Acidovorax radicis sp. nov., a wheat-root-colonizing bacterium

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Strain N35^T was isolated from surface-sterilized wheat roots and is a Gram-negative, aerobic, motile straight rod. Strain N35^T tested oxidase-positive and catalase-negative and grew optimally at pH 7.0, 30 °C and in the absence of NaCl. 16S rRNA gene sequence analysis showed over 97 % sequence similarity to strains of the environmental species *Acidovorax delafieldii*, *A. facilis*, *A. defluvii*, *A. temperans*, *A. caeni* and *A. soli*, as well as *Acidovorax valerianellae*, *A. anthurii* and *Simplicispira metamorpha*. DNA–DNA hybridization between strain N35^T and phylogenetically closely related type strains was 25.3–55.7 %, which clearly separates the strain from these closely related species. Additionally, phenotypic properties, such as substrate metabolism profiles as determined by a Biolog GN2 assay and cell-wall fatty acid profiles, particularly contents of the fatty acids $C_{16:0}$, $C_{16:1}\omega7c/t$, $C_{17:0}$, $C_{17:0}$ cyclo, $C_{18:0}$ cyclo and $C_{19:0}$ cyclo, facilitated the differentiation of the newly isolated strain N35^T from its closest relatives. The isolate underwent phenotypic variation at high frequency in laboratory media. The DNA G+C content was 64.9 mol%. We propose that strain N35^T is classified as a representative of a novel species within the genus *Acidovorax,* and suggest the name *Acidovorax radicis* sp. nov. The type strain is strain N35^T (=DSM 23535^T =LMG 25767^T).

On the basis of 16S rRNA gene sequence analysis, the genus *Acidovorax* belongs to the class *Betaproteobacteria*, family *Comamonadaceae* (Willems & Gillis, 2005). At the time of writing, the genus *Acidovorax* contained 13 recognized species. Species have been isolated from soil and water: *Acidovorax facilis* (the type species), *A. delafieldii*, *A. temperans* (Willems *et al.*, 1990), *A. defluvii* (Schulze *et al.*, 1999), *A. caeni* (Heylen *et al.*, 2008) and *A. soli* (Choi *et al.*, 2010). The genus also includes opportunistic pathogens of clinical origin, as well as the phytopathogenic species *Acidovorax konjaci* (Willems *et al.*, 1992), *A. avenae*, *A. cattleyae*, *A. citrulli*, *A. oryzae* (Schaad *et al.*, 2008), *A. anthurii* (Gardan *et al.*, 2000) and *A. valerianellae* (Gardan *et al.*, 2003).

In this communication, we characterize the rhizosphere bacterial strain N35^T, which was isolated from surface-sterilized wheat roots (*Triticum aestivum*). On the basis of 16S rRNA gene sequence data, DNA–DNA hybridization results, phenotypic features, chemotaxonomic characteristics and other properties, we conclude that the isolate belongs to a novel species within the genus *Acidovorax*.

Strain N35^T was isolated from 4-week-old wheat plants (*T. aestivum* 'Naxos') grown in agricultural soil from Neumarkt (Germany). Surface sterilization of washed roots was carried out with 1 % (w/v) chloramine T for 10 min. Subsequently, the roots were washed three times with 1 × PBS, crushed and plated on NB (nutrient broth no. 4; Fluka) agar to obtain single colonies. In further ecological studies, we showed by using microscopy techniques that this bacterium is truly able to colonize roots endophytically. The bacterium had no plant-pathogenic capacity and, on the contrary, showed plant-growth-promoting properties (D. Li and others, unpublished results).

Reference strains were obtained from the Deutsche Sammlung von Mikroorganismen und Zellkulturen (DSMZ, Braunschweig, Germany).

Optimal culture conditions for strain N35^T were determined in NB medium for 3 days at different temperatures (4, 10, 20, 30, 35 and 37 °C), pH (4.0–10.0 at 30 °C) and NaCl concentrations (0–3 % NaCl, w/v, at 30 °C). Isolate N35^T grew at 10–35 °C, pH 5.0–9.0 and 0–2 % NaCl.

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The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain N35^T is HM027578.

Optimal growth of strain N35^T was observed at 30 °C and pH 7.0 without added NaCl.

