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Enhanced Biomass Production of Various Aquatic Duckweed Species Through the Inoculation of Plant Growth-Promoting Bacteria

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ABSTRACT

Duckweed is a high-protein aquatic plant widely used for various fish feeds. Efforts to increase its biomass production can employ Plant Growth-Promoting Bacteria (PGPB). Therefore, this study aimed to determine the effect of inoculating *Bacillus* sp., *Pseudomonas* sp., and *Rhizobium* sp. on the biomass production of four duckweed species, including *Landoltia punctata*, *Lemna perpusilla, Spirodela polyrrhiza,* and *Wolffia globosa*. The bacterial inoculation was carried out by soaking three fronds of each duckweed species in bacterial suspension for 24 hours. The inoculated fronds were moved into a 50ml Erlenmeyer flask containing Hoagland medium and were incubated in a growth chamber at 25ºC for 14 days with a 16-h/8-h day/night photoperiod at 5000 lux. The number of fronds, fresh weight, and dry weight of each duckweed species were recorded. Data obtained from the observations were analyzed statistically using the ANOVA method (P<0.05). The number of the fronds of four duckweed species co-cultured for 14 days with our PGPB collections increased by 1.2 to 4.6-fold compared with the uninoculated control. In addition, the treated/control ratio biomass for dry weight was around 1.3 to 3.0-fold. Moreover, all bacterial strains exhibited traits of Indole-3-acetic acid synthesis, nitrogen fixation and phosphate solubilization albeit with varying abilities. The co-culture between duckweed and PGPB demonstrated in this study could serve as a promise to increase the biomass production of several duckweed species to support fish feed production.

INTRODUCTION

Duckweed is one of the smallest free-floating aquatic plants that has risen as a promising candidate for sustainable fish feed production. This flowering plant can live on the surface of motionless or slowly flowing freshwater bodies, such as ponds and lakes. Duckweed includes 5 genera, including Landoltia, Lemna, Spirodela, Wolffia, and Wolffiela, with 36 recognized species **(Bog *et al.*, 2019)**. The quick growth, high protein, and carbohydrate content of duckweed, and the ability to efficiently absorb nutrients from the aquatic environment make this tiny plant an attractive choice for aquaculture systems **(Ziegler *et al.*, 2015)**. The increasing popularity of duckweed as a high quality, cost-effective, sustainable, and eco-friendly source of fish feed alternative has been documented in several studies. For example, *Lemna polyrhiza* duckweed has been used for rohu (*Labeo rohita*

Ham.) **(Bairagi *et al.*, 2002)** and tilapia fingerlings **(Qiaoqiao *et al.*, 2023)**. *Lemna paucicostata* has been studied as a supplementary feeding of the the Nile tilapia (*Oreochromis niloticus*) **(Abdullahi, 2023)**. In addition, *Lemna minor* has been used for the Nile tilapia (*Oreochromis niloticus*) in multiple studies **(El-Shafai *et al.*, 2004; Ibrahim *et al.*, 2017; Herawati *et al.*, 2020; Alkhamis, 2024)** and for the silver carp *(Hypophthylmichthys molitrix*) and grass carp (*Ctenopharyngodon idella*) **(Aslam *et al.*, 2018)**. Therefore, to meet the high demand for animal feed production, further research is needed to optimize the biomass yields of duckweed which remains challenging due to several factors, such as water quality, nutrient resources, and aquatic environmental pollution.

One possible strategy to overcome these limitations and increase the duckweed's yield is the utilization of Plant Growth Promoting Bacteria (PGPB). These beneficial microbes, commonly found in the plant rhizosphere, are able to improve plant growth through several mechanisms, including increasing nutrient uptake, improving resistance to environmental stress, and simulating beneficial physiological processes **(Glick, 2012)**. Several PGPB strains have been reported to support duckweed growth. To name but a few, *Acinetobacter calcoaceticus* P23 increased the frond numbers of *Lemna aoukikusa* **(Suzuki *et al.*, 2014)**; *Ensifer* sp. SP4 promoted the *Spirodela polyrhiza* growth by up to 1.5fold **(Toyama *et al.*, 2021)**. In this context, *Exiguobacterium* sp. MH3 doubled the frond number and increased the dry weight of *Lemna minor* by more than 30% **(Tang *et al.*, 2015)**; and *Aquitalea magnusonii* H3 enhanced the growth of *Lemna minor*, with effects on plant growth (EPGs) of up to 20% **(Ishizawa *et al.*, 2017)**.

Numerous researches have documented the mechanisms of PGPB to support and enhance the growth of duckweed through the production of regulatory compounds, such as Indole-3-acetic acid (IAA) produced by *B. amyloliquefaciens*, and FZB42 which significantly increases the fresh weight of *Lemna minor* **(Idris *et al.*, 2007)**. Another study indicates that *Bradyrhizobium* sp. MRB4 containing IAA and siderophore features boosts the growth of *Lemna minor* **(Makino *et al.*, 2022)**. Members of the Betaproteobacteria class, including *Aquitalea magnusonii* H3, *Pelomonas* sp. MRB1, and also *Pelomonas* sp. MRB3 have been reported to produce IAA and to be able to significantly support the growth of *Lemna minor* by increasing frond numbers, dry weight, and total chlorophylls **(Makino *et al.*, 2022)**. Meanwhile, other growth-promoting mechanisms, including phosphate solubilization, have also been reported to be produced by *Pseudomonas otitidis* M12 **(Ishizawa *et al.*, 2017)** *Acinetobacter calcoaceticus* P23 **(Yamakawa *et al.*, 2018; Khairina *et al.*, 2021)**, and *Aquitalea magnusonii* H3 **(Ishizawa *et al.*, 2019; Toyama *et al.*, 2021)** in the studies on *L. minor.* Although various researches have examined the effects of PGPB on duckweed, the majority focused on *Lemna minor.* Therefore, this research aimed to investigate three different PGPB strains, including *Bacillus* sp., *Pseudomonas* sp., and *Rhizobium* sp., and their capacity to promote the biomass production of four different duckweed species, encompassing *Lemna perpusilla, Spirodela polyrrhiza, Landoltia punctata,* and *Wolffia globosa*, through a variety of mechanisms.

MATERIALS AND METHODS

Bacterial and plants conditions

1. Bacterial strains and culture conditions

Three PGPB strains from the culture collection of the Laboratory of Agricultural Microbiology, Faculty of Agriculture, Universitas Gadjah Mada (UGM), Indonesia were used in this study, including *Bacillus* sp., *Pseudomonas* sp., and *Rhizobium* sp. Each PGPB strain was tested for pathogenicity on tobacco (*Nicotiana tabacum*), a well-established model plant, and no pathogenic effects were observed. Each bacterium was grown in a different specific medium, including Potato Agar, Ashby's Mannitol Agar **(Rao, 1995)**, and nitrogen-free medium with 0.5% bromothymol blue **(Baldani *et al.*, 2014)**, respectively. A serial dilution was carried out using spread plate method to confirm the purity of each bacterial strain which was followed by bacterial storage on slant agar. Fresh bacterial cultures were prepared using a shaking incubator at 30^{o}C for 24-48 hours prior to subsequent experiments.

