Aquaculture 563 (2023) 738975

Contents lists available at ScienceDirect

Aquaculture

journal homepage: www.elsevier.com/locate/aquaculture

# An opportunistic pathogenic *Pseudomonas* isolated from healthy brown alga *Saccharina japonica*

Qin Yang [a,b], Yingrui Zhuang [a,b], Mahasweta Saha [c], Qiying Qiu [d], Defu Chen [d], Lirong Chang [e], Luyang Xiao [e], Gaoge Wang [a,b,*]

[a] College of Marine Life Sciences, Ocean University of China, Qingdao 266003, China
[b] Institute of Evolution & Marine Biodiversity, Ocean University of China, Qingdao 266003, China
[c] Marine Ecology and Biodiversity, Plymouth Marine Laboratory, Prospect Place, PL1 3DH, Plymouth, United Kingdom
[d] Fujian Lianjiang Guanwu Seafood Developing Product Co., Ltd., Guanwu 350511, China
[e] Weihai Changqing Ocean Science & Technology Co., Ltd, Rongcheng 264316, China

ARTICLE INFO

*Keywords:*
Disease
Opportunistic pathogenic bacteria
*Saccharina japonica*
*Pseudomonas*

ABSTRACT

Commercially farmed *Saccharina japonica* suffers from various diseases during its cultivation. Isolation and identification of the causative agents are crucial for the diagnosis and control of the diseases. In this study, an opportunistic pathogenic bacterium was isolated from healthy matured *S. japonica* and was identified as *Pseudomonas aeruginosa* MSLj6–25. The pathogenicity of *P. aeruginosa* MSLj6–25 was tested by the infection assay. Microscopic observations indicated that *P. aeruginosa* MSLj6–25 can cause green-rotten disease. Ultrastructural changes showed that the chloroplasts and the nucleus became condensed at 7 h after infection, while at 24 h, the chloroplasts and the nucleus were deformed. Colonies of *P. aeruginosa* MSLj6–25 were found to be flat and bright yellow. The bacterial cells were rod shaped with a single polar flagellum. MSLj6–25 had the highest similarity of 99.93% with *Pseudomonas aeruginosa* strain CZ-45 by comparing 16S rRNA gene sequences. Combining biochemical characterization, we identified the taxonomic classification of MSLj6–25. To the best of our knowledge, this is the first time that *P. aeruginosa* was identified as an opportunistic pathogen in *S. japonica*. These results contribute to the baseline knowledge of pathogenic bacteria in *S. japonica* and lay the foundation for disease diagnosis and control.

## 1. Introduction

Seaweeds are increasingly paid more attention nowadays because of their application as food source and biofuels (Gross, 2008; Gachon et al., 2010; Radulovich et al., 2015; Ward et al., 2020) However, like the land crops, seaweeds suffer from various microbial diseases which are caused by pathogens, including fungi, bacteria, and viruses, especially under the conditions of global climate change (Gachon et al., 2010; Egan et al., 2014; Wang et al., 2014; Zhang et al., 2020; Zhang et al., 2022).

It is well-accepted that most of the causative agents of seaweeds are opportunistic pathogenic bacteria, which has been identified in both farmed and wild seaweeds (Chen et al., 1979, 1981; Case et al., 2011; Egan et al., 2014, Egan and Gardiner, 2016; Wang et al., 2014; Kumar et al., 2016). Opportunistic pathogenic bacteria are those which are present on healthy seaweeds, but can become pathogenic when the environment deteriorates (Chen et al., 1979, 1984; Ding, 1990; Egan et al., 2013, 2014; Wang et al., 2014) or when the hosts are compromised (Egan et al., 2013; Zozaya-Valdes et al., 2015; Kumar et al., 2016). Since the successful cultivation on large scale in 1958, *Saccharina japonica* continues to be threatened by various diseases at both nursery and field cultivation stages, such as green rot disease (Tseng, 1962, 1985; Chen et al., 1979, 1981; Ahmad et al., 2021), hole-rotten disease (Wang et al., 2008), and falling off disease (Ding, 1992; Wang et al., 2014). Chinese researchers have made efforts to identify the pathogenic bacteria for various diseases of farmed *S. japonica* (Chen et al., 1979, 1984; Ding, 1990; Wang, 2003, Wang et al., 2014; Zhang et al., 2022) since 1979. They propose that alginic acid-decomposing bacteria are the pathogenic bacteria for most of the diseases. Further, they found that alginic acid-decomposing bacteria are present on the healthy *S. japonica*, but can become pathogenic bacteria whenever the cultivation environment turns stressful, such as the increased light intensity (Xiang, 2001), the elevated temperature (Chen et al., 1984; Ding, 1990; Xiang, 2001)

* Corresponding author at: College of Marine Life Sciences, Ocean University of China, Qingdao 266003, China.
*E-mail address:* wgaoge@ouc.edu.cn (G. Wang).

https://doi.org/10.1016/j.aquaculture.2022.738975
Received 14 April 2022; Received in revised form 4 September 2022; Accepted 19 October 2022
Available online 8 November 2022
0044-8486/© 2022 Elsevier B.V. All rights reserved.

and the salinity change etc. (Peng and Li, 2013; Wang et al., 2014). Here, alginic acid-decomposing bacteria are not a taxonomic name, but refer to all those bacteria with the ability to degrade alginate, the main component of the cell wall of *S. japonica* (Chen et al., 1979, 1981). Most of the alginic acid-decomposing bacteria belong to the genus *Pseudomonas* (*Pseudomonas* sp. A-2) (Chen et al., 1981; Liu et al., 2002), *Micrococcus* (*Micrococcus* sp.) (Wu, 1990), *Pseudoalteromonas, Vibrio* (*Vibrio* sp. SO-20) (Chen et al., 1979), *Halomonas* (Wang et al., 2008) and *Alteromonas* (*Alteromonas* sp. A-1) (Peng and Li, 2013). So far, isolation and identification of pathogenic bacteria from commercially farmed *S. japonica* has been improved from designating as alginic acid-decomposing bacteria to species level because of the of advances of genomic sequencing technologies (Ahmad et al., 2021; Zhang et al., 2022).

So far, the opportunistic pathogenic bacteria for the bleaching disease in the wild seaweed *Delisea pulchra* have been well studied. It was found that the outbreaks of *D. pulchra* bleaching disease depend on the elevated temperature and the compromised defenses of *D. pulchra* (Case et al., 2011). The bleaching disease frequently occurs in the natural populations of *D. pulchra* in summer time when the sea water temperature reaches at its peak and the chemical defenses of *D. pulchra* are decreased (Case et al., 2011; Fernandes et al., 2011, 2012; Zozaya-Valdes et al., 2015). These results indicate that bleaching disease may be caused by the opportunistic pathogenic bacteria. Further, the bacterial strains *Phaeobacter* sp. LSS9 (LSS9) and *Ruegeria* sp. R11 (R11), which were isolated from healthy *D. pulchra* (Fernandes et al., 2011), were identified to cause bleaching disease in the laboratory (Case et al., 2011; Fernandes et al., 2012). Moreover, R11 only infected *D. pulchra* when the temperature was elevated or the chemical defenses of *D. pulchra* were compromised (Case et al., 2011). LSS9 only induced bleaching disease in the defense-deficient *D. pulchra* (Fernandes et al., 2011). Thus, both LSS9 and R11 were the opportunistic pathogenic bacteria for the bleaching disease of *D. pulchra* (Case et al., 2011; Fernandes et al., 2011). In the meanwhile, because LSS9 and R11 were not abundant on the bleached *D. pulchra*, Fernandes et al. (2012) proposed that there were some other bacterial taxa able to induce the bleaching disease. Then, *Alteromonas* sp. BL110, *Aquimarina* sp. AD1, *Aquimarina* sp. BL5, and *Agarivorans* sp. BL7 on the healthy *D. pulchra* (Fernandes et al., 2012; Zozaya-Valdes et al., 2015; Kumar et al., 2016) were isolated and identified to cause bleaching diseased symptoms on *D. pulchra* under the lab conditions (Kumar et al., 2016). Therefore, these were also the opportunistic pathogenic bacteria for *D. pulchra* bleaching disease (Kumar et al., 2016).

