INTERNATIONAL JOURNAL OF SYSTEMATIC AND EVOLUTIONARY MICROBIOLOGY

TAXONOMIC DESCRIPTION

Tóth *et al.*, *Int. J. Syst. Evol. Microbiol.* 2021;71:004805

DOI 10.1099/ijsem.0.004805

MICROBIOLOGY SOCIETY

Cellvibrio polysaccharolyticus sp. nov., a cellulolytic bacterium isolated from agricultural soil

Ákos Tóth[1,*], Rózsa Máté[2], József Kutasi[2], Ildikó Bata-Vidács[1], Erika Tóth[3], András Táncsics[4], Gábor Kovács[5,6], István Nagy[5,7] and József Kukolya[1]

Abstract

A novel Gram-reaction-negative bacterial strain, designated Ka43^T, was isolated from agricultural soil and characterised using a polyphasic approach to determine its taxonomic position. On the basis of 16S rRNA gene sequence analysis, the strain shows highest similarity (97.1%) to *Cellvibrio diazotrophicus* E50^T. Cells of strain Ka43^T are aerobic, motile, short rods. The major fatty acids are summed feature 3 ($C_{16:1}\omega7c$ and/or iso-$C_{15:0}$ 2-OH), $C_{18:1}\omega7c$ and $C_{16:0}$. The only isoprenoid quinone is Q-8. The polar lipid profile includes phosphatidylethanolamine, phosphatidylglycerol, four phospholipids, two lipids and an aminolipid. The assembled genome of strain Ka43^T has a total length of 4.2 Mb and the DNA G+C content is 51.6 mol%. Based on phenotypic data, including chemotaxonomic characteristics and analysis of the 16S rRNA gene sequences, it was concluded that strain Ka43^T represents a novel species in the genus *Cellvibrio*, for which the name *Cellvibrio polysaccharolyticus* sp. nov. is proposed. The type strain of the species is strain Ka43^T (=LMG 31577^T=NCAIM B.02637^T).

INTRODUCTION

The genus *Cellvibrio* is affiliated with the family *Cellvibrionaceae* [1]. *Cellvibrio* gen. nov. was proposed by Winogradsky with two species [2], but because of the loss of type species the genus was excluded from the Approved List of Bacterial Names [3]. The genus was proposed again by Blackall *et al.* based on numerical taxonomy [4]. Blackall *et al.* described the genus by the following properties: Gram-negative, aerobic, slightly curved rods, oxidase- and catalase-positive, oxidative metabolism of glucose, and capabile of hydrolysing cellulose. The genus description was emended by Humphry *et al.* and Suarez *et al.* [5, 6]. According to the emended descriptions of the genus, the dominant fatty acids of the cells are unsaturated $C_{16:1}$, $C_{18:1}$ and saturated $C_{16:0}$. The G+C content of the DNA is 44.1–53.5 mol%. At the time of writing (December 2020) the genus includes ten species and two subspecies. The type species of the genus is *Cellvibrio mixtus*.

The cellulolytic activity of *Cellvibrio* strains has been highlighted in the genus emended description by Blackall *et al.* [4], and the etymology of *Cellvibrio* (cellulose-degrading vibrio) also indicates the capability of cellulose hydrolysis. *Cellvibrio* strains were isolated from soil, rhizosphere or aquatic environment. The genus and especially *Cellvibrio japonica* is well studied for its hydrolytic enzyme system, which enables these bacteria to degrade various polysaccharides including plant cell walls [5, 7]. These enzymes have great industrial potential for the utilisation of plant biomass. Some strains are capable of nitrogen fixation as described by Suarez *et al.* [6].

Based on its 16S rRNA gene sequence, strain Ka43^T was classified as a novel bacterium in the genus *Cellvibrio*. The aim of

Author affiliations: [1]Research Group for Food Biotechnology, Institute of Food Science and Technology, Hungarian University of Agriculture and Life Sciences, Budapest, Hungary; [2]BioFil Microbiological, Biotechnological and Biochemical Ltd, Budapest, Hungary; [3]Department of Microbiology, Eötvös Loránd University, Budapest, Hungary; [4]Department of Molecular Ecology, Institute of Aquaculture and Environmental Safety, Hungarian University of Agriculture and Life Sciences, Gödöllő, Hungary; [5]SeqOmics Biotechnology Ltd, Szeged, Hungary; [6]University of Sopron, Sopron, Hungary; [7]Institute of Biochemistry, Biological Research Centre, Eötvös Loránd Research Network, Szeged, Hungary.
***Correspondence:** Ákos Tóth, toth.akosgergely@gmail.com
Keywords: *Cellvibrio polysaccharolyticus*; new taxa; *Proteobacteria*; *Cellvibrionaceae*; *Cellvibrionales*.
Abbreviations: ANI, average nucleotide identity; dDDH, digital DNA–DNA hybridization; FAME, fatty acid methyl ester; LB, Luria–Bertani; LBG, locust bean gum; MiGA, Microbial Genomes Atlas; R2A, Reasoner's 2A; RAST, Rapid Annotation using Subsystem Technology; TSA, tryptic soy agar; TSB, tryptic soy broth.
The GenBank accession numbers for the 16S rRNA gene sequence and the whole genome of *Cellvibrio polysaccharolyticus* strain Ka43^T are MF471354 and PRDL00000000, respectively.
The GenBank accession numbers of revealed cellulases are MBE8717239, MBE8716954, xylanases are MBE8716900, MBE8718199, MBE8716193, MBE8716206 and mannanases are MBE8716914, MBE8717822, MBE8718218, MBE8716162.
Three supplementary figures are available with the online version of this article.