2. Molecular identification of PGPB

The Wizard Genomic DNA Purification Kit (Promega, Madison) was used to extract the genomic DNA from a single bacterial colony according to the instructions of manufacturer. To amplify and sequence the 16S rRNA gene, the isolated DNA was used as a template using the MyTaq HS Red Mix PCR Kit (25µl of 2x MyTaq Red Mix; 22 µl of PCR grade water, 1µl of each primer, and 1µl of DNA template). A set of universal primers were employed, including 8F (5'-AGAGTTTGATCCTGGCTCAG-3') and 1492R (5'- GGT TACCTTGTTACGACTT-3') **(Johnson *et al.*, 2019)** with the following thermocycle: 95°C for 2min, 30 cycles of 95°C for 15s, 55°C for 15s, 72°C for 10s, followed by a final extension at 72°C for 5min. The amplified products were visualized by 1.0% Tris-Borate EDTA agarose gel through electrophoresis at 100V for 35 minutes. DNA was stained using ethidium bromide (EtBr) and followed by a visualization using a UV-transluminator with UV light of 260-280nm. Each gel contained a GeneRuler1000 bp DNA marker (ThermoFisher) as a size reference. The amplification products were sequenced and analyzed using SnapGene software. The trimmed sequences were aligned with the available reference on Basic Local Alignment Search Tool National Center for Biotechnology Information through http://blast.ncbi.nlm.nih.gov/. The types of bacteria with sequence similarities approaching 100% were selected, MEGA X was used to perform a phylogenetic analysis to construct the phylogenetic trees by the Neighbor-joining method **(Newman *et al.*, 2016)**.

3. Bacterial plant growth-promoting traits

Phosphate dissolving ability test

The three bacterial cultures from the stock culture were examined for their phosphate solubilizing ability qualitatively. The bacteria were inoculated into a solid Pikovskaya medium **(Pikovskaya, 1948)** in a petri dish with a drop of bacterial culture and were then incubated at 30^{o}C in 7 days. The clear halo zone diameter over each bacterial strain colony was observed and measured as the phosphate solubilization ability **(Edi-Premono *et al.*, 1996)**.

3-Indoleacetic acid production ability test

Bacterial cultures were grown in liquid Luria Bertani (LB) medium and incubated on a shaker at 160rpm at room temperature for 24 hours. One milliliter of the incubated bacterial inoculum was then transferred into 5ml of LB medium to which 200mg/ L L-tryptophan had been added and then incubated on a shaker at 160rpm for 96 hours. Each sample was transferred to a falcon tube and centrifuged at 4000x g for 15 minutes. The supernatant was transferred to a test tube, and 1ml of Salkowski reagent was added. Each test tube was incubated at room temperature for half an hour in the dark. The color changing from pink to dark red indicates a positive reaction of the bacterial culture in producing indole acetic acid **(Gordon & Weber, 1951; Numponsak *et al*., 2018)**.

Nitrogen fixation ability test

The capacity of bacteria to fix the nitrogen was carried out qualitatively by culturing the bacteria in an N-free bromothymol blue semi-solid medium **(Baldani *et al*., 2014)**. A 100µl of bacterial culture was inoculated into an N-free bromothymol blue semi-solid medium and continued by an incubation for 72 hours at room temperature. The color changed from green to blue in the medium **(Cordova-Rodriguez *et al*., 2022)**, and pellicle formation was observed as an indicator of nitrogen-fixing bacterial growth **(Baldani *et al*., 2014)**.

4. Determination of PGP activity of PGPB on duckweed

Duckweed culture

Duckweeds were obtained from their natural habitat in a pond located in Sleman, Yogyakarta, Indonesia (7°40' 54.012" N, 110°19'23.988" E). The species identification was based on morphological traits which were established by **Landolt (1986)** and **Les *et al.* (2002)**, and it has been identified with plant taxonomy experts in the Laboratory of Plant Systematic, Faculty of Biology, Universitas Gadjah Mada, Indonesia resulting in four different species, including *Landoltia* punctata, *Lemna perpusilla*, *Spirodela polyrrhiza,* and *Wolffia globosa*. After the identification, the duckweed was maintained in the Laboratory of Agricultural Microbiology, Faculty of Agriculture, UGM. The acclimatization was performed in a growth chamber with a temperature of 28^{o}C, 5000 lux light intensity, and a 16-hour light photoperiod in a modified Hoagland medium **(Toyama *et al*., 2006; Yamakawa *et al.*, 2018)** with the adjusted pH of 7.0 using sodium hydroxide.

PGPB screening for duckweed

To examine the effect of three different PGPB (*Bacillus* sp., *Pseudomonas* sp., and *Rhizobium* sp.,) on four different duckweeds (*Lemna perpusilla*, *Spirodela polyrrhiza*, *Landoltia punctata*, and *Wolffia globosa*), a completely randomized design (CRD) was applied. In summary, 100ml of liquid LB medium was inoculated with each PGPB strain and incubated with shaking at 120rpm to reach OD600nm = 0.1 **(Wang *et al*., 2019)** at room temperature. After cultivation, the bacterial cell suspension was centrifuged at 4000 × g for 15 minutes at 24^{o}C. The cell pellet was suspended in Hoagland and centrifuged again to wash the cells under the same conditions. The duckweed washing steps were carried out twice,

followed by suspension in 100ml of sterilized Hoagland medium. Twelve individuals of each duckweed species were floated in a 100-ml suspension cell for adhesion and attachment in a growth chamber for 24 hours at 28^{o}C under the same light condition. Three fronds of each duckweed species were then transferred using a sterile tweezer into three 50ml Erlenmeyer flasks. Incubation was carried out for 14 days **(Yamakawa *et al.*, 2018)**. Three single fronds of each duckweed species were also cultivated and incubated in the same condition, without any bacterial treatment, as a control. The number of fronds was observed at 0, 2, 4, 6, 8, 10, and 14 days. After 14 days of incubation, the duckweed biomass was collected and its fresh weight was determined, then incubated at 60^{o}C for 1-2 days to measure the dry weight. Each treatment was repeated four times, with the observed parameters including daily frond numbers, fresh weight, and dry weight. Data were analyzed using the analysis of variance (ANOVA) and Tukey's post hoc test using R-studio software with a significance *P*-value > 0.05.

RESULTS

1. Bacterial identification

An observation was conducted on three different bacterial cultures with distinct morphological characteristics, including shape, color, elevation, consistency, gram staining reaction, colony shape, colony elevation, internal colony structure, and colony margin. The observation of colony morphology was based on the reference of **Pelczar *et al.* (1988)**, and the results of the morphological identification of the three bacterial cultures are shown in Table (1).