Considering that there exist opportunistic pathogenic bacteria on healthy *S. japonica*, in this study, we tried to firstly isolate bacterial strains from healthy matured sporophytes of *S. japonica* with the traditional culture-dependent method; next, to test the pathogenicity of the isolated bacterial strains by infection assays and observe the diseased symptoms via microscopic observations and transmission electron microscope; then, to investigate the growth and morphological characteristics of the opportunistic pathogenic bacteria; finally, to identify the isolated opportunistic pathogenic bacteria using the biochemical characterization test combining with the sequence comparison of 16S rRNA genes. Our results will not only pave the way for further investigation of virulence mechanisms of the pathogenic bacteria, but also contribute to develop disease management and to mitigate disease outbreaks during the cultivation of *S. japonica*.

## 2. Material and methods

### 2.1. Sampling healthy matured S. japonica

Three healthy matured *S. japonica* individuals were collected on 30th June 2019 from the sea off Dinghai (26°16′29.19″N, 119°47′06.59″E, Fujian Lianjiang Guanwu Seafood Developing Product Co., Ltd., Guanwu, 350,511, Fujian province, China). They were transported to the laboratory within 24 h in a cooler box.

### 2.2. Isolation and culture method of epibacteria

Epiphytic bacterial samples were collected by swabbing the surface of *S. japonica* using sterile cotton swabs and then the swabs were suspended in 5 ml of sterile filtered sea water. After that the suspended samples were diluted with tenfold dilution plate method and then spread over modified ZoBell 2216E marine agar medium (5.0 g of Tryptone, Thermo Fisher Scientific, USA; 1.0 g of yeast extract, Thermo Fisher Scientific, USA; 0.1 g of ferric phosphate, Shanghai Macklin Biochemical Co., Ltd., China). 100 μl of each dilution was plated with three replications for each dilution. The plates were incubated at 25 °C for 24 h (GXZ incubator, Ningbo Jiangnan Instrument Company, China). Bacterial samples on the plates were selected according to the colony morphology, such as shape and pigment. Selected strains were purified by three-time-streaking on the modified ZoBell 2216E marine agar medium. Bacteria were grown at 25 °C, 120 rpm (ZQTY-70E Shaker, Shanghai Zhichu Instrument Company, China) in freshly made modified ZoBell 2216E marine broth medium for 24–48 h, or on freshly made modified ZoBell 2216E marine agar plates and were cultured at 25 °C (ZQTY-70E Shaker, Shanghai Zhichu Instrument Company, China). Finally, purified bacterial strains were stored at −81 °C (DW-86 L626 Ultra low temperature freezer, Haier Bio-Medical, China) with 15% (*v*/v) glycerol for further study.

### 2.3. Infection assay

Conserved bacterial strains at −81 °C were re-activated before the infection assay. The bacterial strains were firstly spread on modified ZoBell 2216E marine agar plates and were cultured at 25 °C (ZQTY-70E Shaker, Shanghai Zhichu Instrument Company, China). After single colonies were formed, they were inoculated into 50 ml of freshly made modified ZoBell 2216E marine broth medium, and were grown at 25 °C, 120 rpm (ZQTY-70E Shaker, Shanghai Zhichu Instrument Company, China) for 24–48 h. Then the bacterial cultures were diluted (1:1000) and transferred into new modified ZoBell 2216E marine broth medium and was cultured until the $OD_{600}$ reached 0.5–0.6 (2.5–3.0 × $10^9$ CFU/ml) (TU-1810 Spectrophotometer, PuXi Company, Beijing, China).

Healthy juvenile sporophytes (about 20–30 cm, Fig. 1 A) of *S. japonica* were collected from Weihai Changqing Ocean Science & Technology Co., Ltd. (122°35′56.13″N, 37°12′36.49″E), Rongcheng, Shandong province, China on 8th, 16th, and 17th November 2021. *S. japonica* samples were transported to the laboratory in a cooler box within 4 h and were rinsed by sterilized seawater for at least three times to remove the loosely attached epiphytes (Saha et al., 2016). *S. japonica* tissue sections of 1 $cm^2$ were made as described previously (Zhang et al., 2022), and then were randomly and individually put into 24-well plates (LABSELECT, China). The infection group contained 1 ml of bacterial culture in each cell, while control group contained 1 ml of fresh modified ZoBell 2216E marine broth medium. The 24-well plates (LABSELECT, China) were incubated at 10 °C for 24 h with 12:12 h (light: dark) (GXZ incubator, Ningbo Jiangnan Instrument Company, China) as described by Zhang et al. (2022). Disease symptoms of both groups were monitored, and proportions of healthy replicates were recorded. Microscopic images were recorded at 0 h, 7 h, and 24 h under Nikon microscope (ECLIPSE Ni—U, Nikon, Japan). For the infection assay, three independent experiments were conducted and for each experiment there were 30 tissue sections (independent replicates) individual replicates in both control and infection groups, respectively.

### 2.4. Observations of ultrastructural changes of the infected S. japonica by transmission electron microscope (TEM)

Samples for TEM were processed the same as previously described (Zhang et al., 2022). Tissue sections of both control and infected groups

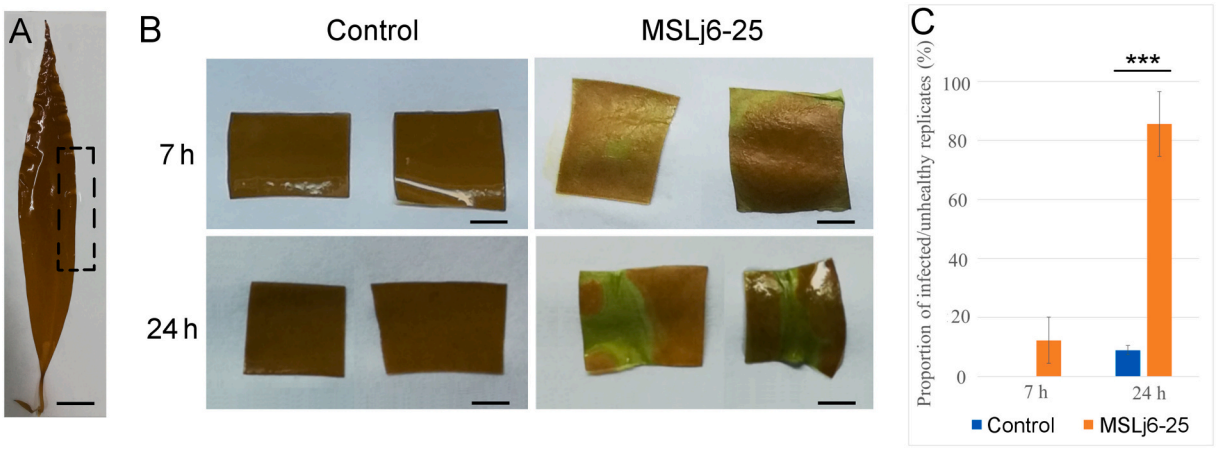


**Fig. 1.** Infection assay by MSLj6–25.
A. Image of representative juvenile sporophytes of *S. japonica*. The box highlighted the area used for the infection assay. B. Representative images of control group and MSLj6–25 infected group in the infection assay at 7 h and 24 h, respectively. C. Proportion of the diseased and unhealthy replicates (diseased or unhealthy samples from the whole samples) in the infected and the control groups. One-way ANOVA statistical analysis applied, *** represent $P < 0.001$. Bars: A = 2.5 cm; B = 0.35 cm.

were collected at 7 h and 24 h after infection and then were fixed in 2.5% glutaraldehyde (Sigma-Aldrich, USA) for 4 h, respectively. Tissue samples were washed three times with 0.1 M PBS (phosphate buffer saline) buffer (Solarbio Life Sciences, USA) for 15 min. One percent osmium tetroxide ($OsO_4$) (provided by the electron microscopic center of Qingdao Medical College) was used to fix the samples for 1.5 h. And then the samples were rinsed again with 0.1 M PBS buffer (Solarbio Life Sciences, USA) and dehydrated using ethanol (Guoyao Chemical Reagent Co., Ltd., China) for 15 min. The samples were embedded into spur's epoxy resin (provided by the electron microscopic center of Qingdao Medical College) and polymerized at 72 °C overnight and were sliced into ultrathin sections. The ultrathin sections were stained with uranyl acetate (provided by the electron microscopic center of Qingdao Medical College) and lead citrate for 15 min and were observed with JEM-1200EX TEM microscope (JEOL, Japan).

