

***Flavobacterium difficile* sp. nov., isolated from a freshwater waterfall**

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Subject category for the Contents list: New Taxa (*Bacteroidetes*).

Running title: *Flavobacterium difficile* sp. nov.

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Supplementary Table S1. Number of genes associated with the eggNOG categories of coding proteins in *Flavobacterium difficle* KDG-16^T genome.

Functional group of genes		Counts	%
Cellular processes and signaling			
D	Cell cycle control, cell division, chromosome partitioning	24	0.9
M	Cell wall/membrane/envelope biogenesis	199	7.7
N	Cell motility	6	0.2
O	Post-translational modification, protein turnover, and chaperones	86	3.3
T	Signal transduction mechanisms	74	2.9
U	Intracellular trafficking, secretion, and vesicular transport	24	0.9
V	Defense mechanisms	34	1.3
W	Extracellular structures	0	0
Y	Nuclear structure	0	0
Z	Cytoskeleton	0	0
Information storage and processing			
A	RNA processing and modification	0	0
B	Chromatin structure and dynamics	0	0
J	Translation, ribosomal structure and biogenesis	141	5.5
K	Transcription	91	3.5
L	Replication, recombination and repair	107	4.1
Metabolism			
C	Energy production and conversion	89	3.4
E	Amino acid transport and metabolism	136	5.3
F	Nucleotide transport and metabolism	56	2.2
G	Carbohydrate transport and metabolism	72	2.8
H	Coenzyme transport and metabolism	85	3.3
I	Lipid transport and metabolism	71	2.8
P	Inorganic ion transport and metabolism	88	3.4
Q	Secondary metabolites biosynthesis, transport, and catabolism	18	0.7
Poorly characterized			
R	General function prediction only	231	8.9
S	Function unknown	159	6.2
Not in eggNOG		795	30.7
Total		2586	100

Supplementary Table S2. Average nucleotide identity and digital DNA-DNA hybridization scores of *Flavobacterium difficile* KDG-16^T in comparison with the type strains of closely related *Flavobacterium* species with whole genome sequence are publicly available.

Strains	Accession no.	ANI (%)	dDDH (%)
<i>Flavobacterium terrigena</i> DSM 17934 ^T	NZ_FNYA000000000	84.2	15.4
<i>Flavobacterium urocaniciphilum</i> JCM 19142 ^T	NZ_FOEI000000000	80.8	18.8
<i>Flavobacterium swingsii</i> DSM 21789 ^T	NZ_FOJT000000000	73.1	21.4
<i>Flavobacterium aquatile</i> DSM 1132 ^T	NZ_JRHH000000000	73.0	21.7
<i>Flavobacterium plurextorum</i> CCUG 60112 ^T	NZ_MUHD000000000	71.8	22.1
<i>Flavobacterium hibernum</i> DSM 12611 ^T	NZ_JPRK000000000	71.8	21.6
<i>Flavobacterium glaciei</i> CGMCC 1.5380 ^T	NZ_VLXX000000000	71.7	22.2
<i>Flavobacterium chryseum</i> P3160 ^T	NZ_SNWW000000000	71.7	22.5
<i>Flavobacterium limicola</i> DSM 15094 ^T	NZ_RBXA000000000	71.6	23.0
<i>Flavobacterium pectinovorum</i> DSM 6368 ^T	NZ_FRBX000000000	71.6	21.9
<i>Flavobacterium saccharophilum</i> DSM 1811 ^T	NZ_FRBY000000000	71.6	22.4
<i>Flavobacterium hydatis</i> ATCC 29551 ^T	NZ_MUGY000000000	71.6	22.0
<i>Flavobacterium ranwuense</i> LB2P22 ^T	NZ_SMLH000000000	71.6	22.9
<i>Flavobacterium glycines</i> Gm-149 ^T	NZ_FNEO000000000	71.5	22.7
<i>Flavobacterium aquidurens</i> DSM 18293 ^T	NZ_MUGR000000000	71.5	22.2
<i>Flavobacterium frigoris</i> DSM 15719 ^T	NZ_FOFZ000000000	71.5	22.2
<i>Flavobacterium chilense</i> LMG 26360 ^T	NZ_LSYT000000000	71.5	21.7
<i>Flavobacterium hercynium</i> DSM 18292 ^T	NZ_MUGW000000000	71.5	21.9
<i>Flavobacterium piscis</i> CCUG 60099 ^T	NZ_MUHC000000000	71.4	21.8
<i>Flavobacterium succinicans</i> DSM 4002 ^T	NZ_FOUT000000000	71.4	23.0
<i>Flavobacterium granuli</i> DSM 17797 ^T	NZ_PVUB000000000	71.3	22.1
<i>Flavobacterium hiemivividum</i> TSA-D2 ^T	NZ_SMFO000000000	71.2	22.8
<i>Flavobacterium collinsii</i> CECT7796 ^T	NZ_CADCST000000000	71.0	22.3
<i>Flavobacterium branchiophilum</i> ATCC 35035 ^T	NZ_MUGT000000000	70.9	21.7
<i>Flavobacterium cupreum</i> CCM 8825 ^T	NZ_QWDM000000000	70.7	21.3