Table 1. Morphological characteristics of bacterial colonies

Bacteria	Shape	Colony elevation	Edge	Colony appearance	Cell form	Gram stain	Color
Bacillus sp.	Round	Low convex	Whole	Coarsely granular	Rod	+	Cream
Pseudomonas sp.	Round	Raised	Crenate	Coarsely granular	Rod	-	White
Rhizobium sp.	Round	Convex	Undulate	Coarsely granular	Rod	-	Transparent

The results of the colony and bacterial cell morphology observation indicate that the three bacterial cultures have different morphological characteristics. *Bacillus* sp. colonies are round, regular, and cream to yellow, with rod-shaped, gram-positive cells. *Pseudomonas* sp. colonies are circular with crenate (wrinkled) edges, white colored, and classified as Gram-negative. *Rhizobium* sp. colonies are circular, colorless/transparent, with rod-shaped, Gram-negative cells.

2. Molecular characterization of bacterial cultures

The results of molecular bacterial characterization are presented in the phylogenetic tree which was developed using the MEGA X software with the Neighbor-joining method and 1,000 bootstrap replications. The phylogenetic tree is presented in Fig. (1). The molecular and phylogenetic analysis, based on a partial sequence of 16S rDNA, shows that the three PGPB strains belong to the *Bacillus* sp., *Pseudomonas* sp., and *Rhizobium* sp.

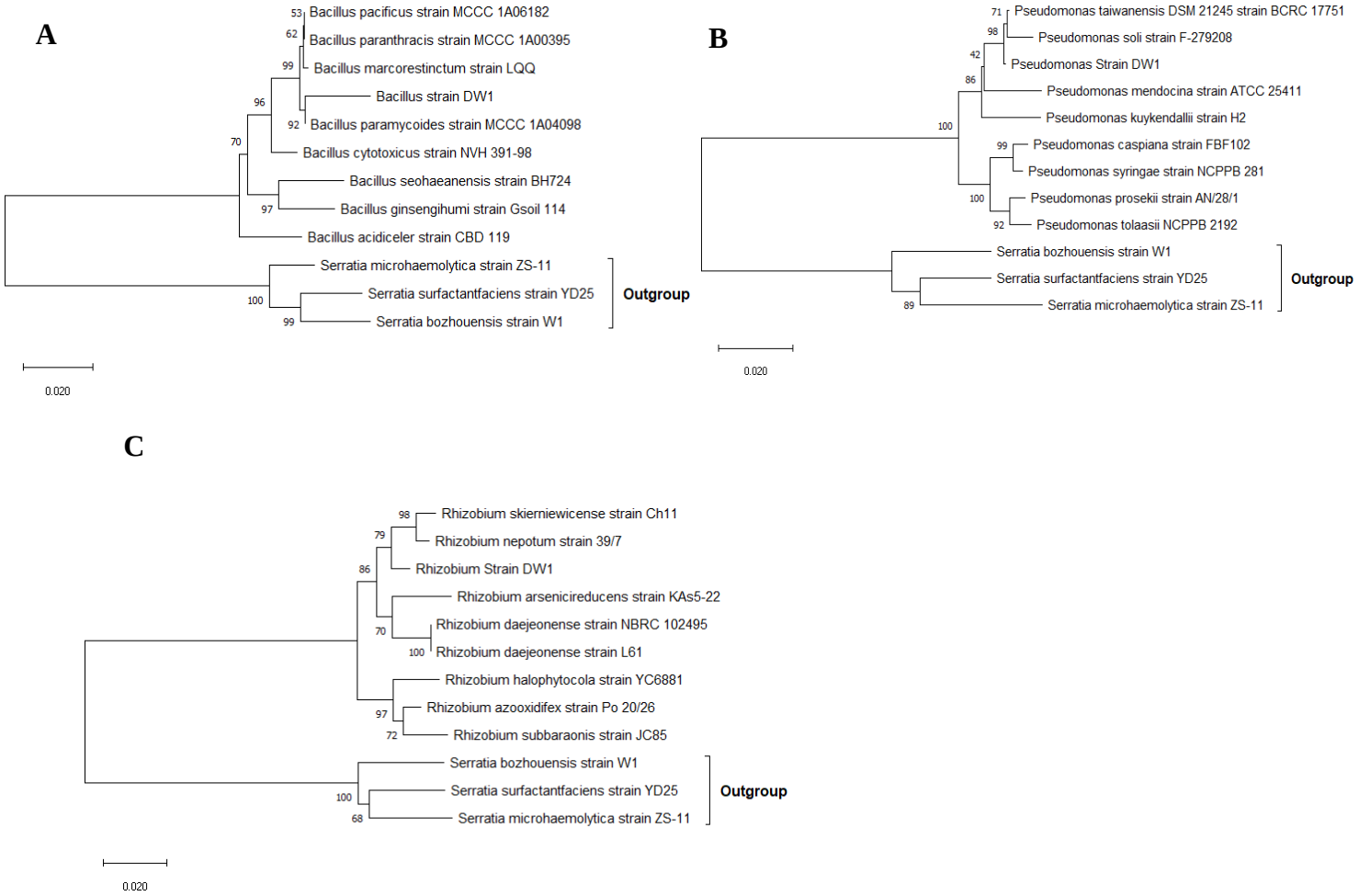

Fig. 1. Phylogenetic tree constructed based on near-complete partial 16S rDNA sequence from three bacterial isolates. Bootstrapping was performed with 1,000 replicates, with (A) *Bacillus* sp. (B) *Pseudomonas* sp., and (C) *Rhizobium* sp. Bacterial sequence samples from the Enterobacteriaceae family used as an outgroup

3. Characterization of bacterial cultures as PGPB

Plant growth-promoting features, including synthesis of IAA, fixation of nitrogen and solubilize of phosphate-related abilities of the three PGPB strains, were observed and recorded, as presented in Table (2). All three bacterial strains proved positive for IAA synthesis, phosphate solubilization, and nitrogen fixation based on the characterization of

PGP components. The highest ability of phosphate solubilization was calculated in the *Bacillus* sp. strain with a phosphate solubilization index (PSI) of 2.40, followed by *Pseudomonas* sp. and *Rhizobium* sp. with the values of 2.36 and 2.00, respectively. All PGPB strains were able to turn red when exposed to Salkowski reagent, form a pellicle on nitrogen-free semi-solid bromothymol blue 0.5% medium, turn to blue, and survive on Pikovskaya medium while forming a clear zone.

Table 2. Plant growth-promoting features of the three PGPB

Bacteria	**IAA synthesis**	***Phosphate Solubilization Index* (PSI)**	**Nitrogen fixation**
Bacillus sp.	+	2.40	+
Pseudomonas sp.	+	2.36	+
Rhizobium sp.	+	2.00	+

Symbol indicates the result: (+) positive ; (-) negative.

4. Evaluation of growth promotion

a. *Duckweed identification*

Identification was carried out to determine the taxonomy, constituting genus and species name, of the duckweed used in this research. Different plant species show different traits that affect their growth and biomass production. Based on the morphological identification, the duckweed used in this study belongs to the species represented in Fig. (2).

