**Pseudomonas oligotrophica sp. nov., a Novel Denitrifying Bacterium Possessing Nitrogen Removal Capability Under Low Carbon–Nitrogen Ratio Condition**

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**Pseudomonas** is a large and diverse genus within the *Gammaproteobacteria* known for its important ecological role in the environment. These bacteria exhibit versatile features of which the ability of heterotrophic nitrification and aerobic denitrification can be applied for nitrogen removal from the wastewater. A novel denitrifying bacterium, designated JM10B5aᵀ, was isolated from the pond water for juvenile *Litopenaeus vannamei*. The phylogenetic, genomic, physiological, and biochemical analyses illustrated that strain JM10B5aᵀ represented a novel species of the genus *Pseudomonas*, for which the name *Pseudomonas oligotrophica* sp. nov. was proposed. The effects of carbon sources and C/N ratios on denitrification performance of strain JM10B5aᵀ were investigated. In addition, the results revealed that sodium acetate was selected as the optimum carbon source for denitrification of this strain. Besides, strain JM10B5aᵀ could exhibit complete nitrate removal at the low C/N ratio of 3. Genomic analyses revealed that JM10B5aᵀ possessed the functional genes including *napA*, *narG*, *nirS*, *norB*, and *nosZ*, which might participate in the complete denitrification process. Comparative genomic analyses indicated that many genes related to aggregation, utilization of alkylphosphonate and tricarballylate, biosynthesis of cofactors, and vitamins were contained in the genome of strain JM10B5aᵀ. These genomic features were indicative of its adaption to various niches. Moreover, strain JM10B5aᵀ harbored the complete operons required for the biosynthesis of vibrioferrin, a siderophore, which might be conducive to the high denitrification efficiency of denitrifying bacterium at low C/N ratio. Our findings demonstrated that the strain JM10B5aᵀ could be a promising candidate for treating wastewater with a low C/N ratio.
INTRODUCTION

_Pseudomonas_ is widely distributed in various natural environments including water, soil, sediment, plants, animals, and clinical samples, and this genus contains a large number of species (Zou et al., 2019; Li et al., 2020; Mamtomin et al., 2021). The genus _Pseudomonas_ was first proposed with the description of _P. aeruginosa_ ICM 5962^1^ by Migula (1894) and belongs to the family _Pseudomonadaceae_ within the class _Gammaproteobacteria_. Recently, Lalucat et al. (2021) proposed the division of _Pseudomonas_ into five new genera: “Halopseudomonas,” “Lifyingimonas,” “Stutzerimonas,” “Alcaligenimonas,” and “Ubiquimonas” based on the phylogenetic and phylogenomic analyses, and the genus _Halopseudomonas_ published by Rudra and Gupta (2021) has been validated. _Pseudomonas_ strains displayed some potential roles, such as the high biodegradation capability of polycyclic aromatic hydrocarbons, sulfate oxidation, phosphate accumulation, and denitrification (Xie et al., 2016, 2021; Sun et al., 2019; Zhang R. C. et al., 2020). For example, a denitrifying phosphorus-accumulating bacterium _P. stutzeri_ ADP-19 could remove 96.9% of nitrate and 73.3% of phosphate under aerobic conditions (Li et al., 2021); a heterotrophic nitrifying–aerobic denitrifying bacterium _P. bauzanensis_ DN13-1 could remove 98.8, 98.9, and 65.9% of nitrite (NO_3^- -N), nitrate (NO_3^- -N), and ammonium (NH_4^+ -N), respectively (Zhang M. et al., 2020). Although the 16S rRNA gene is the basic tool for current bacterial classification, it cannot be used available to differentiate closely related species of _Pseudomonas_ (Wang et al., 2020). Thus, the taxonomy of _Pseudomonas_ strains has evolved with the available methodologies. Up-to-date Bacterial Core Gene set (UBCG, 92 bacterial core gene sets commonly present in all bacterial genomes which are shown on the website of EZBioCloud^1^) together with a phylogenomic pipeline could provide the accurate phylogenomic trees for the taxonomic purpose (Na et al., 2018).

Nitrogen pollution mainly comes from the excessive use of fertilizers, poultry production, domestic sewage, industrial manufacture, and aquaculture wastewater (Rezvani et al., 2019). Excess nitrite and nitrate accumulation can cause eutrophication and pose threat to aquatic animals and human health. For instance, excess nitrate could cause the regressive, circulatory, and inflammatory damages in the post-larvae and juvenile of _Macrobrachium amazonicum_ (Dutra et al., 2020). Among the nitrate-contaminated wastewaters, the low organic carbon-to-nitrogen (C/N) ratio wastewater is a common type such as the polluted groundwater, industrial wastewater, rural sewage, and effluent from wastewater treatment plants (Deng et al., 2016; Hong et al., 2019; Gao et al., 2020).

Biological denitrification is an effective and general method for reducing nitrate to nitrogen gas due to its high efficiency, low cost, and environmental friendliness (Pang and Wang, 2021). Heterotrophic denitrification, which utilizes the organic carbon sources as electron donors, has a higher denitrification rate compared with autotrophic denitrification (Yang et al., 2020). However, the deficiency of available carbon source is an intractable problem for heterotrophic denitrification in the treatment of wastewater with low C/N ratio. To ensure sufficient electron donors for the denitrification process, the conventional solution is to add external organic carbon (often methanol, ethanol, glucose, and acetic acid) into the wastewater with low C/N ratio (Wang et al., 2018). Nevertheless, the addition of external organic carbon may result in the risk of secondary contamination and a high cost (Ling et al., 2021). Therefore, effective and sustainable strategies to enhance heterotrophic denitrification in the treatment of wastewater with a low C/N ratio are needed.

Accumulating approaches have been developed for the nitrate removal from the low C/N ratio wastewater, such as the system coupled with iron-based chemical reduction and autotrophic denitrification (Liu et al., 2020), the heterotrophic denitrification system amended with redox-active biochar (Wu et al., 2019), the fungal pellets immobilized bacterial bioreactor (Zheng et al., 2021), and the utilization of biodegradable and inert carriers in the sequencing batch reactors (Huang et al., 2020). These approaches are focused on generating more electron donors to enhance nitrate removal capability or providing carriers for microorganisms to create a suitable environment. In addition, some heterotrophic denitrifying bacteria that can achieve efficient denitrification at low C/N ratio have been reported, such as _Acinetobacter sp._, _Comamonas sp._, and _Pseudomonas_ sp. (Zhang S. et al., 2020; Chen et al., 2021; Fan et al., 2021). Compared with the abovementioned approaches, these denitrifying bacteria can not only effectively and readily remove nitrate from low C/N ratio wastewater without the utilization of complex engineering but also provide the functional microorganisms possessing excellent nitrate removal ability for the wastewater treatment systems. Among the denitrifying bacteria, _Pseudomonas_ is the dominant bacterial genus in activated sludge and biofilm reactors (Deng et al., 2018; Zhang et al., 2019). Moreover, several _Pseudomonas_ strains possess the denitrification ability and other functions, such as phosphorus removal ability and polyhydroxybutyrate-degrading ability (Di et al., 2019; Li et al., 2021). Therefore, _Pseudomonas_ strains would outcompete other bacteria in the practical application.

