Jurnal Akuakultur Indonesia
Research Article
ISSN: 1412-5269
EISSN: 2354-6700
Vol. 25 No. 2, July 2026
DOI: 10.19027/jai.25.2.232-246

The effectiveness of mangrove endophytic fungal metabolite *Penicillium* sp. KTR58 on the growth and immune response of Pacific white shrimp *Litopenaeus vannamei*

Efektivitas metabolit fungi endofit mangrove *Penicillium* sp. KTR58 terhadap pertumbuhan dan respon imun udang vaname *Litopenaeus vannamei*

Putri Agustina[1], Dinamella Wahjuningrum[1*], Kustiariyah Tarman[2], Munti Yuhana[1], Sri Nuryati[1]

[1]Department of Aquaculture, Faculty of Fisheries and Marine Sciences, IPB University, Bogor, West Java 16680, Indonesia
[2]Department of Aquatic Products Technology, Faculty of Fisheries and Marine Sciences, IPB University, Bogor, West Java 16680, Indonesia
*Corresponding author: dinamellawa@apps.ipb.ac.id

(Received November 3, 2025; Revised December 30, 2025; Accepted February 3, 2026)

ABSTRACT

Mangrove endophytic fungi have the potential to act as immunostimulants for shrimp and antibacterial agents against *V. parahaemolyticus*, including *Penicillium* sp. KTR58. This study aimed to determine the effect of adding *Penicillium* sp. KTR58 metabolites on the growth and immune response of Pacific white shrimp. The research consisted of *in vitro* and *in vivo* tests. The shrimp were maintained for 30 days using a completely randomized design with four treatments, namely K (0), P10 (10), P20 (20), and P30 (30 mL/kg feed), each with three replicates. In vitro test showed that the highest inhibition zone at 30 µL/disc treatment and thin-layer chromatography indicated the presence of steroid, flavonoid, terpenoid, saponin, coumarin, and alkaloid compounds. The compounds showing antibacterial activity from the bioautography test were suspected to be coumarins, flavonoids, alkaloids, saponins, and steroids. Treatment P20 showed the best growth performance and immune response, with a final weight of 8.87 ± 0.16 g, final biomass of 123.79 ± 8.1 g, a specific growth rate of 3.96 ± 0.18 %/day, and a feed conversion ratio of 1.45 ± 0.05, these values were significantly different from the control (P<0.01). The total haemocyte count, phagocytosis activity, respiratory burst, and phenoloxidase values were $9.33\times10^6 \pm 0.31\times10^6$cells/mL, 49.73 ± 1.89%, 0.31 ± 0.01, 0.29 ± 0.002 respectively, and were significantly different from the control (P<0.05). Based on these results, the optimal dose of *Penicillium* sp. KTR58 metabolite addition in Pacific white shrimp feed is 20 mL/kg.

Keywords: acute hepatopancreatic necrosis disease (AHPND), *Penicillium* sp. KTR58, shrimp immunostimulant, *Vibrio parahaemolyticus*

ABSTRAK

Fungi endofit mangrove memiliki potensi sebagai imunostimulan udang dan antibakteri untuk V.parahaemolyticus salah satunya adalah *Penicillium* sp. KTR58. Tujuan penelitian ini adalah untuk mengetahui pengaruh penambahan metabolit *Penicillium* sp. KTR58 terhadap pertumbuhan dan respon imun udang vaname. Tahapan penelitian terdiri dari uji in vitro dan uji in vivo. Tahap pemeliharaan udang selama 30 hari menggunakan rancangan acak lengkap (RAL) empat perlakuan yaitu K (0 mL/kg), P10 (10 mL/kg), P20 (20 mL/kg), P30 (30 mL/kg pakan) masing-masing tiga ulangan. Hasil uji in vitro menunjukkan zona hambat tertinggi pada perlakuan 30 µL/disc, dan hasil uji kromatografi lapis tipis mengindikasikan adanya senyawa steroid, flavonoid, terpenoid, saponin, kumarin dan alkaloid. Senyawa yang menunjukkan aktivitas antibakteri dari hasil uji bioautografi diduga sebagai kumarin, flavonoid, alkaloid, saponin dan steroid. Perlakuan P20 menunjukkan performa pertumbuhan dan respon imun terbaik dengan nilai bobot akhir yaitu 8.87 ± 0.16 g, biomas akhir sebesar 123,79 ± 8,1 g, laju pertumbuhan spesifik 3.96 ± 0.18 %/hari, rasio konversi pakan adalah 1.45 ± 0.05, nilai tersebut berbeda nyata dengan kontrol (P<0.01). Nilai Total hemosit, aktivitas fagositosis, respiratory burst dan phenoloxidase secara berturut-turut yaitu $9.33\times10^6 \pm 0.31\times10^6$cells/mL, 49.73 ± 1.89%, 0.31 ± 0.01, 0.29 ± 0.002 dan berbeda nyata dengan kontrol (P<0.05). Berdasarkan hasil tersebut, dosis terbaik penambahan metabolit *Penicillium* sp. KTR58 pada pakan udang vaname adalah 20 mL/kg.

Kata kunci: acute hepatopancreatic necrosis disease (AHPND), imunostimulan udang, *Penicillium* sp. KTR58, *Vibrio parahaemolyticus*

INTRODUCTION

Pacific white shrimp (*Litopenaeus vannamei*) or white leg shrimp were first introduced in Indonesia in 2001 and now account for more than 75 percent of Indonesia's total shrimp production. Indonesia's total shrimp production in 2022 reached 1.19 million tons (KKP, 2024) and became the world's fifth largest producer in 2022 (FAO, 2023). The value of Indonesia's shrimp exports reached 165,557 tons in 2024 (KKP, 2024). This proves that Indonesia has enormous potential in developing Pacific white shrimp farming. Intensive farming has become one of the efforts to develop Pacific white shrimp production, although various obstacles are still encountered, such as acute hepatopancreatic necrosis disease (AHPND) (Yuhana & Afiff, 2023; Valente & Wan, 2021). AHPND previously known as early mortality syndrome (EMS), is one of the deadly bacterial diseases that commonly attacks various shrimp species (Navaneeth *et al.,* 2020). *Vibrio parahaemolyticus*, the causative agent of the disease, has caused high socioeconomic losses in several countries (Tang & Bondad-Reantaso, 2019), adversely affecting aquaculture production and posing a risk to global food security.

Prevention and treatment strategies have been widely implemented, one of the most common being the use of antibiotics. However, this practice can increase the number of resistant pathogens in aquaculture necessitating new strategies to control pathogens (Mohan *et al.,* 2019a). Potentially more effective, economical, and environmentally safe alternatives to antibiotics include vaccination, water quality control include biofloc technology (Gustilatov *et al.,* 2024), bacteriophage therapy, biosecurity protocols, treatment using plant extracts, immunostimulants (Latif *et al.,* 2022), and natural substances in the form of fungal metabolites (Wahjuningrum *et al.,* 2025). Fungal secondary metabolites are chemical compounds produced by fungi that are not essential for fungal growth, but provide adaptive advantages in their growing environment, and have remarkable chemical diversity including four main families, namely terpenoids, polyketides, non-ribosomal peptides, or combinations thereof (Jinna *et al.* 2025). These secondary metabolites, which include pigments, antibiotics, and bioactive compounds, offer various benefits for aquaculture (Onomu & Okuthe, 2024).

The exploration of natural materials such as fungi is both a challenge and a solution in reducing the impact of AHPND in shrimp through the mechanism of boosting the immune system. Higher fungi are the most important source of various natural products (Mohan *et al.,* 2019a). An example of a secondary metabolite used in aquaculture is the fungus *Nodulisporium* sp. KT29, which is known to improve the production performance of Pacific white shrimp cultivated in the sea (Saputra *et al.,* 2016) and inhibit the growth of *Vibrio harveyi* bacteria (Hariati *et al.,* 2018). Treatment with *Nodulisporium* sp. KT29 supplementation induced by dead *V. harveyi* cells at a dose of 20 mL/kg can increase the resistance and immune system of Pacific white shrimp against vibriosis control in the sea (Wahjuningrum *et al.,* 2020). Furthermore, at low doses, this metabolite can increase the production and innate immunity of Pacific white shrimp against co-infection with WSSV and *V. harveyi* (Wahjuningrum *et al.,* 2022).

