Jurnal Akuakultur Indonesia
Research Article
ISSN: 1412-5269
EISSN: 2354-6700
Vol. 25 No. 2, July 2026
DOI: 10.19027/jai.25.2.164-177

The potential of *Pseudomonas* sp. as a phosphate solubilizer for phytoplankton in *Penaeus vannamei* rearing

Potensi *Pseudomonas* sp. sebagai pelarut fosfat untuk fitoplankton pada pemeliharaan *Penaeus vannamei*

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(Received June 26, 2025; Revised July 30, 2025; Accepted October 21, 2025)

ABSTRACT

Phosphorus is an essential element in aquaculture waters, significantly influencing phytoplankton growth. However, phosphorus is often present in a limited organic form and is rarely found as free dissolved inorganic phosphorus (orthophosphate). The application of *Pseudomonas* AHN.4 offers an alternative approach to enhancing orthophosphate availability for phytoplankton in aquaculture systems through its phosphate-solubilizing capability. This study aimed to evaluate the potential of *Pseudomonas* AHN.4 as a phosphate-solubilizing bacterium and its effect on phytoplankton profile in the *P. vannamei* culture system. The *Pseudomonas* sp. isolate, sourced from the mangrove waters of BLUPP (Balai Layanan Usaha Produksi Perikanan) Karawang, demonstrated phosphate-solubilizing ability by producing a clear zone on Pikovskaya agar with a solubilization index of 2.4 mm. Molecular tests revealed a close genetic relationship to *Pseudomonas oryzihabitans*. Hemolytic assay showed non-pathogenic properties. The bacteria were applied to *P. vannamei* rearing media with three treatments and one control, using bacterial densities of 10^1, 10^3, and 10^5 CFU/mL, each replicated three times. The bacterial application significantly affected phytoplankton growth compared to the control (0 CFU/mL). The highest phytoplankton abundance was recorded in treatment 10^5 CFU/mL, with 173,333 ± 10,006 cell/mL, a diversity index of 1.20, a uniformity index of 0.67, and dominance of 0.43. The increased phytoplankton supported an absolute growth of *P. vannamei* by 1.86 ± 0.02 g with the best survival rate of 86.67%. Although statistically not significantly different between treatments (P>0.05), these results suggest that treatment 10^5 has relevant application potential and is more effective at providing orthophosphate to phytoplankton.

Keywords: aquaculture, orthophosphate availability, phytoplankton, *Pseudomonas* sp., *P. vannamei*

ABSTRAK

Fosfor adalah elemen penting dalam perairan akuakultur, yang secara signifikan memengaruhi pertumbuhan fitoplankton. Namun, fosfor sering kali hadir dalam bentuk organik yang terbatas dan jarang ditemukan sebagai fosfor anorganik terlarut bebas (ortofosfat). Aplikasi bakteri *Pseudomonas* AHN.4 menawarkan alternatif sebagai bakteri pelarut fosfat yang mampu menyediakan ortofosfat secara efisien untuk fitoplankton dalam sistem akuakultur. Penelitian ini bertujuan untuk mengevaluasi potensi *Pseudomonas* AHN.4 sebagai bakteri pelarut fosfat dan pengaruhnya terhadap profil fitoplankton pada sistem kultur *P. vannamei*. Isolat *Pseudomonas* sp. yang berasal dari perairan mangrove BLUPP (Balai Layanan Usaha Produksi Perikanan) Karawang menunjukkan kemampuan melarutkan fosfat dengan menghasilkan zona bening pada media Pikovskaya dengan indeks pelarutan sebesar 2,4 mm. Uji molekuler menunjukkan adanya hubungan genetik yang dekat dengan *Pseudomonas oryzihabitans*. Uji hemolitik menunjukkan sifat nonpatogenik. Bakteri diaplikasikan pada media pemeliharaan *P. vannamei* dengan tiga perlakuan dan satu kontrol, dengan kepadatan bakteri 10^1, 10^3, dan 10^5 CFU/mL, masing-masing diulang tiga kali. Aplikasi bakteri secara signifikan memengaruhi pertumbuhan fitoplankton dibandingkan dengan kontrol (0 CFU/mL). Kelimpahan fitoplankton tertinggi tercatat pada perlakuan 10^5 CFU/mL, yaitu 173.333 ± 10.007 cell/mL, indeks keanekaragaman sebesar 1,20, indeks keseragaman sebesar 0,67, dan dominasi sebesar 0,43. Peningkatan fitoplankton mendukung pertumbuhan mutlak *P. vannamei* sebesar 1,86 ± 0,02 g dengan tingkat kelangsungan hidup terbaik 86,67%. Meskipun secara statistik tidak berbeda nyata antar perlakuan (P>0,05), hasil ini menunjukkan perlakuan 10^5 memiliki potensi aplikasi yang relevan dan lebih efektif dalam menyediakan ortofosfat bagi fitoplankton.

