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Research article

Bacterial Diversity and Abundance in the Rearing Water of Vertical Crab Farming Applied with Microbial-based Portable Urban Filter (P-PUF)

Yuni Puji Hastuti[1]*, Ridwan Affandi[2], Syamsul Bahri Agus[3], Hendra Sugandhi[4], Yuli Siti Fatma[5], Suroso[4], Indra Jaya[3], Bayu Aji Nugroho[1], Wildan Nurussalam[1] and Dudi Wildan Lesmana[2]

[1]Department of Aquaculture, Faculty of Fisheries and Marine Sciences, IPB University, Bogor, Indonesia
[2]Department of Aquatic Resources Management, Faculty of Fisheries and Marine Sciences, IPB University, Bogor, Indonesia
[3]Department of Marine Science and Technology, Faculty of Fisheries and Marine Sciences, IPB University, Bogor, Indonesia
[4]PT Tritunggal Segara Indonesia (TSI), North Jakarta, Indonesia
[5]Research Center for Applied Microbiology, Research Organization for Life Sciences and Environment, National Research and Innovation Agency (BRIN), Bogor, Indonesia

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Abstract

A portable urban filter (P-PUF), equipped with physical filtration materials and specialized aerobic and anaerobic bacteria, was integrated into vertical crab farming systems to maintain water quality throughout crab cultivation. To precisely monitor water quality, an auto-sensor water quality monitoring system (Asor WQM) was employed. The objective of this study was to assess bacterial diversity and abundance in the rearing water of vertical crab farming treated with the P-PUF and Asor WQM (T.P-PUF), in comparison to conventional filtration (C.K) system. Bacterial diversity and abundance were evaluated through 16S rRNA amplicon sequencing and culturable methods. Our results demonstrate a notably higher bacterial diversity in the T.P-PUF treatment compared to the C.K. The top five bacterial phyla in both treatments were *Pseudomonadota*, *Bacteroidota*, *Bacillota*, *Actinomycetota*, and *Acidobacteriota*. The differences were observed in the composition of *Bacillota* and *Bacteroidota* between the two treatments, with T.P-PUF showing an increased abundance of *Bacillota* and C.K exhibiting a higher prevalence of *Bacteroidota*. At the family level, the bacterial community in T.P-PUF was primarily composed of *Vibrionaceae,* whereas *Pseudoalteromadaceae* dominated in C.K. The culturable method revealed that T.P-PUF substantially increased the abundance of total bacteria, nitrifying bacteria, and denitrifying bacteria in the rearing water compared to C.K. These findings indicate that the application of T.P-PUF leads to a distinct and more diverse bacterial

*Corresponding author: E-mail: yuniha@apps.ipb.ac.id
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community in the rearing water of mud crab culture, potentially enhancing the overall health and quality of crab production

Keywords: bacterial composition; metagenomic analysis; mud crab culture; recirculating aquaculture system; water quality

1. Introduction

The demand for mud crabs (*Scylla* spp.) has increased in global markets. Fish Quarantine Inspection Agency (FQIA) of the Ministry of Marine Affairs and Fisheries (MMAF) of the Republic of Indonesia reported that 25,351,249 mud crabs were exported in 2018, and it slightly increased to 27,007,144 and 28,791,824 mud crabs in 2019 and 2020, respectively. China, Malaysia, and Singapore were the top three largest export destination countries in 2020. Due to high demand, mud crabs (*Scylla serrata*) have been overfished in several areas in Indonesia, such as Kendari Bay, Southeast Sulawesi (Suman et al*.,* 2018), Semarang, Central Java (Yudiati et al., 2020), Tarakan City, North Kalimantan (Indarjo et al., 2020), and Asahan Sea, North Sumatera (Pane et al., 2021). The overfishing has led to a decline in the carb population and has threatened crab sustainability in their natural habitat. Increasing mud crabs production has been continuously conducted to meet market demand without population reduction through various techniques of mud crab aquacultures, e.g., aquaculture ponds and recirculating aquaculture systems (RAS).

Most cultivation methods are based on juvenile crab capture and grow-out in an existing pond or pen. However, mud crab cultivation in ponds has some limitations, including large area requirements, cannibalism, crabs' escaping, high sunlight penetration, water pollution exposure, and low security. An advanced aquaculture system is necessary to improve crab production. Gupta et al. (2024) highlighted that RAS enhances water management, promotes eco-friendly farming practices, and improves biosecurity, with its location near consumer markets further boosts the sustainability and profitability of aquaculture operations. Previous studies reported the application of shelters (Hastuti et al., 2020b) and buckets (Yulianto et al., 2019) to cultivate mud crab *S. serrata* under RAS. Vertical crab farming has been continuously developed in several mud crab-producing countries, including Indonesia, Singapore, Malaysia, Philippines, Brunei, Vietnam, and India.

