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Abstract:	Based on genetic, chemotaxonomic and phenotypic characteristics, a novel species belonging to the genus Vibrio is described. The facultative anaerobic strain S2757T was isolated from a mussel collected in the Solomon Sea (Solomon Islands). Phylogenetic analyses based on sequences of 16S rRNA and fur genes indicated the affiliation of the strain to a new species. This observation was supported by a multilocus sequence analysis (MLSA) including sequences of the housekeeping genes 16S rRNA, gyrB, pyrH, recA and topA. In silico DNA-DNA hybridization (DDH) and Average Nucleotide Identity (ANI) values comparing the genomic sequence of strain S2757T with those of closely related type strains were lower than 23 and 82 %, respectively. The DNA G+C content of the strain was 45.3 mol%. Phenotypic and chemotaxonomic analyses clearly differentiated the strain from other Vibrio species. Hence, strain S2757T should be considered a novel species in the genus Vibrio. The name Vibrio galatheae sp. nov. is proposed, with S2757T (= DSM 100497T = LMG 28895T) as the type strain.

Vibrio galatheae sp. nov., a novel member of the *Vibrionaceae* family isolated from the Solomon Sea.

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The GenBank accession number for the *fur* gene sequence of *Vibrio sinaloensis* DSM 21326^T is KT380049**.** GenBank accession numbers for the whole genome sequences of *Vibrio hepatarius* DSM 19134^T, *Vibrio xuii* DSM 17185^T, and *Vibrio nereis* DSM 19584^T are LHPI01, LHPK01, LHPJ01, respectively. Accession numbers of all nucleotide sequences used in this work, including those previously publicly available, are listed in Table S1.

Based on genetic, chemotaxonomic and phenotypic characteristics, a novel species belonging to the genus *Vibrio* is described. The facultative anaerobic strain S2757^T was isolated from a mussel collected in the Solomon Sea (Solomon Islands). Phylogenetic analyses based on sequences of 16S rRNA and *fur* genes indicated the affiliation of the strain to a new species. This observation was supported by a multilocus sequence analysis (MLSA) including sequences of the housekeeping genes 16S rRNA, *gyrB*, *pyrH*, *recA* and *topA*. *In silico* DNA-DNA hybridization (DDH) and Average Nucleotide Identity (ANI) values comparing the genomic sequence of strain S2757^T with those of closely related type strains were lower than 23 and 82 %, respectively. The DNA G+C content of the strain was 45.3 mol%. Phenotypic and chemotaxonomic analyses clearly differentiated the strain from other *Vibrio* species. Hence, strain S2757^T should be considered a novel species in the genus *Vibrio*. The name *Vibrio galatheae* sp. nov. is proposed, with S2757^T (= DSM 100497^T = LMG 28895^T) as the type strain.

Members of the *Vibrionaceae* family are Gram-negative bacteria widespread in aquatic environments (Thompson *et al.*, 2004). Vibrios have been isolated as both planktonic and surface-associated organisms from several ecosystems, including seawater, marine sediments and animals (Thompson *et al.*, 2004). The number of vibrios colonizing different environmental niches can vary over orders of magnitude, depending on factors such as availability of nutrients, temperature and salinity (Takemura *et al.*, 2014). For instance, *Vibrio* species were shown to account for more than 50% of the total microbiota during a bacterial bloom that was possibly due to an increase in the concentration of available nutrients (Gilbert *et al.*, 2012).

A number of vibrios have been intensively studied because of their role as pathogens (Ben-Haim *et al.*, 2003; Faruque *et al.*, 1998; Jones & Oliver, 2009; Ramamurthy *et al.*, 2014) and symbionts (Nyholm *et al.*, 2000). In recent years, *Vibrionaceae* have also emerged as a reservoir of secondary metabolites with therapeutic applications, including antibacterial, anticancer and antifungal activities (Månsson *et al.*, 2011). Here, we report the taxonomic characterization of a strain belonging to the genus *Vibrio*. Strain S2757^T was isolated in 2007

from a mussel collected in the Solomon Sea (Solomon Islands) during the Galathea 3 global research expedition (http://www.galathea3.dk/uk) and was affiliated to the *Vibrionaceae* family based on its 16S rRNA gene sequence, as previously described (Gram *et al.*, 2010).

The type strains included in this study *V. brasiliensis* DSM 17184^T (Thompson *et al.*, 2003a), *V. orientalis* DSM 19136^T (Yang *et al.*, 1983), *V. hepatarius* DSM 19134^T (Thompson *et al.*, 2003b), *V. tubiashii* DSM 19142^T (Hada *et al.*, 1984), *V. sinaloensis* DSM 21326^T (Gomez-Gil *et al.*, 2008), *V. xuii* DSM 17185^T (Thompson *et al.*, 2003a) and *V. nereis* DSM 19584^T (Baumann *et al.*, 1980) were obtained from the German Collection of Microorganisms and Cell Cultures (DSMZ, Braunschweig, Germany). All strains were routinely cultivated on Marine Agar (MA) (212185, Difco) or in Marine Broth (MB) (279110, Difco) at 25 °C. Strain S2757^T grew as small (2-4 mm), round, beige colonies after 48 hours on MA at 25 °C.

Cell morphology of strain S2757^T was observed by means of phase-contrast microscopy (x 1,000 magnification in Olympus BX51) and Scanning Electron Microscopy (FEI Quanta 200 FEG ESEM) after growth in filtered MB for 24 hours at 25 °C. Gram testing and catalase activity were assessed with the 3 % KOH (Gregersen, 1978) and the 3 % H_2O_2 (Cowan, 1974) methods, respectively. Oxidase activity was determined on a BBL™ DrySlide™ Oxidase (231746, BD Diagnostics) following manufacturers' instructions. Test of susceptibility to the vibriostatic agent O/129 (2,4-diamino-6,7-diisopropyl pteridine,10 and 150 µg/disc) was performed on Iso-sensitest agar (CM04741B, Oxoid) supplemented with 1.5 % NaCl and incubation at 25 °C for 48 hours. Salinity requirements of strain S2757^T were determined in synthetic ZoBell medium (5 g/L Bacto peptone, 1 g/L yeast extract, 0.1 g/L ferric citrate) (ZoBell, 1941) with different NaCl concentrations (0 to 9 % w/v) at 28 °C. Growth was assessed using a microplate reader (Spectra Max i3, Molecular Devices). The temperature range for growth was determined on MA. The ability of strain S2757^T to grow in anaerobic conditions was tested on MA at 25 °C using an anaerobic jar and anaerobic atmosphere generation bags (68061, Fluka).

