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Sphingobacterium hungaricum sp. nov. a novel species on the borderline of the genus *Sphingobacterium*

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Abbreviations: AAI, Amino Acid Identity; ANI, Average Nucleotide Identity; dDDH, digital DNA–DNA Hybridisation; DSMZ, Deutsche Sammlung von Mikroorganismen und Zellkulturen (German Collection of Microorganisms and Cell Cultures); GGDC, Genome-to-Genome Distance Calculator; GH, Glycoside Hydrolase; GNL, aminoglycolipid; LB agar, Luria-Bertani agar; L, uncharacterised lipid; MiGA, Microbial Genomes Atlas; TYGS, Type Strain Genome Server; MLSA, MultiLocus Sequence Analysis; RAST, Rapid Annotation using Subsystem Technology; PE, phosphatidylethanolamine; PGL, phosphoglycolipid; PL, phospholipid.

Author notes: The GenBank accession numbers for the 16S rRNA gene sequence and the whole genome of *Sphingobacterium hungaricum* strain Kb22^T are MF471353 and PRDK00000000, respectively.

A Gram-reaction-negative bacterial strain, designated Kb22^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 (94.39%) with *Sphingobacterium nematocida* M-SX103^T. The highest ANI (71.83%) value was found with *Sphingobacterium composti* T5-12^T, and the highest AAI (66.65%) value was found with *Sphingobacterium* olei HAL-9^T. Cells are aerobic, non-motile rods. The isolate was found to be positive for catalase and oxidase tests. The assembled genome of strain Kb22^T has a total length of 4,06 Mb, the DNA G+C content is 38.1 mol%. The only isoprenoid quinone is menaquinone 7 (MK-7). The major fatty acids are iso-$C_{15:0}$ (28.4%), summed feature 3 ($C_{16:1}$ *ω7c* and/or iso-$C_{15:0}$ 2-OH) (25.7%) and iso-$C_{17:0}$ 3-OH (19.7%).

Based on phenotypic characteristics and phylogenetic analysis, it is concluded that strain Kb22^T is a member of the genus *Sphingobacterium* and represents a novel species, for which the name *Sphingobacterium hungaricum* sp. nov. is proposed. The type strain of the species is strain Kb22^T (=LMG 31574 = NCAIM B.02638).

Introduction

The family *Sphingobacteriaceae* was proposed by Steyn *et al.* [1] and emended by García-López *et al.* [2]. *Sphingobacteriaceae* belong to the order *Sphingobacteriales*, class *Sphingobacteriia* and phylum '*Bacteroidetes*'. The type genus of the family is *Sphingobacterium*. At the time of writing, the family includes 15 validly published genera (https://lpsn.dsmz.de/ April, 2021 [3, 4]). According to Steyn *et al.*, members of the family are Gram-stain-negative, non-motile rods, having iso-$C_{15:0}$, iso-$C_{15:0}$ 2-OH, iso-$C_{15:0}$ 3-OH, $C_{16:0}$, $C_{16:1}$ *ω7c*, $C_{16:O}$ 3-OH and iso-$C_{17:O}$ 3-OH as predominant fatty acids; phosphatidylethanolamine as the major polar lipid and menaquinone-7 (MK-7) as the major respiratory quinone [1]. The members of *Sphingobacteriaceae* have been mostly isolated from soil and water. The genus *Sphingobacterium* was proposed by Yabuuchi *et al.* [5] and emended by Wauters *et al.* [6]. At the time of writing, the genus includes 58 taxa with validly published and correct names (https://lpsn.dsmz.de/ April, 2021 [3, 4]), the type species is *Sphingobacterium spiritivorum* [5]. The main characteristics of the genus are the negative result of Gram-staining, positive catalase and oxidase tests, rod shape, non-motility and MK-7

as predominant isoprenoid quinone. Most of the *Sphingobacterium* strains have been isolated from soil, rhizosphere or composts.

Isolation and ecology

Strain Kb22^T was isolated from an agricultural field in the Great Hungarian Plain, Hungary. The approximate geographical coordinates were 47° 11' 56" N and 19° 00' 46" E. 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 xylan 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 xylan; 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 is routinely maintained on LB medium (DSM medium No. 381, www.dsmz.de) at 28 °C and pH 7.5.

16S phylogeny

Sphingobacterium is a relatively large genus, at the time of writing (June of 2021) the genus includes 58 validly published species with correct name. New species in the genus have already been described with 16S rRNA gene similarity of 90.0% [7] and 99.1% [8]. The golden standard for species delineation was DNA-DNA hybridisation, then Stackebrandt and Goebel proposed a boundary of 16S rRNA gene sequence similarity of 97% for species delineation based on a correlation analysis between DDH values and 16S rRNA gene sequence identities [9]. Thus, until recently, taxonomic frameworks primarily based on 16S rRNA gene sequences. However, this threshold value has been updated to 98.7% [10]. As for genera, the generally used genus threshold of 95% 16S rRNA gene identity has been recently revised to a minimal value of 94.5% [11].

DNA was extracted from Kb22^T liquid culture grown in LB medium. Genomic DNA isolation and 16S rRNA gene amplification were performed according to Tóth *et al.* [12]. The partial 16S rRNA gene sequence of strain Kb22^T (MF471353) was compared with the EzBioCloud Database (http://www.ezbiocloud.net/taxonomy) [13] for an approximate phylogenetic affiliation. After Sanger sequencing of the 16S rRNA gene, a genome sequencing project of Kb22^T was carried out. According to the comparisons with the complete 16S rRNA gene sequences in the EzBioCloud Database, the highest level of sequence similarity occurred with

Sphingobacterium nematocida M-SX103^T (94.39%) [14], followed by *Sphingobacterium composti* T5-12^T (94.31%) [15].

Phylogenetic tree based on 16S rRNA gene was inferred by using the neighbor-joining method [16] with Kimura's two-parameter calculation model [17]. Tree topologies and distances were evaluated by bootstrap analysis based on 1000 replicates. Evolutionary analyses were conducted in MEGA X [18]. The phylogenetic tree based on 16S rRNA gene sequences suggested that strain Kb22^T forms a distinct phyletic lineage in the *Sphingobacteriaceae* family.

Genome features

The genome of strain Kb22^T was sequenced with Illumina MiSeq sequencing technology as described previously [19]. Genome assembly was performed by SPAdes v. 3.9.1; CLC NGS Cell v. 11.0. Genome completeness and contamination values were examined by TypeMet tool of MiGA server (http://microbial-genomes.org/) [20]. Annotation of the genome was performed by NCBI Prokaryotic Genome Annotation Pipeline v4.4 with Best-placed reference protein set and GeneMarkS+ methods [21, 22] and Rapid Annotation using Subsystem Technology server v. 2.0 (RAST; https://rast.nmpdr.org) [23]. The completeness and contamination metrics of the genome were found to be 96.2% and 1.9%, respectively. Other quality metrics of genome sequencing and assembly were as follows: 227-fold genome coverage, contig N50=578,756, number of contigs was 10. The genome size and G+C content of Kb22^T were 4,056,205 bp and 38.1 mol%, respectively. According to the annotation, there were 3603 genes, 3548 CDSs and 55 RNA genes in the genome. The coding density was 89.51%.