Kata kunci: akuakultur, fitoplankton, ketersediaan ortofosfat, *Pseudomonas* sp., *P. vannamei*

INTRODUCTION

Phytoplankton are critical components of aquatic ecosystems (Juliyanto *et al.,* 2021). They serve as fertility indicators due to their role in biogeochemical processes supporting nutrient balance. Their role in aquaculture ecosystems is particularly important, especially in relation to phosphate, as they rely on phosphate to transfer energy into cells for growth and to increase biomass (Dyhrman, 2016; Muhammad *et al.,* 2023; Duhamel, 2025). Phytoplankton absorb phosphorus as dissolved inorganic phosphate, mainly as orthophosphate (PO_4^{3-}) in aquatic environments (Whitney & Lomas, 2019; Yakoob *et al.,* 2021). This nutrient is a central component in biogeochemical processes, breaking down into free compounds that influence nutrient distribution and cycling, such as enzyme formation, DNA, RNA, and ATP, and energy transfer processes that then become directly available to phytoplankton (Atiku *et al.,* 2016; Shome *et al.,* 2022).

Phosphorus is a crucial nutrient that often limits productivity in aquaculture systems (Aruna *et al.,* 2024; Solovchenko *et al.,* 2024). In aquaculture, phosphate sources can include the activity of aquatic organisms (Lou, 2022). However, as Aruna *et al.* (2024) noted, phosphorus in aquatic environments usually exists in limited, non-dissolved forms. This limitation is why phosphorus (P) is rarely found in free, dissolved forms as orthophosphate, making it less available for phytoplankton uptake (Jana, 2007; Fitzsimons *et al.,* 2020; Maslukah *et al.,* 2020). Regular phosphate fertilization is therefore necessary to boost water productivity. While phosphorus is present in aquaculture environments, as Aruna *et al.* (2024) state, fertilizers are still often needed to enhance productivity.

According to Jana (2007), phosphate fertilization can improve productivity in ponds. However, it has been estimated that only about 10% of the applied fertilizer effectively raises dissolved phosphate levels in the water, with the remainder precipitating into non-dissolved compounds, making it unavailable to phytoplankton. In Aquatic environments, phosphorus is rarely found in a dissolved form, specifically as orthophosphate (Aruna *et al.,* 2024). Adding phosphate-solubilizing bacteria (PSB) enhances the availability of phosphorus, converting it into a dissolved state within the water, which is readily accessible for phytoplankton use (Kartika *et al.,* 2024). This allows it to function as a primary producer, the foundation of the aquatic food chain, and indirectly supports pond productivity and the growth of *P. vannamei.*

To enhance productivity in aquaculture, ongoing advancements and innovations are continually introduced. One promising approach is using microbial technology to solubilize phosphate in aquaculture waters. Several phosphate-solubilizing bacteria, such as *Bacillus* sp. (Jana, 2007), *Rhizobium* sp. (Yang *et al.,* 2017), *Bacillus cereus* (Mawadah & Prabaningtyas, 2024), and *Pseudomonas* sp. (Luczkiewicz *et al.,* 2015), have been identified. *Rhizobium* and *Bacillus* have limitations; *Rhizobium* is known to be able to solubilize phosphate available to algae (Yang *et al.,* 2017). However, only a small number of *Rhizobium* strains show effectiveness and have limitations in producing enzymes and organic acids needed for efficient phosphate solubilization, especially in aquatic settings (Sridevi & Mallaiah, 2009).

Similarly, some *Bacillus* strains show unstable phosphate solubilization activity in aquaculture, especially in the face of ammonium stress, which is generally found in ponds (Adharani *et al.,* 2019; Lu *et al.,* 2023), which can affect the availability of orthophosphate for phytoplankton. Meanwhile, according to Asril and Lisafitri (2020), species within the *Pseudomonas* sp. genus, such as *P. cepacia*, *P. psychrotolerans*, *P. aeruginosa*, *P. oryzihabitans*, and *P. fluorescens*, are among the best phosphate solubilizers. However, research on the application of these bacteria in aquaculture, particularly their potential effects on phytoplankton in *P. vannamei* farming, remains limited. This study, therefore, focuses on the potential of *Pseudomonas* sp., a bacterium isolated from mangrove water, for application in *P. vannamei* rearing media to examine its impact on phytoplankton profiles, including changes in species composition and abundance.

In this study, *Pseudomonas* sp. was used as a result of previous isolation due to its high efficiency in breaking down complex phosphate compounds into simpler forms (Asril & Lisafitri, 2020), making phosphorus more readily absorbable by phytoplankton for cell growth. However, research on their use in aquaculture, especially their potential impact on phytoplankton in *P. vannamei* farming, remains limited (Vovk *et al.,* 2013; Aruna *et al.,* 2024). Thus, *Pseudomonas* sp. will be applied in *P. vannamei* rearing media to observe

its effects on phytoplankton profiles, including shifts in plankton composition, abundance, and other environmental parameters. Based on this background, this study aims to provide insights into the effect of *Pseudomonas* sp. as phosphate solubilization and its impact on the phytoplankton profile in the *P. vannamei* culture system.