Two different colony types could be observed: one showed characteristic rough colonies on NB agar plates and formed flocks in liquid NB medium, while the other grew as smooth colonies and without flocculation in liquid medium. The rough colony type could switch to the smooth type at a rate of about 5 % of total colony counts when plated on NB agar plates. However, no reversion to the rough colony type could be detected. Because of these observations, N35^T was considered to display phenotypic variation, with the rough colony type being the wild-type and the smooth colony type being a phenotypic variation thereof, named N35^{T}v.

For both growth types, motility was determined by examining 24-h cultures in 1× PBS using an Axioplan 2 epifluorescence microscope (Zeiss). To analyse cell morphology and size, scanning electron microscopy was used (JSM 6300F; JEOL). Briefly, cells were harvested from 1 ml culture grown overnight in NB medium and washed twice with 1× PBS. The cells were then fixed with 2 ml 1 % glutaraldehyde in PBS (pH 7.4) overnight at 4 °C. The cells were dehydrated through a series of ethanol solutions with increasing concentrations (50, 70, 80, 95 and 100 % ethanol). Liquid CO_2 was used to replace the ethanol, and the cells were dried in a critical-point drier. The cells were sputter-coated with platinum and observed in the scanning electron microscope. Cells of the wild-type (N35^T) were characterized by one polar flagellum and rapid movement in 1× PBS, while cells of the phenotypic variant (N35^{T}v) had lost their flagella (Fig. 1) and were not able to swim.

Oxidase activity of strain N35^T was determined by oxidation of 1 % *N*,*N*-dimethyl-*p*-phenylenediamine hemioxalate. Catalase activity was measured by bubble formation in 3 % (v/v) H_2O_2, protease activity by hydrolysis of 1 % (w/v) casein hydrolysate and 2 % (w/v) skimmed milk and lipase activity by the formation of a halo on 1 % (v/v) glyceryl tributyrate agar plates. Siderophore production was tested on CAS agar plates as described by Schwyn & Neilands (1987). Metabolism of various substrates (Table 1) was determined in at least three replicates with GN2 MicroPlates (Biolog) using the standard protocol of the manufacturer. The only difference between the wild-type and variant was that N35^T could readily metabolize L-fucose and formic acid, whereas N35^{T}v lost the ability to utilize these two substrates. However, both forms could clearly be distinguished from phylogenetically closely related type strains of *Acidovorax* species by the utilization of various substrates (Table 1).

The whole genomes of strains N35^T and N35^{T}v were sequenced with a 454 pyrosequencer (Roche) using GS FLX Titanium chemistry and the software package GS FLX 2.0.01 (Roche) for assembly. The average reading coverage for both colony types was 40-fold and the genome size of both types was predicted to be about 5.5 Mb. For determination of the DNA G+C content, a DNA statistics

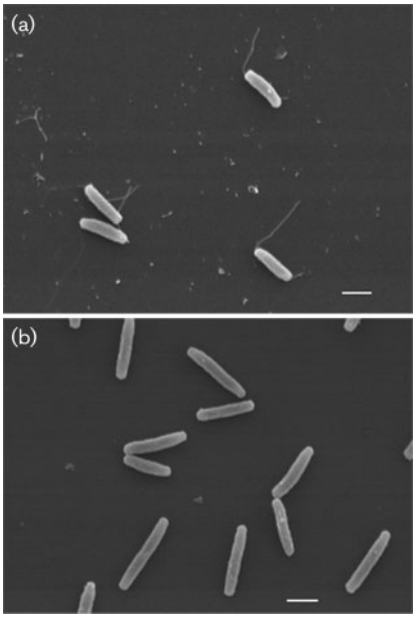

Fig. 1. Scanning electron micrographs of cells of strain N35^T and phenotypic variant N35^{T}v. In (a), single polar flagella can be seen for cells of strain N35^T, while, in (b), cells of the phenotypic variant N35^{T}v have no flagella. Bars, 1 μm.

calculation function of the software Lasergene 7.1.0 (DNASTAR) was used with the whole genome sequence data. The G+C content of genomic DNA of strain N35^T was 64.9 mol%. 16S rRNA gene sequences were also taken from this genomic data and analysed using the ARB software package version 5.1 (Ludwig *et al.*, 2004). Phylogenetic trees were reconstructed using the neighbour-joining (Saitou & Nei, 1987), maximum-likelihood (Olsen *et al.*, 1994) and maximum-parsimony (Felsenstein, 1981) algorithms. The neighbour-joining method is quite efficient for producing a single parsimonious tree and gives more reliable evolutionary distance data in comparison with maximum-parsimony (Saitou & Nei, 1987). In this study, the neighbour-joining tree was selected for illustration and is shown in Fig. 2.