In this study, the exploration of the utilization of fungal secondary metabolites in Pacific white shrimp farming was conducted again using the fungus *Penicillium* sp. KTR58. This fungus is an endophytic fungus of the mangrove *Rhizophora mucronata*, isolated from leaves originating from Gresik, East Java. *Penicillium* sp. is known to have considerable potential for producing bioactive compounds that are beneficial for Pacific white shrimp farming (Prihanto 2012; Mukhlis *et al.* 2018). *Penicillium* sp. KTR58 is an endophytic fungus that is non-toxic to other organisms, making it safe to use and containing active ingredients that are antibacterial. Exploration of mangrove endophytic fungi was conducted to discover substances that can inhibit *Vibrio parahaemolyticus* bacteria, as well as β-glucan compounds that function as immunostimulants. One fungus that has potential as an antibacterial agent against *V. parahaemolyticus* is *Penicillium* sp. KTR58. The purpose of this study was to determine the effect of adding *Penicillium* sp. KTR58 metabolites on the growth and immune response of Pacific white shrimp.

MATERIALS AND METHODS

Materials

The fungal isolation used in this study was the mangrove endophytic fungal isolate *Penicillium* sp. KTR58 from the Aquatic Product Microbiology Laboratory, Department of Aquatic Product Technology (THP), FPIK, IPB. The test animals used were Pacific white shrimp *L.*

vannamei weighing 2.73 ± 0.14 g from the Suri Tani Pemuka Hatchery, Anyer. The bacteria used for the antibacterial test were rifampicin-resistant (Rf[R]) *V. parahaemolyticus* bacteria obtained from the Aquatic Organism Health Laboratory, Department of Aquaculture, Faculty of Fisheries and Marine Sciences, IPB University.

Preparation of *Penicillium* sp. KTR58 metabolites

The rejuvenation of *Penicillium* sp. KTR58 refers to the method described by Tarman *et al.* (2011). The fungal isolates were placed in sterile potato dextrose agar (PDA) medium and incubated at room temperature for seven days. The rejuvenated fungi were precultured in 100 mL of potato dextrose broth (PDB) for seven days at room temperature and shaken at a speed of 120 rpm. The preculture yield was taken at 12.5 mL and transferred to 250 mL of PDB medium, incubated at room temperature for 14 days under shaking conditions at a speed of 120 rpm. The culture was harvested and filtered using Whatman filter paper (mesh size 0.45 µm), then evaporated (at 40°C) using a rotary evaporator for 1 hour until 50 mL of fungal metabolites remained.

The evaporation results were used in the experimental feed, while for the antibacterial activity test, maceration was carried out using ethyl acetate (EA) solvent at a ratio of 1:2 (100 mL of *Penicillium* sp. KTR58 metabolite: 200 mL of ethyl acetate) for 3×24 hours under shaking conditions at 120 rpm. Then evaporated and concentrated to 1 mL. Analysis of saponin and polyphenol content was performed using high performance liquid chromatography (HPLC). It was found that the saponin content was 145.63 mg/g and the polyphenol content was 42.75 mg GAE/g. Analysis of phytosterol content was performed using spectrophotometry, with a result of 14.28 mg/100 g.

Preparation of *V.parahaemolyticus*

Bacterial isolates were cultured in test tubes containing 5 mL of complete seawater agar (SWC) medium and incubated at 28°C for 24 hours. Identification of *V. parahaemolyticus* was performed molecularly using polymerase chain reaction (PCR) referring to Persing *et al.* (2016), by extracting and amplifying *V. parahaemolyticus* bacterial DNA with specific primers for the *pirA* and *pirB* genes. The amplification results were visualized using agar gel electrophoresis.

Antibacterial activity assay

The antibacterial activity assay uses the Kirby-Bauer or paper disc method (Lay, 1994). A total of 50 µL of *V. parahaemolyticus* bacterial suspension that had reached a density of 10^8 CFU/mL was spread evenly on the surface of Mueller Hinton Agar (MHA) (CLSI, 2020). The medium was left for five minutes until the surface of the medium was dry. The test was carried out by placing a 6 mm diameter disc impregnated with *Penicillium* sp. KTR58 metabolite. The treatments used were 10 µL/disc, 20 µL/disc and 30 µL/disc, each with 3 replicates. Oxytetracycline (OTC) 1% was used as a positive control and ethyl acetate 20 µL/disc (the extraction solvent) was used as a negative control. The bacteria were incubated at 37°C for 24 hours. The antibacterial properties were determined by measuring the inhibition zone around the paper disc and measuring the diameter of the inhibition area (Tarman, 2011).

Thin layer chromatography (TLC)

The eluent used in the TLC assay is a mixture of acetone, ethyl acetate, n-hexane in a ratio of 4:25:18 referring to the Tarman (2011) method. Ten microliters of a 0.5 mg extract dissolved in 100 µL EA was spotted on a 20×20 cm alumina oxide silica gel PF254 TLC plate (Merck) to detect spots, then eluted with the selected eluent ratio. The plate was dried and sprayed with an anisaldehyde sulfuric acid color reagent (0.2 mL anisaldehyde, 2 mL glacial acetic acid, 17 mL methanol, 1 mL concentrated sulfuric acid). The plate was then heated at 110°C for 10 minutes. The spots that appeared were observed under 366 nm UV light and the retardation factor (Rf) value was calculated.

TLC was also performed to detect beta-glucan compounds using a saturated acetonitrile:butanol:aquadest (3:6:1) eluent. Plates spotted with samples were developed in a chamber until the eluent reached the end of the elution. The elution results were then observed using UV light at 254 nm and 366 nm, and the Rf values were calculated and compared with the Rf value of the barley standard. The barley standard is a beta-glucan polysaccharide compound obtained from barley plants with an Rf value of 0.625 (Utami *et al.,* 2016).

Bioautography assay

The bioautography assay was performed after the extract was fractionated using TLC. 20

microliters of the extract was spotted on a TLC plate, then eluted with the eluent in the TLC assay. The eluted TLC plate is placed on SWC agar medium mixed with *V. parahaemolyticus* bacterial suspension, incubated at 37°C for 24 hours and the inhibition zone is measured. Thin-layer chromatography (TLC) bioautography is an evolving technology that integrates the separation and analysis technology of TLC with biological activity detection technology (Wang *et al.,* 2021a).

Protease activity assay

The protease activity assay uses the plug agar method. This test uses PDA media made with shrimp broth and PDA made with 1% skim milk (Sedijani *et al.,* 2023). Each pure fungal isolate was cut using a blue tip and dabbed on PDA media, then incubated for 24-48 hours at 37°C. The presence of protease enzyme activity was indicated by the formation of a clear zone around the fungal colony after incubation (Queendy & Roza, 2019). The ability of fungi to produce protease can be indicated by the proteolytic index. The proteolytic index was the ratio between the diameter of the clear zone and the diameter of the colony to obtain potential isolates (Kabense, 2019). The proteolytic index was formulated as follows:

$$IP = \frac{\text{Clear zone diameter}}{\text{Colony diameter}}$$

Experimental feed preparation

The experimental feed consisted of commercial feed coated with *Penicillium* sp. KTR58 metabolites. Commercial feed pellets were spread on trays. The metabolite dosage used was based on the results of antibacterial assay that had been standardized with the metabolite prior to extraction, yielding dosages of 10, 20, and 30 mL/kg of feed supplemented with 2% egg white as a binder (Djauhari *et al.,* 2016). Each was sprayed onto the feed pellets using a spray bottle. The control feed used commercial feed sprayed with PDB and added 2% egg white as a binder. The feed was air-dried for three hours. This method was used to prevent damage to bioactive compounds due to temperature during feed production (Wang *et al.,* 2021b).

Toxicity Test

The purpose of the toxicity test is to determine the toxicity level of *Penicillium* sp. KTR58 metabolites to Pacific white shrimp. The test was conducted by observing the mortality rate of Pacific white shrimp after administration of *Penicillium* sp. KTR58 metabolites within seven days. The shrimp used for the toxicity test were 7 shrimp weighing 2.29 ± 0.14 grams (OECD, 2019). The shrimp were placed in a 7 L container and fed with feed coated with *Penicillium* sp. KTR58 metabolites at concentrations of 0, 10, 30, 50 and 70 mL/kg of feed, each with three repetitions. Feeding was carried out four times a day (07.00, 12.00, 17.00, and 22.00 WIB). Observations were made for 24 hours to seven days after treatment. The LD50 calculation was performed using the Reed & Muench (1938) method below:

$$m = Xi - d\left(\frac{50 - \%Xi}{\%Xi(i-I) - \%xi}\right)$$

Note:
m = Log LD_{50}
Xi = Dose-mortality log below 50%
d = Difference in mortality log below 50% and above 50%
%Xi = Cumulative mortality percentage for doses below 50%
%X(i-I) = Cumulative mortality percentage for doses above 50%
LD_{50} = Antilog interval value “m”

The toxicity test results showed that the administration of metabolites in feed was not toxic at a high dose of 70 mL/kg with a survival rate of 95.24 ± 6.73%.

