Burkholderia heleia sp. nov., a nitrogen-fixing bacterium isolated from an aquatic plant, *Eleocharis dulcis*, that grows in highly acidic swamps in actual acid sulfate soil areas of Vietnam

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Nitrogen-fixing bacteria, strains SA41^T, SA42 and SA53, were isolated from an aquatic plant, *Eleocharis dulcis*, that grows in highly acidic swamps (pH 2–4) in actual acid sulfate soil areas of Vietnam. The isolates were Gram-negative, aerobic, non-spore-forming, rod-shaped bacteria, having a cell width of 0.6–0.7 µm and a length of 1.5–1.7 µm. They showed good growth between pH 3.0 and 7.0, and between 17 and 37 °C. The organisms contained ubiquinone Q-8 as the predominant isoprenoid quinone, and C_{16:0}, C_{17:0} cyclo, C_{18:1} ω7c and summed feature 3 (C_{16:1} ω7c and/or iso-C_{15:0} 2-OH) as major fatty acids. Their fatty acid profiles are similar to those reported for other *Burkholderia* species. The DNA G+C content of these strains was 64 mol%. On the basis of 16S rRNA gene sequence similarity, these strains were shown to belong to the genus *Burkholderia*. Although their calculated 16S rRNA gene sequence similarity values to *Burkholderia silvatlantica*, *Burkholderia mimosarum*, *Burkholderia ferrariae* and *Burkholderia tropica* were 98.5, 98.2, 98.0 and 97.0 %, respectively, the isolates formed a distinct group in phylogenetic trees, and the DNA–DNA relatedness values of strain SA41^T to these species were 39, 41, 39 and 33 %, respectively. The results of physiological and biochemical tests, including whole-cell protein pattern analysis, allowed phenotypic differentiation of these strains from the published *Burkholderia* species. Therefore, strains SA41^T, SA42 and SA53 represent a novel species for which the name *Burkholderia heleia* sp. nov. is proposed. The type strain is SA41^T (=NBRC 101817^T=VTCC-D6-7^T).

Yabuuchi *et al.* first described the genus *Burkholderia* in 1992 by transferring seven species from *Pseudomonas*, with *Burkholderia cepacia* as the type species (Yabuuchi *et al.*, 1992). Although only one N₂-fixing species, *Burkholderia vietnamiensis* (Gillis *et al.*, 1995), was reported in the genus until recently, a number of N₂-fixers in *Burkholderia* have since been isolated from plants (maize, mimosas, sugar cane, teosinte) and soils, and subsequently classified as

novel *Burkholderia* species, including *Burkholderia mimosarum* (Chen *et al.*, 2006), *Burkholderia nodosa* (Chen *et al.*, 2007), *Burkholderia silvatlantica* (Perin *et al.*, 2006), *Burkholderia tropica* (Reis *et al.*, 2004), *Burkholderia unamae* (Caballero-Mellado *et al.*, 2004) and *Burkholderia xenovorans* (Goris *et al.*, 2004). 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 growing in highly acidic aquatic environments (pH 2–4) of AASS in south-east Asia and found and reported a novel bacterial species, *Curtobacterium ammoniigenes*, among them (Aizawa *et al.*, 2007). In the present study we characterized N₂-fixing bacteria SA41^T, SA42 and SA53, isolated from an aquatic plant, *Eleocharis dulcis*, that grows in highly acidic swamps (pH 2–4) in AASS areas in Vietnam. By using a

Abbreviation: AASS, actual acid sulfate soil(s).

The GenBank/EMBL/DDBJ accession numbers for the 16S rRNA gene sequences of strains SA41^T, SA42 and SA53 are AB495123, AB495124 and AB495125, respectively.

An extended phylogenetic tree, SDS-PAGE profiles of whole-cell extracts, and fatty acid compositions of the novel strains and related species are available as supplementary material in IJSEM Online.

polyphasic approach, including 16S rRNA gene analysis, DNA–DNA hybridization, whole-cell protein analysis, fatty acid methyl ester analysis, and phenotypic and biochemical characterization, the strains were shown to be affiliated with the genus *Burkholderia*. The data obtained also suggest that the isolates represent a novel species of the genus *Burkholderia*.