### 2.5. Conduction of the growth curve and $OD_{600}$-CFU (Colony formation unit) correlation curve

The growth curve was conducted based on the measurement of $OD_{600}$ using a UV spectrophotometer (TU-1810, PuXi Company, Beijing, China). The activated bacteria ($OD_{600}$ = 1.037) were inoculated (*v*/v = 1:1000) to freshly made modified ZoBell 2216E marine broth medium and were incubated at 25 °C with rotation at 120 rpm (ZQTY-70E Shaker, Shanghai Zhichu Instrument Company, China). The $OD_{600}$ was measured about every 4 h until the growth reached the stationary phase.

The bacterial culture with different $OD_{600}$ values was diluted in 10 gradient and were spread on ZoBell 2216E marine agar plates. The plates were incubated at 25 °C (GXZ incubator, Ningbo Jiangnan Instrument Company, China) for 2–4 days to obtain the CFU values. Finally, the CFU values (Y-axis) were corresponded to the OD600 values (X axis) to conduct the correlation curve.

### 2.6. Morphological characterization of pathogenic bacteria

The colony shape and pigmentation of the pathogenic bacteria were observed visually on modified ZoBell 2216E marine agar plates. In addition to obtain the cell surface as well as the cell structure characteristics of the pathogenic bacteria, negative staining TEM and ultrathin section TEM were conducted.

To prepare the negative staining samples, the bacteria were activated as described above (section 2.3). Then 2 ml of the bacterial culture was centrifuged at 3000 rpm for 2 min (Centrifuge 5418, Eppendorf, Germany). The bacterial pellet was resuspended and then was fixed in 3% glutaraldehyde (Sigma-Aldrich, USA) for 10 min. A drop of the fixed sample was loaded on a carbon coated copper mesh (provided by the Electron Microscopic Center, Qingdao Medical College) and dried at room temperature for 5 min. Further, the bacterial samples were negatively stained with 1% phosphotungstic acid (Sigma-Aldrich, USA) for 3 min and dried at room temperature for 15 min. Finally, the samples were observed under JEM-1200EX TEM microscope (JEOL, Japan).

The preparation of ultrathin section TEM was the same as mentioned above (section 2.4).

### 2.7. Biochemical and physiological characterizations of bacterial strains by biolog gen $_{III}$ MicroPlate system

Biochemical and physiological characterizations of the pathogenic bacteria were analyzed with the Biolog Gen $_{III}$ MocroPlate (Biolog, Hayward, CA, USA). The activated bacteria were cultured on modified ZoBell 2216E marine agar plates at 25 °C (GXZ incubator, Ningbo Jiangnan Instrument Company, China) for 24 h, and the bacterial colonies were transferred into the Inoculation Fluid A (Biolog, Hayward, CA, USA). The bacterial suspensions were evenly resuspended, and the transmittance was measured by a turbidity meter (Biolog, Hayward, CA, USA) to reach 95–96%. Subsequently, 100 μl of the bacterial suspension was inoculated into each well of the Biolog Gen $_{III}$ MocroPlate device (Biolog, Hayward, CA, USA) and incubated at 25 °C for 24 h. The results were measured at $OD_{590}$ (Synergy LX, BioTek, USA) and analyzed according to the manufacturer's protocol.

### 2.8. Molecular identification and phylogenic analysis

Molecular identification of the pathogenic bacterial strain was achieved using the 16S rRNA gene sequencing method (3730XL DNA Analyzer, Applied Biosystems, USA). The total DNA was extracted with the DNA extraction kit (SK8255, Sangon Biotech (Shanghai) Co., Ltd., China) following the manufacturer's protocol. Concentration of the extracted DNA was measured with Nanodrop 2000 (Thermo Fisher Scientific, USA), and the DNA sample was conserved at −20 °C (Deep freezer, Haier Bio-Medical, China) for further use. PCR amplification of 16S rRNA gene of the pathogenic bacteria were carried out using the universal primers (27F: AGAGTTTGATCMTGGCTCAG; 1492R: TACGGYTACCTTGTTACGACTT) (Solarbio Life Sciences, USA) (Weisburg et al., 1991) in 50 μl high-fidelity PCR systems, containing 25 μl of polymerase mix (I-5 High-Fidelity Master Mix, MCLAB, USA), 2 μl of each

10 μM-primer (Solarbio Life Sciences, USA), 50 ng of gDNA as template, and 20 μl of $ddH_2O$. The PCR procedure was started with an initial denaturation at 98 °C for 2 min; and following 35 cycles of denaturizing at 98 °C for 10 s, annealing at 62 °C for 15 s, extending at 72 °C for 30 s; and was finally ended at 72 °C for 5 min (Life Express Thermal Cycler TC-96G/H(b), Hangzhou Bioer Technology Co., Ltd., China). The PCR products were electrophoresed through 1% agarose gel under 80 V for 1 h, and screened with GelRed (BS354B, Biosharp Company, China) DNA dye in the Gel-imaging system (Tanon-2500, Tanon, China) under 312 nm UV light. The 16S rRNA gene amplicons were purified with a DNA gel extraction kit (TSP601–200, TsingKe Biological Technology, China). The purified DNA fragments were sequenced with sanger method (3730XL DNA Analyzer, Applied Biosystems, USA), by Sangon Biotech Co., Ltd., Shanghai, China. Forward and reverse reads were assembled with BioEdit version 7.2.5. The 16S rRNA gene sequence of the pathogenic bacterial strain was deposited in the National Centre for Biotechnology Information (NCBI) database.

Phylogenic analysis of the pathogenic bacterial strain was obtained as described below. The 16S rRNA gene sequence of the pathogenic bacterial strain was compared against the NT database in the NCBI (nucleotide BLAST search) (https://www.ncbi.nlm.nih.gov/) and in EzBioCloud (http://www. ezbiocloud. net) (Khalifa and Bekhet, 2018). The percent identity/pairwise similarity values of the pathogenic strain and its closely related bacterial strains were calculated. The 16S rRNA gene sequences of related bacterial strains were retrieved from the NCBI and EzBioCloud database and were aligned by using the CLUSTAL X (Larkin et al., 2007). Phylogenetic trees were reconstructed based on the neighbor-joining (NJ) (Saitou and Nei, 1987) using mega software (Kumar et al., 2018).

## 3. Results

### 3.1. Pathogenicity test of the isolated bacterial strains

A total of 90 isolated bacterial strains were isolated in this study. There were 33 bacterial strains exhibited pathogenicity after infection assay. Among these strains, MSLj6–25 had a strong pathogenicity which could lead to green-rotten disease symptoms in the juvenile sporophytes of *S. japonica*. The healthy tissues were brown (Fig. 1 A, B), while the infected tissue of juvenile sporophytes exhibited green and rotten disease symptoms (Fig. 1 B). The green and rotten disease symptoms were obvious and consistent among the infected replicates. The proportion of the diseased tissue sections (meaning diseased tissue samples from the whole samples) were 12.22 ± 7.85% and 85.55 ± 11.00% (difference significant, $P = 0.0006$, from one-way ANOVA statistical analysis) at 7 h and at 24 h after being infected (Fig. 1 C), respectively. There were a few tissue sections that became unhealthy in the control group (symptoms only existed at 24 h). The proportion of unhealthy *S. japonica* (meaning unhealthy tissue samples from the whole samples) in the control group was only 8.89 ± 1.57% (Fig. 1 C). The symptoms of the unhealthy tissue sections in the control group (a little bleaching or slightly green within a small area at the edge of the tissue section) were significantly different to those in the MSLj6–25 infected group. Therefore, the greenish disease symptoms in the infection group were caused by bacterium MSLj6–25.