RAS reuses water through physical, chemical, or biological filter implementation to maintain water quality. These filtrations reduce water pollution in the aquaculture environment, particularly by mitigating nitrogenous waste. Inorganic nitrogenous compounds, such as ammonia/ammonium (NH_3^-/NH_4^+), nitrite (NO_2^-), and nitrate (NH_3^+) are toxic to aquatic animals at certain concentration, adversely affecting their physiology and ecology (de Araújo et al., 2021). Biological filters frequently used to decline the concentration of nitrogen pollution are microbes, especially nitrifying and denitrifying bacteria. Nitrifying bacteria perform autotroph/heterotroph nitrification activity, oxidizing ammonium into nitrite and nitrite into nitrate, which is less toxic than ammonia and nitrite. Ammonia in the water environment decreases the immune response of mud crabs (Cheng et al., 2020a). Meanwhile, nitrite binds to hemoglobin to form methemoglobin in the bloodstream, resulting in hemoglobin not binding to oxygen and oxygen transportation failure (Jensen, 2003). Ammonia and nitrite exposure also induces oxidative stress, which subsequently causes cytological damage, DNA damage, and apoptosis in mud crabs (Cheng et al., 2019a,b). Naturally, nitrifying bacteria exist in the rearing water of recirculating aquaculture systems for mud crabs (Hastuti et al*.,* 2017) and inhabit biofilm in

biofilters (Huang et al*.,* 2020). Denitrifying bacteria, which also inhabit the rearing water and biofilters, play a crucial role in recirculating aquaculture systems by converting nitrate (NO_3^-) into nitrogen gas (N_2), effectively reducing nitrogenous waste accumulation and maintaining water quality (Liu et al., 2022). In addition, the application of nitrifying and denitrifying microbial consortia can reduce ammonia concentration in the rearing water of *Litopenaeus vannamei* (Patil et al., 2021). The microbial-based portable urban filter (P-PUF) in this study contained nitrifying bacteria *Halomonas* sp. and denitrifying bacteria *Stenotrophomonas* sp. in vertical crab farming. The addition of nitrifying bacteria *Halomonas* sp. HIB-F to whiteleg shrimp culture water was reported to lower the ammonium concentration in the *L. vannamei* aquaculture system (Hastuti et al. 2020a). Meanwhile, applying *Stenotrophomonas* sp. HIB_7a in *L.vannamei* culture promoted a higher quality of water, particularly in decreasing nitrite concentration and shrimp growth compared to without application (Lazuardhi, 2019).

Microbes play a crucial role in biological services in the aquaculture environment, such as nitrogen cycling (Hastuti et al., 2018) and organic matter degradation (Bentzon-Tilia et al*.,* 2016). Moreover, bacterial communities inhabiting the rearing water influence microbiome in the cultured biota, including shrimp, a crustacean (Cardona et al*.,* 2016). Little is known about the microbial community in mud crab *S. serrata* rearing water, especially in vertical crab farming after nitrifying and denitrifying bacterial application. Therefore, this study was aimed at determining bacterial diversity and abundance inhabiting the rearing water of mud crab *S. serrata* grown in vertical crab farming with the microbial-based portable urban filter (P-PUF) containing nitrifying bacteria *Halomonas* sp. HIB-F and denitrifying bacteria *Stenotrophomonas* sp. HIB_7a. In this study, next-generation sequencing based on the 16S rRNA gene and the culturable method were used to investigate the bacterial diversity and abundance. The data obtained here would lay basis for further water quality management for disease prevention and growth improvement of mud crab *S. serrata* using the microbial-based portable urban filter.

2. Materials and Methods

2.1 Vertical crab farming condition

In this present study, mud crabs *S. serrata* were reared in vertical crab farming which was set up in a quasi-experimental design with two filtration system, i.e., conventional filter or non-portable filter without replication (C.K), and portable urban filter equipped with auto sensor water quality monitoring or Asor WQM without replication (T.P-PUF).

The microbial-based portable urban filter (PUF) was a fiber tube with a diameter of 64.3 cm, a height of 92.8 cm, and a capacity of 250 L with a total of 4 tubes. Before use, the fiber tubes and filter materials, including filter bag, bio balls, malang sands, zeolite, biofoam, and kaldness, were soaked in a solution of water and calcium hypochlorite ($Ca(ClO)_2$. After three days, fiber tubes were brushed and dried. Each fiber tube was connected with pipes and arranged as shown in Figure 1. One aeration stone was installed in each fiber tube. Fiber tube 1 was applied with a filter bag in the outlet pipe. Fiber tube 2 was filled with filter materials, including bio balls, malang sands, zeolite, biofoam, and 2 pipes, which were added with nitrifying and denitrifying bacteria. Fiber tube 3 was filled with kaldness. Fiber tube 4 was installed with a heater, UV lamp, and a pump connected to a pipe (diameter = 5 cm) in a vertical manner to pump water to culture boxes. Fiber tube 1 was filled with 562.5 L of seawater and 187.5 L of freshwater. Bacterial cultures applied in the portable filter were nitrifying bacteria *Halomonas* sp. HIB-F and denitrifying bacteria