In this study, a novel denitrifying bacterium, designated JM10B5a^T^, was isolated from the pond water for juvenile _Litopenaeus vannamei_. The phylogenetic, genomic, physiological, and biochemical analyses illustrated that strain JM10B5a^T^ represented a novel species of the genus _Pseudomonas_, for which the name _Pseudomonas oligotrophica_ sp. nov. was proposed. This strain performed excellent capability for denitrification under the low C/N ratio condition. Genomic information revealed that the functional genes of napA, narG, nirS, norB, and nosZ

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**Abbreviations:** ANI, average nucleotide identity; CCUG, Culture Collection University of Gothenburg; CDS, protein-coding sequences; CGMCC, China General Microbiological Culture Collection Center; dDDH, digital DNA–DNA hybridization; DPG, diphosphatidylglycerol; DSM, denitrification screening medium; GDMMC, Guangdong Microbial Culture Collection Center; JCM, Japan Collection of Microorganisms; ML, maximum likelihood; NA, nutrient broth agar medium; NB, nutrient broth medium; OGRIs, overall genome relatedness indices; PE, phosphatidylethanolamine; PG, phosphatidylglycerol; RAST, Rapid Annotation using Subsystem Technology; TLC, thin-layer chromatography; UBCG, up-to-date bacterial core gene.

^1^https://help.ezbiocloud.net/ubcg-gene-set/
encoding the enzymatic repertoire for completely denitrification were identified in strain JM10B5a\textsuperscript{T}. Our findings demonstrated that strain JM10B5a\textsuperscript{T} could be a promising candidate for treating wastewater with a low C/N ratio.

**MATERIALS AND METHODS**

**Bacterial Strains**

Strain JM10B5a\textsuperscript{T} was isolated from pond water for juvenile *Litopenaeus vannamei* collected from Jiangmen city, Guangdong Province, P. R. China (N 21° 56’ 31”; E 112° 46’ 16”). To isolate the aerobic denitrifying bacteria, the denitrification screening medium (DSM) was utilized. DSM was formulated as follows (per liter): sodium succinate 0.25 g, sodium citrate dihydrate 0.25 g, Na\textsubscript{2}HPO\textsubscript{4} 1.0 g, KH\textsubscript{2}PO\textsubscript{4} 1.0 g, NaNO\textsubscript{2} 0.069 g, KNO\textsubscript{3} 0.1 g, (NH\textsubscript{4})\textsubscript{2}SO\textsubscript{4} 0.066 g, MgSO\textsubscript{4}·7H\textsubscript{2}O 0.2 g, 2.0 ml of trace element solution (TES) and 1.0 ml of mixed carbon source solution (CSS), pH 7.3, and the solid plate contained the DSM supplemented with 15.0 g/L agar. MgSO\textsubscript{4}·7H\textsubscript{2}O, TES, and CSS were all added to the autoclaved DSM after filtering using 0.22-µm filter membrane. The TES contained (per liter) EDTA-Na\textsubscript{2} 10.0 g, ZnSO\textsubscript{4}·7H\textsubscript{2}O 0.5 g, MnCl\textsubscript{2}·4H\textsubscript{2}O 0.4 g, CoCl\textsubscript{2}·2H\textsubscript{2}O 0.5 g, CuSO\textsubscript{4}·5H\textsubscript{2}O 0.2 g, CaCl\textsubscript{2} 5.5 g, FeSO\textsubscript{4}·7H\textsubscript{2}O 1.1 g, and NaMoO\textsubscript{4}·2H\textsubscript{2}O 0.4 g. The CSS was described in the previous work (Zhang M. et al., 2020) and contained (per liter) D-glucose 13.8 g, D-fructose 13.8 g, D-lactose 13.8 g, sodium acetate 19.0 g, 90% lactic acid 12.8 ml, mannitol 14.0 g, ethyl alcohol 14.0 ml, glyc erin 12.6 ml, sodium benzoate 9.6 g, and salicylic acid 9.2 g. Serial dilutions of 10\textsuperscript{-4} to 10\textsuperscript{-1} of pond water were made, and 0.1 ml of the 10\textsuperscript{-2}, 10\textsuperscript{-3}, and 10\textsuperscript{-4} dilutions was spread on DSM agar plates, respectively. Then, these plates were incubated at 30°C for 5 days. Strain JM10B5a\textsuperscript{T} was isolated and stored at −80°C in the nutrient broth (NB) supplemented with 20% (v/v) glycerol.

*P. stutzeri* CGMCC 1.1803\textsuperscript{T} and *P. balearica* CCUG 44487\textsuperscript{T} were obtained from China General Microbiological Culture Collection Center (CGMCC) and Culture Collection University of Gothenburg (CCUG), respectively. These two type strains were used as the related strains for phenotypic, chemotaxonomic, and genetic analyses.

**Phylogenetic Analysis**

Genomic DNA of strains JM10B5a\textsuperscript{T} was extracted from fresh cells, and the 16S rRNA gene sequence was amplified using the universal primers (27F/1492R) as described previously (Zhang et al., 2021). Sequencing was performed by GENEWIZ, Inc. (Suzhou, China). The BLAST algorithm\textsuperscript{2} and EzBioCloud database\textsuperscript{3} were used to search for similar sequences (Yoon et al., 2017). Pairwise identities of 16S rRNA gene sequences were calculated using the software DNAMAN version 8. Multiple alignments of the 16S rRNA sequences were performed using the software MAFFT version 7.037 under the L-INS-i iterative refinement (Katoh and Standley, 2014). The phylogenetic tree was reconstructed using the software IQ-TREE version 2.1.2 with the maximum likelihood (ML) method under the TN+F+I+G4 nucleotide substitution model (Felsenstein, 1981; Kalyaanamoorthy et al., 2017; Minh et al., 2020). Support for the inferred ML tree was inferred by the ultrafast bootstrapping with 1,000 replicates (Diep Thi et al., 2018). The visualization and annotation of the resulting phylogenetic tree were performed using the software MEGA version X (Kumar et al., 2018).

**Morphological Observations and Analyses of Physiological Characteristics**

Cells and colonies of strain JM10B5a\textsuperscript{T} cultivated on the nutrient broth agar medium (NA) at 30°C for 48 h were observed by a transmission electron microscope (H7650, Hitachi) and naked eyes, respectively. Oxidase activity was determined using oxidase testing strips (HKM), and catalase activity was detected by bubble production after the addition of 3.0% H\textsubscript{2}O\textsubscript{2} (v/v) solution. Growth under an anaerobic environment was determined after 5 days of incubation on NA at 30°C in an anaerobic pouch (MGC, Mitsubishi). Hydrolysis of casein, starch, tyrosine, and Tweens 20, 40, and 80 was investigated according to the protocols described by Lanyi (1987) and Trindall et al. (2007). Cellular motility was examined using the hanging-drop method (Bernardet et al., 2002).

The temperature range for growth was measured on NA at 4, 10, 15, 20, 25, 30, 37, 40, 45, and 50°C, respectively. The pH range for growth was determined after incubation at 30°C and 180 rpm in the modified NB medium with appropriate biological buffers (50 mM): sodium citrate buffer (pH 5.0, 5.5, and 6.0), HEPES buffer (pH 6.5, 7.0, and 7.5), Tris buffer (pH 8.5, 8.0, and 9.0), and Na\textsubscript{2}CO\textsubscript{3}/NaHCO\textsubscript{3} (pH 9.5, 10.0, 11.0, and 12.0). The pH of the medium was adjusted by adding 1.0 M HCl or 1.0 M NaOH before autoclaving. The NaCl tolerance for growth was examined in the modified NB medium (without NaCl) with different NaCl concentrations (w/v, 0, 0.5, 1.0, 2.0, 3.0, 4.0, 5.0, 6.0, 7.0, 8.0, 9.0, and 10.0 %) at 30°C and 180 rpm. After incubation for 72 h, the bacterial growth was estimated by the spectrophotometric measurement of cell density (OD\textsubscript{600}).