The anti-SMASH server was used to identify the secondary metabolite biosynthesis gene clusters [24]. Four putative biosynthetic gene clusters (arylpolyene, resorcinol and two furans) were found in 3 genomic regions.

Strain Kb22^T was isolated on xylan containing minimal agar, and it was also able to grow on media containing mannan or cellulose as sole carbon source. The genome annotation revealed 55 glycoside hydrolases (GHs) in 21 different GH families (Table 1). These enzyme genes may play a role in the breakdown and modification of carbohydrates in soil. Some of these enzymes are active on plant cell wall polysaccharides and potentially have role in the breakdown of lignocelluloses. The main polysaccharide components in lignocellulose are cellulose and xylan. The key enzymes for the decomposition of these two main components

are a xylanases, β-xylosidases, cellulases and β-glucosidases. The presence of these genes was examined in *Sphingobacterium hungaricum* Kb22^T, *Sphingobacterium composti* KCTC 12578^T, *Sphingobacterium olei* HAL-9^T [25] and *Sphingobacterium nematocida* DSM_24091^T (Table 2). According to the annotation of genomes (GCA_015210005, GCA_009829075, GCA_005048855, GCA_900168125), we found the key genes required for xylan degradation in Kb22^T, but in the case of the endophytic *Sphingobacterium nematocida* DSM_24091^T no xylanase or cellulase genes were found. The results revealed differences in the gene set of the compared strains, and it is important to note that lignocellulose degradation in nature is performed by microbial communities. The glycoside hydrolase sequences were identified using the Carbohydrate Active Enzymes database (http://www.cazy.org/) and the Interpro web service (https://www.ebi.ac.uk/interpro/) [26, 27].

Genome based phylogeny

As the number of whole genome sequences increased, it became possible to introduce different overall genome related indexes (OGRI). However, according to Chun et al., OGRI does not have a taxonomic resolution above the species level, so a multigene-based phylogenomic treeing approach should be chosen for defining genera [28]. Others recommend the use of genus-specific ANI and AF (Aligment Fraction) values [29]. Recently, the genomic-scale phylogenetic, AAI and conserved signature indels (CSIs) based classification of broad genera seems to be suitable [30-32].

The 16S rRNA gene similarity found for Kb22^T is at the genus boundary, thus also MLSA and whole genome sequence based methods were used to determine the phylogenetic position of the strain.

Multilocus sequence analyses were performed by autoMLST webserver (https://automlst.ziemertlab.com/) [33]. The concatenated sequences were made from 78 genes (Table S1). Phylogenetic tree based on concatenated sequences was inferred using the neighbor-joining method [16] with Kimura's two-parameter calculation model [17] using MEGA version X [18]. Tree topologies and distances were evaluated by bootstrap analysis based on 1000 replicates. Based on the MLSA tree, Kb22^T was found to be a member of the genus *Sphingobacterium* as a novel species (Fig. 1).

Genome-based relatedness between Kb22^T and *S. spiritivorum* NCTC 11386^T, the type strain of the type species, was determined based on ANI using OrthoANI (https://www.ezbiocloud.net/tools/ani) algorithm [34] and dDDH (identities/HSP length) with

GGDC service of DSMZ (http://ggdc.dsmz.de/) [35]. The OrthoANIu and dDDH values between Kb22^T and *S. spiritivorum* NCTC 11386^T were 70.37 and 19.90%, much lower than the generally accepted species boundary of 95~96 and 70%, respectively [35-37]. The highest OrthoANIu value(71.83%) was found with *Sphingobacterium composti* T5-12^T.

As part of the phylogenomic studies, TYGS (https://tygs.dsmz.de/) [38] and MiGA (http://microbial-genomes.org/) [20] webservers were used. Phylogenomic treeing by TYGS confirmed the MLSA result that Kb22^T represents a new species within genus *Sphingobacterium* (Fig. 2). An AAI matrix with type strains in the *Sphingobacteriaceae* family was calculated by the latest extension of the Microbial Genome Atlas (https://xsede.microbial-genomes.org/). The lowest value was observed with *Anseongella ginsenosidimutans* Gsoil 524^T (52%), the highest with *Sphingobacterium spiritivorum* NCTC 11386^T (66%) (Table 3). Genbank assembly accessions: *Albibacterium bauzanense* DSM 22554^T - GCA_004339765.1 [39]; *Anseongella ginsenosidimutans* Gsoil 524^T - GCA_008033235.1 [40]; *Arcticibacter svalbardensis* MN12-7^T - GCA_000403135.1 [41]; *Mucilaginibacter paludis* DSM 18603^T - GCA_000166195.3 [42]; *Nubsella zeaxanthinifaciens* TDMA-5^T - GCA_003313335.1 [43]; *Olivibacter sitiensis* DSM 17696^T - GCA_000427965.1 [44]; *Parapedobacter koreensis* Jip14^T - GCA_900109365.1 [45]; *Pararcticibacter amylolyticus* FJ4-8^T - GCA_003130405.1 [46]; *Pedobacter heparinus* DSM 2366^T - GCA_000023825.1 [1]; *Pelobium manganitolerans* YS-25^T - GCA_003609575.1 [47]; *Pseudosphingobacterium domesticum* DSM 18733^T - GCA_900109575.1 [48]; *Solitalea koreensis* DSM 21342^T - GCA_900182575.1 [49]; *Sphingobacterium spiritivorum* NCTC 11386^T - GCA_900457435.1 [5].

According to MiGA distance analysis, the closest relatives are *Sphingobacterium olei* HAL-9^T (accession: GCA_005048855) (66.65% AAI) and *Sphingobacterium alkalisoli* Y3L14^T (accession: GCA_005049105**)** (66.39% AAI) [50]. The p-value of taxonomic novelty at genus level is 0.655 and at species level is 0.002. Summarising the phylogenetic assessments, the taxonomic position of Kb22^T is a borderline case. In accordance with the recommendation of Chun *et al*., the phylogenomic results were considered decisive for genus level rank [28].

