Description of *Lujinxingia vulgaris* sp. nov., isolated from coastal sediment via prey-traps

Shuo Wang · Da-Shuai Mu · Guang-Yu Li · Zong-Jun Du

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**Abstract** Two Gram-stain negative, facultative anaerobic, oxidase-negative, catalase-positive bacilli, designated as strains TMQ4\(^{\top}\) and TMQ2, were isolated from Xiaoshi Island, China, using prey-traps. Growth was observed within the ranges 25–45 °C (optimally at 37 °C), 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\(^{\top}\) and TMQ2 contained 184 contigs of 5,609,735 bp with a G+C content of 64.4% and 148 contigs of 5,589,985 bp with a G+C content of 65.0%, respectively. Phylogenetic analysis based on 16S rRNA gene sequences showed that both strains belonged to the genus *Lujinxingia* with the similarity of 98.9%. The phylogenetic and phylogenomic topologies and analyses demonstrated that both strains clustered together and differentiated from the closest neighbour, *Lujinxingia sediminis* SEH01\(^{\top}\). Genomic analyses showed that two strains lost the biosynthesis pathway of several chemical compounds. Iso-C\(_{15:0}\) was contained in the predominant cellular fatty acids in both strains. 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. Both strains predated other bacteria, including *Owenweeksia hongkongensis* JCM 12287\(^{\top}\) and *Paraliobacillus ryukyuensis* DSM 15140\(^{\top}\), and were lured with one prey *Acinetobacter baumannii* ATCC 19606\(^{\top}\) in prey-trap. Combining genomic analyses, two strains had the predatory indices of 2, similar to representative typical bacterial predators. The physiological, biochemical, and phylogenetic properties suggest that the two strains represent a novel species within the genus *Lujinxingia*. The name *Lujinxingia vulgaris* sp. nov. is proposed, with strain TMQ4\(^{\top}\) (= KCTC 62851\(^{\top}\) = MCCC 1H00392\(^{\top}\)) as type strain and strain TMQ2 (= KCTC 72,079 = MCCC 1H00381) as reference strain.

**Keywords** Bacterial predators · *Lujinxingia* · 16S rRNA gene · Phylogenetic analysis · UBCG · GTDB

The GenBank accession numbers for the 16S rRNA gene sequence of *Lujinxingia vulgaris* TMQ4\(^{\top}\)and TMQ2 are MH613067 and MN547342, respectively. The draft genome of *Lujinxingia vulgaris* TMQ4\(^{\top}\) and TMQ2 has been deposited in GenBank under the Accession Numbers VOSM00000000 and VOSL00000000, separately.

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S. Wang · D.-S. Mu · Z.-J. Du (✉) Marine College, Shandong University, Weihai 264209, Shandong, China
e-mail: duzongjun@sdu.edu.cn

G.-Y. Li Third Institute of Oceanography, Ministry of Natural Resources, Xiamen 361005, Fujian, China

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Introduction

Bacterial predators are popular in all environments (Pérez et al. 2016). These bacterial groups live depend on their prey, lysing prey cells (intracellular or extracellular) to uptake the entocyte (Pérez et al. 2016). The classical predatory bacteria represented by *Myxococcus xanthus* grow well without prey cells, which are known for their intricate life cycles and rich secondary metabolism (Arend et al. 2020). In contrast, the growth of *Bdellovibrio* and *Bdellovibrio*-like organisms (BALOs) obligately depend on Gram-stain negative bacteria (Hahn et al. 2017).

Bradymonabacteria is a predatory bacterial group established in 2015 with the discovery of the single representative *Bradymonas sediminis* FA350T (Wang et al. 2015). Four years later, a new genus *Lujinxingia* was proposed as the second member of the family *Bradymonadaceae*, with the description of two novel species, *Lujinxingia litoralis* (type strain was B210T) and *Lujinxingia sediminis* (type strain was SEH01T) (Guo et al. 2019). The following year, *Persicimonas caeni* YN101T was proposed as the representative of the genus *Persicimonas*, with an unusual large genome (compared with that of other members in the order *Bradymonadales*) of 8,047,206 bp in length (Wang et al. 2020).