A number of bacteria were isolated from plants of *E. dulcis* by using one-tenth-strength tryptic soy (1/10 TS) agar plates [3.0 g tryptic soy broth l⁻¹ (Difco) solidified with 15.0 g agar l⁻¹ (Difco); pH 4.0]. For preparation of acidic plates, double-strength medium and 3 % agar solution were autoclaved separately and mixed after autoclaving to prevent hydrolysis of the agar. Among these isolates, N₂-fixing bacteria were selected by performing a *nifH* PCR assay (Ueda *et al.*, 1995; Valdes *et al.*, 2005), followed by an acetylene reduction experiment (Burris, 1972) using Winogradsky's N₂-free mineral soft gel medium containing 1 % mannitol (Hashidoko *et al.*, 2002). Isolates SA41^T, SA42 and SA53 from a plant of *E. dulcis* showed strong acetylene reduction activity. The three isolates formed round, smooth, convex, pale yellow colonies with entire margins on 1/10 TS (pH 4.0) plates. They showed good growth on these plates between 17 and 37 °C with an optimum at 32 °C, but did not grow at 5 or 40 °C. All three showed good growth over the pH range 3.5–9 with an optimum at 5, but no or little growth below pH 3 or above 10 when cultured at 30 °C for 5 days. The isolates showed as good growth on TS (pH 4.0) plates as on 1/10 TS (pH 4.0) plates. Growth under anaerobic conditions was determined after 5 days incubation at 30 °C in an AnaeroPack (Mitsubishi Gas Chemical Co.). The isolates showed good growth on 1/10 TS plates (pH 4.0) under aerobic conditions but not under anaerobic ones, thus suggesting them to be aerobic. The cells grown on 1/10 TS agar plates (pH 4.0) were Gram-negative (Ryu, 1938), non-endospore-forming and non-motile. Morphology was observed by scanning electron microscopy as described by Bogan *et al.* (2003) with slight modifications, including a replacement of gold by platinum–palladium for sputter-coating. The observed cells were irregular rods, 0.6–0.7 µm wide and 1.5–1.7 µm long, when the organisms were cultured on 1/10 TS agar plates (pH 4.0) at 28 °C for 3 days (Supplementary Fig. S1 available in IJSEM Online).

The 16S rRNA genes of the isolates were amplified by PCR using universal primers (Tamura & Hatano, 2001), and nearly complete 16S rRNA gene nucleotide sequences were determined. The sequences showed high similarities to those of species within the genus *Burkholderia*. The 16S rRNA gene sequence similarities of strain SA41^T (AB495123) to strains SA42 (AB495124) and SA53 (AB495125), and to *Burkholderia silvatlantica* (AY965240), *Burkholderia mimosarum* (AY752958), *Burkholderia ferrariae* (DQ514537) and *Burkholderia tropica* (AJ420332) were 99.9, 99.8, 98.5, 98.2, 98.0 and 97.0 %, respectively. The phylogenetic relationship with closely related species was determined by using MEGA version 4 (Tamura *et al.*, 2007) and the PHYLIP 3.65 package

(Felsenstein, 2005) after multiple alignments of the data performed with CLUSTAL_X (Thompson *et al.*, 1997). Evolutionary distances were computed as described by Jukes & Cantor (1969). Phylogenetic trees were constructed by using the maximum-parsimony (Kluge & Farris, 1969), maximum-likelihood (Felsenstein, 1981) and neighbour-joining (Saitou & Nei, 1987) methods. The reliabilities of these tree topologies were evaluated by bootstrap analysis with 1000 replicates (Felsenstein, 1985). The phylogenetic trees constructed by these three methods were topologically similar and showed that isolates SA41^T, SA42 and SA53 belonged to the genus *Burkholderia*. The phylogenetic tree constructed by using the neighbour-joining method is shown in Fig. 1. Although the isolates showed a high degree of 16S rRNA gene sequence similarity to established species of *Burkholderia*, they formed a separate line of descent in the phylogenetic cluster of the genus (Fig. 1 and Supplementary Fig. S2 available in IJSEM Online). Stackebrandt & Goebel (1994) pointed out that a high degree of 16S rRNA gene sequence similarity (97 % or higher) is of limited value for differentiating species and that DNA–DNA hybridization studies must be performed to determine species affiliation under these circumstances. Therefore, we performed DNA–DNA hybridization on these isolates and their neighbours on the phylogenetic tree by using the microplate hybridization method (Ezaki *et al.*, 1988, 1989; Tamura *et al.*, 1999). The DNA–DNA relatedness values of isolates SA41^T with SA42 and SA53 were 94 and 89 %, respectively, which were higher than the recommended threshold value for the delineation of genomic species (70 %; Wayne *et al.*, 1987) and also indicated that these three isolates represent three separate strains. Strain SA41^T exhibited relatively low levels of DNA–DNA relatedness with respect to *Burkholderia silvatlantica* (39 %), *Burkholderia mimosarum* (41 %), *Burkholderia ferrariae* (39 %) and *Burkholderia tropica* (33 %), indicating that strain SA41^T was not related to them at the species level. These data suggest that strains SA41^T, SA42 and SA53 represent a novel species of the genus *Burkholderia*.