Physiologic, morphologic and chemotaxonomic characterisation

Biomass for chemical and molecular studies was obtained by cultivation in shaker flasks (180 r.p.m.) using LB medium at 30 °C for 32 h. Colony morphology of strain Kb22^T was determined on LB agar medium by directly observing single colonies. Cell morphology of

strain Kb22^T was observed under electron microscope. Pigments were investigated as described previously by Daood and Biacs [51] and by Bernardet and Bowman [52]. The Gram reaction was determined with a non-staining method as described by Buck *et al*. [53]. Oxidase activity was studied with OXI oxidase test strip (Diagnostics s.r.o.). Catalase production was demonstrated by the method of Barrow and Feltham [54]. Growth at different temperatures (from 4 to 50 °C), NaCl tolerance (0–5% w/v) and pH tolerance (pH 4–10, using increments of 0.5 pH unit, pH values were adjusted with HCl or NaOH) were determined using LB 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 Kb22^T were investigated with API 50 CH, API 20 NE and API ZYM kits (BioMérieux) according to the manufacturer's instructions. The API 50CH and 20NE tests were read after 24–48 h incubation at 28 °C. Anaerobic and microaerophilic growth was checked on TSA medium using the Anaerocult A and C systems (Merck). The physiological characteristics were examined by side-by-side analysis with *Sphingobacterium composti* T5-12^T [15].

LB medium is used for general laboratory cultivation, but the novel strain also grew well on TSA, nutrient and R2A media. After 72 h growth on LB at 30 °C, colonies were found to be 1 mm in diameter, circular, non-mucoid, smooth and orange. Cells of Kb22^T produced flexirubin-type pigments. Strain Kb22^T was found to be Gram-reaction-negative, aerobic, positive for oxidase and catalase, rod-shaped bacterium (Fig. S1). Cells were observed to be non-motile, grow in 0.0–2.0% (w/v) NaCl, at a pH range 6.5 to 8.5 and at a temperature range between 10 and 35 °C. Optimal growth was observed at 30 °C, 1.0% (w/v) NaCl and pH 7.5. Mean cell size of Kb22^T was found to be 0.5 µm in diameter and 1.0-1.5 µm in length.

According to API 50 CH test, Kb22^T was positive for acid production from D-arabinose, D-glucose, D-fructose, D-mannose, L-rhamnose, methyl-α-D-mannopyranoside, methyl-α-D-xylopyranoside, N-acetyl-glucosamine, amygdaline, arbutine, esculine, salicine, D-cellobiose, D-maltose, D-lactose, D-melibiose, D-saccharose, D-trehalose, D-melezitose, D-raffinose, starch, gentiobiose, D-turanose and L-fucose. β-galactosidase activity, hydrolysis of esculin and assimilation of glucose, mannose, N-acetyl-glucosamine and maltose were demonstrated by using the API 20 NE test. In the API ZYM test, strain Kb22^T was positive for alkaline phosphatase, esterase lipase (C8), leucine arylamidase, naphthol-AS-BI-phosphohydrolase, β-glucosidase, N-acetyl-β-glucosaminidase and trypsin. Differential phenotypic characteristics between strain Kb22^T and *Sphingobacterium composti* T5-12^T are given in Table 4. According to comparative genomics performed on MicroScope platform, the strain specific

CDSs account for 80.577% in Kb22^T genome and 83.065% in *Sphingobacterium composti* T5-12^T genome [55].

Analyses of chemotaxonomic traits were carried out by DSMZ Identification Service, Braunschweig, Germany. For polar lipid and respiratory quinone analyses, the strains were cultivated in LB liquid medium at 30 °C, 180 r.p.m. until exponential growth was reached. The fatty acid profiles of strain Kb22^T and *Sphingobacterium composti* T5-12^T were analysed using active growing cultures on LB at 30 °C.

According to the DSMZ Identification Service, fatty acid methyl esters (FAMEs) were obtained following the method of Miller [56] and Kuykendall *et al*. [57]. FAMEs were separated by gas chromatography, detected by a flame ionisation detector using Sherlock Microbial Identification System (MIS) (MIDI, Microbial ID, Newark, DE 19711 U.S.A.) 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 Kb22^T were observed to be iso-$C_{15:0}$ (28.4%), summed feature 3 ($C_{16:1}\omega 7c$ and/or iso-$C_{15:0}$ 2-OH) (25.7%) and iso-$C_{17:0}$ 3-OH (19.7%). The fatty acid profile was found to be similar to that of related species, in accordance with the description of *Sphingobacterium* genus [5]. However, the ratios of the components were different. The complete fatty acid composition is shown in Table 5.

The respiratory quinones were extracted from freeze dried material and purified by a silica-based solid phase extraction. Purified samples were further analysed by HPLC and UHPLC-ESI-qTOF system [58, 59; dsmz.de]. The only respiratory quinone of Kb22^T was menaquinone-7 (MK-7).

Polar lipids were studied according to Tindall *et al.* [58-60, dsmz.de]. Strain Kb22^T exhibited a complex polar lipid profile consisting of phosphatidylethanolamine (PE) and phosphoglycolipid (PGL) as dominant elements and an uncharacterised aminoglycolipid (GNL), six uncharacterised phospholipids (PL) and five uncharacterised lipids (L) (Fig. S2). Though the domination of PE is characteristic of other species in the genus, the presence and ratio of other components were different [5].

In conclusion, phenotypic, biochemical, chemotaxonomic and phylogenetic information of strain Kb22^T support its classification as a novel species of *Sphingobacterium*, for which the name *Sphingobacterium hungaricum* sp. nov. is proposed. The GenBank accession numbers for the 16S rRNA gene sequence and the whole genome of *Sphingobacterium hungaricum* strain Kb22^T are MF471353 and PRDK00000000, respectively.

Description of *Sphingobacterium hungaricum* sp. nov.

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

Cells are strictly aerobic, Gram-reaction-negative straight rods and non-motile. It grows well on LB, TSA, nutrient and R2A media. Colonies have orange pigmentation on TSA after 48 h of incubation. Cells are 0.5 µm in diameter and 1.0-1.5 µm in length. It grows at 10–35 °C (optimum, 30 °C) and at NaCl concentrations of 0–2 w/v % (optimum, 1 w/v %). It is positive for oxidase, catalase, β-galactosidase, alkaline phosphatase, esterase lipase (C8), leucine arylamidase, naphthol-AS-BI-phosphohydrolase, β-glucosidase, N-acetyl-β-glucosaminidase, trypsin, hydrolysis of esculin and assimilation of glucose, mannose, N-acetyl-glucosamine, and maltose. Strain Kb22^T was found to be positive for acid production from D-arabinose, D-glucose, D-fructose, D-mannose, L-rhamnose, methyl-α-D-mannopyranoside, methyl-α-D-xylopyranoside, N-acetyl-glucosamine, amygdaline, arbutine, esculine, salicine, D-cellobiose, D-maltose, D-lactose, D-melibiose, D-saccharose, D-trehalose, D-melezitose, D-raffinose, starch, gentiobiose, D-turanose, and L-fucose. The major fatty acids are iso-$C_{15:0}$ (28.4%), summed feature 3 ($C_{16:1}$ *ω7c* and/or iso-$C_{15:0}$ 2-OH) (25.7%) and iso-$C_{17:0}$ 3-OH (19.7%). The only respiratory quinone is MK-7. The major polar lipid is phosphatidylethanolamine. The type strain is Kb22^T (=LMG 31574 = NCAIM B.02638), isolated from agricultural field in the Great Hungarian Plain, Hungary. The DNA G+C content of the type strain is 38.1 mol%, the genome is 4.06 Mb. The GenBank accession number of the genome is PRDK00000000.