Additional biochemical characteristics such as the enzyme activities, acid production by fermentation, and utilization of carbon sources were carried out using API ZYM and 20NE kits (bioMérieux) and Biolog GEN III MicroPlate (Biolog) according to the manufacturers’ instructions. The API strips and Biolog results were recorded every 24 h after incubation at 30°C until all reactions were steady. All API and Biolog tests were performed in duplicate under the consistent condition.

**Analyses of Chemotaxonomic Characteristics**

For fatty acid composition assay, the exponentially growing cells of strain JM10B5a\textsuperscript{T} were harvested. Fatty acids were saponified, methylated, and extracted using the standard protocol of the Sherlock Microbial Identification System (MIDI). The prepared fatty acids were analyzed by gas chromatography (model 7890A; Agilent) using the Microbial Identification software package with the Sherlock MIDI 6.1 system and the Sherlock Aerobic Bacterial Database (TSBA 6.1).

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\textsuperscript{2}https://blast.ncbi.nlm.nih.gov/Blast.cgi

\textsuperscript{3}https://www.ezbiocloud.net/
Polar lipids were extracted using the chloroform/methanol system and separated by two-dimensional thin-layer chromatography (TLC, Silica gel 60 F254, Merck) (Minnikin et al., 1984). The plates dotted with samples were subjected to two-dimensional development with the first solvent of chloroform–methanol–water (65:25:4, v/v) and the second solvent of chloroform–methanol–acetic acid–water (80:12:15:4, v/v). Total lipids and specific functional groups were detected using the different spray staining reagents on separate TLC plates: 10% ethanolic molybdophosphoric acid, ninhydrin, molybdenum blue, and α-naphthol–sulfuric acid. The quinone was extracted from the freeze-dried cells and determined using high-performance liquid chromatography (Agilent 1200; ODS 250 × 4.6 mm × 5.0 μm; flow phase, methanol–isopropanol, 2:1; 1.0 ml/min) according to the methods described by Collins and Jones (1981).

**Genome Sequencing and Function Analysis**

The genomic DNA of strain JM10B5aT was extracted using the HiPure Bacterial DNA Kit (Magen Biotech, Guangzhou) according to the manufacturer’s instruction. The genome was sequenced using the Illumina NovaSeq PE150 platform in Shanghai Majorbio Bio-Pharm Technology Co., Ltd. (Shanghai, China). Raw reads were filtered and then de novo assembled using the software SPAdes version 3.14.1 under “--careful” mode and with a k-mer value of 127 (Bankevich et al., 2012). Available genomes of the related type strains *P. stutzeri* CGMCC 1.1803T and *P. balearica* CCUG 44487T were obtained from the GenBank database with the numbers CP002881 and CP007511, respectively. The genome of strain JM10B5aT was in draft status and was quality-checked using the software CheckM version 1.1.2 (Parks et al., 2015). Overall genome relatedness indices (OGRIs) including digital DNA–DNA hybridization values (ddDH) and average nucleotide identity values (ANI) were estimated using the genome-to-genome distance calculator version 2.1 online service with the recommended formula 2a and the software FastANI version 1.31, respectively (Meier-Kolthoff et al., 2013; Jain et al., 2018). The UBCG pipeline which could provide an accurate phylogenomic tree for the taxonomic purpose was used (Chun et al., 2018; Na et al., 2018). The genomes of strain JM10B5aT and the related type strains were annotated using the software Prokka version 1.13 (Seemann, 2014) and Rapid using Subsystem Technology (RAST) version 2.0 with the default parameters (Overbeek et al., 2014).

**Assessment of Nitrogen Removal Characteristics**

The results of the API 20NE test preliminarily indicated that strain JM10B5aT was capable of reducing nitrate and nitrite. Furthermore, the genome annotation revealed that strain JM10B5aT possessed the functional genes including *napA*, *narG*, *nirS*, *norB*, and *nosZ* that participated in the denitrification process. These results suggested that strain JM10B5aT had the potential capability of complete denitrification. To investigate the optimum carbon source of denitrification for strain JM10B5aT, the carbon source was replaced in the DSM-1 (DSM with KNO3 0.36 g/L as the sole nitrogen source) or DSM-2 (DSM with NaNO3 0.25 g/L as the sole nitrogen source) by sodium acetate, sodium succinate, sodium citrate, glucose, sucrose, and starch, respectively, with the C/N ratio of 10 (the molar mass of carbon to the molar mass of nitrogen). Furthermore, the initial C/N ratios of 2, 3, 4, and 5 were controlled by changing the addition amount of the optimum carbon source in the DSM-1. Before the experiments, strain JM10B5aT was incubated in NB at 30°C and 180 rpm for 24 h. The suspension was centrifuged and then washed 3 times with sterile physiological saline to remove the residual medium. The biomass was suspended in sterile water with the initial OD600 adjusting to 1.0. The bacterial suspension was inoculated into a conical flask containing sterile medium with the inoculum amount of 4.0% and incubated at 30°C statically. Samples taken from flasks after 48 h of incubation were used for determining the OD600 and chemical analyses. The medium without bacterial inoculation was used as control treatment. All the experiments were performed in biological quadruplicate.

**Analytical Methods and Statistical Analyses**

The concentrations of NO3-N and NO2-N were measured using *N-*(1-naphthyl) ethylenediamine dihydrochloride and UV spectrophotometry, respectively, according to the standard analytical procedures (Association et al., 2005). All the data were presented as mean and standard error (SE). One-way ANOVA (multiple range test) was performed with Tukey’s HSD test (*P <* 0.05) using SPSS Statistics 19.

**RESULTS AND DISCUSSION**

**16S rRNA Gene Sequence Analyses and Chemotaxonomic Characterization**

The almost complete 16S rRNA gene sequence of strain JM10B5aT obtained by amplification (1,375 bp) was included in the complete 16S rRNA gene sequence assembled from genomic sequences (1,537 bp). The sequence comparison showed that strain JM10B5aT fell into the genus *Pseudomonas* and shared the highest similarity with *P. stutzeri* CGMCC 1.1803T (98.0%). All the other type strains showed similarities lower than 98.0% with strain JM10B5aT. Based on phylogenetic analysis using the ML algorithm, strain JM10B5aT was stably located in the genus *Pseudomonas* and formed a clade with *P. balearica* CCUG 44487T (the similarity to JM10B5aT was 97.0%) at the 79.0% bootstrap confidence level (Figure 1). The similarity and phylogenetic analysis based on the 16S rRNA gene sequences indicated that strain JM10B5aT should represent a novel member of the genus *Pseudomonas*. Therefore, the type strains *P. stutzeri* CGMCC 1.1803T and *P. balearica* CCUG 44487T were purchased from the culture collection centers and used as references for further comparisons of phenotypic and chemotaxonomic characteristics.

