Acidocella aluminiidurans sp. nov., an aluminium-tolerant bacterium isolated from *Panicum repens* grown in a highly acidic swamp in actual acid sulfate soil area of Vietnam

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An aluminium-tolerant bacterium, strain AL46^T, was isolated from a waterweed, *Panicum repens*, grown in a highly acidic swamp (pH 3) at an actual acid sulfate soil area of Vietnam. Cells were Gram-negative, aerobic, non-spore-forming, non-motile rods (0.3 µm wide and 1.2–1.6 µm long). 16S rRNA gene sequence analysis indicated that strain AL46^T belongs to the genus *Acidocella*, class *Alphaproteobacteria*. Strain AL46^T was related most closely to the type strains of *Acidocella facilis* and *Acidocella aminolytica* (99.4 and 97.8 % 16S rRNA gene sequence similarity, respectively). Levels of DNA–DNA relatedness between strain AL46^T and the above type strains were 40 %. The results of physiological and biochemical tests allowed the novel strain to be differentiated phenotypically from the two recognized *Acidocella* species. Data for predominant cellular fatty acids (cyclopropyl C_{19:0} and C_{18:1}), major isoprenoid quinone (Q-10) and DNA G + C content (65.6 mol%) were in accordance with those reported for the genus *Acidocella*. Therefore, strain AL46^T is considered to represent a novel species of the genus *Acidocella*, for which the name *Acidocella aluminiidurans* sp. nov. is proposed. The type strain is AL46^T (=NBRC 104303^T =VTCC-D9-1^T).

The genus *Acidocella* was described by Kishimoto *et al.* (1995) and, at the time of writing, comprises two recognized species, *Acidocella facilis* (the type species; Wichlacz *et al.*, 1986; Kishimoto *et al.*, 1995) and *Acidocella aminolytica* (Kishimoto *et al.*, 1993, 1995), both of which were isolated from acidic coal mine drainage. During the course of a study to develop bioremediation measures for actual acid sulfate soils (AASS) (Sasaki *et al.*, 2008), we isolated a number of bacteria associated with plants grown in aquatic environments of AASS in south-east Asia, among which were previously undescribed species (Aizawa *et al.*, 2007, 2008). The present paper describes an aluminium-tolerant bacterium, strain AL46^T, isolated from a waterweed, *Panicum*

repens L., in a highly acidic swamp (pH 3) at an AASS area of the city of Can Tho in Vietnam. Combined phenotypic, genotypic, chemotaxonomic and phylogenetic analyses indicated that the strain was affiliated to the genus *Acidocella*. The data obtained also suggest that strain AL46^T represents a novel species of the genus *Acidocella*.

Strain AL46^T grew well on MT liquid medium [per litre distilled water: 0.1 g tryptone (Difco), 0.2 g MgCl₂ · 7H₂O, 0.1 g CaCl₂ · 2H₂O, 0.1 g NaCl, 0.02 g FeCl₂ · 6H₂O, 0.5 g (NH₄)₂SO₄ (pH 4.0)] containing 500 mM aluminium sulfate or 200 mM aluminium chloride at 28 °C under aerobic conditions after 7 days. Strain AL46^T showed growth in MT liquid medium at pH 3.0–7.0, with optimum growth at pH 4–5. It showed good growth in MT liquid medium at 17–42 °C, with optimum growth at 37 °C, but did not grow at 5 or 45 °C. On one-tenth-strength trypticase soy (1/10 TS) agar plates [per litre: 3.0 g

Abbreviation: AASS, actual acid sulfate soil.

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain AL46^T is AB362219.

trypticase soy broth (Difco) solidified with 15.0 g agar (Difco)] (pH 4.0), the organism formed smooth, round, convex, white colonies. For preparation of acidic plates, double-strength medium (pH 4.0) and 3 % agar solution were autoclaved separately and then mixed. Cells grown on 1/10 TS agar plates were Gram-negative (Ryu, 1938), non-endospore-forming and non-motile. Growth under anaerobic conditions was determined after 5 days incubation at 30 °C in an AnaeroPak (Mitsubishi Gas Chemical Co., Ltd). Morphology was observed by scanning electron microscopy as described by Bogan *et al.* (2003) with slight modifications, including replacement of gold with platinum–palladium for sputter-coating. When cultured on 1/10 TS agar plates (pH 4.0) at 28 °C for 3 days, cells were irregular rods, 0.3 µm wide and 1.2–1.6 µm long.

