Flavobacterium hungaricum sp. nov. a novel soil inhabitant, cellulolytic bacterium isolated from plough field

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Abstract
A Gram-negative bacterial strain, named Kb82, was isolated from agricultural soil and a polyphasic approach was used for characterisation and to determine its taxonomic position. Based on 16S rRNA gene sequence analysis, the highest similarity was found with Flavobacterium artemisiae SYP-B1015 (98.2%). The highest ANI (83.3%) and dDDH (26.5%) values were found with Flavobacterium ginsenosidimutans THG 01 and Flavobacterium fluviale HYN0086T, respectively. The isolate is aerobic with rod-shaped cells, positive for catalase and negative for oxidase tests. The DNA G+C content is 34.7 mol%. The only isoprenoid quinone is menaquinone 6 (MK-6). The major fatty acids are iso-C15:0, summed feature 3 (C16:1 ω7c/C16:1 ω6c) and iso-C17:0 3OH. The major polar lipid is phosphatidylethanolamine. On the bases of phenotypic characteristics and analysis of 16S rRNA gene sequences, it is concluded that strain Kb82T represents a novel species in the Flavobacterium genus, for which the name Flavobacterium hungaricum sp. nov. is proposed. The type strain of the species is strain Kb82T (= LMG 31576T = NCAIM B.02635T).

Keywords Bacteroidetes · Flavobacterium · New taxa · Cellulolytic · glycoside hydrolase

Abbreviations
ANI Average Nucleotide Identity
dDDH Digital DNA–DNA hybridisation
DDGS Distillers Dried Grains with Solubles
DSMZ Deutsche Sammlung von Mikroorganismen und Zellkulturen (German Collection of Microorganisms and Cell Cultures)
GGDC Genome-to-Genome Distance Calculator
GNL Aminoglycolipid
L Lipid
LB agar Luria–Bertani agar
MiGA Microbial Genomes Atlas
MLSA MultiLocus Sequence Analysis
PE Phosphatidylethanolamine
PGL Phosphoglycolipid
PL Phospholipid
RAST Rapid Annotation using Subsystem Technology
TYGS Type Strain Genome Server
Introduction

The genus Flavobacterium was described by Bergey et al. (1923) emended by Bernardet et al. (1996), Dong et al. (2013), Kang et al. (2013) and Kuo et al. (2013). The genus belongs to the family Flavobacteriaceae, order Flavobacterales, class Flavobacteria, phylum ‘Bacteroidetes’.

This group of Bacteria is very diverse and strains have been isolated from a wide variety of habitats. The genus includes 257 validly published species with correct names (https://lpsn.dsmz.de/ February 2022) (Parte et al. 2020) at the time of writing, the type species is Flavobacterium aquatile (Frankland and Frankland 1889). Culture-based and culture-independent studies indicate that Flavobacteria are one of the most abundant group in soil, especially in the rhizosphere. Members of the Flavobacterium are often associated with the capacity to degrade complex organic compounds in soil. Many recent studies suggest that these bacteria have a plant growth-promoting properties, particularly in the early and intermediate growth stages (Kolton et al. 2016). Organisms that are able to break down biopolymers play a pivotal role in the turnover of various organic matter in the soil. Lignocellulose is a complex biopolymer consisting of cellulose, hemicellulose and lignin. In the background of chemical and biological resistance stand chemical complexity and an arranged structure. The phenotypic, chemotaxonomic and genotypic properties indicate that strain Kb82T represents a novel species within the genus Flavobacterium, for which the name Flavobacterium hungaricum sp. nov. is proposed. The type strain of the species is strain Kb82T (=LMG 31576T = NCAIM B.02635T).