Additionally, strains SA41^T, SA42 and SA53 could be distinguished from closely related species of *Burkholderia* on the basis of physiological characteristics (Table 1). These three strains were also phenotypically distinguishable from each other by acid production from melibiose, utilization of D-serine and resistance to gentamicin, oxolinic acid and enrofloxacin (Supplementary Table S1 available in IJSEM Online). Standard physiological tests were carried out according to the methods described by Smibert & Krieg (1994). Acid production from carbon sources, enzyme activities and resistance to antibiotics were assessed by using the API 50 CH system, API ZYM and API 20 E systems, and the ATB VET system (bioMérieux), respectively, according to the manufacturer's instructions. The utilization of various substrates as sole carbon sources was tested by using Biolog GN2 Microplates (Biolog Identification System) in accordance with the manufacturer's instructions. Analyses of DNA G + C content and cellular fatty acids were performed as described by Tamura

Fig. 1. Neighbour-joining tree based on nearly complete 16S rRNA gene sequences (positions 58–1440 of the *Escherichia coli* 16S rRNA gene), showing the positions of strains SA41^T, SA42 and SA53 among closely related species of the genus *Burkholderia*. Numbers at branch nodes are values based on 1000 resamplings; only values over 50% are given. The sequence of *Burkholderia kururiensis* KP23^T was used as an outgroup. Bar, 0.005 nucleotide substitutions per nucleotide position.

et al. (1994). The major isoprenoid quinone was prepared as described previously (Tamura *et al.*, 1994) and analysed by using a Quattro premier mass spectrometer coupled to an ACUITY UPLC (UPLC/MS/MS, Waters; Aizawa *et al.*, 2010). The DNA G + C content of strains SA41^T, SA42 and SA53 was 64 mol%. Major fatty acids of all three strains grown on 1/10 TS agar plates containing 1% glucose (pH 5.0) at 28 °C for 3 days were C_{16:0}, C_{17:0} cyclo, C_{18:1} ω 7c and summed feature 3 (C_{16:1} ω 7c and/or iso-C_{15:0} 2-OH; Supplementary Table S2 available in IJSEM Online), and similar patterns were obtained from cells of the type strains of the closely related species in the genus. The major isoprenoid quinone of all three strains was determined to be Q-8, as in the case for other species of the genus *Burkholderia* (Yamada *et al.*, 1982; Zhang *et al.*, 2000; Yang *et al.*, 2006; Valverde *et al.*, 2006).

Whole-cell protein extracts were prepared from the three strains and the type strains of several related species, and analysed by SDS-PAGE as described by Pot *et al.* (1994); the results are shown in Supplementary Fig. S3 in IJSEM Online. The three newly isolated strains showed almost identical protein profiles among themselves (lanes 1–3), but their protein patterns were clearly different from those of other related species of *Burkholderia* (lanes 4–10). Since it is a well-known fact that bacteria with identical or very similar protein patterns possess high levels of genome similarity (Vandamme *et al.*, 1996), the SDS-PAGE results also support the notion that strains SA41^T, SA42 and SA53 represent a novel N₂-fixing *Burkholderia* species.

Therefore, based on the physiological, biochemical, chemotaxonomic and molecular genetic results described above, strains SA41^T, SA42 and SA53 represent a novel species of the genus *Burkholderia*, for which the name *Burkholderia heleia* sp. nov. is proposed.

Description of *Burkholderia heleia* sp. nov.

Burkholderia heleia (he.le'ia. Gr. n. *heleios* a marsh, low ground, meadow; N.L. fem. adj. *heleia* of the marsh, referring to the isolation of this species from a plant growing in a marsh).

