Description of Lujinxingia Vulgaris Sp. Nov., Isolated From Coastal Sediment via Prey-traps

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Research Article

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Abstract

Two Gram-stain-negative, facultatively anaerobic, oxidase-negative, catalase-positive bacilli, designated strain TMQ4^T and TMQ2, were isolated from Xiaoshi Island in Weihai, PR China. The two strains had the abilities of predating other bacteria and were lured out with the prey *Acinetobacter baumannii* ATCC 19606^T. Growth was observed within the ranges 25–45 ^oC (optimally at 37 ^oC), pH 6.5–9.0 (optimally at pH 7.5–8.0) and 1–8 % NaCl (optimally at 3–4 %, w/v). The draft genome sequences of strains TMQ4^T and TMQ2 contained 184 contigs of 5,609,735 bp with a G+C content of 64.4 mol% and 148 contigs of 5,589,985 bp with a G+C content of 65.0 mol%, respectively. Phylogenetic analysis based on 16S rRNA gene sequences showed two strains belonged to the genus *Lujinxingia*, and their closest neighbour was *Lujinxingia sediminis* SEH01^T, both with the similarity of 98.9 %. The phylogenomic topologies and analyses also demonstrated the taxonomic status of the two strains. Based on genomic analyses, the two strains lost biosynthesis pathway of several kinds of important chemical compounds and possessed the predatory indices of 2. Iso-$C_{15:0}$ was contained in the predominant cellular fatty acids for strains TMQ4^T and TMQ2. The major polar lipids of both strains consisted of diphosphatidylglycerol, phosphatidylglycerol, phosphatidylethanolamine and unidentified lipids, and the respiratory quinone was menaquinone MK-7 for both strains. The physiological, biochemical and phylogenetic properties suggest that two strains represent a novel species within the genus *Lujinxingia*. The name *Lujinxingia vulgaris* sp. nov. is proposed, with strain TMQ4^T (= KCTC 62851^T = MCCC 1H00392^T) as type strain and strain TMQ2 (=KCTC 72079 = MCCC 1H00381) as reference strain.

Introduction

The order *Bradymonadales* was established by Wang *et al*. at 2015 with a single representative *Bradymonas sediminis* FA350^T (Wang et al. 2015). Four years later, a new genus *Lujinxingia* was proposed as the second member in the family *Bradymonadaceae*, with description of two novel species, *Lujinxingia litoralis* (type strain was B210^T) and *Lujinxingia sediminis* (type strain was SEH01^T) (Guo et al. 2019). The following year, *Persicimonas caeni* YN101^T was proposed as the representative of the genus *Persicimonas*, which yields an unusual big genome (compared with other members in the order *Bradymonadales*) of 8,047,206 bp in length (Wang et al. 2020).

The order *Bradymonadales* live a facultative anaerobic growth, disparate from other obligate aerobic and anaerobic taxa in *Deltaproteobacteria*, including *Myxococcales*, which are famous for their intricate life cycles and rich secondary metabolism, and those orders can utilize sulfate, sulfite, thiosulfate, sulfur or some other inorganic compounds as their terminal electron acceptors and reduce protons in the presence of a methanogen partner (Wang et al. 2015). Moreover, the order *Bradymonadales* featured being able to prey on some other bacteria. The classical predatory bacteria represent by *Myxococcus xanthus* could grow well without prey cells, on the contrary, growth of *Bdellovibrio* and *Bdellovibrio*-like organisms (BALOs) depended on Gram-stain-negative bacteria obligately (Hahn et al. 2017). Multiple nutritional deficiencies in cells of bradymonabacteria made them relying on prey cells to a certain degree. Though

these predators could live without prey cells, the existence of prey allowed bradymonabacteria a better growth. Therefore, bradymonabacteria was suggested as facultatively prey-dependent predators for all its predatory features (Mu et al. 2020), which may allow it a unique ecological niche in marine environment.

By now, all culturable bradymonabacteria were isolated from offshore sediment samples. However, further investigation indicated that the bradymonabacteria were abundant not only in marine environments, but also in high-salinity water environments, and even in intestines of pregnant pigs (Bachran et al. 2019; Cheng et al. 2018). Considering the wide distribution and predatory characteristics of this kind of bacteria, it may play important roles in microbial community structure and dynamics.

In this study, we introduce a novel species of the genus *Lujinxingia*, with predatory characteristics, which indicating further extension of this bacterial group.

Material And Methods

Prey-trap, isolation and maintenance conditions

A coastal sediment sample (collected in October 2017) in Xiaoshi Island, China (122°01' E, 37°31' N) was used to screening for predatory bacteria. Suspension of living *Acinetobacter baumannii* ATCC 19606^T was spread on marine agar 2216 (MA; BD), then a spot of sediment sample (as the same size as a grain of rice) was inoculated on the plate for two weeks. Predatory bacteria diffused from the mud around to the plate for its predation on *A. baumannii* ATCC 19606^T. Similar to inhibition zone, predation zone formed and could be observed. However, it was disparate from usual inhibition zone that predators grew and formed a thin layer of biofilm all over the whole predation zone. The two strains were in respective predation zones, and they were isolated and purified apart from the prey bacteria by the three quadrants streak method, then pure isolates were stored at -80 ^{o}C in sterile 15 % (v/v) glycerol supplemented with 3 % (v/v) NaCl. *Lujinxingia litoralis* B210^T and *Lujinxingia sediminis* SEH01^T were used as related strains, which was isolated and published by our laboratory.