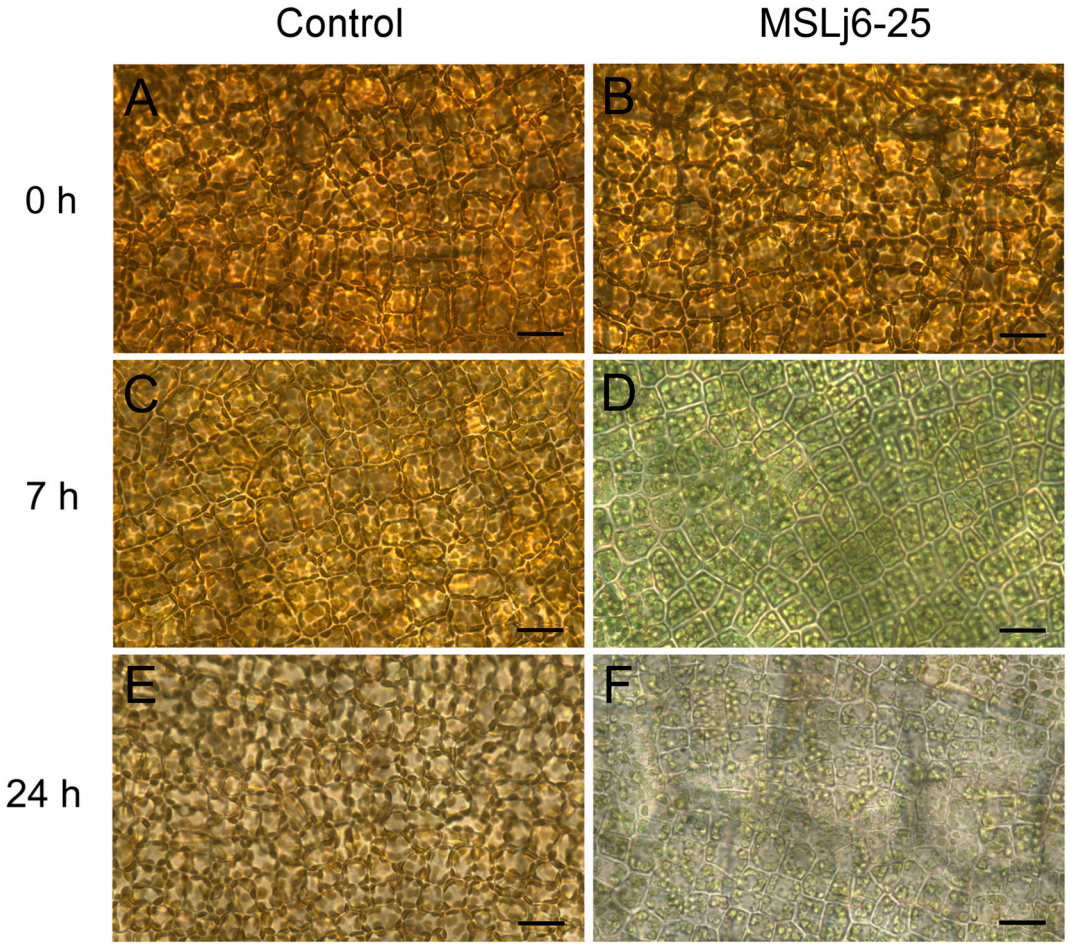


**Fig. 2.** Microscopic observation after infected by MSLj6–25 in *S. japonica*.
A, C, E. Representative images of microscopic observation in control group at 0 h, 7 h, and 24 h respectively. B, D, F. Microscopic images of MSLj6–25 infected groups at 0 h, 7 h, and 24 h respectively. Bars = 20 μm.

### 3.2. Microscopic observation and ultrastructural changes caused by MSLj6–25

When compared to the *S. japonica* tissues which were brown in the control group (Fig. 2 A – C, E), MSLj6–25 infected tissues lost pigmentation (turned obvious greenish) at 7 h (Fig. 2 D). The chloroplasts lost their pigmentation (turned green) and gathered around the cell wall. At 24 h, the loss of pigmentation became even worse and the color of the tissues became whitish. In the infected cells, the amount of the chloroplasts reduced, the chloroplasts lost pigmentation but still gathered around the cell wall (Fig. 2 F).

TEM observations were conducted at 0 h, 7 h, and 24 h after infected by MSLj6–25, respectively (Fig. 3). The normal ultrastructure in the control group was shown in Fig. 3 A – D. The chloroplasts were in rod shapes with integrated membranes, The nuclear membranes were clear, and the nucleoles were visual. The heterochromatins were evenly distributed in the nucleus. The mitochondria and cell walls were integrated. The ultrastructure of the infected cells changed after infected by MSLj6–25. At 7 h after the infection, the chloroplasts were lightly concentrated, and the nucleus became condensed. Nuclear membranes became deeply stained and the nucleoles and heterochromatins disappeared (Fig. 3 E). But the mitochondria and cell wall were integrated (Fig. 3 E). At 24 h after the infection, the chloroplasts were deformed (Fig. 3 F) and the nucleus were completely degraded (Fig. 3 F). There were some bacterial cells on the outside of the cell wall, which were degraded (Fig. 3 F). Mitochondria kept intact during the infection process.

### 3.3. Growth characteristics of MSLj6–25

The $OD_{600}$-CFU correlation curve (Fig. 4 A) and the growth curve (Fig. 4 B) of MSLj6–25 were conducted respectively. The correlation curve showed good linear trend ($R^2 = 0.99$). For example, according to the correlation curve, the calculated CFU of MSLj6–25 ($OD_{600} = 1.037$) was $5.2 \times 10^9$ CFU/ml, while the measured value was $5.6 \times 10^9$ CFU/ml.

The growth of MSLj6–25 in modified ZoBell 2216E marine broth medium was monitored for 71 h in this study (Fig. 4 B). The activated bacteria ($OD_{600} = 1.037$) were inoculated ($v/v = 1{:}1000$) to freshly made modified ZoBell 2216E marine broth medium and it grew slowly during the first 8 h. The growth rate was lower than $3.10 \times 10^7$ CFU/(ml•h). But the MSLj6–25 started to grow rapidly from 10 h to 41 h, meaning it reached its logarithmic phase (Fig. 4 B). The highest growth rate was $3.02 \times 10^8$ CFU/(ml•h), occurring at 16 h (Fig. 4 B). Subsequently, MSLj6–25 turned to its stationary phase after 41 h, during which the bacterial biomass had small fluctuations (Fig. 4 B). The $OD_{600}$ of the stationary phase was around 1.10, which is equal to about $5.5 \times 10^9$ CFU/ml (Fig. 4 B).

### 3.4. Morphological characterization of MSLj6–25

Colonies of MSLj6–25 on ZoBell 2216E marine agar were bright yellow (Fig. 5A). The visible colonies were normally formed after a 2-day-incubation at 25 °C (Fig. 5 A). The colonies were flat with round shapes and smooth surfaces, and some of them had lightly concave edges or/and convex centers on the agar plates (Fig. 5A).

Negative staining TEM indicated that the cell of MSLj6–25 was in a rod shape with a single polar flagellum (Fig. 5 B). The bacterial cells were about 1.9–2.3 μm (mean = 2.1 ± 0.1 μm, $p < 0.0001$) in length and about 0.4–0.5 μm (mean = 0.5 ± 0.1 μm, $p < 0.0001$) in width. The length of flagella was about 3.0–3.5 μm (mean = 3.2 ± 0.1 μm, $p < 0.0001$). Ultrathin section of TEM confirmed that the cells of MSLj6–25 had rod-like shapes (Fig. 5 C).