the present work was to determine the taxonomic position of strain Ka43^T and its description using a polyphasic approach.

ISOLATION

Strain Ka43^T was isolated from an agricultural field on the Great Hungarian Plain as part of an isolation campaign of bacteria with polysaccharide-degrading ability. The approximate geographical coordinates are 47° 11′ 56″ N 19° 00′ 46″ E. The sample was collected in October 2016, from circa 30 cm depth. Before sampling, maize was harvested from the field. The soil was fertilised and its pH was moderately alkaline. After sampling, the soil particles were homogenised by vortexing and serially diluted with peptone water (9 g peptone, 1 g NaCl, in 1000 ml dH_2O). It was subsequently spread onto locust bean gum (LBG)-containing agar (1 g $NaNO_3$, 1 g K_2HPO_4, 3 g NaCl, 0.5 g $MgCl_2$, 0.5 g yeast extract, 0.5 g peptone, 3 g LBG, 25 g agar, 1000 ml dH_2O) and incubated at 10 °C for 5 days. Single colonies on the plates were purified on the same medium. The isolate was routinely maintained on tryptic soy agar (TSA) medium (DSM medium No. 545, dsmz.de) at 28 °C and pH 7.5.

16S rRNA GENE PHYLOGENY

DNA was extracted from Ka43^T liquid culture grown in tryptic soy broth (TSB) medium. Genomic DNA isolation and 16S rRNA gene amplification were performed according to Tóth *et al.* [8]. The partial 16S rRNA gene sequence of strain Ka43^T was compared with the EzBioCloud Database (www.ezbiocloud.net/taxonomy) [9] for an approximate phylogenetic affiliation. After Sanger sequencing of the 16S rRNA gene, a genome sequencing project of Ka43^T was carried out, which revealed three 16S rRNA gene copies in the genome. The three copies have the same sequence. The 16S rRNA gene sequence of strain Ka43^T obtained by the Sanger method was compared with the extracted 16S rRNA gene sequence from the genome assembly and showed 100% similarity.

According to the comparisons with the full 16S rRNA gene sequences, the highest level of sequence similarity occurred with *Cellvibrio diazotrophicus* E50^T (97.16%) [6], followed by *Cellvibrio zantedeschiae* TPY-10^T (96.43%) [10], *Cellvibrio gandavensis* R-4069^T (96.36%) [11] and *Cellvibrio mixtus* subsp. *mixtus* ACM 2601^T (96.01%) [4].

Phylogenetic trees were reconstructed using the neighbour-joining [12] and maximum-likelihood [13] methods with Kimura's two-parameter calculation model and the maximum-parsimony algorithm [14] using MEGA version 7.0 [15]. Tree topologies and distances were evaluated by bootstrap analysis based on 1000 replicates. The overall topology of the maximum-likelihood tree is similar to those of the neighbour-joining and maximum parsimony trees. Results of phylogenetic analysis based on 16S rRNA gene sequences suggest that strain Ka43^T forms a distinct phyletic lineage within the genus *Cellvibrio* (Fig. 1).

GENOME FEATURES

The genome of strain Ka43^T was sequenced with Illumina MiSeq sequencing technology as described previously [16]. Genome assembly was performed by SPAdes version 3.9.1 and CLC NGS Cell version 11.0. Genome completeness and contamination values were examined by TypeMet tool of the Microbial Genomes Atlas (MiGA) server (http://microbial-genomes.org/) [17]. Annotation of the genome was performed by NCBI Prokaryotic Genome Annotation Pipeline version 4.6 with the Best-placed reference protein set and GeneMarkS+ [18, 19] and Rapid Annotation using Subsystem Technology server version 2.0 (RAST; https://rast.nmpdr.org) [20]. The completeness and contamination value