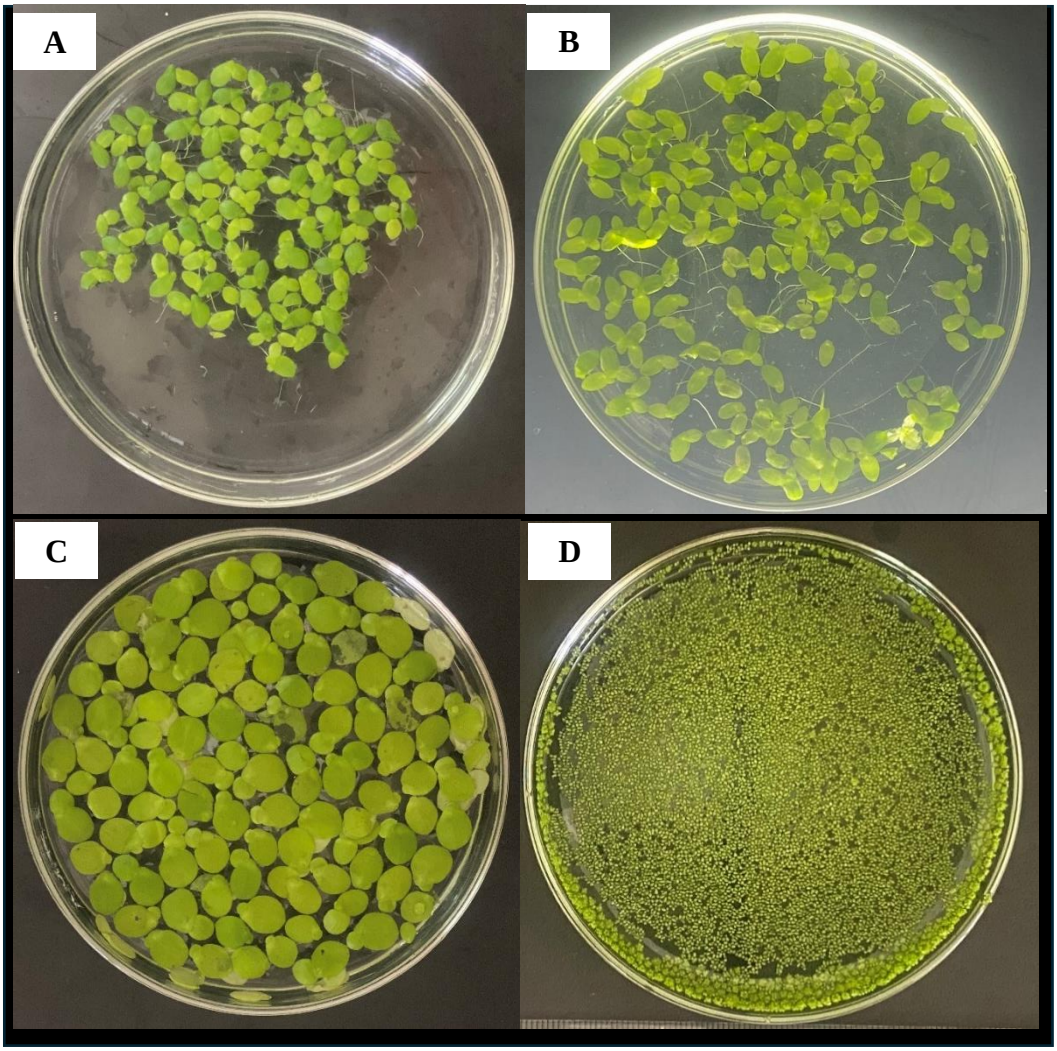

Fig. 2. The morphological identification results of four different duckweed species isolated from ponds in Sleman, Yogyakarta, Indonesia: A. *Landoltia punctata*, B. *Lemna perpusilla*, C. *Spirodela polyrrhiza*, D. *Wolffia globosa*

b. *Duckweed growth promotion by PGPB*

The effect of the three PGPB strains on biomass production was evaluated based on several parameters, namely frond number, fresh weight, and dry weight. The number of duckweed fronds increased by 1.2 to 4.6 times with all three PGPB strains inoculations. After 14 days of incubation, the frond number of *Landoltia punctata* co-cultivated with *Bacillus* sp., *Pseudomonas* sp., and *Rhizobium* sp. markedly enhanced from three fronds as the starting number to 38.5±4.7; 37.25±3.9; and 43.25±2.4 fronds, respectively. In contrast, the control group showed an increase to only 17.75±1.1 fronds. In addition, *Lemna perpusilla* co-cultivated with those three bacterial strains also exhibited a significant increase in the number of fronds to 24.75±1.2; 30.5±1.5; and 25.25±1.5 fronds, respectively. Similarly, *Spirodela polyrrhiza* also displayed a noticeable increase to 20±0.7; 16.5±0.9; and 16.75±0.6 fronds, while the control increased only to 11.5±0.9 fronds. Furthermore, *Wolffia globosa* demonstrated an increase in its frond number reaching 41.25±2.8; 29±0.4; and 24.25±3.0

fronds, compared to the control which only increased to 19.25±1.2 fronds. Clearly, *Lemna perpusilla* showed the highest treated/control frond number ratios (3.8 to 4.7), followed by *Landoltia punctata* (2.1 to 2.4), *Spirodela polyrrhiza* (1.4 to 1.7), and *Wolffia globosa* (1.2 to 2.1).

Further evaluation was conducted on the biomass of each duckweed species based on fresh weight and dry weight in co-culture with or without PGPB strains. The fresh weight of *Landoltia punctata*/*Bacillus* sp., *Landoltia punctata*/*Pseudomonas* sp.; *Landoltia punctata*/*Rhizobium* sp. were 73.42±11.1mg, 68.95±10.55mg, and 82.12±9.54mg, respectively (All calculated relative to the control). These numbers were 1.3 to 1.6-fold higher than those of control treatment. Even though the fresh weight of *Lemna perpusilla* biomass was lower than that of *Landoltia punctata* biomass, if compared to that of the control treatment, the three bacterial strains were able to increase by up to 2.2 until 3.3-fold. *Spirodela polyrrhiza* known as giant duckweed showed the highest production of biomass among the four-duckweed species. The fresh weight of co-cultivation between *Spirodela polyrrhiza*/*Bacillus* sp. was 191.92±5.8mg; *Spirodela polyrrhiza*/*Pseudomonas* sp. was 147/92 ± 7.0mg; and *Spirodela polyrrhiza*/ *Rhizobium* sp. was 149.35±9.75mg. Meanwhile, the control showed only 66.17±4.5mg of fresh weight. *Wolffia globosa*, known as the smallest duckweed species, showed the lowest fresh weight values. Co-cultivated with *Bacillus* sp., *Pseudomonas* sp., and *Rhizobium* sp., its fresh weight was 3.27±0.11mg, 2.37±0.24mg, and 2.05±0.42mg, respectively. However, these values corresponded to the increases of 2.9; 2.1, and 1.86-fold respectively compared to the uninoculated, without bacterial inoculation.