Shrimp feeding trial

Shrimp feeding trial using a completely randomized design (CRD) consisted of 4 treatments, namely C (control), P10 (10 mL/kg feed), P20 (20 mL/kg feed), and P30 (30 mL/kg feed), each with 3 replicates. Feed was administered at a feeding rate of 8% and a feeding frequency of four times a day at 07.00, 12.00, 17.00, and 22.00 WIB. A feeding trial was carried out for 30 days in an aquarium measuring 60×40×40 cm^3 with a density of 80 shrimp/m^2 (15 shrimp/aquarium), shrimp weight of 2.73 ± 0.14 g (Hasyimi *et al.* 2020). During the feeding trial, observations of research variables and water quality measurements (dissolved oxygen, salinity, pH, temperature, and total ammonia nitrogen) were carried out for each treatment. Water changes during maintenance were carried out every three days, replacing 5-10% of the water.

Water quality

Water quality during Pacific white shrimp cultivation is maintained within the optimal range namely temperature (28.0-29.2°C), pH (7.52-7.72), DO (6.2–7.4 mg/L), salinity (25–30 g/L), and TAN (0.022-0.094 mg/L) in accordance with Permen (2016).

Research Variables

Specific growth rate (SGR)

SGR measurements following Li *et al.* (2022) were conducted on day 0 and day 30 of feeding trial. SGR calculations used the following formula:

$$SGR = \left(\frac{\text{Ln Wt} - \text{Ln W0}}{t}\right) \times 100$$

Note:
Wt = Average weight at the end of treatment (g)
Wo = Average weight at the beginning of treatment (g)
T = Cultivation period (days)

Weight and final biomass

Measurements of weight and final biomass of shrimp were taken on day 0 and day 30. Biomass measurements were taken by weighing the shrimp using digital scales.

Feed Conversion Ratio

The feed conversion ratio (FCR) of Pacific white shrimp during feeding trial (day 0 and day 30) was calculated according to Foysal *et al.* (2020) using the following formula:

$$FCR = \frac{Fi}{Fw - Lw}$$

Note:
FCR = Feed conversion ratio
Fi = Amount of feed given (g)
Fw = Shrimp biomass at the end of treatment (g)
Lw = Shrimp biomass at the beginning of treatment (g)

Survival Rate (SR)

The survival rate of Pacific white shrimp was observed daily until the end of the treatment (day 30) using the following formula (Aftabuddin *et al.*, 2017):

$$SR\ (\%) = \frac{N_t}{N_0} \times 100$$

Note:
KH = Survival rate (%)
Nt = Number of shrimp at the end of treatment
N0 = Number of shrimp at the beginning of treatment

Total haemocyte count (THC)

Total haemocyte count (THC) measurement refers to the method of Hamsah *et al.* (2019). Hemolymph and anticoagulant ratio was 1:3. Hemolymph was dripped onto a hemocytometer and observed using a microscope with 100× magnification. THC calculation was based on the following formula:

$$THC = \frac{\text{Number of cells observed}}{\text{Number of observed fields}} \times 25 \times \frac{1}{\text{Hemocytometer volume}} \times \text{diluting factor}$$

Phagocytosis activity (PA)

Phagocytosis activity (PA) was measured by mixing 100 µl of shrimp hemolymph sample with 25 µl of *Staphylococcus aureus* suspension (10^7 CFU/mL) and incubating for 20 minutes. A smear preparation was made, dried, fixed with methanol for five minutes and dried again. Then stained with Giemsa stain for 20 minutes and observed using a microscope with 400x magnification (Chen *et al.,* 2014).

Phenoloxidase (PO)

Phenoloxidase activity was measured using a spectrophotometer by recording the change in dopachrome formed from *L-dihydroxyphenylalanine* (L-DOPA). The optical density of phenoloxidase activity in the experimental shrimp was expressed by the dopachrome formed in 100 µL of hemolymph. The optical density was set at 490 nm using a microplate reader. Phenoloxidase (PO) activity was measured based on the formation of dopachrome produced by *L-dihydroxyphenylalanine* (L-DOPA) according to Zhao *et al.* (2017).

Respiratory burst (RB)

A mixture of hemolymph and anticoagulant (50 µL) was incubated for 30 minutes at room temperature. It was centrifuged at 3,000 rpm for 20 minutes. The supernatant was discarded and 100 µL of NBT (0.3%) was added and incubated for 2 hours at room temperature. The solution was centrifuged at 3,000 rpm in 10 minutes. The supernatant was discarded and 100 µL of absolute methanol was added and centrifuged again at 3,000 rpm for 10 minutes. The resulting pellet was washed twice with 70% methanol and dried. 120 µL of 2M *potassium hydroxide* (KOH) and 140 µL of *dimethylsulfoxide* (DMSO) were added. The sample was placed in a microplate titer well and the respiratory burst value was measured using a spectrophotometer at a wavelength of 630 nm with KOH or DMSO as the standard solution (blank). The respiratory burst is expressed as NBT reduction per 10 µL hemolymph. Respiratory burst is characterized by the rapid release of reactive oxygen species, predominately from neutrophils, for pathogen killing (Hampton *et al.,* 2020).

Data analysis

Data were analyzed using Microsoft Excel 365 and IBM SPSS 25 software for analysis of variance ANOVA) with a 95% confidence interval followed by Duncan's multiple range test.

RESULTS AND DISCUSSION

Antibacterial activity assay

The highest inhibition zone diameter (26.50 ± 0.5 mm) was obtained from treatment with a dose of 30 µL/disc. The inhibition zone diameter decreased at a dose of 20 µL/disc (24.2 ± 0.76 mm) (Table 1). This inhibition zone value was used as the basis for determining the addition dose of *Penicillium* sp. KTR58 metabolites in Pacific white shrimp feed.

Thin-layer chromatography fractions

The TLC fractions of *Penicillium* sp. KTR58 extract in the form of compound spots on the TLC plate detected physically (UV light) and chemically (reagent staining) are presented in Table 2. Table 2 shows that the separation of compounds was successful with the compound fractions clearly visible under UV light at λ 254 nm and λ 366 nm, as well as after spraying with $FeCl_3$ and anisaldehyde. The Rf values of *Penicillium* sp. KTR58 ranged from 0.97 to 0.1. The $FeCl_3$ reagent identified two blue spots with a retardation factor (Rf) value of 0.3 (tannins) and red spots with an Rf value of 0.1, namely flavonoids. The *Penicillium* sp. KTR58 extract showed nine spots with the anisaldehyde reagent. The blue spots were suspected to be steroid compounds, the red and weak violet spots were suspected to be flavonoids and terpenoids and the yellow spots were suspected to be saponin compounds. The high Rf value (0.93) indicates that these steroid compounds had low polarity so they move further in the nonpolar mobile phase.

Observation under UV light λ 254 nm detected four dark spots with Rf 0.54, 0.34, 0.14, 0.1 indicating compounds that absorb UV light, such as coumarin, flavonoids, and alkaloids. Observation under UV light at λ 366 nm showed five green spots with Rf values of 0.9, 0.8, 0.4, and 0.66 as flavonoids and a blue spot with an Rf value of 0.5 as a coumarin compound. The results of TLC using acetonitrile:butanol:aquadest (3:6:1) eluent were compared with the standard Rf value of barley, which is 0.625 (Utami *et al.,* 2016). The Rf value of the ethyl acetate extract of *Penicillium* sp. KTR58 fungal metabolites were 0.7. Based on the comparison with the standard Rf value, there was no significant difference, indicating that the compounds were almost the same.