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Conflict of interest

The authors declare that there are no conflicts of interest.

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Figures and Tables

Table 1. Glycoside hydrolases coded in *Sphingobacterium hungaricum* Kb22^T genome

GH family	NCBI accession number	Activities in Family
GH2	MBE8713017; MBE8713638; MBE8712575; MBE8715037	β-galactosidase (EC 3.2.1.23) ; β-mannosidase (EC 3.2.1.25); β-glucuronidase (EC 3.2.1.31); α-L-arabinofuranosidase (EC 3.2.1.55); exo-β-glucosaminidase (EC 3.2.1.165); α-L-arabinopyranosidase (EC 3.2.1.-); β-galacturonidase (EC 3.2.1.-); β-xylosidase (EC 3.2.1.37); β-D-galactofuranosidase (EC 3.2.1.146); β-glucosidase (EC 3.2.1.21) and others
GH3	MBE8715078; MBE8713443; MBE8713973	β-glucosidase (EC 3.2.1.21); xylan 1,4-β-xylosidase (EC 3.2.1.37); α-L-arabinofuranosidase (EC 3.2.1.55); glucan 1,4-β-glucosidase (EC 3.2.1.74); β-1,2-glucosidase (EC 3.2.1.-); β-1,3-glucosidase (EC 3.2.1.-); xyloglucan-specific exo-β-1,4-glucanase / exo-xyloglucanase (EC 3.2.1.155) and others
GH5	MBE8712452	endo-β-1,4-glucanase / cellulase (EC 3.2.1.4); endo-β-1,4-xylanase (EC 3.2.1.8); β-glucosidase (EC 3.2.1.21); β-mannosidase (EC 3.2.1.25); glucan β-1,3-glucosidase (EC 3.2.1.58); exo-β-1,4-glucanase / cellodextrinase (EC 3.2.1.74); glucan endo-1,6-β-glucosidase (EC 3.2.1.75); mannan endo-β-1,4-mannosidase (EC 3.2.1.78); cellulose β-1,4-cellobiosidase (EC 3.2.1.91); steryl β-glucosidase (EC 3.2.1.104); endo-β-1,6-galactanase (EC 3.2.1.164); mannan transglycosylase (EC endo-β-1,3-glucanase / laminarinase (EC 3.2.1.39); β-N-acetylhexosaminidase (EC 3.2.1.52); chitosanase (EC 3.2.1.132); β-D-galactofuranosidase (EC 3.2.1.146); β-galactosylceramidase (EC 3.2.1.46); α-L-arabinofuranosidase (EC 3.2.1.55) and others
GH13	MBE8713329; MBE8712407	α-amylase (EC 3.2.1.1); pullulanase (EC 3.2.1.41); cyclomaltodextrinase (EC 3.2.1.54); trehalose-6-phosphate hydrolase (EC 3.2.1.93); α-glucosidase (EC 3.2.1.20); maltotetraose-forming α-amylase (EC 3.2.1.60); isoamylase (EC 3.2.1.68); maltotriose-forming α-amylase (EC 3.2.1.116); trehalose synthase (EC 5.4.99.16); amylo-α-1,6-glucosidase (EC 3.2.1.33); oligosaccharide α-4-glucosyltransferase (EC 2.4.1.161) and others
GH15	MBE8712115	glucoamylase (EC 3.2.1.3); glucodextranase (EC 3.2.1.70); α,α-trehalase (EC 3.2.1.28); dextran dextrinase (EC 2.4.1.2)
GH16	MBE8713572; MBE8714674	xyloglucan:xyloglucosyltransferase (EC 2.4.1.207); endo-1,3-β-glucanase / laminarinase (EC 3.2.1.39); endo-1,3(4)-β-glucanase (EC 3.2.1.6); licheninase (EC 3.2.1.73); β-agarase (EC 3.2.1.81); xyloglucanase (EC 3.2.1.151); endo-β-1,3-galactanase (EC 3.2.1.181); hyaluronidase (EC 3.2.1.35); endo-β-1,4-galactosidase (EC 3.2.1.-); chitin β-1,6-glucanosyltransferase (EC 2.4.1.-); β-glycosidase (EC 3.2.1.-); β-carrageenase (EC 3.2.1.-) and others
GH20	MBE8715038	β-hexosaminidase (EC 3.2.1.52); lacto-N-biosidase (EC 3.2.1.140); β-1,6-N-acetylglucosaminidase (EC 3.2.1.-); β-6-SO3-N-acetylglucosaminidase (EC 3.2.1.-)
GH25	MBE8715245	lysozyme (EC 3.2.1.17)
GH26	MBE8712451; MBE8712453	β-mannanase (EC 3.2.1.78); exo-β-1,4-mannobiohydrolase (EC 3.2.1.100); β-1,3-xylanase (EC 3.2.1.32); lichenase / endo-β-1,3-1,4-glucanase (EC 3.2.1.73); mannobiose-producing exo-β-mannanase (EC 3.2.1.-)
GH31	MBE8712408; MBE8713639	α-glucosidase (EC 3.2.1.20); α-galactosidase (EC 3.2.1.22); α-mannosidase (EC 3.2.1.24); α-1,3-glucosidase (EC 3.2.1.84); α-xylosidase (EC 3.2.1.177); α-glucan lyase (EC 4.2.2.13); isomaltosyltransferase (EC 2.4.1.-); α-N-acetylgalactosaminidase (EC 3.2.1.49) and others
GH32	MBE8713030	invertase (EC 3.2.1.26); endo-inulinase (EC 3.2.1.7); exo-inulinase (EC 3.2.1.80); fructan β-(2,6)-fructosidase/6-exohydrolase (EC 3.2.1.154); sucrose:fructan 6-fructosyltransferase (EC 2.4.1.10); fructan:fructan 6G-fructosyltransferase (EC 2.4.1.243); levan fructosyltransferase (EC 2.4.1.-); cycloinulo-oligosaccharide fructanotransferase (EC 2.4.1.-) and others
GH36	MBE8712331	α-galactosidase (EC 3.2.1.22); α-N-acetylgalactosaminidase (EC 3.2.1.49); stachyose synthase (EC 2.4.1.67); raffinose synthase (EC 2.4.1.82)