The dry weight of *Landoltia punctata* co-cultivated with *Bacillus* sp., *Pseudomonas* sp., and *Rhizobium* sp. was 4.9±0.6mg, 4.6±0.7mg; 5.6±0.4mg, respectively. The treated/control ratio ranged from 1.3 to 1.6-fold. Furthermore, *Lemna perpusilla* treated with *Bacillus* sp., *Pseudomonas* sp., and *Rhizobium* sp. resulted in an increase of dry weight by 3.0, 1.9, and 1.9 times higher than the control. Additionally, the dry weight of *Spirodela polyrrhiza* treated with *Bacillus* sp., *Pseudomonas* sp., and *Rhizobium* sp. was 11.1±0.3mg; 9.9±0.4mg, and 9.9±0.4mg, respectively (Fig. 3).

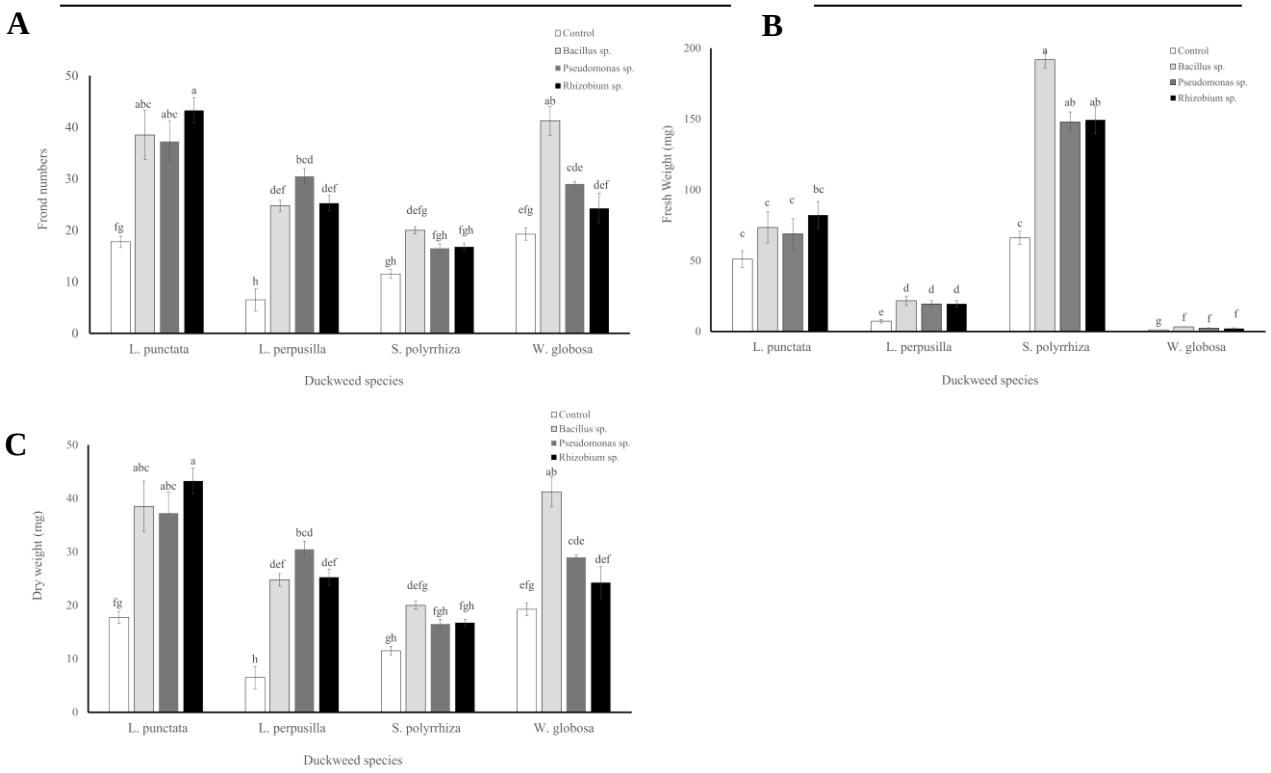

Fig. 3. Comparison of four duckweed species (*Landoltia punctata, Lemna perpusilla, Spirodela polyrrhiza*, and *Wolffia globosa*) after cultivated with three strains of PGPB, including *Bacillus* sp., *Pseudomonas* sp., *Rhizobium* sp., and no bacteria control in sterile Hoagland medium in 14 days. (A) Number of fronds each duckweed species, (B) fresh weight (mg) of each duckweed after 14 days, and (C) dry weight (mg) of each duckweed species after 14 days. Error bar represents standard error (SE). a-h showed significant differences ($P< 0.05$).

The PGP activity was examined by observing the frond number of four different Lemnaceae species every two days. The data on *Landoltia punctata*, after four days of incubation, showed considerable differences ($P<0.05$) in frond numbers following the inoculation with all three bacterial strains (Fig. 4a). In addition, in co-culture conditions with *Lemna perpusilla*, significant differences have already been shown from day 2 (Fig. 4 b). In contrast, *Wolffia globosa* started to show significant differences in the number of fronds after 8 days of incubation. However, across all co-culture conditions for 14 days of incubation, the results (Fig. 4a-d) revealed that all three PGPB noticeably increased the frond numbers by 1.2 to 4.6-fold compared to the control ($P< 0.05$).