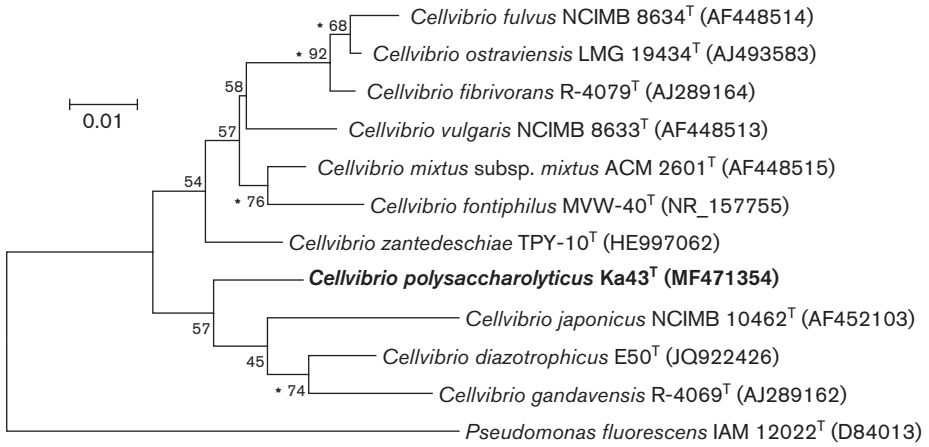

Fig. 1. Maximum-likelihood tree based on 16S rRNA gene sequences showing the phylogenetic relationships between strain Ka43^T and related taxa. Bootstrap values are shown as percentages of 1000 replicates; only values over 50% are shown. Branches signed with an asterisk occurred with every tree-making algorithm used in the study. *Pseudomonas fluorescens* IAM 12022^T was used as an outgroup. Bar, 0.01 substitution per nucleotide position.

of the genome are 100.0 and 0.9%, respectively. Other quality labels of genome sequencing and assembly are as follows: 306-fold genome coverage; N50 value, 4.258.256; and number of contigs, 2. The genome size and G+C content of Ka43^T are 4.261.962 bp and 51.6 mol%. According to the annotation there are 3637 genes, 3575 CDSs and 62 RNA genes in the genome. The coding density is 87.54%. The phylogenetically closest neighbours with sequenced genomes were determined using the Type Strain Genome Server (https://tygs.dsmz.de/) [21] and MiGA [17] servers. Genome-based relatedness between Ka43^T and *C. japonicus* UDEA107^T [5] was determined based on average nucleotide identity (ANI) using the OrthoANI (www.ezbiocloud.net/tools/ani) algorithm [22] and digital DNA–DNA hybridization (dDDH; identities/HSP length) with the Genome-to-Genome Distance Calculator (DSMZ, http://ggdc.dsmz.de/) [23]. The OrthoANIu and dDDH values between Ka43^T and *C. japonicus* UDEA107^T are 71.03 and 20.50%, much lower than the generally accepted species boundaries of 95~96 and 70%, respectively [23–25].

The RAST analysis revealed the presence of 291 subsystems and the subsystem coverage is 29% (Fig. S1, available in the online version of this article). The genus *Cellvibrio*, especially *C. japonicus*, has been intensively studied for the degradation of polysaccharides. Several genes involved in plant cell-wall polysaccharide degradation have been identified [7, 26, 27]. Strain Ka43^T was isolated on LBG-containing minimal agar, and proved to be a cellulose decomposer. The genome annotation with the NCBI Prokaryotic Genome Annotation Pipeline reveald 10 glycoside hydrolases that may play a role in the degradation of cellulose (GenBank accession numbers: MBE8717239, MBE8716954), xylan (MBE8716900, MBE8718199, MBE8716193, MBE8716206) and mannan (MBE8716914, MBE8717822, MBE8718218, MBE8716162).

The anti-SMASH server was used to identify the secondary metabolite biosynthesis gene clusters [28]. The genome of Ka43^T contains five putative biosynthetic gene clusters (arylpolyene, resorcinol ectoine, RiPP-like and butyrolactone) in three secondary metabolite regions.

PHYSIOLOGY AND CHEMOTAXONOMY

Biomass for chemical and molecular studies was obtained by cultivation in shaker flasks (180 r.p.m.) using TSB medium at 28 °C for 32 h. Colony morphology of strain Ka43^T was tested on TSA medium by directly observing single colonies. Cell morphology of strains Ka43^T was observed by electron microscopy (Fig. S2). The Gram reaction was determined with a non-staining method as described by Buck *et al.* [29]. Oxidase activity was studied with an OXI oxidase test strip (Diagnostics s.r.o.). Catalase production was demonstrated by the methods of Barrow and Feltham [30]. Growth at different temperatures (4–50 °C), NaCl tolerance (0.5–6 % w/v) and pH tolerance (pH 4–10, at increments of 0.5 pH unit, pH values were adjusted with HCl or NaOH) were determined using TSB medium. Growth at pH 4–10 was examined in flasks and 96-well plates with continuous monitoring of optical density. Acid production from different carbon sources, the assimilation of different substrates and the enzymatic activities of strain Ka43^T were investigated with API 50 CH, API 20 NE and API ZYM kits (bioMérieux) according to the manufacturer's instructions. The API 50 CH and 20 NE tests were read after 24–48 h incubation at 25 °C. Hydrolysis of mannan, xylan and cellulose was tested by Congo red staining. Anaerobic and microaerophilic growth was checked on TSA medium using the Anaerocult A and C systems (Merck). The physiological characteristics were examined in side- by- side analysis of the two related strains, *C. gandavensis* LMG 18551^T [11] and *C. diazotrophicus* LMG 27267^T [6].