The nucleotide sequences of the 16S rRNA genes of N35^T and N35^{T}v were 100 % identical and showed 97.2–99.0 % similarity to sequences from other environmental *Acidovorax* species (Fig. 2). The closest phylogenetic neighbour of strain N35^T was *A. defluvii* BSB411^T (Schulze *et al.*, 1999), with a sequence similarity of 99.0 %; other close relatives were *A. facilis* CCUG 2113^T (98.5 %), *A. delafieldii* ATCC 17505^T (98.2 %), *A. temperans* CCUG 11779^T (98.1 %; Willems *et al.*, 1990), *A. soli* BL21^T (97.6 %; Choi *et al.*, 2010), *A. caeni* R-24608^T (97.2 %; Heylen *et al.*, 2008), *A. anthurii* CFBP 3232^T (97.1 %; Gardan *et al.*, 2000), *A. valerianellae* CFBP 4730^T (97.1 %; Gardan *et al.*, 2003) and *Simplicispira metamorpha* DSM 1837^T (97.1 %; Grabovich *et al.*, 2006). As Ludwig *et al.* (1998) stated that a value of 16S rRNA gene sequence

Table 1. Utilization of different carbon sources in the Biolog GN2 microplate by strain N35^T, phenotypic variant N35^{T}v and type strains of phylogenetically closely related species

Strains: 1, *Acidovorax radicis* sp. nov. N35^T; 2, *A. radicis* sp. nov. N35^{T}v (phenotypic variant); 3, *A. defluvii* DSM 12644^T; 4, *A. facilis* DSM 649^T; 5, *A. delafieldii* DSM 64^T; 6, *A. temperans* DSM 7270^T; 7, *A. caeni* DSM 19327^T; 8, *A. soli* KCTC 22399^T; 9, *A. valerianellae* DSM 16619^T; 10, *A. anthurii* DSM 16745^T; 11, *Simplicispira metamorpha* DSM 1837^T. All of the tested strains were examined under the same experimental conditions with at least three replicates. +, Positive; −, negative; w, weakly positive. All strains were positive for utilization of Tweens 40 and 80, methyl pyruvate, β-hydroxybutyric acid and DL-lactic acid. All strains were negative for the negative control (water) and utilization of α-cyclodextrin, glycogen, *N*-acetyl-D-galactosamine, adonitol, cellobiose, i-erythritol, gentiobiose, *myo*-inositol, lactulose, melibiose, methyl β-D-glucoside, raffinose, L-rhamnose, citric acid, D-galactonic acid lactone, D-glucosaminic acid, D-glucuronic acid, itaconic acid, D-saccharic acid, glucuronamide, uridine, phenylethylamine, putrescine, 2-aminoethanol and glucose 1-phosphate.