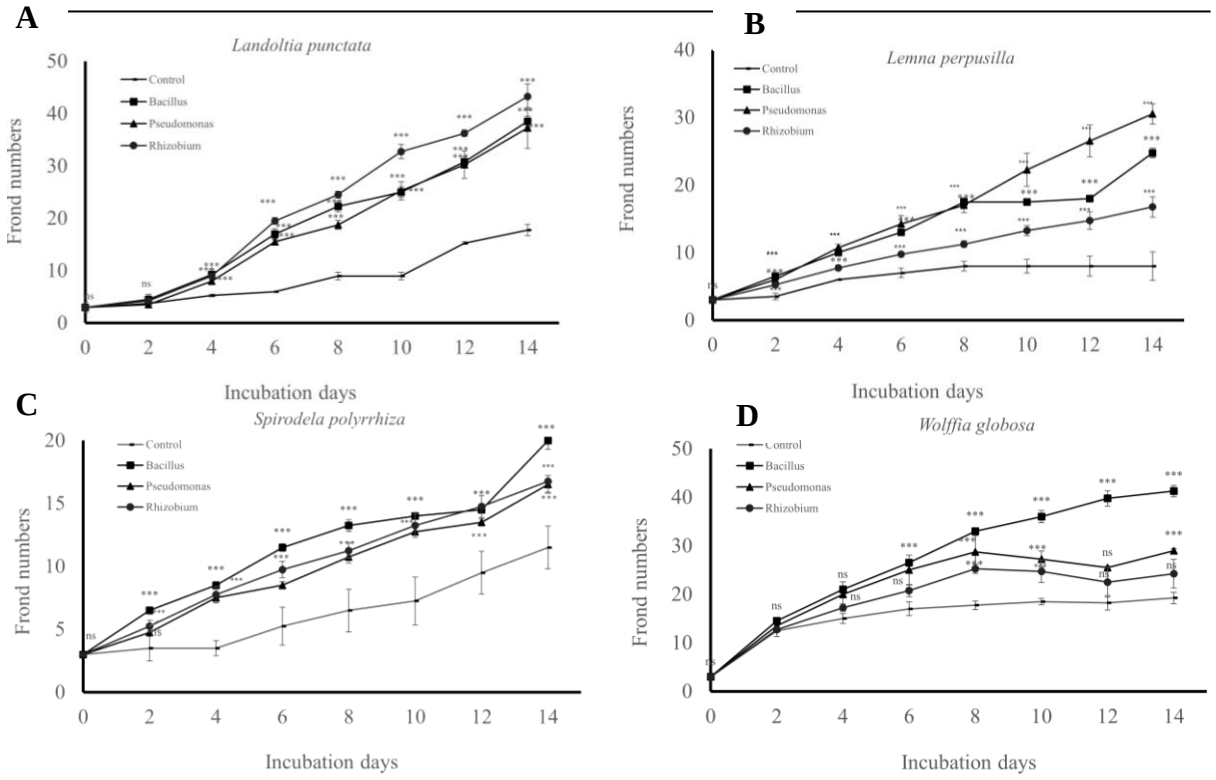

Fig. 4. Effects of PGPB, *Bacillus* sp., *Pseudomonas* sp., *Rhizobium* sp. and no bacteria control treatments on the growth of four different duckweed species in Hoagland medium. The increase in the number of fronds is shown for (A) *Landoltia punctata*, (B) *Lemna perpusilla*, (C) *Spirodela polyrrhiza*, and (D) *Wolffia globosa*. All the duckweed cultivation started with a single frond. Symbols represent the following: control, *Bacillus* sp., *Pseudomonas* sp., and *Rhizobium* sp. Asterisks indicate the significant differences between values with and without bacterial inoculation, ns means no significant difference (T-test, $P< 0.05$)

DISCUSSION

The results of colony and bacterial cell morphology observations indicate that the three bacterial cultures have different morphological characteristics, which are consistent with the previous studies. These findings corroborate the study by **Tarangini and Mishra (2013)** reporting that *Pseudomonas* has a circular colony shape, crenate margins, white and transparent color, rod-shaped cells, and is Gram-negative. Meanwhile, the result of the observation on *Bacillus* sp. colonies aligns with the **Ashwini *et al*. (2011)** investigation which reveals that the shape of the colonies is round, regular, cream and yellow in color, with rod-shaped cells and are Gram-positive. Moreover, the colony morphology results of *Rhizobium* sp. correspond with the research findings of **Ribeiro *et al*. (2012)** who found that *Rhizobium* sp. has the following morphological characteristics: circular shape, colorless/transparent, rod-shaped cells and Gram-negative.

The observation of PGP factors indicates that all three bacterial strains can perform fixation of nitrogen. It is known that *Rhizobium* sp. is a group of symbiotic bacteria capable

of nitrogen fixation **(Prasad *et al*., 2014)**, while *Bacillus* sp. and *Pseudomonas* sp. are groups of non-symbiotic bacteria that can also perform fixation of nitrogen **(Kumar *et al*., 2019)**. The results support the findings which revealed that *Bacillus* sp. can produce IAA, solubilize the phosphate, and fix nitrogen **(Yousuf *et al*., 2017; Lebrazi *et al*., 2020)**. In addition, it has been suggested that IAA-producing *Bacillus amylolyiquefaciens* support the growth of *L. minor* **(Idris *et al*., 2007)**. However, it is early to wrap up that this growth-promoting element is the only cause of the increase of duckweed, as has been reported that *L. minor* growth was unaffected by exogenous IAA **(Utami *et al*., 2018)**. While *Pseudomonas* sp. **(De La Torre-Ruiz *et al*., 2016; Ishizawa *et al*., 2017)** and *Rhizobium* sp. **(Purwaningsih *et al*., 2021)** have been reported to have the same abilities although with varying phosphate solubilization capabilities. In terrestrial plants, nitrogen and phosphate frequently restrict plant growth, thus, P-solubilizing and Nitrogen-fixing microbes are important to supply nutrients and promote the plant growth **(Rodríguez & Fraga, 1999; Raymond *et al*., 2004)**. Additionally, this research found that three PGPB strains increased the four-duckweed species, reporting that the mechanisms and nature of duckweed growth promotion might be different from those of soil PGPB. Hence, future research is important to understand the strategies unique of PGPB to aquatic plants.

In an aquatic environment, the plant body of duckweed provides bacterial cells with an ecological niche and an ideal habitat to live where nutrients are available **(Ishizawa *et al*., 2020)**. This study also indicates that particular bacteria groups can actively multiply and promote growth in host plants, resulting in a mutualistic relationship that supports the growth of both plant and PGPB. To verify our hypothesis, we compared the PGPB on the growth of four different duckweed species and observed their growth through frond number, fresh weight, and dry weight records. The frond number of the duckweed is used as the first parameter to see the effect of bacterial inoculation since it can be as an indication of active growth and replication in this aquatic plant **(Suzuki *et al*., 2014)**. One limitation of this research is that the use of non-sterilized duckweed, which might contain indigenous microbial community which can interact with our inoculated PGPB strains. Future research can occupy the surface sterilizing duckweed before inoculation. Additionally, the microbial communities profiling such as 16S rRNA sequence can evaluate and confirm the domination community. This approach can be used to strengthen the interpretation of PGPB strains effect and clarify the plant-microbe interactions.

In this study, although co-cultivating with our three different PGPB strains promoted the growth of four distinct duckweed species, co-cultivation with *Bacillus* sp. was found the highest promotion compared to two other bacteria, *Pseudomonas* sp. and *Rhizobium* sp. Previous study also established that *Lemna minor* inoculated by the rhizobacterium group increased the frond numbers by 37% **(Tang *et al*., 2015)**. In addition, our observation on daily frond numbers showed that it doubled the growth rate of all four-duckweed species on day six after incubation with the PGPB. This finding is higher than that observed from *Acinetobacter calcoaceticus* P23 inoculation on *L. minor* which increased the frond number after 7 days cultured by 1.7-fold **(Toyama *et al*., 2017)**. The beneficial effect of PGPBs on

duckweed growth have been reported in other genera, suggesting the broader application of the PGPBs inoculation method. For instance, *Wolffia globosa* has been shown to increase their biomass significantly over 15 days after being inoculated with PGPB including *Azospirillum brasilense Burkholderia vietnamiensis, Azospirillum brasilense* and *Gluconacetobacter diazotrophicus* **(Akkarajeerawat *et al.*, 2025)**. This finding is consistent with our studies and indicates that the diverse duckweed species receive the benefit of PGPB applications.

CONCLUSION

Here, we reported that *Bacillus* sp., *Pseudomonas* sp., and *Rhizobium* sp. notably promoted the growth of four duckweed species (*Landoltia punctata, Lemna perpusilla, Spirodela polyrrhiza,* and *Wolffia globosa*) and accelerated their biomass production, including frond numbers, fresh weight, and dry weight. All three bacterial strains exhibited traits of Indole-3-acetic acid synthesis, nitrogen fixation, and phosphate solubilization albeit with varying abilities. This study's demonstration of duckweed and PGPB co-culture has potential to enhance the biomass production of several duckweed species to support fish feed production. Further research will focus on the analysis of bacterial communities in the duckweed and their impact on the biomass production. The exploration of PGPB strains combination and culture environments will be also an interesting study toward effective and promising PGPB applications. In addition, assessing the impact of duckweed on the enhancement of nutritional quality, particularly as influenced by the biomass yield promoted by PGPR strains is essential to understand its potential application in aquaculture feed system.