The 16S rRNA gene of strain AL46^T was amplified by PCR by using universal primers (Tamura & Hatano, 2001), and the nearly complete 16S rRNA gene nucleotide sequence (1474 bp) was determined. Strain AL46^T showed high levels of 16S rRNA gene sequence similarity to the type strains of *Acidocella facilis* (99.4 %) and *Acidocella aminolytica* (97.8 %). The phylogenetic relationship between strain AL46^T and its closest relatives was determined by using MEGA version 4 (Tamura *et al.*, 2007) after multiple alignment of data via the CLUSTAL X program (Thompson *et al.*, 1997). Evolutionary distances were computed as described by Jukes & Cantor (1969). Phylogenetic trees were constructed with the maximum-parsimony (Kluge & Farris, 1969) and neighbour-joining (Saitou & Nei, 1987) methods. The reliability of the tree topology was evaluated by bootstrap analysis of 1000 replicates (Felsenstein, 1985). The phylogenetic trees constructed with the two methods were topologically similar and showed that strain AL46^T belonged to the genus *Acidocella*. Although the novel strain showed a high level of 16S rRNA gene sequence similarity to the type strains of the two recognized species of the genus, it formed a separate line of descent in the phylogenetic cluster of the genus (Fig. 1).

Fig. 1. Neighbour-joining tree, based on nearly complete 16S rRNA gene sequences (positions 19–1487 of the *Escherichia coli* 16S rRNA gene), showing the position of strain AL46^T among its phylogenetic neighbours. Numbers at branch nodes are bootstrap percentages based on 1000 resamplings; only values >50 % are given. The sequence of *Acetobacter acetii* NCIB 8621^T was used as an outgroup. Bar, 0.01 substitutions per nucleotide position.

DNA–DNA hybridization between strain AL46^T and its closest phylogenetic neighbours was performed, as Stackebrandt & Goebel (1994) have pointed out that a high level of 16S rRNA gene sequence similarity (≥ 97 %) is of limited value for differentiating between bacterial species and that DNA–DNA hybridization studies need to be performed to determine species affiliation. Hybridization was performed according to the microplate method (Ezaki *et al.*, 1988, 1989; Tamura *et al.*, 1999). Levels of DNA–DNA relatedness between strain AL46^T and *Acidocella facilis* NCCB 86038^T and *Acidocella aminolytica* JCM 8796^T were 40 %, well below the 70 % recommended threshold value for the delineation of genomic species (Wayne *et al.*, 1987). On this basis, strain AL46^T is considered to represent a novel species of the genus *Acidocella*.

Strain AL46^T could be distinguished from the two recognized *Acidocella* species on the basis of physiological characteristics (Table 1). Standard physiological tests were carried out according to the methods described by Smibert & Krieg (1994). Growth inhibition experiments with acetate, lactate and succinate were carried out as described by Wakao *et al.* (1994) and Kishimoto *et al.* (1995). Acid production from carbon sources, enzyme activities and resistance to antibiotics were assessed by using the API 50 CH system, the API ZYM and API 20 E systems and the ATB VET system (all bioMérieux), respectively, according to the manufacturer's instructions. Utilization of various substrates as sole carbon sources was tested by using Biolog GN2 MicroPlates in accordance with the manufacturer's instructions. Analyses of DNA G+C content and cellular fatty acids were performed as described by Tamura *et al.* (1994). Major isoprenoid quinones were analysed by using a Quattro premier mass spectrometer coupled to an ACUITY UltraPerformance liquid chromatograph (UPLC/MS/MS; Waters). The major isoprenoid quinone of strain AL46^T was ubiquinone-10 (Q-10), which is also the case for *Acidocella aminolytica* (Kishimoto *et al.*, 1993) and *Acidocella facilis* (Kishimoto *et al.*, 1995). The DNA G+C content of strain AL46^T was 65.6 mol%. The major fatty acids of strain AL46^T when grown on 1/10 TS agar plates containing 1 % glucose (pH 5.0) at 28 °C for 7 days were cyclopropyl C_{19:0} (49.1 % of the total), C_{18:1} (25.4 %), C_{16:0} (12.2 %), C_{16:0} 2-OH (6.7 %), C_{14:0} 3-OH (4.3 %) and C_{18:0} (2.2 %). Similar profiles were obtained for *Acidocella facilis* NCCB 86038^T and *Acidocella aminolytica* JCM 8796^T under the same growth conditions: the major fatty acids were cyclopropyl C_{19:0} (39.6 and 52.3 %, respectively), C_{18:1} (35.8 and 22.6 %), C_{16:0} (13.2 and 11.2 %), C_{16:0} 2-OH (2.9 and 5.2 %), C_{14:0} 3-OH (5.8 and 6.1 %), and C_{18:0} (2.0 and 1.8 %).