MATERIALS AND METHODS

Bacterial preparation

Pseudomonas sp. was isolated from water samples taken from the mangrove area at BLUPP (Balai Layanan Usaha Produksi Perikanan) Karawang. Samples were diluted by the serial dilution technique from 10^{-1} to 10^{-6}. Then inoculated into ISP 4 media using the and incubated at 28°C for 24 to 48 hours (Duman *et al.,* 2021). Isolates were inoculated into Pikovskaya agar to assess the phosphate solubilization index based on the clear zones produced by the bacteria, following the spot inoculation method described by Ulfiyati and Zulaika (2015). The plates were then incubated at room temperature for seven days, with observations made every 24 hours. Phosphate solubilization was determined by the formation of clear zones around the bacterial colonies in Pikovskaya agar, and the diameters of both the clear zones and the colonies were measured using the formula provided by Asril and Lisafitri (2020).

$$\text{Phosphate solubility index} = \frac{\text{Clear zone} - \text{colony diameter}}{\text{Colony diameter}}$$

Molecular identification

Molecular identification of the bacteria was performed to determine their species. DNA extraction was carried out using the Presto™ Mini gDNA Bacterial Kit, followed by PCR amplification with primer pairs 16S rRNA F (5'-AGA-GTT-TGA-TCC-TGG-CTC-AG-3') and 16S rRNA R (5'-GGT-TAC-CTT-GTT-ACG-ACT-T-3'). The PCR products were then purified and verified using agarose gel electrophoresis, which was observed under a UV transilluminator. The extracted DNA was sent to First Base Company in Singapore for sequencing. The 16S rRNA gene sequences were analyzed using BioEdit software to assemble contigs (complementary sequences). The homology levels of these sequences were assessed against the database using the BLAST (Basic Local Alignment Search Tool) program available on the NCBI (National Center for Biotechnology Information).

Preparation and maintenance of the container

For 21 days, twelve containers, each measuring 50×30×22 cm^3. Before use, the containers were cleaned, washed, and dried. Each container was filled with 15 L of seawater and equipped with two aeration devices. *P. vannamei*, with an average weight of approximately ±2 g, were stocked at a density of 15 individuals per container (the shrimp were fed four times a day with pellet feed containing 40% protein).

Experimental design

The experimental design of this study was to evaluate the potential of *Pseudomonas* sp. bacteria as phosphate solubilizers in relation to phytoplankton growth in *P. vannamei* rearing media. This study was carried out over a 21-day observation period, consisting of four treatment doses: 10^1, 10^3, 10^5 CFU/mL, and one control group, each with three replicates.

Hemolytic activity test

The hemolytic activity test was conducted using Blood Agar media to determine whether the isolated bacteria are pathogenic to animals. The test bacteria were inoculated onto Blood Agar plates and incubated for 24 to 48 hours at 30°C. The presence of a clear zone around the colonies indicates that the bacteria are pathogenic to animals, while the absence of a clear zone suggests that the bacteria are not pathogenic (Hawaz, 2014).

Application of *Pseudomonas* sp. as phosphate-solubilizing bacteria in *P. vannamei* rearing water

Bacterial propagation was carried out following the method described by Paul and Sinha (2017). Pure isolates were transferred into Erlenmeyer flasks containing Pikovskaya broth and incubated in a rotary shaker at 180 rpm for 24 to 48 hours at 28°C. The cultures were then centrifuged at 10,000 rpm for 10 minutes. The application was conducted in each observation container according to the predetermined treatments. The containers were connected to aerators to ensure an adequate oxygen supply. Each container was filled with 15 liters of rearing seawater. The water used already contained naturally occurring phytoplankton.

Experimental parameters

Orthophosphate

This observation, orthophosphate measurements were taken by collecting 25 mL of water from the *P. vannamei* rearing tank and transferring it into a test tube (sample water in a filtered state using membrane paper). Each sample received 0.5 mL of ammonium molybdate solution along with four drops of $SnCl_2$ solution. The samples were left to stand for ±10 minutes before being tested with a spectrophotometer set to a wavelength of 690 nm. The method and formula used are based on APHA (2012), as follows:

$$\text{Phosphate (mg/L)} = \frac{\text{Abs sample} - \text{Abs blanko}}{\text{Abs standard} - \text{Abs blanko}} \times \text{Cst}$$

Note:
Abs sample = Absorbance of sample (mg/L)
Abs blank = Absorbance of blank (mg/L)
Abs standard = Absorbance of standard (mg/L)
Cst = Concentration of standard solution (mg/L)

Phytoplankton identification

Phytoplankton in this study developed naturally from the seawater used as rearing water without any phytoplankton inoculation. Observations were conducted at the beginning and end of the experiment using a hemocytometer. The sample was then covered with a cover glass for qualitative and quantitative analysis under a microscope with 100× magnification (Ferreira *et al.,* 2017; Katmoko *et al.,* 2021).

Phytoplankton abundance

The calculation of phytoplankton abundance is defined as the number of cells per liter of water, expressed in cell/mL, which indicates the number of cells found per mL of water. This is determined using the following equation (Risjani *et al.,* 2021):

$$\text{PD} = \frac{nA + nB + nC + nD}{4} \times 10{,}000$$

Note:
PD = Phytoplankton density
nA+nB+nC+nB = Number of cells block*s* A, B, C, and D

Diversity index

Describe the diversity of the phytoplankton population systematically using the Shannon-Wiener formula (Odum, 1993) as follows:

$$H' = \sum (i = 1) \text{ ^ } n \, (Pi \ln Pi)$$

Note:
H´ = Diversity index
P = ni/N
Ni = Number of individuals of the "i" species
N = Total number of individuals of all species