The predominant cellular fatty acids (>10%) of strain JM10B5aT were C16:0 (22.0%), summed feature 3 (C16:1ω6c

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1.http://ggdc.dsmz.de/ggdc.php/
**FIGURE 1** | ML tree based on 16S rRNA gene sequences of strain JM10B5aT and the related type strains of genus *Pseudomonas*. Type strain *Entomomonas moraniae* QZS01T was used as an outgroup. There were a total of 1,303 positions used to construct the phylogenetic tree. Bootstrap values higher than 60% were shown at branch points. Bar, 0.05 represents the number of substitutions per site.

### 16S rRNA Gene Sequences

- **Pseudomonas balearica** CCUG 44487T (CP007511)
- **Pseudomonas oligotrophica** JM10B5aT (OM341414)
- **Pseudomonas zhaodongensis** NEAU-ST5-21T (RFFM01000015)
- **Pseudomonas xanthomarina** DSM 18231T (MN901207)
- **Pseudomonas kunmingensis** HL22-2T (JQ246444)
- **Pseudomonas nitrititolerans** GL14T (MH917718)
- **Pseudomonas stuiteri** CGMCC 1.1803T (CP002881)
- **Pseudomonas otitidis** MCC10330T (AY953147)
- **Azotobacter bryophylli** L461T (MF078077)
- **Azotobacter beijerinckii** ATCC 19360T (AJ308319)
- **Azotobacter nigricans** subsp. *nigricans* IAM 15005T (AB175651)
- **Azotobacter armeniacus** DSM 2284T (AB175655)
- **Azotobacter salinestris** ATCC 49674T (AB175656)
- **Azotobacter vinelandii** IAM 15004T (AB175657)
- **Azotobacter paspali** ATCC 23833T (AJ308318)
- **Pseudomonas benzenivorans** DSM 8628T (FNCT01000040)
- **Thiopseudomonas denitrificans** X2T (KJ567598)
- **Oblitimonas alkaliphila** B4199T (CP012358)
- **Entomomonas moraniae** QZS01T (CP029822)

### Comparative Fatty Acid Analysis

And/or C_{16:1} ω7c (21.3%), and summed feature 8 (C_{18:1} ω6c and/or C_{18:1} ω7c, 29.1%), which were also detected in other *Pseudomonas* species (Mamtimin et al., 2021). Comparative fatty acid profiles between strain JM10B5aT and the two related type strains are shown in Table 1. The fatty acid profile of JM10B5aT was similar to that of other related strains, although there were minor quantitative differences observed. The predominant respiratory quinone of strain JM10B5aT was ubiquinone-9 (Q-9) which was consistent with that of other members of the genus *Pseudomonas* (Zou et al., 2019; Mamtimin et al., 2021). The major polar lipids were phosphatidylethanolamine (PE), phosphatidylglycerol (PG), and diphosphatidylglycerol (DPG), which were consistent with the previously published data for *Pseudomonas* species (Zou et al., 2019; Li et al., 2020). In addition, minor amounts of two unidentified aminophospholipids (APLs)
The morphological features of strain JM10B5aT were observed to be gram-stain-negative, aerobic, rod-shaped (0.6–1.4 × 1.5 μm), facultative anaerobic, and motile with a single polar flagellum (Supplementary Figure 2B). Growth was determined at 10–45°C (optimum, 25–30°C), at pH 5.5–11.0 (optimum, 6.0), and in 0–4.0% (w/v) NaCl (optimum, 0.5–2.0%). The activities of catalase and oxidase were positive, and the further details differentiating strain JM10B5aT from the two related type strains are shown in Table 2. For instance, strain JM10B5aT could not grow in the NB with a NaCl concentration of 5.0 while the two related type strains could grow; the optimum pH of strain JM10B5aT was 6.0, which was inconsistent with that of P. stutzeri CGMCC 1.1803T and P. balearica CCUG 44487T of 6.5–7.5 and 8.5–9.5, respectively; the growth of strain JM10B5aT was weaker than that of the two related type strains at 45°C. Besides, stain JM10B5aT could be distinguished from the reference type strains by positive for adipic acid, trisodium citrate, esterase (C4), and lipase (C14) in the API 20NE and ZYM tests; and positive for D-galacturonic acid, D-glucuronic acid, gentiobiose, L-arginine, L-aspartic acid, L-galactonic acid lactone, mucic acid, quinic acid, α-hydroxybutyric acid, and γ-aminobutyric acid in the Biolog GNE III MicroPlate system. Importantly, strain JM10B5aT exhibited the capability for complete denitrification that liberated copious amounts of nitrogen gas from nitrate. The denitrification characteristic of this strain was consistent with that of P. stutzeri and P. balearica strains (Bennasar et al., 1996; Li et al., 2021).

### Morphological Observations and Physiological Characterization

#### Table 1 | Cellular fatty acid profiles of strain JM10B5aT and the closely related type strains of genus Pseudomonas.

| Fatty acid | JM10B5aT | P. stutzeri CGMCC 1.1803T | P. balearica CCUG 44487T |
|------------|---------|--------------------------|--------------------------|
| Straight-chain saturated | | | |
| C12:0 | 8.3 | 8.1 | 8.3 |
| C14:0 | 1.4 | 1.0 | 0.9 |
| C16:0 | 22.0 | 20.8 | 23.1 |
| Hydroy | | | |
| C10:0 3-OH | 2.8 | 2.8 | 3.2 |
| C12:0 3-OH | 4.0 | 3.8 | 4.1 |
| Branched saturated | | | |
| C17:0 cyclo | 5.2 | 2.0 | 6.0 |
| iso C17:0 | TR | TR | 0.8 |
| Unsaturated | | | |
| C19:0 cyclo w8c | 4.2 | 2.3 | 5.5 |
| Summed Feature 3* | 21.3 | 22.8 | 18.9 |
| Summed Feature 8* | 29.1 | 34.1 | 27.4 |

All data were obtained from this study. Constituents <0.5% in three strains were not shown. TR, trace (<0.5%). *Summed features contain two or more fatty acids that cannot be separated by the MIDI system; summed feature 3 comprises C16:1 ω6c and/or C16:1 ω7c; summed feature 8 comprises C18:1 ω6c and/or C18:1 ω7c.

### Table 2 | Differential characteristics of strain JM10B5aT and the closely related type strains of genus Pseudomonas.

| Characteristic | JM10B5aT | P. stutzeri CGMCC 1.1803T | P. balearica CCUG 44487T |
|----------------|---------|--------------------------|--------------------------|
| Growth | | | |
| NaCl concentration (optimum, w/v) | 0–4.0 (0.5–2.0) | 0–5.0 (0.5) | 0–5.0 (1.0) |
| pH range (optimum) | 5.5–11.0 (6.0) | 6.0–11.0 (8.5–9.5) | 5.5–11.0 (6.5–7.5) |
| Temperature (optimum, °C) | 10–45 (25–30) | 10–45 (30–37) | 10–45 (30–37) |
| 45°C | w | + | + |
| API ZYM and 20NE tests | | | |
| Adipic acid | + | + | – |
| Trisodium citrate | + | + | – |
| Esterase (C4) | + | – | – |
| Lipase (C14) | + | – | + |
| Biolog (GEN III) tests | | | |
| Dextrin | – | + | + |
| D-Galacturonic acid | – | + | – |
| D-Glucuronic acid | + | w | – |
| D-Maltose | – | + | + |
| D-Mannitol | – | + | – |
| D-Saccharic acid | + | – | – |
| Formic acid | – | + | – |
| Gentiobiose | + | – | – |
| L-Ariginne | + | – | – |
| L-Aspartic acid | + | – | + |
| L-Galactonic acid lactone | + | – | – |
| L-Pyroglutamic acid | – | + | – |
| Mucic acid | – | – | – |
| Quinic acid | – | – | – |
| α-Hydroxybutyric acid | – | – | + |
| γ-Amino butyric acid | + | – | – |
| G + C content (%) | 67.2 | 63.6† | 64.7† |
| Genome size (Mb) | 4.0 | 4.5† | 4.4† |

All data were obtained from this study unless indicated otherwise.