Stenothropomonas sp. HIB_7a, which was previously grown in a specific medium containing $CaCl_2 \cdot 6H_2O$ 0.0184 $g \cdot L^{-1}$, KH_2PO_4 0.2 $g \cdot L^{-1}$, Na_2HPO_4 0.9 $g \cdot L^{-1}$, $FeCl_3 \cdot 6H_2O$ 0.005 $g \cdot L^{-1}$, $MgSO_4 \cdot 7H_2O$ 0.1 $g \cdot L^{-1}$, NH_4Cl 1 $g \cdot L^{-1}$, yeast extract 0.25 $g \cdot L^{-1}$, and $C_4H_6O_4$ 5 $g \cdot L^{-1}$ and CH_3COONa 5 $g \cdot L^{-1}$ as carbon source, respectively (Hastuti et al., 2019). Bacterial cultures were incubated for 24-48 h. Bacterial application was performed by adding tthe bacterial culture (10^9 $CFU \cdot mL^{-1}$) to the fiber tube 2 at day 7 and 21 of the experimental period.

A conventional filter or non-portable filter (C.K) was a concrete tube with a size of 3.5x1.5x1 m^3 and a total capacity of 5,325 L, which was partitioned into 4 parts (Figure 2). Each part was given one aeration stone. Part 1 had a size of 0.6x1.5x1 m^3 and contained a filter bag and Dacron in the outlet pipe, and ginger coral and biofoam in the bottom. Part 2 had a size of 0.6x1.5x1 m^3 and was filled with bio balls. Part 3, with a size of 1.4x1.5x1 m^3, was filled with bio balls at the bottom and layered with filter cloth on the surface. Part 4 had a size of 0.9x1.5x1 m^3 and was equipped with three UV lamps and heaters. The tube was filled with 3,994 L of seawater and 1,331 L of freshwater.

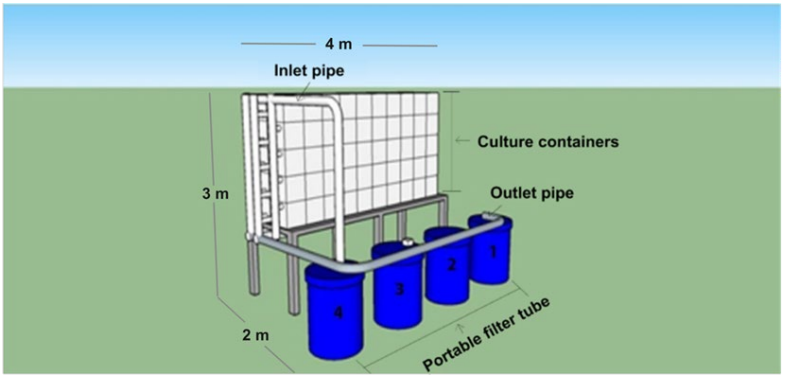

Figure 1. The illustration depicts the vertical crab farming equipped with a portable urban filter (P-PUF). P-PUF is composed of four filter tubes, each serving a specific function. Filter tube 1 is responsible for collecting dirty water; filter tube 2 is designated for water treatment using bacteria; filter tube 3 is allocated for disinfection; and filter tube 4 is designed to collect clean and ready-to-use water.

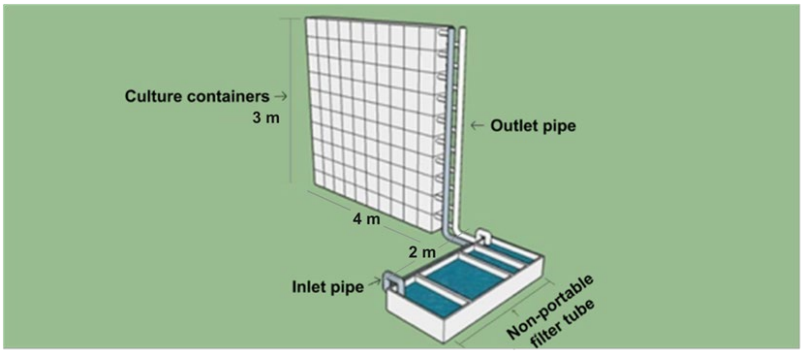

Figure 2. The illustration portrays the vertical crab farming with a non-portable filter, or conventional filter (C.K).

Mud crab *S. serrata* was obtained from Pemalang, Central Java, with an average weight of 112±16 g per individual. The mud crabs were first acclimatized for five days. Each mud crab was then reared for 30 days in a culture box (32.5x40x16 cm^3) with a total of 100 boxes per treatment set up transversely on several levels. Each culture box was filled with 6.2 L of water and was installed with one aeration stone. Feeding was given once a day at 5 p.m. with a feeding rate of 3% using a restricted method biomass (Hastuti et al., 2016).

2.2 Metagenomic analysis of bacterial diversity

2.2.1 Water sample collection

The rearing water samples of mud crab cultivation were separately collected from each treatment on day 30 of the crab cultivation period, consisting of inlet and outlet water samples with a total volume of 1 L, respectively. Water sample collection was conducted using sterile bottles and was transported to the laboratory in a cooler box.