Materials and methods

Isolation and cultivation

Strain Kb82T was isolated in the Great Hungarian Plain, from an agricultural field, after the maize was harvested. The soil with a pH moderately alkaline was fertilised. For the study, soil particles were homogenised by vortexing and serial dilutions were prepared with peptone water (1 g peptone, 9 g NaCl, in 1000 ml dH2O). 100–100 µl of the third to the fifth member of the dilution series was spread onto Distillers Dried Grains with Solubles (DDGS) containing agar (1 g NaNO3; 1 g K2HPO4; 3 g NaCl; 0.5 g MgCl2; 0.5 g yeast extract; 0.5 g peptone; 3 g DDGS; 25 g agar; 1000 ml dH2O). The plated were incubated at 10 °C for 5 days. Single colonies were taken from the plates and purified on the same medium. All similar phenotypes’ 16S rRNA gene were sequenced. The isolate was maintained on LB medium (DSM medium No. 381, www.dsmz.de) at 28 °C and pH 7.5. but the novel strain also grows well on TSA, nutrient, R2A and minimal media with xylan, mannan and carboxymethyl cellulose (CMC) as the sole carbon source (1 g NaNO3; 1 g K2HPO4; 3 g NaCl; 0.5 g MgCl2; 4 g xylan/mannan/CMC; 25 g agar; 1000 ml dH2O).

Physiology and chemotaxonomy

For the chemical and molecular studies, biomass was prepared by cultivation in shaker flasks in LB medium at 28 °C for 32 h. The colony morphology of the strain was studied on LB agar medium by directly observing single colonies. Presence of flexirubin type pigment (by 20% KOH), production of brown diffusible pigment on L-tyrosine agar, absorption of Congo red degradation of agar, casein, chitin, pectin, DNA, L-tyrosine, production of H2S and gliding motility (by the hanging-drop technique) were estimated according to the minimal standards for describing novel taxa in the family Flavobacteriaceae (Bernardet et al. 2002) and Barrow and Feltham (2004). Cell morphology of the strain was observed by electron microscopy. Gram reaction was studied with the non-staining method of Buck (1982). Oxidase activity was determined with OXI oxidase test strip (Diagnostics s.r.o.). Catalase production was shown according to Barrow and Feltham (2004). The effects of different temperatures (from 4 to 50 °C) on the growth of the bacterium, NaCl (0–4% w/v) and pH (pH 4–10, using increments of 0.5 pH units) tolerances were determined in LB medium. API 50 CH, API 20 NE and API ZYM kits (BioMérieux) were used according to the manufacturer’s instructions for determining acid production from different carbon sources, the assimilation of different substrates and the enzymatic activities of the strain. The API 50 CH and 20 NE tests were read after 24–48 h incubation at 28 °C. Growth under anaerobic and microaerophilic conditions was checked on LB medium with the help of Anaerocult A and C systems (Merck). The physiological characteristics were compared to the closely related Flavobacterium compostarboris JCM 16527T by side-by-side analysis.

Chemotaxonomic traits were analysed by DSMZ Identification Service (DSMZ, Braunschweig, Germany). Active growing cultures of the strain on LB agar were used in the analysis of the fatty acid profiles of the strain. According to the DSMZ Identification Service, fatty acid methyl esters (FAMEs) were obtained following the methods of Miller (1982) and Kuykendall et al. (1988). Gas chromatography was used for the separation of FAMEs, which were detected by a flame ionisation detector using the Sherlock Microbial Identification System (MIS) (MIDI, Microbial ID, Newark, DE 19711 U.S.A.). FAMEs were identified by using the TSBA6 6.10 database of the Microbial Identification
System. GC/MS was used for the identification of summed feature components thereafter.

Respiratory quinones were extracted from freeze-dried material and silica-based solid phase extraction method was used for purification. The purified samples were further analysed by HPLC and UHPLC-ESI-qTOF systems (Tindall 1990a, b; dsmz.de). Polar lipids were determined based on the methods of Tindall et al. (1990a, b; Tindall et al. 2007; dsmz.de).