### 3.5. Biochemical characterization of MSLj6–25

The biochemical characterization of MSLj6–25 was examined with the Biolog Gen III MicroPlate system. The bacterium MSLj6–25 positively reacted with 19 carbon sources and had borderline reactions with another 19 carbon sources among the 71 in the carbon source utilization assays (Table 1). It could metabolize a comparatively wide range of carbohydrates, such as sucrose, D-trehalose, α-D-glucose, D-mannose, D-galactose etc. In addition, MSLj6–25 metabolized a number of amino acids like L-serine, L-aspartic acid, L- and D-glutamic acid. The chemical

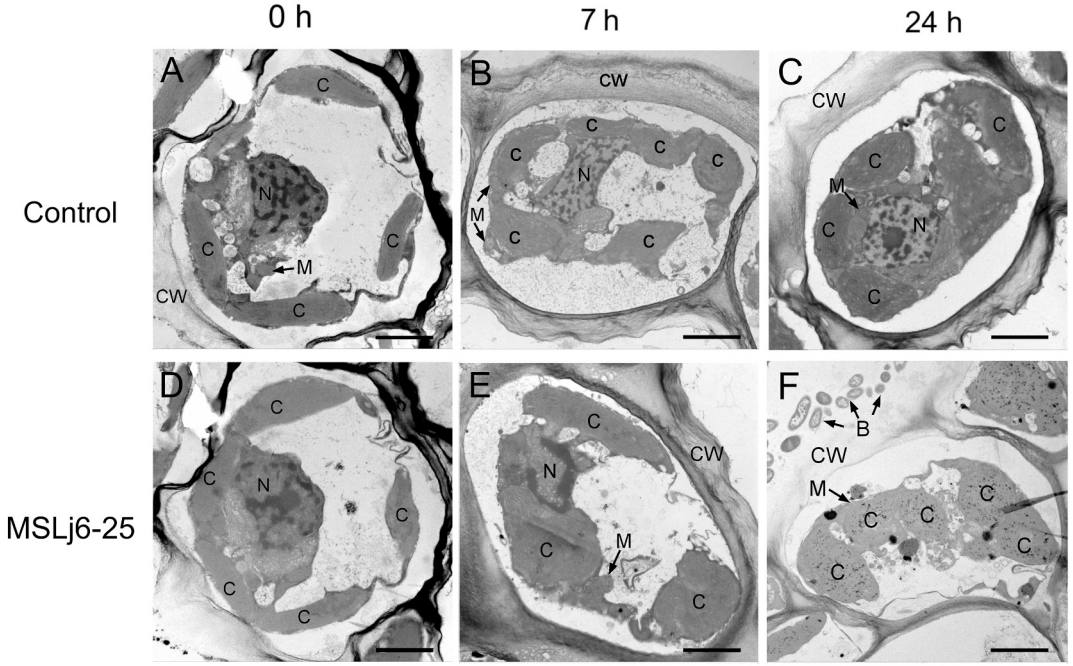


**Fig. 3.** Ultrastructural changes after infected by MSLj6–25 in *S. japonica*.
A – C. TEM images of *S. japonica* in control group at 0 h, 7 h, and 24 h respectively. D – F. TEM images of *S. japonica* infected by MSLj6–25 at 0 h, 7 h, and 24 h respectively. B: bacteria; C: chloroplast; CW: cell wall; M: mitochondria; N: nucleus. Bars: A, C, D = 2 μm; B, E = 2.5 μm; F = 3.5 μm.

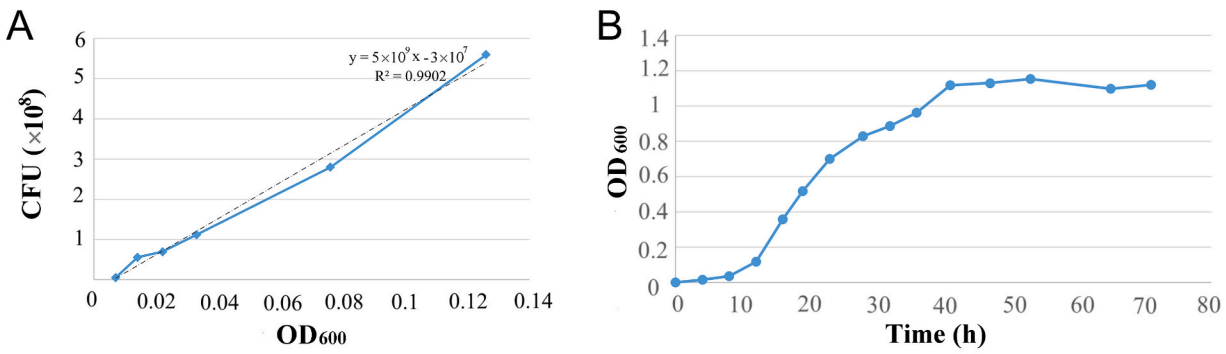


**Fig. 4.** The $OD_{600}$-CFU correlation curve and the growth curve of MSLj6–25.
A. The $OD_{600}$-CFU correlation curve of MSLj6–25 growing in modified ZoBell 2216E marine broth medium, $R^2 = 0.99$. B. The growth curve of MSLj6–25 in modified ZoBell 2216E marine broth medium at 25 °C.

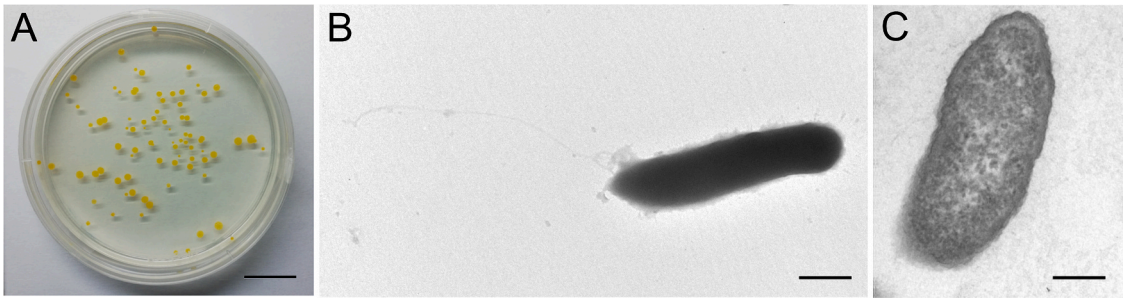


**Fig. 5.** Morphology of MSLj6–25.
A. Image of MSLj6–25 colonies on modified ZoBell 2216E marine agar plates. B. A negative staining TEM image of a cell of MSLj6–25. C. An ultrathin section TEM image of the MSLj6–25 cell. Bars: A = 2.0 cm; B = 0.5 μm; C = 0.25 μm.

**Table 1**
Biochemical characterization of MSLj6–25 via Biolog Gen III MicroPlate.

| | Carbon source utilization assays | | Chemical sensitivity assays |
|---|---|---|---|
| Positive | Sucrose<br>D-Trehalose<br>α-D-Glucose<br>D-Mannose<br>D-Galactose<br>L-Serine<br>Mucic Acid<br>Quinic Acid,<br>Citric Acid | D-Malic Acid,<br>L-Malic Acid,<br>L-Aspartic Acid,<br>L-Glutamic Acid,<br>D-Gluconic Acid,<br>D-Saccharic Acid,<br>D-Glucuronic Acid,<br>D-Galacturonic Acid,<br>α-Keto-Glutaric Acid,<br>L-Galactonic Acid Lactone, | PH 6,<br>1% NaCl,<br>Niaproof 4,<br>Lincomycin,<br>Vancomycin,<br>Rifamycin SV,<br>Troleandomycin,<br>Tetrazolium Blue<br>Tetrazolium Violet,<br>1% Sodium Lactate, |
| Total | 19 | | 10 |
| Moderate | Glyceerol,<br>D-Fucose,<br>L-Fucose,<br>Tween 40,<br>D-Sorbitol,<br>Acetic Acid,<br>L-Alanine,<br>Formic Acid,<br>D-Arabitol, | D-Fructose,<br>D-Mannitol,<br>L-Rhamnose,<br>myo-Inositol,<br>L-Lactic Acid,<br>Methyl Pyruvate,<br>Bromo-Succinic Acid<br>L-Pyroglutamic Acid<br><br>γ-Amino-Butryric Acid,<br>β-Hydroxy-D,L-Butyric Acid, | Lithium Chloride |
| Total | 19 | | 1 |

sensitivity assays indicated that MSLj6–25 exhibited 10 positive reactions and 1 borderline reaction out of the 23 inhibitory chemicals (Table 1). It can grow under the condition of subacidity (pH 6.0) and hypotonicity, but cannot grow in acid condition (pH 5.0) and hypertonic conditions (NaCl ≥4%).