Phylogenetic and phylogenomic analyses

The 16S rRNA gene was amplified from boiled cells by PCR with the universal primers 27F and 1492R (Liu et al. 2014). PCR products were purified using a PCR product purification kit (TaKaRa) and then ligated to the pMD18-T vector (Takara) according to the manufacturer's instructions. Sequencing was performed by Ruibiotech Co. Ltd (Qingdao) using universal primers M13-R and M13-F. Genomic DNA were extracted using DNA extraction kit (Takara Bio) according to the manufacturer's instructions with cells cultivated under the temperature of 37 ^{o}C with modified MB (30 g sea salt [Sigma] l^{-1}, 1 g yeast extract l^{-1}, 5 g peptone l^{-1}, and 0.1 g ferric citrate l^{-1}; pH 7.5). The genome was sequenced by Novogene Biotechnology Co., China, using the Illumina HiSeq platform. Raw sequencing reads were assembled using Newbler (version 2.8, 20110517_1502) and GapCloser (http://soap.genomics.org.cn/soapdenovo.html) (Li et al. 2010).

To further detect the taxonomic relationship between the two strains, within members in the genus *Lujinxingia* as well, the average nucleotide identity (ANI) values and *in silico* DNA-DNA hybridization (dDDH) were calculated pairwise (Lee et al. 2016; Klappenbach et al. 2007) (details are list in Supplementary Table S1). The ANI values (including OrthoANIu, ANIb, ANIm and Tetra values) between genomes were calculated using online ANI calculators of EzGenomes and JSpiecesWS (http://www.ezbiocloud.net/tools/ani & http://jspecies.ribohost.com/jspeciesws) (Yoon et al. 2017; Richter *et al*. 2016).

Moreover, phylogenetic trees (including neighbour-joining, maximum-likelihood and maximum-parsimony trees) were built with the software MEGA 7.0 (Kumar et al. 2016). Genetic distances were calculated using the Kimura two-parameter model (Kimura *et al*. 1980). A phylogenomic tree was constructed on the basis of 92 core genes using UBCG (https://www.ezbiocloud.net/tools/ubcg) (Na et al. 2018). Moreover, phylogenomic analyses based on genomes inferred the bac120 marker set (https://gtdb.ecogenomic.org) were also performed with IQ-TREE (Chaumeil et al. 2019; Nguyen et al. 2015).

Genomic analyses

Genomic analyses were performed with annotation using RAST (http://rast.nmpdr.org) and antiSMASH (https://antismash.secondarymetabolites.org). Predatory index of two genomes were calculated according to the method described by Pasternak to confirm whether they were over-looked bacterial predators (Pasternak et al. 2013). Indicator proteins summarized by genomes of predators and non-predators were proposed. In this index, each species received a + 1 point for each of the predatory indicator proteins that it contained, and a − 1 point for each of the non-predatory indicator proteins that it contained (Supplementary Table S2).

Morphological, physiological and biochemical analysis

The morphological and physiological features of strains TMQ4^T and TMQ2 were examined after incubation at 37°C with modified MA (modified MB with agar) for 3 d. Motility was examined using the hanging-drop experiment, and the plate-cultures (cultured with modified MA) diffused in 3 % (w/v) NaCl solution were prepared for observation. Gliding was examined with modified MB supplement with 0.3 % (w/v) agar according to the method described by Bernardet JF (Bernardet et al. 2002). A scanning electron microscope (Nova NanoSEM 450; FEI) was used to observe the cell size and morphology. A four-days-culture (late exponential phase, Fig. S1 in online Supplementary Material) with modified MB was collected for fixation with 2.5 % glutaraldehyde solution. Then gradient dehydration was performed with 30 %, 50 %, 70 %, 90 %, and 100 % ethanol before observation (Castejón 2003).

The effects of different growth temperatures were assessed upon incubation at 15, 20, 25, 28, 30, 33, 37, 40, 45, and 50°C on modified MA (pH adjusted with 1 M NaOH solution) until cell lawn were visible. To determine the effect of pH on growth, the pH of modified MB was adjusted to different levels with buffers (MES [pH 5.5 and 6.0], PIPES [pH 6.5 and 7.0], HEPES [pH 7.5 and 8.0], Tricine [pH 8.5], and CAPSO [pH 9.0 and 9.5; Sangon]) at 20 mM. The pH of the medium was adjusted with 1 M HCl or NaOH before

autoclaving, and OD_{600} values of the culture were measured after 96 h of incubation at 37°C (according to growth curves in Fig. S1 in online Supplementary Material). Growth under different NaCl concentrations, from 0 % to 10 % (w/v) at 1 % intervals, was assessed using modified MA without sea salt and recorded every 12 h, where in purified water was substituted for artificial seawater (0.32 % [w/v] $MgSO_4$, 0.12 % [w/v] $CaCl_2$, 0.07 % [w/v] KCl, and 0.02 % [w/v] $NaHCO_3$), and NaCl concentrations were accordingly adjusted. Growth conditions were recorded every 12 h, and the time spent for the growth of the first area was used as the evaluating criterion for temperature and salt tolerance tests.