2.2.2 DNA extraction

Water samples from the inlet and outlet were mixed to obtain a pooled water sample with a total volume of 2 L per treatment. The water sample was filtered through the polycarbonate membrane filter with a pore size of 0.22 µm. DNA was extracted from the water samples using the PowerWater DNA Isolation Kit (MO BIO Laboratories, Inc., Carlsbad, CA, US) following the manufacturer's instructions. DNA concentration was measured using agarose gel electrophoresis and Agilent 5400 (Agilent Technologies, Inc., Santa Clara, CA, US).

2.2.3 DNA sequencing

The metagenomic analysis was performed using an Illumina HiSeq platform (Illumina, Inc., San Diego, CA, US). We used a universal primer set for amplifying the V3-V4 hypervariable region of bacterial 16S rRNA, i.e., 341F (5'-CCTACGGGAGGCAGCAG-3') (Muyzer et al*.,* 1993) and 806R (5'-GGACTACHVGGGTWTCTAAT-3') (Caporaso et al., 2011). PCR amplification was carried out using the Phusion® High-Fidelity PCR Master Mix (New England Biolabs, Ipswich, MA, US) following the manufacturer's manual. PCR products with a size of 400-450 bp were chosen for the subsequent step and purified using the Qiagen Gel Extraction Kit (Qiagen, Hilden, DE) according to the manufacturer's instructions. The sequencing library generation was carried out using the NEBNext® Ultra™ DNA Library Prep Kit (NEB, Ipswich, MA, US) for the Illumina platform. The quality and quantity of the DNA library were examined by Qubit® dsDNA assays (Thermo Fisher Scientific, Waltham, MA, US) and qPCR. DNA sequencing was performed on an Illumina HiSeq 2500 PE250. The DNA library preparation and sequencing were outsourced to NovogeneAIT Genomics, Singapore.

2.2.4 Sequences analysis

The paired-end reads were obtained from the sequencing process. Barcode and adapter sequences were removed from the paired end reads. Then, the paired-end reads were merged using FLASH v1.2.7 (Magoč et al., 2011), producing the raw tags. The quality of the raw tags was filtered under specific filtering conditions using Quantitative Insights Into

Microbial Ecology (QIIME) v1.7.0 to obtain high-quality clean tags (Caporaso et al., 2010; Bokulich et al*.,* 2013). Subsequently, the obtained tags were compared to a reference database (GOLD database) using the UCHIME algorithm (Edgar et al., 2011) to detect and remove chimera sequences (Haas et al., 2011). This step generated effective tags.

The effective tags were analyzed with UPARSE software v7.0.1001 for OTU clustering and species annotation (Edgar et al., 2013). The same operational taxonomic unit (OTU) was defined as the sequences with a similarity of ≥97%. The representative sequences, the sequence with the highest frequency in each OTU, were screened and chosen for further steps. Each representative sequence was aligned against the SSU rRNA database of the SILVA database (Wang et al., 2007). The alignment process was conducted using MOTHUR software to obtain species annotation at each taxonomic level with a Threshold of 0.8~1 (Quast et al*.,* 2013). OTU abundance information was normalized using a standard sequence number corresponding to the sample with the fewest number of sequences. Alpha diversity, bacterial diversity within a sample, was analyzed on the normalized data using QIIME v.1.7.0 and displayed by R software v.2.15.3 (R Development Core Team, 2010). Alpha diversity was exhibited through 6 indices, including observed species, species richness estimates (Chao1 and ACE), diversity indices (Shannon and Simpson), and Good's coverage.

2.3 Analysis of culturable bacterial abundance

Water samples were obtained from C.K and T.P-PUF on day 7 and day 21. Total bacterial abundance in the rearing water was calculated using total plate count (TPC). A 1 mL of water sample was serially diluted using seawater complete (SWC) medium (tryptone 5 $g \cdot L^{-1}$, yeast extract 3 $g \cdot L^{-1}$, glycerol 3 $mL \cdot L^{-1}$, aquadest 250 mL, and seawater 750 $mL \cdot L^{-1}$), then 0.1 mL sample was cultured in SWC agar with 2 replicates. Incubation was performed at room temperature for 24-48 h. The bacterial colony was calculated within a range of 25-250 colonies. Meanwhile, nitrifying and denitrifying bacterial abundance were enumerated using the most probable number (MPN) method. A 1 mL of water sample was serially diluted using NaCl 0.85%. A total of 1 mL diluted water sample was inoculated to 9 mL of liquid medium containing Na_2HPO_4 0.9 $g \cdot L^{-1}$, KH_2PO_4 0.2 $g \cdot L^{-1}$, $MgSO_4 \cdot 7H_2O$ 0.1 $g \cdot L^{-1}$, $FeCl_3 \cdot 6H_2O$ 0.005 $g \cdot L^{-1}$, $CaCl_2 \cdot 6H_2O$ 0.184 $g \cdot L^{-1}$, yeast extract 0.25 $g \cdot L^{-1}$, succinate 5 $g \cdot L^{-1}$ as carbon source, and NH_4Cl 1 $g \cdot L^{-1}$ and KNO_3 1 $g \cdot L^{-1}$ were added to nitrifying and denitrifying bacterial growth media, respectively. Incubation was conducted at room temperature for 24 h. To verify nitrifying and denitrifying bacteria, bacterial culture was tested using sulfanilamide 1% and naphthalene ethylene diamine (NED) 0.1%, respectively, exhibiting pink to purple color (purplish color). Bacterial abundance was calculated using the MPN formula.