Carbon source	1	2	3	4	5	6	7	8	9	10	11
Dextrin	−	−	+	−	−	−	−	+	−	−	−
N-Acetyl-D-glucosamine	−	−	−	+	−	−	−	−	−	−	−
L-Arabinose	+	+	+	+	+	−	+	−	−	−	−
D-Arabitol	+	+	−	+	+	+	+	−	−	−	−
D-Fructose	+	+	−	+	+	+	+	−	+	+	−
L-Fucose	+	−	−	−	−	−	−	−	−	−	−
D-Galactose	+	+	+	+	+	−	+	−	−	−	−
α-D-Glucose	+	+	−	+	+	+	−	+	−	+	−
Lactose	−	−	−	−	−	−	−	−	−	+	−
Maltose	−	−	−	−	−	−	−	−	−	+	−
D-Mannitol	+	+	+	+	+	+	+	−	−	+	−
D-Mannose	+	+	w	+	+	−	−	−	−	−	−
D-Psicose	+	+	−	+	+	+	−	−	−	−	−
D-Sorbitol	+	+	−	+	+	+	−	−	−	−	−
Sucrose	−	−	+	−	−	−	−	−	−	+	−
Trehalose	−	−	−	−	−	−	−	−	−	+	−
Turanose	−	−	+	−	−	−	−	−	−	+	−
Xylitol	−	−	−	−	−	−	−	−	−	+	−
Monomethyl succinate	+	+	+	+	+	+	+	+	+	−	+
Acetic acid	+	+	−	−	+	+	+	−	+	−	−
cis-Aconitic acid	−	−	−	−	+	−	+	+	−	−	−
Formic acid	+	−	−	−	+	−	−	−	−	−	−
D-Galacturonic acid	−	−	+	−	−	−	−	−	−	−	−
D-Gluconic acid	+	+	−	−	+	−	+	−	+	+	−
α-Hydroxybutyric acid	+	+	+	−	+	+	−	−	w	−	−
γ-Hydroxybutyric acid	−	−	+	+	−	+	−	+	−	−	−
p-Hydroxyphenylacetic acid	+	+	−	−	+	−	w	−	w	−	w
α-Ketobutyric acid	+	+	+	+	+	+	−	−	−	−	+
α-Ketoglutaric acid	−	−	+	w	w	−	−	w	−	−	−
α-Ketovaleric acid	−	−	+	+	−	+	w	−	+	−	−
Malonic acid	−	−	−	w	−	−	+	−	−	−	−
Propionic acid	+	+	−	−	+	+	−	−	+	−	+
Quinic acid	+	+	−	−	+	−	−	−	−	−	−
Sebacic acid	+	w	+	w	+	+	+	−	+	−	+
Succinic acid	+	+	−	w	+	+	+	w	+	+	−
Bromosuccinic acid	+	+	−	+	+	+	+	−	+	−	−
Succinamic acid	+	w	+	+	+	+	+	−	+	−	−
L-Alaninamide	−	−	+	−	w	−	+	−	−	−	−
D-Alanine	+	+	−	w	w	−	+	−	−	−	−
L-Alanine	+	+	+	w	+	−	w	+	−	+	−
L-Alanyl glycine	w	w	−	+	+	−	−	+	−	w	−
L-Asparagine	+	+	w	−	+	+	+	+	+	−	−
L-Aspartic acid	+	+	w	−	+	+	+	+	+	+	−
L-Glutamic acid	+	+	+	−	+	w	+	+	+	+	+
Glycyl L-aspartic acid	−	−	−	+	−	−	−	−	−	+	−
Glycyl L-glutamic acid	+	+	−	−	+	−	−	−	−	+	−

Table 1. cont.

Carbon source	1	2	3	4	5	6	7	8	9	10	11
L-Histidine	+	+	−	−	+	−	+	−	w	−	−
Hydroxy-L-proline	+	+	−	−	+	−	−	−	−	−	−
L-Leucine	+	+	−	w	+	w	+	−	+	−	−
L-Ornithine	−	−	+	−	−	−	−	−	−	−	−
L-Phenylalanine	+	+	−	+	+	+	+	−	+	−	−
L-Proline	+	+	+	+	+	+	+	+	+	+	−
L-Pyroglutamic acid	−	−	−	+	+	+	+	+	+	+	−
D-Serine	−	−	−	−	−	−	w	−	−	−	−
L-Serine	−	−	+	+	+	−	+	−	+	−	−
L-Threonine	+	+	−	−	+	−	−	w	−	−	−
DL-Carnitine	−	−	−	+	−	−	−	−	−	−	−
γ-Aminobutyric acid	+	+	−	+	+	−	+	w	−	−	−
Urocanic acid	+	+	−	−	+	+	+	−	+	−	−
Inosine	−	−	−	−	−	−	−	−	−	+	−
Thymidine	−	−	−	−	−	−	−	−	−	+	−
2,3-Butanediol	−	−	+	−	−	−	−	−	−	−	−
Glycerol	+	+	+	+	+	+	+	−	+	+	−
DL-α-Glycerol phosphate	−	−	w	−	w	w	−	−	−	−	+
Glucose 6-phosphate	−	−	−	−	+	−	−	−	−	−	−

