar using the MIDI microbial identification system (Microbial ID). Cellular fatty acids for strain 1a22^T and the type strain of *I. dechloratans* are given in Table 2 and are consistent with prior observations (Lechner *et al.*, 2007). The diagnostic hydroxy fatty acids $C_{12:0}$ 2-OH and $C_{12:0}$ 3-OH were found in both strain 1a22^T and *I. dechloratans*, but not in species of related genera, supporting inclusion of strain 1a22^T in the genus *Ideonella*. However, the hydroxy fatty acid $C_{14:0}$ 2-OH, which is present in the type strain of *I. dechloratans*, was not present in strain 1a22^T or strains of related genera. In addition, the fatty acid $C_{17:0}$ cyclo was present in both strain 1a22^T and *Aquincola tertiaricarbonis*, but absent from the type strain of *I. dechloratans* and species of related genera. This evidence is consistent with data from 16S rRNA gene sequence analysis, which show that strain 1a22^T is closely related to *I. dechloratans* and occupies an intermediate position between this species and *Aquincola tertiaricarbonis*.

The genomic DNA G+C content of strain 1a22^T was determined by DNA melting temperature assessed in the presence of SYBR green dye in an iCycler iQ Detection System (Bio-Rad) as described by Gonzales & Saiz-Jimenez (2004) using the type strain of *I. dechloratans* as a reference species (Table 1). DNA–DNA relatedness was calculated by using the microplate hybridization method developed by Ezaki *et al.* (1989). The DNA–DNA relatedness of strain 1a22^T and the type strain of *I. dechloratans* was 56 %, thus supporting the creation of a novel species within the genus *Ideonella*.

Tests for growth in N-free media were conducted in both liquid and solid dNfb supplemented with 10 mM substrate. Growth of strain 1a22^T on N-free liquid media was inconsistent in the presence of air, but was stimulated in an atmosphere containing 1–5 % oxygen. Vigorous growth was obtained on dNfb media solidified with washed agar (15 g l^{-1}) and to which 10 mM carbon source (glucose, malate, mannitol or mannose) was added. In contrast, although the type strain of *I. dechloratans* was shown by PCR to possess a *nifH* gene, growth was not observed consistently on any N-free solid or liquid media and nitrogenase activity was not observed. Nitrogenase activity was assayed by acetylene reduction as described by Paerl (1998). Briefly, cultures were grown for 3 days on 10 ml dNfb slants (in 18 × 150 mm Balsch tubes) with mannitol (for strain 1a22^T) or glucose (for *I. dechloratans*), after which ethylene production in the presence of 10 % acetylene was measured with a Shimadzu GC-MS QP2010S equipped with a carboxen 1010 PLOT column. Acetylene reduction was standardized to measurements of total protein made through use of the Bradford assay. Strain 1a22^T reduced acetylene to ethylene at 42.2 ± 7.5 μmol (mg protein)$^{-1}$ h^{-1} [corresponding to an N_2 fixation rate of 10.6 ± 1.9 μmol (mg protein)$^{-1}$ h^{-1}, given a 4 : 1 ratio for ethylene : N_2 (Postgate, 1982)], a rate that is somewhat higher than those observed for other obligately aerobic diazotrophs such as *Azotobacter vinelandii* [6.7 ± 0.4 μmol (mg protein)$^{-1}$ h^{-1}; (Linkerhagner & Oleze, 1997)], *Azospirillum brasilense* [5 μmol (mg protein)$^{-1}$ h^{-1}; (Nelson & Knowles, 1978)] and *Azospirillum lipoferum* [2.68 ± 0.25 μmol (mg protein)$^{-1}$ h^{-1}; (Haahtela *et al.*, 1982)].

Several strains that tentatively belong to the genus *Ideonella* on the basis of 16S rRNA gene sequence similarity have been isolated from soils. Strain 0-0013 was isolated from soil on media containing the quorum-sensing signal molecule 3-hydroxypalmitic acid methyl ester as the sole

Table 1. Phenotypic and genotypic properties that differentiate strain 1a22^T (*I. azotifigens* sp. nov.) from the type strains of *I. dechloratans* and species of related genera

Strains: 1, 1a22^T; 2, *I. dechloratans* ATCC 51718^T; 3, *Aquincola tertiaricarbonis* DSM 18512^T; 4, *Rubrivivax gelatinosus* ATCC 17011^T; 5, *Leptothrix discophora* ATCC 43182^T; 6, *Roseateles depolymerans* DSM 11813^T. Results for strain 1a22^T and *I. dechloratans* were either determined or independently confirmed in this study. Results not determined in this study are from Lechner *et al.* (2007), Suyama *et al.* (1999), Ramana *et al.* (2006), Malmqvist *et al.* (1994) and Amakata *et al.* (2005). NA, Not available.