Physiological and biochemical characterization using API 20 NE strips (20050, Biomerieux), API ZYM strips (25200, Biomerieux) and Biolog GN2 plates (Biolog Inc., USA) was done on strain S2757^T and on the closely related species *V. tubiashii* DSM 19142^T, *V. brasiliensis* DSM 17184^T, *V. orientalis* DSM 19136^T and *V. hepatarius* DSM 19134^T. Bacterial suspensions were prepared in 1.5 % w/v NaCl using biomass grown overnight on MA at 25 °C. Inoculation of strips and plates was done in agreement with the manufacturers' instructions. Cellular fatty acid of strain S2757^T and related species were analyzed as methyl esters (FAME) by gas chromatography. The analysis was performed in duplicates by the DSMZ using biomass grown for 24 hours on MA at 25 °C and according to the instructions of the Microbial Identification System (MIDI Inc., USA). Cell morphology of strain S2757^T and related species was observed on thiosulfate-citrate-bile-sucrose (TCBS, CM0333, Oxoid) agar plates. Detailed morphological, physiological and biochemical features distinguishing strain S2757^T from related species are summarized in Table 1 and in the species description. The complete list of the results of the performed tests and analyses is available in Table S2.

Cells of S2757^T were Gram negative, slightly curved rod shaped (1.5 ± 0.4 μm in length) and motile by means of one polar flagellum (0.7 ± 0.2 μm in length) (Figure S1). Strain S2757^T was catalase and oxidase positive, and sensitive to the vibriostatic agent O/129. NaCl was required for growth and tolerated up to a concentration of 8 % w/v. Strain S2757^T grew as green, small (2-3 mm) colonies on TCBS agar. Strain S2757^T produced α-glucosidase but not acid phosphatase, N-acetyl-β-glucosaminidase and lipase. The strain could utilize D-glucose-6-phosphate and D-alanine, but not L-threonine, L-proline and sucrose. The major cellular fatty acids of strain S2757^T were summed feature 3 (16:1 ω7c and/or 16:1ω6c and/or 15 iso 2OH), 16:0, and summed feature 8 (18:1 ω7c and/or 18:1 ω6c). These values were comparable to those of the closely related species; however, the fatty acid pattern of strain S2757^T was distinct due to the presence of a relatively high amount (9.8% in total) of the

fatty acids 15:0 iso, 16:0 iso and 17:0 iso compared to the patterns of the other analyzed species, for which values were lower than 1.2 %.

For strains *V. hepatarius* DSM 19134^T, *V. xuii* DSM 17185^T and *V. nereis* DSM 19584^T no whole genome sequence was publicly available at the time this study was started. Therefore, high purity genomic DNA was obtained as described previously (Sambrook & Russel, 2001) by repeated phenol:chloroform:isoamyl alcohol purification steps followed by RNase treatment and DNA precipitation. Quantification was performed on a NanoDrop Spectrometer (Saveen Werner, Sweden) and a Qubit 2.0 Analyser (Invitrogen, United Kingdom). Genome sequencing was carried out at the NovoNordisk Foundation Center for Biosustainability (Hørsholm, Denmark). Libraries of 300-400 bp were prepared and used for 151 bp paired-end sequencing by Illumina sequencing technology on a MiSeq sequencer. Data were assembled to contigs using the *de novo* assembly algorithm of CLC Genomic Workbench, version 7 (CLC Bio, Aarhus, Denmark). The list of the GenBank/EBI accession numbers of the nucleotide sequences used in this study, including those herein generated, is available in Table S1. For the *in silico* phylogenetic analysis, sequences of the single genes were obtained directly from the GenBank database or extracted from whole genome sequences based on their PGAP (NCBI Prokaryotic Genome Annotation Pipeline) annotation (Tatusova *et al.*, 2013) or by BLAST search using CLC Main Workbench Version 7.6.2 (CLC Bio, Aarhus, Denmark).

The comparison of the 1487 bp long 16S rRNA gene sequence obtained from the complete genome sequence of the new isolate with those from type strains available in the GenBank database using the BLASTN algorithm (https://blast.ncbi.nlm.nih.gov) and the Ez-Taxon-e service (http://www.ezbiocloud.net/eztaxon) confirmed that strain S2757^T belongs to the genus *Vibrio*, as previously established (Gram *et al.*, 2010). Pairwise alignment of the almost complete 16S rRNA gene sequences was carried out using CLC Main Workbench. A phylogenetic tree was constructed in MEGA6 (Tamura *et al.*, 2013) using the Neighbor-Joining method. The robustness of the tree topology was tested with 1000 bootstrap iterations (Fig.1). Based on the 16S rRNA gene sequences, strain

S2757^T was phylogenetically closely related to *V. hepatarius* DSM 19134^T, *V. brasiliensis* DSM 17184^T, *V. maritimus* R 40493^T and *V. tubiashii* DSM 19142^T sharing 98.5%, 98.3%, 98.2% and 97.8% 16S rRNA gene sequence similarity, respectively. However, due to the low interspecies resolution which can be obtained in *Vibrionaceae* by using the 16S rRNA gene sequence (Sawabe *et al.*, 2007), two phylogenetic trees based on complete sequences of the recently proposed *Vibrionaceae* phylogenetic marker *fur* gene (Machado & Gram, 2015) (Fig. 2) and on the concatenated sequences of five housekeeping genes (Fig. 3) were constructed. These phylogenetic trees were obtained as described above and elsewhere (Machado & Gram, 2015; Sawabe *et al.*, 2013; Thompson *et al.*, 2005). For the *fur* gene phylogenetic tree, gene sequences were obtained either by PCR based gene amplification followed by sequencing as described previously (Machado & Gram, 2015), or from whole genome sequences as described above. For the multilocus sequence analysis (MLSA), sequences of the 16S rRNA, DNA gyrase subunit B (*gyrB*), uridylate kinase (*pyrH*), recombinant protein RecA (*recA*) and DNA topoisomerase I (*topA*) genes were retrieved from the GenBank database or from whole genome sequences, as described above. Sequences were trimmed to a common length and concatenated to a final length of 3800 bp. Both phylogenetic trees showed that strain S2757^T was clearly separated from the other analyzed *Vibrio* species.