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

Multiple nutritional deficiencies in cells of bradymonabacteria made them rely on prey cells to a certain degree. Although these predators live without prey cells, the presence of prey allowed bradymonabacteria to grow better. Therefore, bradymonabacteria have been suggested as facultatively prey-dependent predators for all its predatory features (Mu et al. 2020), attributing it a unique ecological niche in marine environments.

In this study, we present a novel species of the genus *Lujinxingia*, which was isolated from the sediments collected from the littoral zone, indicating further extension of this predatory group. Considering the special ecological niche where bradymonabacteria may locate, identification of new taxa may assist in analysing the role of this group in the material cycling in the ecosystem. More physiological characteristics and genomic information will be collected for further analyses.

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 screen for predatory bacteria. A suspension of living *Acinetobacter baumannii* ATCC 19606T was spread on marine agar 2216 (MA; BD), and a spot of sediment sample, the same size as a grain of rice, was inoculated onto the plate and incubated for two weeks. Predatory bacteria diffused from the mud around to the plate to predate on *A. baumannii* ATCC 19606T. Similar to an inhibition zone, a predation zone was observed. However, it was different from the usual inhibition zone where predators grew and produced a thin layer of biofilm throughout the entire predation zone. The two strains were in their respective predation zones and were isolated and purified apart from the prey bacteria by using the three-quadrant streak method. Isolates were stored at −80 °C in 15% (v/v) sterile glycerol supplemented with 3% (v/v) NaCl. *Lujinxingia litoralis* B210T and *Lujinxingia sediminis*
SEH01T, isolated and published by our laboratory, were used as reference strains.

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 into 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 was extracted with a DNA extraction kit (Takara) according to the manufacturer’s instructions using cells cultured at 37 °C within 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 2500 platform (Illumina, San Diego, CA, USA). Raw sequencing reads were assembled using ABySS v. 2.0.2 (http://www.bcgsc.ca/platform/bioinfo/software/abyss) (Simpson et al. 2009).

Phylogenetic trees (including neighbour-joining, maximum-likelihood, and maximum-parsimony trees) were built with the software MEGA 7.0 based on the complete 16S rRNA gene retrieved from the genome sequences, with genetic distances calculated using the Kimura two-parameter model (Kumar et al. 2016, Kimura 1980). To further detect the taxonomic relationship between the two strains and within members in the genus Lujinxingia, 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, available within the Supplementary Material). The ANI values (including OrthoANIu, ANIb, ANIm and TETRA values) between genomes were calculated using online ANI calculators of EzGenomes and JSpeciesWS (http://www.ezbiocloud.net/tools/ani and http://jspecies.ribohost.com/jspeciesws) (Yoon et al. 2017; Richter et al. 2016).

Additionally, 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 bac120 marker set (GTDB, 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).

Morphological, physiological and biochemical analysis

The morphological and physiological features of strains TMQ4T and TMQ2 were examined after incubation at 37 °C with modified MA (modified MB with agar) for 3 d. Motility was examined via the hanging-drop experiment, and the plate-cultures (cultured with modified MA) diffusion was prepared in 3% (w/v) NaCl solution for observation. Gliding was examined with a modified MB supplemented with 0.3% (w/v) agar according to the method described by Bernardet et al. (2002). A scanning electron microscope (Nova NanoSEM 450, FEI) was used to observe the cell size and morphology. A four-day culture (late exponential phase, according to growth curves in Supplementary Fig. S1, available within online Supplementary Material) in 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 (Castejon 2003). The effects of different growth temperatures were assessed after 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 lawns were visible. To determine 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 OD600 values of the culture was determined after 96 h of incubation at 37 °C (according to growth curves in Supplementary Fig. S1). 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] MgSO4, 0.12% [w/v] CaCl2, 0.07% [w/v] KCl, and 0.02% [w/v] NaHCO3), and NaCl concentrations were adjusted accordingly. Growth conditions were recorded every 12 h, and the time spent on the growth of the first area was used as the evaluation criterion for the temperature and salt tolerance tests.

