Fuscibacter Oryzae Gen. nov., sp. nov., A Phosphate-Solubilizing Bacterium Isolated from the Rhizosphere of Rice Plant

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Abstract

An ovoid to rod shaped, white tobrown pigmented, facultative anaerobic, mesophilic, non-phototrophic, Gram-staining-negative, non-motile, multiply by binary fission designated strain KVB23^T, which was isolated from root of rice plant, near Ilsan, South Korea, was investigated for its taxonomic position by polyphasic approach. Optimal growth was found to occur at 30˚C, at pH 6.5 and in the absence of NaCl on R2A. Phylogenetic analysis based on the 16S rRNA gene sequence of strain KVB23^{T}revealed that it formed a distinct lineage, as a separate deep branch within the family *Rhodobacteriaceae*, with <96.5% sequence similarity to representatives of the genera *Rhodobacter*, *Xinfangfangia*, *Tabrizicola*, *Falsirhodobacter*, *Haematobacter*, *Paenirhodobacter*, *Pseudorhodobacter* and *Pararhodobacter*. Based in 16S rRNA sequences strain KVB23^T was most closely related to *Tabrizicola fusiformis* KCTC 62105^T (96.5%) and *Rhodobacter thermarum*KCTC 52712^T(96.2%).The draft genome of strain KVB23^{T}was 3.80 bp long with a DNA G + C content of 63.1 %. Genome of strain KVB23^T harboured gene clusters for tryptophan and cobalaminbiosynthesis. The strain contained Q-10 as the sole respiratory quinone. The predominant fatty acids were found to consist of $C_{16:0}$, $C_{18:0}$ and summed feature 8 (comprising $C_{18:1}$ ω7c and / or $C_{18:1}$ ω6). The polar lipids were identified as diphosphatidylglycerol, phosphatidylethanolamine,seven unidentified phosphoglycolipids, two unidentified aminophosphoglycolipid, one unidentified glycolipid and four unidentified lipids.Phosphate-solubilizing bacteria have the ability to dissolve insoluble phosphates and enhance the soil fertility. Strain KVB23^{T}can solubilize calcium phosphate tribasic. Phosphate solubilizing and tryptophan biosynthesis property of strain KVB23^T could be a possible factor for the increase in growth of rice plant. Differential phenotypic, chemotaxonomic and genotypic properties, together with the phylogenetic distinctiveness, demonstrated that strain KVB23^T was found to represent a novel genus in the *Rhodobacteriaceae*family, for which the name *Fuscibacteroryzae* gen. nov., sp. nov. is proposed, with the type strain KVB23^T(=KACC 21711^T =NBRC 114716^T).

Repositories

The draft genome and 16S rRNA gene sequences of strain KVB23T have been deposited at GenBank/EMBL/DDBJ under accession numbers JAESVP000000000 and MN955430 respectively.

Introduction

The *Rhodobacteriaceae* familywas first established by Garrity et al. (2005) as a sole member of the order *Rhodobacterales* within the class *Alphaproteobacteria* andphylum *Proteobacteria.Rhodobacteriaceae*is one of the most widely distributed bacterial lineages in marine habitats such as seawater, sediments, marine snails, marine sponges and marine phytoplankton etc. Theyare mainly aerobic photoheterotrophs and chemoheterotrophs however, they can also exist as purple non-sulfur bacteria, which perform photosynthesis in anaerobic environments (Garrity et al. 2005).The members are Gram-stain-negative, oval-shaped and non-spore-forming bacteria. Most of the species are positive for oxidase activity.

Pigmentation occurs not only in photosynthetic members but also in non-phototrophic members. Some membersproduce polyhydroxyalkanoates (PHBs). The G+C content of genomic DNA ranges from 58.5 to 65.0 mol% (Hetharua et al. 2018). Q-10 is the predominant isoprenoid quinone and the major fatty acid is unsaturated fatty acid C18:1 *ω7c*. There are approximately 220 recognized genera in the family at the time of writing (https://lpsn.dsmz.de/family/rhodobacteraceae).In the course of screening the bacterial diversity in the roots of rice plants near Dongguk university, Ilsan, South Korea, strain KVB23T was isolated from a paddy field in the, Republic of Korea.Phosphate solubilizing bacteria play important role in biogeochemical phosphorus cycling in both terrestrial and aquatic environments (Das et al. 2007). In the present study, we introduce a novel non-phototrophic and phosphate-solubilizingbacterium isolated from the roots of rice plants that belongs to a new genus associated with the family *Rhodobacteriaceae*.