Genome features

DNA was extracted from Kb82T liquid culture grown in LB medium. Genomic DNA isolation and 16S rRNA gene amplification were performed according to Tóth et al. (2017). Sequencing of the genome of the strain was done with Illumina MiSeq sequencing technology according to Szuroczki et al. (2019). Genome assembly was performed by SPAdes v. 3.9.1; CLC NGS Cell v. 11.0. Genomic completeness and contamination values were studied by TypeMet tool of MiGA server (http://microbial-genomes.org/) (Rodriguez-R et al. 2018). ANI and digital DNA–DNA hybridisation (dDDH; identities/HSP length) values were determined using the OrthoANI algorithm (www.ezbiocloud.net/tools/ani) (Yoon et al. 2017) and Genome-to-Genome Distance Calculator service of DSMZ (http://ggdc.dsmz.de/) (Meier-Kolthoff et al. 2013). Annotation of the genome was performed by RAST analysis revealed the presence of 277 subsystems, the subsystem coverage was 17% (Online resource 4). The genome of Kb82T contains 10 putative biosynthetic gene clusters (Non-ribosomal peptide synthetase, Type I polyketide synthase, Type III polyketide synthase, betalactone, arylypolyene, resorcinol, proteusin, siderophore, terepene, Class I lanthipeptide clusters like nisin) in 9 genomic regions. Based on the RAST and the anti-SMASH server the strain encodes genes required for siderophore production.

Whole-genome sequence analysis

The completeness and contamination values of the genome are 97.2 and 1.9%, respectively. Other quality labels of genome sequencing and assembly are as follows: 155-fold genome coverage, contig N50 = 1,261,348 bp, number of contigs are 12. The genome size and G+C content of Kb82T are 5,872,517 bp and 34.7 mol%, respectively. According to the annotation, there are 5178 genes, 5088 CDSs and 90 RNA genes in the genome. The coding density is 87.0%. The genomic traits of the strain and related type strains are summarised in Table 2.

To identify the secondary metabolite biosynthesis gene clusters, the anti-SMASH server was used (Blin et al. 2019).

Phylogeny

The partial 16S rRNA gene sequence of the strain was compared with the EzTaxon EzBioCloud Database (http://www.ezbiocloud.net/taxonomy) (Kim et al. 2012a, b) for an approximate phylogenetic affiliation. After Sanger sequencing of the 16S rRNA gene, a genome sequencing project of Kb82T was carried out, which revealed that there is only one 16S rRNA gene copy in the genome. Phylogenetic trees were built by using the neighbor-joining (Saitou and Nei 1987) and maximum-likelihood (Felsenstein 1981) methods with Kimura’s two-parameter calculation model and the maximum-parsimony algorithm (Kimura 1980) using MEGA version 10.0 (Kumar et al. 2018). Tree topologies and distances were evaluated by bootstrap analysis based on 1000 replicates. For phylogenomic studies TYGS (https://tygs.dsmz.de/) (Meier-Kolthoff and Göker 2019), MiGA

Results and discussion

Phenotypic and biochemical characterisation

Distinctive physiological and biochemical characteristics of the isolate are given in Table 1. List of all negative traits from API tests is presented in Online resource 1. The other morphological and physiological characteristics are listed in the species description.

Chemotaxonomic characteristics

The predominant cellular fatty acids of the strain were found to be iso-C15:0 (32.6%), summed feature 3 (C16:1ω7c/C16:1ω6c, 14.6%) and iso-C17:0 3OH (11.6%). The fatty acid profile is similar to that of related strains, in accordance with the description of Flavobacterium genus (Kang et al. 2013), though the ratios of the different components are different. The complete fatty acid composition is shown in Online resource 2. The only respiratory quinone of Kb82T is menaquinone-6 (MK-6). Strain Kb82T exhibits a complex polar lipid profile consisting of one phosphatidylethanolamine (PE) as the dominant element, two aminolipids (AL), three phospholipids (PL), one aminoglycolipid (GNL) and seven uncharacterised lipids (L) (Online resource 3).

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The RAST analysis revealed the presence of 277 subsystems, the subsystem coverage was 17% (Online resource 4). The genome of Kb82T contains 10 putative biosynthetic gene clusters (Non-ribosomal peptide synthetase, Type I polyketide synthase, Type III polyketide synthase, betalactone, arylypolyene, resorcinol, proteusin, siderophore, terpene, Class I lanthipeptide clusters like nisin) in 9 genomic regions. Based on the RAST and the anti-SMASH server the strain encodes genes required for siderophore production.
The production of siderophores can promote plants health by the suppression of pathogens (Rana et al. 2020).