Bioautography assay

Fractions in the metabolite extract of *Penicillium* sp. KTR58 showed clear inhibition zones in the middle of the chromatogram with Rf values of 0.14–0.66 which were identified as coumarin, flavonoids, alkaloids, saponins, and steroids (Figure 1). The upper part (Rf 0.80–0.98) with weaker antibacterial activity consists of flavonoids, terpenoids, and steroids. while the lower fraction of the chromatogram showed no antibacterial activity. The bioautography test results are presented in Figure 1.

Table 1. The inhibition zone diameter of *Penicillium* sp. KTR58 fungal isolate against *V.parahaemolyticus*.

Treatment	Dose	Inhibition zone diameter (mm)
Oxytetracycline (OTC) 1% (+)	20 µL/disc	36.67 ± 1.53
Ethyl acetate (-)	20 µL/disc	0.00 ± 0.00
P1	10 µL/disc	15.67 ± 0.58
P2	20 µL/disc	24.20 ± 0.76
P3	30 µL/disc	26.50 ± 0.50

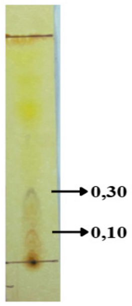
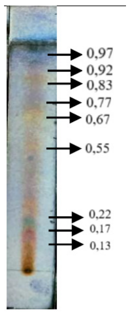
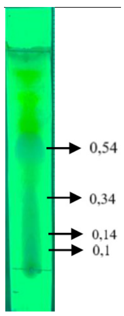
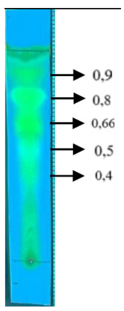
Protease activity assay

The test results showed that a thin clear zone formed around the *Penicillium* sp. KTR58 isolate using PDA medium with shrimp broth and PDA medium with 1% skim milk, indicating that *Penicillium* sp. KTR58 produce protease enzymes (Figure 2).

Growth performance and survival

Feeding trial containing *Penicillium* sp. KTR58 metabolites showed positive effects on growth performance, survival rate, and feed conversion ratio in Pacific white shrimp during a 30-day feeding trial. The results of this study indicate that supplementation with *Penicillium* sp. KTR58

Table 2. Chromatogram of *Penicillium* sp. KTR58 extract on $FeCl_3$ reagent, anisaldehyde, UV light λ 254 nm, and UV light λ 366 nm.

UV light/ Reagen	Number of spots	*Retardation factor* (Rf)	Color	Compound (Wagner & Bladt, 1996)
 $FeCl_3$	2	0.30	Blue	Tannin
		0.10	Red	Flavonoid
 Anisaldehid	9	0.97	Blue	Steroid
		0.92	Weak-violet	Terpenoid
		0.83	Yellow	Terpenoid
		0.77	Weak-violet	Flavonoid
		0.67	Yellow	Saponin
		0.55	Yellow	Saponin
		0.22	Blue-green	Steroid
		0.17	Brown	Saponin
		0.13	Red	Terpenoid
 UV light λ 254 nm	4	0.54	Dark	Coumarin, flavonoid. and alkaloid
		0.34	Dark	
		0.14	Dark	
		0.10	Dark	
 UV light λ 366 nm	5	0.90	Green	Flavonoid
		0.80	Green	Flavonoid
		0.66	Green	Flavonoid
		0.50	Blue	Coumarin
		0.40	Green	Flavonoid

significantly improved growth parameters, including final weight, final biomass, specific growth rate (SGR), and feed conversion ratio (FCR) compared to the control group (P<0.05). However, no significant differences were found in survival rate (SR) between treatments (P>0.05). The P20 treatment showed the best growth performance compared to other treatments with

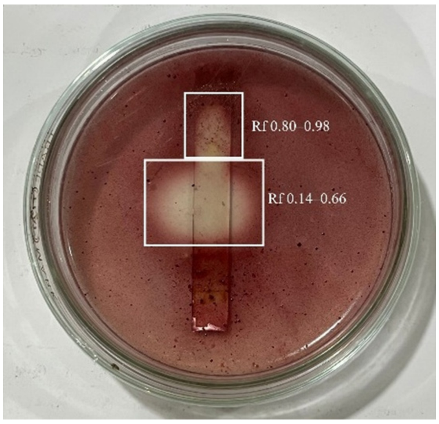


Figure 1. Bioautography assay results of *Penicillium* sp. KTR58 metabolite extract.

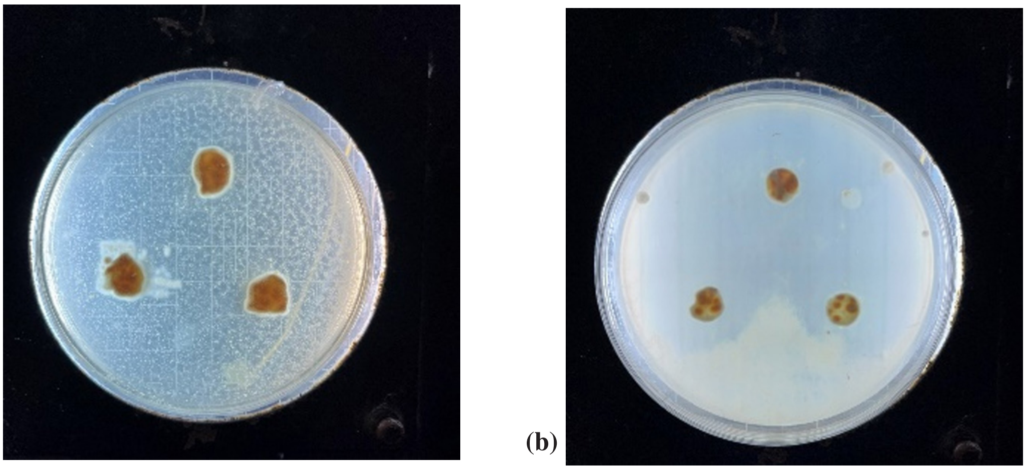


Figure 2. Protease activity assay using PDA medium with shrimp broth (a) and PDA medium with 1% skim milk (b).

Table 3. Growth performance of Pacific white shrimp in *Penicillium* sp. KTR58 metabolite supplementation during 30 days of feeding trial.

Variables	Treatment			
	Control	P10	P20	P30
W_0 (g)	2.71 ± 0.13[a]	2.76 ± 0.13[a]	2.71 ± 0.15[a]	2.73 ± 0.17[a]
W_t (g)	6.59 ± 0.12[a]	8.39 ± 0.24[c]	8.87 ± 0.16[d]	7.57 ± 0.17[b]
B_0 (g)	40.56 ± 0.09[a]	40.67 ± 0.13[a]	40.54 ± 0.10[a]	40.64 ± 0.10[a]
B_t (g)	90.35 ± 5.22[a]	112.09 ± 10.62[bc]	123.79 ± 8.13[c]	105.75 ± 7.50[b]
SGR (%/day)	2.96 ± 0.16[a]	3.71 ± 0.17[c]	3.96 ± 0.18[d]	3.4 ± 0.22[b]
FCR	2.16 ± 0.14[c]	1.73 ± 0.18[b]	1.45 ± 0.05[a]	1.84 ± 0.09[b]
SR(%)	93.33 ± 6.66[a]	91.11 ± 10.18[a]	93.33 ± 6.66[a]	93.33 ± 6.66[a]

Note: W0 = initial individual weight; Wt = final individual weight; B_0 = initial biomass; B_t = final biomass; SGR = specific growth rate; FCR = feed conversion ratio; SR = survival rate. Different superscript letters on the same row (mean values ± standard deviation) indicate statistically significant differences (P<0.05).

final weight, final biomass, specific growth rate, and feed conversion ratio values of 8.87 ± 0.16 g, 123,79 ± 8,1 g, 3.96 ± 0.18 %/day, 1.45 ± 0.05 respectively (Table 3). Growth performance in the P30 treatment was significantly lower ($P<0.05$) compared to P20 and P30 (Figure 3).

Immune response of Pacific white shrimp

The results of immune response observations showed that the administration of *Penicillium* sp. KTR58 metabolites through feed for 30 days produced immune responses (THC, PA, PO and RB) values that were significantly ($P<0.05$) higher than those of the control (Figure 4). The immune response trend over 30 days showed an increase. The highest immune response values were found in the P20 treatment, with THC, PA, RB, and PO values were $9.33\times10^6 \pm 0.31\times10^6$ cells/mL, 49.73 ± 1.89%, 0.31 ± 0.01, 0.29 ± 0.002 respectively. The immune response value of the P30 treatment was significantly lower than P20 ($P>0.05$). So overall, the administration of *Penicillium* sp. KTR58 metabolite treatment P20 was the best dose for improving shrimp health status.