Characteristic	1	2	3	4	5	6
16S rRNA gene similarity (%)*	(100)	97.7	96.8	96.3	95.9	94.6
DNA G+C content (mol%)	67	68	69–71	71–75	71	66
Nitrogenase activity	+	−	NA	+	NA	NA
Sheath formation	−	−	−	−	+	−
Bacteriochlorophyll *a*	−	−	−	+	−	+
pH for growth	6–8	5–9	6–8	NA	NA	5–8
Temperature range for growth (°C)	4–35	12–42	4–40	35†	15–33	5–43
Enzyme activities						
Catalase	+	+	+	+	−	−
Urease	−	+	−	−	NA	−
Arginine dihydrolase	−	+	−	−	NA	NA
Nitrate respiration	−	+	−	−	−	−
Chlorate respiration	−	+	−	NA	NA	NA
Carbon source utilization						
2-Hydroxyisobutyrate	−	−	+	−	NA	NA
Cellobiose	+	−	NA	NA	NA	NA
Mannitol	+	−	+	−	NA	+
Mannose	+	+	+	−	NA	NA
N-Acetylglucosamine	+	−	+	−	−	−
Isolation source	Soil	Sludge	Aquifer	Mud	Water	Water

*Similarities between strain 1a22^T and each type strain.
†Optimum temperature for growth.

carbon source and was shown to degrade this compound (Shinohara *et al.*, 2007). Three strains, B508-1, B511 and B513, isolated from the stems of cultivated rice plants on an N-free medium containing mannitol, sucrose, lactate and malate, have been shown to reduce acetylene and have been proposed as possible endophytes (Elbeltagy *et al.*, 2001). All three strains were motile and degraded pectin and two strains could degrade cellulose (Elbeltagy *et al.*, 2001). Based on 16S rRNA gene sequence analyses, strains B508-1, B511 and B513 are tightly associated with the type strain of *I. dechloratans* (Fig. 1). Another strain tentatively identified as a member of *Ideonella* is strain Long 7, which has been shown to possess a *nifH* gene (GenBank accession no. AY231580). Analysis of the *nifH* gene from *Ideonella* strain Long 7 shows that it is more similar to genes from the type strain of *I. dechloratans* (96.9 % nucleotide and 98.7 % amino acid similarities) and strain 1a22^T (92.2 % nucleotide and 98.7 % amino acid similarities) than to any other known *nifH* genes. This strain is identified as being isolated from roots of pot-grown rice, but no other information or characterization is currently available in the literature. Finally, a recent survey of *nifH* diversity in maize rhizosphere soil has identified a number of *Ideonella*-like *nifH* gene sequences (Roesch *et al.*, 2008). *Ideonella*-like *nifH* gene sequences were the most abundant sequences detected within surface-sterilized maize stems (27 % of the total recovered) and represented 11 % of the *nifH* genes recovered from maize rhizosphere soil. Gene sequences from other suspected endophytes such as *Azospirillum* and *Herbaspirillum* were also abundant in surface-sterilized maize stems (at 20 % and 7.4 % of the total, respectively). These data suggest that *Ideonella* strains are widespread in soils, commonly contain *nifH* genes and should be investigated as potential endophytes of graminaceous plants.

Although 16S rRNA and *nifH* gene sequences, genomic DNA G+C content, fatty acid analysis and DNA–DNA hybridization values all suggest a close relationship between the type strain of *I. dechloratans* and strain 1a22^T, there are substantial differences between these strains to justify the creation of a novel species (Table 1). Most notable is the inability of strain 1a22^T to grow anaerobically with nitrate or chlorate as electron acceptor. It is also interesting to note the relationship of the strains to nitrogen. Strains 1a22^T, B508-1, B511 and B513 and the type strain of *Rubrivivax gelatinosus* all fix dinitrogen and these and strains of related genera lack both urease and arginine dihydrolase activities, suggesting that these organisms are adapted to environments where organic nitrogen sources are somewhat uncommon. In contrast, urease and arginine

Table 2. Fatty acid composition of strain 1a22^T (*I. azotifigens* sp. nov.) and related strains

Strains: 1, 1a22^T (data from this study); 2, *I. dechloratans* ATCC 51718^T (this study); 3, *Aquincola tertiaricarbonis* DSM 18512^T (Lechner *et al.*, 2007). Values are percentages of the total fatty acids. Empty cells, fatty acid not detected.