The cells are Gram-negative, strictly aerobic, non-spore-forming, non-motile rods (0.6–0.7 μ m $\times$ 1.5–1.7 μ m). Colonies are smooth, round, convex and pale yellow with entire margins after cultivation for 3 days at 32 °C on 1/10 tryptic soy agar plates (pH 4.0). Growth occurs between 17 and 42 °C with an optimum at 32 °C. The pH range for growth is 3.5–9.0, with optimum growth at pH 5.0. The bacteria grow on MacConkey agar at 29 °C, but not at 37 °C. Growth and acetylene reduction to ethylene occur on N₂-free soft gel medium. Tweens 20, 40 and 60 are hydrolysed, but Tween 80 is not. Positive for esterase (C4), esterase lipase (C8), β -glucosidase and urease activities, but negative for valine arylamidase, cystine arylamidase and arginine dihydrolase activities (API ZYM, API 20E and API 20NE systems). Positive for acid production from erythritol, D-xylose, D-adonitol, L-sorbose, dulcitol, aesculin, salicin, D-lactose, trehalose, xylitol and gentiobiose, but negative for acid production from D-mannose, L-rhamnose, arbutin, maltose, sucrose, raffinose, L-arabitol, potassium gluconate, potassium 2-ketogluconate and potassium 5-ketogluconate (API 50CH system). Can utilize adonitol, cellobiose, *i*-erythritol, L-rhamnose, trehalose, quinic acid, L-ornithine, 2-aminoethanol and D-glucose 6-phosphate as sole carbon source, but not N-acetyl-D-galactosamine, gentiobiose, α -D-lactose, lactulose, maltose, melibiose, raffinose, sucrose, turanose, xylitol, D-galacturonic acid, D-glucuronic acid, γ -hydroxybutyric acid, itaconic acid, α -ketovaleric acid, malonic acid, D-saccharic acid, glucuronamide, glycyl-L-aspartic acid, DL-carnitine, inosine, phenylethylamine, 2,3-butanediol or α -D-glucose 1-phosphate (Biolog GN2 Microplate system). Resistant to streptomycin (8 μ g ml⁻¹), chloramphenicol (8 μ g ml⁻¹), erythromycin (1 μ g ml⁻¹), tylosin (2 μ g ml⁻¹), colistin (4 μ g ml⁻¹), flumequin (4 μ g ml⁻¹) and rifampicin (4 μ g ml⁻¹), but sensitive to kanamycin (8 μ g ml⁻¹), apramycin (16 μ g ml⁻¹) and cotrimoxazole (2 μ g trimethoprim ml⁻¹ plus 38 μ g ml⁻¹ sulfamethoxazole) (API VET system). Additional physiological characteristics are summarized in Table 1. The major isoprenoid quinone is Q-8, as described for the genus. The predominant cellular fatty acids are C_{16:0}, C_{17:0} cyclo, C_{18:1} ω 7c and summed feature 3 (C_{16:1} ω 7c and/or iso-C_{15:0} 2-OH). The DNA G + C content of these strains is 64 mol%.

Table 1. Physiological characteristics of *Burkholderia heleaia* sp. nov. and closely related type strains of *Burkholderia* species