Materials And Methods

Isolation of the novel strain and cultivation

For assessing the diversity of culturable bacteria in the roots of rice plant, root samples were collected from a paddy field near Dongguk University, Ilsan, South Korea (GPS positioning of the sample collection site;37° 40’ 26.4” N 126° 48’ 20.88” E). The samples were placed in sterile polyethylene bags and brought back to the laboratory. For the isolation of strain KVB23^T, the root samples were thoroughly washed with sterilized water, to remove the external soil that was clinging to the surface of roots. The roots were cut into small fragments and macerated using a sterile pestle and mortar in sterile distilled water. The macerated samples were serially diluted using 0.85% NaCl as described previously (Chhetri et al. 2020a). Isolation was achieved using R2A agar (Difco) at 28℃ for 1 week. A single colony chosen on the plates was purified by transferring it to new R2A plates. Pure culture of strain KVB23^T was obtained by their repeated transfer on R2A agar plates and the purified colonies were sent to Bionics (Daejeon, Republic of Korea) for 16S rRNA gene analysis. From the purified bacterial colonies, a novel strain of the genus was identified to be a member of *Fuscibacter*and designated as KVB23^T.The isolate was preserved in R2A broth (Difco) supplemented with 50% (v/v) glycerol at -80℃.The reference strains *Tabrizicola fusiformis*KCTC62105^T, *Rhodobacter thermarum*KCTC52712^T and *Falsirhodobacter deserti*KCTC32408^T were purchased from KCTC (Korean Collection for Type Cultures).

Morphological characteristics

Colony morphology was studied on R2A medium at 30℃ for 5 days. The cell morphology and dimension were visualised by negative staining with 1% (w/v) phosphotungstic acid and viewing under a transmission electron microscope (TEM) (LIBRA 120, Carl Zeiss, Germany) after 3 days of incubation in R2A agar at 30℃.

Physiological and biochemical characteristics

To determine the optimal temperature range for growth, the growh of the strain was assessed at a temperature range of 4-40℃ (0, 2, 4, 10, 15, 20, 25, 28, 30, 35, 37 and 40℃) was measured by observing

the formation of colonies on R2A to estimate the optimal temperature range for growth. The growth of the strains on different media was assessed at 30°C for 10 days using R2A agar (Difco), Trypticase soy agar, Marine agar, nutrient agar, Luria-Bertani agar and MacConkey. The requirement for NaCl was determined using R2A broth containing various concentrations of NaCl (0.2 increments, w/v) was tested in R2A medium at pH 7.0 by incubation for 10 days at 30°C. Cell growth at various pH values in R2A broth (pH 4.0-10.0, in intervals of 1.0 pH unit) was examined using the following buffer systems as described previously: citrate/NaH_2PO_4 buffer (for pH 4.0-5.0), phosphate buffer (for pH 6.0-8.0) and Tris buffer (for pH 9.0-10.0) (Chhetri et al. 2018). Anaerobic growth was assessed by checking for colony formation on R2A agar at 30°C for two weeks in a GasPak jar (BBL, Cockeysville, MD, USA). Motility was assessed in R2A medium containing 0.4% agar. Gliding motility was tested using the hanging-drop method after growing the cells in R2A broth (Difco) for 48 h at 30°C(Bernardt et al. 2002). Gram reaction was determined using the non-staining KOH lysis method (Fautz and Reichenbach, 1980). Test for catalase and oxidase activities were performed using 3% (v/v) hydrogen peroxide solution and oxidase reagent, respectively as described previously (Kim et al. 2020).The presence of flexirubin-type pigments was investigated by performing the bathochromic shift test with 20% KOH (w/v) (Kim et al. 2019). The hydrolysis of Tween 20, 40, 60 and 80 was assessed according to the method described by Simbert & Krieg (1994). Moreover, the hydrolysis of chitin, carboxymethyl-cellulose, starch, and casein was also assessed according to a previously described method (Chhetri et al. 2019a). DNase activity was detected on DNase test agar by using toluidine blue. Additional enzyme activities, biochemical features and physiological characteristics were tested using the API 20NE and API ZYM kits (bioMérieux) according to the manufacturer's instructions. Since the strain KVB23^T was isolated from the roots of rice plantsits nitrification ability was assessed.Jensen's nitrogen free medium was used for this purpose, and bromothymol blue (BTB) was used as an indicator. Growth of strain KVB23^T in nitrogen free medium was observed for one week. Pikovakaya's (PVK) media was used to check the phosphate solubilizing activity of strain KVB23^T.

Phylogenetic and genotypic analysis

Genomic DNA was extracted using the TaKaRa MiniBEST Bacteria Genomic DNA extraction Kit version 3.0 (TaKaRa) following the manufacturer's instructions. The 16S rRNA gene of the isolate was directly amplified by colony-PCR using the universal bacterial primers pairs 27F, 518F, 805R and 1492R; the PCR products were commercially sequenced (Solgent, Korea). The nearly complete sequence (1415bp) of the 16S rRNA gene was deposited to NCBI GenBank under accession number MN955430 after assembled with SeqMan software (DNASTAR). The pairwise 16S rRNA gene sequence similarities, were calculated and phylogenetic neighbours were identified based on sequences of bacterial type strains from the EzBiocloud server database (Kim et al. 2012). Multiple sequences were aligned using MEGA 7.0 software (Kumar et al. 2016) and analyzed using the CLUSTALX2.1 (Thompson et al. 1997). A phylogenetic tree was constructed according to the neighbour-joining (NJ)(Felsenstein, 1985), maximum-parsimony (MP) and maximum-likelihood (ML) methods with the Kimura two-parameter model (Kimura,1980). Minimum-evolution tree was also constructed using the MEGA 7.0 software in order to estimate the confidence of

tree topologies (Rzhetsky and Nei 1992). MEGA 7.0 software was used to construct a phylogenetic tree by bootstrap analysis with 1000 replications (Felsenstein, 1985).

