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Original Article

The characteristics of the novel bacterial strain *Pseudomonas mendocina* isolated from freshwater aquaculture farm

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Abstract

Plastic contamination can cause damage to the water quality of fish farm ponds, and also affect the quality of the final product. *Pseudomonas mendocina* was found to biodegrade plastics. Our study aimed to investigate the physicochemical properties and drug resistance of *P. mendocina* isolated from local freshwater aquaculture farms. Firstly, the strain was isolated from aquaculture water and then identified by matrix-assisted flight mass spectrometry and 16S rDNA sequencing. Then, biochemical and antibiotic resistance analyses were performed, and a microbial high-throughput growth detector was used to assess the growth of the strain. Finally, PCR and proteomics analyses were conducted to determine drug-resistance-related genes/proteins. According to the results of the spectrum diagram and sequencing, the isolated bacteria were identified as *P. mendocina*, and were positive for reactions of ADH, MTE, LAC, MNE, FRU, CIT, MLT, ONPG, and ACE. *P. mendocina* was sensitive to most of the antibiotics, and its resistance to CHL, MIN, and TIC/CLA was intermediate. Additionally, *gyrB* was the resistance gene, and mdtA2, mdtA3, mdaB, and emrK1 were closely related to the drug resistance of *P. mendocina*. Our results show the biochemical properties of *P. mendocina* in isolated aquaculture water, and provide a new perspective for *P. mendocina* involved in the biological removal of plastics or microplastics in freshwater aquaculture farms.

Keywords: *Pseudomonas mendocina*; Proteomics; Antibiotic; Freshwater aquaculture farm; Mass spectrometry

1. Introduction

Plastic is a versatile, flexible, durable, and inexpensive material, and widely used in modern society. The increase in plastic production is attributed to the exponential growth of the world's population, with an estimated 81,200–70,000 tons of plastic entering the rivers each year [1]. At present, plastic pollution is an important issue facing the world, and microplastics make up a large proportion of plastic debris [2]. Microplastic pollution can come from many sources and is widely distributed in watersheds. This water pollution can reach aquaculture systems, such as beaches, seawater columns, sediments, soil, atmosphere, freshwater rivers and lakes, and even aquaculture farms [3]. The latter is a very serious situation; for example, China is the world's largest producer of aquaculture, accounting for more than 60% of global production by volume [4]. Microplastic contamination can cause damage to the water quality of fish farm ponds and also affect the quality of the final product [5]. Therefore, the degradation of plastics or microplastics has become a pivotal point in relation to the ecological system.

Much research has tried to solve the problem of plastic pollution, especially microorganisms, as an important method to control environmental pollution. In soil containing polybutylene adipate-co-terephthalate (PBAT), co-culture with *Priestia megaterium*, *Pseudomonas mendocina* (*P. mendocina*), and *P. pseudoalcaligenes* can improve the biodegradative process of PBAT [6, 7]. A previous study isolated two types of polycaprolactone (PCL)-degrading bacteria from seawater at a depth of 320 m in Toyama Bay, which was identified as *Pseudomonas* and capable of decomposing PCL at 4°C [8]. Another study isolated bacterial and fungal strains with the ability to degrade bioplastics from the Arctic region and found that *Rhodocladobacillus*, *Trichoderma*, *Pseudomonas*, and *Rhodococcus* could effectively degrade PCL, poly(lactic acid) (PLA), poly(butylene succinate) (PBS), and poly(butylene succinate-co-butylene adipate) (PBSA) [9]. These reports indicated that microorganisms isolated from different environments have the potential to biodegrade plastic waste in ecosystems.

P. mendocina is a Gram-negative rod bacterium belon-

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ging to *Pseudomonadaceae*. *P. mendocina*, as a highly efficient aerobic denitrifying microbe, can be isolated from sewage sludge, shows good growth and can remove all types of inorganic nitrogen without the accumulation of the intermediate products nitrite and nitrous oxide [10]. A recent study has shown that a single bacterium, identified as *P. mendocina,* can remove nitrogen and phosphorous, as well as improve biological reaction efficiency and reduce operating costs and complexity in wastewater treatment plants [11]. A further study showed *P. mendocina* has a high performance regarding heterotrophic nitrification and aerobic denitrification during the total process, without intermediate accumulation [12]. A previous study demonstrated that *P. mendocina* was found on the outer surface of freshwater fish that were imported into Australia [13]. Therefore, we speculated that *P. mendocina* may exist in freshwater aquaculture water. Zhang et al. illustrated that the aerobic denitrifying bacterium *P. mendocina* had high denitrification potential for a deep improvement in effluent quality in the wastewater treatment plant [14]. The co-culture of *P. mendocina* and *Actinomucor elegans* could degrade the PLA/PBAT and secrete proteases and lipases, which could catalyze the ester bonds of PLA1 and PBAT in PLA/PBAT films, then further hydrolyze them into different monomers and oligomers as nutrition sources [15]. However, the physicochemical properties and drug resistance of *P. mendocina* remain unknown. In this study, *P. mendocina* was isolated from the water samples in a freshwater aquaculture farm and then employed for the identification, sequencing, and determination of drug-resistant genes, and proteomics. This work will provide the foundation for the potential functions of *P. mendocina* in the management of freshwater aquaculture farms.