Oxidase activity was tested using a bioMérieux oxidase reagent kit according to the manufacturer's instructions. Catalase activity was detected by bubble production in 3 % (v/v) H_2O_2. Cells of late exponential growth phase (Fig. S1) were collected for both oxidase and catalase tests. Anaerobic growth was determined by inoculating on modified MA with or without 0.1 % (w/v) KNO_3, $NaNO_2$, $Na_2S_2O_3$ or $FeSO_4$ in a micro-anaerobic and anaerobic incubation system. Nitrate reduction was tested using sulfanilic acid and α-naphthylamine with cells cultured in modified MB containing 0.1 % (w/v) KNO_3. Hydrolysis of starch, lipid, alginate, cellulose, casein, Tweens 20, 40, 60 and 80 were determined as described by Weinberg *et al.* (Weinberg *et al*. 1965). DNase activity was examined by using DNase test agar with methyl green (Difco) supplemented with 3 % NaCl. Each experiment was repeated for the confirmation of results.

Antibiotic sensitivity was assessed as described by the Clinical and Laboratory Standards Institute (CLSI, 2012): a cell suspension (McFarland standard 0.5) was swabbed over the surface of modified MA plates to form a uniform lawn before aseptic placement of antibiotic discs onto the agar surface. Inoculated plates were incubated at 37°C until visible lawn could be observed. Three replicates were performed for each kind of antibiotic discs.

Tests for other physiological and biochemical characteristics were performed using API 20E, API ZYM (all from bioMérieux) in accordance with the manufacturer's instructions, except that the salinity was adjusted to 3 % (w/v) with sea salt (Sigma). The oxidising potential of the strain for various carbon sources was assessed using Biolog GEN III in accordance with the manufacturer's instructions. Acid productions from carbohydrates were assessed using the API 50CHB fermentation kit (bioMérieux). The salinity of supplementary medium of both kits was adjusted to 3 % (w/v) with sea salt (Sigma). These rapid identification systems except for API ZYM were read every 12 h while being cultured at 37 ^{o}C, and all the API and Biolog tests were repeated for confirmation.

Chemotaxonomic analyses

Cells of exponential phase (Fig. S1) in liquid modified MB (100 mL for each culture) at 37 ^{o}C were used to determine the polar lipids by two-dimentional TLC (10 × 10 cm, no. 5554; Merck) (Xu et al. 2007). Total lipid materials were detected using molybdatophosphoric acid and specific functional groups were detected by using spray staining reagents (Sigma-Aldrich) on four separate TLC plates, including

phosphomolybdic acid solution (total lipids), molybdenum blue solution (phosphates), *α*-naphthol sulfuric solution (carbohydrates) and ninhydrin (amines).

Respiratory quinones were analysed by using cells of the exponential phase (according to the growth curves in Fig. S1 online Supplementary Material) under optimal physiological conditions. Extraction from 300 mg of freeze-dried cell material was separated into different classes by TLC on silica gel (Tindall 1990a; Tindall 1990b). In accordance with the spots on the silica gel plate, the effective components were removed from the plate and analysed further by using reverse-phase HPLC, then eluted with a mixture of acetonitrile and isopropanol (3:2, v/v) at a flow rate of 1 mL min^{-1} (Kroppenstedt 1982).

To assess the cellular fatty acid composition, about 40 mg wet cells of each strain were collected after cultured in modified MB at 37°C until they approached the exponential phase of growth (Fig. S1) in accordance with the four quadrants streak method. Fatty acids were saponified, methylated, and extracted using the standard protocol of Sherlock microbial identification system (MIDI) version 6.1 equipped with Agilent model 6890N gas chromatograph. Peaks were automatically integrated, fatty acids named, and percentages calculated using the MIS standard software using the TSBA40 database (Buyer 2002).

Predatory assays

Cross-streaking incubation assays (Fig. S2A, available with the online Supplementary Material) were performed to demonstrate the predation of strains TMQ4^T and TMQ2. A suspension (McFarland standard 5.0) of predatory bacteria was prepared and inoculated on modified MA in the first direction, followed by the suspension of prey bacteria (McFarland standard 5.0) inoculated in the second direction across the first inoculation line. The plate was then incubated until the growth of both bacteria was indicated by visible lawn.

Results And Discussion

Phylogenetic and phylogenomic analyses

Strains TMQ4^T and TMQ2 yielded genomes of 5,609,735 bp and 5,589,985 bp in length, separately. The calculated G + C mol% of the two strains were 64.4 mol% and 65.0 mol% as calculated from the draft genome sequences, which were similar with two related strains (Table 1). There were 184 and 148 contigs in the genomes of strains TMQ4^T and TMQ2, respectively. All contigs in two genomes were larger than 500 bp, of which the largest were of 917,808 bp and 568,134 bp. Genome of strain TMQ2 encoded 4,316 genes, including 53 tRNAs and 16 rRNAs, and 4,229 genes accompanied with 53 tRNAs and 12 rRNAs were encoded by strain TMQ4^T. N50 values were 568,134 and 97,137, and the sequencing depths of coverage were 346 × and 120 × for the type strain and another strain, respectively. The genomic information of two strains were both listed in Table 1. It seemed that most culturable members in *Bradymonadales* yielded similar size of genomes except *Persicimonas caeni* YN101^T (Wang et al. 2020),

which is more closely related to *Bradymonas sediminis* FA350^T (Table 1). Similar number of genes, tRNA and G + C mol% were also found in these predatory genomes belonged to different genera (Table 1).