The comparison yields a value for H' that falls between 0 and 1:
H'<1 = Low (Unstable)
H' 1<E<3 = Medium (Quite stable)
H'>3 = High (Prime/Stable)

Uniformity index

The phytoplankton uniformity index is calculated using the following equation (Odum, 1993):

$$E = \frac{H'}{H \text{ max}}$$

Note:
E = Uniformity index
H' = Diversity index
H maks = Maximum diversity
S = Total number of species found

The comparison yields a value for E that falls between 0 and 1:
E<0.4 = Low uniformity
0.4<E<0.6 = Medium uniformity
E>0.6 = High uniformity

Dominance index

The dominance index is used to identify the dominant species in a body of water using the following formula (Odum, 1993):

$$c = \sum_{i=1}^{n} \left(\frac{ni}{N}\right)^2$$

Note:
C = Dominance index
ni = Number of individuals of the ith species
N = Total number of individuals

The ratio is considered high if C = 1. The interpretations for dominance are as follows:

C<0.50 = Low dominance
0.50<C<0.75 = Medium dominance
0.75<C<1 = High dominance

Absolute weight growth rate of P. vannamei

The calculation of absolute weight growth uses the formula according to Effendie (1997):

$$Wm = W_t - W_0$$

Note:
Wm = Absolute weight growth rate (g)
Wt = Shrimp weight at the end of the study (g)
Wo = Shrimp weight at the start of the study (g)

Data analysis

The analysis of bacterial application data utilized a Completely Randomized Design, and the results were tabulated in Microsoft Excel. Subsequently, the data were analyzed using SPSS 26. ANOVA test results that showed significant differences (P<0.05) were further examined using the Duncan test with a 95% confidence interval.

RESULT AND DISCUSSION

Result

Pseudomonas sp.

The Pseudomonas sp. isolate in this study was labeled with the sample code AHN.4. The bacteria were successfully isolated on ISP 4 medium agar, displaying a unique colony morphology with a yellow color. The bacteria were tested for their ability to dissolve phosphate using Pikovskaya agar, where the isolate successfully produced a clear zone with an index of 2.4 mm (Figure 1).

Molecular identification

The phylogenetic tree analysis indicates that the isolated bacteria for application are closely related to MN894061.1 *P. oryzihabitans* (bootstrap value 89), as they share the same branch. In contrast, MT184863.1 *P. aeruginosa*, MT367856.1 *P. psychrotolerans*, and MK685113.1 *P. putida* exhibit relatively long branches, suggesting a longer evolutionary distance. However, these species have higher bootstrap values compared to MN894061.1 *P. oryzihabitans*, indicating an evolutionary relationship among the three species (Figure 2).

Hemolytic activity test

According to Hawaz (2014), the presence of a clear zone around bacterial colonies indicates pathogenicity to animals, whereas its absence suggests that the bacteria are non-pathogenic. In this study, no hemolytic activity was found, nor was there a clear zone around the colony, indicating that the bacteria are safe for use in *P. vannamei* rearing media.

Application of Pseudomonas sp. as a phosphate solubilizer

Orthophosphate

The dynamics of orthophosphate concentrations in the *P. vannamei* rearing media are shown in Figure 4. The graph indicates fluctuations throughout the observation period. On D6, the orthophosphate levels in all treatments increased significantly (P<0.05). By D11, the concentrations in the control and 10^5 treatments rose, while those in 10^1 and 10^3 decreased. On D16, most treatments showed a decline, except for 10^3, which experienced an increase. Finally, on day 20, all treatments recorded a significant decrease (P<0.05). Although all treatments

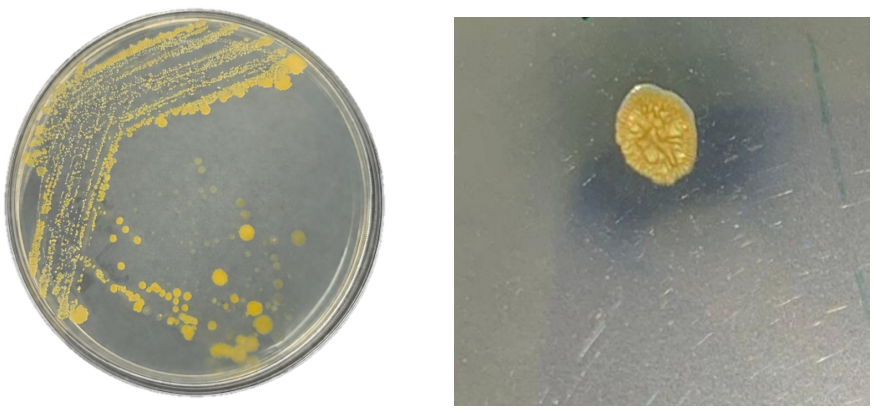

Figure 1. A. *Pseudomonas* AHN.4 in ISP 4 agar; B. clear zone on Pikovskaya agar.