Supplementary Table S3. Genome characteristics of *Flavobacterium difficile* KDG-16^T and three type strains of the genus *Flavobacterium* used for comparison.

Strain	Genome size (Mbp)	Scaffolds	G+C content (%)	GenBank/EMBL/DDBJ accession number
<i>F. difficile</i> KDG-16 ^T	2.96	33	31.6	NZ_JAAJBT000000000
<i>F. terrigena</i> DSM 17934 ^T	3.17	18	31.2	NZ_FNYA000000000
<i>F. urocaniciphilum</i> JCM 19142 ^T	2.85	23	30.6	NZ_FOEI000000000
<i>F. aquatile</i> DSM 1132 ^T	3.49	7	32.2	NZ_JRHH000000000

Supplementary Table S4. The related genes presented only in *F. difficile* KDG-16^T and *F. aquatile* DSM 1132^T but not in *F. terrigena* DSM 17934^T and *F. urocaniciphilum* JCM 19142^T annotated by Rapid Annotation of microbial genomes using Subsystem Technology.

Genes putatively encoding
Cell wall and capsule
Capsular polysaccharide biosynthesis and assembly
Tyrosine-protein kinase Wzc (EC 2.7.10.2)
Oligosaccharide repeat unit polymerase Wzy
O-antigen flippase Wzx
Regulation and cell signaling
Toxin-antitoxin replicon stabilization system
ParE toxin protein
ParD protein (antitoxin to ParE)
Photosynthesis
Proteorhodopsin
Predictet Brp-like protein Blh
Sulfur metabolism
Galactosylceramide and sulfatide metabolism
Beta-galactosidase (EC 3.2.1.23)
Lipid metabolism
Glycerolipid and glycerophospholipid metabolism
Aldehyde dehydrogenase B (EC 1.2.1.22)
Alcohol dehydrogenase (EC 1.1.1.1)
1-acyl-sn-glycerol-3-phosphate acyltransferase (EC 2.3.1.51)
Acyl carrier protein, diacylglycerol kinase (EC 2.7.1.107)
Glycerol-3-phosphate dehydrogenase [NAD(P)+] (EC 1.1.1.94)
Phosphatidate cytidyltransferase (EC 2.7.7.41)
CDP-diacylglycerol-serine O-phosphatidyltransferase (EC 2.7.8.8)
Phosphatidylserine decarboxylase (EC 4.1.1.65)
Carbohydrate metabolism
Trehalose biosynthesis and trehalose uptake and utilization
Trehalose phosphorylase (EC 2.4.1.64)
Beta-phosphoglucomutase (EC 5.4.2.6)
Lactose and galactose uptake and utilization
Beta-galactosidase (EC 3.2.1.23)

Aldose 1-epimerase (EC 5.1.3.3)

Galactokinase (EC 2.7.1.6)

Galactose-1-phosphate uridylyltransferase (EC 2.7.7.10)