Whole genomes sequences of strain S2757^T and closely related species were compared by DNA-DNA Hybridization (DDH) and Average Nucleotide Identity (ANI) values obtained *in silico* using the Genome-to-Genome Distance calculator 2.0 (GGDC) provided by the DSMZ (http://ggdc.dsmz.de/) (Meier-Kolthoff *et al.*, 2013) and the Average Nucleotide Identity calculator (http://enve-omics.ce.gatech.edu/ani/) developed by the Kostas Lab (Goris *et al.*, 2007). All DDH and ANI values were below the thresholds used for species definition (70 % for DDH and 95 % for ANI) and identified *V. tubiashii* ATCC 19109^T as the closest relative of strain S2757^T, with DDH = 22.50 % and ANI = 81.13% (Table 2). The G + C content of S2757^T calculated *in silico* using CLC Main Workbench was 45.3 mol%, which is in agreement with values reported in literature for *Vibrio* species.

The presented results indicate that strain S2757^T should be classified as a novel species in the genus *Vibrio*, for which the name *Vibrio galatheae* sp. nov. is proposed.

Description of *Vibrio galatheae* sp.nov.

Vibrio galatheae (ga.la.the'ae. N.L. gen. n. galatheae, referring to the name of the Danish research expedition Galathea 3 during which the type strain was isolated).

Cells are slightly curved rods, Gram-negative and motile by means of one polar flagellum. Colonies are circular, beige in color and 2–4 mm in size after 48 hours at 25 °C on MA and round, green and 2–4 mm in size after 48 hours at 25 °C on TCBS. Growth occurs in presence of 0.5–8 % (w/v) NaCl in synthetic ZoBell medium, with optimal growth at 2–5 %. The strain grows at 15–40 °C, with optimal growth at 25–30 °C. Growth is observed under anaerobic conditions. The strain is positive for catalase and oxidase and sensitive to the vibriostatic agent O/129. Strain S2757^T reduces nitrates to nitrites, produces indole and hydrolyzes esculin. Positive for alkaline phosphatase, esterase lipase, leucine arylamidase, valine arylamidase and cysteine arylamidase but not for lipase and acid phosphatase. Strain S2757^T can utilize as sole carbon sources: N-acetyl-D-glucosamine, D-cellobiose, D-fructose, α-D-glucose, maltose, D-mannitol, D-mannose, D-trehalose, D-gluconic acid, D,L-lactic acid, D-alanine, L-alanine, L-alanyl-glycine, L-asparagine, L-aspartic acid, L-glutamic acid, glycil-L-glutamic acid, inosine, uridine thymidine, α-D-glucose-1-phosphate and D-glucose-6-phosphate. It cannot utilize: N-acetyl-D-galactosamine, sucrose, succinic acid, glycil-L-aspartic acid, L-proline, L-threonine amd glycerol. The most abundant fatty acids of strain S2757^T are summed feature 3 (comprising 16:1 ω7c and/or 16:1ω6c and/or 15 iso 2OH), 16:0, and summed feature 8 (comprising 18:1 ω7c and/or 18:1 ω6c).

The type strain, S2757^T (= DSM 100497^T = LMG 28895^T), was isolated from a mussel collected in the Solomon Sea, Solomon Islands. The DNA G+C content of the type strain is 45.3 mol%.

Acknowledgements

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Figure 1. Phylogenetic tree based on partial 16S rRNA gene sequences, obtained using the Neighbor-Joining method. Numbers at nodes indicate the level of bootstrap based on 1000 replicates; only values >50 % are shown. *Photobacterium aquae* was used as outgroup. Bar, 0.5 % estimated sequence divergence.

Figure 2. Phylogenetic tree based on complete *fur* gene sequences, obtained using the Neighbor-Joining method. Numbers at nodes indicate the level of bootstrap based on 1000 replicates; only values >50 % are shown. *Photobacterium aquae* was used as outgroup. Bar, 5 % estimated sequence divergence.

Figure 3. Phylogenetic tree based on concatenated sequences of five genes (16S rRNA, *gyrB, pyrH, recA* and *topA*; approximately 3800 bp) obtained using the Neighbor-Joining method. The sizes of the gene sequences were: 16S RNA, 1439 bp; *gyrB*, 738 bp; *pyrH*, 530 bp; *recA*, 554 bp and *topA*, 552 bp. Numbers at nodes indicate the level of bootstrap based on 1000 replicates; only values >50 % are shown. *Photobacterium aquae* was used as outgroup. Bar, 2 % estimated sequence divergence.

Table 1. Features differentiating strain S2757^T from closely related *Vibrio* species.
Species are identified as: (1) *Vibrio galatheae* sp. nov., (2) *V. brasiliensis* DSM 17184^T, (3) *V. orientalis* DSM 19136^T, (4) *V. hepatarius* DSM 19134^T, and (5) *V. tubiashii* DSM 19142^T. G, green; Y, yellow; +, positive; -, negative. All data were generated in this work in biological duplicates.

Table 2. Comparison of the genomic sequences of S2757^T and related species based on DNA-DNA Hybridization (DDH) and two-way Average Nucleotide Identity (ANI) values obtained with *in silico* methods. Taxa: (1) *V. galatheae*; (2) *V. hepatarius* DSM 19134^T; (3) *V. xuii* DSM 17185^T; (4) *V. nereis* DSM 19584^T; (5)

V. brasiliensis LMG 20546^{T} = DSM 17184^{T}; (6) *V. orientalis* DSM 19136^{T}; (7) *V. tubiashii* ATCC 19109^{T} = DSM 19142^{T}.