Nearly complete 16S rRNA gene sequences of strains TMQ4^T (1,501 bp) and TMQ2 (1,500 bp) obtained by amplification were included in the 16S rRNA gene sequences assembled from genomic sequences (1,524 bp and 1,536 bp, separately). Only one complete 16S rDNA were identified, even if there were three pieces in the genome of strain TMQ4^T, and all of four 16S rDNA in the genome of strain TMQ2 were found to be partial. The 16S rRNA gene sequence annotated from genome sequences was submitted to GenBank databases, and similar sequences were searched using BLAST algorithm. The EzTaxon-e server (http://eztaxone.ezbiocloud.net/) (Kim et al. 2012) was used to achieve the similarity values of sequences. Based on the 16S rRNA gene sequence (from genome sequences), the strain closely related to *Lujinxingia sediminis* SEH01^T with the highest similarity values of 98.9 %, following *Lujinxingia litoralis* B210^T with the similarity values of 98.1 %. Additionally, the similarity value between strains TMQ4^T and TMQ2 was 99.8 %.

Strains TMQ4^T and TMQ2 had the OrthoANIu value of 87.7 %, and *Lujinxingia sediminis* SEH01^T had the OrthoANIu value of 85.7 % with strain TMQ4^T and 87.0 % with strain TMQ2. Moreover, the ANIb and ANIm values between each pair of strains were all below 90.0 %. However, the Tetra value between strains TMQ4^T and TMQ2 was 0.998, higher than the threshold of 0.990, which meant the strain TMQ4^T and TMQ2 belonged to the same species (Richter and Rosselló-Móra 2009). The dDDH values between strain TMQ4^T and the two members in *Lujinxingia* were both under the threshold of 70 %, illustrating each of the pair formed deep lineages (Li et al. 2010). Due to an ANI value of 95.0 % and a Tetra value of 0.990, as well as a dDDH value of 70 %, have been proposed as species boundaries, our data indicates that strain TMQ4^T (TMQ2) represents a novel species distinct from *L. sediminis* SEH01^T (Richter and Rosselló-Móra 2009).

As the topology shown in Fig. 1, two strains were clustered with *L. sediminis* SEH01^T at the bootstrap confidence level of 92 % and 99 % (Fig. 1). Furthermore, the same taxonomic status with phylogenetic trees was also demonstrated by two phylogenomic trees (Fig. 2).

Genomic analyses

According to the genetic analyses using RAST (http://rast.nmpdr.org) and NCBI genome database (https://www.ncbi.nlm.nih.gov/genome), strains TMQ4^T and TMQ2 lost biosynthesis pathway of several amino acids, including glycine, serine, threonine, valine, leucine, isoleucine and lysine. Thiamine and vitamin B6 biosynthesis, as well as biotin metabolism were absent in the two genomes. All above indicated that uptakes of these substrates maybe occurred during their predations. As the genetic annotations using antiSMASH (https://antismash.secondarymetabolites.org), lassopeptide (berninamycin) and NRPS-like (VEPE/AEPE/TG-1/crocaginA/crocaginB) gene clusters were encoded by the genome (Medema et al. 2011; Wang et al. 2019). Predatory index of two strains were 2 (higher than 0), which indicated these two strains were hypothetic bacterial predators. Predatory index of “2” was

common among culturable bradymonabacteria (Supplementary Table S2). Actually, all of the genomic features described above could also be found in the other culturable members of *Bradymonadales* (Mu et al. 2020). Furthermore, as one qualitative and predictive parament, predatory index could not be criteria to evaluate predation of bacterial predators. It was also not relevant to the taxonomic status, actually, it reflected the genomic features affiliated with predation more.

Morphological, physiological and biochemical analysis

No visible colonies but transparent lawn formed for both strains on modified MA after incubation at 37 ^{o}C for 144 h. Cells of two strains were both Gram-stain-negative rods without flagella, 0.3–0.5 × 1.0–4.5 μm for strain TMQ4^{T} and 0.3–0.5 × 1.0–3.5 μm for strain TMQ2 in size (Fig. S3, available with the online Supplementary Material). Cells were motile through gliding for they could spread into a big circle culture from a spot inoculation with modified MB with 0.3 % agar.

The optimal NaCl concentration was defined as the one under which the lawn in the first arear on agar medium grew taking least time. No growth happened in salt-free modified MA. Optimal growth of strains TMQ4^{T} and TMQ2 was both observed with 3–4 % (w/v) NaCl (range, 1–8 % [w/v]), at 37°C (range, 25–45°C) and pH 7.5–8.0 (range, pH 6.5–8.0). The physiological characteristics of two strains and phylogenetic neighbours were list in Table 2.