Discussion

The highest inhibition zone diameter (26.50 ± 0.50 mm) was obtained from treatment with a dose of 30 µL/disc. The inhibition zone diameter decreased at a dose of 20 µL/disc (24.20 ± 0.76 mm). These inhibition zone values fall within the very strong inhibition zone category (diameter ≥21 mm) (Surjowardojo, 2015). Previous studies have shown that *Penicillium* sp. isolated from mangroves exhibit significant antibacterial activity against pathogenic bacteria. *Penicillium* sp. R1M isolated from *Sonneratia caseolaris* showed superior antibacterial effects compared to

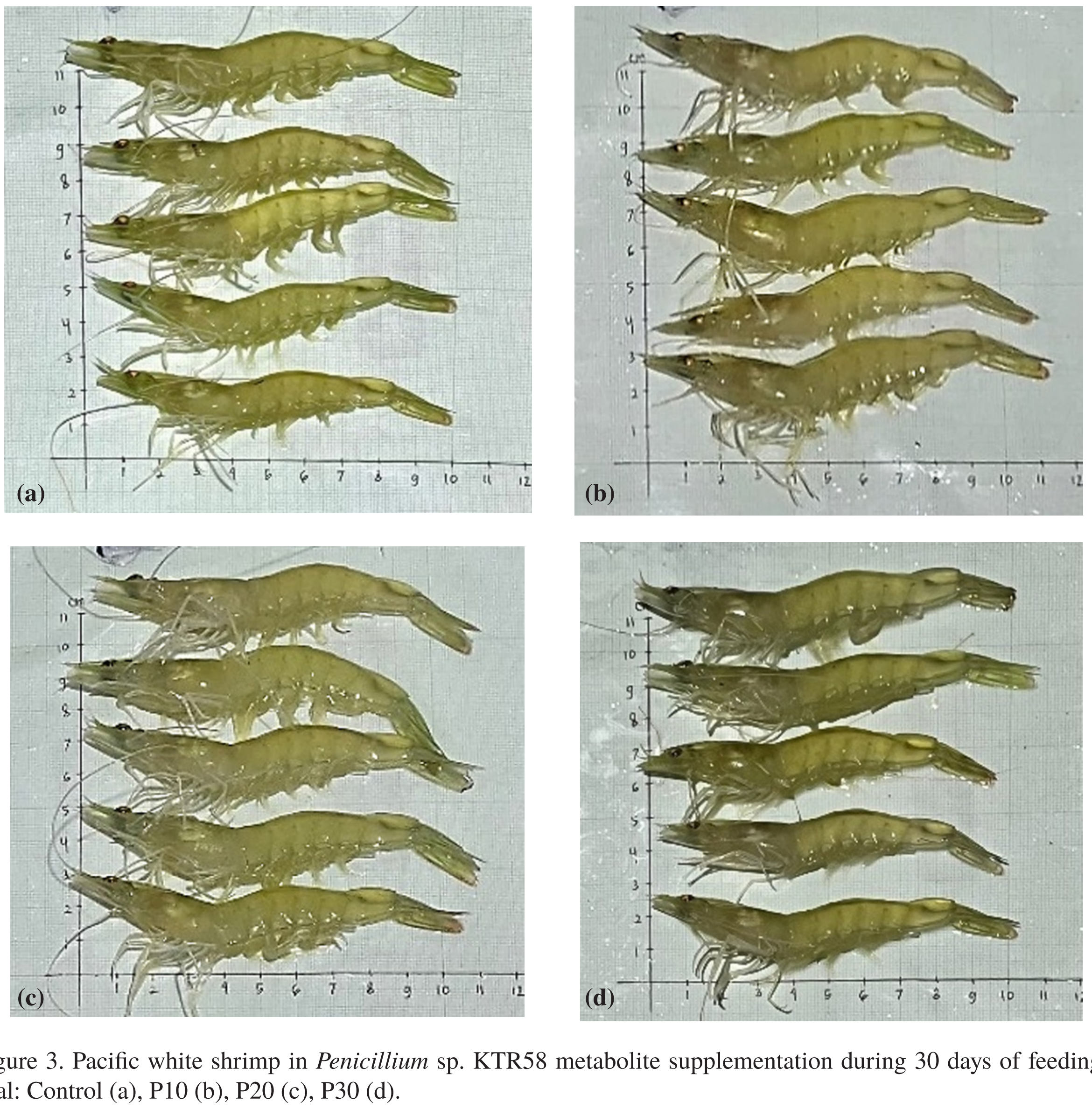


Figure 3. Pacific white shrimp in *Penicillium* sp. KTR58 metabolite supplementation during 30 days of feeding trial: Control (a), P10 (b), P20 (c), P30 (d).

the standard *Penicillium notatum*, with inhibition zones of 11.50 ± 0.60 mm against *Staphylococcus aureus* and 10.60 ± 0.40 mm against *Escherichia coli* (Prihanto *et al.,* 2012). Similarly, *Penicillium* sp. from *Rhizophora apiculata* showed strong inhibitory activity with 14.62 mm against *S. aureus* and 12.07 mm against *E. coli* (Mukhlis *et al.,* 2018).

The highest inhibition zone value was used as the basis for determining the addition dose of *Penicillium* sp. KTR58 metabolites in Pacific white shrimp feed in line with Pung *et al.* (2025) that *Lignosus rhinocerotis* methanol extract was selected over *Lignosus rhinocerotis* aqueous extract for incorporation into shrimp feed for *in vivo* experiments due to its higher bacterial inhibitory effects and lower cytotoxicity. Thin-layer chromatography testing of *Penicillium* sp. KTR58 extracts showed suspected steroid, flavonoid, terpenoid, saponin, coumarin and alkaloid compounds. An Rf value close to 0 indicates that the compound tends to be more polar because it interacts strongly with the stationary phase, while an Rf value close to 1 indicates that the compound tends to be more nonpolar because it interacts more strongly with the mobile phase (solvent) than with the stationary phase. The content of these compounds is consistent with the results of Zhou *et al.* (2025) study which found that a total of 417 secondary metabolites were obtained from *Penicillium* fungi derived from mangrove ecosystems. A total 170 compounds were identified as new compounds and 126 exhibited bioactivities. These compounds were structurally categorized into alkaloids (121 compounds), polyketides (146 compounds), terpenoids (84 compounds), benzene derivatives (38 compounds), steroids (8 compounds) and other compounds (20 compounds).

Thin-layer chromatography (TLC) is a method of separating chemical compounds chemically and physically based on the migration speed or distribution ratio of the components of the stationary phase and mobile phase mixture.