†Data from draft genomes in the NCBI genome database.

+, positive; –, negative; w, weakly positive.

Genome Data and Comparative Genomic Analysis

The draft genome of strain JM10B5aT was assembled into 20 contigs (>500 bp) with a genome size of 3,978,222 bp, and the estimated completeness and contamination were 100 and 0.1%, respectively, indicating that this genome was high quality according to the standards (Parks et al., 2015). Genomes of strains P. stutzeri CGMCC 1.1803T and P. balearica CCUG 44487T were studied on NA medium and formed irregular, filmy sheet, non-transparent, and ecru white colonies after 48 h of incubation at 30°C (Supplementary Figure 2A). Cells of strain JM10B5aT were observed to be gram-stain-negative, aerobic, rod-shaped (0.6–1.4 × 1.5 μm), facultative anaerobic, and motile with a single polar flagellum (Supplementary Figure 2B). Growth was determined at 10–45°C (optimum, 25–30°C), at pH 5.5–11.0 (optimum, 6.0), and in 0–4.0% (w/v) NaCl (optimum, 0.5–2.0%). The activities of catalase and oxidase were positive, and the further details differentiating strain JM10B5aT from the two related type strains are shown in Table 2. For instance, strain JM10B5aT could not grow in the NB with a NaCl concentration of 5.0 while the two related type strains could grow; the optimum pH of strain JM10B5aT was 6.0, which was inconsistent with that of P. stutzeri CGMCC 1.1803T and P. balearica CCUG 44487T of 6.5–7.5 and 8.5–9.5, respectively; the growth of strain JM10B5aT was weaker than that of the two related type strains at 45°C. Besides, stain JM10B5aT could be distinguished from the reference type strains by positive for adipic acid, trisodium citrate, esterase (C4), and lipase (C14) in the API 20NE and ZYM tests; and positive for D-galacturonic acid, D-glucuronic acid, gentiobiose, L-arginine, L-aspartic acid, L-galactonic acid lactone, mucic acid, quinic acid, α-hydroxybutyric acid, and γ-aminobutyric acid in the Biolog GNE III MicroPlate system. Importantly, strain JM10B5aT exhibited the capability for complete denitrification that liberated copious amounts of nitrogen gas from nitrate. The denitrification characteristic of this strain was consistent with that of P. stutzeri and P. balearica strains (Bennasar et al., 1996; Li et al., 2021).
were obtained from the GenBank database with the numbers CP002881 and CP007511, respectively. Based on the Prokka annotation, a total of 3,744 genes, 3,679 protein-coding sequences (CDS), 60 tRNA genes, and 3 rRNA genes were found in the genome of strain JM10B5aT. The genomic DNA G+C content of JM10B5aT was 67.2%. Compared with the genomes of related type strains, JM10B5aT had ANI values of 78.9–88.1% and dDDH values of 20.8–30.8% (Supplementary Table 1), which were all below 95.0 and 70% cutoff commonly used to define a bacterial species, respectively (Stackebrandt and Goebel, 1994; Richter and Rossello-Mora, 2009). To further determine the taxonomic position of strain JM10B5aT, the phylogenomic tree based on the 92 bacterial core gene sets was reconstructed (Na et al., 2018). As shown in Figure 2, strain JM10B5aT and P. balearica CCUG 44487T formed a clade with 100% bootstrap value, which was consistent with the phylogenetic tree based on the 16S rRNA gene sequences. Therefore, the comprehensive analyses of OGRIs and phylogenomic tree further indicated that strain
**FIGURE 3** | Genomic analyses based on the RAST annotation. (A) Comparative analyses of the functional genes assigned to nitrogen metabolisms of strain JM10B5a<sup>T</sup> and the related type strains of genus Pseudomonas. (B) Relative arrangements of the denitrification genes on the draft genome of strain JM10B5a<sup>T</sup>. (C) The biosynthetic gene cluster for vibrioferin on the draft genome of strain JM10B5a<sup>T</sup>. Parallel double lines indicate a break in locus organization among scaffolds, and dotted black lines indicate where unrelated continuity loci are not shown. Numbers below the line symbolize the locations.

JM10B5a<sup>T</sup> should represent a novel species within the genus of *Pseudomonas*.

According to the subsystem category distribution of RAST annotation, there were 190, 128, 402, and 336 genes associated with "RNA metabolism," "DNA metabolism," "amino acid and derivatives," and "carbohydrates," respectively, which might be important to the bacterial growth. In addition, 75 genes were identified in the nitrogen metabolism, including the functional genes related to denitrification and assimilation of nitrite and nitrate (Figures 3A,B). Nitrate reductase catalyzing the reduction in nitrate to nitrite plays an important role in the nitrogen cycle (Kuypers et al., 2018). Previous studies have shown that bacterial dissimilatory nitrate reduction could be catalyzed by two different enzymes: a membrane-bound nitrate...
reductase (NAR, the catalytic subunit NarG encoded by *narG*) and periplasmic nitrate reductase (NAP, the catalytic subunit NapA encoded by *napA*) (Moreno-Vivian et al., 1999). Genes *narG* and *napA* were both contained in strain JM10B5aT, which was consistent with *Pseudomonas* sp. JP-H3 and *Paracoccus denitrificans* (Moreno-Vivian et al., 1999; Wang et al., 2019). Genes *nirS* and *nosZ* encoding for nitrous oxide reductase (NAR, the catalytic subunit NarG encoded by *narG*), nitric oxide reductase (NorB, the key enzyme for reducing nitric oxide to nitrous oxide) and *nosZ* encoding for nitrous oxide reductase (NosZ, the key enzyme for reducing nitrous oxide to nitrogen gas) were also identified. Therefore, it was speculated that strain JM10B5aT could perform the complete denitrification pathway: \( \text{NO}_3^- \rightarrow \text{NO}_2^- \rightarrow \text{NO} \rightarrow \text{N}_2 \). Hence, strain JM10B5aT was a novel denitrifying bacterium.

In addition, comparative genomic analyses showed that strain JM10B5aT encoded 126 and 147 functional genes more than *P. ballecica* CCUG 44487 and *P. stutzeri* CGMCC 1.1803, respectively, and 67 genes of them were the shared differential genes (Supplementary Table 2). These genes were related to type I secretion system for aggregation, utilization of alkylphosphonate and tricarballylate, histidine ABC transporter, LysR family transcriptional regulator, and many other proteins. Most of these proteins were mainly affiliated with dehydratase, transferase, and biosynthesis of cofactors and vitamins. The genes encoding arginase and galactarate dehydratase made strain JM10B5aT capable of performing the positive for L-arginine and mucic acid (Table 2). Moreover, strain JM10B5aT was found to harbor the complete operon *pvuABCDE* and vibrioferrin receptor gene *pvuA* (Figure 3C) that are required for the production of a siderophore vibrioferrin. This siderophore was shown to be responsible for vibrioferrin-mediated iron uptake in the terrestrial bacteria *Azotobacter vinelandii*, *Pseudomonas* sp., and the marine bacterium *Vibrio paraheamolyticus* (Yamamoto et al., 1994; Baars et al., 2016; Stanborough et al., 2018; Sood et al., 2019). It was reported that siderophore had a very high affinity to iron with the formation of Fe-siderophore which could facilitate the transport of iron through the membrane directly (Stintzi et al., 2000). Moreover, the denitrification efficiency might be enhanced with the improvement of iron transport from extracellular to intracellular (Jiang et al., 2020). Therefore, the biosynthesis of vibrioferrin might partly explain that the strain JM10B5aT performed the high denitrification efficiency under the low C/N ratio condition.