2. Materials and methods

2.1. Isolation and cultivation of bacteria

Water samples (500 mL) were collected from a freshwater aquaculture farm (Kaifeng, Henan), and then bacteria were isolated from the water samples using an inoculation ring, and incubated on a nutrient agar medium (Hopebiol, Qingdao, China) in an incubator at 37 °C. After that, a fresh single colony was chosen for inoculation in a nutritional broth medium (Hopebiol, Qingdao, China), and then cultured at 240 rpm for 16 hours.

2.2. Extraction of genomic DNA andplasmids

DNA was extracted from bacterial fluid using the Genome and Plasmid Extraction Kit (Tiangen, Shanghai, China), and 16S rDNA gene PCR amplification was performed using universal primers 27F (5 '- AGAGTTTGATCCTGGCTCAG-3') and 1492R (5 '- GGTTACCTTGTTACGATT-3'). The PCR conditions were shown as follows: 98 °C for 5 min, a total of 40 cycles at 98 °C for 15 s, 55 °C for 25 s, 72 °C for 20 s, and then 72 °C for 5 min. Gelred (Tsingke Biotechnology Co., Ltd., Beijing, China), a nucleic acid dye, was used to produce 1% agarose gel, and the size of the PCR product was confirmed by electrophoresis.

2.3. Establishment of a phylogenetic tree

A phylogenetic tree was constructed by the neighbor-joining method with Max Seq difference 0.5 through the NCBI Website (https://blast.ncbi.nlm.nih.gov/Blast.cgi?PROGRAM=blastn). GenBank accession numbers for the sequences used in the study are shown in parentheses. B9 was indicated as the novel isolated sequence in this study.

2.4. The identification of matrix-assisted laser-desorption ionization time-of-flight mass spectrometry

The matrix-assisted laser-desorption ionization time-of-flight mass spectrometry (M-Discover 100 Excellence, MEIHUA, Zhuhai, China) and mass spectrometry detector microbial sample pretreatment reagents (2 × 50 model, MEIHUA, Zhuhai, China) for sample processing were used for bacterial identification. Firstly, 1 μL of bacterial suspension was transferred to the target sample plate of the mass spectrometry and then smeared to form a thin film. After that, 1 μL of lysis solution M1 (MEIHUA, Zhuhai, Chia) was added to the bacterial membrane and dried at room temperature. Finally, 1 μL of matrix liquid was added to cover the same target, and after drying at room temperature, matrix-assisted laser-desorption ionization time-of-flight mass spectrometry was employed for detection.

2.5. Biochemical Identification of Microorganisms

The microbial identification and drug-sensitivity analysis system (MA120, MEIHUA, Zhuhai, China) was used for the biochemical identification of microorganisms and antibiotic resistance experiments. The biochemical experiment operation was performed as follows: firstly, a single colony was selected for pure culture, and then the bacterial solution was diluted with the sterile physiological saline to make 0.5 McFarland standard bacterial suspension. Subsequently, 100 μL bacterial suspension was added to the biochemical identification wells, and after incubation at 37 ℃ for 24 h, the samples were employed for detection and interpretation.

2.6. Analysis of antibiotic resistance

The antibiotic resistance test was conducted as follows: 50 μL of 0.5 McFarland standard bacterial suspension was added to Mueller–Hinton Broth culture medium (Hopebiol, Qingdao, China), and a drop of drug-sensitivity chromogenic solution (cat. no. A2060058, MEIHUA, Zhuhai, China) was added. After mixing, 100 μL mixture was added to each drug-sensitivity test well and put on the drug-sensitivity identification plate. After incubation at 37 ℃ for 24 h, the drug-sensitivity analysis system (MA120, MEIHUA, Zhuhai, China) was employed to measure and analyze the MIC values based on the regulations of the Clinical and Laboratory Standards Institute (CLSI).

2.7. Detection of the bacterial growth

A total of 200 μL of bacterial suspension was added to each well of a 96-well plate, and the absorbance value at OD_{600nm} was detected using a microplate reader (Sunrise, Tecan (Shanghai) Trading Co., Ltd, Shanghai, China). In brief, the bacterial suspension was diluted to 1 OD, and growth experiments were conducted with different concentrations of bacterial suspension (0, 1, 10, 20, 40, 60, 80, and 100 μL). A total of 1 mL of culture medium with different concentrations of bacteria was added to a 48-deep well plate, 1 mL, and the plate was placed in a microbial high-throughput growth detector (MicroScreen HT, JIELING, Tianjin, China). The cultivation conditions were set at 600 rpm, 37 ℃, and OD values were collected once an hour. After 87 h of growth, the OD values collec-

ted every hour were plotted into a growth curve.