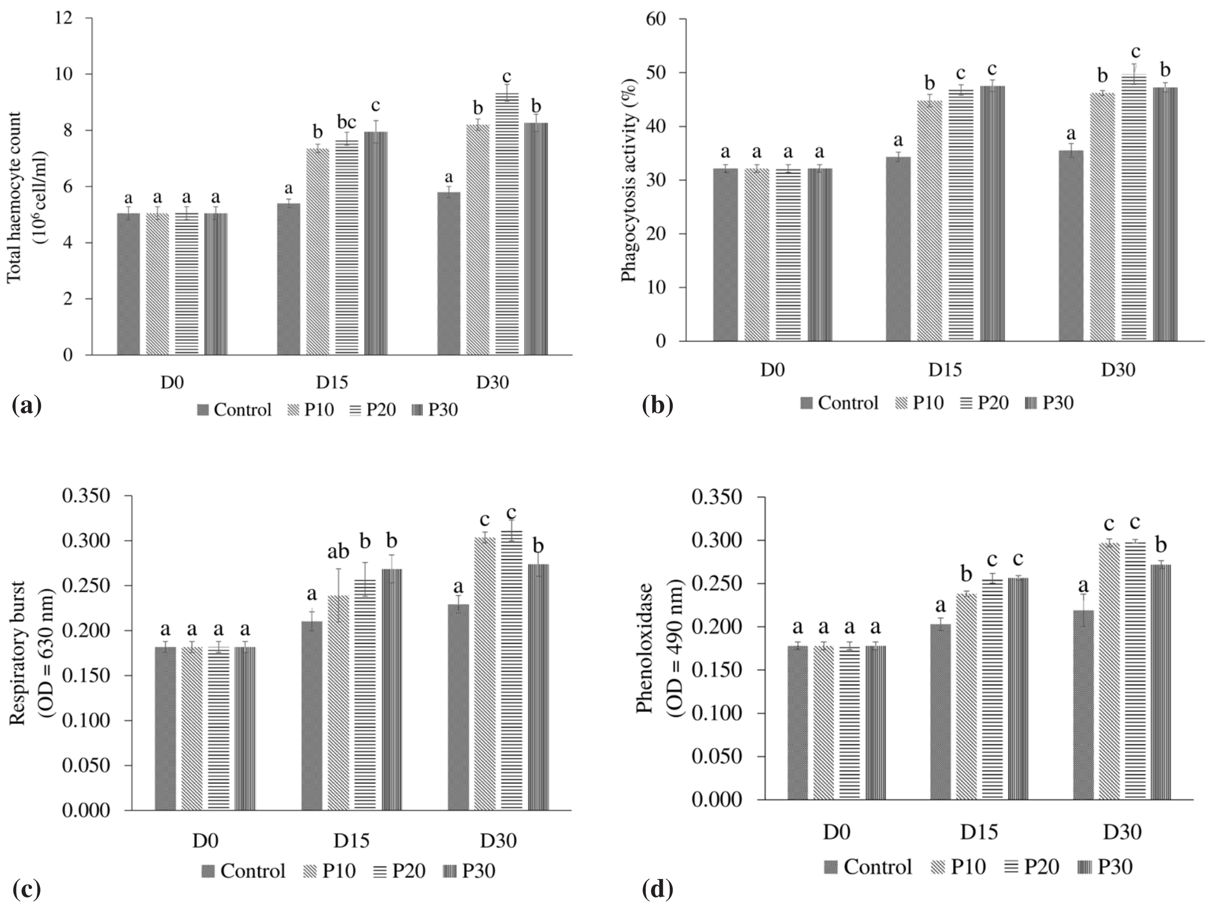


Figure 4. Total haemocyte count (THC) (a), phagocytosis activity (PA) (b), phenoloxidase (PO) (c), and respiratory burst (RB) (d) in Pacific white shrimp fed different doses of *Penicillium* sp. KTR58 metabolite. Different letters on each bar at the same observation time indicate statistically significant differences (P<0.05). Day 0 (D0), day 15 (D15), day 30 (D30).

TLC is a relatively simple, fast and commonly used chromatography method for identifying pharmaceutical substances. Based on TLC assay, bioautography was performed to identify compounds with specific antibacterial activity. The bioautography method was used to visualize compounds with antimicrobial activity directly on the chromatogram plate. Extracts that had been fractionated using thin layer chromatography (TLC) were coated with agar media that had been inoculated with test bacteria. Clear zones that formed indicated the presence of inhibitory power on bacterial growth.

Red color indicated microbial growth, while areas that did not change color (without formazan crystals) indicate the presence of antimicrobial compounds that inhibited the activity of these enzymes. The test results showed that the fraction in the metabolite extract of *Penicillium* sp. KTR58 exhibited a clear inhibition zone in the middle of the chromatogram with an Rf value of 0.14–0.66 which was identified as coumarin, flavonoids, alkaloids, saponins, and steroids. The upper part (Rf 0.80–0.98) with weaker activity from flavonoids, terpenoids, and steroids, while the lower fraction of the chromatogram showed no antibacterial activity. The fractions resulting from the separation of the *Penicillium* sp. KTR58 extract showed lower activity and some showed no activity at all, compared to the ethyl acetate extract. This finding indicated the presence of synergistic interactions between compounds in the extract.

Synergistic interactions occur when a combination of several compounds produced a stronger effect than the individual effects of each compound. It was suggested that this was responsible for the high antibacterial activity in the whole extract. *Penicillium* sp. metabolites are known to contain 26 compounds that exhibit biological activity, such as cytotoxic, antifungal, antibacterial, anti-inflammatory, and α-glucosidase-inhibitory activities and 11 compounds exhibited diverse biological activities (Zhou *et al.,* 2025). *Penicillium* sp. KTR58 exhibited a weak ability to produce protease enzymes, as evidenced by the formation of a thin clear zone around the isolate in the protease activity assay. In line with most studies showing widespread protease production. De Souza *et al.* (2024) highlighted protease production across multiple *Penicillium* species, including *P. citrinum. P. oxalicum.* and *P. sumatraense*.

The protease activity test was conducted to determine the ability of fungi to produce protease enzymes which are enzymes that hydrolyze (break down) proteins into peptides or amino acids. Based on their location and function, enzymes are divided into two types, intracellular and extracellular enzymes. One of the extracellular enzymes that was commonly used was protease, and the formation of a clear zone around the colony indicated the presence of protease activity (Sedijani *et al.,* 2023). Feeding with *Penicillium* sp. KTR58 metabolites for 30 days showed a significant increase in the growth performance of Pacific white shrimp compared to the control group (Table 3). This increase in growth rate was associated with higher feed utilization efficiency as indicated by the low feed conversion ratio in the treatment group.

The improved growth in the *Penicillium* sp KTR58 metabolite treatment group was believed to be due to the phytochemical content of the metabolite. Phytosterols and saponins are known to increase feed efficiency (Couto *et al.,* 2014). Flavonoid compounds can aid the digestive system of shrimp, thereby optimizing protein absorption (Naban *et al.,* 2024). In addition, flavonoid compounds can protect the shrimp's body from infections that attack its digestive system, thereby enhancing the growth of white shrimp (Anggawati *et al.,* 2020). The administration of β-glucan in shrimp causes the intestinal surface structure to become more extensive, resulting in better nutrient absorption. Improvements in digestion and nutrient absorption will lead to better feed efficiency and protein absorption which in turn will result in better growth performance. This occurs because betaglucan entering the digestive glands is degraded by glucanase for energy production leading to greater protein utilization for feed conversion and growth (Dawood *et al.,* 2015).

Furthermore, Uengwetwanit *et al.* (2025) observed that higher concentrations of betaglucan (0.4%) resulted in elevated gene expression, increased metabolite levels, and improved survival rates when shrimp were exposed to pathogens. However, it was crucial to consider that administering too much betaglucan might harm shrimp by potentially worsening immune reactions, inflammation, and stress. The addition of *Penicillium* sp. KTR58 metabolites at a dose of 30 mL/kg of feed showed lower growth compared to a dose of 20 mL/kg. This means that the optimum dose for growth is 20 mL/kg of feed. The administration of an active ingredient especially one that functions as an immunostimulant must be given at the optimum dose because administering immunostimulants at too high a dose was not expected to increase the immune response (Saputra *et al.,* 2016).

The results of THC immune response observations showed that on day 30 the THC values of all metabolite treatments were significantly different from the control (P<0.05). The highest THC value after maintenance was found in the P20 treatment with a value of $9.33\times10^6 \pm 0.31\times10^6$ cells/mL and were significantly different from other treatments (P<0.05). The health condition of Pacific white shrimp can be assessed through their hemocyte profile. Hemocytes play a role in recognizing and neutralizing incoming pathogens as well as mediating immune responses through various mechanisms such as phagocytosis, nodule formation, melanization, intercellular communication, and cytotoxic activity which vary depending on the characteristics of the pathogen (Eleftherianos *et al.,* 2021).

The increase in THC was also in line with the increase in RB, PO, and PA. All treatments showed an increase in immune response over 30 days. Jannah *et al.* (2018) stated that total haemocyte count (THC) plays an important role in maintaining resistance to pathogens. The higher the THC value, the higher the ability of hemocytes to phagocytose. Additionally, high THC values can stimulate the activity of *Prophenoloxidase* (ProPO) in producing *phenoloxidase* (PO) activity which can then defend against pathogen attacks. Mushroom and fungal components such as glucan, nucleotides, vitamins, chitin, and proteins serve as immunostimulants by improving shrimp immunity. They do this by encouraging the development of intestinal gut microbiota, stimulating phagocytic cells and the prophenoloxidase (proPO) system, and enhancing antibacterial activity in the hemolymph (Mohan *et al.,* 2019b; Ceseña *et al.,* 2021).

