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Research Article

Characterization of bacterial communities and fish growth in biofloc-based tanks for rearing Eastern catfish (*Silurus asotus*) or Japanese eel (*Anguilla japonica*)

Kim Y.G.[1], Niu K.M.[2], Hwang J.A.[3], Kim H.S.[3], Park J.S.[3], Lee J.H.[4], Ga G.W.[1], Jeon S.W.[1], Lee A.R.[5], Kim S.K.[1*]

1Department of Animal Science and Technology, Konkuk University, Seoul 05029, Republic of Korea
2Institute of Biological Resources, Jiangxi Academy of Sciences, Nanchang 330029, China
3Advanced Aquaculture Research Center, National Institute of Fisheries Science, Changwon 51688, Republic of Korea
4Fish Genetics and Breeding Research Center, National Institute of Fisheries Science, Geojero 53334, Republic of Korea
5Animal Resources Research Center, Konkuk University, Seoul 05029, Republic of Korea
*Correspondence: sookikim@konkuk.ac.kr

Keywords

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Microbiota,
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BFT

Abstract

The present study investigated the effects of water temperature and culture duration on *Silurus asotus* or *Anguilla japonica* growth performance and bacterial community in a biofloc technology (BFT)-based system. A temperature-dependent higher growth performance was observed in *S. asotus* or *A. japonica* with the highest growth rate at 30°C culture temperature. In 25°C-BFT tanks, the bacterial diversity achieved the highest value before rearing the fish, while the highest bacterial diversity was observed in 30°C-BFT tanks after rearing eel or catfish. The bacterial communities were differentially clustered depending on the temperature, culture duration, and fish species. Proteobacteria and bacteroidetes were the most dominant phyla and 45 predominant genera were identified. Among the predominant genera, *Cetobacterium* was the most abundant which accounts for 16-38% in the BFT tanks of catfish with different water temperatures. *Nannocystis* (22.3%), *Cetobacterium* (10.2%), and *Bacillus* (13.3%) were observed the most abundant in the 20°C, 25°C, or 30°C maintained BFT tanks of eel, respectively. Notably, the correlation analysis demonstrated *Devosia* abundance could affect catfish growth positively, while the *Flavobacterium* abundance could affect eel growth negatively. Additionally, *Exiguobacterium acetylicum* SK4913 exhibited the most probiotic potential among the 27 isolates from all the BFT tanks considering its multi-enzymatic activities. In summary, the culture temperature at the biofloc-preparation stage and fish growth stage should be maintained at 20°C and 25-30°C, respectively.

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Introduction

Increasing density in aquaculture operations and deteriorating cultural environment have led to the frequent occurrence of fish diseases, which threatens sustainable aquaculture production (Mugimba *et al*., 2021). Biofloc technology (BFT) is an emerging aquaculture technology that relies on microbiota within the biofloc to achieve nutritional recycling, water purifying, reduction of external pathogen inflow, as well as growth and health improvements of farm animals (Kumar *et al.*, 2021). A BFT system shows the advantages of being cost-effective and user-friendly by using a single-culture tank to satisfy aquatic animals rearing and degradation of waste compounds produced from feed debris, animal feces, toxic nitrogen sources, and other beneficial features (Dauda *et al.*, 2018; El-Sayed, 2021; Azim *et al*., 2022).

The microbial community plays a fundamental role in a BFT system, especially the heterotrophic bacteria, which are capable of reusing waste nutrients to produce microbial proteins and absorbing surrounding pollutants to improve the water quality (Kim *et al.*, 2021). The microbial flora is highly dependent on its capacity to produce digestive enzymes such as amylase, lipase, protease, and cellulase (Panigrahi *et al.*, 2021). Studies have shown that preying on bioflocs could help fish and shrimp elevate digestion and feed intake due to the rich digestive enzymes within the biofloc (Wang *et al.*, 2016; Long *et al.*, 2015). In addition, self-components of indigenous microorganisms can serve as immunostimulants and antioxidants to promote immunity and antioxidant defense of fish and crustaceans (Chen *et al.*, 2018; Le Xuan *et al.*, 2022). Undoubtedly, the microbial community is the key to achieving the benefits of a BFT aquaculture system.

Next-generation sequencing (NGS) has been widely used in exploring environmental and intestinal microbiota. Several studies have reported that external carbon sources, culture period, water temperature, and culture fish species all could change microbial diversity and composition in BFT-based aquaculture (Cardona *et al.*, 2016; Liu *et al.*, 2019; Kim *et al.*, 2021; Sontakke *et al.*, 2021). Artificial manipulation of the indigenous microbiome is likely to improve water purification efficiency and total productivity in BFT aquaculture (Mueller and Sachs, 2015). In a BFT system, operating parameters including water temperature, mixing intensity, dissolved oxygen (DO), pH, organic carbon sources, and organic loading rate all can impact microbial activities, and further influence properties and qualities of formed bioflocs (Dauda *et al.*, 2018).

Studies have investigated the performance of BFT systems for culturing various aquaculture fish species such as common carp (Azim *et al*., 2022), tilapia (Green *et al.*, 2021; Suarez-Puerto *et al.*, 2021), Pacific white shrimp (Zhang *et al*., 2025), catfish (Battisti *et al.*, 2020; Chen *et al.*, 2020; Dauda, 2020), and eel (Jiang *et al.*, 2019). However, to the best of our knowledge, no studies have investigated the effects of culture temperature on influencing the bacterial community in catfish- or eel-rearing BFT aquaculture systems. It is known that environmental

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factors such as water temperature, culture period, and rearing fish species all could impact water quality, bacterial community, and the growth performance of farmed fish (Dauda, 2020; El-Sayed, 2021). More importantly, the dynamic change of indigenous microbiota and its relationship with fish growth need to be better understood. Therefore, the objective of the present study was to characterize the bacterial community, and fish growth performance in biofloc-based tanks for catfish (*Silurus asotus*) or eel (*Anguilla japonica*) culture. The probiotic potential of bacteria isolates from the BFT tanks was also evaluated.