3. Results and Discussion

Vertical crab farming has been developed in several mud crab-producing countries. Rearing water becomes an essential part of the cultivation. The microbial community in the rearing water plays pivotal roles in maintaining water quality. Several bacteria in culture water are involved in nitrogen waste degradation, such as nitrifying and denitrifying bacteria. This study applied nitrifying bacteria *Halomonas* sp. HIB-F and denitrifying bacteria *Stenotrophomonas* sp. HIB_7a in a portable urban filter for vertical crab farming. After a 30-day rearing period, we investigated the bacterial diversity inhabiting culture water

in vertical crab farming with a microbial-based portable filter (T.P-PUF) compared to a conventional filter (C.K).

3.1 Bacterial diversity in *S. serrata* rearing water

We obtained 430,433 reads from the V3-V4 hypervariable region of the bacterial 16S rRNA gene from two samples (Table 1). All clean reads were clustered into 3,750 OTUs (Table 1). In general, C.K had a higher number of observed species, Chao1, and ACE, yet a lower Shannon and Simpson index compared to T.P-PUF. The rarefaction curves for the samples reached the plateau (Figure 3), suggesting sufficient coverage of the samples in describing the microbial community. According to the Shannon index, bacterial diversity, in which a higher value means higher bacterial diversity, showed a higher diversity in T.P-PUF than C.K (Table 1). The Simpson index, for which a high value (close to 1) denotes high diversity, also validated this result.

Table 1. The summary of bacterial diversity in the rearing water of the control (C.K) and portable urban filter (T.P.PUF) in vertical crab farming, as measured by bacterial richness (OTUs), diversity index (Shannon and Simpson), estimated OTUs richness (Chao1 and ACE), and sample coverage (Good's coverage)

Sample Name	Raw Tags	High-Quality Tags	OTU Number	Observed Species	Chao1	ACE	Shannon	Simpson	Good's Coverage
C.K	216,256	143,613	1,972	1,912	1,993	1,977	5.978	0.941	0.999
T.P-PUF	214,177	143,194	1,778	1,703	1,784	1,794	6.326	0.963	0.999
Total	430,433	286,807	3,705	-	-	-	-	-	-

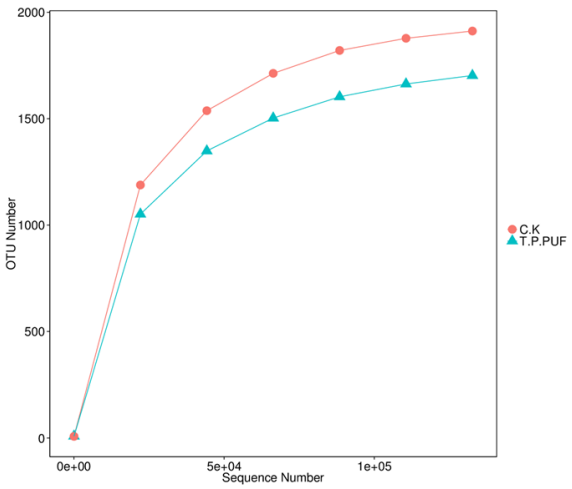

Figure 3. The rarefaction curves of bacterial diversity in the rearing water of vertical crab farming with the conventional filter (C.K) and the portable urban filter (T.P.PUF) which reached the plateau, indicating sufficient sample coverage for describing the microbial community in both samples. OTUs were defined with a similarity threshold of ≥97%.

The Venn Diagram showed that 1,207 OTUs were commonly found in the bacterial community of the rearing water of C.K and T.P.PUF, which corresponds to 50.12% of the total OTUs (Figure 4). C.K. had greater amount of unique OTUs (705 OTUs or 29.28%) in comparison with T.P.PUF (496 OTUs or 20.60%) (Figure 4). The Venn diagram confirms that C.K.'s bacterial OTUs are substantially more diverse than T.P-PUF (Figure 4).

Abundant bacterial phyla, families, and genera are shown in a relative abundance of >1%. Figure 5 shows the relative abundance of bacterial phyla in the rearing water of vertical crab farming between C.K and T.P.PUF. The result showed that the bacterial composition was different between the rearing water in C.K and T.P.PUF. The bacterial phylum detected in C.K was dominated by *Pseudomonadota* (63.95%), *Bacteroidota* (16.29%), *Bacillota* (7.92%), *Actinomycetota* (6.44%), and *Acidobacteriota* (1.2%). Meanwhile, in T.P.PUF, the bacterial community was dominated by *Pseudomonadota* (62.95%), *Bacillota* (20.10%), *Bacteroidota* (9.85%), *Actinomycetota* (4.85%), and *Acidobacteriota* (0.86%). *Thaumarchaeota*, belonging to archaea, was also detected in C.K (2.55%), which was higher than in T.P.PUF (0.07%).