Strains: 1, *Burkholderia heleaia* sp. nov. SA41^T, SA42 and SA53; 2, *Burkholderia silvatlantica* LMG 23149^T; 3, *Burkholderia ferrariae* LMG 23612^T; 4, *Burkholderia nodosa* LMG 23741^T; 5, *Burkholderia tropica* LMG 22274^T; 6, *Burkholderia unamae* CIP 107921^T; 7, *Burkholderia sacchari* LMG 19450^T; 8, *Burkholderia mimosarum* LMG 23256^T. +, Positive; –, negative; (+), weakly positive. All strains had the following characteristics: positive for hydrolysis of Tweens 20, 40 and 60; positive for oxidase, catalase; positive for acid and alkaline phosphatase, leucine arylamidase, naphthol-AS-BI-phosphohydrolase and nitrate reduction (API ZYM and API 20NE systems); negative for Gram stain and growth on MacConkey agar at 37 °C; negative for hydrolysis of DNA and gelatin; negative for lipase (C4), trypsin, α -chymotrypsin, α -galactosidase, β -glucuronidase, α -glucosidase, *N*-acetyl-D-glucosaminidase, α -mannosidase, α -fucosidase, proteinase, indole production and nitrate reduction to N₂ (API ZYM and API 20NE systems); positive for acid production from glycerol, D- and L-arabinose, D-ribose, D-xylose, D-galactose, D-glucose, D-fructose, inositol, D-mannitol, D-sorbitol, *N*-acetylglucosamine, cellobiose, D-lyxose, D- and L-fucose and D-arabitol (API 50CH system); negative for acid production from methyl- β -D-xylopyranoside, methyl- α -D-mannopyranoside, methyl- α -D-glucopyranoside, amygdalin, inulin, melizitose, starch, glycogen and turanose (API 50CH system); positive for utilization of Tweens 40 and 80, *N*-acetyl-D-glucosamine, L-arabinose, D-arabitol, D-fructose, L-fucose, D-galactose, α -D-glucose, *m*-inositol, D-mannitol, D-mannose, D-sorbitol, pyruvic acid methylester, succinic acid monomethylester, acetic acid, *cis*-aconitic acid, citric acid, formic acid, D-galactonic acid lactone, D-gluconic acid, D-glucosaminic acid, β -hydroxybutyric acid, *p*-hydroxyphenylacetic acid, α -ketobutyric acid, α -ketoglutaric acid, DL-lactic acid, propionic acid, sebacic acid, succinic acid, bromosuccinic acid, succinamic acid, L-alaninamide, D- and L-alanine, L-alanylglycine, L-asparagine, L-aspartic acid, L-glutamic acid, glycyl-L-glutamic acid, L-histidine, hydroxy-L-proline, L-leucine, L-phenylalanine, L-proline, L-pyroglyutamic acid, L-serine, L-threonine, γ -aminobutyric acid, urocanic acid and glycerol (Biolog GN2 Microplate system); negative for utilization of α -cyclodextrin, β -methyl-D-glucoside, uridine and thymidine (Biolog GN2 Microplate system); resistant to penicillin (0.25 μ g ml⁻¹), amoxicillin (4 μ g ml⁻¹), amoxicillin (4 μ g ml⁻¹) plus clavulanic acid (2 μ g ml⁻¹), oxacillin (2 μ g ml⁻¹), cephalothin (8 μ g ml⁻¹), cefoperazone (4 μ g ml⁻¹), lincomycin (2 μ g ml⁻¹), pristinamycin (2 μ g ml⁻¹), sulfamethizole (100 μ g ml⁻¹), nitrofurantoin (25 μ g ml⁻¹), fusidic acid (2 μ g ml⁻¹) and metronidazole (4 μ g ml⁻¹) (API VET system); sensitive to spectinomycin (64 μ g ml⁻¹), tetracycline (4 μ g ml⁻¹) and doxycycline (4 μ g ml⁻¹) (API VET system).

Characteristic	1	2	3	4	5	6	7	8
Growth on:								
MacConkey agar at 29 °C	+	–	–	–	+	+	–	–
Hydrolysis of:								
Tween 80	–	+	+	+	+	+	+	+
Enzyme activity*								
Esterase (C4)	+	+	+	+	–	+	+	+
Esterase lipase (C8)	+	+	+	+	–	+	+	+

Table 1. cont.