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Characteristic	**1**	**2**	**3**	**4**	**5**
Citrate‡	+	+	-	-	-
Malic acid‡	+	+	-	-	+
Growth in/on:					
8 % NaCl	+	-	+	-	-
TCBS (colour)	G	Y	Y	Y	Y
Production of:					
Lipase†	-	+	+	+	+
α-glucosidase†	+	-	-	-	+
Acid phosphatase†	-	+	+	-	-
N-acetyl-β-glucosaminidase†	-	-	+	+	+
Utilization of:					
D-glucose-6-phosphate	+	+	+	-	+
L-threonine	-	+	-	+	-
L-proline	-	+	+	-	-
D-alanine	+	+	-	-	-
Sucrose	-	+	-	+	+
FAME:					
16:0	19.5 ± 0.2	24.8 ± 0.3	23.3 ± 0.4	22.6 ± 0.2	22.2 ± 0.2
15:0 iso	2.1	0.3	0.2	0.1	0.1
16:0 iso	3.7	0.5	0.6	1.1	0.4
17:0 iso	4.0	0.3	-	0.1	0.2
Summed feature 3*	34.7 ± 0.4	35.9 ± 0.4	44.7 ± 0.4	39 ± 0.4	35.8 ± 0.3
Summed feature 8*	15.8 ± 0.2	19.5 ± 0.2	10.6 ± 0.2	18.3 ± 0.2	23.0 ± 0.2

† API ZYM

‡ API 20 NE

* Summed feature 3: one or more of 16:1 ω7c, 16:1ω6c and/or 15:0 iso 2OH. Summed feature 8: 18:1 ω7c and/or 18:1 ω6c.

Table 2. Comparison of the genomic sequences of S2757^T and related species based on DNA-DNA Hybridization (DDH) and two-way Average Nucleotide Identity (ANI) values obtained with *in silico* methods. Taxa: (1) *V. galatheae*; (2) *V. hepatarius* DSM 19134^T; (3) *V. xuii* DSM 17185^T; (4) *V. nereis* DSM 19584^T; (5) *V. brasiliensis* LMG 20546^T = DSM 17184^T; (6) *V. orientalis* DSM 19136^T; (7) *V. tubiashii* ATCC 19109^T = DSM 19142^T.

		ANI ± SD (%)						
		1	**2**	**3**	**4**	**5**	**6**	**7**
DDH ± SD (%)	**1**		80.22 ± 6.17	80.51 ± 5.90	79.81 ± 5.89	80.32 ± 5.73	80.56 ± 5.81	81.13 ± 5.48
	2	20.00 ± 2.31		80.35 ± 5.76	82.56 ± 6.48	80.45 ± 6.00	80.24 ± 5.79	80.53 ± 6.16
	3	20.80 ± 2.33	20.90 ± 2.33		80.41 ± 6.06	81.29 ± 6.06	88.74 ± 4.55	81.61 ± 6.13
	4	19.70 ± 2.30	22.30 ± 2.36	35.50 ± 2.48		80.06 ± 5.91	80.06 ± 5.72	80.26 ± 5.90
	5	20.60 ± 2.32	20.30 ± 2.32	21.20 ± 2.34	19.90 ± 2.30		81.28 ± 6.01	81.14 ± 5.90
	6	20.70 ± 2.33	20.30 ± 2.31	38.00 ± 2.49	20.30 ± 2.32	21.40 ± 2.34		81.83 ± 6.99
	7	22.50 ± 2.36	20.60 ± 2.32	21.80 ± 2.35	20.30 ± 2.31	21.60 ± 2.34	21.80 ± 2.35	