UDP-glucose 4-epimerase (EC 5.1.3.2)

Arabinogalactan endo-1,4-beta-galactosidase (EC 3.2.1.89)

Cellulosome

Alpha-amylase (neopullulanase) SusA (EC 3.2.1.135)

Alpha-glucosidase SusB (EC 3.2.1.20)

SusC (outer membrane protein involved in starch binding)

SusD (outer membrane protein)

1,4-alpha-glucan branching enzyme (EC 2.4.1.18)

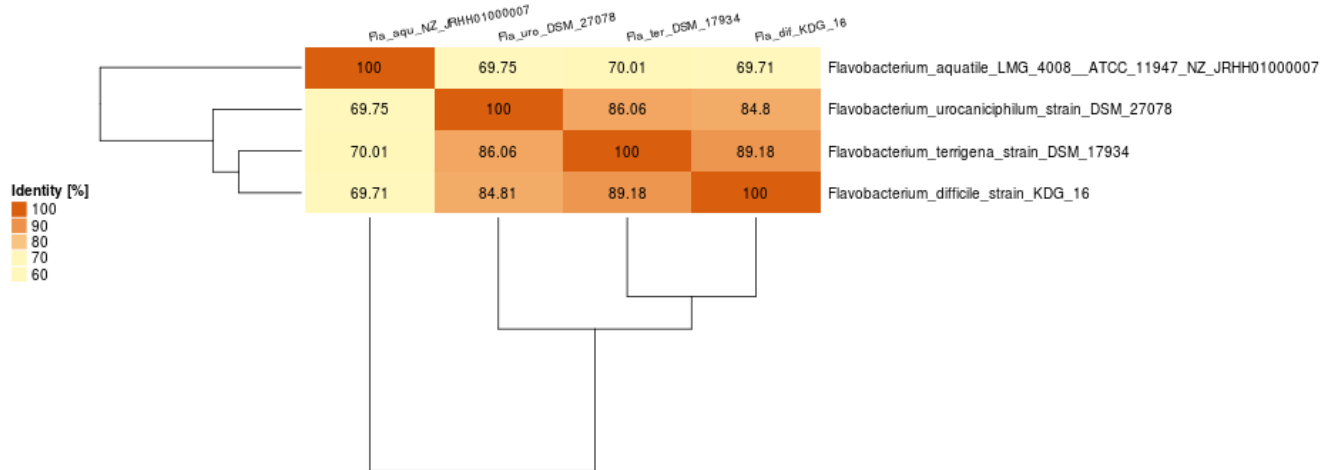
Supplementary Table S5. Negative reactions in API ZYM, API 20NE and API 50CH kits and carbon source utilization for *Flavobacterium diffcile* KDG-16^T.

API ZYM kit
α -galactosidase, β -galactosidase (ONPG), β -glucuronidase, β -glucosidase, <i>N</i> -acetyl- β -glucosaminidase, α -mannosidase and α -fucosidase activities
API 20NE kit
nitrate reduction, indole production, glucose acidification, arginine dihydrolase, urease and β -galactosidase (PNPG) activities, esculin hydrolysis, and assimilation of glucose, arabinose, mannose, mannitol, <i>N</i> -acetyl-glucosamine, maltose, gluconate, caprate, adipate, malate, citrate and phenyl-acetate
API 50CH kit
glycerol, erythritol, D-arabinose, L-arabinose, D-ribose, D-xylose, L-xylose, D-adonitol, methyl β -xyloside, D-galactose, D-glucose, D-fructose, D-mannose, L-sorbose, L-rhamnose, dulcitol, inositol, D-mannitol, D-sorbitol, methyl α -mannoside, methyl α -glucoside, <i>N</i> -acetylglucosamine, amygdalin, arbutin, salicin, D-cellobiose, maltose, lactose, melibiose, sucrose, trehalose, inulin, melezitose, raffinose, xylitol, gentiobiose, turanose, D-lyxose, D-tagatose, D-fucose, L-fucose D-arabitol, L-arabitol, gluconate and 2-ketogluconate
Carbon source utilization
D-fructose, maltose, dextrin, D-cellobiose, sucrose, D-trehalose, L-rhamnose, <i>N</i> -acetyl-glucosamine, D-mannitol, D-sorbitol, gluconate, citrate, L-glutamic acid, L-leucine, L-histidine, L-serine, L-proline and Tween 80