Using the genome annotation and the Pfam database (Mistry et al. 2020; http://pfam.xfam.org/), several glycoside hydrolase (GH) genes in various GH families were found, which indicates that the strain Kb82T specialises in the breakdown of complex plant-associated carbohydrates. Genome sequence analysis also revealed three genes from glycoside hydrolase families GH78 (GenBank accession: MBE8726264, MBE8726269 and MBE8726274) and one rhamnogalacturonan acetylemesterase gene (GenBank accession: MBE8726158) which may play a role in rhamnogalacturonan utilisation. Because this polymer is present only in terrestrial plants, these genes can only be found in the genomes of the terrestrial clade (Kolton et al. 2013). Cellulose degradation was proven by Congo red staining and the strain is able to grow on a minimal medium with polysaccharides as the sole carbon source. In the genome of the strain enzyme genes have been identified that may play a role in the breakdown of lignocellulose (http://www.cazy.org/) (Lombard et al. 2014). We identified 100 GH genes in 32 GH families (Online resource 5).

| Isolation source | Soil | Compost | Rhizosphere | Freshwater | Activated sludge |
|------------------|------|---------|-------------|------------|-----------------|
| Temperature range for growth (°C) (optimum) | 10–30 (25) | 15–30 (30) | 5–34 (24–30) | 4–40 (30) | 15–37 (25) |
| pH range for growth (optimum) | 5.5–8.5 (7.0) | 5.5–8.0 (6.5) | 5.0–8.0 (7.0) | 5.0–11.5 (7.5) | 5.0–9.0 (7.0) |
| Indole production | − | − | + | − | − |
| Hydrolysis of Esculin | + | + | − | + | + |
| Cellulose | + | + | − | − | − |
| Starch | + | + | + | − | − |
| Acid production from α-trehalose | − | + | nd | nd | nd |
| α-arabinose | − | − | + | nd | + |
| Maltose | + | + | − | nd | + |
| Potassium 5-ketoglucuronate | + | − | nd | nd | nd |
| Activity of N-acetyl-β-glucosaminidase oxidase | − | + | nd | + | + |
| DNA G+C content (mol%) | 34.7 | 33.6* | 33.5 | 34.0 | 36.1 |

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As a result of genome analysis, several genes involved in flavobacterial gliding motility (GldN, GldK, GldL, GldM, GldI, GldA, GldE, GldD, GldJ, GldB, GldC, GldH, GldG, GldF, SprA, SprE, SprF, SprT, ChiA, RemB) have been identified in Kb82T genome (McBride and Nakane 2015; Penttinen et al. 2018). A subset of these genes has been found to form a protein translocation system called type IX secretion system (T9SS) restricted to ‘Bacteroidetes’. T9SS has an important role in the secretion of gliding motility adhesins. Several studies indicate the significant role of Flavobacterium strains in soil and especially in the rhizosphere. In such highly competitive ecosystems, the large numbers of glycoside hydrolase genes and the special gliding mobility may help in the successful colonisation of niches (Kolton et al. 2016).

Fig. 1 Maximum-likelihood tree based on 16S rRNA gene sequences showing the phylogenetic relationships between strain Kb82T and related taxa. Bootstrap values (>50%) are shown as percentages of 1000 replicates. Branches with lower bootstrap values than 50% are uncertain. Branches signed with an asterisk occurred with every tree-making algorithm used in the study. Bar, 0.05 substitution per nucleotide position.
Phylogenetic analysis

According to the comparisons with the complete 16S rRNA gene sequences in the EzTaxon database, the highest level of sequence similarity occurred with Flavobacterium artemisiae SYP-B1015T (98.2%) (Zhao et al. 2018), followed by Flavobacterium crocinum HYN0056T (97.4%) (Baek et al. 2018) and Flavobacterium compostarboris 15C3T (97.3%) (Kim et al. 2012a, b). The 16S rRNA gene based phylogeny tree suggests that strain Kb82T forms a distinct phyletic lineage within Flavobacterium genus (Fig. 1).

According to genome-based analysis, the closely related taxa found by MiGA are Flavobacterium ginsenosidimutans THG01T (Yang et al. 2011) (GenBank assembly accession: GCA_003254625) (83.3% ANI) and Flavobacterium sharifuzzamanii A7.6T (Debnath et al. 2019) (GenBank assembly accession: GCA_003254585) (83.2%). The p-value of taxonomic novelty at the species level is 0.00269.