### 3.6. Molecular identification and phylogenetic analysis

The 16S rRNA gene sequence (1443 bp) of MSLj6–25 was uploaded to NCBI GeneBank with the accession number of ON075453. After comparing the 16S rRNA gene sequence against the database in both NCBI and in EzBioCloud, it displayed the highest identity of 99.93% with *Pseudomonas aeruginosa* strain CZ-45. The phylogenetic analysis was performed according to the 16S rRNA gene sequences of other related species (Fig. 6). MSLj6–25 was clearly clustered with the *Pseudomonas aeruginosa* strain CZ-45 in the phylogenetic tree (Fig. 6).

Based on the morphology, the chemical characterization, the full length of 16S rRNA gene and the phylogenetic analysis, the isolated pathogenic bacterial strain MSLj6–25 was designated as *Pseudomonas aeruginosa* MSLj6–25.

## 4. Discussion

In this study, an opportunistic pathogenic bacterial strain was isolated from healthy mature sporophytes of *S. japonica* and was identified as *Pseudomonas aeruginosa* MSLj6–25 by combining culture-dependent and molecular identification methods. *P. aeruginosa* MSLj6–25 can infect healthy juvenile *S. japonica* and cause visual green disease symptoms within 24 h in laboratory conditions. The ultrastructural changes of the diseased *S. japonica* cells indicated that chloroplasts were the most sensitive organelle to the infection of *P. aeruginosa* MSLj6–25. To the best of our knowledge, this is the first study identifying an opportunistic pathogenic bacterium of *P. aeruginosa* from farmed healthy *S. japonica*.

So far, opportunistic pathogenic bacteria have been identified in

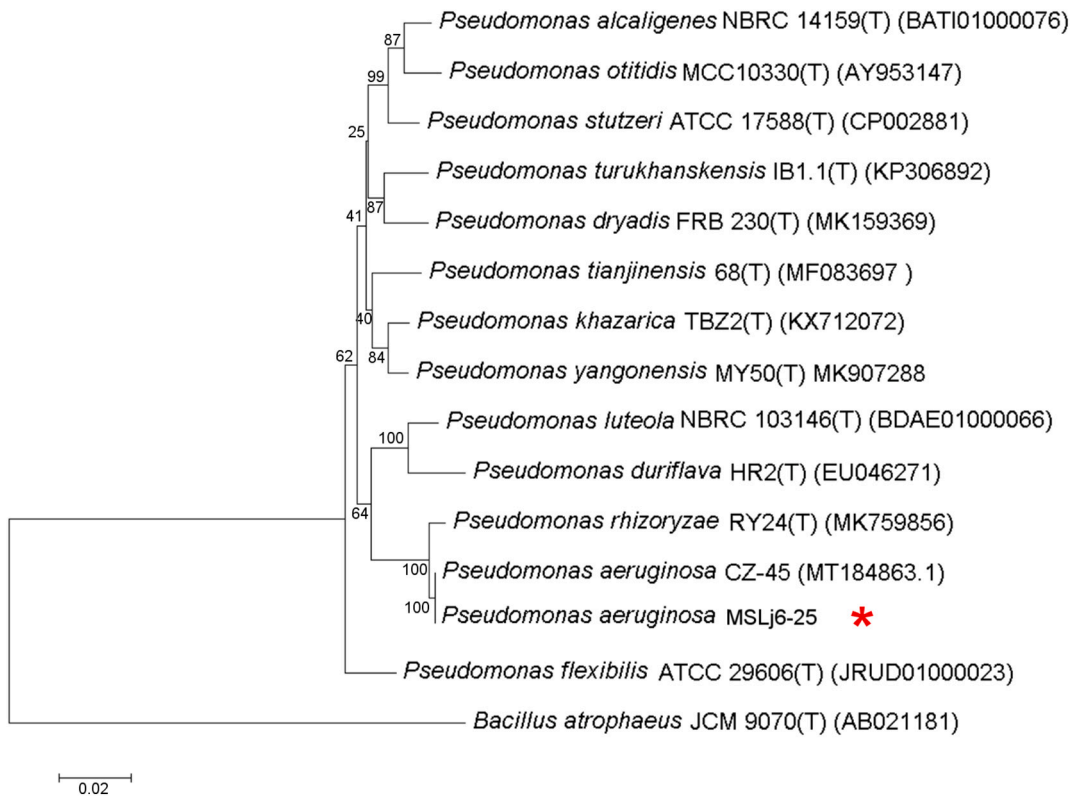


**Fig. 6.** Neighbor-joining phylogenetic tree based on the full length of 16S rRNA gene sequences.
Percentage bootstrap values above 50% (1500 replicates) are shown at branch nodes. *Bacillus atrophaeus* JCM 9070 $^{T}$ belonging to family Bacillaceae was used as an out group. The other strains belong to family Pseudomonadaceae. The asterisk points out *P. aeruginosa* MSLj6–25. Bar = 0.02.

both farmed and wild seaweeds (see reviews by Egan et al., 2013, Wang et al., 2014). However, although alginic acid-decomposing bacteria are supposed to be the opportunistic pathogenic bacteria for various diseases of farmed *S. japonica* (Chen et al., 1979, 1981; Ding, 1992; Wang et al., 2008, 2014; Ahmad et al., 2021), none of them were identified to species. On the other hand, identification of opportunistic pathogenic bacteria has been well studied in the wild red seaweed *D. pulchra,* such as *Aquimarina* sp. AD1, *Aquimarina* sp. BL5, and *Agarivorans* sp. BL7 (Case et al., 2011; Kumar et al., 2016; Li et al., 2022). The difference in opportunistic pathogenic bacterial species between *D. pulchra* and *S. japonica* may result from the different seaweed hosts.

Due to the high salinity of the ocean, it is supposed that *P. aeruginosa* could not grow in seawater and thus cannot be a marine bacterium (Botzenhart and Döring, 1993). However, it has been reported that very few strains of this microorganism may grow in the seawater near sewage outfalls or polluted river outlet (Botzenhart and Rueden, 1987; Cheung et al., 1991). Therefore, we speculate that the *P. aeruginosa* MSLj6–25 in *S. japonica* probably originates from the cultivating seawater polluted by human activities. Usually, *S. japonica* farms are located quite near the seashore, thus could be easily affected by anthropogenic pollution.

So far, *P. aeruginosa* is well known for its opportunistic pathogenicity in immunocompromised patients in hospital (Yeung et al., 2009; Gellatly and Hancock, 2013; Acebrón-García-de-Eulate et al., 2022). *P. aeruginosa* was also found to be a pathogen in plants (Elamin et al., 2017; Schroth et al., 2018; Zhang et al., 2021). For example, *P. aeruginosa* can cause soft rot disease in *Arabidopsis thaliana* (Djonović et al., 2013) and basal stem rot disease in *Solanum lycopersicum* (Zhang et al., 2021), respectively. Nevertheless, *P. aeruginosa* was rarely reported to cause diseases in seaweeds to date.

The identification of pathogens is of great importance to diagnose and to prevent the outbreaks of algal diseases. Currently the culture-independent methods such as 16S rRNA gene amplicon sequencing, the traditional culture-dependent approaches and infection assays are applied to identify pathogens (Egan et al., 2014; Kumar et al., 2016; Li et al., 2020; Zhang et al., 2020; Ahmad et al., 2021). However, culturing methods cannot adequately reflect the true microbial diversity, because only a very small proportion (about 1%) of the epiphytic bacteria might be isolated with the traditional culture-dependent method (Kumar et al., 2016; Ahmad et al., 2021). The dominant bacteria identified in the culture-independent analysis are difficult to be isolated and identified by the culture-dependent methods. For example, in the farmed seaweed *S. japonica,* the composition, diversity, and temporal dynamics of epiphytic bacterial communities associated with both healthy and green rotten disease tissue have been analyzed using the 16S rRNA gene amplicon sequencing method. The results indicated that the bacteria of the genera *Granulosicoccus, Litorimonas, Tenacibaculum, Blastopirellula,* and *Sulfitobacter* were enriched in diseased tissues (Zhang et al., 2020; Li et al., 2020). However, these bacteria were rarely isolated from *S. japonica* by the culture-dependent method. There are two main reasons that lead to such a difficulty on isolation of pathogenic bacteria using culture-dependent method. First of all, most of the pathogenic bacteria are unculturable (Zhang et al., 2020; Wang et al., 2021), which definitely increases the inconvenience to isolate opportunistic pathogenic bacteria. Secondly, even though a few proportion of pathogenic bacteria were successfully isolated and cultured in laboratory, many of them may lose their virulence under laboratory conditions (Wang et al., 2014). Therefore, innovative methods, such as investigation of new bacterial isolation medium, need to be developed to isolate the culture-independent-method-identified dominant bacterial strains associated with diseased algal hosts, and identify their pathogenicity.