Supplementary Fig. S1. *Flavobacterium difficle* KDG-16^T was isolated during the investigations on the biodiversity in the Dajin Waterfall (GPS location: 22° 51' 40" N 120° 38' 43" E; 20 °C, pH 7, 0% NaCl) in the Sandimen Township of Pingdong County, Taiwan on 5 April 2015.

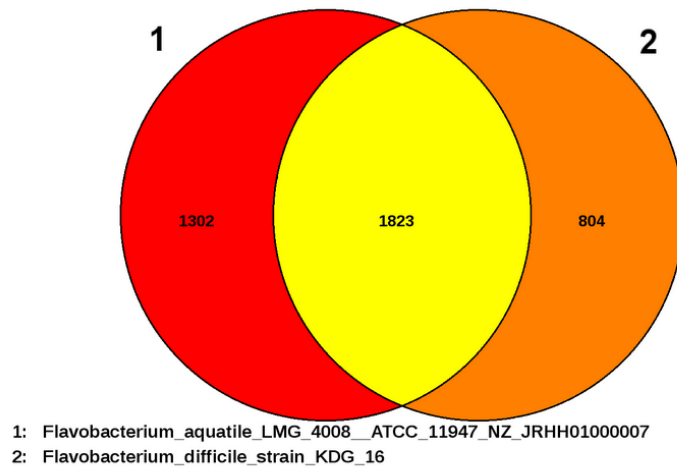


Supplementary Fig. S2. Average amino acid identities (AAI) between *F. difficile* KDG-16^T and *F. terrigena* DSM 17934^T, *F. urocaniciphilum* JCM 19142^T and *F. aquatile* DSM 1132^T.