The highest dDDH value (identities/HSP length) between Kb82T and related strains was found with Flavobacterium fluviale HYN0086T (Baek et al. 2020) (GenBank assembly accession: GCA_003312915) (26.5%). Whole genome based tree generated by TYGS also confirmed the taxonomic position of Kb82T within Flavobacterium genus as a novel species (Online resource 6).

According to the 16S rRNA based and whole genome based phylogenetic analyses, Kb82T represents a novel species in genus Flavobacterium. The generally accepted species boundary for 16S rRNA gene similarity, ANI and dDDH values are 98.7, 95–96 and 70%, respectively (Chun et al. 2018). Obtained values for Kb82T (98.2% for 16S rRNA gene similarity, 83.3% for ANI and 26.5% for dDDH) are all lower, confirming the results of phylogenetic treeing.

In conclusion, the phenotypic, biochemical, chemotaxonomic and phylogenetic information of strain Kb82T support its classification as a novel species of Flavobacterium, for which the name Flavobacterium hungaricum sp. nov. is proposed.

Description of Flavobacterium hungaricum sp. nov.

Flavobacterium hungaricum (hun.ga’ri.cum. M.L. neut. adj. hungaricum of or belonging to Hungary, where the type strain was isolated).

Grows well on LB, TSA, nutrient and R2A plates. Colonies are circular, non-mucoid, smooth and have orange pigmentation on LB after 72 h incubation. Flexirubin-type pigment are present. Congo red is not absorbed by colonies. Cells are motile by gliding, strictly aerobic, Gram-reaction-negative, oxidase negative and catalase-positive straight rods. Cells are 0.5 µm in width and 1.5–2.0 µm in length. Individual cells form filaments. It grows at 10–30 °C (optimum, 25 °C), pH 5.5–9.0 (optimum, 7.0) and at NaCl concentrations of 0.0–2.0 w/v % (optimum, 0 w/v %). Able to degrade cellulose, casein, L-tyrosine and esculin, Positive for H2S production and acid production from α-xylene, β-galactose, β-glucose, β-fructose, N-mannose, N-acetyl-glucosamine, amygdalin, arbutin, esculin, salicin, β-cellooligoside, β-maltose, β-lactose, starch, glycopycin, gentobiose, L-fucose, potassium 5-ketogluconate, assimilation of β-glucose, L-arabinose, N-mannose, N-acetyl-glucosamine, α-maltose and β-galactosidase, alkaline phosphatase, leucine arylamidase, valin arylamidase, acid phosphatase naphthol-AS-BI-phosphoehydrolase, β-glucosidase activity. The major fatty acids are iso-C15:0 summed feature 3 (C16:1ω7c/C16:1ω6c) and iso-C17:0 3OH. The DNA G+C content is 34.7 mol%.

The type strain is Kb82T (= LGM 31576T = NCAIM B.02635T) isolated from an agricultural field in the Great Hungarian Plain.

Supplementary Information  The online version contains supplementary material available at https://doi.org/10.1007/s00203-022-02905-x.

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Availability of data and material  The GenBank accession numbers for the 16S rRNA gene sequence and the whole genome of Flavobacterium hungaricum strain Kb82T are MF471352 and PRDM01000000, respectively. The type strain of the species is deposited in Belgian Coordinated Collections of Microorganisms (LMG 31576T) and Hungarian National Collection of Agricultural and Industrial Microorganisms (NCAIM B.02635T).

Declarations

Conflict of interest  The authors have no relevant financial or non-financial interests to disclose.