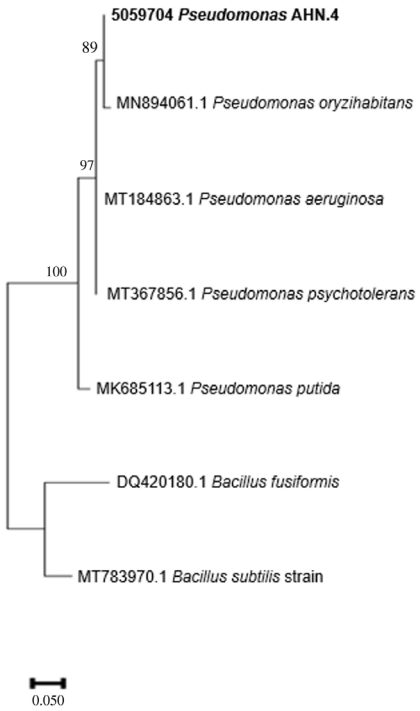

Figure 2. Phylogenetic analysis of the 16S rRNA gene in *Pseudomonas* sp. as phosphate solubilizers for application in *P. vannamei* rearing.

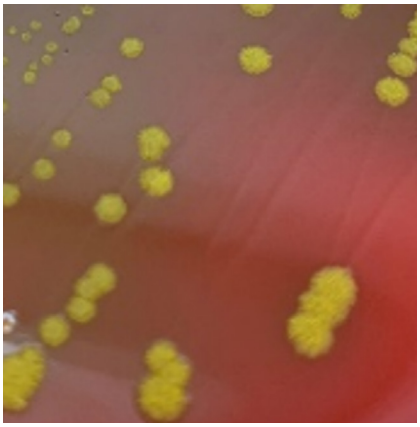

Figure 3. Hemolytic activity of *Pseudomonas* AHN.4 on blood agar media as a phosphate-solubilizing bacterium for application in *P. vannamei* rearing.

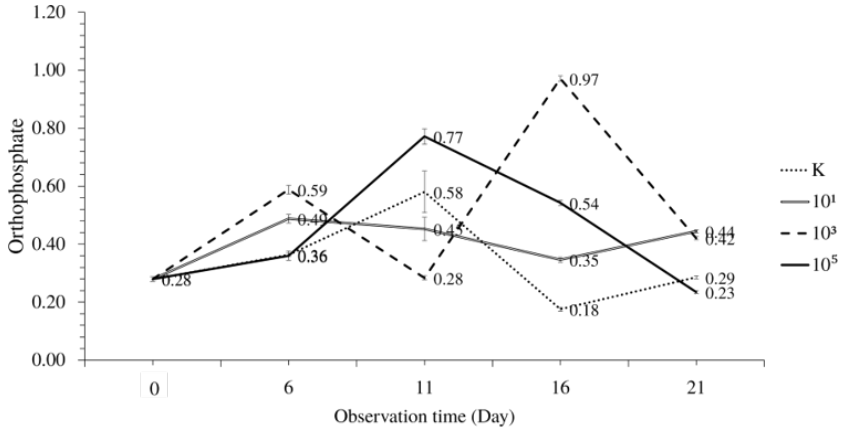

Figure 4**.** Orthophosphate dynamics in *P. vannamei* rearing media treated with the phosphate-solubilizing bacterium *Pseudomonaas* AHN.4.

showed fluctuations, the 10^5 treatment showed more stable levels of orthophosphate availability compared to the others because it was utilized by phytoplankton. The concentration values of orthophosphate in the *P. vannamei* rearing media are shown in Table 1.

Phytoplankton abundance

During the observations, seven genera of phytoplankton were identified: 3 from Chlorophyceae (*Chlorella*, *Tetrasentrum*, *Coelastrum*), 2 from Cyanophyceae (*Oscillatoria* and *Spirulina*), and 2 from Bacillariophyceae (*Cymbella* and *Navicula*) (Figure 5). The graph shows that Chlorella was more abundant than the other genera. Overall, the 10^5 CFU/mL treatment resulted in the highest abundance at the end of the observation, followed by 10^3 CFU/mL, while the lowest abundance was found in the control and 10^1 CFU/mL treatments.

Phytoplankton index

During the observation, phytoplankton index, including uniformity, diversity, and dominance index were calculated. The index values obtained are presented in the following Table 3.

Survival rate of *P. vannamei*

The survival rate of vannamei shrimp during the observation period is presented in Table 4.

Table 1. Orthophosphate availability in *P. vannamei* rearing media treated with the phosphate-solubilizing bacterium *Pseudomonas* AHN.

Treatment (CFU/mL)	D0	D6	D11	D16	D21
K	0.28 ± 0.01^{a}	0.36 ± 0.01^{a}	0.58 ± 0.07^{c}	0.18 ± 0.01^{a}	0.29 ± 0.005^{b}
10^1	0.28 ± 0.01^{a}	0.49 ± 0.02^{b}	0.45 ± 0.04^{b}	0.35 ± 0.01^{b}	0.44 ± 0.005^{d}
10^3	0.28 ± 0.01^{a}	0.59 ± 0.02^{c}	0.28 ± 0.01^{a}	0.97 ± 0.01^{c}	0.42 ± 0.005^{c}
10^5	0.28 ± 0.01^{a}	0.36 ± 0.02^{a}	0.77 ± 0.03^{d}	0.54 ± 0.01^{d}	0.23 ± 0.005^{a}

Note: Different superscript letters indicate significantly different results between treatments on the same observation based on the Duncan test (P<0.05).