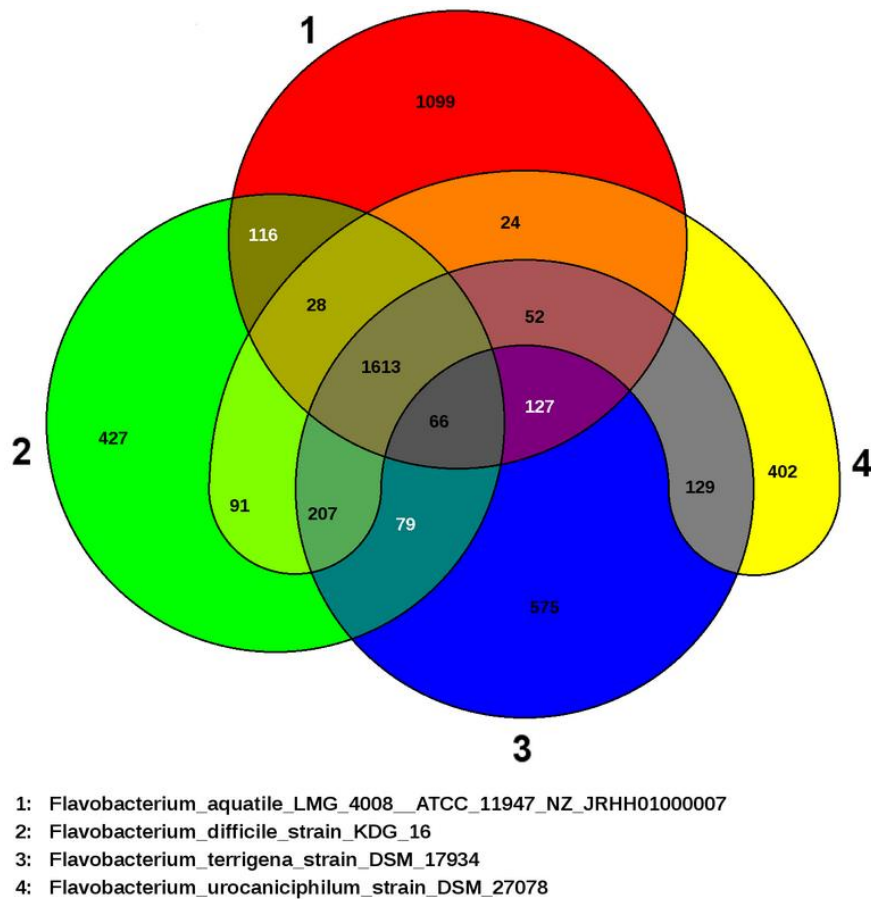


Supplementary Fig. S3. Comparative gene content analyses. (A) *F. difficile* KDG-16^T and *F. aquatile* DSM 1132^T; (B) *F. difficile* KDG-16^T, *F. terrigena* DSM 17934^T, *F. urocaniciphilum* JCM 19142^T and *F. aquatile* DSM 1132^T.

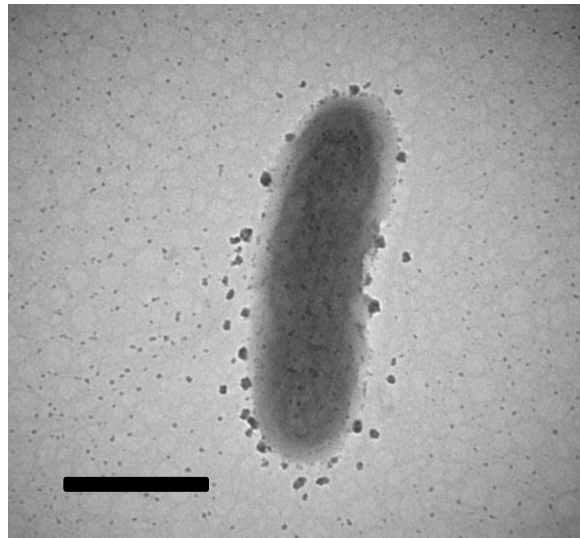
(A)