Oxidase activity was tested using a bioMérieux (Craponne, France) oxidase reagent kit according to the manufacturer’s instructions. Catalase activity was detected through bubble production using 3% (v/v) H2O2. Cells in the late exponential growth phase (Supplementary 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) KNO3, NaNO2, Na2S2O3, or FeSO4 in a micro-anaerobic and anaerobic incubation systems. Nitrate reduction was tested using sulfanilic acid and α-naphthylamine with cells cultured in modified MB containing 0.1% (w/v) KNO3. Hydrolysis of starch, lipid, alginate, cellulose, casein, and Tweens 20, 40, 60 and 80 were determined as described by 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 to confirm the 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 onto the surface of modified MA plates to produce a uniform lawn before aseptic placement of antibiotic discs onto the agar surface. Inoculated plates were incubated at 37 °C until visible lawns were observed. Three replicates were performed for each kind of antibiotic discs.

Tests for other physiological and biochemical characteristics were performed using API 20E, API 20NE, and 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 according to the manufacturer’s instructions. Acid production from carbohydrates was assessed using the API 50CHB fermentation kit (bioMérieux). The salinity of the supplementary medium of both kits was adjusted to 3% (w/v) with sea salt (Sigma). Except for API ZYM, these rapid identification systems were read every 12 h while being cultured at 37 °C, and all the API and Biolog tests were repeated for confirmation.

Chemotaxonomic analyses

Cells in the exponential phase (Supplementary Fig. S1) in liquid modified MB (100 ml for each culture) at 37 °C were used to determine the polar lipids through two-dimensional thin layer chromatography (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 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 in the exponential phase (according to the growth curves in Supplementary Fig. S1) under optimal physiological conditions. Extracts from 300 mg of freeze-dried cell material was separated into different classes by TLC on silica gel (Tindall 1990a, b). 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 and 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, approximately 40 mg wet cells of each strain were collected after culturing in modified MB at 37 °C until they approached the exponential phase of growth (Supplementary Fig. S1), according to the four-quadrant streak method. Fatty acids were saponified, methylated, and extracted using the standard protocol of the Sherlock Microbial Identification System (MIDI) version 6.1, equipped with Agilent model 6890 N gas chromatograph. Peaks were automatically integrated, fatty acids determined, and percentages calculated using the MIS standard software using the TSBA40 database (Buyer 2002).

Predatory assays

Predatory indice 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.

Spot plate assays were performed to evaluate the predation of two strains. Suspensions of prey bacteria (McFarland standard 5.0) were inoculated onto the modified MA with 0.3% agar as a long streak, and then a suspension of predatory bacteria (McFarland standard 5.0) was spot-inoculated beside the prey line to maintain an appropriate distance (Fig. 1a). *Owenweeksia hongkongensis* JCM 12287T (Lau et al. 2005) was chosen as the test strain. The plate was then incubated until growth of both bacteria was evidenced by visible colonies or lawns.

Cross-streaking incubation assays (Fig. 2a) were performed to demonstrate the predation of strains TMQ4T and TMQ2. *Paraliobacillus ryukyuensis* DSM 15140T (Ishikawa et al. 2002) and *Brumimicrobium aurantiaca* N62T (Zhang et al. 2017) were selected as prey strains according to previous studies. Suspensions of predatory bacteria and test bacteria (both of McFarland standard 5.0) were prepared, and the suspensions of strains TMQ4T and TMQ2 were inoculated onto modified MA in the first direction, followed by the suspension of test bacteria (McFarland standard 5.0) inoculated in the second direction across the first inoculation line (Fig. 2a). The plate was then incubated until the growth of both bacteria was indicated by visible lawns.

**Results and discussion**

Phylogenetic and phylogenomic analyses

Nearly complete 16S rRNA gene sequences of strains TMQ4T (1501 bp) and TMQ2 (1500 bp) obtained by PCR amplification were included in the 16S rRNA gene sequences assembled from genomic sequences (1524 bp and 1536 bp, respectively). Only one complete 16S rDNA was identified, even if there were three pieces in the genome of strain TMQ4T, and all four 16S rDNA in the genome of strain TMQ2 were found to be partial. The 16S rRNA gene sequence annotated from the genome sequences was submitted to GenBank databases, and similar sequences were searched using the BLAST algorithm. The EzTaxon-e server (http://eztaxon.ezbiocloud.net/) (Kim et al. 2012) was used to achieve sequence similarities. Based on the 16S rRNA gene sequence (from genome sequences), the strain closely related to *Lujinxingia sediminis* SEH01T with the highest similarity values of 98.9%, following *Lujinxingia litoralis* B210T, with a
similarity of 98.1%. Additionally, the similarity value between strains TMQ4T and TMQ2 was 99.8%.