GH43	MBE8714090; MBE8715079; MBE8714498; MBE8712801; MBE8712802; MBE8714088; MBE8714089; MBE8715346	β-xylosidase (EC 3.2.1.37); α-L-arabinofuranosidase (EC 3.2.1.55); xylanase (EC 3.2.1.8); α-1,2-L-arabinofuranosidase (EC 3.2.1.-); β-1,3-xylosidase (EC 3.2.1.-); exo-β-1,3-galactanase (EC 3.2.1.145) and others
GH65	MBE8713327; MBE8713430	α,α-trehalase (EC 3.2.1.28); maltose phosphorylase (EC 2.4.1.8); trehalose phosphorylase (EC 2.4.1.64); trehalose-6-phosphate phosphorylase (EC 2.4.1.216) and others
GH76	MBE8713771; MBE8715179; MBE8713770	α-1,6-mannanase (EC 3.2.1.101); α-glucosidase (EC 3.2.1.20)
GH78	MBE8713718; MBE8713719; MBE8713720; MBE8713061; MBE8713026; MBE8715340	α-L-rhamnosidase (EC 3.2.1.40); rhamnogalacturonan α-L-rhamnohydrolase (EC 3.2.1.174); L-Rhap-α-1,3-D-Apif -specific α-1,3-L-rhamnosidase (EC 3.2.1.-)
GH88	MBE8713573	d-4,5-unsaturated β-glucuronyl hydrolase (EC 3.2.1.-)
GH92	MBE8715180; MBE8715337; MBE8713866; MBE8712429; MBE8713427; MBE8712806; MBE8714415; MBE8715336	mannosyl-oligosaccharide α-1,6-mannosidase (EC 3.2.1.-); α-mannosidase (EC 3.2.1.24); α-1,2-mannosidase (EC 3.2.1.-); α-1,3-mannosidase (EC 3.2.1.-); α-1,4-mannosidase (EC 3.2.1.-) and others
GH106	MBE8713721	α-L-rhamnosidase (EC 3.2.1.40); rhamnogalacturonan α-L-rhamnohydrolase (EC 3.2.1.174)
GH125	MBE8714531	exo-α-1,6-mannosidase (EC 3.2.1.-)
GH29/GH95	MBE8713785; MBE8713440; MBE8714269; MBE8715039	α-L-fucosidase (EC 3.2.1.51); α-1,3/1,4-L-fucosidase (EC 3.2.1.111); α-1,2-L-fucosidase (EC 3.2.1.63) α-L-galactosidase (EC 3.2.1.-)

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Table 2. Genes annotated as xylanase, β-xylosidase, cellulase or β-glucosidase in the genome of Kb22^T and related strains. GenBank accession numbers are GCA_015210005 (*Sphingobacterium hungaricum* Kb22^T), GCA_009829075 (*Sphingobacterium composti* KCTC 12578^T), GCA_005048855 (*Sphingobacterium olei* HAL-9^T) and GCA_900168125 (*Sphingobacterium nematocida* DSM_24091^T). The GH family numbers are in parentheses.

	S. hungaricum Kb22^T	*S. composti* KCTC 12578^T	*S. olei* HAL-9^T	*S. nematocida* DSM_24091^T
isolation source of the strain	soil	compost	soil	leaf tissue
xylanase	MBE8715079(GH43) MBE8714090(GH43)	-	WP_136902756 (GH10) WP_136902760 (GH10)	-
β-xylosidase	MBE8712801(GH43)	-		-
cellulase	-	WP_159637017 (GH5) WP_159637239 (GH5)	WP_136901870 (GH5)	-
β-glucosidase	MBE8715078(GH3) MBE8713443(GH3)	-	WP_136900542 (GH1) WP_136902682 (GH3)	-

Table 3. AAI matrix with Kb22^T and type strains in the *Sphingobacteriaceae* family. Genbank assembly accessions: *Albibacterium bauzanense* DSM 22554^T - GCA_004339765.1; *Anseongella ginsenosidimutans* Gsoil 524^T - GCA_008033235.1; *Arcticibacter svalbardensis* MN12-7^T - GCA_000403135.1; *Mucilaginibacter paludis* DSM 18603^T - GCA_000166195.3; *Nubsella zeaxanthinifaciens* TDMA-5^T - GCA_003313335.1; *Olivibacter sitiensis* DSM 17696^T - GCA_000427965.1; *Parapedobacter koreensis* Jip14^T - GCA_900109365.1; *Pararcticibacter amylolyticus* FJ4-8^T - GCA_003130405.1; *Pedobacter heparinus* DSM 2366^T - GCA_000023825.1; *Pelobium manganitolerans* YS-25^T - GCA_003609575.1; *Pseudosphingobacterium domesticum* DSM 18733^T - GCA_900109575.1; *Solitalea koreensis* DSM 21342^T - GCA_900182575.1; *Sphingobacterium spiritivorum* NCTC 11386^T - GCA_900457435.1.

Sphingobacterium hungaricum Kb22^T	*Albibacterium bauzanense* DSM 22554^T	*Parapedobacter koreensis* Jip14^T	*Sphingobacterium spiritivorum* NCTC 11386^T	*Anseongella ginsenosidimutans* Gsoil 524^T	*Olivibacter sitiensis* DSM 17696^T	*Solitalea koreensis* DSM 21342^T	*Pararcticibacter amylolyticus* FJ4-8^T	*Pelobium manganitolerans* YS-25^T	*Pedobacter heparinus* DSM 2366^T	*Mucilaginibacter paludis* DSM 18603^T	*Arcticibacter svalbardensis* MN12-7^T	*Pseudosphingobacterium domesticum* DSM 18733^T	*Nubsella zeaxanthinifaciens* TDMA-5^T	
100	58	60	66	52	59	54	58	56	57	57	57	60	57	*Sphingobacterium hungaricum* Kb22^T
58	100	59	59	52	59	54	58	56	56	57	57	61	57	*Albibacterium bauzanense* DSM 22554^T
60	59	100	62	53	60	54	58	56	56	57	57	61	57	*Parapedobacter koreensis* Jip14^T
66	59	62	100	52	60	54	59	56	57	57	57	61	57	*Sphingobacterium spiritivorum* NCTC 11386^T
52	52	53	52	100	52	54	53	53	52	53	52	53	53	*Anseongella ginsenosidimutans* Gsoil 524^T
59	59	60	60	52	100	54	58	57	57	57	57	64	57	*Olivibacter sitiensis* DSM 17696^T
54	54	54	54	54	54	100	55	55	55	55	54	55	55	*Solitalea koreensis* DSM 21342^T

58	58	58	59	53	58	55	100	58	58	59	63	60	58	*Pararcticibacter amylolyticus* FJ4-8^{T}
56	56	56	56	53	57	55	58	100	58	57	57	57	58	*Pelobium manganitolerans* YS-25^{T}
57	56	56	57	52	57	55	58	58	100	58	58	57	65	*Pedobacter heparinus* DSM 2366^{T}
57	57	57	57	53	57	55	59	57	58	100	58	58	57	*Mucilaginibacter paludis* DSM 18603^{T}
57	57	57	57	52	57	54	63	57	58	58	100	58	57	*Arcticibacter svalbardensis* MN12-7^{T}
60	61	61	61	53	64	55	60	57	57	58	58	100	57	*Pseudosphingobacterium d omesticum* DSM 18733^{T}
57	57	57	57	53	57	55	58	58	65	57	57	57	100	*Nubsella zeaxanthinifaciens* TDMA-5^{T}