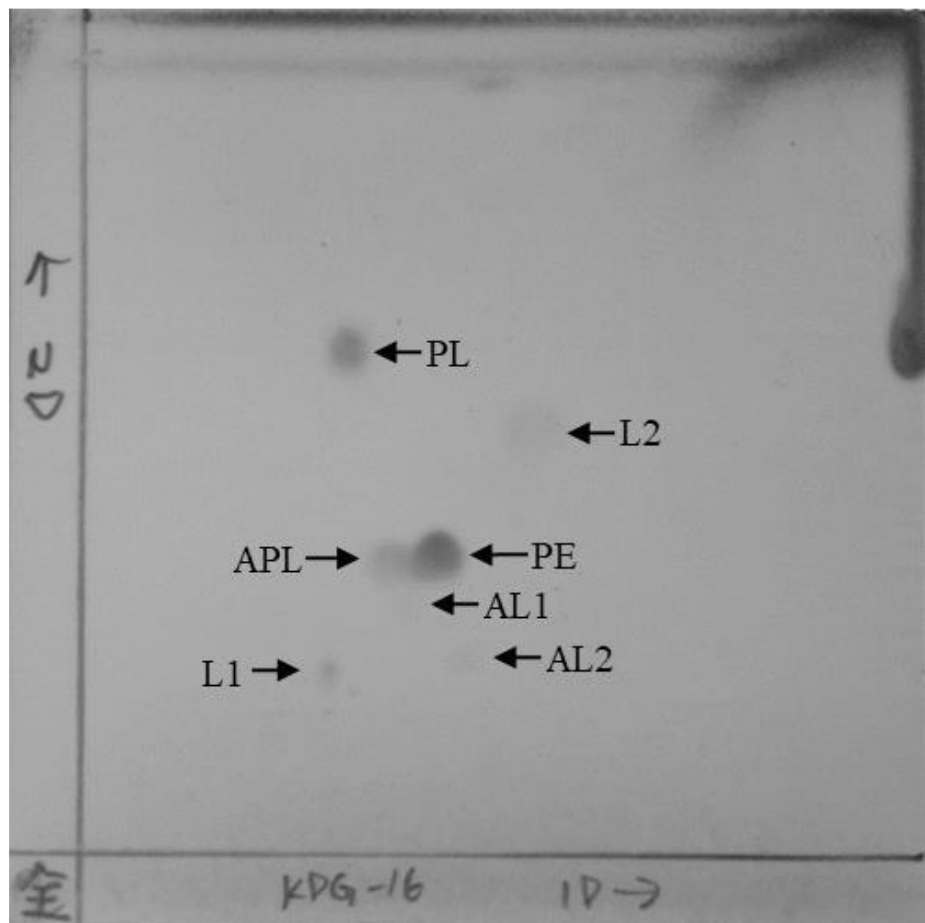
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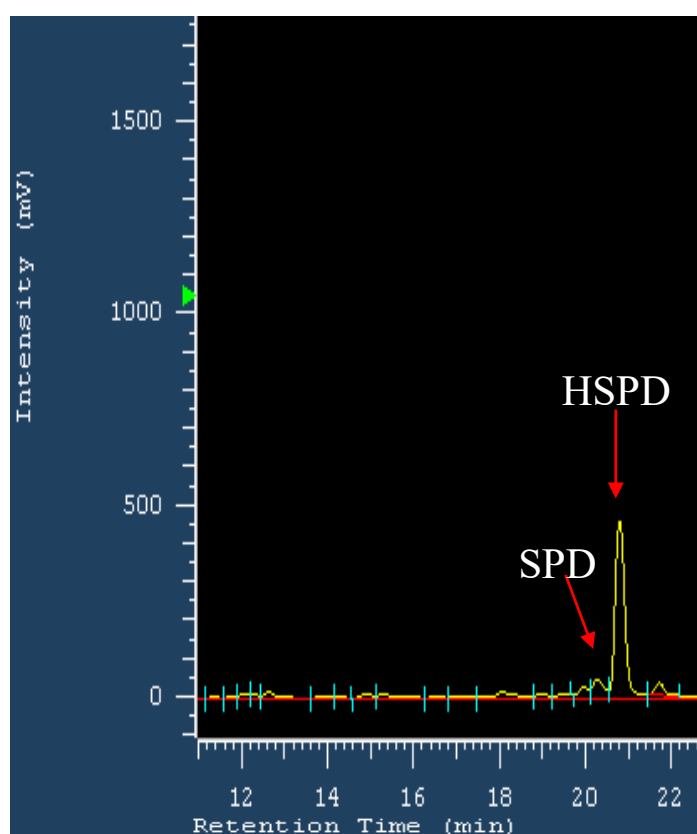
Supplementary Fig. S4. Transmission electron micrograph of cells of *Flavobacterium difficile* KDG-16^T. Cells were aerobically grown on R2A agar for 3 days at 25 °C. Bar, 0.5 µm.



Supplementary Fig. S5. Two-dimensional thin-layer chromatograms of polar lipids of *Flavobacterium difficile* KDG-16^T. Components were revealed by staining the preparations with 10% molybdophosphoric acid in ethanol and heating them at 150 °C for 10 min. Abbreviations: PE, phosphatidylethanolamine; APL, uncharacterized aminophospholipid; PL, uncharacterized phospholipid; AL1-AL2 uncharacterized aminolipids; L1-L2 uncharacterized lipids.



Supplementary Fig. S6. HPLC chromatogram of polyamines of *Flavobacterium difficile* KDG-16^T. Strain KDG-16^T contained homospermidine (HSPD, 88.5%) as the major polyamine and spermidine (SPD, 11.5%) as the minor component. Abbreviations: HSPD, homospermidine; SPD, spermidine.



Supplementary Fig. S7. HPLC chromatogram of respiratory quinone of *Flavobacterium difficile* KDG-16^T. Strain KDG-16^T had menaquinone-6 (MK-6) as major isoprenoid quinone.