The increase in immune response indicated that *Penicillium* sp. KTR58 metabolites can act as immunostimulants that stimulate hemocyte proliferation. This increase in immunity was likely due to the bioactive compounds contained in *Penicillium* sp. KTR58 metabolites, namely flavonoids, alkaloids, saponins, betaglucan, and steroids, which play an important role in enhancing the nonspecific immune response of Pacific white shrimp. Flavonoids function as antioxidants and immunostimulants that can suppress oxidative stress and increase the activity of immune enzymes such as superoxide dismutase (SOD), catalase (CAT), and total hemocyte count (THC). Saponins have been shown to enhance humoral and cellular immune responses by increasing the number of hemocytes, phagocytic activity, and phenoloxidase enzyme activity, this significantly improves shrimp resistance to infection (Lin *et al.,* 2024). The synergistic combination of bioactive compounds can strengthen the immune system of Pacific white shrimp, reduce oxidative stress, and ultimately improve the resilience and productivity of aquaculture.

CONCLUSION

Metabolic extract of *Penicillium* sp. KTR58 showed strong inhibitory activity with 26.50 ± 0.50 mm against *V.parahaemolyticus. Penicillium* sp. KTR58 metabolite consisted of steroid, flavonoid, terpenoid, saponin, coumarin, alkaloid, and betaglucan compounds. In the in vivo test, the best dose of *Penicillium* sp. KTR58 metabolite addition to Pacific white shrimp feed was 20 mL/kg because it significantly increased the growth and immune response of Pacific white shrimp compared to the control.

ACKNOWLEDGMENTS

This research was funded by the Ministry of Education, Culture, Research, and Technology of the Republic of Indonesia through the PTM Research Grant 2025 under contract number [No. 006/C3/DT.05.00/PL/2025].

REFERENCES

Aftabuddin S, Siddique MAM, Romkey SS, Shelton WL. 2017. Antibacterial function of herbal extracts on growth, survival and immunoprotection in the black tiger shrimp *Penaeus monodon*. Fish & Shellfish Immunology 65: 52–58.

Anggawati, Hilyana S, Marzuki M. 2020. Pengaruh Penambahan Ekstrak Kulit Manggis (*Garcinia mangostana*) Dengan Konsentrasi Berbeda Terhadap Pertumbuhan dan Kelangsungan Hidup Udang Vaname (*Litopanaeus vannamei*). Jurnal Perikanan Unram 9: 172–179. (In Indonesian).

Ceseña EC, Vega-Villasante F, Aguirre-Guzman G, Luna-Gonzalez A, Campa-Cordova A.

2021. Update on the use of yeast in shrimp aquaculture: a minireview. International Aquatic Research 13: 1–16.
Chen YY, Chen JC, Lin YC, Putra DF, Kitikiew S, Li CC, Hsieh JF, Liou CH, Yeh ST. 2014. Shrimp that have received carrageenan via immersion and diet exhibit immunocompetence in phagocytosis despite a post-plateau in immune parameters. Fish Shellfish Immunol 36: 352–366.
[CLSI] Clinical and Laboratory Standards Institute. 2020. Methods for Antimicrobial Dilution and Disk Susceptibility Testing of Infrequently Isolated or Fastidious Bacteria 3th ed. United States of America: Clinical and Laboratory Standards Institute.
Couto A, Kortner TM, Penn M, Bakke AM, Krogdahl A, Oliva-Teles A. 2014. Effects of dietary phytosterols and soy saponins on growth, feed utilization efficiency and intestinal integrity of gilthead sea bream (*Sparus aurata*) juveniles. Aquaculture 432: 295-303.
Dawood MAO, Koshio S, Ishikawa M, Yokoyama S, El Basuini MF, Hossain MS, Nhu TH, Moss AS, Dossou S, Wei H. 2015. Dietary supplementation of β-glucan impro-vees growth performance the innate immune response and stres resistance of red sea bream *Pagrus major*. Aquaculture Nutrition 23: 148–159.
De Souza MMM, Santiago PAL, Da Silva Santiago SRS, Birth ATR, Barbosa AN. 2024. Comparative analysis of the effects of different substrates on protease production by *Penicillium* spp. isolated from the Amazon. Curitiba 22: 1–16.
Djauhari R, Widanarni, Sukenda, Suprayudi MA, Zairin JrM. 2016. Characterization of *Bacillus* sp. NP5 and its application as probiotic for common carp *Cyprinus carpio*. Research Journal of Microbiology 11: 101–111.
Eleftherianos I, Heryanto C, Bassal T, Zhang W, Tettamanti G, Mohamed A. 2021. Haemocyte-mediated immunity in insects: cells processes and associated components in the fight against pathogens and parasites. Immunology 164: 401–432.
[FAO] Food and Agriculture Organization. 2023. Globefish highlights-International markets for fisheries and aquaculture products-Second issue 2023, with January-December 2022 statistics. Rome, Italy: FAO.
Foysal MJ, Fotedar R, Siddik MAB, Tay A. 2020. *Lactobacillus acidophilus* and *L. plantarum* improve health status, modulate gut microbiota and innate immune response of marron (*Cherax cainii*). Scientific reports 10: 1–13.
Gustilatov M, Widanarni, Ekasari J, Julyantoro PGS, Waturangi DE. 2024. The influence of biofloc system on Vibrio composition, the growth and the gut microvilli performance of the Pacific white shrimp *Penaeus vannamei*. Jurnal Akuakultur Indonesia 23: 12-23.
Hamsah H, Widanarni W, Alimuddin A, Yuhana M, Junior MZ, Hidayatullah D. 2019. Immune response and resistance of Pacific white shrimp larvae administered probiotic, prebiotic, and synbiotic through the bio-encapsulation of *Artemia* sp.. Aquaculture International 27: 567–580.
Hampton LMT, Jeffries MKS, Venables BJ. 2020. A practical guide for assessing respiratory burst and phagocytic cell activity in the fathead minnow, an emerging model for immunotoxicity. Method Article 7: 100992.
Hariati S, Wahjuningrum D, Yuhana M, Tarman K, Effendi I, Saputra F. 2018. Aktivitas antibakteri ekstrak kapang laut *Nodulisporium* sp. KT29 terhadap *Vibrio harveyi.* Jurnal Pengolahan Hasil Perikanan Indonesia 21: 250–257. (In Indonesian).
Jannah M, Junaidi M, Setyowati DN, Azhar F. 2018. Pengaruh pemberian *Lactobacillus* sp. dengan dosis yang berbeda terhadap sistem imun udang vaname (*Litopenaeus vannamei*) yang diinfeksi bakteri *Vibrio parahaemolyticus*. Jurnal Kelautan Indonesian Journal of Marine Science and Technology 11: 140–150. (In Indonesian).
Jinna Z, Khan T, Kanwal N, Zhenji W. 2025. Diversity and biological functions of fungal secondary metabolites: Biocontrol agents for sustainable agriculture A review. Cellular and Molecular Biology 71: 43–48.
Kabense R. 2019. Penapisan bakteri proteolitik yang bersimbiosis dengan alga *Gracillaria* sp.. Jurnal Ilmiah Platax 7: 413–418.
[KKP] Kementerian Kelautan dan Perikanan. 2024. Produksi perikanan: rekapitulasi data. https://portaldata.kkp.go.id/portals/data-statistik/prod-ikan/summary [29 January 2025].
Latif HMRA, Yilmaz E, Dawood MAO, Ringo E, Ahmadifar E, Yilmaz S. 2022. Shrimp vibriosis and possible control measures using probiotics, postbiotics, prebiotics, and

synbiotics: a review. Aquaculture 551: 737951.

Lay BW. 1994. Analisis Mikroba di Laboratorium, 1st edition. Jakarta, Indonesia: PT. Raja Grafindo Persada.

Li X, Li S, Yu Y, Zhang X, Xiang J, Li F. 2022. The immune function of a NLR like gene, LvNLRPL1, in the Pacific whiteleg shrimp *Litopenaeus vannamei*. Dev Comp Immunol 128: 104311.

Lin S, Yao R, Cui X, Guo Y, Hu D, Zhou B, Zhou Z, He X, Liao Z, Tan B, Niu J. 2024. Optimizing shrimp nutrition and health: ginseng saponins as functional additives in low-fishmeal diets on *Litopenaeus vannamei*. Frontiers in Marine Science 11: 104311.

Mohan K, Ravichandran S, Muralisankar T, Uthayakumar V, Chandirasekar R, Seedevi P, Rajan DK. 2019a. Potential uses of fungal polysaccharides as immunostimulants in fish and shrimp aquaculture: a review. Aquaculture 500: 250–263.