523

524 **Table 4.** Differential phenotypic characteristics between strain Kb22^T and *Sphingobacterium*
525 *composti* T5-12^T. Data are from this study, except G+C content of *Sphingobacterium*
526 *composti* T5-12^T [9]
527 Strains: 1, Kb22^T; 2, *Sphingobacterium composti* T5–12^T

	1	2
Isolation source	soil	compost
Temperature range for growth (°C) (optimum)	10-35 (30)	10-40 (35)
Growth with NaCl (%) (optimum)	0-2 (1)	0.5-3 (0.5)
pH range for growth (optimum)	6.0-8.5 (7.5)	5.5-8.5 (7.0)
G+C content (mol%)	38.1	35.2
API 50CH		
D-arabinose	+	-
D-galactose	-	+
rhamnose	+	-
mannitol	-	+
methyl-α-D-mannopyranoside	+	-
methyl-α-D-glucopyranoside	+	-
N-acetyl glucosamine	+	-
amygdalin	+	-
arbutin	+	-
salicin	+	-
D-cellobiose	+	-
D-maltose	+	-
D-lactose	+	-
D-melibiose	+	-
D-saccharose	+	-
D-trehalose	+	-
melesitose	+	-
D-raffinose	+	-
starch	+	-
gentiobiose	+	-
turanose	+	-
API 20 NE		
hydrolysis of esculin	+	-
assimilation of mannitol	-	+
assimilation of maltose	+	-
API ZYM		
esterase lipase (C8)	+	-
β-glucosidase	+	-
α-chymotrypsin	-	+

528

Table 5. Cellular fatty acid composition of *Sphingobacterium hungaricum* Kb22^T and *Sphingobacterium composti* T5-12^T. Strains: 1, Kb22^T; 2, *Sphingobacterium composti* T5-12^T; tr, trace amount (<1%). Summed Features are fatty acids that cannot be resolved reliably from another fatty acid using the chromatographic conditions chosen. The MIDI system groups these fatty acids together as one feature with a single percentage of the total. The unknown fatty acids have no name listed in the peak library file of the MIDI system. ECL, equivalent chain length.

	1	**2**
iso-$C_{15:0}$	28.4	39.7
iso-$C_{15:0}$ 3-OH	2.7	1.8
iso-$C_{15:1}$ F	-	2.0
iso-$C_{15:1}$ G	5.1	
iso-$C_{17:0}$ 3-OH	19.7	17.6
iso-$C_{17:1}$ ω9*c*	10.1	10.6
summed feature 1 (iso-$C_{15:1}$ H/$C_{13:0}$ 3-OH)	-	1.2
summed feature 3 ($C_{16:1}$ ω7*c*/iso-$C_{15:0}$ 2-OH)	25.7	20.6
summed feature 4 (iso-$C_{17:1}$ I/anteiso-$C_{17:1}$ B)	1.3	-
unknown (ECL 13.565)	1.1	1.1
unknown (ECL 16.582)	1.6	1.3

Fig. 1. MLSA tree showing the relationship of strain Kb22^T to closely related species. Genes for MLSA were selected by autoMLST webserver. The phylogenetic tree based on concatenated genes was inferred using the neighbor-joining method with Kimura's two-parameter calculation model.

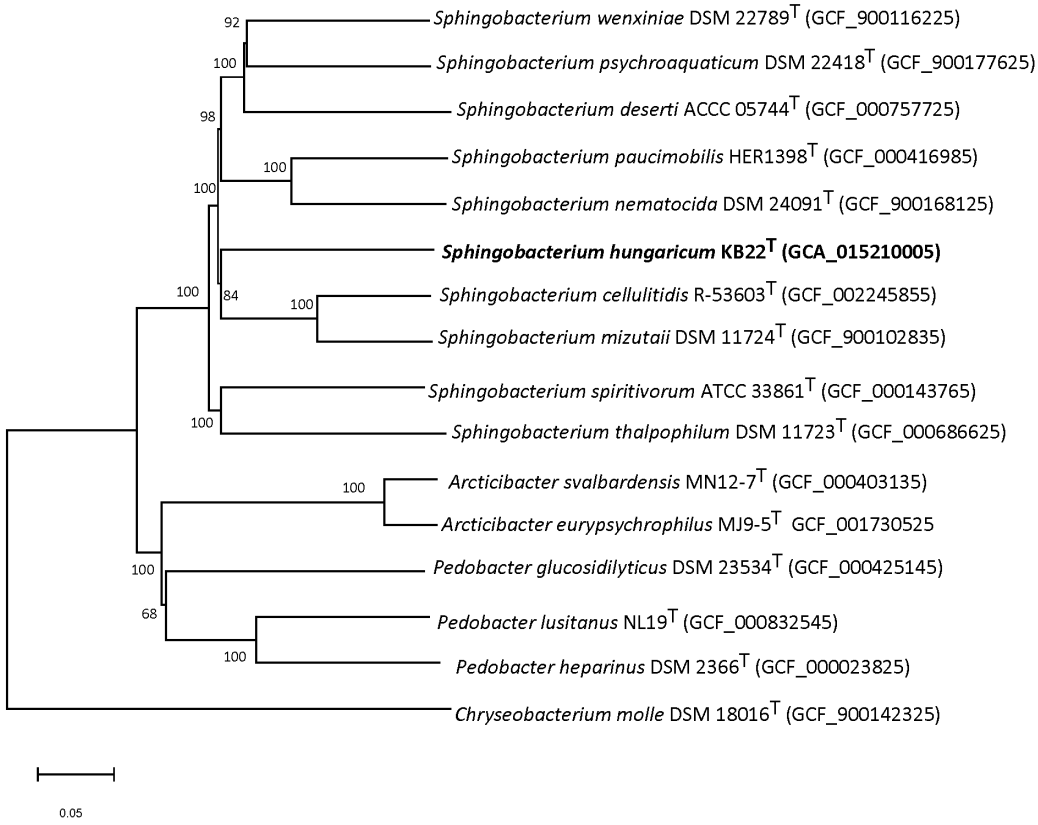
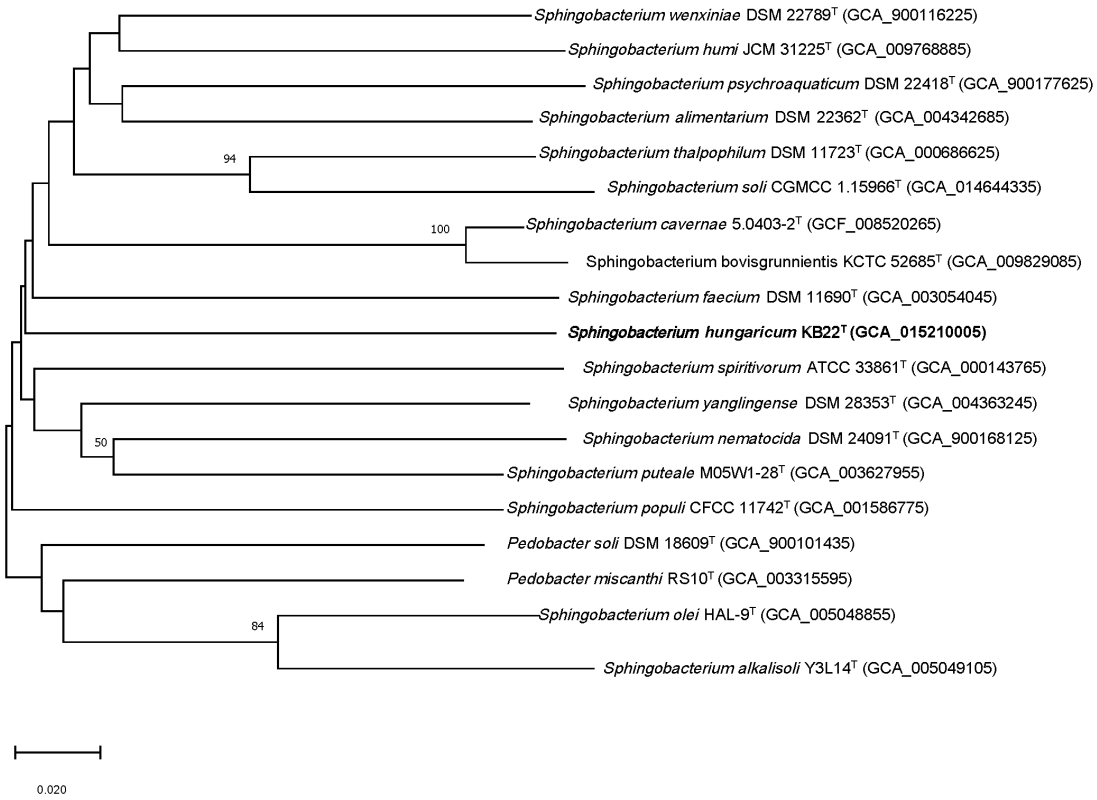