For two strains, nitrate but not nitrite, thiosulfate or sulfate could be used as electron acceptors when in anaerobic growth, and nitrate reductions were positive. Strains TMQ4^{T} and TMQ2 were positive for catalase, DNase and starch, Tweens 20, 40 and 60 hydrolases, but negative for oxidase and alginate hydrolase. Additionally, strain TMQ4^{T} was also positive for Tween 80 hydrolase.

Consequently, cells of strains TMQ4^{T} and TMQ2 were both sensitive to lincomycin (2 μg), norfloxacin (10 μg), neomycin (30 μg), clindamycin (30 μg), kanamycin (30 μg), tetracycline (30 μg), ceftriaxone (30 μg) and erythromycin (15 μg), and both resisted to penicillin (10 μg), vancomycin (30 μg) and tobramycin (10 μg).

Both strains showed positive activities for the reactions of alkaline phosphatase, esterase lipase (C8), leucine arylamidase, cystine arylamidase and naphthol-AS-BI-phosphohydrolase in API ZYM kits (Supplementary Table S3). Additionally, strain TMQ4^{T} was positive for esterase (C4), which was different from other bradymonabacteria. Moreover, negative for acid phosphatase distinguished strain TMQ4^{T} from strain TMQ2 and two members in *Lujinxingia*. According to the API 20E tests, both two strains and *L. sediminis* SEH01^{T} produced tryptophane deaminase and gelationase. However, acetoin production occurred in *L. litoralis* B210^{T} rather than other bradymonabacteria. The results of API 50CHB indicated that acids could be produced by the both two strains with d-ribose, esculin, d-tagatose and potassium 5-ketogluconate. However, acids were not produced by strain TMQ4^{T} with l-sorbose but by strain TMQ2 and the two members in *Lujinxingia*. According to Biolog GEN III kits, strains TMQ2 and TMQ4^{T} oxidized l-fucose, d-fructose-6-PO_4, l-glutamic acid, glucuronamide, mucic acid and α-keto-glutaric acid. However,

oxidations of d-arabitol, N-acetyl-d-galactosamine and d-glucose-6-PO_4 occurred in strain TMQ2, and d-raffinose, d-lactose, d-salicin, N-acetyl-d-galactosamine oxidized by strain TMQ4^T. Strains TMQ4^T, SEH01^T and B210^T but not strain TMQ2 oxidized the substrate of acetoacetic acid. Phenotypic characteristics that differentiate two strains from the closest phylogenetic neighbours are given in Table 2.

Chemotaxonomic analyses

Diphosphatidylglycerol (DPG), phosphatidylglycerol (PG) and phosphatidylethanolamine (PE) were detected in strains TMQ4^T and TMQ2, as well as two members in *Lujinxingia* as the major polar lipids. These three lipids were also main components in other culturable members in *Bradymonadales*. Moreover, unidentified lipids existed as the moderate and or minor components in strain TMQ4^T (L1, L2, L3 and L4) and strain TMQ2 (L1, L2, L4 and L5). What made strains TMQ4^T and TMQ2 differed from the two species of the genus *Lujinxingia* was the different contents of unidentified lipids (the absence of L2, L3 and L4 in strain SEH01^T and the presence of L7 and L8 in strain B210^T). Therefore, the polar lipid profile supports that the two strains represented one novel species of the genus *Lujinxingia*. Further details of contents of polar lipids of strain TMQ4^T from *L. litoralis* B210^T and *L. sediminis* SEH01^T were shown in Fig. S4 (available with the online Supplementary Material).

The sole quinone of strains TMQ4^T and TMQ2 were menaquinone MK-7, which was consistent with all culturable bradymonabacteria.

High content of iso-$C_{15:0}$ was detected in strains TMQ4^T (75.3 %) and TMQ2 (71.5 %) in conjunction with the members in the genus *Lujinxingia*. Iso-$C_{17:0}$ contained by strain TMQ4^T (11.9 %) tested as one major component in two of the three repeats (one in three repeats for strain TMQ2), which was higher than that in two members of the genus *Lujinxingia*. Four minor components, $C_{16:0}$, $C_{18:0}$, iso-$C_{16:0}$ and $C_{18:1}$ $\omega 9c$, existed in all four strains. Moreover, sum in feature 3 detected in strain SEH01^T was absent in strain TMQ4^T and strain B210^T. All the variances referred suggested that strain TMQ4^T (accompanied with strain TMQ2) presented a novel species in the genus *Lujinxingia*, and the details of the discrepancy between strains TMQ4^T and TMQ2, as well as the related strains were listed in Table S4 (available with the online Supplementary Material).