2.8. Detection of drug resistance genes

The sequences of all primers for AAC (3) - II, cmlA, *CTX-M-l*, *gyrA*, *gyrB*, *blaKPC*, *NDM-1*, *oqxA*, *oqxB*, *OXA*, *parC*, *qepA*, *qnrA*, *qnrB*, *qnrC*, *qnrD*, *qnrS*, and *Sul2* genes were synthesized and obtained from Tsingke Biotechnology Co., Ltd. (Beijing, China). AAC(3)-II: GGCGACTTCACCGTTTCT, GGACCGATCACCCTACGAG, 412bp; cmlA: GGGTGGCGGGCTATCTTT, GCGACACCAATACCCACTAG, 467bp; CTX-M-l: CAGCGCTTTTGCCGTCTAAG, GGCCCATGGTTAAAAAATCACTC, 94bp; gyrA: CGATGTCGGTCATTGTTG, ACTTCCGTCAGGTTGTGC, 496bp; gyrB: GAAATGACCCGCCGTAA, CTTGCCTTTCTTCACTTTGT, 456bp; blaKPC: GCTACACCTAGCTCCACCTTC, TCAGTGCTCTACAGAAAACC, 1050bp; NDM-1: ATTAGCCGCTGCATTGAT, CATGTCGAGATAGGAAGTG, 151bp; OqxA: CTCGGCGCGATGATGCT, CCACTCTTCACGGGAGACGA, 393bp; oqxB: TTCTCCCCCGGCGGGAAGTAC, CTCGGCCATTTTGGCGCGTA, 512bp; OXA: ACAGAAGCATGGCTCGAAAGT, TTGCTGTGAATCCTGCACCA, 190bp; ParC: CTGAATGCCAGCGCCAAAT, GCGCATACGCACTGAAC, 567bp; qepA: GCAGGTCCAGCAGCGGGTAG, CTTCCTGCCCGAGTATCGTG, 218; qnrA: ATTTCTCACGCCAGGATTTG, GATCGGCAAAGGTTAGGTCA, 516bp; qnrB: GATCGTGAAAGCCAGAAAGG, ATGAGCAACGATGCCTGGTA, 476bp; qnrC: GGGTTGTACATTTATTGAATC, TCCACTTTACGAGGTTCT, 447; qnrD: CGAGATCAATTTACGGGGAATA, AACAAGCTGAAGCGCCTG, 582bp; qnrS: ACGACATTCGTCAACTGCAA, TAAATTGGCACCCTGTAGGC, 417bp.Su12: GATGGCATTCCCGTCTC, TTCTTGCGGTTTCTTTCAGC, 577bp. The resistance genes were amplified by PCR (C1000 Touch, BIORAD, Hercules, CA, USA), and products were confirmed by gel electrophoresis (Powerpac Basic, BIORAD, Hercules, CA, USA). The molecular weight of the gene was identified by gel imaging (GelGel Go, BIORAD, Hercules, CA, USA). The correct molecular weight sequence was determined and compared with the NCBI database. Real-time PCR was performed in an QuantStudio5 real-time system (ABI) with ssoAdvanced Universal SYBR Green supermix (BioRad) for gyrB level. Real-time PCR conditions were as follows: 98℃ for 2 min, then 98℃ for 5 s, 60℃ for 20 S, 40 cycles. We used 16S gene as an internal reference in order to normalize the gyr B level. The generation of specific PCR products wasconfirmed by melting curve analysis. The $2^{-\triangle\triangle ct}$ method was used to quantify the gyrB relative level.

2.9. Pre-processing of protein samples

Sample preparation contained the processes of protein extraction, denaturation, reduction, alkylation and tryptic digestion and peptide cleanup. Commercially available iST Sample Preparation kit (PreOmics, Germany) was used for sample preparation according to the manufacturer's protocols. In brief, 50 µL of lyse buffer was added and heated at 95 °C for 10 min at 1000 rpm with agitation. After cooling to room temperature, trypsin digestion buffer was added and then incubated at 37 °C for 2 h at 500 rpm with shaking. The digestion process was stopped with a stop buffer. The process of sample clean-up and desalting was carried out with the recommended wash buffer in the iST cartridge. Peptides were eluted with elution buffer (2 × 100 µL) and then lyophilized by SpeedVac. After that, the peptides were re-dissolved in solvent A (A: 0.1% formic acid in water) and then analyzed by Q-Exactive Plus coupled with an EASY-nanoLC 1200 system (Thermo Fisher Scientific, MA, USA. In brief, a 3 µL peptide sample was loaded onto a 25 cm analytical column (75 µm inner diameter, 1.9 µm resin, Dr Maisch), and separated with a 60 min gradient starting at 2% buffer B (80% acetonitrile with 0.1% formic acid), followed by a stepwise increase to 30% in 47 min and 100% in 1 min, remaining at the same temperature for 12 min. The column flow rate was maintained at 300 nL/min with a column temperature of 40 °C. The electrospray voltage was set to 2 kV. The mass spectrometer was run under data-dependent acquisition (DDA) mode, and automatically switched between MS and MS/MS mode. The survey of full-scan MS spectra (350-1800 m/z) was acquired in the Orbitrap with 70,000 resolutions, with the automatic gain control (AGC) target of 3e6 and the maximum injection time of 50 ms. Then, the precursor ions were selected for insertion into the collision cell for fragmentation by higher-energy collision dissociation (HCD), and the normalized collection energy was 28. The MS/MS resolution was set at 17,500, with the automatic gain control (AGC) target of 1e5, maximum injection time of 45 ms, and dynamic exclusion of 30 s.