TSA medium was used for general laboratory cultivation, but the novel strain also grows well on Luria–Bertani (LB) and Reasoner's 2A (R2A) media, with no growth observed on nutrient agar and nitrogen-free medium. After 48 h at 25 °C, colonies were found to be 2.0–2.5 mm in diameter, circular, non-mucoid, smooth and creamy when grown on TSA. Cells of strain Ka43^T were observed to be Gram-reaction-negative, aerobic, positive for oxidase and catalase, and rod-shaped. Cells were found to be motile by the means of single polar flagellum (Fig. S2), grow in 0.5–3.0% (w/v) NaCl, at a pH range from pH 6.5 to 9.5 and at a temperature range between 10 and 35 °C. Optimal growth was observed at 25 °C, 0.5% (w/v) NaCl and pH 8.5. The mean cell size of Ka43^T is 0.5 µm in diameter and 1.5–2.0 µm long.

According to the API 50 CH test, Ka43^T produces acid from D-arabinose, methyl β-D-xylopyranoside, D-galactose, salicin, melibiose and trehalose but not from potassium 5-ketogluconate. β-Galactosidase activity, hydrolysis of aesculin, assimilation of glucose, arabinose, *N*-acetyl-glucosamine and maltose were demonstrated by using the API 20 NE test. In the API ZYM test, strain Ka43^T shows activities of esterase (C4), leucine arylamidase, naphthol-AS-BI-phosphohydrolase, α-glucosidase and *N*-acetyl-β-glucosaminidase. Mannan and cellulose hydrolysis were proven by Congo red staining on solid medium. More detailed phenotypic characteristics for side-by-side comparison are given in Table 1.

Analyses of chemotaxonomic traits were carried out by the DSMZ Identification Service (Braunschweig, Germany).

The fatty acid profiles of strain Ka43^T, *C. gandavensis* LMG 18551^T and *C. diazotrophicus* LMG 27267^T were analysed on active growing cultures on TSA.

According to the DSMZ Identification Service, fatty acid methyl esters (FAMEs) were obtained following the method of Miller [31] and Kuykendall *et al.* [32]. FAMEs were separated by gas chromatography, detected by a flame ionization detector using the Sherlock Microbial Identification System (MIDI) and identified by using the TSBA40 4.10 database of the Microbial Identification System. Summed feature components were identified thereafter by GC/MS.

The predominant cellular fatty acids of strain Ka43^T are summed feature 3 ($C_{16:1}$ *ω7c*/iso-$C_{15:0}$ 2-OH; 34.8%), $C_{18:1}$ *ω7c* (22.0%) and $C_{16:0}$ (19.4%). The fatty acid profile is similar to that of related strains, in accordance with the

Table 1. Differential characteristics of strain Ka43^T and type strains of phylogenetically closely related *Cellvibrio* species

Strains: 1, Ka43^T; 2, *Cellvibrio diazotrophicus* LMG 27267^T; 3, *Cellvibrio gandavensis* LMG 18551^T. Data are from this study.

Characteristic	1	2	3
Isolation source	Soil	Rhizosphere	Soil
Cell morphology	Straight rod	Curved rod	Straight rod
Yellow pigment on TSA	+	–	–
Mucoid growth on TSA	–	–	+
Temperature range (optimum) for growth (°C)	10–35 (25)	15–35 (30)	10–30 (25)
Growth with NaCl (optimum) (%)	0.5–3.0 (0.5)	0.5–5.0 (1.0)	0.5–1.0 (1.0)
Acid from (API 50 CH):			
D-Arabinose	+	–	–
L-Arabinose	+	+	–
D-Xylose	+	+	–
Methyl β-D-xylopyranoside	+	–	–
D-Galactose	+	–	–
D-Fructose	–	–	+
N-Acetyl glucosamine	+	+	–
Amygdalin	+	–	+
Arbutin	+	–	–
Salicin	+	–	–
Maltose	+	–	+
Lactose	+	–	+
Melibiose	+	–	–
Sucrose	+	–	+
Trehalose	+	–	–
Inulin	–	–	+
Raffinose	–	–	+
Starch	+	–	+
Gentiobiose	+	–	+
K-5-Ketogluconate	–	+	+
API 20 NE assay:			
β-Galactosidase	+	+	–
Glucose	+	+	–
Arabinose	+	+	–
N-Acetyl-glucosamine	+	+	–
Maltose	+	+	–
API ZYM assay:			
Esterase (C4)	+	–	–
Leucine arylamidase	+	+	–
α-Glucosidase	+	–	–
N-Acetyl-β-glucosaminidase	+	+	–