Figure 1

Click here to download Figure 4. Figure 1.eps

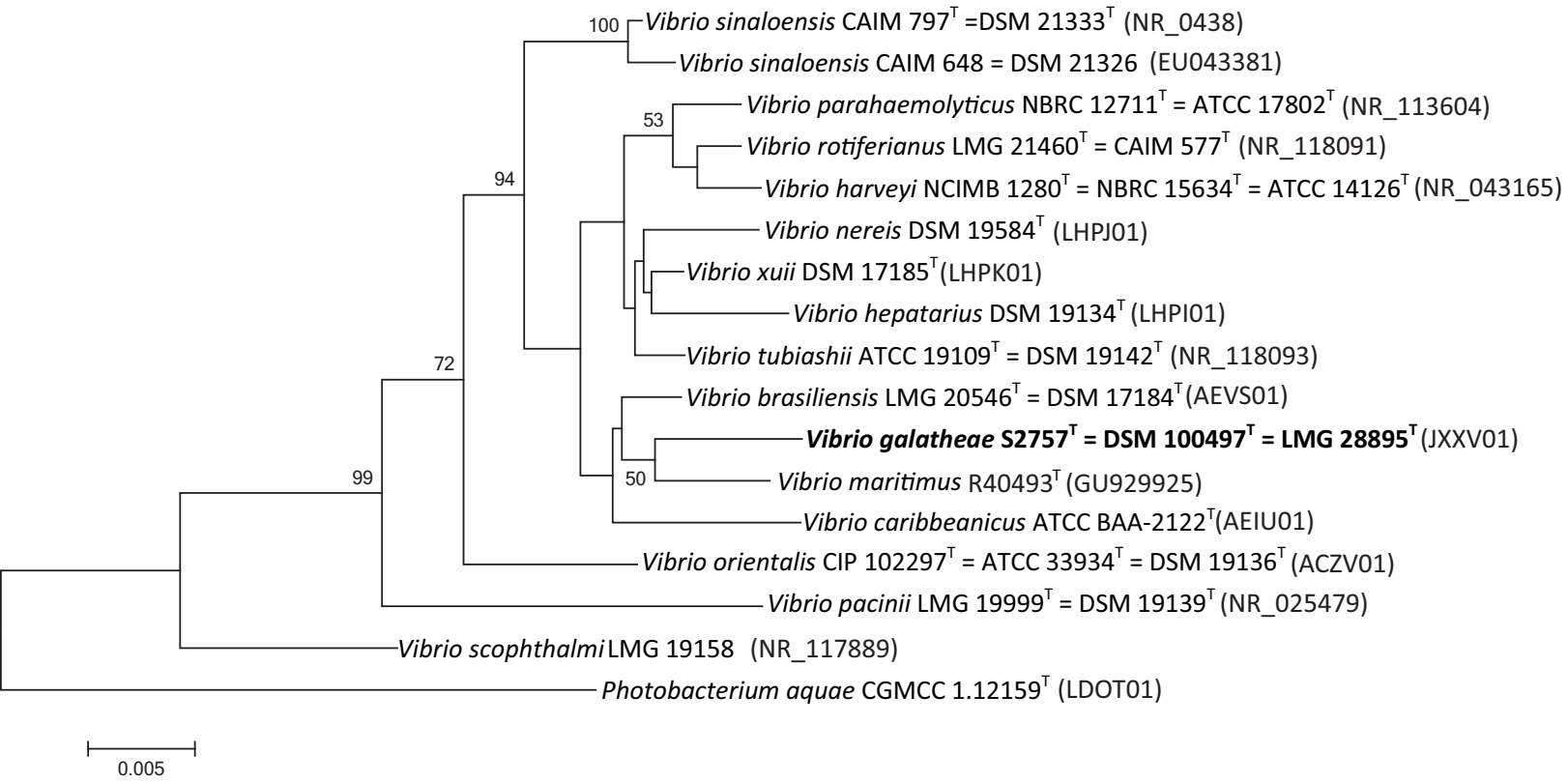


Figure 2

Click here to download Figure 5. Figure 2.eps

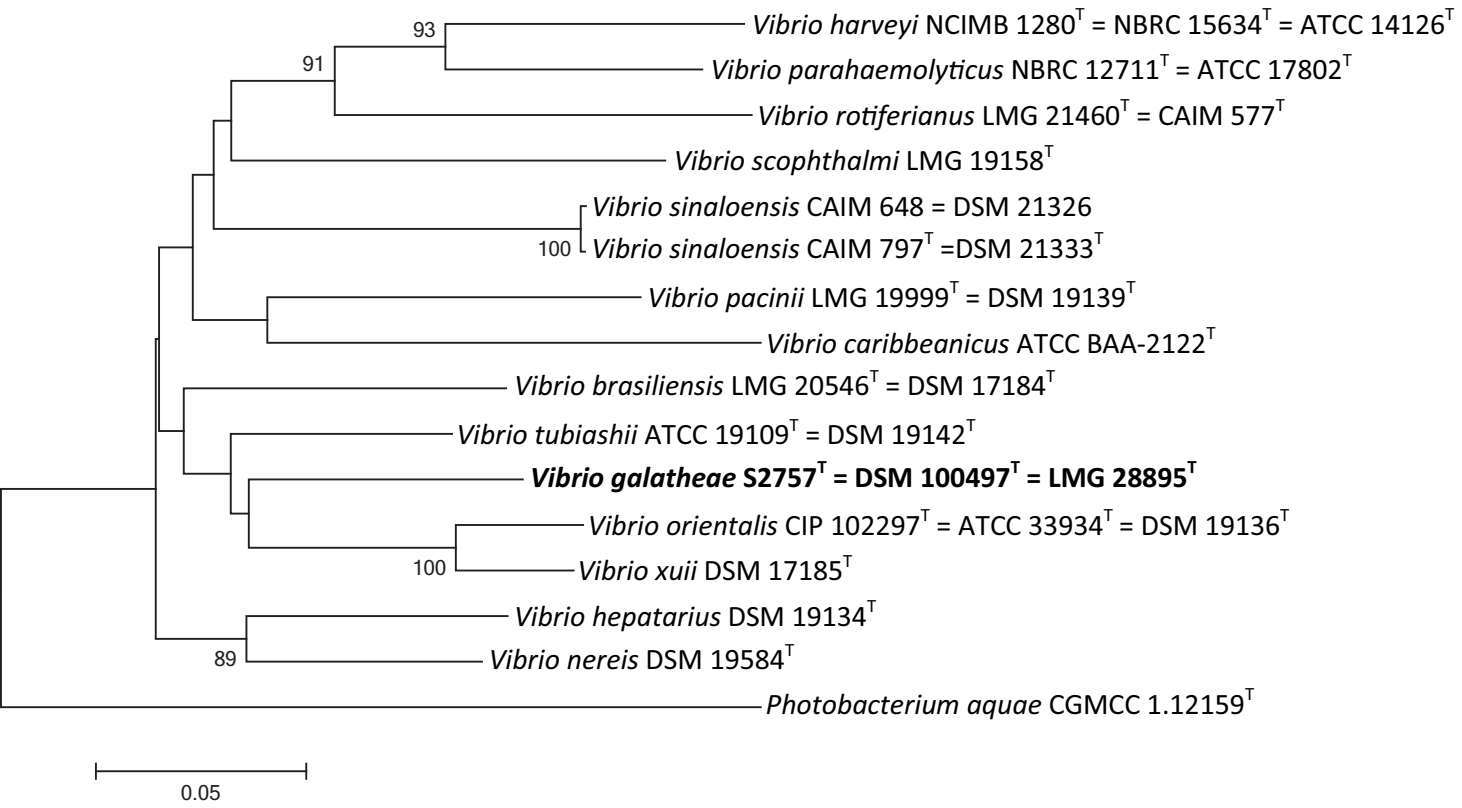
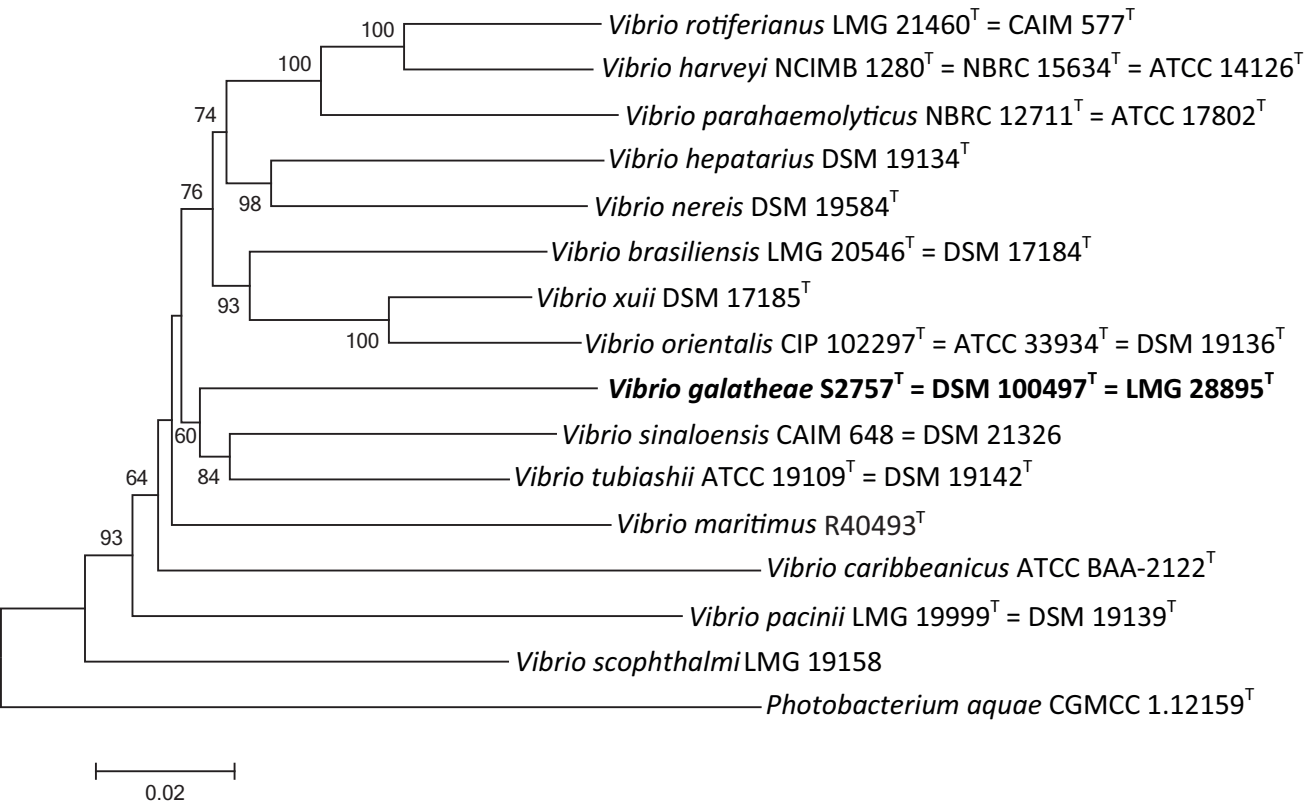