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REFERENCES

Abdullahi, A. I. (2023). Apparent digestibility coefficient and carcass composition of Nile tilapia (Oreochromis niloticus) fed processed duckweed (Lemna paucicostata) meals. *Int. J. Fish. Aquac.*, *13*(4), 1–6.

Akkarajeerawat, S.; Chauvanasmith, T. and Keatsirisart, C. (2025). The optimization of high-protein duckweed cultivation in eutrophicated water with mutualistic bacteria. *JEI.*, *8*(1), 1-7.

Alkhamis, Y. A. (2024). Effect of Aquaponically Grown Duckweed as a Sustainable Feed on Growth Indices, Water Quality, and Digestive Activities, for the Nile Tilapia Reared in Aquaponic Culture. *Egypt. J. Aquat. Biol. Fish.*, *28*(2), 631–646.

Ashwini, K.; Gaurav, K.; Karthik, L. and Bhaskara Rao, K. V. (2011). Optimization,

production and partial purification of extracellular α-amylase from Bacillus sp. marini. *Arch. Appl. Sci. Res.,* *3*(1), 33–42.

Aslam, S.; Zuberi, A. and Shoaib, A. (2018). Effect of experimental diets on the activities of intestinal digestive enzymes of Grass carp (Ctenopharyngodon idella) and Silver carp (Hypophthylmichthys molitrix). *Int. J. Fish. Aquac.,* *9*(1), 51–57.

Bairagi, A.; Ghosh, K. S.; Sen, S. K. and Ray, A. K. (2002). Duckweed (Lemna polyrhiza) leaf meal as a source of feedstuff in formulated diets for rohu (Labeo rohita Ham.) fingerlings after fermentation with a fish intestinal bacterium. *Bioresour. Technol.,* *85*(1), 17–24.

Baldani, J. I.; Reis, V. M.; Videira, S. S.; Boddey, L. H. and Baldani, V. L. D. (2014). The art of isolating nitrogen-fixing bacteria from non-leguminous plants using N-free semi-solid media: a practical guide for microbiologists. *Plant Soil*, *384*, 413–431.

Bog, M.; Appenroth, K. and Sree, K.S. (2019). Duckweed (Lemnaceae): Its Molecular Taxonomy. *Front. Sustain. Food Syst.,* *3*(117), 1-7.

Cordova-Rodriguez, A.; Rentería-Martínez, M. E.; López-Miranda, C. A.; Guzmán-Ortíz, J. M. and Moreno-Salazar, S. F. (2022). Simple and sensitive spectrophotometric method for estimating the nitrogen-fixing capacity of bacterial cultures. *MethodsX*, *9*(101917), 1-5.

De La Torre-Ruiz, N.; Ruiz-Valdiviezo, V. M.; Rincón-Molina, C. I.; Rodríguez-Mendiola, M.; Arias-Castro, C.; Gutiérrez-Miceli, F. A.; Palomeque-Dominguez, H. and Rincón-Rosales, R. (2016). Effect of plant growth-promoting bacteria on the growth and fructan production of Agave americana L. *Braz. J. Microbiol.*, *47*, 587–596.

Edi-Premono.; Moawad, M.A. and Vleck L.G. (1996). Effect of phosphate solubilizing Pseudomonas putida on the growth of maize and its survival in the rhizosphere. *Indones. J. Crop Sci.,* *11*, 13–23.

El-Shafai, S. A.; El-Gohary, F. A.; Verreth, J. A. J.; Schrama, J. W. and Gijzen, H. J. (2004). Apparent digestibility coefficient of duckweed (Lemna minor), fresh and dry for Nile tilapia (Oreochromis niloticus L.). *Aquac. Res.,* *35*(6), 574–586.

Glick, B. R. (2012). Plant growth-promoting bacteria: mechanisms and applications. *Scientifica.,* *2012*, 1-15.

Gordon, S. A. and Weber, R. P. (1951). Colorimetric estimation of indoleacetic acid. *Plant Physiol.,* *26*(1), 192.

Herawati, V. E.; Pinandoyo, P.; Darmanto, Y. S.; Rismaningsih, N.; Windarto, S. and Radjasa, O. K. (2020). The effect of fermented duckweed (Lemna minor) in feed on growth and nutritional quality of tilapia (Oreochromis niloticus). *Biodiversitas.,* *21*(7), 3350-3358.

Ibrahim, W.M.; Mohamed, K. and Abd-elfattah, B. (2017). Effect of Replacement of Soybean Meal with Duckweed (Lemna minor) Meal on the Growth Performance and Feed Utilization in Nile Tilapia Fingerlings. *J. Anim. Poult. Fish. Prod.,* *6*(1), 7–12.

Idris, E. S. E.; Iglesias, D. J.; Talon, M. and Borriss, R. (2007). Tryptophan-dependent production of Indole-3-Acetic Acid (IAA) affects level of plant growth promotion by Bacillus amyloliquefaciens FZB42. *Mol. Plant Microbe. Interact.,* *20*(6), 619–626.

Ishizawa, H.; Kuroda, M.; Morikawa, M. and Ike, M. (2017). Evaluation of environmental bacterial communities as a factor affecting the growth of duckweed Lemna minor. *Biotechnol. Biofuels.*, *10*, 1–10.

Ishizawa, H.; Tada, M.; Kuroda, M.; Inoue, D. and Ike, M. (2019). Performance of plant growth-promoting bacterium of duckweed under different kinds of abiotic stress factors. *Biocatal. Agric. Biotechnol.*, *19*(101146), 1-7.

Ishizawa, H.; Tada, M.; Kuroda, M.; Inoue, D.; Futamata, H. and Ike, M. (2020). Synthetic bacterial community of duckweed: a simple and stable system to study plant-microbe interactions. *Microbes Environ.*, *235*(4), 1-5.

Johnson, J. S.; Spakowicz, D. J.; Hong, B. Y.; Petersen, L. M.; Demkowicz, P.; Chen, L.; Leopold, S. R.; Hanson, B. M.; Agresta, H. O.; Gerstein, M.; Sodergren, E. and Weinstock, G. M. (2019). Evaluation of 16S rRNA gene sequencing for species and strain-level microbiome analysis. *Nat. Commun.*, *10*(1), 1–11.

Khairina, Y.; Jog, R.; Boonmak, C.; Toyama, T.; Oyama, T. and Morikawa, M. (2021). Indigenous bacteria, an excellent reservoir of functional plant growth promoters for enhancing duckweed biomass yield on site. *Chemosphere.*, *268*, 1-8.

Landolt, E. (1986). The Family of Lemnaceae - A Monographic Study: Vol 1: Morphology, Karyology, Ecology, Geographic Distribution, Systematic Position, Nomenclature, Descriptions. *Veroffentlichungen des Geobotanischen Institutes der ETH, Stiftung Rubel, Zurich*, pp. 566.