Table 2. Cellular fatty acid composition of strain Ka43^T and type strains of phylogenetically closely related *Cellvibrio* species

Strains: 1, Ka43^T; 2, *Cellvibrio diazotrophicus* LMG 27267^T; 3, *Cellvibrio gandavensis* LMG 18551^T; 4, *Cellvibrio zantedeschiae* TPY-10^T; 5, *Cellvibrio mixtus* ACM 2601^T. Data for Ka43^T, *Cellvibrio diazotrophicus* LMG 27267^T and *Cellvibrio gandavensis* LMG 18551^T are from this study. Data for *Cellvibrio zantedeschiae* TPY-10^T and *Cellvibrio mixtus* ACM 2601^T are from Shue *et al.* [10]. –, Not detected; TR, trace amount (<1%).

Fatty acid	1	2	3	4	5
$C_{10:0}$	TR	2.7	1.3	1.5	2.9
$C_{12:0}$	7.2	4.8	4.5	5.8	4.0
$C_{14:0}$	1.3	1.2	TR	6.3	–
$C_{16:0}$	19.4	16.9	22.9	34.1	23.5
$C_{17:0}$	1.7	2.0	1.6	2.3	1.0
$C_{18:0}$	2.3	3.8	1.0	1.9	2.0
$C_{10:0}$ 3-OH	2.7	3.5	2.1	1.8	4.9
$C_{12:0}$ 2-OH	1.4	2.5	–	– or TR	– or TR
$C_{12:0}$ 3-OH	3.6	4.3	3.3	1.5	2.8
$C_{12:1}$ 3-OH	–	–	3.0	5.8	–
$C_{18:1}$ $\omega 7c$	22.0	16.4	20.4	12.3	15.1
$C_{18:1}$ $\omega 9c$	–	–	–	1.6	1.5
anteiso-$C_{14:0}$	–	–	–	2.2	2.5
anteiso-$C_{17:1}$ $\omega 9c$	–	–	–	–	2.5
cyclo-$C_{19:0}$ $\omega 8c$	–	1.7	–	– or TR	– or TR
Summed feature 3 ($C_{16:1}$ $\omega 7c$/iso-$C_{15:0}$ 2-OH/ $C_{16:1}$ $\omega 6c$)	34.8	37.7	37.7	19.8	34.8

emended description of the genus *Cellvibrio* [5, 6, 10, 11]. However, the ratios of the different components are different. The complete fatty acid composition is shown in Table 2.

The respiratory quinones were extracted from freeze-dried material and purified by silica-based solid phase extraction. Purified samples were further analysed by HPLC and a UHPLC-ESI-qTOF system [33, 34]. The only respiratory quinone of Ka43^T is ubiquinone-8 (Q-8).

Polar lipids were studied according to Tindall *et al.* [33–35]. Strain Ka43^T exhibits a complex polar lipid profile consisting of phosphatidylethanolamine and phosphatidylglycerol as dominant elements and an uncharacterized aminolipid, four uncharacterized phospholipids and two uncharacterized lipids (Fig. S3). However, the domination of phosphatidylethanolamine and phosphatidylglycerol are characteristic of the other species in the genus, the presence and ratio of minor components are different [5, 10, 36]. Regarding polar lipids, the presence of only one aminolipid in the profile is a distinctive characteristic.

ECOLOGICAL ROLE AND POLYSACCHARIDE DEGRADATION

Members of the genus *Cellvibrio* are well known for their cellulolytic activity and can be isolated from soil, the rhizosphere or aquatic environments [5, 7]. According to metabolic tests and its ability to grow in different culture media, strain Ka43^T is a chemoorganotrophic organism. The isolation of Ka43^T was performed on LBG-containing agar plates, and the strain can also use xylan and cellulose as sole carbon and energy sources. Cellulose and mannan degradation was proved by Congo red staining; however, hydrolization of xylan could not be detected.