Predatory assays

Preys of strain TMQ4^T distributed in different category of bacterial species isolated from marine and saltern environments, which included Gram-stain-negative bacteria and Gram-stain-positive bacteria (data not shown). *Paraliobacillus ryukyuensis* DSM 15140^T and *Brumimicrobium aurantiaca* N62^T were further analysed as representative strains in this study. After cross-streaking incubation of predators and the prey, plates were incubated until the growth of both bacteria was indicated by visible lawn. Predatory bacteria could be detected by 16S rRNA gene sequencing in the second line, if the test strain was not prey

(Fig. S2E). However, when the predator fed on the test bacteria, such as *P. ryukyuensis* DSM 15140^T, strain TMQ4^T could be detected in the second line (Fig. S2B, C and D). Fig. S2D and E indicated that the growth of predators increased when provided with appropriate prey strains. For most culturable bradymonabacteria, the similar predation pattern could be observed. Results of cross-streaking tests incubating strain TMQ4^T and the test bacteria are also shown in Fig. S2.

Conclusions

The phenotypic and chemotaxonomic analyses, integrating the results of phylogenetic trees and phylogenomic trees based on 16S rRNA gene sequences and genomic sequences, respectively, lead to the conclusion that strain TMQ4^T (as well as TMQ2) belong to the genus *Lujinxingia* but differs from *L. litoralis* B210^T and *L. sediminis* SEH01^T. Therefore, strains TMQ4^T and TMQ2 represent a novel species, for which the name *Lujinxingia vulgaris* sp. nov. is proposed.

Description of Lujinxingia vulgaris **sp. nov.**

Lujinxingia vulgaris (vul.ga'ris. L. fem. adj. *vulgaris* common, referring to the lack of specific characteristics).

Cells are rods in size of 0.3–0.5 μm wide and 1.0–4.5 μm long. No flagella are found around the cells. Cells motile by the means of gliding. After incubation for 5 days at 37 ^oC on modified MA, no typical colonies but orange-colored and transparent lawn is formed. Growth occurs in presence of NaCl 1–8 % (w/v; optimum 3–4 %), at pH of 6.5–9.0 (optimum 7.5–8.0), and at temperature of 25–45 ^oC (optimum 37 ^oC). Anaerobic growth occurs. Indole, acetoin (Voges-Proskauer reaction) and H_2S are not produced. Negative for oxidase, and positive for nitrate reduction, catalase, alkaline phosphatase, esterase lipase (C8), leucine arylamidase, valine arylamidase, cystine arylamidase, trypsin, naphthol-AS-BI-phosphohydrolase, tryptophane deaminase and gelationase. Hydrolyses starch, lipase (Tweens 20, 40 and 80) and DNA. The polar lipid pattern consists of diphosphatidylglycerol (DPG), phosphatidylglycerol (PG), phosphatidylethanolamine (PE) and unidentified lipids (L). The sole respiratory quinone is MK-7. The cellular fatty acid profile contained iso-$C_{15:0}$.

The type strain, TMQ4^T (= KCTC 62851^T = MCCC 1H00392^T), was isolated from coastal sediments. The G□C content of the genomic DNA of type strain is 64.4 mol%. The GenBank accession number for the 16S rDNA sequence of *Lujinxingia vulgaris* TMQ4^T is MH613067 and the draft genome has been deposited in GenBank under the accession number VOSM00000000. Acids can be produced from d-ribose, esculin, d-tagatose and potassium-5-ketogluconate. d-Raffinose, d-lactose, d-salicin, N-acetyl- d-galactosamine, l-fucose, d-fructose-6-PO_4, l-glutamic acid, glucuronamide, mucic acid, α-keto-glutaric acid and acetoacetic acid can be oxidized.

The reference strain TMQ2 (= KCTC 72079 = MCCC 1H00381) was also isolated from coastal sediments. The G□C content of the genomic DNA of strain TMQ2 is 65.0 mol%. The GenBank accession number for

the 16S rDNA sequence of *Lujinxingia vulgaris* TMQ2 is MN547342 and the draft genome has been deposited in GenBank under the accession number VOSL00000000. Acids can be produced from d-ribose, L-sorbose, esculin, d-tagatose and potassium-5-ketogluconate. l-Fucose, d-arabitol, d-fructose-6-PO_4, l-glutamic acid, glucuronamide, mucic acid and *α*-keto-glutaric acid can be oxidized.

Abbreviations

HPLC, High Performance Liquid Chromatography; MA, Marine agar 2216; MB, Marine broth 2216; MCCC, Marine Culture Collection of China; MEGA, Molecular Evolutionary Genetics Analysis; MIDI, Microbial Identification System; NCBI, National Centre of Biotechnology Information; RAST, Rapid Annotation using Subsystem Technology; TLC, Thin layer chromatography.

The GenBank accession numbers for the 16S rRNA gene sequence of *Lujinxingiavulgaris* TMQ4^{T}and TMQ2 are MH613067 and MN547342, respectively. The draft genome of *Lujinxingiavulgaris* TMQ4^T and TMQ2 has been deposited in GenBank under the accession number VOSM00000000 and VOSL00000000, separately.

Four supplementary figures and three supplementary tables are available with the online version of this article.

Declarations

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Conflicts of interests

The authors declare that they have no conflicts of interest.

Ethical statements

This article does not contain any studies with animals performed by any of the authors.

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Tables

Table 1. Genomic information of strain TMQ4^T (accompanied with strain TMQ2) from the closely neighbours in the genus *Lujinxingia* and culturable bradymonabacteria.