This result aligns with a previous study by Cardona et al. (2016), who reported that *Pseudomonadota* (formerly known as *Proteobacteria*) and *Bacteroidota* (formerly known as *Bacteroidetes*) were the predominant and second most abundant phyla in the Clear seaWater system (CW) and BioFloc Technology (BFT) rearing water of whiteleg shrimp. Similarly, in shrimp aquaculture systems in Banyuwangi, Pangkajene, and Wonogiri, Indonesia, *Pseudomonadota*, *Bacteriodota*, as well as *Bacillota* were identified as the prevalent bacterial phyla (Hastuti et al., 2021a,b). *Pseudomonadota*, which are widely distributed in marine ecosystems, exhibit diverse metabolic capabilities, including phototrophy, autotrophy, and heterotrophy. They play key roles in biogeochemical cycles by utilizing sulfur compounds, C1 substrates, fatty acid, aromatic compounds, carbohydrates, and peptides, as well as contributing to denitrification processes (Zhou et al., 2020). *Bacteroidota* is known as a large group in the marine environment with adhesion and gliding motility capacity. This phylum grows attached to particles or other surfaces (adhesion capacity) where it could move by gliding motility and degrade high molecular weight compounds or polymers using degrading enzymes, including peptidases, glycoside hydrolases (GHs), and glycosyl transferases (Fernández-Gómez et al., 2013). *Bacillota* in seawater has been reported as spore-forming bacterium and antimicrobial-producing bacterium (Stincone & Brandelli, 2018). Autotrophic and heterotrophic nitrifiers, belonging to α, β, γ, δ *Pseudomonadota*, and aerobic denitrifiers, such as *Bacteroidota*, *Actinomycetota* and *Bacillota* were detected in marine and brackish water (Preena et al., 2021).

Bacterial families that were dominant in C.K were *Pseudoalteromonadaceae* (24.97%), *Vibrionaceae* (19.94%), *Cryomorphaceae* (13.91%), *Bacillaceae* (4.98%), *Mycobacteriaceae* (4.40%), *Rhodobacteraceae* (3.70%), *Nitrosopumilaceae* (2.50%), and *Saccharospirillaceae* (1.09%). On the other hand, abundant bacterial families in T.P.PUF were *Vibrionaceae* (20.45%), *Bacillaceae* (14.19%), *Rhodobacteraceae* (12.39%), *Pseudoalteromonadaceae* (10.00%), *Chitinophagaceae* (5.90%), *Mycobacteriaceae* (2.63%), *Planococcaceae* (2.07%), *Saccharospirillaceae* (2.04%), and *Cryomorphaceae* (1.14%).

Abundant bacterial genera in C.K were *Vibrio* (19.75%), *Pseudoalteromonas* (19.24%), *Cryomorphaceae*-uncultured (13.85%), *Bacillus* (4.56%), *Mycobacterium* (4.40%), *Candidatus_Nitrosopumilus* (2.36%). We observed that the bacterial community in T.P.PUF was dominated by *Vibrio* (20.31%), *Bacillus* (13.56%), *Pseudoalteromonas* (9.45%), *Marivita* (8.64%), *Chitinophagaceae*-uncultured (5.84%), *Mycobacterium* (2.63%), *Salinisphaera* (1.95%), *Lysinibacillus* (1.59%), *Cryomorphaceae*-uncultured (1.07%), and *Marinomonas* (1.04%).

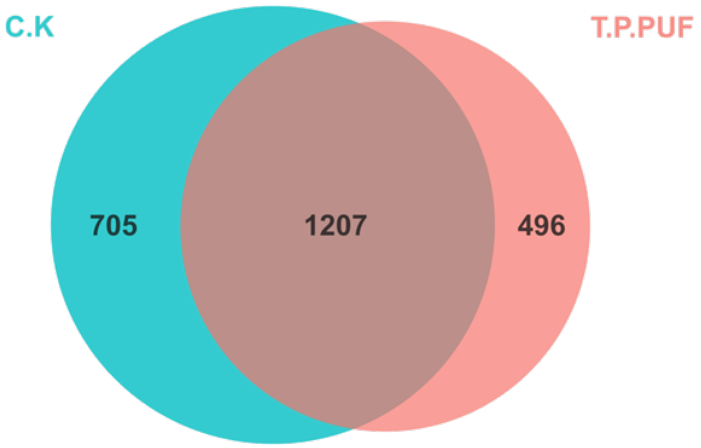

Figure 4. A Venn diagram illustrating the specific and shared bacterial OTUs between the rearing water of the conventional filter (C.K) and the portable urban filter (T.P.PUF) in vertical crab farming