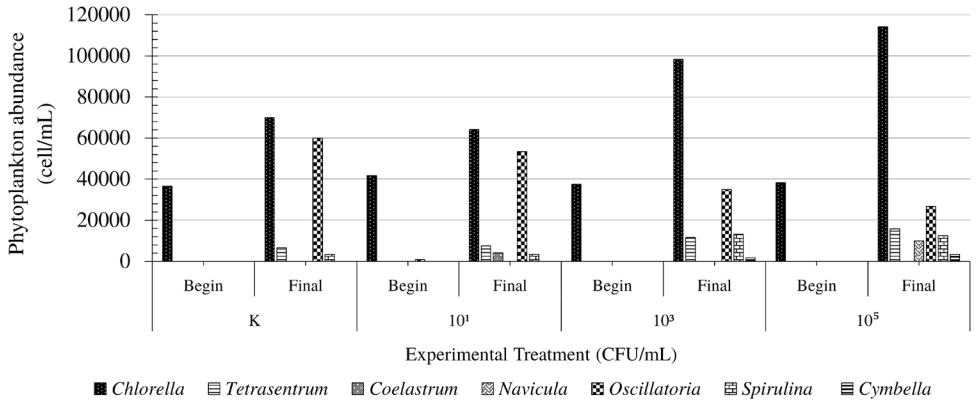

Figure 5. Phytoplankton abundance (cell/mL) in *P. vannamei* rearing media treated with the phosphate-solubilizing bacterium AHN.4.

Table 2. Total phytoplankton abundance (cell/mL) in *P. vannamei* rearing media with application of *Pseudomonas* AHN.4 as phosphate solubilizer.

Treatment (CFU/mL)	Begin (cell/mL)	Final (cell/mL)
K	36,667 ± 8,036^{a}	140,000 ± 5,00^{0a}
10^1	42,500 ± 11,456^{a}	132,500 ± 2,50^{0a}
10^3	37,500 ± 2,500^{a}	162,500 ± 13,22^{8b}
10^5	38,333 ± 3,828^{a}	173,333 ± 10,00^{6c}

Note: Different superscript letters indicate significantly different results between treatments on the same observation based on the Duncan test (P<0.05).

Treatment 10^5 showed a significant difference (86.67 ± 6.65%; P<0.05) compared to the control (53.33 ± 13.35%) and treatment 10^1 (60.00 ± 6.70%) but was not significantly different from treatment 10^3 (60.00 ± 6.70%; P>0.05). Although treatment 10^3 was not significantly different from 10^5, treatment 10^5 showed the lowest mortality rate, as indicated by the highest survival percentage (86.67 ± 6.65%).

Absolute weight growth rate of *P. vannamei*

Observation of *P. vannamei* growth during the 21-day period showed an increase in growth in all treatments, as shown in Table 5. However, the ANOVA test results showed no significant difference (P>0.05) among the treatments.

Discussion

Pseudomonas sp. is a bacterium that effectively dissolves phosphate and is commonly found in terrestrial and aquatic environments (Asril & Lisafitri, 2020). In this study, molecular identification was performed to determine the bacterial species, revealing a close relationship with *P. oryzihabitans*. The isolate was also able to solubilize phosphate in Pikovskaya medium, which contains the compound $Ca_3(PO_4)_2$. The formation of a clear zone around the bacterial colony on the medium indicates that the isolate is capable of producing extracellular organic acids that react with calcium ions (Ca^{2+}) in $Ca_3(PO_4)_2$, thereby releasing $H_2PO_4^-$ ions (Tarigan *et al.,* 2023; Zulkifli *et al.,* 2020). This reaction leads to the formation of a transparent area around the colony, serving as an indicator of phosphate-solubilizing activity (Figure 1) (Sudewi *et al.,* 2020; Sonia & Setiawati, 2022).

Morphologically, the isolate exhibits a rough and wrinkled colony surface, which is one of its distinguishing characteristics. Furthermore, the hemolysis test showed that this bacterium does not possess hemolytic activity, indicating that it is non-pathogenic and safe for use with research biota (Putri *et al.,* 2024). The phosphatase enzyme and organic acid ability possessed by phosphate-solubilizing bacteria can convert insoluble

Table 3. Phytoplankton index in *P. vannamei* rearing with application of *Pseudomonas* AHN.4 as phosphate solubilizer.

Treatment	H'	Category	E	Category	D	Category
K	0.94	Medium	0.68	High	0.44	Low
10^1	1.08	Medium	0.67	High	0.40	Low
10^3	1.08	Medium	0.67	High	0.44	Low
10^5	1.20	Medium	0.67	High	0.43	Low

Note: H' (Diversity index), E (Uniformity index), D (Dominance index).

Table 4. Survival rate of *P. vannamei* shrimp in rearing media with the application of *Pseudomonas* AHN.4 as phosphate solubilizers.

Treatment	Survival Rate (%)
K	53.33 ± 13.35^{a}
10^1	60.00 ± 6.70^{ab}
10^3	73.33 ± 6.65^{bc}
10^5	86.67 ± 6.65^{c}

Different superscript letters indicate significant differences in treatment effects within each observation based on the Duncan test (P<0.05).

Table 5. *P. vannamei* absolute weight growth with application of *Pseudomonas* AHN.4 as phosphate solubilizer.