1, Strain TMQ4^T; 2, Strain TMQ2; 3, *Lujinxingia sediminis* SEH01^T; 4, *Lujinxingia litoralis* B210^T; 5, *Bradymonas sedimnis* FA350^T; 6, *Persicimonas caeni* YN101^T.

Genomic data	1	2	3*	4*	5¶	6#
Genome size (bp)	5,609,735	5,589,985	5,329,124	5,083,303	5,045,683	8,047,206
G+C content (mol%)	64.4	65.0	64.1	64.7	61.1	63.8
Number of genes	4,229	4,316	4,099	4,050	3,992	6,072
Number of tRNAs	53	53	52	54	54	53
Number of rRNAs	12	16	7	8	15	9
Number of scaffolds	184	148	38	30	-	-
N50 values	568,134	97,137	331,157	491,720	-	-
Sequencing coverage	346 ×	120 ×	129 ×	390 ×	669 ×	144 ×

*: Guo *et al.*, 2019

¶: Wang *et al.*, 2019

#: Wang *et al.*, 2020

Table 2. Characteristics that differentiate strain TMQ4^T and TMQ2 from the closely neighbours.

1, Strain TMQ4^T; 2, Strain TMQ2; 3, *Lujinxingia sediminis* SEH01^T; 4, *Lujinxingia litoralis* B210^T; 5, *Bradymonas sediminis* FA350^T.

Characteristics	1	2	3	4	5¶
Optimal NaCl concentration required (%, w/v)	3–4 (1–8)	3–4 (1–8)	3–4 (0–8)	3–4 (0–8)	4 (1–9)
Optimal pH	7.5–8.0 (6.5–9.0)	7.5–8.0 (6.5–9.0)	8.0 (7.5–9.5)	8.0 (7.5–9.5)	8.5 (6.5–9.5)
Optimal temperature	37 (25–45)	37 (25–45)	37 (20–42)	37 (20–42)	33(20–37)
G + C content (mol%)	64.4	65.0	63.6*	64.7*	62.0
Cell size (μm)	0.3–0.5 × 1.0–4.5	0.3–0.5 ×1.0–3.5	0.4–0.5 × 1.8–3.2*	0.3–0.5 × 2.2–5.1*	0.5 × 1.0–2.5
Enzyme activities					
Esterase (C4)	+	−	−	−	−
Lipase (C14)	−	−	+	+	+
Acid phosphatase	−	+	+	+	−
Leucine arylamidase	+	+	−	+	+
Acids production					
d-Xylose	−	−	−	+	−
d-Fructose	−	−	+	+	−
l-Sorbose	−	+	+	+	−
Hydrolysis					
Starch	+	+	−	−	−
Tween 80	+	−	+	+	−
Substrate oxidation					
Gentiobiose	−	−	−	+	−
d-Raffinose	+	−	−	−	+
d-Lactose	+	−	−	−	−
d-Salicin	+	−	−	−	−
N-Acetyl-d-galactosamine	+	+	−	−	−
d-Arabitol	−	+	−	−	−
d-Glucose-6-PO_4	−	+	−	+	−

d-Gluconic acid	−	−	+	+	+
Acetoacetic acid	+	−	+	+	+

+, Positive; −, negative;

Data obtained from:

*: Guo *et al*, 2019.

¶: Wang *et al*, 2015.