Our results showed that *Vibrio* (*Vibrionaceae;* as *Pseudomonadota*) and *Pseudoalteromonas* (*Pseudoalteromonadaceae; Pseudomonadota*), were the top two dominant genera in C.K (Figures 5b, 5c). While *Vibrio* (*Vibrionaceae; Pseudomonadota*) and *Bacillus* (*Bacillaceae; Bacillota)* were frequently detected in T.P-PUF (Figures 5b, 5c). *Vibrio* abundance was slightly higher in T.P-PUF (20.45%) in comparison with C.K (19.94%). *Vibrio* is naturally found as normal bacterial flora in the rearing water of cultured biota, including crustaceans. The dominance of *Vibrio* in C.K and P-PUF might be from crab seeds used in this study. All these crabs came from natural catches suspected of carrying *Vibrio*. However, many Vibrios are known as common opportunistic pathogens of the portunid crab, both at larval and juvenile/adult stage, including *V. alginolyticus, V. harveyi, V. metschnikovii, V. natriegens,* and *V. parahaemolyticus* (Gunasekaran et al., 2019; Coates et al., 2022). *Vibrio* causes shell disease in cultured mud crabs, leading to mass mortality and economic losses. *Vibrio parahaemolyticus* reduces total haemocyte counts of mud crabs, resulting in cytological damage and high mortality (Cheng et al*.,* 2020b). *Pseudoalteromonas flavipulchra* were reported as a dual-functional probiotic in aquaculture, exhibiting bacterial activity against *Vibrio* pathogens (*V. vulnificus, V. parahaemolyticus* and *V. cholerae*) while also promoting microalgae growth (Wu et al., 2024). Also, marine *Bacillus* has a potential activity as aquaculture probiotics. As a probiotic, *Bacillus* is capable of performing some actions, including organic pollutants degradation, antibacterial and quorum quenching activity against fish pathogen *Aeromonas hydrophila* and *V. parahemolyticus*, acid-tolerant activity (up to 3 h in pH 2.0), and bile-tolerant activity (up to 20% concentration after 12 h of exposure) (Zhou et al., 2018). In addition, *Bacillus* possesses several antagonistic mechanisms, including the production of bacteriocins, lytic enzymes, antibiotics, and organic acids; repression of virulence gene expression; competition for adhesion site, nutrients, and energy; and immunostimulation (Kuebutornye et al*.,* 2020). In addition to rearing water, *Bacillus* was also one of the dominant genera in the gut of *S.serrata,* whereas *V. parahaemolyticus* accounted for less than 0.1% of the observed OTUs (Apine et al., 2021).

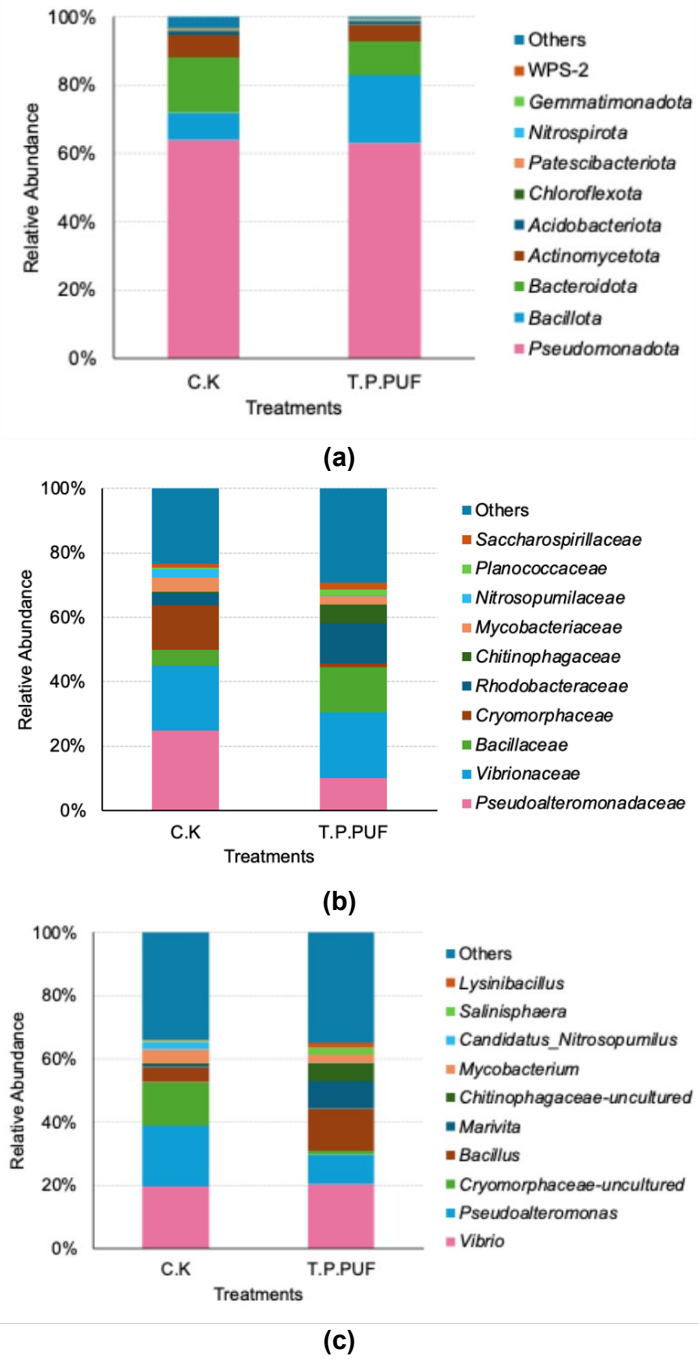

Figure 5. The relative abundance of bacterial OTUs highlighting the top ten phyla (a), families (b), and genera (c) in the rearing water of vertical crab farming with the conventional filter (C.K) and portable urban farming (T.P.PUF)