Treatment	Growth (g)
K	1.79 ± 0.06
10^1	1.80 ± 0.06
10^3	1.82 ± 0.02
10^5	1.86 ± 0.02

The results between treatments on the same observation based on the Duncan test indicated no significant differences (P>0.05).

phosphate into a dissolved form (Asril & Lisafitri, 2020; Aruna *et al.,* 2024), making it readily available for phytoplankton to support their cell growth and productivity. However, phosphorus in aquatic environments is often scarce and plays a critical role.

According to Jana (2007) and Aruna *et al.* (2024), the availability of phosphorus in water is limited but essential, as it significantly influences the natural productivity of aquatic ecosystems. One source of phosphorus in aquaculture environments comes from residual organic matter, such as uneaten feed, which quickly settles at the bottom and forms insoluble compounds. Even with the application of fertilizers, Jana (2007) notes that phosphate levels may remain insufficient because only a small portion dissolves in water, while the rest accumulates at the bottom. By utilizing PSB, phosphate bound in insoluble forms can be transformed into a dissolved state through enzymatic activity. The phosphatase enzyme plays a key role in this process by breaking down complex phosphate compounds into orthophosphate, which phytoplankton can easily absorb and use for growth (Jana, 2007).

The application of *Pseudomonas* AHN.4 in *P. vannamei* rearing media showed that orthophosphate concentration increased in all treatments, from the D0 to D6 periods, likely due to organic acid secretion and phosphatase enzyme activity by bacteria that dissolve phosphate, making it more accessible to phytoplankton (Aruna *et al.,* 2024). At D6, treatment 10^3 had the highest orthophosphate levels among the treatments, indicating that bacteria may have been functioning optimally with few limiting factors. In contrast, treatment 10^5 showed the lowest orthophosphate levels, similar to the control. This could be due to competition among the higher density of bacteria, which may limit nutrient availability, affecting bacterial metabolism and enzyme production necessary for phosphate solubilization (Satyantini *et al.,* 2020). Between the D6 and D11 periods, treatment 10^5 experienced a significant increase in orthophosphate, suggesting that bacterial activity had started converting available phosphate into a usable form.

According to Satyantini *et al.* (2020) and Zhou *et al.* (2023), microbial applications can enhance nutrient availability in *P. vannamei* media, influenced by microbial metabolic activities. Conversely, treatment 10^3 showed a decrease during this period, potentially due to earlier uptake of phosphorus by phytoplankton, which led to limited phosphate availability in the medium by D11, reducing bacterial activity as they competed with phytoplankton for orthophosphate. Satyantini *et al.* (2020) and Luckwambe *et al.* (2019) both support that nutrient reduction can result from bacteria and phytoplankton fulfilling their metabolic needs. From D11 to D16, orthophosphate levels in treatment 10^5 began to decrease, indicating that phytoplankton effectively utilized dissolved phosphate, corresponding with a significant increase in phytoplankton abundance by the end of the study. Meanwhile, treatment 10^3 showed a sharp increase in D16, possibly reflecting resumed bacterial phosphate solubilization activity after a temporary slowdown. Effendi (2003) notes that phosphate transformation into dissolved orthophosphate occurs faster in water containing bacteria, as observed here with the higher bacterial dose in treatment 10^5, accelerating phosphate solubilization and availability in the shrimp media by the D11 period.

This study, although orthophosphate levels in treatment 10^3 were higher during the D5 period, while the other treatments remained relatively low, all treatments still showed a significant increase in the next period. According to Satyantini *et al.* (2020), low initial nutrient levels are often due to bacterial adaptation to new surroundings. Orthophosphate levels in the control and treatment 10^1 showed minor fluctuations, resulting in lower nutrient availability for phytoplankton compared to other treatments, underscoring the role of *Pseudomonas* AHN. 4 in promoting phosphate solubilization. At the end of the observation (D21), orthophosphate concentrations declined across all treatments, with treatment 10^5 showing the lowest final concentration at 0.23 mg/L. This decline is likely due to phytoplankton utilizing available nutrients, as Aziz *et al.* (2015) report a drop in phosphorus due to phytoplankton uptake.

Satyantini *et al.* (2020) and Hastuti *et al.* (2023) similarly note that reduced nutrient and phosphorus levels may result from microbial and phytoplankton phosphorus uptake for metabolism. Additionally, Mawadah and Prabaningtyas (2024) suggest that phosphate-solubilizing bacteria possess phosphatase enzymes that break down organic phosphate compounds, thereby enhancing phosphate availability for microalgae growth.

This is consistent with the highest phytoplankton growth observed in treatment 10^5 CFU/mL. The application of *Pseudomonas* AHN.4 as PSB resulted in the presence of phytoplankton from the genera Chlorophyceae (*Chlorella, Tetrasentrum, Coelastrum, Navicula*), Cyanophyceae (*Oscillatoria, Spirulina*), and Bacillariophyceae (*Cymbella*) in nearly every treatment (Figure 5). Among these, the Chlorophyceae genus was the most abundant phytoplankton group. Overall, the highest total abundance of phytoplankton was observed in the 10^5 CFU/mL treatment, reaching 173,333 cell/mL, followed by the 10^3 CFU/mL treatment with 162,500 cell/mL. These results were significantly different ($P<0.05$), but no significant difference ($P>0.05$) was found between the control (140,000 cell/mL) and the 10^1 CFU/mL treatment (132,500 cell/mL).