**Effect of Carbon Source on Denitrification by Strain JM10B5aT**

Heterotrophic denitrifying bacteria require organic carbon for cell growth and as the electron donor in the denitrification process (Rajita et al., 2020). Thus, carbon source was considered to be an important factor influencing denitrification. As expected, significant differences were observed using different carbon sources. As shown in Figure 4A, the strain JM10B5aT could grow well with the OD600 of 0.42 when glucose was used as the sole carbon source. However, it exhibited a higher NO2−-N removal efficiency of 99.5% when sodium acetate served as carbon source than that of 58.6% when glucose served as carbon source. As shown in Figure 4B, strain JM10B5aT could grow quite well when glucose served as the carbon source (OD600 of 0.52), but it performed the relatively high NO2−-N removal of 100% when sodium acetate served as the carbon source. These results indicated that sodium acetate was the optimum carbon source for the performance of denitrification for strain JM10B5aT, which was consistent with *Bacillus pumilus*, *Arthrobacter* sp., and *Streptomyces lusitanus* (Elkarrach et al., 2021). Therefore, sodium acetate was employed in the following experiments.

**Effect of Carbon/Nitrogen Ratio on Nitrate Removal by Strain JM10B5aT**

C/N ratio is a measure of the electron donor to acceptor ratio in the biological denitrification process. This factor can influence the denitrification efficiency and accumulation of NO2−-N. To investigate the influence of C/N ratio on denitrification of strain JM10B5aT, the initial concentration of NO2−-N was fixed at ~50.0 mg/L and the different initial C/N ratios (2, 3, 4, and 5) were controlled. As shown in Figure 5, strain JM10B5aT grew poorly with the OD600 range of 0.10–0.15 at these low C/N ratios. The NO2−-N was reduced completely at the C/N ratios of 3–5 which was distinctly higher than that of 84.4% at the C/N ratio of 2. Furthermore, there was no NO2−-N accumulation observed at the C/N ratios of 3–5, but the NO2−-N concentration of 19.3 mg/L was detected at the C/N ratio of 2. The low NO2−-N removal efficiency and NO2−-N accumulation at the C/N ratio of 2 were mainly due to the limited carbon source that could not provide sufficient energy for bacterial growth and electron donors for denitrification (Fan et al., 2021). These results were consistent with previous reports that denitrification efficiency could decrease under the extremely low carbon concentration (Zhao et al., 2018). Numerous studies have suggested that the optimum C/N ratios for most heterotrophic denitrifying bacteria were in the range of 8–15 (Guo et al., 2016; Liu et al., 2018; Zhao et al., 2018). For example, the C/N ratios of 10 and 15 were the most suitable for strains *P. stutzeri* XL-2 and *P. taiwanensis* to achieve efficient nitrate removal, respectively (He et al., 2018; Zhao et al., 2018). In addition, some denitrifying bacteria could achieve complete denitrification at the relatively low C/N ratios. For example, Rout et al. (2017) used *Bacillus cereus* to remove nitrogen from domestic wastewater with 7.5 as the optimum C/N ratio; Zhang S. et al. (2020) reported that strain *Comamonas* sp. YSF15 could achieve complete denitrification at C/N of 3. Our results showed that strain JM10B5aT could achieve the complete nitrate removal without accumulation of nitrite at the low carbon–nitrogen ratio of 3 (COD/C/N ratio of 2.6), indicating that this strain could be a promising candidate for treating the oligotrophic wastewater.
FIGURE 4 | Growth (OD$_{600}$) and NO$_3^-$-N and NO$_2^-$-N removal performance of strain JM10B5a$^T$ under various common carbon sources. (A) NO$_3^-$-N as the sole nitrogen source. (B) NO$_2^-$-N as the sole nitrogen source. Values are mean ± SE (standard error) for four replicates.

FIGURE 5 | Influence of C/N ratio on NO$_3^-$-N removal characteristic of strain JM10B5a$^T$. Values are mean ± SE (standard error) for four replicates.

Description of *Pseudomonas oligotrophica* sp. nov.

*Pseudomonas oligotrophica* (o.li.go.tro’phi.ca. Gr. adj. oligos, few; Gr. adj. trophikos, nursing, tending, or feeding; N.L. fem. adj. oligotrophica, eating little, referring to a bacterium living on low-nutrient media).

Cells are rods (1.4–1.5 µm in length and 0.6–0.7 µm in width), gram-stain-negative, facultative anaerobic, and motile.
by the polar flagellum. Colonies are irregular, filmy sheet, non-transparent, and ecru white after 48 h of incubation on NA agar (C8), lipase (C14), leucine arylamidase and naphtol-AS-B1- and hydrolysis of Tween 20 and 60. In the API ZYM and MicroPlate system, it is positive for gentiobiose, α-D-glucose, D-fructose, glycerol, L-alanine, L-arginine, L-aspartic acid, L-glutamic acid, D-galacturonic acid, L-galactonic acid lactone, D-glucuronic acid, D-glucuronic acid, glucuronamide, mucic acid, quinic acid, D-saccharic acid, L-lactic acid, citric acid, α-ketoglutaric acid, D-malic acid, L-malic acid, bromosuccinic acid, γ-aminobutyric acid, α-hydroxybutyric acid, β-hydroxy-D-L- butyric acid, α-ketobutyric acid, propionic acid, and acetic acid. The major fatty acids are C16:0, summed feature 3 (C16:1 ω6c and/or C16:1 ω7c), and summed feature 8 (C18:1 ω6c and/or C18:1 ω7c). The major polar lipids are PE, PG, and DPG, and/or C16:0-3-hydroxybutyric acid, β-glucosidase, and/or C18:0. The DNA G+C content of strain JM10B05aT is 67.2%. Accession numbers of the complete 16S rRNA gene sequence and draft genome in DDBJ/ENA/GenBank are OM341414 and JAKJRU000000000000, respectively. The type strain, JM10B05aT (= GDMCC 1.2828T = JCM 35033T), was isolated from pond water for juvenile Litopenaeus vannamei collected from Jiangmen city Guangdong Province, China.

DATA AVAILABILITY STATEMENT

The datasets presented in this study can be found in online repositories. The names of the repository/repositories and accession number(s) can be found in the article/Supplementary Material.

AUTHOR CONTRIBUTIONS

MZ, AL, and QY were involved in conceptualization and project administration. MZ involved in data curation, software, visualization, and writing—original draft. MZ and BX designed the final analysis and methodology. AL, HZ, and BX were involved in funding acquisition. MZ, AL, QY, BX, and HZ investigated the study. HZ collected the resources and supervised the study. MZ and AL validated the study. MZ, QY, and BX were involved in writing—review and editing. All authors contributed to the article and approved the submitted version.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fmicb.2022.882890/full#supplementary-material

REFERENCES

Association, A. P. H., Association, A. W. W., and Federation, W. E. (2005). Standard Methods for the Examination of Water and Wastewater. Washington, DC: American Public Health Association Press.