3.2 The abundance of culturable total bacteria and nitrifying and denitrifying bacteria

The results showed that total bacterial abundance in C.K was lower than that of T.P-PUF on days 7 and 21. In general, total bacterial abundance increased from day 7 to day 21 either in C.K or T.P-PUF (Table 2). The abundance of nitrifying and denitrifying bacteria was enhanced from day 7 to day 21 in C.K. and T.P-PUF. In C.K, nitrifying bacterial abundance was higher than denitrifying bacteria on days 7 and 21. Meanwhile, in T.P-PUF, denitrifying bacteria were more abundant compared to C.K on days 7 and 21 (Table 2).

Total bacteria in T.P-PUF was higher than in C.K. (Table 2). The microbial-based potable urban filter might increase the number of total bacteria in the rearing water and/or stimulate indigenous bacterial proliferation. This might probably be due to an increase in organic compounds, in terms of nitrogen excretion, in the rearing water along with the increase in mud crab growth. Meanwhile, the abundance of nitrifying and denitrifying bacteria differed between C.K. and T.P-PUF. In C.K., nitrifying bacteria were more abundant than denitrifying bacteria, suggesting a more extensive ammonium content in that rearing water. This finding is confirmed by a higher concentration of total ammonia nitrogen (TAN), ammonia, and nitrite on week 4 in C.K. (Hastuti et al., 2024). Qiu et al. (2025) reported that a greater abundance of functional microorganisms and related genes was correlated with ammonia-oxidizing activity and ammonia removal contribution rate. In contrast, in T.P-PUF, denitrifying bacteria had a greater number compared to nitrifying bacteria, meaning a higher nitrate and/or nitrite concentration. Generally, after a 21-day rearing period, T.P-PUF had more abundant nitrifying and denitrifying bacteria than in C.K.

Table 2. Total bacterial abundance in the rearing water of vertical crab farming with conventional filter (C.K) and microbial-based portable urban filter (T.P-PUF)

Bacterial Group	Day	Bacterial Abundance (CFU·mL^{-1})	
		CK	T.P-PUF
Total bacteria	7	3.35 x 10^4	5.90 x 10^5
	21	4.10 x 10^5	7.15 x 10^6
Nitrifying bacteria	7	230	460
	21	940	1,700
Denitrifying bacteria	7	20	2,400
	21	40	3,500

4. Conclusions

Vertical crab farming of mud crab *S. serrata* in this study was performed utilizing the microbial-based portable urban filer (T.P-PUF) that had been inoculated with nitrifying and denitrifying bacteria. More diverse bacteria in the rearing water of T.P-PUF were detected in comparison with conventional filter (C.K). Water microbiota inhabiting T.P-PUF and C.K was dominated by the top five bacterial phyla, i.e., *Pseudomonadota, Bacteroidota, Bacillota, Actinomycetota*, and *Acidobacteriota*, with different relative abundance.

Bacteroidota was higher than *Bacillota* in C.K, while *Bacillota* was relatively more abundant in T.P-PUF than in C.K. Applying microbial-based portable urban filter (T.P-PUF) in the vertical crab farming of mud crab increased the abundance of total bacteria, nitrifying and denitrifying bacteria in the rearing water. The bacterial community in both filter systems resulted from evaluating the nitrifying and denitrifying bacterial application provides the basis of knowledge for the maintenance of water quality during mud crab rearing period.

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6. Authors' Contributions

Yuni Puji Hastuti: Conceptualization, Data curation, Visualization, Formal analysis, Supervision, Funding acquisition, Project Administration, Writing – review and editing. Ridwan Affandi: Conceptualization, Writing – review and editing. Syamsul Bahri Agus: Conceptualization, Writing – review and editing. Hendra Sugandhi: Conceptualization, Funding acquisition. Yuli Siti Fatma: Data curation, Formal analysis, Software, Visualization, Writing – original draft, Writing – review and editing. Suroso: Conceptualization, Funding acquisition. Indra Jaya: Conceptualization. Bayu Aji Nugroho: Data curation, Formal analysis, Writing – original draft. Wildan Nurussalam: Data curation. Dudi Wildan Lesmana: Data curation.

ORCID
Yuni Puji Hastuti https://orcid.org/0000-0002-9068-5204
Ridwan Affandi https://orcid.org/0000-0003-2881-2142
Syamsul Bahri Agus https://orcid.org/0000-0002-9562-3790
Yuli Siti Fatma https://orcid.org/0000-0002-2651-3598
Indra Jaya https://orcid.org/0000-0002-1007-0657
Wildan Nurussalam https://orcid.org/0000-0002-7957-8301
Dudi Wildan Lesmana https://orcid.org/0000-0002-7910-0164

7. Conflicts of Interest

The authors declare that they have no known competing financial or non-financial, professional, or personal conflicts that could have appeared to influence the work reported in this paper.

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