Characteristic	1	2	3	4	5	6	7	8
Valine arylamidase	–	+	+	–	–	–	+	–
Cystine arylamidase	–	(+)	+	–	–	–	+	–
β -Galactosidase	–	–	–	+	–	–	–	–
β -Glucosidase	+	+	–	–	–	–	–	–
Arginine dihydrolase	–	+	+	+	–	–	(+)	–
Urease	+	+	+	+	–	–	–	+
Acid production from:†								
Erythritol	+	+	–	(+)	–	(+)	–	–
L-Xylose	+	–	(+)	+	(+)	+	–	–
D-Adonitol	+	+	+	+	+	+	+	–
D-Mannose	–	+	+	+	+	+	+	+
L-Sorbose	+	–	–	–	(+)	(+)	–	–
L-Rhamnose	–	+	+	+	+	+	–	–
Dulcitol	+	–	+	–	–	–	–	–
Arbutin	–	–	–	(+)	–	(+)	–	(+)
Aesculin	+	–	(+)	(+)	(+)	(+)	–	(+)
Salicin	+	(+)	(+)	(+)	+	(+)	–	(+)
Maltose	–	–	+	–	–	+	–	–
D-Lactose	+	–	+	–	(+)	+	–	+
Melibiose	v	–	–	–	+	+	–	–
Sucrose	–	+	–	–	–	–	+	–
Trehalose	+	–	+	+	+	+	–	–
Raffinose	–	+	–	–	–	–	+	–
Xylitol	+	–	–	+	(+)	–	–	–
Gentiobiose	+	+	(+)	(+)	+	+	–	–
D-Tagatose	(+)	–	+	–	–	(+)	–	–
L-Arabitol	–	–	–	–	–	(+)	(+)	–
Potassium gluconate	–	(+)	–	–	–	(+)	–	–
Potassium 2-ketogluconate	–	(+)	–	–	–	(+)	(+)	–
Potassium 5-ketogluconate	–	–	–	–	–	–	(+)	–
Utilization of:‡								
Dextrin	(+)	–	(+)	–	(+)	–	–	+
Glycogen	(+)	(+)	(+)	+	(+)	–	(+)	+
<i>N</i> -Acetyl-D-galactosamine	–	+	+	–	–	(+)	(+)	–
Adonitol	+	+	+	+	+	+	+	–
Cellobiose	+	+	+	+	(+)	+	–	–
<i>i</i> -Erythritol	+	+	–	+	–	+	+	+
Gentiobiose	–	+	+	–	(+)	–	–	–
α -D-Lactose	–	–	(+)	–	–	–	–	–
Lactulose	–	–	+	–	+	–	+	+
Maltose	–	–	(+)	–	–	(+)	–	–
Melibiose	–	–	–	–	(+)	–	–	–
D-Psicose	(+)	–	(+)	+	+	(+)	(+)	(+)
Raffinose	–	+	–	–	–	–	+	–
L-Rhamnose	+	+	+	+	+	+	+	–
Sucrose	–	+	–	–	–	(+)	+	–
Trehalose	+	–	+	+	+	+	–	–
Turanose	–	–	–	–	–	(+)	–	–
Xylitol	–	–	(+)	+	–	–	–	–
D-Galacturonic acid	–	–	+	+	+	+	–	+

Table 1. cont.

Characteristic	1	2	3	4	5	6	7	8
D-Glucuronic acid	–	–	+	–	–	(+)	–	–
α -Hydroxybutyric acid	(+)	+	+	–	+	(+)	+	+
γ -Hydroxybutyric acid	–	–	+	+	–	+	(+)	+
Itaconic acid	–	+	–	+	(+)	+	+	+
α -Ketovaleic acid	–	(+)	+	–	(+)	(+)	+	+
Malonic acid	–	+	–	–	–	(+)	(+)	+
Quinic acid	+	–	+	+	+	+	+	+
D-Saccharic acid	–	(+)	+	+	+	+	+	+
Glucuronamide	–	+	+	–	+	(+)	(+)	–
Glycyl-L-aspartic acid	–	+	+	+	–	–	+	+
L-Ornithine	+	+	+	–	(+)	(+)	+	+
DL-Carnitine	–	–	–	–	(+)	–	+	–
Inosine	–	–	–	–	(+)	+	–	–
Phenylethylamine	–	–	–	+	–	–	–	+
Putrescine	(+)	–	–	–	–	–	–	–
2-Aminoethanol	+	+	–	–	+	+	+	+
2,3-Butanediol	–	–	–	–	(+)	(+)	–	–
DL- α -Glycerol phosphate	(+)	+	+	–	(+)	(+)	+	–
α -D-Glucose 1-phosphate	–	–	–	–	(+)	(+)	+	–
D-Glucose 6-phosphate	+	+	+	+	+	+	+	–
Resistance to (μ g ml ⁻¹):§								
Streptomycin (8)	+	(+)	(+)	–	–	–	+	+
Kanamycin (8)	–	(+)	–	–	–	–	–	–
Apramycin (16)	–	(+)	–	–	–	–	(+)	–
Chloramphenicol (8)	+	+	+	+	–	–	+	+
Erythromycin (1)	+	+	+	+	–	–	+	+
Tylosin (2)	+	+	+	+	–	+	+	+
Colistin (4)	+	+	+	+	–	–	+	+
Cotrimoxazole (2/38)	–	(+)	+	+	–	–	+	+
Flumequin (4)	+	+	+	–	–	–	+	–
Rifampicin (4)	+	+	+	+	–	(+)	+	+

*Results obtained by using the API ZYM and API 20 NE systems.

†Results obtained by using the API 50CH system.

‡Results obtained by using the Biolog GN2 Microplate system.

§Results obtained by using the API VET system.

The type strain, SA41^T (=NBRC 101817^T=VTCC-D6-7^T), was isolated from an aquatic plant, *Eleocharis dulcis*, that grows luxuriantly in a highly acidic swamp (pH 3) in an AASS area in Vietnam.

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