similarity of 95 % or more indicated membership of the same genus, these results provide evidence that isolate N35 belongs to the genus *Acidovorax*. However, 16S rRNA gene sequences could not provide sufficient resolution at the species level (Rosselló-Mora & Amann, 2001). Thus, we compared DNA–DNA relatedness between strain N35^T, variant N35^{T}v and the phylogenetically closely related type strains *A. defluvii* DSM 12644^T, *A. facilis* DSM 649^T, *A. delafieldii* DSM 64^T, *A. temperans* DSM 7270^T, *A. caeni* DSM 19327^T, *A. soli* KCTC 22399^T, *A. valerianellae* DSM 16619^T, *A. anthurii* DSM 16745^T and *S. metamorpha* DSM 1837^T.

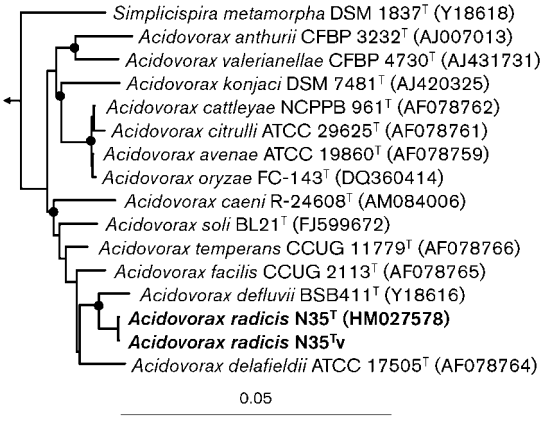

Fig. 2. Neighbour-joining phylogenetic tree based on 16S rRNA gene sequence data. GenBank accession numbers for sequences are given in parentheses. The tree includes all members of the genus *Acidovorax* with validly published names as well as *Simplicispira metamorpha*. Filled circles indicate that the corresponding nodes were also recovered in the maximum-likelihood tree. Bar, 5 % sequence divergence.

For DNA–DNA hybridizations, about 3 g wet cell material of all 11 tested strains was grown in NB medium, harvested and washed twice with 1× PBS. Finally, the cells were suspended in 10 ml 50 % 2-propanol. All the other steps were accomplished by the DSMZ as described in the protocols of Cashion *et al.* (1977) and De Ley *et al.* (1970) and the modified protocol of Huß *et al.* (1983). DNA–DNA hybridization between N35^T and N35^{T}v was 100 %. According to 16S rRNA gene sequence data and DNA–DNA hybridization, the two phenotypes were genetically identical. These results confirmed that N35^{T}v was not a different strain, but instead a phenotypic variation of the wild-type N35^T. The mechanism behind this phenotypic variation is not yet known. The values for DNA–DNA hybridization with strain N35^T were 24.8 % for *A. caeni* DSM 19327^T, 25.3 % for *A. defluvii* DSM 12644^T, 25.7 % for *A. valerianellae* DSM 16619^T, 25.9 % for *A. anthurii* DSM 16745^T, 39.0 % for *S. metamorpha* DSM 1837^T, 40.3 % for *A. delafieldii* DSM 64^T, 42.9 % for *A. soli* KCTC 22399^T, 45.9 % for *A. temperans* DSM 7270^T and 55.7 % for *A. facilis* DSM 649^T. According to the accepted criterion for species definition (Wayne *et al.*, 1987), values of over 70 % in DNA–DNA hybridizations are characteristic of members of the same species. Therefore, isolate N35^T belongs to none of the nine species compared.