Fig. 2. Tree inferred with FastME 2.1.6.1 [61] from GBDP distances calculated from genome sequences. The branch lengths are scaled in terms of GBDP distance formula $_{\delta}5$. The numbers above branches are GBDP pseudo-bootstrap support values from 100 replications, with an average branch support of 39.9%. The tree was rooted at the midpoint [62]. Assembly accessions are in parentheses.



555 **Table S1.** Genes selected for MLSA by autoMLST

TIGFRAM entries	Gene abbreviation	Biological process	Gene name
TIGR01798	cit_synth_I	Energy metabolism	citrate (Si)-synthase
TIGR00055	uppS	Cell envelope	di-trans,poly-cis-decaprenylcistransferase
TIGR03953	rplD_bact	Protein synthesis	50S ribosomal protein uL4
TIGR00635	ruvB	DNA metabolism	Holliday junction DNA helicase RuvB
TIGR00447	pth	Protein synthesis	aminoacyl-tRNA hydrolase
TIGR00114	lumazine-synth	Biosynthesis of cofactors, prosthetic groups, and carriers	6,7-dimethyl-8-ribityllumazine synthase
TIGR00138	rsmG_gidB	Protein synthesis	16S rRNA (guanine(527)-N(7))-methyltransferase RsmG
TIGR01394	TypA_BipA	Regulatory functions	GTP-binding protein TypA/BipA
TIGR00059	L17	Protein synthesis	ribosomal protein bL17
TIGR00150	T6A_YjeE	Protein synthesis	tRNA threonylcarbamoyl adenosine modification protein YjeE
TIGR01520	FruBisAldo_II_A	Energy metabolism	fructose-bisphosphate aldolase, class II
TIGR00482	TIGR00482	Biosynthesis of cofactors, prosthetic groups, and carriers	nicotinate (nicotinamide) nucleotide adenylyltransferase
TIGR00242	TIGR00242	Regulatory functions	division/cell wall cluster transcriptional repressor MraZ
TIGR00420	trmU	Protein synthesis	tRNA (5-methylaminomethyl-2-thiouridylate)-methyltransferase
TIGR00575	dnlj	DNA metabolism	DNA ligase, NAD-dependent
TIGR00422	valS	Protein synthesis	valine--tRNA ligase
TIGR00461	gcvP	Energy metabolism	glycine dehydrogenase
TIGR01169	rplA_bact	Protein synthesis	ribosomal protein uL1
TIGR00928	purB	Purines, pyrimidines, nucleosides, and nucleotides	adenylosuccinate lyase
TIGR00096	TIGR00096	Protein synthesis	16S rRNA (cytidine(1402)-2'-O)-methyltransferase
TIGR01163	rpe	Energy metabolism	ribulose-phosphate 3-epimerase
TIGR00510	lipA	Biosynthesis of cofactors, prosthetic groups, and carriers	lipoyl synthase
TIGR01071	rplO_bact	Protein synthesis	ribosomal protein uL15
TIGR00487	IF-2	Protein synthesis	translation initiation factor IF-2
TIGR03284	thym_sym	Purines, pyrimidines, nucleosides, and nucleotides	thymidylate synthase

TIGR00922	nusG	Transcription	transcription termination/antitermination factor NusG
TIGR00628	ung	DNA metabolism	uracil-DNA glycosylase
TIGR01066	rplM_bact	Protein synthesis	ribosomal protein uL13
TIGR01063	gyrA	DNA metabolism	DNA gyrase, A subunit
TIGR00967	3a0501s007	Protein fate	preprotein translocase, SecY subunit
TIGR01009	rpsC_bact	Protein synthesis	ribosomal protein uS3
TIGR01021	rpsE_bact	Protein synthesis	ribosomal protein uS5
TIGR00043	TIGR00043	Protein synthesis	rRNA maturation RNase YbeY
TIGR00042	TIGR00042	DNA metabolism	non-canonical purine NTP pyrophosphatase, RdgB/HAM1 family
TIGR00445	mraY	Cell envelope	phospho-N-acetylmuramoyl-pentapeptide-transferase
TIGR01051	topA_bact	DNA metabolism	DNA topoisomerase I
TIGR00382	clpX	Protein fate	ATP-dependent Clp protease, ATP-binding subunit ClpX
TIGR02013	rpoB	Transcription	DNA-directed RNA polymerase, beta subunit
TIGR00065	ftsZ	Cellular processes	cell division protein FtsZ
TIGR00064	ftsY	Protein fate	signal recognition particle-docking protein FtsY
TIGR00048	rRNA_mod_RlmN	Protein synthesis	23S rRNA (adenine(2503)-C(2))-methyltransferase
TIGR00165	S18	Protein synthesis	ribosomal protein bS18
TIGR00166	S6	Protein synthesis	ribosomal protein bS6
TIGR02729	Obg_CgtA	Protein synthesis	Obg family GTPase CgtA
TIGR00414	serS	Protein synthesis	serine--tRNA ligase
TIGR00431	TruB	Protein synthesis	tRNA pseudouridine(55) synthase
TIGR00416	sms	DNA metabolism	DNA repair protein RadA
TIGR00228	ruvC	DNA metabolism	crossover junction endodeoxyribonuclease RuvC
TIGR02386	rpoC_TIGR	Transcription	DNA-directed RNA polymerase, beta' subunit
TIGR00212	hemC	Biosynthesis of cofactors, prosthetic groups, and carriers	hydroxymethylbilane synthase
TIGR00755	ksgA	Protein synthesis	ribosomal RNA small subunit methyltransferase A
TIGR00952	S15_bact	Protein synthesis	ribosomal protein uS15
TIGR00436	era	Protein synthesis	GTP-binding protein Era
TIGR01302	IMP_dehydrog	Purines, pyrimidines, nucleosides, and nucleotides	inosine-5'-monophosphate dehydrogenase
TIGR00615	recR	DNA metabolism	recombination protein RecR