Baars, O., Zhang, X., Morel, F. M. M., and Seyedsayamdost, M. R. (2016). The siderophore metabolome of Azotobacter vinelandii. Appl. Environ. Microbiol. 82, 27–39. doi: 10.1128/AEM.03160-15

Bankevich, A., Nurk, S., Antipov, D., Gurevich, A. A., Dvorkin, M., Kulikov, A. S., et al. (2012). SPAdes: a new genome assembly algorithm and its applications to single-cell sequencing. J. Comput. Biol. 19, 455–477. doi: 10.1089/cmb.2012.0021

Bennasar, A., RosselloMora, R., Lalucat, J., and Moore, E. R. B. (1996). 16S RNA gene sequence analysis relative to genomovars of Pseudomonas stutzeri and proposal of Pseudomonas balearica sp. nov. Int. J. Syst. Bacteriol. 46, 200–205. doi: 10.1099/00227713-46-1-200

Bernardet, J. F., Nakagawa, Y., and Holmes, B. (2002). Proposed minimal standards for describing new taxa of the family Flavobacteriaceae and emended description of the family. Int. J. Syst. Evol. Microbiol. 52, 1049–1070. doi: 10.1099/ijs.0.02136-0

Chen, C., Ali, A., Su, J., Wang, Y., Huang, T., and Gao, J. (2021). Pseudomonas stutzeri GF2 augmented the denitrification of low carbon to nitrogen ratio.
Li, B., Jing, F., Wu, D., Xiao, B., and Hu, Z. (2021). Simultaneous removal of nitrogen and phosphorus by a novel aerobic denitrifying phosphorus-accumulating bacterium, *Pseudomonas stutzeri* ADP-19. *Bioresour. Technol.* 321, 124445. doi: 10.1016/j.biortech.2020.124445

Li, J., Wang, L. H., Xiang, F. G., Ding, W. L., Xi, L. J., Wang, M. Q., et al. (2020). *Pseudomonas phragmitis* sp. nov., isolated from petroleum polluted river sediment. *Int. J. Syst. Evolution. Microbiol.* 70, 364–372. doi: 10.1099/ijsem.0.053763

Ling, Y., Yan, G., Wang, H., Dong, W., Wang, H., Chang, Y., et al. (2021). Release mechanism, secondary pollutants and denitrification performance comparison of six kinds of agricultural wastes as solid carbon sources for nitrate removal. *Int. J. Environ. Res. Public Health* 18, 1232. doi: 10.3390/ijerph18013232

Liu, S., Chen, Q., Ma, T., Wang, M., and Ni, J. (2018). Genomic insights into metabolic potentials of two simultaneous aerobic denitrification and phosphorus removal bacteria, *Achromobacter* sp. GAD3 and *Agrobacterium* sp. LAD9. *FEMS Microbiol. Ecol.* 94, 4. doi: 10.1093/femsyc/fty020

Liu, X., Huang, M., Bao, S., Tang, W., and Fang, T. (2020). Nitrate removal from low carbon-to-nitrogen ratio wastewater by combining iron-based chemical reduction and autotrophic denitrification. *Bioresour. Technol.* 301, 122731. doi: 10.1016/j.biortech.2019.122731

Mammitin, T., Anvar, N., Abdurahman, M., Kurban, M., Rozahon, M., Mammitin, H., et al. (2021). *Pseudomonas lognresinis* sp. nov., an endophytic bacterium isolated from *Populus euphratica* at the ancient Ugan river. *Antonie Van Leeuwenhoek* 114, 399–410. doi: 10.1007/s10482-021-01524-8

Meier-Kolthoff, J. P., Auch, A. F., Klenk, H. P., and Göker, M. (2013). Genome sequence-based species delimitation with confidence intervals and improved distance functions. *BMCCBioinform.* 14, 60. doi: 10.1186/1471-2105-14-60

Migula, W. (1894). Über ein neues system der bakterien. *Arb. Bakteriol. Inst. Karlsruhe*. 1, 235–238.

Minnikin, D. E., Odonnell, A. G., Goodfellow, M., Alderson, G., Athalye, M., Schaal, A., et al. (1984). An integrated procedure for the extraction of bacterial isoprenoid quinones and polar lipids. *J. Microbiol. Methods.* 2, 233–241. doi: 10.1016/0167-7012(84)90018-6

Moreno-Vivian, C., Cabello, P., Martinez-Luque, M., Blasco, R., and Castillo, F. (1999). Prokaryotic nitrate reduction: molecular properties and functional distinction among bacterial nitrate reductases. *J. Bacteriol.* 181, 6573–6584. doi: 10.1128/JB.181.11.6573-6584.1999

Na, S. I., Kim, Y. O., Yoon, S. H., Ha, S. M., Baek, I., and Chun, J. (2018). UBCG: Up-to-date bacterial core gene set and pipeline for phylogenomic reconstruction. *J. Microbiol. Method.* 56, 280–285. doi: 10.1016/j.mimet.2018.01.014-6

Overbeek, R., Olson, R., Pusch, G. D., Olsen, G. J., Davis, J. J., Disz, T., et al. (2014). The SEED and the rapid annotation of microbial genomes using subsystems technology (RAST). *Nucleic Acids Res.* 42, D206–D214. doi: 10.1093/nar/gkt1226

Pang, Y., and Wang, J. (2021). Various electron donors for biological nitrate removal: a review. *Sci. Total. Environment.* 794, 148699. doi: 10.1016/j.scitotenv.2021.148699

Parks, D. H., Imelfort, M., Skennerton, C. T., Hugenholtz, P., and Tyson, G. W. (2015). CheckM: assessing the quality of microbial genomes recovered from isolates, single cells, and metagenomes. *Genome Res.* 25, 1043–1055. doi: 10.1101/gr.186072.114

Rajta, A., Bhata, R., Setia, H., and Pathania, P. (2020). Role of heterotrophic aerobic denitrifying bacteria in nitrate removal from wastewater. *J. Appl. Microbiol.* 128, 1261–1278. doi: 10.1111/jam.14476

Razvani, F., Sarrafzadeh, M. H., Ebrahimi, S., and Oh, H. M. (2019). Nitrate removal from drinking water with a focus on biological methods: a review. *Environ. Sci. Pollut. Res. Int.* 26, 1124–1141. doi: 10.1007/s11356-017-9185-0

Richter, M., and Rossello-Mora, R. (2009). Shifting the genomic gold standard for the prokaryotic species definition. *Proc. Natl. Acad. Sci. U. S. A.* 106, 19126–19131. doi: 10.1073/pnas.0906412106

Rout, P. R., Bhunia, P., and Dash, R. R. (2017). Simultaneous removal of nitrate and dissolved organic matter removal from wastewater treatment plant effluent in a solid-phase denitrification biofilm reactor. *Bioresour. Technol.* 314, 123714. doi: 10.1016/j.biortech.2020.123714

Schaal, A., et al. (1984). An integrated procedure for the extraction of bacterial ribonucleic acid from isolates, single cells, and metagenomes. *Int. J. Syst. Evol. Microbiol.* 44, 19126–19131. doi: 10.1073/pnas.0906412106