Figure 3

Click here to download Figure 6. Figure 3.eps



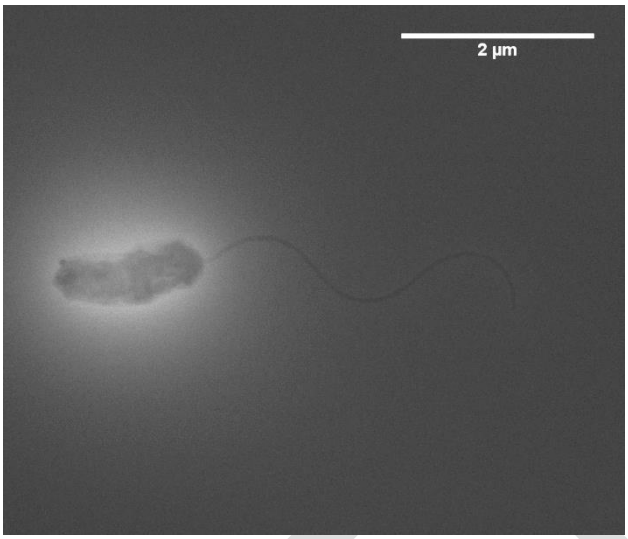


Figure S1. Wet scanning transmission electron Micrograph in Scanning Electron Microscope (wet-STEM SEM) image of uranyl acetate stained strain S2757^{T} grown in MB for 24 hours at 25 °C.

Table S1. GenBank/EBI Accession numbers of the nucleotide sequences used in this study. For gene sequences that were extracted from whole genome sequences, the WGS accession number is listed.

Species	Strain	GenBank/EBI accession number						
		WGS	*16S rRNA*	*fur*	*gyrB*	*pyrH*	*recA*	*topA*
V. galatheae	S2757^T = DSM 100497^T = LMG 28895^T	JXXV01	JXXV01	JXXV01	JXXV01	JXXV01	JXXV01	JXXV01
V. brasiliensis	LMG 20546^T = DSM 17184^T	AEVS01	AEVS01	AEVS01	AEVS01	AEVS01	AEVS01	AEVS01
V. orientalis	CIP 102297^T = ATCC 33934^T = DSM 19136^T	ACZV01	ACZV01	ACZV01	ACZV01	ACZV01	ACZV01	ACZV01
V. hepatarius	DSM 19134^T	LHPI01*	LHPI01*	LHPI01*	LHPI01*	LHPI01*	LHPI01*	LHPI01*
V. tubiashii	ATCC 19109^T = DSM 19142^T	AFWI01	NR_118093	AFWI01	AFWI01	AFWI01	AFWI01	AFWI01
V. caribbeanicus	ATCC BAA-2122^T	AEIU01	AEIU01	AEIU01	AEIU01	AEIU01	AEIU01	AEIU01
V. xuii	DSM 17185^T	LHPK01*	LHPK01*	LHPK01*	LHPK01*	LHPK01*	LHPK01*	LHPK01*
V. nereis	DSM 19584^T	LHPJ01*	LHPJ01*	LHPJ01*	LHPJ01*	LHPJ01*	LHPJ01*	LHPJ01*
V. harveyi	NCIMB 1280^T = NBRC 15634^T = ATCC 14126^T	BAOD01	NR_043165	BAOD01	BAOD01	BAOD01	BAOD01	BAOD01
V. pacinii	LMG 19999^T = DSM 19139^T	JONH01	NR_025479	JONH01	JONH01	JONH01	JONH01	JONH01
V. parahaemolyticus	NBRC 12711^T = ATCC 17802^T	LATW01	NR_113604	LATW01	LATW01	LATW01	LATW01	LATW01
V. rotiferianus	LMG 21460^T = CAIM 577^T	BAOI01	NR_118091	BAOI01	BAOI01	BAOI01	BAOI01	BAOI01
V. scophthalmi	LMG 19158^T	AFWE01	NR_117889	AFWE01	AFWE01	AFWE01	AFWE01	AFWE01
V. sinaloensis	CAIM 797^T=DSM 21333^T		NR_043858	KT380049*				
V. sinaloensis	CAIM 648 = DSM 21326	AEVT01	EU043381	AEVT01	AEVT01	AEVT01	AEVT01	AEVT01
V.maritimus	R 40493^T		GU929925		GU929929	GU929933	GU929935	GU929939
P. aquae	CGMCC 1.12159^T	LDOT01	LDOT01	LDOT01	LDOT01	LDOT01	LDOT01	LDOT01