For genome sequencing, a standard DNA library was prepared using the TruSeq DNA PCR-Free kit library (Illumina). Subsequently, whole genome sequencing was performed by de novo sequencing analysis using an Illumina Hiseq 4000 sequencerwith a paired-end read length of 151 bp and assembled with the SPAdes Analysis v.3.10.1 at Macrogen (Republic of Korea). Average nucleotide identity (ANI) and digital DNA-DNA hybridization values between the strain KVB23^T and closely related strain were calculated using ANI calculator (www.ezbiocloud.net/tools/ani), and the Genome-to-Genome Distance Calculator (GGDC 2.1; https://ggdc.dsmz.de/ggdc.php) (Meier-Kolthoff et al. 2013). To obtain more sufficient taxonomic evidence, UBCG phylogenomic tree based on the core genomes was constructed (Na et al. 2018). Publicly available genomes of closely related taxa were used.The DNA G+C content of strain KVB23^T was calculated from the genome data.Genes involved in secondary metabolism were predicted by antibiotics and Secondary Metabolite analysis shell (antiSMASH) version 5.0 (Blin et al. 2019) and RAST annotation pipeline was carried out using the SEED platform (Aziz et al. 2008).The Venn diagram was constructed by OrthoVenn2 (https://orthovenn2.bioinfotoolkits.net/home), using the generated protein sequences.The draft genome and 16S rRNA gene sequences of strain KVB23^T have been deposited at GenBank/EMBL/DDBJ under accession numbers JAESVP000000000 and MN955430respectively. The DNA G+C content of strain KVB23^T was calculated from the genome data.

Chemotaxonomy

For determining the chemotaxonomic characteristics, the cells were grown in R2A agar at 30℃ untill the late exponential phase and then harvested by centrifugation. Cellular fatty acids were acquired by saponification, methylation and extraction as reported previously (Kuykendall et al. 1988). The extract was analysed using the Sherlock Microbial Identification system V6.01 (MIS, database TSBA6, MIDI Inc., Newark, DE, USA) and was subsequently compared with the extracts of other type strains.

For the purification of isoprenoid quinones, Sep-Pak Vac cartridges (Waters Associates Inc., Milford MA USA) were used and the extract was analysed by using high-performance lipid chromatography as reported previously (Hiraishi et al. 1996; Collins and Jones 1981).

For carotenoid analysis, the cells were extracted using 10 ml mixture of methanol/acetone (1:1, v/v) (Chhetri et al. 2019b). The absorption spectrum of the pigments was assessed with a spectrophotometer (Multiskan GO; Thermo Fisher Scientific).

Polar lipids were extracted as described previously (Minnikin et al. 1984) and analyzed by two-dimensional thin-layer chromatography using chloroform/methanol/water (65:25:4; v/v/v) in the first dimension and chloroform/methanol/acetic acid/water (80:15:12:4; v/v/v/v) in the second dimension (Minnikin et al. 1984). Appropriate detection reagents (Komagata and Suzuki 1987) were used to identify the spots; molybdophosphoric acid (phosphomolybdic acid reagent, 5% v/v solution in ethanol; Sigma-Aldrich, Germany) was used to detect total lipids, ninhydrin reagent (0.2% solution; Sigma life Science,

USA) was used to detect amino lipids, Zinzadze reagent (molybdenum blue spray reagent, 1.3%; Sigma Life Science) was used to detect phospholipids, and α-naphthol reagent was used to detect glycolipids.