TIGR03263	guanyl_kin	Purines, pyrimidines, nucleosides, and nucleotides	guanylate kinase
TIGR00430	Q_tRNA_tgt	Protein synthesis	tRNA-guanine transglycosylase
TIGR01050	rpsS_bact	Protein synthesis	ribosomal protein uS19
TIGR02012	tigrfam_recA	DNA metabolism	protein RecA
TIGR00521	coaBC_dfp	Biosynthesis of cofactors, prosthetic groups, and carriers	phosphopantothenoylcysteine decarboxylase / phosphopantothenate--cysteine ligase
TIGR01059	gyrB	DNA metabolism	DNA gyrase, B subunit
TIGR00337	PyrG	Purines, pyrimidines, nucleosides, and nucleotides	CTP synthase
TIGR03594	GTPase_EngA	Protein synthesis	ribosome-associated GTPase EngA
TIGR00499	lysS_bact	Protein synthesis	lysine--tRNA ligase
TIGR00088	trmD	Protein synthesis	tRNA (guanine(37)-N(1))-methyltransferase
TIGR00086	smpB	Protein synthesis	SsrA-binding protein
TIGR00496	frr	Protein synthesis	ribosome recycling factor
TIGR00479	rumA	Protein synthesis	23S rRNA (uracil-5-)-methyltransferase RumA
TIGR01171	rplB_bact	Protein synthesis	ribosomal protein uL2
TIGR02348	GroEL	Protein fate	chaperonin GroL
TIGR00959	ffh	Protein fate	signal recognition particle protein
TIGR01379	thiL	Biosynthesis of cofactors, prosthetic groups, and carriers	thiamine-phosphate kinase
TIGR00033	aroC	Amino acid biosynthesis	chorismate synthase
TIGR01135	glmS	Central intermediary metabolism	glutamine-fructose-6-phosphate transaminase (isomerizing)
TIGR01357	aroB	Amino acid biosynthesis	3-dehydroquinate synthase
TIGR01032	rplT_bact	Protein synthesis	ribosomal protein bL20
TIGR03631	uS13_bact	Protein synthesis	ribosomal protein uS13
TIGR01011	rpsB_bact	Protein synthesis	ribosomal protein uS2

558 **Fig. S1.** Transmission electron micrograph of strain Kb22^{T}

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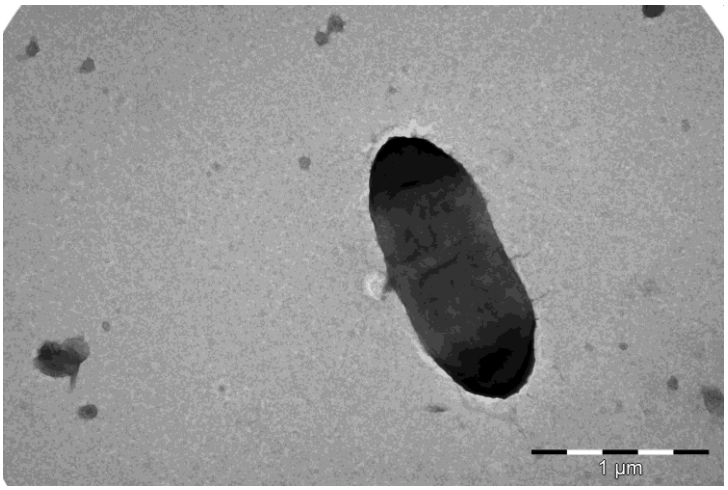

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562 **Fig S2.** Polar lipids of strain Kb22^{T} after two-dimensional thin-layer chromatography (TLC)
563 PE, phosphatidylethanolamine; PGL, phosphoglycolipid; GNL, aminoglycolipid; PL,
564 phospholipid; L, uncharacterised lipid

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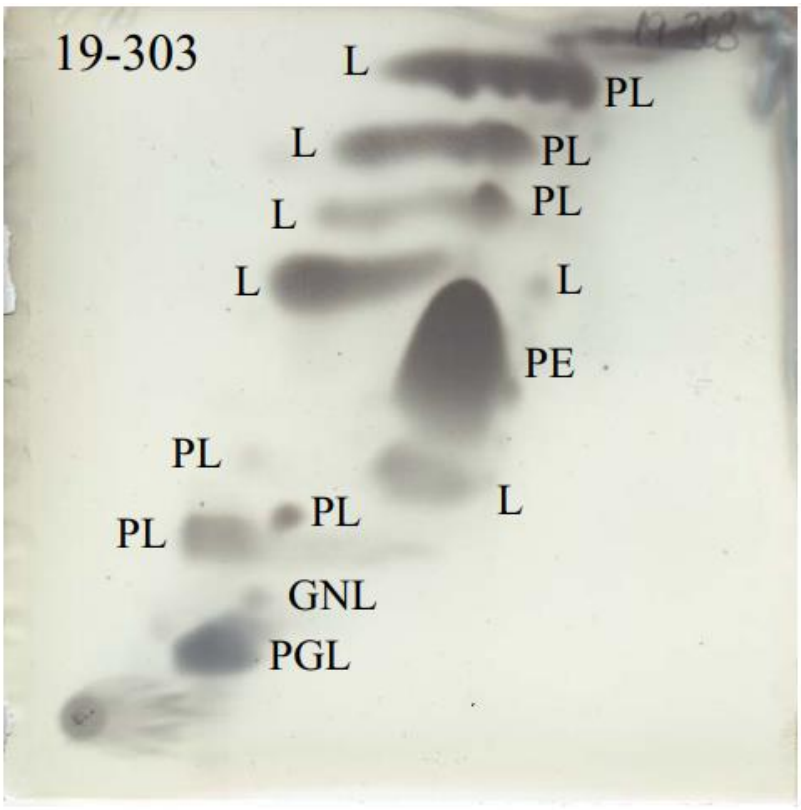

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