Stutzerimonas, S., Minh, B. Q, Wong, T. K. F., von Haeseler, A., and Jermiin, L. S. (2017). ModelFinder: fast model selection for accurate phylogenetic estimations. *Nat. Methods.* 14, 587–589. doi: 10.1038/nmeth.4285

Katoh, K., and Standley, D. M. (2014). MAFFT: iterative refinement and additional methods. *Methods Mol. Biol.* 1079, 131–146. doi: 10.1007/978-1-62703-646-7_8

Kumar, S., Stecher, G., Li, M., Knyaz, C., and Tamura, K. (2018). MEGA X: molecular evolutionary genetics analysis across computing platforms. *Mol. Biol. Evol.* 35, 1547–1549. doi: 10.1093/molbev/mmy966

Kuypers, M. M. M., Marchant, H. K., and Karpel, B. (2018). The microbial nitrification-cycling network. *Nat. Rev. Microbiol.* 16, 263–276. doi: 10.1038/nrmicro.2018.9

Lalucat, J., Gomila, M., Mulet, M., Zaruma, A., and Garcia-Valdes, E. (2021). Past, present and future of the boundaries of the *Pseudomonas* genus: proposal of *Stutzerimonas* gen. *Nov. Syst. Appl. Microbiol.* 45, 126289. doi: 10.1016/j.syapm.2021.126289

Lanyi, B. (1987). Classical and rapid identification methods for medically important bacteria. *Method. Microbiol.* 19, 1–67. doi: 10.1016/S0380-9517(08)70047-0

Li, R., Jing, F., Wu, D., Xiao, B., and Hu, Z. (2021). Simultaneous removal of nitrogen and phosphorus by a novel aerobic denitrifying phosphorus-accumulating bacterium, *Pseudomonas stutzeri* ADP-19. *Bioresour. Technol.* 321, 124445. doi: 10.1016/j.biortech.2020.124445

Ling, Y., Yan, G., Wang, H., Dong, W., Wang, H., Chang, Y., et al. (2021). Release mechanism, secondary pollutants and denitrification performance comparison of six kinds of agricultural wastes as solid carbon sources for nitrate removal. *Int. J. Environ. Res. Public Health* 18, 1232. doi: 10.3390/ijerph18013232

Li, S., Chen, Q., Ma, T., Wang, M., and Ni, J. (2018). Genomic insights into metabolic potentials of two simultaneous aerobic denitrification and phosphorus removal bacteria, *Achromobacter* sp. GAD3 and *Agrobacterium* sp. LAD9. *FEMS Microbiol. Ecol.* 94, 4. doi: 10.1093/femsyc/fty020

Liu, X., Huang, M., Bao, S., Tang, W., and Fang, T. (2020). Nitrate removal from low carbon-to-nitrogen ratio wastewater by combining iron-based chemical reduction and autotrophic denitrification. *Bioresour. Technol.* 301, 122731. doi: 10.1016/j.biortech.2019.122731
misclassified species of the genus Pseudomonas into other genera. Int. J. Syst. Evol. Microbiol. 71, 005011. doi: 10.1099/ijsem.0.005011
Seemann, T. (2014). Prokka: rapid prokaryotic genome annotation. Bioinformatics 30, 2068–2069. doi: 10.1093/bioinformatics/btu153
Sood, U., Hira, P., Kumar, R., Bajaj, A., Rao, D. L. N., Lal, R., et al. (2019). Comparative genomic analyses reveal core-genome-wide genes under positive selection and major regulatory hubs in outlier strains of Pseudomonas aeruginosa. Front. Microbiol. 10, 53. doi: 10.3389/fmicb.2019.00053
Stackebrandt, E., and Goebel, B. M. (1994). Taxonomic Note: a place for DNA-DNA reassociation and 16s rRNA sequence analysis in the present species definition in bacteriology. Int. J. Syst. Bacteriol. 44, 846–849. doi: 10.1099/00207713-44-4-846
Stanborough, T., Fegan, N., Powell, S. M., Tamplin, M., and Chandy, P. S. (2018). Vibrioferdin production by the food spoilage bacterium Pseudomonas fragi. FEMS Microb. Lett. 365, 6. doi: 10.1093/femsec/fvx279
Stintzi, A., Barnes, C., Xu, L., and Raymond, K. N. (2000). Microbial iron transport via a siderophore shuttle: a membrane ion transport paradigm. Proc. Natl. Acad. Sci. U. S. A. 97, 10691–10696. doi: 10.1073/pnas.200318797
Sun, S., Wang, Y., Zang, T., wei, J., and Wu, H., Wei, C., et al. (2019). A biosurfactant-producing Pseudomonas aeruginosa S5 isolated from coking wastewater and its application for bioremediation of polycyclic aromatic hydrocarbons. Bioreour. Technol. 281, 421–428. doi: 10.1016/j.biortech.2019.02.087
Tindall, B., Sikorski, J., Smibert, R., and Krieg, N. (2007). “Phenotypic characterization and the principles of comparative systematics,” in Methods for General and Molecular Microbiology. eds C. Reddy, T. Beveridge, J. Breznak, G. Marzluf, T. Schmidt, and L. Snyder, (Washington, DC: ASM Press), 330–393.
Wang, J. W., Cai, M., Nie, Y., Hu, B., Yang, Y., and Wu, X. L. (2020). Simultaneous heterotrophic nitrification and aerobic denitrification by a novel isolated Pseudomonas mendocina strain XL1 to achieve efficient nitrate removal at low C/N wastewater. Bioreour. Technol. 305, 122626. doi: 10.1016/j.biortech.2019.122626
Wang, R. C., Chen, C., Wang, W., Shao, B., Xu, X. J., Zhou, X., et al. (2020). The stimulating metabolic mechanisms response to sulfide and oxygen in typical heterotrophic sulfate-oxidizing nitrate-reducing bacteria Pseudomonas C27. Bioreour. Technol. 309, 123451. doi: 10.1016/j.biortech.2020.123451
Zhang, S., Lang, Z., and Yang, S. (2020). Denitrification strategies of strain YSF15 in response to carbon scarcity: based on organic nitrogen, soluble microbial products and extracellular polymeric substances. Bioreour. Technol. 314, 123733. doi: 10.1016/j.biortech.2020.123733
Zhao, B., Cheng, D. Y., Tan, P., An, Q., and Guo, J. S. (2018). Characterization of an aerobic denitrifier Pseudomonas stutzeri strain XL-2 to achieve efficient nitrate removal. Bioreour. Technol. 250, 564–573. doi: 10.1016/j.biortech.2017.11.038
Zhang, Z., Li, A., Su, J., Huang, T., Wang, Y., and Zhang, S. (2021). Fungal pellets immobilized bacterial bioreactor for efficient nitrate removal at low C/N wastewater. Bioreour. Technol. 332, 125113. doi: 10.1016/j.biortech.2021.125113
Zou, Y., He, S., Sun, Y., Zhang, X., Liu, Y., and Cheng, Q. (2019). Pseudomonas uramigenes sp. nov., isolated from rhizosphere soil of Alhagi sparsifolia. Int. J. Syst. Evol. Microbiol. 69, 1760–1766. doi: 10.1099/ijsem.0.003390

Conflict of Interest: AL was employed by Guangdong BOWOTE BioSciTech, Co. Ltd.

The remaining authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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