2.10. Proteomic analysis

Tandem mass spectra were processed by PEAKS Studio version 10.6 (Bioinformatics Solutions Inc., Waterloo, Canada). The database was Pseudomonas sp. THAF187a (version 2023,4811entries), downloaded from UniProt. Trypsin was set as the digestion enzyme, and semi-specific was specified as the digestion type. PEAKS DB was searched with a fragment ion mass tolerance of 0.02 Da and a parent ion tolerance of 10 ppm. The max missed cleavage was 2. Cysteine carbamidomethylation was specified as the fixed modification. Oxidation on methionine, deamination on asparagine and glutamine, and acetylation on protein N-term were specified as the variable modifications. The peptides with 1% FDR, and the proteins with 1% FDR and containing at least 1 unique peptide were filtered.

2.11. Statistical analysis

All data were expressed as the means ± S.E.M. (standard error of the mean). Student's t-test was used to determine the significance of differences between two groups, P-values of less than 0.05 were considered to be statistically significant.

3. Results

3.1. *P. mendocina* identified by matrix-assisted flight mass spectrometry

Through the matrix-assisted laser-desorption ionization time-of-flight mass spectrometry and its own supporting software, the detected ion peak (intensity) was used as the vertical axis and the ion mass charge ratio (m/z) was used as the horizontal axis. The baseline of the isolated bacterial protein fingerprint was stable, and the main protein peak was obvious, which showed good and reliable results. After being cultured for 16 and 24 h, the main ion intensities of the isolated bacteria were around 2578, 4128, 5157, 6010, and 6030 (Figure 1A, B). The isolated

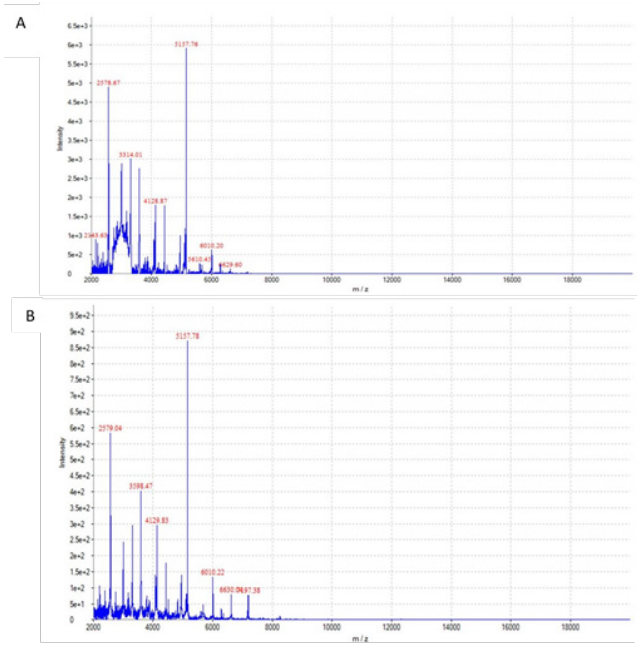

Fig. 1. Flight mass spectrometry identification of isolated strains at different growth times. The colony flight mass spectrometry identification results of the strain after 16 h (A) and 24 h (B) of cultivation.

bacterial fingerprint is shown in Figure 1. Through comparison with the standard spectrum diagram in the supporting software (M-Discover 100 Excellence), we confirmed that the isolated bacteria were identified as *P. mendocina*.

3.2. 16S rDNA sequencing of *P. mendocina*, and establishment of a phylogenetic tree

The purified amplification product of the 16S rDNA gene has an expected size of approximately 1500bp and exhibits good specificity, with only one specific DNA fragment appearing (Figure 2A). According to the 16S rDNA sequencing results and comparison with the NCBI database, the isolate was confirmed as *P. mendocina*. We analyzed the molecular evolution of *P. mendocina* isolated from aquaculture water, and it was observed to have a high homology with *P. mendocina* in the gene database, as shown in Figure 2B.

3.3. Biochemical identification results of *P. mendocina*

Biochemical identification experiments showed that the reactions of ADH, MTE, LAC, MNE, FRU, CIT, MLT, ONPG, and ACE were all positive, whereas those of GLUf, H_2S, ODC, LDC, C, URE, ESC, GEL, NIT, IND, GLU, MAN, SAC, MAL, and XYL were all negative (Table 1).

3.4. Analysis of antibiotic resistance in *P. mendocina*

Antibiotic sensitivity experiments showed that *P. mendocina* isolated from aquaculture water was sensitive to most of the antibiotics (ceftazidime, gentamicin, tobramycin, levofloxacin, ciprofloxacin, and cefepime, etc.), but its resistance to chloramphenicol (CHL), minocycline (MIN), and ticarcillin/clavulanic acid (TIC/CLA) was intermediate. This indicates that *P. mendocina* is still sensitive to most antibiotics (Table 2).

3.5. Growth trend of *P. mendocina*

We evaluated the growth of *P. mendocina* with different dilutions after 90 hours. According to the results of

Table 1. Biochemical identification of *P. mendocina*.