Regarding cellulose degradation, the strategies of aerobic and anaerobic microorganisms differ. Aerobic microbes like Ka43^T secrete extracellular enzymes with cellulose-binding modules into the environment, but anaerobic microbes have cell-associated cellulases. Due to the complex structure of a plant cell wall, its deconstruction needs the collective work of several enzymes. The majority of these enzymes belong to the family of glycoside hydrolases (for example endoglucanases, exoglucanases, xylanases, etc.), but effectiveness can be increased by representatives of other enzyme families (for example carbohydrate esterases, polysaccharide liases) [37]. Based on genome sequence data, ten glycoside hydrolase (GH) genes were identified in Ka43^T. According to the genome annotation and the Interpro database (https://www.ebi.ac.uk/interpro/), one glycosid hydrolase family 9 (GH9; locus tag: C4F51_07085) and one GH6 (locus tag: C4F51_08575) enzyme gene are related to cellulose degradation. Four genes were found to be associated with mannan degradation, two of these belong to the GH26 family (locus

tags: C4F51_06885 and C4F51_11565) and two to the GH5 family (locus tags: C4F51_13570 and C4F51_03065). Despite the fact that xylan degradation could not be detected, three GH43 (C4F51_13475, C4F51_03220 and C4F51_03290) and one GH10 (Ka43xyl1; locus tag: C4F51_06815) glycoside hydrolases related to xylan degradation were also identified. Further investigating the ability of strain Ka43^T to degrade xylan, we cloned and expressed the GH family 10 xylanase as described previousliy [38]. The xylanase activity of Ka43xyl1 on arabinoxylan was proved by reducing sugar assay [39].

In nature, lignocellulose utilization is carried out by multiple coexisting cellulolytic species together with many noncellulolytic species. The interaction of enzymes produced by several microorganisms assures high effectiveness [40]. Ramson-Jones *et al.* examined the microbial communities in a waste disposal dump, and they could identify the genes of more than 8000 enzymes participating in the modification of carbohydrates [41]. In light of these, strain Ka43^T can play a role in the breakdown of lignocellulosic plant residues, thereby promoting the biogeochemical cycle of carbon in agricultural soil.

In soils, the lack of fixed nitrogen and other nutrients may limit the microbial growth. *Cellvibrio diazotrophicus* E50^T, the closest relative of Ka43^T, is able to fix molecular nitrogen [6], but strain Ka43^T was unable to grow on nitrogen-free medium and the *nifH* gene was not present in its genome.

PROTOLOGUE

In conclusion, strain Ka43^T is a member of the genus *Cellvibrio* and shows a number of characteristics that differentiate it from other *Cellvibrio* species. According to 16S rRNA gene based phylogenetic trees, strain Ka43^T occupies a separate lineage in the genus. The 16S rRNA gene sequence similarities to the closest relatives (*C. diazotrophicus* E50^T, 97.16%; *C. gandavensis* R-4069^T, 96.36%) also indicate its distance from other species. Distinctness of Ka43^T is confirmed by the following phenotypic traits: only one aminolipid in the polar lipid profile; yellow pigment on TSA; and acid production from methyl β-D-xylopyranoside, arbutin and salicin, but not from K-5-ketogluconate. Phenotypic, biochemical, chemotaxonomic and phylogenetic data of strain Ka43^T support its classification as representing a novel species of *Cellvibrio*, for which the name *Cellvibrio polysaccharolyticus* sp. nov. is proposed.

DESCRIPTION OF *CELLVIBRIO POLYSACCHAROLYTICUS* SP. NOV.

Cellvibrio polysaccharolyticus (po.ly.sac.cha.ro.ly'ti.cus. Gr. masc. adj. *polys* many; Gr. neut. n. *sakcharon* sugar; Gr. masc. adj. *lytikos* dissolving; N.L. masc. adj. *polysaccharolyticus* many sugars dissolving).

Cells are strictly aerobic, Gram-reaction-negative straight rods and motile by a single polar flagellum. Grows well on TSA, LB and R2A plates, but no growth is observed on nutrient agar. Colonies have yellow pigmentation on TSA after 48 h incubation. Cells are 0.5 µm in diameter and 1.5–2.0 µm long. Grows at 10–35 °C (optimum, 25 °C) and NaCl concentrations of 0.5–3 w/v % (optimum, 0.5 w/v %). Positive for oxidase, catalase, C4 esterase, leucine arylamidase, naphthol-AS-BI-phosphohydrolase, *N*-acetyl-β-glucosaminidase, β-galactosidase, α-glucosidase, CM-cellulose and mannan hydrolysis. Acid is produced from D-arabinose, L-arabinose, D-xylose, methyl β-D-xylopyranoside, D-galactose, D-glucose, *N*-acetyl glucosamine, amygdalin, arbutin, aesculin, salicin, cellobiose, maltose, lactose, melibiose, sucrose, trehalose, starch and gentiobiose. The major fatty acids are summed feature 3 ($C_{16:1}$ *ω7c* and/or iso-$C_{15:0}$ 2-OH), $C_{18:1}$ *ω7c* and $C_{16:0}$. The only respiratory quinone is Q-8. The major polar lipids are phosphatidylethanolamine and phosphatidylglycerol. The type strain is Ka43^T (=LMG 31577^T=NCAIM B.02637^T), which was isolated from an agricultural field on the Great Hungarian Plain, Hungary. The DNA G+C content of the type strain is 51.6 mol%.