The observations also revealed that the phytoplankton species composition was similar across treatments. However, the 10^5 CFU/mL treatment exhibited the greatest species diversity, followed by the 10^3 CFU/mL treatment. According to Palupi *et al.* (2022), greater phytoplankton diversity in aquaculture waters indicates stable water conditions, which can enhance productivity and shrimp growth. The highest abundance of phytoplankton in the 10^5 CFU/mL treatment can be attributed to the sufficient number of bacteria applied to the *P. vannamei* rearing medium. This is consistent with the findings of Dong *et al.* (2022), who reported that the application of *Paenibacillus xylanexedens* cultured alongside *Chlorella pyrenoidosa* led to an increase in algal biomass.

According to Fuentes *et al.* (2016) and Satyantini *et al.* (2020), the high abundance of phytoplankton can be attributed to the application of bacteria that degrade organic matter, making nutrients and phosphates more accessible for phytoplankton cell growth. These bacteria effectively degraded organic matter, providing nutrients and phosphorus that supported phytoplankton growth (Khan *et al.,* 2018; Satyantini *et al.,* 2020). The diversity index (H') was calculated periodically to assess the environmental conditions for aquatic biota (Akbarurrasyid *et al.,* 2022). The range from a low of 0.94 in the control treatment to a high of 1.20 in the 10^5 treatments. According to Samadan and Andriani (2020), an H' range of 1–3 is considered moderate, indicating a stable presence of phytoplankton and favorable water quality for *P. vannamei* rearing, despite the dominance of *Chlorella*. The lower H' value in the control suggests that the rearing water may be slightly polluted, leading to a disturbed phytoplankton community due to environmental factors (Samadan & Andriani, 2020).

According to Akbarurrasyid *et al.* (2022), the uniformity index (E) is calculated to assess the uniformity of phytoplankton in the water. Observations shown in Table 3 indicate that all treatments fall within a high uniformity category, with values ranging from 0.67 to 0.68 ($E>0.6$). This suggests that the phytoplankton distribution across treatments is relatively even, with no significant differences. The dominance index (D) indicates the level of dominance of specific species within the aquatic environment. The observed D values for each treatment ranged from 0.40 to 0.44 (Table 3), showing only slight variations. These values fall into the low category ($0<D\leq0.5$). According to Akbarurrasyid *et al.* (2022), a lower D value suggests a more stable phytoplankton community.

The application of *Pseudomonas* AHN.4 as a phosphate-solubilizing agent in this study resulted in the highest survival rate in the 10^5 treatment, reaching 86.67%. ANOVA results indicated that the 10^3 treatment was not significantly different from 10^5 ($P>0.05$); however, the highest survival percentage was still observed in the 10^5 group. The low survival rates in the control and 10^1 treatments are likely associated with the high dominance of *Cyanophyceae*, particularly the genus *Oscillatoria*, which accounted for 42.86% and 40.25%, respectively. This genus is known to produce toxic compounds that can lead to the mortality of aquatic organisms. As reported by Aliviyanti *et al.* (2017) and Aklakur *et al.* (2023), the presence of *Cyanophyceae* may exert sublethal effects on growth, physiology, and survival, as well as contribute to both abiotic and biotic stress in aquatic environments.

Overall, the observed trends confirm a strong functional relationship between orthophosphate availability and phytoplankton development and reinforce the role of *Pseudomonas* sp. as a biofertilizer in enhancing primary productivity in aquaculture systems. The presence of phytoplankton in the rearing medium also plays an important role, because it can naturally be a source of nutrients for *P. vannamei*, which has the potential to stimulate their growth. However, this growth was not significantly different ($P>0.05$)

between the control and treatment using bacteria, this is because shrimp tend to consume, filter, and absorb all edible particles found in the sediment and water column of rearing containers (Widowati *et al.,* 2022). In addition, as reported by Arifin *et al.* (2017) and Kamilia *et al.* (2021), members of the Chlorophyceae and Bacillariophyceae groups contain nutrients that can support shrimp growth. Although the difference was not statistically significant, the 10^5 treatment shows relevant application potential and warrants further investigation over a longer experimental period.

CONCLUSION

Based on this research, *Pseudomonas* AHN.4 was closely related to *P. oryzihabitans* and can dissolve phosphate with an index of 2.4 mm in Pikovskaya agar. *Pseudomonas* AHN.4, as a phosphate-solubilizing bacterium, effectively increased orthophosphate availability in the rearing medium over a 21-day period, supporting both the growth and diversity of phytoplankton, particularly Chlorella. The treatment with 10^5 CFU/mL yielded the best outcomes in terms of phosphate concentration (0.23 mg/L), phytoplankton abundance (173,333 ± 10,006 cell/mL), diversity index (H' = 1.20), evenness index (E = 0.67), and dominance index (D = 0.43). These findings suggest that Pseudomonas AHN.4 has strong potential as a biofertilizer to enhance productivity in *P. vannamei* culture systems.

ACKNOWLEDGMENTS

We would like to express our gratitude to the laboratory team at the Laboratory of Aquatic Organism Health and the Environmental Laboratory at IPB University for their assistance during this research.

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