Item	Name	Abbreviation	Results
1	Anaerobic glucose fermentation	GLUf	Negative
2	Hydrogen sulfide production	H2S	Negative
3	Ornithine decarboxylase	ODC	Negative
4	Arginine dihydrolase	ADH	Positive
5	Lysine decarboxylase	LDC	Negative
6	Amino acid control	C	Negative
7	Urease	URE	Negative
8	Aescin hydrolysis	ESC	Negative
9	Gelatin hydrolysis	GEL	Negative
10	Nitrate reduction	NIT	Negative
11	Production of indole	IND	Negative
12	Malic acid utilization	MTE	Positive
13	Acid production of aerobic glucose	GLU	Negative
14	Acid production of mannitol	MAN	Negative
15	Acid production of sucrose	SAC	Negative
16	Acid production of lactose	LAC	Positive
17	Acid production of mannose	MNE	Positive
18	Acid production of maltose	MAL	Negative
19	Acid production of fructose	FRU	Positive
20	Acid production of xylose	XYL	Negative
21	Citrate utilization	CIT	Positive
22	Malonic acid salt utilization	MLT	Positive
23	Galactosidase	ONPG	Positive
24	Acetamide	ACE	Positive

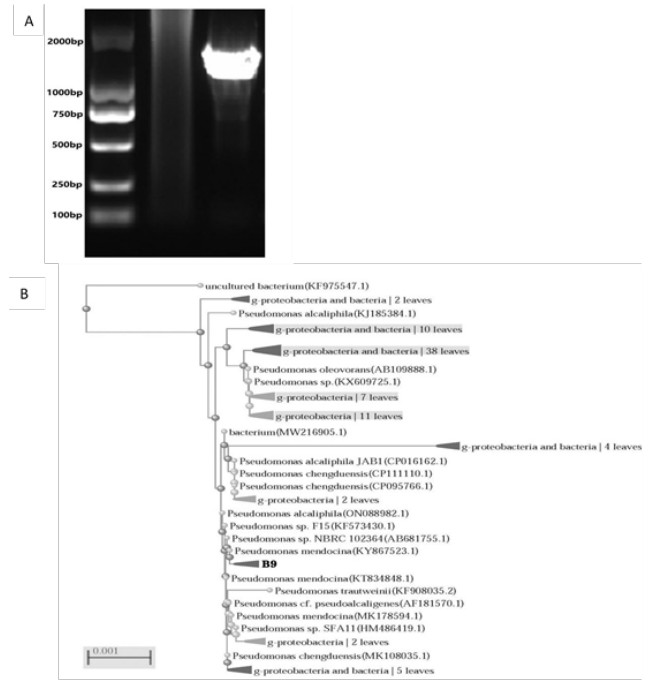

Fig. 2. 16S rDNA sequencing. Molecular biology identification of *P. mendocina* from aquaculture water and establishment of a phylogenetic tree. A: The 16S primers of bacteria were used to amplify the genes of the colony, and the amplified products were displayed by gel electrophoresis. Lane 1: DNA marker; lane 2: blank control; and lane 3: the isolated strain. B: The phylogenetic tree based on 16S rDNA gene sequences of *P. mendocina*. B9 represents the strain that we isolated from aquaculture water.

Figure 3, it was found that, within 0-20 hours of culture, the growth of *P. mendocina* first gradually increased and then decreased, and dropped to a lower point at around 20 h. After 20 h of culture, the growth of *P. mendocina* continued to increase, reached a peak at about 22 hours after cultivation, and then gradually decreased. However, there are differences in the time points at which *P. mendocina* reached its peak with different dilutions of bacterial solution. The time to reach its peak with a small dilution of bacterial solution was longer than that with a large dilution of bacterial solution. However, the OD value at the peak with the smallest dilution of bacterial solution was lower than other dilutions of bacterial solution. The higher the OD value, the more bacteria there are. It was found that the highest OD value was obtained after a dilution of bacterial solution with 20 µL, while the lowest OD value was obtained after a dilution of bacterial solution with 1 µL (Table 3).

3.6 Drug-resistance-related gene detection test

Using the bacterial suspension, extracted bacterial genomes, and blank plasmid as the templates, PCR amplification was carried out using the primers of *AAC (3) - II*, *cmlA*, *CTX-M-l*, *gyrA*, *gyrB*, *blaKPC*, *NDM-1*, *oqxA*, *oqxB*, *OXA*, *parC*, *qepA*, *qnrA*, *qnrB*, *qnrC*, *qnrD*, *qnrS*, and *Sul2*. After that, the PCR production was visualized by gel electrophoresis. It was found only *gyrB* was specifically expressed in the bacteria and genomic, which showed the presence of a *gyrB* resistance gene in the isolated strain of *P. mendocina* (Figures 4 and 5).

3.7. Proteomics analysis

Table 2. Antibiotic sensitivity experiment of *P. mendocina*.