Results And Discussion

Morphology, physiology and biochemical analysis

Cells of the strain KVB23^T were facultative anaerobic, Gram-stain-negative, rod-shaped, oxidase-negative, catalase-positive and devoid of flagella. Colonies were white, convex and circular with entire edges. The colour of colonies were white after culture for 5 days and it became slightly brown at the center of the colonies after ten days of incubation at 30℃ (Fig.1). The strain grew in the presence of 0-2% (w/v) NaCl (optimum 0%), at 7-35℃ (optimum 30℃) and at pH 6.5–7.5 (optimum pH 7.0). It did not show photoautotrophic and photoheterotrophic growth under anaerobic conditions. No absorption maxima were detected at 377, 590, 803 and 860 nm confirmig that the strain KVB23^T did not contain any photosynthetic pigments. In addition, the genome of strain KVB23^T did not have photosynthetic genes. Growth occurred at temperatures ranging from 7-35℃ (optimum, 30℃), at pH 6.0-8.0 (optimum, pH 7.0) and in the presence of 0-03% NaCl (w/v: optimum, 0%). The new isolated was unable to perform anoxygenic photosynthesis or to grow phototrophically under anoxic conditions, and this character can clearly distinguish strain KVB23^T from the species of genus *Tabrizicola* and *Rhodobacter*.Growth of strain KVB23^T was not occured in Jensen's nitrogen free medium. Occurrence of halo zone around the colonies in phosphate-solubilizing agar showed the solubilizing ability of strain KVB23^T (Fig. S1).In the API ZYM system, the strain KVB23^T displayed positive results for alkaline phosphatase, esterase, esterase lipase, leucine arylamidase, valine arylamidase, trypsin, acid phosphatase, naphthol-AS-BI-phosphohydrolase and *α*-glucosidase activities however it displayed negative results for lipase, cystine arylamidase, *α*-chymotrypsin, *α*-galactosidase, *β*-galactosidase, *β*-glucuronidase, *β*-glucosidase, N-acetyl-*β*-glucosaminidase, *α*-mannosidase and *α*-fucosidase activities.In the API 20NE system, the strain did not reduce nitrate and did not produce indole. Moreover, it did not ferment glucose and did not hydrolyse arginine and gelatin. It only assimilated malic acid but did not assimilate $_D$-glucose, $_L$-arabinose, $_D$-mannose, *N*-Acetyl-$_D$-glucosamine, $_D$-maltose, potassium gluconate, capric acid, adipic acid, trisodium citrate and phenylacetic acid. KVB23^T could be differentiated from other closely related members by using several phenotypic and biochemical properties, such as able to grow in low temperature of 7℃, could grow in anaerobic condition and able to hydrolyse esculin. Most importantly, strain KVB23^T showed brown pigmentation at the center of the colony after ten days of incubation but the close relatives were not shown the pigmentation.The differentiatingcharacteristics between strain KVB23^T and the members of its related genera within the family *Rhodobacteriaceae* and also the reference strains were presented in Table 1.

Phylogenetic and genome analysis

16S rRNA gene sequence of strain KVB23^T indicated that strain was most closely related to *Tabrizicola fusiformis* SY72^T (96.5%), *Rhodobacter thermarum* YIM 73036^T (96.2%) and *Tabrizicola alkalilacus* DJC^T (96.0%). Phylogenetic analysis based on neighbour-joining tree (Fig. 2)further revealed that the novel strain KVB23^T, formed a distinct lineage within the family *Rhodobacteriaceae*, clustering with the clade comprising phototrophic species belonging to the genus *Tabrizicola and Rhodobacter*, and non-phototrophic species belonging to *Falsirhodobacter* and *Xinfangfangia*. Similar results were obtained in both maximum-likelihood (Fig. S2) and maximum parsimony trees (Fig. S3). The topologies of all tree trees were almost same and available in the online version of this article.Phylogenomic tree also gave the similar results (Fig. S4).

The genome size of strain KVB23^{T}was found to be 3,808,773 bp. The complete genome of strain KVB23^T consisted of 3,720 coding genes, 3 rRNA and 46 tRNA genes and 32 pseudogenes. The number of contigs was 21 and N50 value was 446,226. The G + C content of genomic DNA is 63.1 % which is within the range for the members of the family *Rhodobacteriaceae*. The ANI values between strain KVB23^T and the closely related reference strains *T. fusiformis* KCTC 62105^T, *R. thermarum* KCTC 52712^T and *F. deserti* KCTC 32408^T were 78.2, 74.4 and 72.9% and the corresponding dDDH values were 21, 19.2 and 19.4%. These ANI and dDDH values are clearly below the species-delineating thresholds (95% and 70%, respectively) (Chun et al. 2018; Richter and Rossello-Mora 2009; Stackebrandt and Ebers 2006), supporting the conclusion that strain KVB23^T represents a novel genus in the family *Rhodobacteriaceae*. The antiSMASH server revealed eight gene clusters for the biosynthesis of several secondary metabolites; one gene cluster each for redox-cofactor, terpene, Type I polyketide synthase (T1PKS), non-ribosomal peptide synthetase, three gene clusters for hserlactone, and one gene cluster for RRE-element.The presence of three striking hserlactone gene clusters may have potential ecological roles, which may be related to the communication between fungi and bacteria (Shiner et al. 2005).The comparison of biosynthetic gene clusters between strain KVB23^T and its reference strains is provided in Table S1. According to RAST annotation, 1455 protein encoding genes in whole genome of strain KVB23^T were classified into 27 functional categories (Table 2). Interesting point was all strains have genes for motility (Flagellar) and chemotaxis except *F. deserti* KCTC 32408^T. However, all strains were found to be non-flagellated. Strain KVB23^T, also had five gene clusters for motility and chemotaxis however it was not flagellated.The genome of strain KVB23^T was compared with those of phylogenetically related species belonging to the family *Rhodobacteriaceae*. Four gene clusters for auxin biosynthesis were also annotated in the genome of strain KVB23^T. The main precursor for the synthesis of IAA is typtophan, four genes encoding for tryptophan biosynthesis were also found: tryptophan-rich sensory protein (JAESVP010000001.1), tryptophan 2, 3-dioxygenase (kynA; JAESVP010000002.1), tryptophan synthase subunitbeta (trpB; JAESVP010000003.1) and tryptophan synthase subunit alpha (JAESVP010000005.1).Cobalamin has been suggested to stimulate plant development and could be synthesized either *via de novo* or salvage pathways. Five gene clusters for cobalamin biosynthesis were also found in the genome of strain KVB23^T. Based on Venn diagrams of protein clusters, Fig S5 showed the number of orthologous clusters shared among strain KVB23^T and other closely related members.