Lebrazi, S.; Niehaus, K.; Bednarz, H.; Fadil, M.; Chraibi, M. and Fikri-Benbrahim, K. (2020). Screening and optimization of indole-3-acetic acid production and phosphate solubilization by rhizobacterial strains isolated from Acacia cyanophylla root nodules and their effects on its plant growth. *J. Genet. Eng. & and Biotechnol.*, *18*, 1-12.

Les, D.H.; Crawford, D.J.; Landolt, E.; Gabel, J. and Kimball, R.T. (2002). Phylogeny and systematics of Lemnaceae, the duckweed family. *Syst. Bot.*, *27*, 221-240.

Makino, A.; Nakai, R.; Yoneda, Y.; Toyama, T.; Tanaka, Y.; Meng, X.-Y.; Mori, K.; Ike, M.; Morikawa, M. and Kamagata, Y. (2022). Isolation of aquatic plant growth-promoting bacteria for the floating plant duckweed (Lemna minor). *Microorganisms*, *10*(8), 1564.

Newman, L.; Duffus, A. L. J. and Lee, C. (2016). Using the free program MEGA to build phylogenetic trees from molecular data. *Am. Bio. Teac.*, *78*(7), 608–612.

Numponsak, T.; Kumla, J.; Suwannarach, N.; Matsui, K. and Lumyong, S. (2018). Biosynthetic pathway and optimal conditions for the production of indole-3-acetic acid by an endophytic fungus, Colletotrichum fructicola CMU-A109. *PloS One.*, *13*(10), 1-17.

Pelczar, M.J.; Chan, E.C.S. and Krieg, N.R. (1988) *Microbiology*. McGraw-Hill, New York.

Pikovskaya, R. (1948). Mobilization of phosphorus in soil in connection with vital activity of some microbial species. *Mikrobiologiya* *17*, 362–370. *Plant Soil.*, *287*, 77–84.

Prasad, R.; Kumar, M. and Varma, A. (2014). Role of PGPR in soil fertility and plant health. In: "Plant-Growth-Promoting Rhizobacteria (PGPR) and Medicinal Plants." **Egamberdieva, D.; Shrivastava, S. and Varma, A** (Eds). *Springer.*, Cham,

pp 247–260.

Purwaningsih, S.; Agustiyani, D. and Antonius, S. (2021). Diversity, activity, and effectiveness of Rhizobium bacteria as plant growth promoting rhizobacteria (PGPR) isolated from Dieng, central Java. *Iran. J. Microbiol.*, *13*(1), 130.

Qiaoqiao, Y.; Bingying, H.; Bohan, Y.; Hanggui L. and Jiaming, Z. (2023). Effects of giant duckweed DW2602 as feed supplement on the growth of tilapia fingerling *J. Trop. Biol.*, *14*(4), 451-457.

Raymond, J.; Siefert, J.L.; Staples, C.R. and Blankenship, R.E. (2004). The Natural History of Nitrogen Fixation. *Mol. Biol. Evol.*, *21*, 541–554.

Rao, N. S. S. (1995). *Soil microorganisms and plant growth*, third ed. Science Publisher, Lebanon NH, 335pp.

Ribeiro, R. A.; Roge, M. A.; López-López, A.; Ormeño-Orrillo, E.; Barcellos, F. G.; Martínez, J.; Thompson, F. L.; Martínez-Romero, E. and Hungria, M. (2012). Reclassification of Rhizobium tropici type A strains as Rhizobium leucaenae sp. nov. *Int. J. Sys. Evol. Microbiol.*, *62*(5), 1179–1184.

Rodríguez, H. and Fraga, R. (1999). Phosphate Solubilizing Bacteria and their Role in Plant Growth Promotion. *Biotechnol. Adv.*, *17*, 319–339.

Suzuki, W.; Sugawara, M.; Miwa, K. and Morikawa, M. (2014). Plant growth-promoting bacterium Acinetobacter calcoaceticus P23 increases the chlorophyll content of the monocot Lemna minor (duckweed) and the dicot Lactuca sativa (lettuce). *J. Biosci. Bioeng.*, *118*(1), 41–44.

Tang, J.; Zhang, Y.; Cui, Y. and Ma, J. (2015). Effects of a rhizobacterium on the growth of and chromium remediation by Lemna minor. *Environ. Sci. Pollut. Res.*, *22*, 9686–9693.

Tarangini, K. and Mishra, S. (2013). Production, characterization and analysis of melanin from isolated marine Pseudomonas sp. using vegetable waste. *Res. J. Engineering Sci.*, *2*(5), 2278–9472.

Toyama, T.; Yu, N.; Kumada, H.; Sei, K.; Ike, M. and Fujita, M. (2006). Accelerated aromatic compounds degradation in aquatic environment by use of interaction between Spirodela polyrrhiza and bacteria in its rhizosphere. *J. Biosci. Bioeng.*, *101*(4), 346–353.

Toyama, T.; Kuroda, M.; Ogata, Y.; Hachiya, Y.; Quach, A.; Tokura, K.; Tanaka, .; Mori, K.; Morikawa, M. and Ike, M. (2017). Enhanced biomass production of duckweeds by inoculating a plant growth-promoting bacterium, Acinetobacter calcoaceticus P23, in sterile medium and non-sterile environmental waters. *Water Sci. Technol.*, *76*, 1418-1428.

Toyama, T.; Mori, K.; Tanaka, Y,; Ike, M. and M. Morikawa, (2022). Growth promotion of giant duckweed Spirodela polyrhiza (Lemnaceae) by Ensifer sp. SP4 through enhancement of nitrogen metabolism and photosynthesis. *MPMI.*, *35*(1), 28-38.

Utami, D.; Kawahata, A.; Sugawara, M.; Jog, N.R.; Miwa, K. and Morikawa, M. (2018). Effect of exogenous general plant growth regulators on the growth of the duckweed Lemna minor. *Front Chem.*, *6*(251), 1-9.

Wang, Q.; Ma, L.; Zhou, Q.; Chen, B.; Zhang, X.; Wu, Y.; Pan, F.; Huang, L.; Yang, X.

and Feng, Y. (2019). Inoculation of plant growth promoting bacteria from hyperaccumulator facilitated non-host root development and provided promising agents for elevated phytoremediation efficiency. *Chemosphere.*, *234*, 769–776.

Yamakawa, Y.; Jog, R. and Morikawa, M. (2018). Effects of co-inoculation of two different plant growth-promoting bacteria on duckweed. *Plant Growth Regul.*, *86*(2), 287–296.

Yousuf, J.; Thajudeen, J.; Rahiman, M.; Krishnankutty, S.; P. Alikunj, A. and A. Abdulla, M. H. (2017). Nitrogen fixing potential of various heterotrophic Bacillus strains from a tropical estuary and adjacent coastal regions. *J. Basic Microbiol.*, *57*(11), 922–932.

Ziegler, P.; Adelmann, K.; Zimmer, S.; Schmidt, C. and Appenroth, K.J. (2015). Relative in vitro growth rates of duckweeds (Lemnaceae)—the most rapidly growing higher plants. *Plant Biol.*, *17*, 33-41.