Item	Drug Name	Abbreviation	Group	MIC Value	Results
1	Ceftazidime	CAZ	A	≤1	Susceptible
2	Gentamicin	GEN	A	≤2	Susceptible
3	Tobramycin	TOB	A	≤1	Susceptible
4	Levofloxacin	LEV	B	≤2	Susceptible
5	Ciprofloxacin	CIP	B	≤1	Susceptible
6	Cefepime	FEP	B	≤2	Susceptible
7	Imipenem	IPM	B	≤1	Susceptible
8	Aztreonam	ATM	B	=8	Susceptible
9	Piperacillin/tazobactam	P/T	B	≤4/4	Susceptible
10	Sulfamethoxazole/trimethoprim	SMZ/TMP	B	≤2/38	Susceptible
11	Amikacin	AMK	B	≤4	Susceptible
12	MeropeneM	MRP	B	≤1	Susceptible
13	Cefotaxime	CTX	C	=8	Susceptible
14	Ceftriaxone	CRO	C	=4	Susceptible
15	Chloramphenicol	CHL	C	=32	Resistant
16	Cefoperazone/sulbactam	CEF/SUL	I	≤4/2	Susceptible
17	Polymyxin E	CT	I	≤2	-
18	Polymyxin B	PB	I	≤2	-
19	Ampicillin/sulbactam	AMP/SUL	I	=32/16	-
20	Minocycline	MIN	O	=8	Intermediate
21	Piperacillin	PIP	O	≤8	Susceptible
22	Ticarcillin/clavulanic acid	TIC/CLA	O	=32/2	Intermediate
23	Doxycycline	DOX	O	≤4	Susceptible
24	Tetracycline	TET	U	≤4	Susceptible

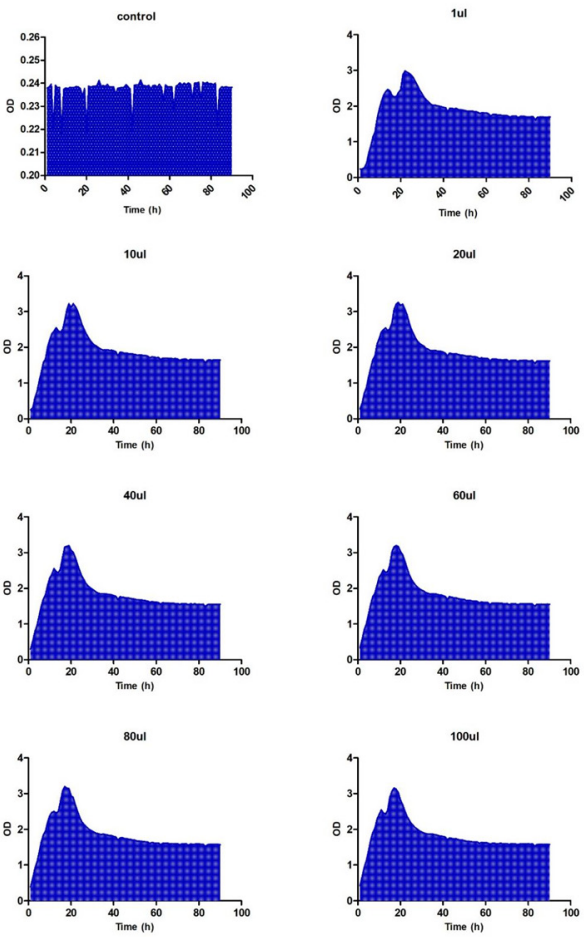

Fig. 3. Growth mode of *P. mendocina*. The grow period of 90 h was evaluated the growth of *P. mendocina* under different dilutions of bacterial suspension (control, 1 µL, 10 µL, 20 µL, 40 µL, 60 µL, 80 µL, and 100 µL).

In consideration of the limitations of gene amplification, we further screened the possible drug-resistance-related proteins by protein detection and database scanning alignment. In order to further screen possible drug-resistant components, we found more than 1700 proteins of *P. mendocina* through proteomics analysis. After screening, we found four proteins related to drug resistance (mdtA2, mdtA3, mdaB, and emrK1), as shown in Table 4.

4. Discussion

Microplastics can absorb substances and act as carriers for the transportation of pollutants in aquatic systems due to their high stability, mobility in water and hydrophobicity, allowing for them to transfer and accumulate at the trophic level [16]. Microplastic pollution can seriously affect the water and product quality of freshwater farms. *P. mendocina* was found to be a strain that produces a medium-chain-length PHA (mcl-PHA) isolated from farmland soil,