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References

Aziz RK, Bartels D, Best AA, DeJongh M, Disz T et al (2008) The RAST Server: rapid annotations using subsystems technology. BMC Genomics 9:75. https://doi.org/10.1186/1471-2164-9-75

Baek C, Shin SK, Yi H (2018) Flavobacterium magnum sp. nov., Flavobacterium pallidum sp. nov., Flavobacterium crociatum sp. nov. and Flavobacterium album sp. nov. Int J Syst Evol Microbiol 68:3837–3843. https://doi.org/10.1099/ijsem.0.0303067

Baek MG, Shin SK, Yi H (2020) Gemmobacter aquarius sp. nov., Runella rosea sp. nov. and Flavobacterium fluviale sp. nov., isolated from the Namhangang River system. Int J Syst Evol Microbiol 70:5640–5647. https://doi.org/10.1099/ijsem.0.004455

Barrow GI, Feltham RKA (2004) Cowan and Steel’s manual for the identification of medical bacteria, 3rd edn. Cambridge University Press, Cambridge

Berger JD, Harrison FC, Breed RS, Hammer BW, Huntoon FM (1923) Bergey’s manual of determinative bacteriology, 1st edn. Baltimore, The Williams & Wilkins Co

Bernardet JF, Segers P, Vancanneyt M, Berthe F, Kersters K et al (1996) Cutting a Gordian knot: emended classification and proposal of the family Flavobacteriaceae, and proposal of Flavobacterium hydatis nom. nov. (basonym, Cytophaga aquatilis Strohl and Tait 1978). Int J Syst Bacteriol 46:128–148. https://doi.org/10.1099/00207133-46-1-128

Bernardet JF, Nakagawa Y, Holmes B, Subcommittee on the taxonomy of Flavobacterium and Cytophaga-like bacteria of the International Committee on Systematics of Prokaryotes (2002) Proposed minimal standards for describing new taxa of the family Flavobacteriaceae and emended description of the family. Int J Syst Evol Microbiol 52:1049–1070. https://doi.org/10.1099/00207133-52-3-1049

Blin K, Shaw S, Steinke K, Villebro R, Ziemert N et al (2019) antiSMASH 5.0: updates to the secondary metabolite genome mining pipeline. Nucleic Acids Res 47:81–87. https://doi.org/10.1093/nar/gkz310

Buck JD (1982) Nonstaining (KOH) method for determination of Gram reactions of marine bacteria. Appl Environ Microbiol 44:992–993. https://doi.org/10.1128/aem.44.4.992-993.1982

Chun J, Oren A, Ventosa A, Christensen H, Arahal DR et al (2018) Proposed minimal standards for the use of genome data for the taxonomy of prokaryotes. Int J Syst Evol Microbiol 68:461–466. https://doi.org/10.1099/ijsem.0.025516

Debath SC, Chen C, Liu SX, Di YN, Zheng DQ et al (2019) Flavobacterium sharifuzzamantii sp. nov., isolated from the Sediments of the East China Sea. Curr Microbiol 76:297–303. https://doi.org/10.1007/s00284-018-1609-7

Dong K, Chen F, Du Y, Wang G (2013) Flavobacteriumensis sp. nov., isolated from soil, and emended descriptions of the genus Flavobacterium and Flavobacterium cauense, Flavobacterium saliperosum and Flavobacterium sunchoonense. Int J Syst Evol Microbiol 63:886–892. https://doi.org/10.1099/ijsem.0.039974-0

Farris JS (1972) Estimating phylogenetic trees from distance matrices. Am Nat 106:645–667. https://doi.org/10.1086/282802

Felsenstein J (1981) Evolutionary trees from DNA sequences: a maximum likelihood approach. J Mol Evol 17:368–376. https://doi.org/10.1007/BF01754359

Frankland GC, Frankland PF (1889) Über einige typische Mikroorganismen im Wasser und im Boden. Z Hyg Infektionskr 6:373–400. https://doi.org/10.1007/BF02188159

Kang JY, Chun J, Jahng KY (2013) Flavobacterium acidulophilum sp. nov., isolated from freshwater, and emended description of the genus Flavobacterium. Int J Syst Evol Microbiol 63:1633–1638. https://doi.org/10.1099/ijsem.0.044495-0 (Epub 2012 Aug 17 PMID: 22904226)

Kim JJ, Kanaya E, Weon HY, Koga Y, Takano K et al (2012a) Flavobacterium compostarboris sp. nov., isolated from leaf-and-branch compost, and emended descriptions of Flavobacterium hercynium, Flavobacterium resistens and Flavobacterium johnsoniae. Int J Syst Evol Microbiol 62:2018–2024. https://doi.org/10.1099/ijsem.0.032920-0 (Epub 2011 Oct 21 PMID: 22021577)