Chemotaxonomic characterization

The major respiratory quinone of the strain KVB23^T was ubiquinone Q-10, which is common in *Rhodobacteriaceae* family.The polar lipid of strain KVB23^T were diphosphatidylglycerol (DPG), phoshatidylethanolamine (PE), seven unidentified phosphoglycolipid (PGL), two unidentified aminophosphoglycolipid (APGL), one unidentified glycolipid (GL) and four unidentified lipids (L) which characteristically differentiated KVB23^T from other recognized families (Fig. S6).The fatty acid profiles of strain KVB23^T and its closely related members were presented in Table 3. The drastic difference in the major fatty acids also differentiate strain KVB23^T from other close relatives. Difference in the percentage of major fatty acid $C_{16:0}$, $C_{18:0}$ and summed feature 8 (comprising $C_{18:1}$ ω7c and / or $C_{18:1}$ ω6c) distinguish the strain KVB23^T from its close relatives. Absence of anteiso $C_{15:0}$, iso $C_{16:0}$, presence of $C_{18:1}$ ω7c and some other qualitative and quantitative differences in the fatty acid composition between the novel strain KVB23^T and other members of the family *Rhodobacteriaceae*could considered as a distinguishing characteristics for the novel genus.

Strain KVB23^T has the ability to solubilize phosphate and the genome of strain KVB23^T contains striking genes that may contribute to rice plant growth stimulation and has good application potential in sustainable agriculture.Based on theaforementioned distinct phylogenetic, phenotypic, biochemical, chemotaxonomic and genomic data the novel isolate KVB23^T cannot be assigned to any previously recognized bacterial taxa and therefore, we propose that strain KVB23^T represents a novel species belonging to a novel genus *Fuscibacteroryzae*gen. nov., sp. nov., within a novel family, *Rhodobacteriaceae* fam. Nov.

Description of *Fuscibacteroryzae*gen. nov.

Fuscibacter gen. nov. (Fus.ci.bac'ter. L. adj. *fuscus*, brown; L. n. *bacter* (from Gr. *bakterion*) a rod; N.L. masc. n. *Fuscibacter*, a brown rod).

Cells are Gram-stain-negative, facultative anaerobic, catalase- and oxidase-negative, asporogenous, short-rod shaped, non-flagellated and non-motile. Flexirubin-type pigments are absent. They contained Q-10 as the sole respiratory quinone. The main cellular fatty acids are $C_{16:0}$, $C_{18:0}$ and summed feature 8 (comprising $C_{18:1}$ ω7c and / or $C_{18:1}$ ω6).The polar lipids include diphosphatidylglycerol, phoshatidylethanolamine, seven unidentified phosphoglycolipid, two unidentified aminophosphoglycolipid, one unidentified glycolipid and four unidentified lipids. The DNA G+C content of the type strain of the type species is 63.1%. Based on phylogenetic analysis, the genus belongs to the family *Rhodobacteriaceae* within the phylum *Proteobacteria*. The type species is *Fuscibacteroryzae.*

Description of *Fuscibacteroryzae*sp. nov

*F. oryzae*sp. nov. (o.ry'zae. L. fem. n.*oryzae*, of rice, referring to the isolation of the type strain from the root of a rice plant)

Cells are Gram-stain negative, aerobic, ovoid to rod-shaped, non-motile, asporogenous, 0.4-0.5μm long and 0.8-1.4μm wide after 3 days of culture on R2A. Colonies on R2A agar are white to brown pigmented, smooth, circular, convex and have an entire margin. Cells are non-motile, multiply by binary fissionand negative for catalase and oxidase activities. Growth occurs at 7-35 ˚C (optimum 25-30 ˚C) and pH 6.0-8.0 (optimum 6.5-7.0). Hardly tolerates NaCl (w/v) upto 2% (optimum 0% NaCl). Good growth occurs on R2A agar and NA, weak growth on TSA and no growth on MA and LB. Strain KVB^T was able to grow in the absence of oxygen and showed negative activities for catalase and oxidase reaction.Strain KVB23^T able to hydrolyze esculin and Tween 20, but not Tween 40 and 60, starch, casein and CM-cellulose. It does not have a vesicular photosynthetic membrane. Moreover, it does not contain bacteriochlorophyll a, carotenoids and flexirubin. Photoautotrophic and photoheterotrophic growth does not occur. Furthermore, photosynthetic pigments are not produced and apparently photosynthetic genes were also not found in the genome of strain KVB23^T. Strain KVB23^T was able to dissolve phosphate when grown in Pikovakaya's medium. The predominant respiratory quinone is ubiquinone Q-10 and the G + C content of the genomic DNA of the type strain is 63.1%. The main cellular fatty acids are $C_{16:0}$, $C_{18:0}$ and summed feature 8 (comprising $C_{18:1}$ ω7c and / or $C_{18:1}$ ω6).The polar lipids include diphosphatidylglycerol, phoshatidylethanolamine, seven unidentified phosphoglycolipid, two unidentified aminophosphoglycolipid, one unidentified glycolipid and four unidentified lipids.