Funding information
This work was supported by the NVKP_16-1-2016-0009 project. Ákos Tóth was supported by the János Bolyai Research Scholarship of the Hungarian Academy of Sciences.

Acknowledgements
We thank Professor Aharon Oren (The Hebrew University of Jerusalem, Israel) for the assistance with the nomenclature. The authors are grateful to Károly Bóka (Eötvös Loránd University, Hungary) for the EM pictures.

Conflicts of interest
The authors declare that there are no conflicts of interest.

References

1. Spring S, Scheuner C, Göker M, Klenk H-P. A taxonomic framework for emerging groups of ecologically important marine Gammaproteobacteria based on the reconstruction of evolutionary relationships using genome-scale data. *Front Microbiol* 2015;6:281.
2. Winogradsky S. Etudes sur La microbiologie Du sol. sur La degradation de la cellulose dans Le sol. *Ann Inst Pasteur* 1929;43:549–633.
3. Skerman VBD, Sneath PHA, McGowan V. Approved lists of bacterial names. *Int J Syst Evol Microbiol* 1980;30:225–420.
4. Blackall LL, Hayward AC, Sly LI. Cellulolytic and dextranolytic Gram-negative bacteria: revival of the genus *Cellvibrio*. *J Bacteriol* 1985;59:81–97.
5. Humphry DR, Black GW, Cummings SP. Reclassification of '*Pseudomonas fluorescens* subsp. cellulosa' NCIMB 10462 (Ueda *et al.* 1952) as *Cellvibrio japonicus* sp. nov. and revival of *Cellvibrio vulgaris* sp. nov., nom. rev. and *Cellvibrio fulvus* sp. nov., nom. rev. *Int J Syst Evol Microbiol* 2003;53:393–400.
6. Suarez C, Ratering S, Kramer I, Schnell S. *Cellvibrio diazotrophicus* sp. nov., a nitrogen-fixing bacteria isolated from the rhizosphere of salt meadow plants and emended description of the genus *Cellvibrio*. *Int J Syst Evol Microbiol* 2014;64:481–486.
7. DeBoy RT, Mongodin EF, Fouts DE, Tailford LE, Khouri H *et al.* Insights into plant cell wall degradation from the genome sequence of the soil bacterium *Cellvibrio japonicus*. *J Bacteriol* 2008;190:5455–5463.
8. Tóth Á, Baka E, Bata-Vidács I, Luzics S, Kosztik J *et al.* Micrococcoides hystricis gen. nov., sp. nov., a novel member of the family Micrococcaceae, phylum Actinobacteria. *Int J Syst Evol Microbiol* 2017;67:2758–2765.