Figures

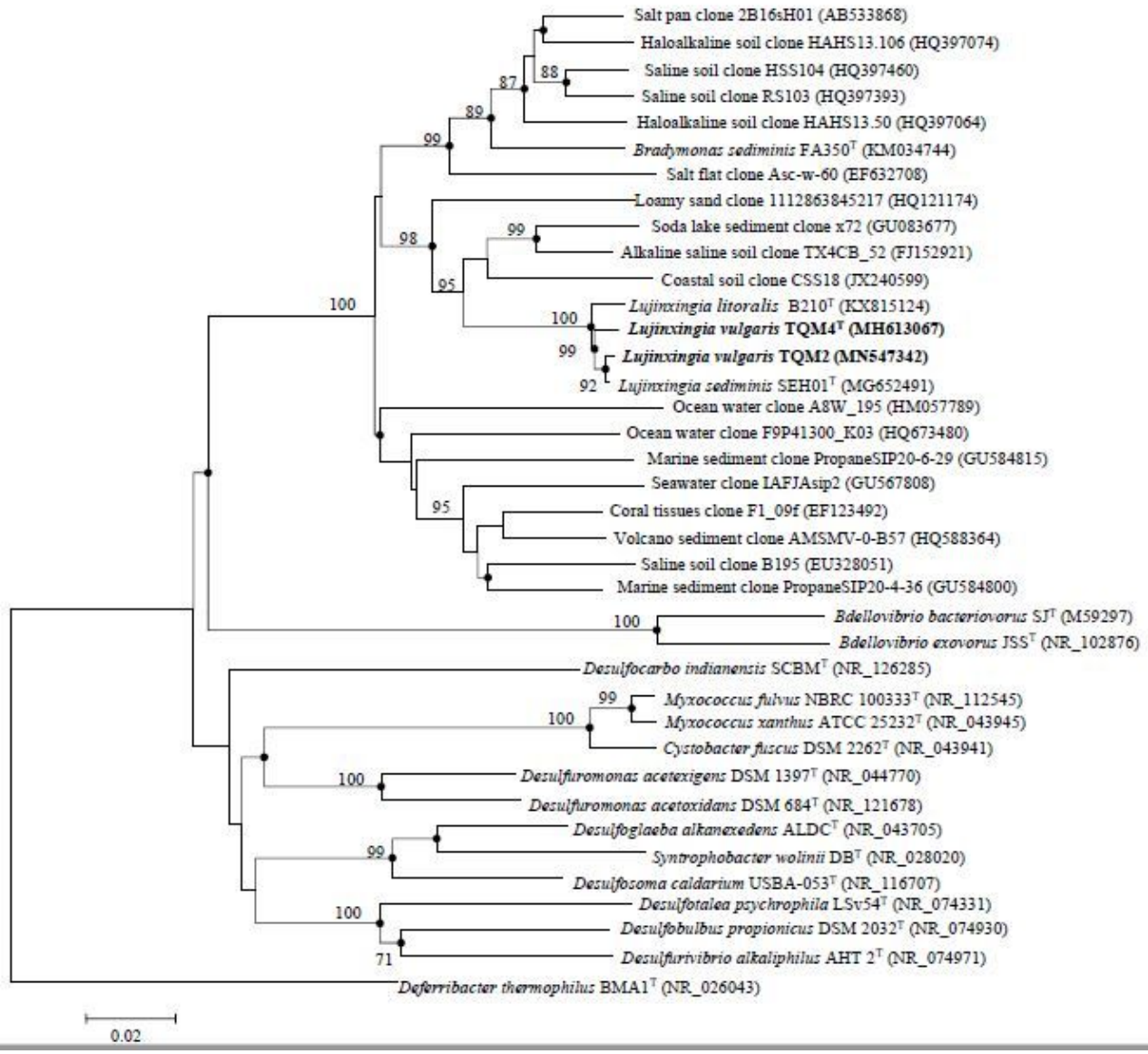

Figure 1

Phylogenetic tree constructed from 16S rRNA gene sequences, showing the position of strain TMQ4T (including strain TMQ2), as well as the related taxa within the class Deltaproteobacteria. The tree was constructed using the neighbor-joining algorithm. The filled circles indicate that the corresponding nodes were also found in the trees generated with the maximum-likelihood and maximum-parsimony algorithms. Bootstrap values (expressed as percentages of 1000 replications) > 70 % are shown at branch points. GenBank accession numbers of 16S rRNA gene sequences are given in parentheses. Deferribacter thermophilus BMA1T (NR_026043) was used as an outgroup. Bar, 0.02 substitutions per nucleotide position.

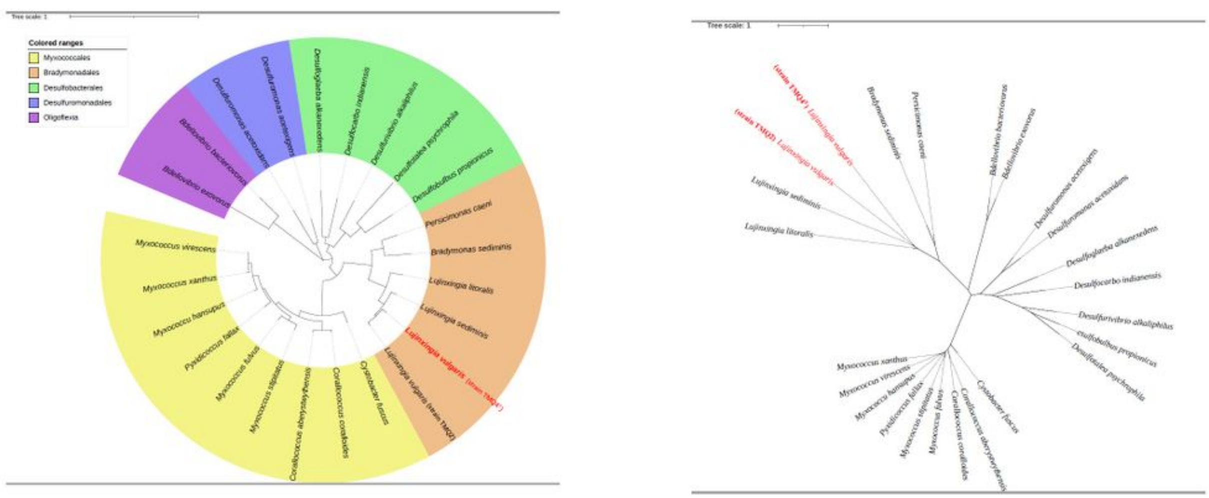

Figure 2

Phylogenomic trees based on up-to-date bacterial core gene sequences and genomes inferred the bac120 marker set of strain TMQ4T, as well as strain TMQ2 and related taxa within the class Deltaproteobacteria. Both trees were constructed using maximum-likelihood algorithm. Gene support indices (GSIs) and percentage bootstrap values are given at branching points. Bars, substitutions per nucleotide position of 1.0 for a b. a, Phylogenomic tree based on up-to-date bacterial core gene sequences; b, phylogenomic tree based on genomes inferred the bac120 marker set.