The type strain KVB23^T (=KACC 21711^T =NBRC 114716^T) was isolated from root of rice plant collectedfromrice field near Ilsan, South Korea. The GenBank accession number of the 16S rRNA gene sequence of the strain KVB23^T is MN955430 and its draft genome sequence accession number is JAESVP000000000.

Abbreviations

KCTC: Korean Collection for Type Cultures

MEGA: Molecular Evolutionary Genetics Analysis

UBCG: up-to-date bacterial core gene

NJ: Neighbour-joining

ML: Maximum-likelihood

MP: Maximum-parsimony

DPG: Diphosphatidylglycerol

PE: Phosphatidylethanolamine

Declarations

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Conflicts of interest

All the authors declare that there is no conflict of interest.

Authors' contributions

GC isolated the bacterium, designed the study, performed the phenotypic and biochemical characterization, and wrote the original draft; MK, JK, IK, YS helped with the analysis of taxonomic data; TS designed and supervised the study, and edited the original draft.

Ethics approval

This study does not describe any experimental work related to human.

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Tables

Table 1. Physiological and biochemical characteristics of strain KVB23^T and closely related type strains of the family *Rhodobacteriaceae*. All data were examined in this study unless otherwise indicated.

Characteristics	**1**	**2**	**4**	**3**
Isolation source	Plant root	Industrial wastewater	Sediment	Sandy soil
Cell shape	Ovoid to rod	fusiform	Ovoid to rod	rod
Colony color	white to brown	Translucent white	Brown	Lemon yellow
Catalase/oxidation	-/-	+/+	+/+	+/+
Anaerobic growth	+	-	-	+
Optimal growth	25-30	30-37	37-45	30
Temperature range for growth	7-35	15-40	20-55	10-40
Growth in 3 % NaCl	-	-	+	+
NaCl range for growth	0-2	0-1.5	0-3.5	0-10
Media for optimum growth	R2A	R2A	R2A	TSA
Optimal pH	6.5-7.0	6.0-7.0	7.0-7.5	7
Phototrophic growth	-	-	+	-
Photosynthetic pigments	-	-	Bacteriochlorophyll *a*	-
Internal membrane system	-	-	+	-
β-Caroten production	-	-	+	
pufL and *pufM* genes	-	-	+	-
Enzyme activities (API ZYM)				
Lipase	-	+	+	+
Cystine arylamidase	-	-	+	+
Trypsin	+	-	-	+
Acid phosphatase	+	+	-	-
Naphtol-AS-BI-phosphohydrolase	+	-	-	+
β-galactosidase	-	-	+	+
β-galactosidase	+	+	+	+
β-glucosidase	-	+	-	+

N-acetyl-*β*-glucosaminidase	-	+	-	-
API 20NE				
Arginine dihydrolase	-	+	-	+
Urease	-	+	-	+
Esculin hydrolysis	+	+	-	-
D-glucose	-	-	+	-
L-arabinose	-	+	+	+
D-mannose	-	+	+	+
D-mannitol	-	+	+	-
N-Acetyl-D-glucosamine	-	+	-	-
D-maltose	-	+	+	-
malic acid	+	-	-	+
DNA G+C content (mol%)	63.5	63.7+0.2[a]	66[b]	67.3[c]

Strains: 1, KVB23^T; 2, *Tabrizicola fusiformis* KCTC 62105^T; 3, *Rhodobacter thermarum* KCTC 52712^T; 4, *Falsirhodobacter deserti* KCTC 32408^T.

*Data taken from: a (Ko et al. 2018); b (Khan et al 2018) and c (Wang et al 2015); +, Positive; –, negative.

Table 2. Comparison of numbers of genes associated with functional categories in genomes of strain KVB23^T and phylogenetically related species in the family *Rhodobacteriaceae*.

Strains	1	2	3	4
Cofactors, Vitamins, Prosthetic Groups, Pigments	136	137	119	38
Cell Wall and Capsule	23	31	36	5
Virulence, Disease and Defense	34	63	35	5
Potassium metabolism	4	8	4	-
Photosynthesis	-	-	2	-
Miscellaneous	15	28	19	6
Phages, Prophages, Transposable elements, Plasmids	20	33	28	1
Membrane Transport	37	77	35	19
Iron acquisition and metabolism	4	9	4	0
RNA Metabolism	36	46	35	10
Nucleosides and Nucleotides	98	91	91	33
Protein Metabolism	170	191	181	58
Cell Division and Cell Cycle	-	-	-	-
Motility and Chemotaxis	5	16	15	0
Regulation and Cell signaling	25	19	17	6
Secondary Metabolism	4	5	5	0
DNA Metabolism	71	66	69	28
Fatty Acids, Lipids, and Isoprenoids	52	75	62	3
Nitrogen Metabolism	43	42	22	7
Dormancy and Sporulation	1	1	1	0
Respiration	106	110	93	13
Stress Response	65	66	55	10
Metabolism of Aromatic Compounds	9	50	39	2
Amino Acids and Derivatives	243	259	228	82
Sulfur Metabolism	3	18	3	3
Phosphorus Metabolism	24	21	23	12
Carbohydrates	227	252	188	34

Strains: 1, KVB23^T; 2, *Tabrizicola fusiformis* KCTC 62105^T; 3, *Rhodobacter thermarum* KCTC 52712^T; 4, *Falsirhodobacter deserti* KCTC 32408^T.