Kim OS, Cho YJ, Lee K, Yoon SH, Kim M et al (2012b) Introducing EzTaxon-e: a prokaryotic 16S rRNA gene sequence database with phylotypes that represent uncultured species. Int J Syst Evol Microbiol 62:716–721. https://doi.org/10.1099/ijsem.0.038075-0

Kimura M (1980) A simple method for estimating evolutionary rate of base substitutions through comparative studies of nucleotide sequences. J Mol Evol 16:111–120. https://doi.org/10.1007/BF01731581

Kolton M, Sela N, Elad Y, Cytryn E (2013) Comparative genomic analysis indicates that niche adaptation of terrestrial Flavobacteria is strongly linked to plant glycan metabolism. PLoS One 8(9):e76704. https://doi.org/10.1371/journal.pone.0076704

Kolton M, Erlacher A, Berg G, Cytryn E (2016) The Flavobacterium genus in the plant holobiont: ecological, physiological, and applicative insights. Microbial models: from environmental to industrial sustainability. Springer, Singapore, pp 189–207

Kumar S, Stecher G, Li M, Knyaz C, Tamura K (2018) MEGA X: molecular evolutionary genetics analysis across computing platforms. Mol Biol Evol 35:1547–1549. https://doi.org/10.1093/molbev/msy096

Kuo I, Saw J, Kapan DD, Christensen S, Kaneshiro KY et al (2013) Flavobacterium akiainiivens sp. nov., from decaying wood of Wikstroemia oahuensis, Hawai‘i, and emended description of the genus Flavobacterium. Int J Syst Evol Microbiol 63:3280–3286. https://doi.org/10.1099/ijsem.0.047217-0

Kuykendall LD, Roy MA, O’Neill JJ, Devine TE (1988) Fatty acids, antibiotic resistance, and deoxyribonucleic acid homology groups of Bradyrhizobium japonicum. Int J Syst Evol Microbiol 38:358–361. https://doi.org/10.1099/00207133-38-4-358

Lefort V, Desper R, Gascuel O (2015) FastME 2.0: A comprehensive, accurate, and fast distance-based phylogeny inference program. Mol Biol Evol 32:2798–2800. https://doi.org/10.1093/molbev/msv150

Lombard V, Golconda RH, Drula E, Coutinho PM, Henrissat B (2014) The Carbohydrate-active enzymes database (CAZy) in 2013. Nucleic Acids Res 42:D490–D495. https://doi.org/10.1093/nar/gkt1178

McBride MJ, Nakane D (2015) Flavobacterium gliding motility and the type IX secretion system. Curr Opin Microbiol 28:72–77. https://doi.org/10.1016/j.mib.2015.07.016

Meier-Kolthoff JP, Göker M (2019) TYGS is an automated high-throughput platform for state-of-the-art genome-based taxonomy. Nat Commun 10:2182. https://doi.org/10.1038/s41467-019-10210-3

Meier-Kolthoff JP, Göker M (2013) Genome sequence-based species delimitation with confidence intervals and improved distance functions. BMC Bioinform 14:60. https://doi.org/10.1186/1471-2105-14-60

Miller LT (1982) A single derivatization method for bacterial fatty acid methyl esters including hydroxyc acids. J Clin Microbiol 16:584–586. https://doi.org/10.1128/jcm.16.3.584-586.1982
Mistry J, Chuguransky S, Williams L, Qureshi M, Salazar GA et al (2020) Pfam: The protein families database in 2021. Nucleic Acids Res 49:D412–D419. https://doi.org/10.1093/nar/gkaa913

O’Leary NA, Wright MW, Brister JR, Ciufo S, Haddad D et al (2016) Reference sequence (RefSeq) database at NCBI: current status, taxonomic expansion, and functional annotation. Nucleic Acids Res 44:D733–D745. https://doi.org/10.1093/nar/gkv1189

Parte AC, Sardà Carbasse J, Meier-Kolthoff JP, Reimer LC, Göker M (2020) List of Prokaryotic names with Standing in Nomenclature (LPSN) moves to the DSMZ. Int J Syst Evol Microbiol 70:5607–5612. https://doi.org/10.1099/ijsem.0.004332