* Sequences generated in this study.

Table S2: Results from the phenotypic and chemotaxonomic analyses. +, positive; -, negative, ND, not determined; w, weak reaction.

		Strains				
	Characteristic	***V. galathea***	***V. brasiliensis***	***V. orientalis***	***V. hepatarius***	***V. tubiashii***
Biolog GN2	water	-	-	-	-	-
	a-cyclodextrin	-	-	-	-	-
	dextrin	+	+	+	+	+
	glycogen	+	+	+	+	+
	Tween 40	w	+	+	w	+
	Tween 80	w	+	+	w	+
	N-acetyl-D-galactosamine	-	+	-	-	-
	N-acetyl-D-glucosamine	+	+	+	+	+
	adonitol	-	-	-	-	-
	L-arabinose	-	+	-	-	-
	D-arabitol	-	-	-	-	-
	D-cellobiose	+	+	-	+	+
	i-erythritol	-	-	-	+	-
	D-fructose	+	+	-	+	+
	L-fucose	-	+	-	-	-
	D-galactose	-	-	-	w	-
	Gentibiose	-	-	-	-	-
	a-D-glucose	+	+	+	+	+
	m-inositol	-	-	-	-	-
	a-D-lactose	-	-	-	-	-
	lactulose	-	-	-	-	-
	maltose	+	+	+	+	+
	D-mannitol	+	+	+	+	+
	D-mannose	+	+	-	+	+
	D-melibiose	-	-	-	-	-
	b-methyl-D-glucoside	w	-	-	-	-
	D-psicose	w	+	-	+	+
	D-raffinose	-	-	-	-	-
	L-rhamnose	-	-	-	-	-
	D-sorbitol	-	+	-	-	-
	sucrose	-	+	-	+	+
	D-trehalose	+	+	+	+	+
	turanose	-	-	-	-	-
	xylitol	-	-	-	-	-
	pyruvic acid methyl ester	w	+	-	w	-
	succinic acid mono-methyl ester	-	+	-	-	-
	acetic acid	-	+	-	-	w
	cis-aconitic acid	-	-	-	-	-
	citric acid	-	-	-	-	-
	formic acid	-	-	-	-	-
	D-galactonic acid lactone	-	-	-	-	-
	D-galacturonic acid	-	-	-	-	-
	D-gluconic acid	+	+	-	+	+
	D-glucosamininc acid	-	-	-	-	-
	D-glucuronic acid	-	-	-	-	+
	a-hydroxybutyric acid	-	-	-	-	-
	b-hydroxybutyric acid	-	+	-	-	w
	g-hydroxybutyric acid	-	-	-	-	-
	p-hydroxyphenylacetic acid	-	-	-	-	-
	itaconic acid	-	-	-	-	-
	a-ketobutyric acid	-	-	-	-	-

	Characteristic	Strains				
		V. galathea	*V. brasiliensis*	*V. orientalis*	*V. hepatarius*	*V. tubiashii*
	a-ketoglutaric acid	-	+	-	-	-
	a-ketovaleric acid	-	-	-	-	-
	D,L-lactic acid	+	+	-	+	+
	malonic acid	-	-	-	-	-
	proprionic acid	-	-	-	-	-
	quinic acid	-	-	-	-	-
	D-saccharic acid	-	-	-	-	-
	sebacic acid	-	-	-	-	-
	succinic acid	-	+	+	+	+
	bromosuccinic acid	-	+	-	-	-
	succinamic acid	-	-	-	-	-
	glucuronamide	-	-	-	-	w
	L-alaninamide	-	-	-	-	-
	D-alanine	+	+	-	-	-
	L-alanine	+	+	+	-	+
	L-alanyl-glycine	+	+	+	+	+
	L-asparagine	+	+	+	+	+
	L-aspartic acid	+	+	+	+	+
	L-glutamic acid	+	+	+	+	+
	glycil-L-aspartic acid	-	+	-	+	+
	glycil-L-glutamic acid	+	+	-	+	+
	L-histidine	-	-	-	-	-
	hydroxy-L-proline	-	-	-	-	-
	L-leucine	-	-	-	-	-
	L-ornithine	-	+	-	-	-
	L-phenylalanine	-	-	-	-	-
	L-proline	-	+	+	-	-
	L-pyroglutamic acid	-	-	+	-	-
	D-serine	w	-	-	-	-
	L-serine	w	+	-	+	+
	L-threonine	-	+	-	+	-
	D,L-carnitine	-	-	-	-	-
	g-aminobutyric acid	-	-	-	-	-
	urocanic acid	-	-	-	-	-
	inosine	+	+	+	+	+
	uridine	+	+	+	+	+
	thymidine	+	+	+	+	+
	phenylethylamine	-	-	-	-	-
	putrescine	-	-	-	-	-
	2-aminoethanol	-	-	-	-	-
	2,3-butanediol	-	-	-	-	-
	glycerol	-	+	+	+	+
	D,L,a-glycerol phosphate	-	+	+	-	-
	a-D-glucose-1-phosphate	+	-	-	-	-
	D-glucose-6-phosphate	+	+	+	-	+
API ZYM	alkaline phosphatase	+	+	+	+	+
	esterase	+	+	+	+	+
	esterase lipase	+	+	+	+	+
	lipase	-	+	+	+	+
	leucine arylamidase	+	+	+	+	+
	valine arylamidase	+	+	-	+	-
	cystine arylamidase	+	+	-	+	-
	trypsin	-	-	-	-	-
	a-chymotripsin	-	-	-	-	-
	acid phosphatase	-	+	+	-	-
	naphtol-AS-BI-phosphohydrolase	+	+	+	+	+
	a-galactosidase	-	-	-	-	-