Table 3. Cellular fatty acid compositions (%) of strain KVB23^T and reference strains of the closely related species.

Fatty acids	1	2	3	4
$C_{14:0}$	1	TR	-	1.2
$C_{16:0}$	13.1	6.8	25.1	6.8
$C_{17:0}$	1.5	1.6	TR	TR
$C_{18:0}$	16.9	3.5	22	10.6
iso $C_{16:0}$	-	1.5	-	-
iso $C_{11:0}$ 3OH	1.7	TR	-	-
$C_{10:0}$ 3OH	5.5	3.5	6.9	3.8
anteiso $C_{15:0}$	-	1.2	1.3	-
$C_{18:1}$ ω7c	4.8	-	38.9	60.8
$C_{16:0}$ N alcohol	TR	-	2.5	1.8
$C_{18:1}$ ω7c 11-methyl	7.4	1.8	1.2	15
Summed features*				
3	TR	-	1	TR
7	-	1.2	TR	TR
8	48	78.3	-	-

Strains: 1, KVB23^T; 2, *Tabrizicola fusiformis* KCTC 62105^T; 3, *Rhodobacter thermarum* KCTC 52712^T; 4, *Falsirhodobacter deserti* KCTC 32408^T. Prior to fatty acid extraction, all strains were grown on R2A agar at 30℃ for 3 days except *R. thermarum* KCTC 52712^T, it is grown at 40℃ on R2A. Values are percentages of total fatty acids, and only fatty acids representing more than 1% for at least one of the strains are shown. -, Not detected; TR, trace amounts (<1%).

*Summed features represent groups of two or three fatty acids that cannot be separated using MIDI system. Summed feature 3 (composed of $C_{16:1}\omega7c$ and/or $C_{16:1}$ $\omega7c$ and/or iso-$C_{15:0}$ 2-OH), summed

feature 7 (composed of $C_{19:1}\omega7c$ and / or $C_{19:1}\omega6c$) and summed feature 8 comprises $C_{18:1}\omega7c$ and/or $C_{18:1}\omega6c$.