Our study is significant in two ways. Firstly, it helps to develop a technique for predicting diseases and diagnosis by monitoring the relative abundance of *P. aeruginosa* during the cultivation of *S. japonica.* An increase in the relative abundance of *P. aeruginosa,* will lead to an

outbreak of the disease. Thus, monitoring the relative abundance of *P. aeruginosa* can be a useful tool that can be employed by the nurseries. Secondly, our work will help to establish *P. aeruginosa–S. japonica* as an infection model, which may encourage the investigation of virulence mechanisms. Understanding the virulence mechanisms and the virulence factors will help with disease management, which in turn will effectively prevent or mitigate disease outbreaks in the farmed *S. japonica*.

## 5. Conclusions

We isolated and identified a marine *P. aeruginosa* strain as an opportunistic pathogen in the commercially farmed brown seaweed *S. japonica*. To the best of our knowledge, this is the first time that *P. aeruginosa* was found to cause disease in *S. japonica*, although under laboratory conditions. Our results indicate that opportunistic pathogenic bacteria can also be present on healthy *S. japonica*. We highly advocate development of new culture-dependent approaches for pathogenic bacteria isolation, for example, the innovation of isolation medium and cultural technique, to isolate and identify the dominant bacteria from the culture-independent analysis. Under this condition, the culture-dependent and -independent methods could be best combined and lead to a more efficient identification of pathogenic bacteria in the near future.

## CRediT authorship contribution statement

**Qin Yang:** Methodology, Investigation, Formal analysis, Writing – original draft, Funding acquisition. **Yingrui Zhuang:** Investigation. **Mahasweta Saha:** Writing – review & editing. **Qiying Qiu:** Resources. **Defu Chen:** Resources. **Lirong Chang:** Resources. **Luyang Xiao:** Resources. **Gaoge Wang:** Conceptualization, Resources, Writing – review & editing, Supervision, Funding acquisition.

## Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

## Data availability

Data will be made available on request.

## Acknowledgement

This study was sponsored by National Key R & D Program of China (2018YFD0900305); the National Natural Science Foundation of China (42076106; 41576158); Sino-German Center for Research Promotion (GZ1357) and the Qingdao Postdoctoral Foundation for Application Research.

## References

Acebrón-García-de-Eulate, M., Mayol-Llinàs, J., Holland, M.T.O., Kim, S.Y., Brown, K.P., Marchetti, C., et al., 2022. Discovery of novel inhibitors of uridine diphosphate-N-acetylenolpyruvylglucosamine reductase (MurB) from *Pseudomonas aeruginosa*, an opportunistic infectious agent causing death in cystic fibrosis patients. J. Med. Chem. 65, 2149–2173. https://doi.org/10.1021/acs.jmedchem.1c01684.

Ahmad, R., Chen, Y., Zhuang, Y., Qiu, Q., Chen, D., Saha, M., et al., 2021. Isolation and identification of a pathogenic bacterium, *Exiguobacterium oxidotolerans* XP-2, from the abnormal diseased mature sporophytes of a commercially cultivated brown seaweed *Saccharina japonica*. J. Appl. Phycol. 33, 3239–3249. https://doi.org/10.1007/s10811-021-02499-9.

Botzenhart, K., Döring, G., 1993. Ecology and epidemiology of *Pseudomonas aeruginosa*. In: Campa, M., Bendinelli, M., Friedman, H. (Eds.), *Pseudomonas aeruginosa* as an opportunistic pathogen. Springer US, Boston, MA, pp. 1–18. https://doi.org/10.1007/978-1-4615-3036-7_1.

Botzenhart, K., Rueden, H., 1987. Hospital infections caused by *Pseudomonas aeruginosa*. Antibiot. Chemother. 1–15. https://doi.org/10.1159/000414328.

Case, R.J., Longford, S.R., Campbell, A.H., Low, A., Tujula, N., Steinberg, P.D., et al., 2011. Temperature induced bacterial virulence and bleaching disease in a chemically defended marine macroalga. Environ. Microbiol. 13, 529–537. https://doi.org/10.1111/j.1462-2920.2010.02356.x.

Chen, D., Lin, G., Shen, S., 1979. Studies on alginic acid decomposing bacteria I. action of alginic acid decomposing bacteria and alginase on *Laminaria japonica*. Oceanol. Limnol. Sin. Deep Sea Res. B 10, 329–333 (In Chinese with English abstract).

Chen, D., Lin, G., Shen, S., 1981. Studies on alginic acid decomposing bacteria II. Rot disease of *Laminaria* summer sporelings caused by alginic acid decomposing bacteria. Oceanol. Limnol. Sin. 12, 133–137 (In Chinese with English abstract).

Chen, D., Liu, X.Y., Liu, X.Z., Yu, Y.D., Yang, Z.H., Qiu, S.H., 1984. Studies on alginic acid-de-composing bacteria, III. The cause of the rot disease and detaching of *Laminaria* sporophytes in sporeling culture stations and their preventive measures. Oceanol. Limnol. Sinica 15, 581–589 (In Chinese with English abstract).

Cheung, W.H.S., Chang, K.C.K., Hung, R.P.S., 1991. Variations in microbial indicator densities in beach waters and health-related assessment of bathing water quality. Epidemiol. Infect. 106, 329–344. https://doi.org/10.1017/S0950268800048482.

Ding, M., 1990. Effect of environmental factors on alginic acid decomposing bacteria leading to disease and decay of kelp. Acta Oceanol. Sin. 12 (2), 224–229 (In Chinese with English abstract).

Ding, M., 1992. The effects of the environmental factors on *Laminaria* disease caused by alginic acid decomposing bacteria. Acta Oceanol. Sin. 11, 123–130 doi: CNKI:SUN:SEAE.0.1992-01-010.

Djonović, S., Urbach, J.M., Drenkard, E., Bush, J., Feinbaum, R., Ausubel, J.L., et al., 2013. Trehalose biosynthesis promotes *Pseudomonas aeruginosa* pathogenicity in plants. PLoS Pathog. 9, e1003217 https://doi.org/10.1371/journal.ppat.1003217.

Egan, S., Gardiner, M., 2016. Microbial dysbiosis: rethinking disease in marine ecosystems. Front. Microbiol. 7, 1–8. https://doi.org/10.3389/fmicb.2016.00991.

Egan, S., Harder, T., Burke, C., Steinberg, P., Kjelleberg, S., Thomas, T., 2013. The seaweed holobiont: understanding seaweed–bacteria interactions. *FEMS Microbiol.* Rev. 37, 462–476. https://doi.org/10.1111/1574-6976.12011.

Egan, S., Fernandes, N.D., Kumar, V., Gardiner, M., Thomas, T., 2014. Bacterial pathogens, virulence mechanism and host defense in marine macroalgae. Environ. Microbiol. 16, 925–938. https://doi.org/10.1111/1462-2920.12288.

Elamin, A.A., Steinicke, S., Oehlmann, W., Braun, Y., Wanas, H., Shuralev, E.A., et al., 2017. Novel drug targets in cell wall biosynthesis exploited by gene disruption in *Pseudomonas aeruginosa*. PLoS One 12, e0186801. https://doi.org/10.1371/journal.pone.0186801.

Fernandes, N., Case, R.J., Longford, S.R., Seyedsayamdost, M.R., Steinberg, P.D., Kjelleberg, S., et al., 2011. Genomes and virulence factors of novel bacterial pathogens causing bleaching disease in the marine red alga *Delisea pulchra*. PLoS One 6. https://doi.org/10.1371/journal.pone.0027387.

Fernandes, N., Steinberg, P., Rusch, D., Kjelleberg, S., Thomas, T., 2012. Community structure and functional gene profile of Bacteria on healthy and diseased thalli of the red seaweed *Delisea pulchra*. PLoS One 7, e50854. https://doi.org/10.1371/journal.pone.0050854.