	Characteristic	Strains				
		V. galathea	*V. brasiliensis*	*V. orientalis*	*V. hepatarius*	*V. tubiashii*
	b-galactosidase	-	-	-	-	-
	b-glucuronidase	-	-	-	-	-
	a-glucosidase	+	-	-	-	+
	b-glucosidase	-	-	-	-	-
	N-acetyl-b-glucosaminidase	-	-	+	+	+
	a-mannosidase	-	-	+	-	-
	a-fucosidase	-	-	-	-	-
FAME analysis	Sum in Feature 2 12:0 aldehyde					
	10:0 3OH		0,06			0,06
	unknown 11.799					
	12:0 iso	0,1				
	12:0	3,4	3,2	3,6	3,6	3,4
	unknown 12,484	0,4		0,5	0,3	
	13:0 ISO	0,7	0,3	0,1	0,1	0,2
	13:1 at 12-13		0,3		0,2	0,2
	13:0		0,1	0,2	0,1	
	13:0 iso 3 OH	0,3				
	12:0 2OH	0,1	0,1	0,1	0,1	0,0
	12:0 3OH	1,8	2,0	1,9	2,1	2,6
	14:0 ISO	0,4	0,1	0,2	0,1	
	14:0	6,5	6,4	9,1	6,3	6,5
	unknown 14,502		0,1			
	14:1 w5c			0,1		
	15:0 ISO	2,1	0,3	0,2	0,1	0,1
	15:0 ANTEISO	0,3	0,1	0,1	0,1	0,1
	15:0	0,4	0,3	0,7	0,4	0,3
	15:1 w8c					0,2
	15:0 iso 3 OH	0,3				
	14:0 ISO 3 OH	0,4	0,1		0,2	
	Sum in feature 2 14:0 3OH/16:1 ISO I					
	16:0 ISO	3,7	0,5	0,6	1,1	0,4
	16:1 w9c		0,5	0,5	0,5	
	16:1 w7c alcohol	0,6		0,3		
	Sum in feature 3 16:1 w7c/16:1w6c					
	Sum in feature 3 16:1 w7c/15 iso 2OH					
	sum in feature 3 15:0 ISO 2OH/16:1w7c					
	sum in feature 3 16:1 w6c/16:1 w7c					
	16:1 w5c	0,2	0,2	0,3	0,3	0,3
	16:0	19,5	24,8	23,3	22,6	22,2
	17:0 ISO	4,0	0,3		0,1	0,2
	17:0 anteiso	0,6			0,1	0,1
	17:1 w8c	0,3	0,2	0,2	0,2	0,2
	17:1 w9c	0,3				
	17:0	0,4	0,2	0,4	0,3	0,3
	16:0 3OH		0,3		0,3	0,4
	Sum in feature 8 18:1 w7c	15,9			18,5	
	Sum in feature 8 18:1 w6c					
	18:1 w9c		0,3		0,3	0,3
	18:1 w7c 11-methyl					0,2
	18:1 w7c		19,5			
	18:1 w5c				0,1	
	18:0	0,3	0,4	0,3	0,4	0,4
	18:0 iso	0,4				
	sum in feature 7 un 18.846/19:1 w6c		0,4			
	20:1 w7c				0,2	
	Summed feature 2 *	2,1	2,6	2,0	2,2	2,4
	Summed feature 3 *	34,7	35,9	44,7	39,0	35,8

	Characteristic	Strains				
		V. galathea	*V. brasiliensis*	*V. orientalis*	*V. hepatarius*	*V. tubiashii*
	Summed feature 7 *		0,4		0,4	
	Summed feature 8 *	15,8		10,6	18,3	23,0
API 20 NE	Nitrates--> nitrites	+	+	+	+	+
	L-tryptophane	+	+	+	+	+
	D-glucose	-	+	-	-	+
	L-arginine	-	-	-	-	-
	urea	-	-	-	-	-
	esculin ferric citrate (b-glucosidase)	+	+	-	-	-
	gelatin (protease)	-	-	-	+	-
	b-galactosidase	+	+	-	ND	+
	D-glucose	-	-	-	-	-
	L-arabinose	-	-	-	-	-
	D-mannose	-	-	-	-	-
	D-mannitol	-	-	-	-	-
	N-acetylglucosamine	-	-	-	-	-
	D-maltose	-	-	-	-	-
	potassium gluconatecapric acid	-	-	-	-	-
	capric acid	-	-	-	-	-
	adipic acid	-	-	-	-	-
	malic acid	+	+	-	-	+
	trisodium citrate	-	-	-	-	-
	phenylacetic acid	-	-	-	-	-
	oxidase	-	-	-	-	-

*Summed feature 2: one or more among 12:0 aldehyde, 14:0 3OH and/or 16:1 iso; summed feature 3: one or more among 16:1 w7c, 16:1w6c and/or 15:0 iso 2OH; summed feature 7: one or more among unknown 18.846 and/or 19:1 w6c; summed feature 8: one or more among 18:1 w7c and/or 18:1 w6c.