9. Kim O-S, Cho Y-J, Lee K, Yoon S-H, Kim M *et al*. Introducing EzTaxon-e: a prokaryotic 16S rRNA gene sequence database with phylotypes that represent uncultured species. *Int J Syst Evol Microbiol* 2012;62:716–721.
10. Sheu S-Y, Huang C-W, Hsu M-Y, Sheu C, Chen W-M. *Cellvibrio zantedeschiae* sp. nov., isolated from the roots of *Zantedeschia aethiopica*. *Int J Syst Evol Microbiol* 2017;67:3615–3621.
11. Mergaert J, Lednická D, Goris J, Cnockaert MC, De Vos P *et al*. Taxonomic study of *Cellvibrio* strains and description of *Cellvibrio ostraviensis* sp. nov., *Cellvibrio fibrivorans* sp. nov. and *Cellvibrio gandavensis* sp. nov. *Int J Syst Evol Microbiol* 2003;53:465–471.
12. Saitou N, Nei M. The neighbor-joining method: a new method for reconstructing phylogenetic trees. *Mol Biol Evol* 1987;4:406–425.
13. Felsenstein J. Evolutionary trees from DNA sequences: a maximum likelihood approach. *J Mol Evol* 1981;17:368–376.
14. Kimura M. A simple method for estimating evolutionary rates of base substitutions through comparative studies of nucleotide sequences. *J Mol Evol* 1980;16:111–120.
15. Kumar S, Stecher G, Tamura K. MEGA7: molecular evolutionary genetics analysis version 7.0 for bigger datasets. *Mol Biol Evol* 2016;33:1870–1874.
16. Szuróczki S, Khayer B, Spröer C, Toumi M, Szabó A *et al*. *Arundinibacter roseus* gen. nov., sp. nov., a new member of the family *Cytophagaceae*. *Int J Syst Evol Microbiol* 2019;69:2076–2081.
17. Rodriguez-R LM, Gunturu S, Harvey WT, Rosselló-Mora R, Tiedje JM *et al*. The Microbial Genomes Atlas (MiGA) webserver: taxonomic and gene diversity analysis of *Archaea* and *Bacteria* at the whole genome level. *Nucleic Acids Res* 2018;46:W282–W288.
18. Tatusova T, DiCuccio M, Badretdin A, Chetvernin V, Nawrocki EP *et al*. NCBI prokaryotic genome annotation pipeline. *Nucleic Acids Res* 2016;44:6614–6624.
19. O'Leary NA, Wright MW, Brister JR, Ciufo S, Haddad D *et al*. Reference sequence (RefSeq) database at NCBI: current status, taxonomic expansion, and functional annotation. *Nucleic Acids Res* 2016;44:D733–D745.
20. Aziz RK, Bartels D, Best AA, DeJongh M, Disz T *et al*. The RAST server: rapid annotations using subsystems technology. *BMC Genomics* 2008;9:75.
21. Meier-Kolthoff JP, Göker M. TYGS is an automated high-throughput platform for state-of-the-art genome-based taxonomy. *Nat Commun* 2019;10:2182.
22. Yoon SH, SM H, Lim JM, Kwon SJ. Chun J a large-scale evaluation of algorithms to calculate average nucleotide identity. *Antonie van Leeuwenhoek* 2017;10:1281–1286.
23. Meier-Kolthoff JP, Auch AF, Klenk H-P, Göker M. Genome sequence-based species delimitation with confidence intervals and improved distance functions. *BMC Bioinformatics* 2013;14:60.
24. Goris J, Konstantinidis KT, Klappenbach JA, Coenye T, Vandamme P *et al*. DNA–DNA hybridization values and their relationship to whole-genome sequence similarities. *Int J Syst Evol Microbiol* 2007;57:81–91.
25. Richter M, Rosselló-Móra R. Shifting the genomic gold standard for the prokaryotic species definition. *Proc Natl Acad Sci USA* 2009;106:19126–19131.
26. Gardner JG. Polysaccharide degradation systems of the saprophytic bacterium *Cellvibrio japonicus*. *World J Microbiol Biotechnol* 2016;32:121.
27. Wu Y-R, Lin B, Yu Y. Draft genome sequence of a xylanase-producing bacterial strain, *Cellvibrio mixtus* J3-8. *Genome Announc* 2014;2:e01281–14.
28. Blin K, Shaw S, Steinke K, Villebro R, Ziemert N *et al*. antiSMASH 5.0: updates to the secondary metabolite genome mining pipeline. *Nucleic Acids Res* 2019;47:W81–W87.
29. Buck JD. Nonstaining (KOH) method for determination of Gram reactions of marine bacteria. *Appl Environ Microbiol* 1982;44:992–993.
30. Barrow GI, Feltham RKA. *Cowan and Steel's Manual for the Identification of Medical Bacteria*, 3rd ed. Cambridge: Cambridge University Press; 2004.
31. Miller LT. A single derivatization method for bacterial fatty acid methyl esters including hydroxy acids. *J Clin Microbiol* 1982;16:584–586.
32. Kuykendall LD, Roy MA, O'Neill JJ, Devine TE. Fatty acids, antibiotic resistance, and deoxyribonucleic acid homology groups of *Bradyrhizobium japonicum*. *Int J Syst Bacteriol* 1988;38:358–361.
33. Tindall BJ. A comparative study of the lipid composition of *Halobacterium saccharovorum* from various sources. *Syst Appl Microbiol* 1990a;13:128–130.
34. Tindall BJ. Lipid composition of *Halobacterium lacusprofundi*. *FEMS Microbiol Lett* 1990b;66:199–202.
35. Tindall BJ, Sikorski J, Smibert RM, Kreig NR. Phenotypic characterization and the principles of comparative systematics. *Methods for General and Molecular Microbiology*, 3rd; 2007. pp. 330–393.
36. Chen W-M, Liu L-P, Sheu S-Y. *Cellvibrio fontiphilus* sp. nov., isolated from a spring. *Int J Syst Evol Microbiol* 2017;67:2532–2537.
37. Lynd LR, Weimer PJ, van Zyl WH, Pretorius IS. Microbial cellulose utilization: fundamentals and biotechnology. *Microbiol Mol Biol Rev* 2002;66:506–577.
38. Tóth Á, Barna T, Szabó E, Elek R, Hubert Á *et al*. Cloning, expression and biochemical characterization of endomannanases from Thermobifida species isolated from different niches. *PLoS One* 2016;11:e0155769.
39. Miller GL. Use of dinitrosalicylic acid reagent for determination of reducing sugar. *Anal Chem* 1959;31:426–428.
40. Montella S, Amore A, Faraco V. Metagenomics for the development of new biocatalysts to advance lignocellulose saccharification for bioeconomic development. *Crit Rev Biotechnol* 2016;36:998–1009.
41. Ransom-Jones E, McCarthy AJ, Haldenby S, Doonan J, McDonald JE. Lignocellulose-degrading microbial communities in landfill sites represent a repository of unexplored biomass-degrading diversity. *mSphere* 2017;2:e00300–00317.