Strains TMQ4T and TMQ2 yielded genomes of 5,609,735 bp and 5,589,985 bp in length, respectively. The calculated G+C% of the two strains were 64.4% and 65.0% as calculated from the draft genome sequences, which were similar to those of two related strains (Table 1). There were 184 and 148 contigs in the genomes of strains TMQ4T 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. The genome of strain TMQ2 encoded 4316 genes, including 53 tRNAs and 16 rRNAs, and 4229 genes accompanied with 53 tRNAs and 12 rRNAs were encoded by strain TMQ4T. The N50 values were 568,134 and 97,137, and the sequencing depths of coverage were 346× and 120× for the type and reference strain, respectively. The genomic information of two strains were both listed in Table 1. It seemed that most culturable members of Bradymonadales yielded similar genome sizes, except Persicimonas caeni YN101T (Wang et al. 2020), which is

**Table 1** Genomic information of strain TMQ4T (accompanied with strain TMQ2) from the closely neighbours in the genus *Lujinxingia* and culturable bradymonabacteria

| Genomic data             | 1          | 2          | 3a         | 4a         | 5b         | 6c         |
|--------------------------|------------|------------|------------|------------|------------|------------|
| 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          | 4229       | 4316       | 4099       | 4050       | 3992       | 6072       |
| 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×       |

1. Strain TMQ4T; 2. Strain TMQ2; 3. *Lujinxingia sediminis* SEH01T; 4. *Lujinxingia litoralis* B210T; 5. *Bradymonas sediminis* FA350T; 6. *Persicimonas caeni* YN101T  

Guo et al. (2019)  
Wang et al. (2019)  
Wang et al. (2020)
more closely related to *Bradymonas sediminis* FA350\(^T\) (Table 1). A similar number of genes, tRNA, and G+C mol\% were also found in these predatory genomes belonging to different genera (Table 1).

Strains TMQ4\(^T\) and TMQ2 had OrthoANIu value of 87.7%, and *Lujinxingia sediminis* SEHO1\(^T\) had an 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%. The dDDH values between strain TMQ4\(^T\) and the two members in *Lujinxingia* were both below the threshold of 70%, demonstrating that

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**Fig. 3** Phylogenetic tree constructed from 16S rRNA gene sequences, showing the position of strain TMQ4\(^T\) (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* BMA1\(^T\) (NR_026043) was used as an outgroup. Bar, 0.02 substitutions per nucleotide position.
each of the pair formed deep lineages (Li et al. 2010). However, the TETRA value between strains TMQ4^T and TMQ2 was 0.998, higher than the threshold of 0.990, indicating the two strains belonged to the same species (Richter and Rossello-Móra 2009). Details about ANI and dDDH values are list in Supplementary Table S1 (available within the Supplementary Material).

**Table 2** Characteristics that differentiate strains TMQ4^T and TMQ2 from the closely neighbours

| Characteristics                        | 1              | 2              | 3              | 4              | 5\(^b\)          |
|----------------------------------------|----------------|----------------|----------------|----------------|-----------------|
| **Optimal NaCl concentration required** | 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        | 7.5–8.0 (6.5–9.0) | 8.0            | 8.0            | 8.5             |
| **Optimal temperature**                | 37 (25–45)     | 37 (25–45)     | 37 (20–42)     | 37 (20–42)     | 33 (20–37)      |
| **G+C content (%)**                    | 64.4           | 65.0           | 63.6\(^a\)     | 64.7\(^a\)     | 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\(^a\) | 0.3–0.5 × 2.2–5.1\(^a\) | 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