Fatty acid	1	2*	3
$C_{16:1}$ *cis*-9	33.1	39–40	39
$C_{16:0}$	27.3	25–37	37
$C_{12:0}$	10.4	1–4	4
$C_{18:1}$	9.4	6–19	6
$C_{10:0}$ 3-OH	4.1	2.0–2.4	2
$C_{12:0}$ 3-OH	6.7	4	
$C_{12:0}$ 2-OH	1.1	2–3	
$C_{14:1}$ *cis*-5	0.4	0–0.4	
$C_{17:0}$ cyclo	4.0		2
$C_{18:0}$	0.5		1
$C_{14:0}$		1–2	2
$C_{17:0}$		0.4–0.5	2
$C_{16:0}$ N alcohol	0.5		
$C_{14:0}$ 2-OH		2–3	
$C_{15:1}$ *cis*-6		0–0.7	
$C_{17:1}$ *cis*-6		0–0.6	
$C_{15:0}$			3
$C_{15:1}$			2

*Trace amounts (<0.25 %) of the fatty acids $C_{18:0}$, $C_{16:1}$ *cis*-5, $C_{17:1}$ *cis*-8, $C_{11:0}$ 3-OH and iso-$C_{16:0}$ were detected in the type strain of *I. dechloratans*.

dihydrolase activities are present in the type strain of *I. dechloratans* and, although this organism has a *nifH* gene, it appears unable to grow in media lacking nitrogen. Broadly interpreted, this evidence suggests that the type strain of *I. dechloratans*, which was isolated from sludge, has become adapted to growth in nutrient-rich and frequently anaerobic environments, whereas closely related ancestral species are uniformly restricted to aerobic growth and are commonly found in soils. Species of the genus *Ideonella* can be resolved from related genera on the basis of 16S rRNA gene sequence analysis and by the presence of the hydroxy fatty acids $C_{12:0}$ 2-OH and $C_{12:0}$ 3-OH. In addition, species of *Ideonella* can be further resolved from species of *Leptothrix* on the basis of sheath formation and iron oxidation, from species of *Aquincola* on the basis of growth with 2-hydroxyisobutyric acid and from species of *Rubrivivax* by the presence of bacteriochlorophyll *a* and phototrophic growth (Table 1).

Emended description of the genus *Ideonella* Malmqvist, Welander, Moore, Ternstrom, Molin and Stenstrom 1994

According to the original description, members of the genus are able to grow anaerobically by reducing chlorate to chloride. However, *Ideonella azotifigens* 1a22^T is unable to grow via reduction of chlorate. As a result, chlorate reduction is not a distinguishing characteristic of the genus. In addition, the cellular fatty acids of *Ideonella* were not given in the original genus description. Major cellular fatty acids are $C_{16:1}$ *cis*-9 and $C_{16:0}$. The hydroxy fatty acids include $C_{10:0}$ 3-OH, $C_{12:0}$ 2-OH and $C_{12:0}$ 3-OH, with the latter two fatty acids being diagnostic for *Ideonella* among species of related genera. Other characteristics are consistent with the original description of Malmqvist *et al.* (1994). The type species is *Ideonella dechloratans*.

Description of *Ideonella azotifigens* sp. nov.

Ideonella azotifigens [a.zo.ti.fi′gens. N.L. n. *azotum* (from french noun *azote*) nitrogen; L. v. *figere* to fix, attach; N.L. part. adj. *azotifigens* nitrogen-fixing].

Cells are motile, Gram-negative, rod-shaped, 0.5–1.0 μm in width and 2–6 μm in length and contain polyhydroxybutyrate-like granules when grown on dNfb media with mannitol. Cells grown in nutrient broth are generally smaller (0.5 × 2.0 μm) and do not contain granules. Able to fix nitrogen. Colonies are 1 mm diameter, generally round, convex with entire margins, mucoid, shiny and cloudy to opaque when grown on N-free media with mannitol at 30 °C for 2 days. Colonies continue to grow on prolonged incubation (1 week or more), spreading, merging together and producing prodigious amounts of slime which drips off the surface of inverted plates. Growth occurs at 4 and 35 °C, but not at temperatures higher than 35 °C. Optimum pH for growth is between 6 and 7. NaCl is not required for growth and growth does not occur at NaCl concentrations of 0.5 % or greater. Positive for oxidase and gelatinase and weakly catalase-positive. Does not produce urease, arginine dihydrolase, indole or dihydroxyacetone. Growth is strictly aerobic and neither chlorate nor nitrate can be reduced. Compounds that can be used as sole carbon sources for growth include acetate, cellobiose, glucose, malate, mannitol, mannose and *N*-acetylglucosamine, but not cellulose or 2-hydroxyisobutyrate. Amino acids that can be used as sole carbon sources for growth include glutamic acid, but not arginine or glycine. Lipolytic against Tweens 20, 40, 60, 80 and 85. Proteinolytic against casein.

The type strain is 1a22^T (=JCM 15503^T=DSM 21438^T), isolated from soil in association with the rhizosphere of a perennial grass. The genomic DNA G+C content of the type strain is 67.4 mol%.

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