Figures

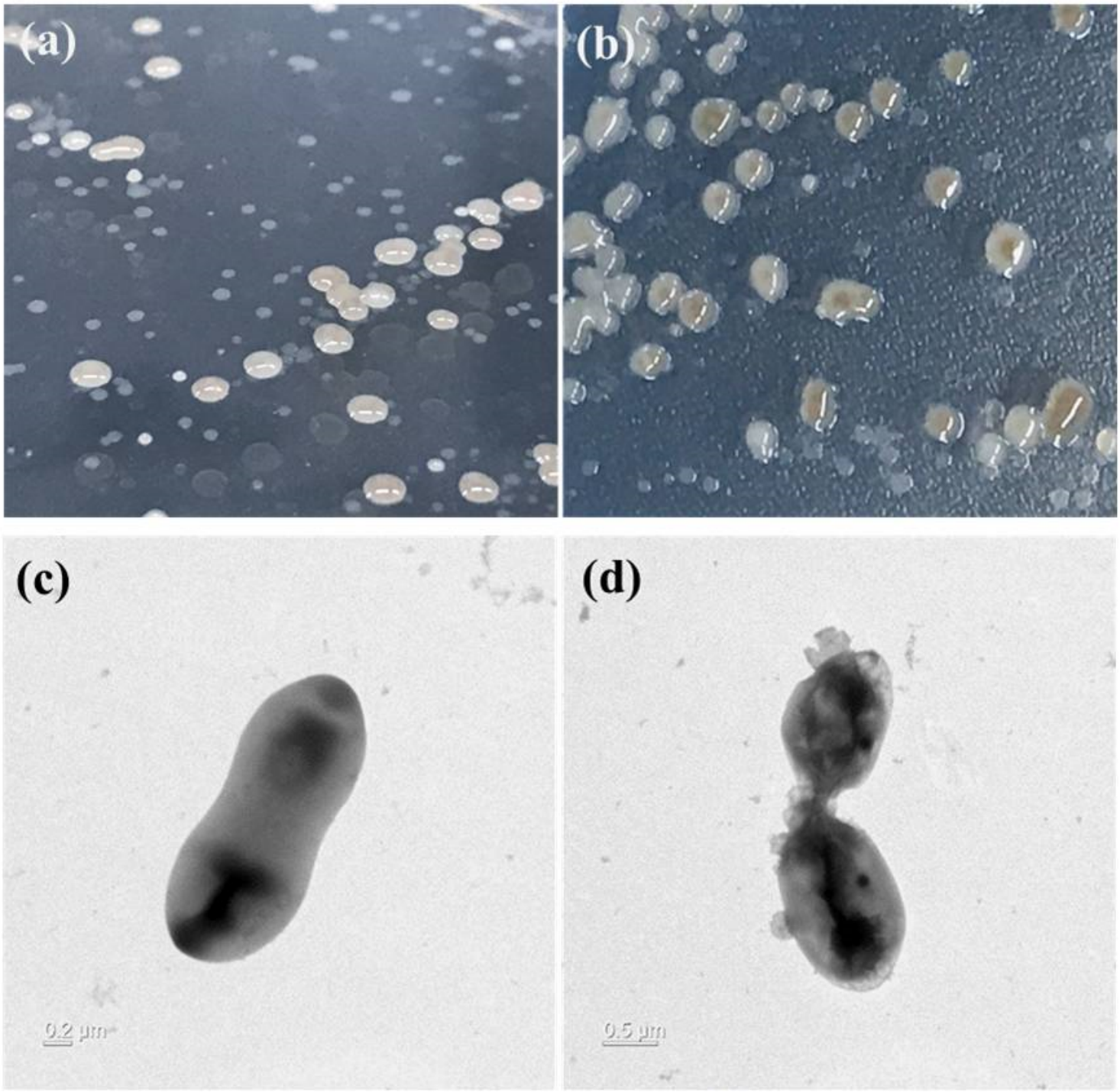

Figure 1

Creamy white colonies (a) turns to brown at the center (b) after 10 days of incubation at 30˚C. Transmission electron microscopy of strain KVB23T, four days old.Cells were grown for four days and

seven days and negatively stained with phosphotungstic acid after growth for one week at 30℃ on R2A agar. Bar (c) and (d) 0.2 μm and 0.5 μm. Figure (d) showing budding cells.

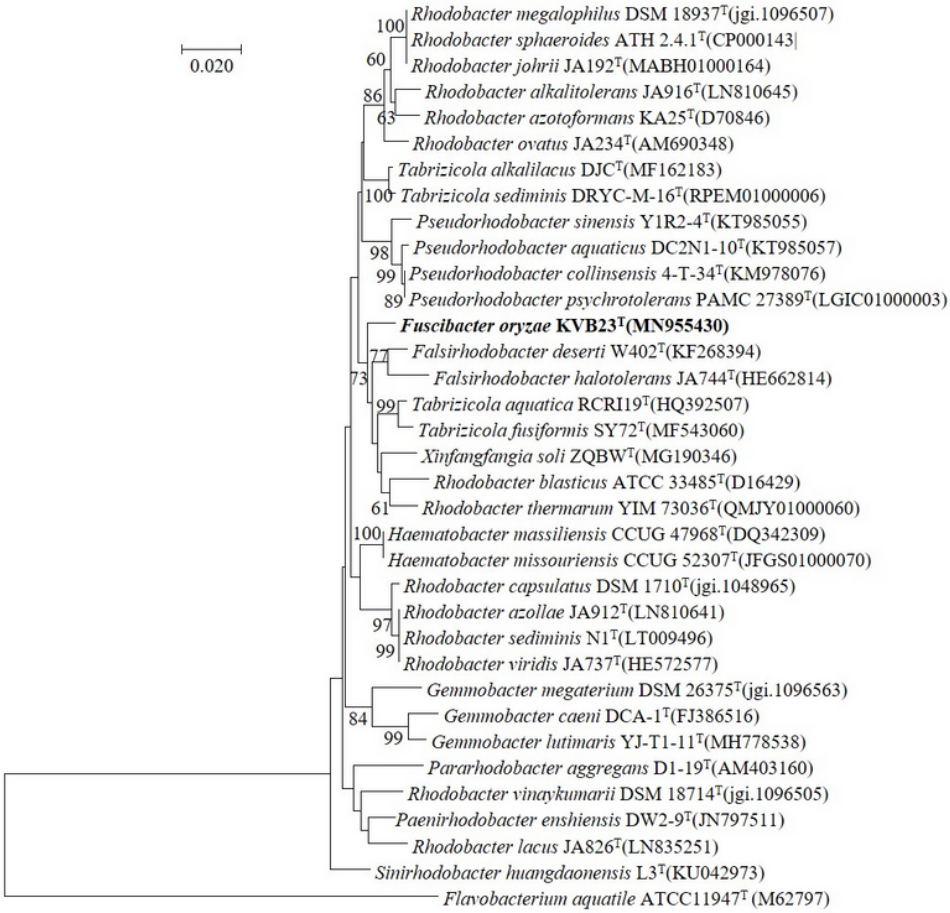

Figure 2

Neighbour-joining tree based on 16S rRNA gene sequences showing the relationship between strain KVB23Tand related species. Bootstrap values (based on 1000 replications) greater than 50% are shown at branch points. Flavobacterium aquatile ATCC11947T (M62797) was used as an out-group. Bar, 0.020 substitutions per nucleotide position.

Supplementary Files

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