Mohan K, Muralisankar T, Uthayakumar V, Chandirasekar R, Rajan DK. 2019b. Dietary *Ganoderma lucidum* polysaccharides to enhance the growth, immune response, and disease resistance of freshwater prawn *Macrobrachium rosenbergii*. Aquaculture Reports 14: 100203.

Mukhlis DK, Rozirwan R, Hendri M. 2018. Isolasi dan aktivitas antibakteri jamur endofit pada mangrove *Rhizophora apiculata* dari kawasan mangrove Tanjung Api-Api Kabupaten Banyuasin Sumatera Selatan. Maspari Journal 10: 151–160. (In Indonesian).

Naban SHF, Lumbessy SY, Rahmadani TBC. 2024. Optimization of Vaname Shrimp Growth (*Litopenaeus vannamei*) in Feeding with The Addition of Moringa Leaf Extract (*Moringa oleifera* Lam). Jurnal Biologi Tropis 24: 763-777. (In Indonesian).

Navaneeth KA, Bhuvaneswari T, Rajan JJS, Alavandi SV, Vijayan KK, Otta SK. 2020. Characterization of *Vibrio parahaemolyticus* isolates from shrimp farms of Southeast coast of India with special reference to Acute Hepatopancreatic Necrosis Disease (AHPND) status. Aquaculture 518: 734813.

[OECD] Organisation for Economic Co-operation and Development. 2019. Guideline for testing of chemicals, test guideline no. 203 fish, acute toxicity testing. Paris, Prancis: OECD.

Onomu AJ, Okuthe GE. 2024. The Application of Fungi and Their Secondary Metabolites in Aquaculture. J. Fungi 711: 1-21.

[Permen] Peraturan Menteri. 2016. Peraturan Menteri Kelautan Dan Perikanan Republik Indonesia Nomor 75/Permen-KP/2016 Tentang Pedoman Umum Pembesaran Udang Windu (*Penaeus monodon*) dan Udang Vaname (*Litopenaeus vannamei*). Kementerian Kelautan dan Perikanan Republik Indonesia.

Persing DH, Tenover FC, Hayden RT, Ieven M, Miller MB, Nolte FS, Tang YW, Belkum A. 2016. Molecular microbiology diagnostic principles and practice, 3rd edition. Washington DC: ASM Press.

Prihanto AA. 2012. Perbandingan aktivitas antibakteri *Penicillium notatum* ATCC 28089 dengan *Penicillium* sp. R1M yang diisolasi dari mangrove *Sonneratia caseolaris*. Jurnal Pengolahan Hasil Perikanan Indonesia 15: 66–70.

Pung QE, Chen Y, Chong CM, Lee PT, Peng K, Liew HJ, Loh JY. 2025. Exploring the potential of tiger milk mushroom (*Lignosus rhinocerotis*) crude extracts as immunostimulants for Pacific white shrimp (*Litopenaeus vannamei*): effects on survival and immunological responses against *Vibrio parahaemolyticus*. Journal of Applied Aquaculture 37: 449–467.

Queendy V, Roza RM. 2019. Aktivitas antifungi isolat *Aktinomisetes arboretum* Universitas Riau terhadap jamur *Fusarium oxysporum* f.sp *lycopersici* dan *Ganoderma boninense*. Jurnal Biologi. 12: 73–88. (In Indonesian).

Reed LJ, Muench H. 1938. A simple method of estimating fifty percent endpoints. American Journal of Epidemiology 27: 493–497.

Saputra F, Wahjuningrum D, Tarman K, Effendi I. 2016. Utilization of marine fungal *Nodulisporium* sp. KT29 metabolites to improve the production performance of marine culture of white shrimp. Jurnal Ilmu dan Teknologi Kelautan Tropis. 8 :747–755.

Sedijani P, Lestari DP, Rasmi DAC, Kusmiyati. 2023. Protease enzyme activity of fungal isolates from avocado and coconut fleshes on different pH and temperature. Jurnal Biologi Tropis 23: 218–225.

Surjowardojo, Susilorini TE, Sirait GR. 2015. Daya hambat dekok kulit Apel Manalagi (*Malus sylvestris* Mill) terhadap pertumbuhan *Staphylococcus aureus* dan *Pseudomonas* sp. penyebab mastitis pada sapi perah. Jurnal Ternak Tropika 16: 40–48. (In Indonesian).

Tang KFJ, Bondad-Reantaso MG. 2019. Impacts of acute hepatopancreatic necrosis disease on commercial shrimp aquaculture. Revue

Scientifique et Technique (International Office of Epizootics) 38: 477–490.

Tarman K, Lindequist U, Wende K, Porzel A, Arnold N, Wessjohann LA. 2011. Isolation of a new product and cytotoxic and antimicrobial activities of extracts from fungi of Indonesian marine habitats. Marine Drugs 9: 294–306.

Tarman K. 2011. Biological and chemical investigations of Indonesian marine derived fungi and their secondary metabolites. Greifswald: Cuvillier.

Uengwetwanit T, Uawisetwathana U, Angthong P, Phanthura M, Phromson M, Tala S, Thepsuwan T, Chaiyapechara S, Prathumpai W, Rungrassamee W. 2025. Investigating a novel β-glucan source to enhance disease resistance in Pacific white shrimp (*Penaeus vannamei*). Scientific Reports 15: 1–18.

Utami NF, Wardatun S, Suri FA. 2016. Identifikasi kandungan polisakarida betaglukan pada jamur ganoderma (*Ganoderma lucidum*). Jurnal Ilmiah Farmasi 6: 71–77. (In Indonesian).

Valente CDS, Wan AHL. 2021. Vibrio and major commercially important vibriosis diseases in decapod crustaceans. Journal of Invertebrate Pathology 181: 107527.

Wagner H, Bladt S. 1996. Plant drug analysis: a thin layer chromatography atlas, 2nd edition. Germany: Berlin Heidelberg-Springer.

Wahjuningrum D, Efiandi TR, Tarman K, Yuhana M, Effendi I, Saputra F. 2020. Supplementation of *Nodulisporium* sp. KT29 induced by *Vibrio harveyi* as an immunostimulant for controlling vibriosis in vannamei white shrimp under marine culture system. Jurnal Akuakultur Indonesia 19: 95–105.

Wahjuningrum D, Hariati S, Yuhana M, Effendi I, Citarasu T, Utami DAS. Tarman K. 2022. Low dose of *Nodulisporium* sp. KT29 metabolite promotes production performance and innate immunity of pacific white leg shrimp (*Litopenaeus vannamei*) against co-infection of white spot syndrome virus and *Vibrio harveyi*. Aquaculture International 30: 2611–2628.

Wahjuningrum D, Gustilatov M, Tarman K, Sukenda, Effendi I, Umam K, Ulzanah N, Miranti S, Kumala FB. 2025. Dietary comparison of algal, herbal, and fungal ingredients fed to Pacific white shrimp across cultivation sites: Impacts on growth performance, immune response, and *Vibrio harveyi* infection resistance. Comparative Immunology Reports 9: 1-8.

Wang M, Zhang Y, Wang R, Wang Z, Yang B, Kuang H. 2021a. An evolving technology that integrates classical methods with continuous technological developments: thin-layer chromatography bioautography. Molecules 26: 1–21.

Wang AJ, Lin, Q Zhong. 2021b. Spray-coating as a novel strategy to supplement broiler feed pellets with probiotic *lactobacillus salivarius*. LWT-Food Science & Technology 137: 110419.

Yuhana M, Afiff U. 2023. Mini-review: utilization of *Vibrio parahaemolyticus* virulence coding genes for early detection of acute hepatopancreatic necrosis disease (AHPND). Jurnal Akuakultur Indonesia 22: 87-96.

Zhao Z, Xie J, Liu B, Ge X, Song C, Ren M, Zhou Q, Miao L, Zhang H, Zhang H, Shan F. 2017. The effects of emodin on cell viability, respiratory burst and gene expression of *Nrf2-Keap1* signaling molecules in the peripheral blood leukocytes of blunt snout bream (*Megalobrama amblycephala*). Fish Shellfish Immunol 62: 75–85.

Zhou G, Cai J, Wang B, Diao W, Zhong Y, Pan S, Xiong W, Huang G, Zheng C. 2025. Secondary metabolites from the mangrove ecosystem-derived fungi *Penicillium* spp.: chemical diversity and biological activity. Marine Drugs 23: 1–61.