1, Strain TMQ4^T; 2, Strain TMQ2; 3, Lujinxingia sediminis SEH01^T; 4, Lujinxingia litoralis B210^T; 5, Bradymonas sediminis FA350^T
+ , Positive; −, negative;

Data obtained from:
\(^a\) Guo et al. (2019)
\(^b\) Wang et al. (2015)
As the topology shown in Fig. 3, strains TMQ4\textsuperscript{T} and TMQ2 were clustered together at a bootstrap confidence level of 99%, apart from \textit{L. sediminis} SEH01\textsuperscript{T}. Combined with the TETRA values calculated from genomic data, strains TMQ4\textsuperscript{T} and TMQ2 may represent a novel species distinct from \textit{L. sediminis} SEH01\textsuperscript{T}. Furthermore, the same taxonomic status with phylogenetic trees was also demonstrated by two phylogenomic trees (Fig. 4a, b). The two strains clustered together in the clade containing the two members of \textit{Lujinxingia}. Combined with the clades and the length of branches differentiating species of other taxa in the two phylogenomic trees, strains TMQ4\textsuperscript{T} and TMQ2 should be considered one novel species distinct from \textit{L. sediminis} SEH01\textsuperscript{T}.

Genomic analyses

Comparing to previous genomic and proteomic studies on the predation of validly published \textit{bradymonabacteria}, the genomic features of strains TMQ4\textsuperscript{T} and TMQ2 had many characteristics indicating predation. According to the genetic analyses using RAST (http://rast.nmpdr.org) and NCBI genome database (https://www.ncbi.nlm.nih.gov/genome), strains TMQ4\textsuperscript{T} and TMQ2 lost the biosynthesis pathway of several amino acids, including glycine, serine, threonine, valine, leucine, isoleucine, and lysine. Thiamine and vitamin B6 biosynthesis and biotin metabolism were absent in the two genomes. These results indicated that the uptakes of these substrates may have occurred during their predation. As the genomic annotations using antiSMASH (https://antismash.secondarymetabolites.org), lassopeptide (berninamycin) and NRPS-like (VEPE/AEPE/\textit{TG-1/crocaginA/crocaginB}) gene clusters were encoded by the genome, which showed the potential of two strains to produce antibiotics (Medema et al. 2011; Wang et al. 2019).

Morphological, physiological, and biochemical analysis

No visible colonies but a transparent lawn formed for both strains on modified MA after incubation at 37 °C for 144 h. Cells of both strains were Gram-stain negative rods without flagella, 0.3–0.5 × 1.0–4.5 μm for strain TMQ4\textsuperscript{T} and 0.3–0.5 × 1.0–3.5 μm for strain TMQ2 (Supplementary Fig. S2, available within the online Supplementary Material). Cells were motile by gliding, as they spread into a large circle 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 area on the agar medium took the least time to be produced. No growth was observed in the salt-free modified MA. Optimal growth of strains TMQ4\textsuperscript{T} and TMQ2 was 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 their phylogenetic neighbours were list in Table 2.