Based on the physiological, biochemical, chemotaxonomic and molecular genetic data presented, strain AL46^T is considered to represent a novel species of the genus *Acidocella*, for which the name *Acidocella aluminiidurans* sp. nov. is proposed.

Table 1. Differential characteristics between strain AL46^T and type strains of recognized *Acidocella* species

Strains: 1, AL46^T; 2, *Acidocella facilis* NCCB 86038^T; 3, *Acidocella aminolytica* JCM 8796^T. +, Positive; –, negative; (+), weakly positive. Data were generated in this study. All three strains were negative for growth in the presence of 0.25 mM acetate, 2 mM lactate and 4 mM succinate, acid production (API 50CH) from D-arabinose, D-xylose, L-xylose, D-fructose, N-acetylglucosamine, amygdalin, salicin, cellobiose, maltose, lactose, melibiose, trehalose, inulin, melezitose and xylitol, activities (API ZYM/API 20E) of/for oxidase, H₂S production, indole production, nitrate reduction, lysine decarboxylase, ornithine decarboxylase, tryptophan deaminase, alkaline phosphatase, lipase (C4), trypsin, chymotrypsin, α -galactosidase, β -galactosidase, β -glucuronidase, α -glucosidase, β -glucosidase, N-acetyl- β -glucosaminidase, α -mannosidase and α -fucosidase, assimilation (Biolog GN2) of α -cyclodextrin, N-acetyl-D-galactosamine, N-acetyl-D-glucosamine, adonitol, L-arabinose, D-arabitol, cellobiose, *i*-erythritol, L-fucose, D-galactose, gentiobiose, *myo*-inositol, α -lactose, lactulose, maltose, D-mannitol, melibiose, methyl β -D-glucoside, raffinose, L-rhamnose, D-sorbitol, sucrose, trehalose, turanose, xylitol, acetic acid, *cis*-aconitic acid, citric acid, D-galactonic acid lactone, D-galacturonic acid, D-gluconic acid, D-glucosaminic acid, D-glucuronic acid, γ -hydroxybutyric acid, itaconic acid, α -ketovaleric acid, malonic acid, propionic acid, quinic acid, D-saccharic acid, sebacic acid, glucuronamide, L-alanyl glycine, L-aspartic acid, glycyl L-aspartic acid, glycyl L-glutamic acid, hydroxy-L-proline, L-leucine, L-phenylalanine, L-proline, L-pyrroglutamic acid, D-serine, L-serine, L-threonine, DL-carnitine, inosine, uridine, thymidine, phenylethylamine, 2,3-butanediol, DL- α -glycerol phosphate, α -D-glucose 1-phosphate and D-glucose 6-phosphate and antibiotic resistance (ATB VET) to amoxicillin/clavulanic acid, streptomycin, spectinomycin, kanamycin, gentamicin, apramycin, tetracycline, doxycycline and rifampicin. All three strains were positive for acid production (API 50CH) from aesculin, D-tagatose and potassium 5-ketogluconate, activities (API ZYM/API 20E) of catalase, esterase (C4), esterase lipase (C8), leucine arylamidase, valine arylamidase, acid phosphatase and naphthol-AS-BI-phosphohydrolase, assimilation (Biolog GN2) of α -D-glucose, pyruvic acid methyl ester, formic acid, β -hydroxybutyric acid, DL-lactic acid, L-alaninamide, L-alanine, L-asparagine and γ -aminobutyric acid and antibiotic resistance (ATB VET) to penicillin, oxacillin, cephalothin, cefoperazone, chloramphenicol, erythromycin, lincomycin, pristinamycin, tylosin, colistin, flumequine, oxolinic acid, enrofloxacin, nitrofurantoin, fusidic acid and metronidazole.