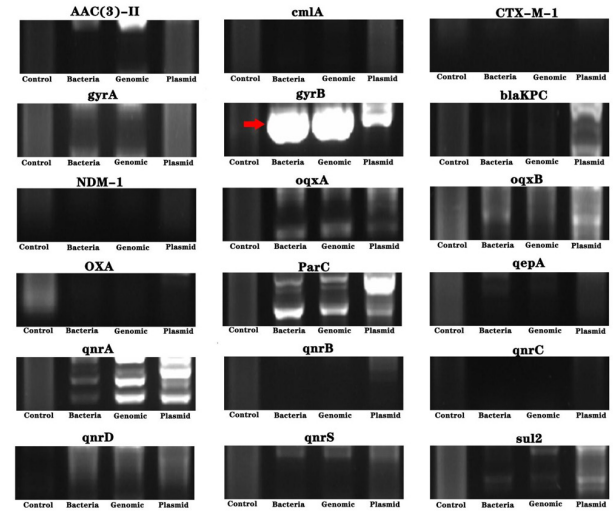

Fig. 4. Analysis of drug-resistance genes of *P. mendocina* in aquaculture water. PCR amplification was carried out using different primers, and electrophoresis bands were sequenced. The nucleic acid electrophoresis images of the resistance genes of *AAC(3)-II*, *cmlA*, *CTX-M-1*, *gyrA*, *gyrB*, *blaKPC*, *NDM-1*, *oqxA*, *oqxB*, *OXA*, *ParC*, *qepA* , *qnrA*, *qnrB*, *qnrC*, *qnrD*, *qnrS*, and *sul2*. Red arrow indicated *gyrB* gene.

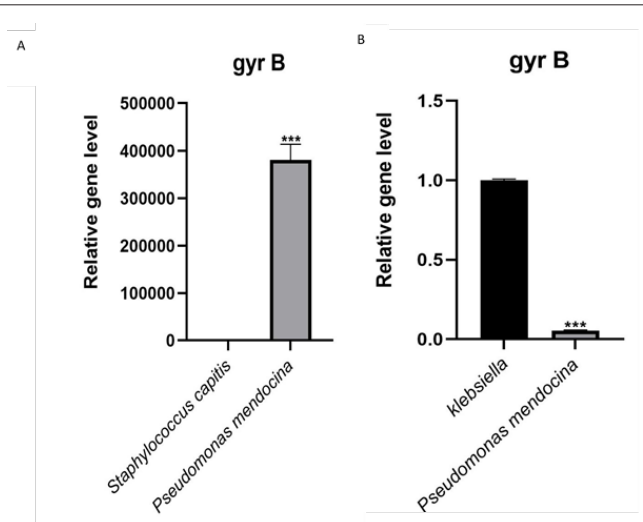

Fig. 5. The analysis of gyrB gene in *P. mendocina*. Realtime PCR technology analyzed the levels of gyrB gene in three types of bacteria. A: The gyrB level of *Staphylococcus capitis* and *Pseudomonas mendocina.* B: The gyrB level of *klebsiella* and Pseudomonas mendocina.

Table 3. The growth peak point of *P. mendocina* with different concentrations.

Test group	Time (h)	Peak point (OD)
P. mendocina-1 µL	22	2.99
P. mendocina-10 µL	19	3.22
P. mendocina-20 µL	19	3.26
P. mendocina-40 µL	19	3.2
P. mendocina-60 µL	18	3.2
P. mendocina-80 µL	17	3.2
P. mendocina-100 µL	17	3.16

Table 4. Proteomics screened out proteins related to drug resistance.

Item	Proteomics analysis
1	Multidrug-resistance protein MdtA (mdtA2)
2	Multidrug-resistance protein MdtA (mdtA3)
3	Modulator of drug activity B (mdaB)
4	Putative multidrug-resistance protein EmrK (emrK1)

and biodegrades plastics [17], but it has not been investigated in the water of a freshwater aquaculture farm. Therefore, our study successfully isolated a strain of *P. mendocina* from the water samples of a freshwater farm, and it was found that *P. mendocina* was positive for reactions of ADH, MTE, LAC, MNE, FRU, CIT, MLT, ONPG, and ACE, and was sensitive to most antibiotics. The growth experiment showed that the *P. mendocina* strain had a good growth rate in nutrient broth. After detecting the drug-resistance-related genes and proteomics, *gyrB* was the resistance gene, while mdtA2, mdtA3, mdaB, and emrK1 were closely related to the drug resistance of *P. mendocina. P. mendocina* is a new type of aerobic bacteria, which has the advantages of assimilation and dissimilation while simultaneously efficiently removing nitrate [18]. A previous study isolated a strain of *P. mendocina* S16 from freshwater aquaculture ponds and showed significant heterotrophic nitrification-aerobic denitrification abilities, which indicated the potential application of the strain in aquaculture tail water treatment [19]. Another study isolated two *P. mendocina* strains from a healthy Anas platyrhynchos fecal sample (Ps542) and lettuce (Ps799), and whole genome analysis showed that both strains lacked antimicrobial-resistance genes, but the Ps799 genome showed a MOBP3 family relaxase; however, it possessed an important number of virulence factors, including flagella, pili, leukotoxin, and the Type 2 and Type 6 Secretion Systems, which could be responsible for their pathogenesis [20]. In the present research, *P. mendocina* was successfully isolated from the water of a freshwater aquaculture farm, and its biochemical properties were further explored. In our study, we found that *P. mendocina* was positive to reactions of ADH, MTE, LAC, MNE, FRU, CIT, MLT, ONPG, and ACE. Arginine is a semi-essential amino acid and participates in various metabolic processes and pathways [21]. Arginine dihydrolase (ADH) can convert arginine to ornithine, accompanied by the release of carbon dioxide and ammonia, as well as playing a crucial role in nitrogen storage and remobilization [22]. Malic acid (MTE) is a dicarboxylic acid mainly used in the food industry and can be produced by microbial fermentation oxidation/reduction TCA and glyoxylic acid pathways, as well as from fossil resources [23]. By shaker fermentation, lactose is effectively converted to lactose acid (LAC), which is widely used in the pharmaceutical, food, nanotechnology and chemical industries [24]. Cork hydrolysate contains a high mannose content, and it has been reported that two strains can effectively convert mannose to L- and D-lactic acid isomers (MNE), thus providing important knowledge for the production of chiral lactic acid from lignocellulosic feedstock using the spectrum of fermentable sugars in cork [25]. Hyperuricemia is characterized by the excessive production and deposition of urate crystals, and the acid produced by fructose metabolism (FRU) is strongly associated with an increased risk of developing hyperuricemia [26]. Understanding the mechanism of citric acid utilization (CIT) helps in the selection of conditions that promote the formation of citric acid flavor [27]. Acetam (ACE) is primarily used as a stabilizer and plasticizer, and can also be used in organic synthesis, the pharmaceutical industry, and dye preparation [28]. Malonic acid salt (MLT) can be used as the main carbon source for bacterial growth. Galactosidase (ONPG) is mainly divided into α-galactosidase (α-ONPG) and β-galactosidase (β-ONPG), which has great potential for application in the food and pharmaceutical industries [29, 30]. In addition, our study observed that *P. mendocina* was sensitive to most antibiotics, with intermediate levels of resistance to CHL, MIN and TIC/CLA. Taken together, we hypothesize that *P. mendocina* isolated from water in a freshwater farm may participate in ADH, MTE, CIT, MLT, ONPG and ACE processes, and have good sensitivity to most antibiotics.