Gachon, C.M.M., Sime-Ngando, T., Strittmatter, M., Chambouvet, A., Kim, G.H., 2010. Algal diseases: spotlight on a black box. Trends Plant Sci. 15, 633–640. https://doi.org/10.1016/j.tplants.2010.08.005.

Gellatly, S.L., Hancock, R.E.W., 2013. *Pseudomonas aeruginosa:* new insights into pathogenesis and host defenses. Pathog. Dis. 67, 159–173. https://doi.org/10.1111/2049-632X.12033.

Gross, M., 2008. Algal biofuel hopes. Curr. Biol. 18, R46–R47. https://doi.org/10.1016/j.cub.2007.12.034.

Khalifa, A.Y.Z., Bekhet, G., 2018. First isolation and characterization of the pathogenic *Aeromonas veronii* bv. *Veronii* associated with ulcerative syndrome in the indigenous *Pelophylax ridibundus* of Al-Ahsaa, Saudi Arabia. Microb. Pathog. 117, 361–368. https://doi.org/10.1016/j.micpath.2017.10.019.

Kumar, S., Stecher, G., Li, M., Knyaz, C., Tamura, K., 2018. MEGA X: molecular evolutionary genetics analysis across computing platforms. *Mol. Biol.* Evol. 35, 1547–1549. https://doi.org/10.1093/molbev/msy096.

Kumar, V., Zozaya-Valdes, E., Kjelleberg, S., Thomas, T., Egan, S., 2016. Multiple opportunistic pathogens can cause a bleaching disease in the red seaweed *Delisea pulchra*. Environ. Microbiol. 18, 3962–3975. https://doi.org/10.1111/1462-2920.13403.

Larkin, M.A., Blackshields, G., Brown, N.P., Chenna, R., McGettigan, P.A., McWilliam, H., et al., 2007. Clustal W and Clustal X version 2.0. Bioinformatics 23, 2947–2948. https://doi.org/10.1093/bioinformatics/btm404.

Li, J., Pang, S., Shan, T., Su, L., 2020. Changes of microbial community structures associated with seedlings of *Saccharina japonica* at early stage of outbreak of green rotten disease. J. Appl. Phycol. 32, 1323–1327. https://doi.org/10.1007/s10811-019-01975-7.

Li, J., Majzoub, M.E., Marzinelli, E.M., Dai, Z., Thomas, T., Egan, S., 2022. Bacterial controlled mitigation of dysbiosis in a seaweed disease. ISME J. 16, 378–387. https://doi.org/10.1038/s41396-021-01070-1.

Liu, C.S., Wang, L.L., Wang, M., Tang, X.X., 2002. Difference analysis of infection activity of alginic acid decomposing bacteria infecting *Laminaria japonica*. Mar. Sci. 26, 44–47 (In Chinese with English abstract).

Peng, Y., Li, W., 2013. A bacterial pathogen infecting gametophytes of *Saccharina japonica (Laminariales, Phaeophyceae)*. Chin. J. Oceanol. Limnol. 31, 366–373. https://doi.org/10.1007/s00343-013-2136-9.

Radulovich, R., Neori, A., Valderrama, D., Reddy, C.R.K., Cronin, H., Forster, J., 2015. Farming of seaweeds. In: Seaweed Sustainability. Elsevier, pp. 27–59. https://doi.org/10.1016/B978-0-12-418697-2.00003-9.

Saha, M., Wiese, J., Weinberger, F., Wahl, M., 2016. Rapid adaptation to controlling new microbial epibionts in the invaded range promotes invasiveness of an exotic seaweed. J. Ecol. 104, 969–978. https://doi.org/10.1111/1365-2745.12590.

Saitou, N., Nei, M., 1987. The neighbor-joining method: a new method for reconstructing phylogenetic trees. Mol. Biol. Evol. 4, 406–425. https://doi.org/10.1093/oxfordjournals.molbev.a040454.

Schroth, M.N., Cho, J.J., Green, S.K., Kominos, S.D., 2018. Epidemiology of *Pseudomonas aeruginosa* in agricultural areas. J. Med. Microbiol. 67, 1191–1201. https://doi.org/10.1099/jmm.0.000758.

Tseng, C.K., 1962. Manual of cultivation of Haidai (*Laminaria Japonica*), 99-122. Science Press, Beijing, pp. 146–154 (In Chinese with English abstract).

Tseng, C.K., 1985. Manual of Cultivation of Seaweeds. Shanghai Science and Technics Press, Shanghai, pp. 82–96 (In Chinese with English abstract).

Wang, F., Li, M., Huang, L., Zhang, X.-H., 2021. Cultivation of uncultured marine microorganisms. Mar. Life Sci. Technol. 3, 117–120. https://doi.org/10.1007/s42995-021-00093-z.

Wang, G., Shuai, L., Li, Y., Lin, W., Zhao, X., Duan, D., 2008. Phylogenetic analysis of epiphytic marine bacteria on hole-rotten diseased sporophytes of *Laminaria japonica*. J. Appl. Phycol. 20, 403–409. https://doi.org/10.1007/s10811-007-9274-4.

Wang, G., Lu, B., Shuai, L., Li, D., Zhang, R., 2014. Microbial diseases of nursery and field-cultivated *Saccharina japonica* (Phaeophyta) in China. Arch. Hydrobiol. Suppl. Algol. Stud. 145–146, 39–51. https://doi.org/10.1127/1864-1318/2014/0167.

Wang, G.G., 2003. Studies on Defense Mechanisms of *Laminaria* to Pathogens. Post-Doctoral Thesis. Institute of Oceanology, Chin. Academy of Sciences, Qingdao (In Chinese with English abstract).

Ward, G.M., Faisan, J.P., Cottier-Cook, E.J., Gachon, C., Hurtado, A.Q., Lim, P.E., et al., 2020. A review of reported seaweed diseases and pests in aquaculture in Asia. J. World Aquacult. Soc. 51, 815–828. https://doi.org/10.1111/jwas.12649.

Weisburg, W.G., Barns, S.M., Pelletier, D.A., Lane, D.J., 1991. 16S ribosomal DNA amplification for phylogenetic study. J. Bacteriol. 173, 697–703. https://doi.org/10.1128/jb.173.2.697-703.1991.

Wu, C.Y., 1990. Cultivation of Temperate Seaweeds in the Asia Pacific Region. In regional workshop on the culture and utilization of seaweeds, Cebu City, pp. 27–31.

Xiang, J.H., 2001. Disease Occurrence and Control Strategies of Mariculture Organisms, 1. Ocean Press, Beijing, pp. 65–69 (In Chinese with English abstract).

Yeung, A.T.Y., Torfs, E.C.W., Jamshidi, F., Bains, M., Wiegand, I., Hancock, R.E.W., et al., 2009. Swarming of *Pseudomonas aeruginosa* is controlled by a broad spectrum of transcriptional regulators, including MetR. J. Bacteriol. 191, 5592–5602. https://doi.org/10.1128/JB.00157-09.

Zhang, R., Chang, L., Xiao, L., Zhang, X., Han, Q., Li, N., et al., 2020. Diversity of the epiphytic bacterial communities associated with commercially cultivated healthy and diseased *Saccharina japonica* during the harvest season. J. Appl. Phycol. 32, 2071–2080. https://doi.org/10.1007/s10811-019-02025-y.

Zhang, X., Chen, Y., Saha, M., Zhuang, Y., Chang, L., Xiao, L., et al., 2022. *Pseudoalteromonas piscicida* X-8 causes bleaching disease in farmed *Saccharina japonica*. Aquaculture 546, 737354. https://doi.org/10.1016/j.aquaculture.2021.737354.

Zhang, Z., Yao, B., Huang, R., 2021. First report of *Pseudomonas aeruginosa* causing basal stem rot of *Solanum lycopersicum* L. in China. Plant Dis. 95, 10–11. https://doi.org/10.1094/PDIS-08-21-1830-PDN.

Zozaya-Valdes, E., Egan, S., Thomas, T., 2015. A comprehensive analysis of the microbial communities of healthy and diseased marine macroalgae and the detection of known and potential bacterial pathogens. Front. Microbiol. 6, 1–9. https://doi.org/10.3389/fmicb.2015.00146.