Characteristic	1	2	3
Acid production (API 50CH) from:			
Glycerol	–	–	+
Erythritol	–	+	–
L-Arabinose	–	+	–
D-Ribose	–	(+)	–
D-Adonitol	–	+	–
Methyl β -D-xylopyranoside	+	–	–
D-Galactose	–	+	–
D-Glucose	–	+	+
D-Mannose	–	+	–

Table 1. cont.

Characteristic	1	2	3
L-Sorbose	–	(+)	+
L-Rhamnose	–	+	+
Dulcitol	–	+	–
Inositol	–	+	+
D-Mannitol	–	+	+
D-Sorbitol	(+)	(+)	–
Methyl α -D-mannopyranoside	–	+	+
Methyl α -D-glucopyranoside	–	–	+
Arbutin	–	+	–
Sucrose	–	–	(+)
Raffinose	–	+	–
Starch	–	+	+
Glycogen	–	+	+
Gentiobiose	+	–	–
Turanose	+	–	+
D-Lyxose	–	–	+
D-Fucose	–	–	+
L-Fucose	–	–	+
D-Arabitol	+	–	+
L-Arabitol	–	(+)	+
Potassium gluconate	–	–	+
Potassium 2-ketogluconate	–	(+)	–
Activity (API ZYM/API 20E) of:			
Arginine dihydrolase	(+)	–	–
Urease	–	+	+
Acetoin production	–	+	+
Gelatinase	+	–	–
Cystine arylamidase	–	(+)	(+)
Assimilation (Biolog GN2) of:			
Dextrin	(+)	(+)	–
Glycogen	(+)	(+)	–
Tween 40	–	+	(+)
Tween 80	–	+	(+)
D-Fructose	–	+	+
D-Mannose	–	(+)	–
D-Psicose	–	+	(+)
Succinic acid monomethyl ester	+	–	+
α -Hydroxybutyric acid	–	+	–
<i>p</i> -Hydroxyphenylacetic acid	–	+	–
α -Ketobutyric acid	–	+	–
α -Ketoglutaric acid	(+)	–	+
Succinic acid	(+)	–	+
Bromosuccinic acid	+	–	+
Succinamic acid	+	–	+
D-Alanine	(+)	–	–
L-Glutamic acid	(+)	–	–
L-Histidine	–	+	–
L-Ornithine	–	+	–
Urocanic acid	+	+	–
Putrescine	–	(+)	–
2-Aminoethanol	–	–	(+)
Glycerol	–	+	–

Table 1. cont.

Characteristic	1	2	3
Antibiotic resistance (ATB VET)			
Amoxicillin	+	–	–
Cotrimoxazole	+	–	+
Sulfamethazole	+	–	+

Description of *Acidocella aluminidurans* sp. nov.

Acidocella aluminidurans [a.lu.mi.ni.i.du'rans. N.L. n. *aluminium* (from L. n. *alumen* alum) aluminium; L. part. adj. *durans* enduring, being insensible; N.L. part. adj. *aluminidurans* aluminium-tolerant].

Gram-negative, strictly aerobic, non-spore-forming, non-motile rods ($0.3 \times 1.2\text{--}1.6\ \mu\text{m}$). Colonies are smooth, round, convex and white on 1/10 TS agar plates. Growth occurs at 17–42 °C but not at 5 or 45 °C. The pH range for growth is 3.0–7.0 with optimum growth at pH 4–5. Growth occurs on MT medium (pH 4.0) containing 500 mM aluminium sulfate or 200 mM aluminium chloride. Physiological characteristics are summarized in Table 1. The major quinone is Q-10. The predominant cellular fatty acids are cyclopropyl C_{19:0} and C_{18:1}. The DNA G+C content of the type strain is 65.6 mol%.

The type strain, AL46^T (=NBRC 104303^T =VTCC-D9-1^T), was isolated from *Panicum repens* inhabiting an acidic swamp in an AASS area in Vietnam.

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