For two strains, nitrate, but not nitrite, thiosulfate, or sulfate could be used as electron acceptors during anaerobic growth, and nitrate reduction was positive. Strains TMQ4\textsuperscript{T} and TMQ2 were positive for catalase, DNase, and starch, and Tweens 20, 40 and 60 hydrolyses, but negative for oxidase and alginate hydrolase. Additionally, strain TMQ4\textsuperscript{T} was positive for Tween 80 hydrolyse. Consequently, cells of strains TMQ4\textsuperscript{T} and TMQ2 were 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 were resistant 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 S2, available within the Supplementary Material). Additionally, strain TMQ4\textsuperscript{T} was positive for esterase (C4), which was different from other \textit{bradymonabacteria}. Moreover, negative for acid phosphatase differentiated strain TMQ4\textsuperscript{T} from strain TMQ2 and the two members of \textit{Lujinxingia}. According to the API 20E tests, both strains and \textit{L. sediminis} SEH01\textsuperscript{T} produced tryptophane deaminase and gelatinase. However, acetoin production was present in \textit{L. litoralis} B210\textsuperscript{T} rather than in other \textit{bradymonabacteria}. Based on the results of 20NE, no assimilation of any substrate included in the kit occurred in the cells of the two strains, as well as related strains (Supplementary Table S3, available within the Supplementary Material). The results of API 50CHB indicated that acids were produced with d-ribose, esculin, d-tagatose and potassium 5-ketogluconate by both strains. However, acids were not produced by strain TMQ4\textsuperscript{T} with l-sorbose but by strain TMQ2 and the two members of \textit{Lujinxingia}.
According to Biolog GEN III kits, strains TMQ2 and TMQ4\textsuperscript{T} oxidized L-fucose, D-fructose-6-PO\textsubscript{4}, L-glutamic acid, glucuronamide, mucic acid and \(-\)keto-glutaric acid. However, oxidation of D-arabitol, N-acetyl-D-galactosamine, and D-glucose-6-PO\textsubscript{4} occurred in strain TMQ2, and D-raffinose, D-lactose, D-salicin, and N-acetyl-D-galactosamine were oxidized by strain TMQ4\textsuperscript{T}. Strains TMQ4\textsuperscript{T}, SEH01\textsuperscript{T}, and B210\textsuperscript{T}, but not strain TMQ2, oxidized the substrate of acetoacetic acid. Phenotypic characteristics that differentiate the two strains from the closest phylogenetic neighbours were shown in Table 2, and biochemical characteristics that existed within both strains TMQ4\textsuperscript{T} and TMQ2 were also shown in Supplementary Table S4 (available within the Supplementary Material).

### Chemotaxonomic analyses

Diphosphatidylglycerol (DPG), phosphatidylglycerol (PG), and phosphatidylethanolamine (PE) were the major polar lipids detected in strains TMQ4\textsuperscript{T} and TMQ2, as well as the two members in \textit{Lujinxingia}. These three lipids are also the main components of other culturable members of \textit{Bradymonadales}. Moreover, unidentified lipids were present as the moderate and or minor components in strain TMQ4\textsuperscript{T} (L1, L2, L3, and L4) and strain TMQ2 (L1, L2, L4, and L5). The strains TMQ4\textsuperscript{T} and TMQ2 differed between the two species of the genus \textit{Lujinxingia} because of the different contents of unidentified lipids (the absence of L2, L3, and L4 in strain SEH01\textsuperscript{T} and the presence of L7 and L8 in strain B210\textsuperscript{T}). Therefore, the polar lipid

### Table 3 Predatory index of strains TMQ4\textsuperscript{T} and TMQ2 with related species

| Protein function                        | 1 | 2 | 3 | 4 | 5 |
|-----------------------------------------|---|---|---|---|---|
| **Predatory indicator proteins**        |   |   |   |   |   |
| Mevalonate pathway                      |   |   |   |   |   |
| Diphosphomevalonate decarboxylase       | +1| +1| +1| +1| +1|
| Acetyl-CoA acetyltransferase            | +1| +1| +1| +1| +1|
| Cross-membrane signal transduction     |   |   |   |   |   |
| Histidine kinase                        | +1| +1| +1| +1| +1|
| Various functions                       |   |   |   |   |   |
| N-linked GlcNAc transferase             |   |   |   |   |   |
| Adhesion                                |   |   |   |   |   |
| Hypothetic protein                      | +1| +1| +1|   |   |
| von-Willebrand factor                   |   |   | +1| +1| +1|
| Polypeptide degradation                 |   |   |   |   |   |
| Serine protease                         |   | +1| +1| +1| +1|
| Benzoid degradation                     |   |   |   |   |   |
| \(\beta\)-Ketoacidipate enol-lactone hydrolase |   |   |   |   | +1|
| Tryptophane metabolism                  |   |   |   |   |   |
| Tryptophane 2,3-dioxygenase             | +1| +1| +1| +1| +1|
| Pyrimidine metabolism                   |   |   |   |   |   |
| Phosphoribulosine kinase/Uridine kinase | +1| +1| +1| +1| +1|
| Flavin metabolism                       |   |   |   |   |   |
| NADPH-dependent FMN reductase           |   |   |   |   |   |
| Glycerolphospholipid metabolism         |   |   |   |   |   |
| Glycerophosphoryl diester phosphodiesterase | +1| +1|   |   |   |
| **Non-predatory indicator proteins**    |   |   |   |   |   |
| Riboflavin biosynthesis                 |   |   |   |   |   |
| Riboflavin biosynthesis protein RibD    |   |   |   |   |   |
| Riboflavin synthase subunit \(\alpha\)   |   |   |   |   |   |
| GTP cyclohydrolase II                   |   |   |   |   |   |
| Tryptophane biosynthesis                |   |   |   |   |   |
| Tryptophane synthase subunit \(\beta\)  |   |   |   |   |   |
| Tryptophane synthase subunit \(\alpha\) |   |   |   |   |   |
| Argininosuccinate synthesis             |   |   |   |   |   |
| Argininosuccinate synthase              |   |   |   |   |   |
| Valine, leucine and isoleucine biosynthesis |   |   |   |   |   |
| Acetolactate synthase, large subunit    |   |   |   |   |   |
| Glycine, serine and threonine metabolism|   |   |   |   |   |
| Glycyl-tRNA synthetase \(\alpha\) subunit|   |   |   |   |   |
| Phospholipid biosynthesis               |   |   |   |   |   |
| Phosphatidyglycerophosphate synthase    |   |   |   |   |   |
| Predatory index                         | 2 | 2 | 2 | 1 | 2 |