Fatty acid analysis of all 11 tested strains was carried out using a procedure described by Zelles (1997). All fractions were analysed on a GC/MS (Trace MS; Thermo Finnigan) according to Zelles (1999). Fatty acids were identified using FAME and BAME standards (Supelco) as well as established fatty acid libraries (CH 6500 Solvit). The

Table 2. Fatty acid compositions of strain N35^T and type strains of phylogenetically closely related species

Strains: 1, *A. radicis* sp. nov. N35^T; 2, *A. radicis* sp. nov. N35^{T}v (phenotypic variant); 3, *A. defluvii* DSM 12644^T; 4, *A. facilis* DSM 649^T; 5, *A. delafieldii* DSM 64^T; 6, *A. temperans* DSM 7270^T; 7, *A. caeni* DSM 19327^T; 8, *A. soli* KCTC 22399^T; 9, *A. valerianellae* DSM 16619^T; 10, *A. anthurii* DSM 16745^T; 11, *S. metamorpha* DSM 1837^T. All data were obtained in this study under the same experimental conditions. Values are means ± ranges of three measurements, given as mol%. ND, Not detected; tr, trace component (<0.1 %).

Fatty acid	1	2	3	4	5	6	7	8	9	10	11
$C_{14:0}$	0.8±0.1	1.2±0.4	0.4±0.0	1.0±0.1	0.5±0.1	ND	ND	0.1±0.0	0.3±0.0	ND	ND
$C_{14:1}\omega5c$	ND	ND	ND	tr	0.6±0.0	ND	ND	ND	ND	ND	ND
$C_{14:0}$ 3-OH	ND	0.6±0.1	ND	tr	ND	ND	ND	ND	ND	ND	ND
$C_{15:0}$	ND	1.7±0.1	7.9±0.1	2.1±0.1	ND	ND	18.3±6.5	0.2±0.0	4.4±0.0	ND	ND
$C_{15:0}$ α	0.6±0.2	0.1±0.0	0.4±0.0	ND	ND	ND	ND	ND	ND	ND	ND
iso-$C_{15:0}$	0.5±0.0	ND	0.1±0.0	ND	ND	ND	ND	ND	ND	ND	ND
$C_{15:1}\omega5c$	ND	0.3±0.0	ND	0.5±0.0	ND	ND	ND	ND	ND	ND	ND
$C_{15:1}\omega6c$	ND	ND	1.9±0.1	ND	0.2±0.0	ND	4.6±3.7	ND	1.5±0.0	ND	ND
$C_{16:0}$	50.7±2.8	49.0±2.9	36.2±0.6	34.5±1.5	24.0±1.4	25.8±2.3	20.2±0.6	33.0±0.2	35.2±0.2	28.1±2.1	35.2±2.0
$C_{16:1}\omega7c/t$*	36.8±1.3	35.9±1.7	38.7±0.5	5.7±0.4	26.0±0.6	49.6±3.0	27.2±6.5	50.0±0.3	45.7±0.2	47.9±4.7	55.5±3.0
$C_{17:0}$	0.4±0.0	0.5±0.0	3.9±0.1	1.2±0.1	0.4±0.0	ND	10.9±1.0	0.1±0.0	2.8±0.0	ND	ND
iso-$C_{17:0}$	ND	0.1±0.0	0.2±0.0	tr	ND	ND	ND	0.1±0.0	ND	ND	ND
br-$C_{17:0}$†	ND	ND	ND	tr	ND	ND	ND	ND	ND	ND	ND
$C_{17:0}$ cyclo	ND	ND	0.6±0.1	37.9±0.4	0.5±0.0	ND	ND	0.1±0.0	0.7±0.0	ND	ND
$C_{17:1}\omega6c$	ND	ND	0.3±0.1	tr	ND	ND	2.5±0.4	ND	ND	ND	ND
$C_{17:1}\omega7c$	0.2±0.1	0.7±0.0	0.4±0.1	ND	ND	0.6±0.0	1.3±0.3	0.2±0.1	0.4±0.3	ND	ND
$C_{17:1}\omega8c$	ND	ND	0.1±0.0	ND	ND	ND	ND	ND	ND	ND	ND
$C_{18:0}$	0.4±0.0	1.2±0.1	0.3±0.0	2.5±0.2	0.2±0.0	ND	0.3±0.0	ND	ND	ND	ND
br-$C_{18:0}$†	ND	ND	ND	ND	ND	0.8±0.0	ND	0.2±0.1	0.2±0.0	1.0±0.0	ND
$C_{18:0}$ cyclo	ND	ND	ND	ND	34.7±0.5	ND	ND	ND	ND	ND	ND
$C_{18:1}\omega7c/t$*	9.5±1.5	8.9±1.0	8.6±0.3	7.7±0.5	12.8±0.2	23.8±0.5	16.2±1.1	16.2±0.2	9.2±0.0	23.6±2.3	9.3±1.1
$C_{19:0}$ cyclo	ND	ND	ND	5.9±0.9	ND	ND	ND	ND	ND	ND	ND