After that, *P. mendocina* was found to have a good growth rate in nutrient broth medium and then was used for the measurement of drug-resistance-related genes and proteins. We observed that *gyrB* was the resistance gene, and mdtA2, mdtA3, mdaB, and emrK1 were closely related to the drug resistance of *P. mendocina.* The *gyrB* gene is a powerful molecular marker that effectively distinguishes the most closely related genera of *Myxococcus*, *Pyxidicoccus*, and *Corallococcus* [31]. Feng et al. reported that *gyrB* mutation plays an important role in in vitro screenings for fluoroquinolones resistance in *Pseudomonas aeruginosa*. The multidrug transporter ABC (mdtABC), formerly known as yegMNO, genes, which encode a resistance-nodulation-cell division (RND) drug efflux system, are responsible for resistance [32]. A previous study showed that the mdtABC system comprised the transmembrane mdtB/mdtC heteromultimer and mdtA membrane fusion protein [33]. mdtAC also confers bile salt, but not novobiocin resistance, which indicates that the evolution from an mdtC homomultimer to an mdtBC heteromultimer contributed to extending the drug resistance spectrum [34]. Wang et al. found *P. mendocina,* a novel variant with a plasmid-carried tigecycline-resistance gene cluster, tmexCD-toprJ, located on the chromosome of environmental-origin P. mendocina [35]. Further research used proteomic analysis to explore the mechanism of the resistance of *Enterococcus faecalis* isolates to linezolid and found that the OptrA, Sea1, TraB and RepA proteins were involved in the resistance of *E. faecalis* to linezolid [36]. Combined with our results, it can be inferred that *P. mendocina* could grow well in nutrient broth medium, and the *gyrB* gene, and mdtA2, mdtA3, mdaB, and emrK1 proteins may have a close connection with the drug resistance of *P. mendocina*. From our recent research, we can find that *P. oleovorans* and *P. mendocina* have some similar results. For example, the growth mode of bacteria is very familiar and the detection of drug-resistance genes can also seen that these two bacteria have the same gene expression profiling. Proteomics found that *P. oleovorans* has 1781 proteins and *P. mendocina* has 1745 proteins, but the two are different in mass spectrometry, 16S genes, and several biochemical drug resistance indicators. Considering the close genetic relationship between these two bacteria and the limited selection of genes, it is understandable that there are some similarities, but the two bacteria bring us a very good topic to further study the evolution and function of *Pseudomonas oleovorans* and *Pseudomonas mendocina* [37].

5. Conclusion

P. mendocina was successfully isolated from water in a freshwater aquaculture farm and was found to be positive to reactions of ADH, MTE, LAC, MNE, FRU, CIT, MLT, ONPG, and ACE, and have good sensitivity to most antibiotics. In addition, *gyrB* was found to be the resistance gene, while mdtA2, mdtA3, mdaB, and emrK1 may be the drug-resistance proteins in *P. mendocina*. However, the potential roles of *P. mendocina* in the biodegradation of plastics and the management of freshwater aquaculture farms need to be further explored. These findings provide preliminary knowledge regarding the next step in the biological removal of plastics or microplastics in freshwater aquaculture farms.

Conflicts of Interest

The authors declare no conflicts of interest.

Consent for publications

The author read and approved the final manuscript for publication.

Ethics approval and consent to participate

No human or animals were used in the present research.

Informed Consent

The authors declare that no patients were used in this study.

Availability of data and material

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Authors' contributions

Methodology, Kai Yan, Shuai Wei, and Haifeng Wang; editing, Kai Yan and Shuai Wei; conceptualization and review, Haifeng Wang.

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