Predatory index calculated according to the number of protein families specific to predators and non-predators

1, Strain TMQ4\textsuperscript{T}; 2, Strain TMQ2; 3, \textit{Lujinxingia sediminis} SEH01\textsuperscript{T}; 4, \textit{Lujinxingia litoralis} B210\textsuperscript{T}; 5, \textit{Bradymonas sediminis} FA350\textsuperscript{T}
profile suggested that the two strains represented a novel species of the genus *Lujinxingia*. Further details of the polar lipids content of strain TMQ4<sup>T</sup> from *L. litoralis* B210<sup>T</sup> and *L. sediminis* SEH01<sup>T</sup> were shown in Supplementary Fig. S3 (available within the online Supplementary Material).

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

High content of iso-C<sub>15:0</sub> was detected in strains TMQ4<sup>T</sup> (75.3%) and TMQ2 (71.5%) in conjunction with the members of the genus *Lujinxingia*. Iso-C<sub>17:0</sub> contained by strain TMQ4<sup>T</sup> (11.9%) was one of the major components in two of the three repeats (one in three repeats for strain TMQ2), which was higher than that in the two members of the genus *Lujinxingia*. Four minor components, C<sub>16:0</sub>, C<sub>18:0</sub>, iso-C<sub>16:0</sub>, and C<sub>18:1 ω9c</sub>, were present in the four strains. Moreover, sum in feature 3 detected in strain SEH01<sup>T</sup> was absent in strains TMQ4<sup>T</sup> and B210<sup>T</sup>. All the variances suggested that strain TMQ4<sup>T</sup> (accompanied with strain TMQ2) represented a novel species in the genus *Lujinxingia*, and the details of the discrepancy between strains TMQ4<sup>T</sup> and TMQ2, as well as the related strains, were listed in Supplementary Table S5 (available within the online Supplementary Material).

**Predatory behaviour of strains TMQ4<sup>T</sup> and TMQ2**

As a qualitative parameter, the predatory index is considered a criterion to predict bacterial predators. Thus, the predatory index of the two strains calculated according to genomic information was 2 (higher than 0), indicating that these two strains were hypothetic bacterial predators. The predatory index of “2” was common among culturable bradymonabacteria, but it seemed to reflect the genomic features associated with predation (Table 3).