Penttinen R, Hoikkala V, Sundberg LR (2018) Gliding motility and expression of motility-related genes in spreading and non-spreading colonies of Flavobacterium columnare. Front Microbiol 26(9):525. https://doi.org/10.3389/fmicb.2018.00525

Rana KL, Kour D, Kaur T, Devi R, Yadav AN et al (2020) Endophytic microbes: biodiversity, plant growth-promoting mechanisms and potential applications for agricultural sustainability. Antonie Van Leeuwenhoek 113(8):1075–1107. https://doi.org/10.1007/s10482-020-01429-y

Rodriguez-R LM, Gunturu S, Harvey WT, Rosselló-Mora R, Tiedje JM et al (2018) The Microbial Genomes Atlas (MiGA) webserver: taxonomic and gene diversity analysis of Archaea and Bacteria at the whole genome level. Nucleic Acids Res 46:W282–W288. https://doi.org/10.1093/nar/gky467

Saitou N, Nei M (1987) The neighbor-joining method: a new method for reconstructing phylogenetic trees. Mol Biol Evol 4:406–425. https://doi.org/10.1093/oxfordjournals.molbev.a040454

Szuroczki S, Khayer B, Sproer C, Toumi M, Szabo A et al (2019) Arundinibacter roseus gen. nov., sp. nov., a novel member of the family Cytophagaceae. Int J Syst Evol Microbiol 69:2076–2081. https://doi.org/10.1099/ijsem.0.003436

Tatusova T, DiCuccio M, Badretdin A, Chetverin V, Nawrocki EP et al (2016) NCBI prokaryotic genome annotation pipeline. Nucleic Acids Res 44:6614–6624. https://doi.org/10.1093/nar/gkw569

Tindall BJ (1990a) A comparative study of the lipid composition of Halobacterium saccharovorum from various sources. Syst Appl Microbiol 13:128–130. https://doi.org/10.1016/S0723-2020(11)80158-X

Tindall BJ (1990b) Lipid composition of Halobacterium lacusprofundi. FEMS Microbiol Lett 66:199–202. https://doi.org/10.1111/j.1574-6968.1990.tb03996.x

Tindall BJ, Sikorski J, Smibert RM, Kreig NR (2007) Phenotypic characterization and the principles of comparative systematics. In: Reddy CA, Beveridge TJ, Breznak JA, Marzluf GA, Schmidt TM, Snyder LR (eds) Methods for general and molecular microbiology, 3rd edn. Wiley, Hoboken, pp 330–393

Tóth Á, Baka E, Bata-Vidács I, Luzics S, Kosztik J et al (2017) Micrococcoides hystricis gen. nov., sp. nov., a novel member of the family Micrococccaceae, phylum Actinobacteria. Int J Syst Evol Microbiol 67:2758–2765. https://doi.org/10.1099/ijsem.0.002018

Yang JE, Kim SY, Im WT, Yi TH (2011) Flavobacterium ginsenosidumutans sp. nov., a bacterium with ginsenoside converting activity isolated from soil of a ginseng field. Int J Syst Evol Microbiol 61:1408–1412. https://doi.org/10.1099/ijss.0.025700-0

Yoon SH, Ha S, Lim J, Kwon S, Chun J (2017) A large-scale evaluation of algorithms to calculate average nucleotide identity. Antonie Van Leeuwenhoek 110:1281–1286. https://doi.org/10.1007/s10482-017-0844-4

Zhang B, Liu ZQ, Zheng YG (2017) Flavobacterium quisquiliarum sp. nov., isolated from activated sludge. Int J Syst Evol Microbiol 67(10):3965–3970. https://doi.org/10.1099/ijsem.0.002230

Zhao JC, Cheng J, Zhang Q, Gao ZW, Zhang MY et al (2018) Flavobacterium artemisiae sp. nov., isolated from the rhizosphere of Artemisia annua L. and emended descriptions of Flavobacterium compostarboris and Flavobacterium procerum. Int J Syst Evol Microbiol 68:1509–1513. https://doi.org/10.1099/ijsem.0.002701

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