*Unable to resolve *cis* and *trans* isomers.
†Position of methyl branching not determined.

dominant fatty acids detected in strain N35^T were $C_{16:0}$ (51 mol%), $C_{16:1}\omega7c/t$ (37 mol%) and $C_{18:1}\omega7c/t$ (9.5 mol%) (Table 2); N35^{T}v had a fatty acid profile identical to that of wild-type N35^T. All of the other fatty acids detected were present at <2 mol% (Table 2). We could not resolve $C_{16:1}\omega7c$ and $C_{16:1}\omega7t$ or $C_{18:1}\omega7c$ and $C_{18:1}\omega7t$. However, comparison of fatty acid data for the genus *Acidovorax* in the literature (Willems & Gillis, 2005) and our data suggested $C_{16:1}\omega7c$ and $C_{18:1}\omega7c$ as the dominant fatty acids. Strain N35^T was found to have a different fatty acid profile from type strains of phylogenetically closely related species. Differences were found in the amounts of $C_{16:0}$, $C_{16:1}\omega7c/t$, $C_{17:0}$, $C_{17:0}$ cyclo, $C_{18:0}$ cyclo and $C_{19:0}$ cyclo.

Description of *Acidovorax radicis* sp. nov.

Acidovorax radicis (ra′di.cis. L. gen. n. *radicis* of a root, the source of isolation of the type strain).

Root-colonizing bacterium without apparent phytopathogenic potential. In contrast, it exhibits plant-growth-promoting activity. Cells are Gram-negative, motile, straight rods, 1.5–2 μm long and 0.3–0.5 μm in diameter. Forms rough colonies after 48 h growth on NB agar at 30 °C and flocculates in liquid NB medium after overnight growth at 30 °C. One polar flagellum can be detected with scanning electron microscopy. The type strain undergoes phenotypic variation in NB medium, showing smooth colonies on NB agar, no flocculation in liquid NB medium and no flagellum. Oxidase-positive, catalase-negative and grows at 10–35 °C (optimum 30 °C), 0–2 % NaCl (optimum 0 %) and pH 5.0–9.0 (optimum pH 7.0). Glyceryl tributyrate can be hydrolysed, but casein is not. Produces siderophores. The following sugars, alcohols, fatty acids and amino acids can be metabolized: Tweens 40 and 80, L-arabinose, L-arabitol, D-fructose, L-fucose, D-galactose, α-D-glucose, D-mannitol, D-mannose, D-psicose, D-sorbitol, methyl pyruvate, monomethyl succinate, acetic acid, formic acid, D-gluconic acid, α-hydroxybutyric acid, β-hydroxybutyric acid, *p*-hydroxyphenylacetic acid, α-ketobutyric acid, DL-lactic acid, propionic acid, quinic acid, sebacic acid, succinic acid, bromosuccinic acid, succinamic acid, D- and L-alanine, L-alanyl glycine, L-asparagine, L-aspartic acid, L-glutamic acid, glycyl L-glutamic acid, L-histidine, hydroxy-L-proline, L-leucine, L-phenylalanine, L-proline, L-threonine, γ-aminobutyric acid, urocanic acid and glycerol. Major

cellular fatty acids are $C_{16:0}$, $C_{16:1}\omega7c/t$ and $C_{18:1}\omega7c/t$. The type strain can be differentiated from the type strains of its phylogenetically closest neighbours, *A. defluvii*, *A. facilis*, *A. delafieldii*, *A. temperans*, *A. soli*, *A. caeni*, *A. valerianellae*, *A. anthurii* and *S. metamorpha*, through DNA–DNA relatedness analysis, fatty acid composition and by the utilization of different substrates.

The type strain, N35^T (=DSM 23535^T =LMG 25767^T), was isolated from surface-sterilized roots of wheat (*Triticum aestivum*). Its DNA G+C content is 64.9 mol%.

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