The results of spot plate assays demonstrated the predation of strains TMQ2 and TMQ4<sup>T</sup>. As shown in Fig. 1b, both strains could invade and kill the lawn of *O. hongkongensis* JCM 12287<sup>T</sup> lawn through cell motility. However, prey-taxis motility was hardly observed in colonies of strains TMQ2 and TMQ4<sup>T</sup>. Gliding of these two strains, as well as other bradymonabacteria, showed a radial shape with central symmetry (Fig. 1c). Additionally, the predation of bradymonabacteria was confirmed to be contact-dependent, which meant that the prey cells were killed only when they touched the surface of predator cells (Mu et al. 2020). Moreover, as the distance was not well mastered, it was difficult to observe predation in spot plate assays. As Fig. 1d shown, when prey cells (*Bacillus subtilis* SDUM 301771, deposited in the Engineering Research Centre for Marine Technological Deposition and Application of the Shandong Province) with intense motility were too close to cells of strain TMQ4<sup>T</sup> cells, they spread to the colony of predatory bacteria, making it difficult to observe predation. Meanwhile, improperly adjusting the distance from the predator (strain TMQ2) to the prey (*Bacillus subtilis* SDUM 301771) prevented the contact between two bacteria; therefore, predation could not be triggered.

Comparing spot plate assays mentioned above, the crossing of streaks mimicked the contact between prey and predator cells, which occurred at the spot “a” in Fig. 2a. After cross-streaking incubation of predators and prey, plates were incubated until the growth of both bacteria was evidenced through a visible lawn. Predatory bacteria were not detected by 16S rRNA gene sequencing in the second line when the test strain was not predated (Fig. 2e). In other words, when the predator fed on the potential prey, such as *P. ryukyuensis* DSM 15140<sup>T</sup>, strains TMQ4<sup>T</sup> or TMQ2 could be detected in the second line (Fig. 2b–d). Figure 2d, e, indicated that the growth of predators increased when provided with appropriate prey strains. For most culturable bradymonabacteria, a similar predation pattern was observed.

Meanwhile, all the genomic features found in the two strains were also found in other culturable members of *Bradymonadales* (Mu et al. 2020). Based on the previous studies, genes encoding the type III secretion system inner-membrane protein complex were significantly up-regulated during the predation of *B. sediminis* FA350<sup>T</sup>. Considering genes affiliated with the main structure of type III secretion system (including SctJ/YscJ, SctRSV/YscRSV, and SctN/YscN) were also found in the genomes of strain TMQ2 and TMQ4<sup>T</sup>, we predicted that type III secretion system may play an important role in the process of predation. However, the function of the type III secretion system during predation remains unclear.
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 TMQ4T and TMQ2 belong to the genus *Lujinxingia* but differs from *L. litoralis* B210T and *L. sediminis* SEH01T. Therefore, strains TMQ4T and TMQ2 represent a novel species, for which the name *Lujinxingia vulgaris* sp. nov. is proposed. Strain TMQ4T is proposed as the type strain and strain TMQ2 as a reference strain. Cells of *Lujinxingia vulgaris* sp. nov. are facultative anaerobic Gram-stain negative rods without flagella, with the characteristics of predating other bacteria.

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 Gram-stain negative, oxidase-negative, and catalase-positive rods without flagella around and are motile by the means of gliding. Anaerobic growth occurs within cells. After incubation for 5 days at 37 °C on modified MA, few typical colonies or orange-coloured and transparent lawns forms. Growth occurs in presence of 1–8% NaCl (w/v; optimum 3–4%), at pH of 6.5–9.0 (optimum 7.5–8.0), and at temperature of 25–45 °C (optimum 37 °C). Anaerobic growth occurs. The polar lipid pattern consists of diphosphatidylglycerol (DPG), phosphatidylglycerol (PG), phosphatidylethanolamine (PE), and unidentified lipids (L). The sole respiratory quinone is MK-7. The major cellular fatty acid profile contains iso-C_{15:0}.

The type strain, TMQ4T (= KCTC 62851T = MCCC 1H00392T), was isolated from coastal sediments. The G+C content of the genomic DNA of type strain is 64.4%. The GenBank accession number for the 16S rDNA sequence of *Lujinxingia vulgaris* TMQ4T is MH613067, and the draft genome has been deposited in GenBank under the accession number VOSM0000000.

Lujinxingia vulgaris TMQ2 is MN547342, and the draft genome has been deposited in GenBank under the Accession Number VOSL0000000.

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Declarations

Conflict of interest The authors declare that they have no conflicts of interest.

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

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