Materials and methods

Experimental design, fish, and rearing conditions

The fish feeding experiment followed the guidelines and regulations of the Care and Use of Laboratory Animals of the National Institute of Fisheries Science (NIFS) and was approved as 2021-NIFS-IACUC-4. This study was conducted at the Advanced Aquaculture Research Center (AARC), NIFS, Changwon, Republic of Korea. Each 3 indoor circular fiber reinforced plastic (FRP) tanks (diameter 1.2 m, depth 1 m, volume 1000 L) were used for eel and catfish culture, respectively.

The experiment comprised a biofloc-preparation stage (35 days) and a subsequent fish-rearing stage (28 days). The water in the 20°C, 25°C, or 30°C adjusted tanks was heated using submerged heaters with thermostats. All tanks were filled with sterilized freshwater with a UV pipe tube. Nearby groundwater located in the AARC was the freshwater source. At the biofloc-preparation stage, 5 L of molasses (carbon source, C) and 1 kg of the commercial feed (Sajo-Dongaone Co., Seoul, Republic of Korea) (nitrogen source, N) containing 44% protein were added into tanks with a C/N ratio of 15:1. A commercial probiotic BFT-ST product (30 mL/ton, EgeeTech, Texas, USA) containing *Bacillus subtilis*, *Bacillus amuloliquefaciens*, *Bacillus licheniformis*, *Cellumomona* sp., *Cellulomanas biazotea*, *Pseudomonas stutzeri*, *Pseudomonas denitrificans*, *Rhodopseudomonas palustris*, *Nitrobacter winogradskyi*, and *Nitrosomonas europaea* and a nutritional BFT-CT product (30 mL/ton, egeeTech, Texas, USA) containing vitamins and essential amino acids were also added into tanks. A total of 30 mL of BFT-ST was added in 1 m^3 of water in a BFT-tank to achieve $3x10^9$ CFU/m^3. Continuous aeration was supported to keep the biofloc always suspended using 1.5 Hp high-speed centrifugal motor pumps (60 W, CT-50, PhiGreen Ltd., China). The water temperature in BFT tanks was maintained and monitored using an automatic thermometer (1 KW, DH-1000ACW, DH electronics Ltd., Republic of Korea).

The water volume in the culture tank was maintained at about 1 ton (perimeter 1.2 m, depth 0.6 m) throughout the whole experimental period. No external water was supplemented except for the amount of water lost due to evaporation. In the fish rearing stage, a total of 165 eels (*Anguilla japonica*) with an initial average body length of 375.97±13.28 mm and an initial body weight of 104.01±5.99 g were assigned to three tanks with 55 fish per tank (8 kg/m^3). A total of 180 catfish (*Silurus*

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asotus) with an initial average body length of 176.39±4.37 mm and an initial body weight of 66.47±4.32 g were assigned to another three tanks with 60 fish per tank (6 kg/m^3). The commercial extruded diets containing 44% protein content (Sajo Dongaone Co., Seoul, Republic of Korea) or 54% protein content (Purina feed, Gyeonggi-do, Republic of Korea) were supplied to feed the catfish and eel, respectively. The fish was fed 5% of their body weight daily.

Before the fish inputting, we measured the biolfoc volume (mm) by the Imhoff for 30 days and when the biofloc volume reached 15.5 mm we placed the fish in each tank. We also measured the total ammonia nitrogen (TAN), nitrite (NO_2-N), and nitrate (NO_3-N) within the BFT tanks before the fish culture.

Fish growth performance

BFT systems were maintained for 35 days to prepare bioflocs. A total of 30 fish (catfish or eel) from a tank were then randomly selected. The body weight (BW) and body length (BL) of each fish were then measured after 28 days of rearing. Growth was determined based on average BL gain (BLG) and BW gain (BWG) using the following equations as follows:

BWG (g, %) = (final BW-initial BW)/initial BW × 100
BLG (mm, %) = (final BL-initial BL)/fish number
Specific growth rate (SGR, %/day) = [(ln final body weight- ln initial body weight)/days] × 100
Feed gain ratio = feed intake/wet weight gain
Survival (%) = (final number of fish/initial number of fish) × 100

Bacterial community analysis

Three water samples taken from each BFT tank at the biofloc-preparation stage of 0 W, 2 W, and 4 W and the fish-rearing stage of 4 W were analyzed for bacterial community by 16S rDNA sequencing. Samples were designated as 0W, 2W20°C, 2W25°C, 2W30°C, 4W20°C, 4W25°C, 4W30°C, CATFISH20°C, CATFISH25°C, CATFISH30°C, EEL20°C, EEL 25°C, and EEL 30°C. Genomic DNA extraction, PCR, sequencing, and bioinformatic analyses were conducted according to our previous methods with minor modifications (Niu *et al.*, 2019).

Briefly, a PowerSoil DNA isolation kit (Mo Bio Laboratories, Inc., Carlsbad, CA, USA) was used to isolate genomic DNA. The V3-V4 region of bacterial 16S rDNA gene was amplified using 341F (5′-CCTACGGGNGGCWGCAG-3′) and 785R (5′-GACTACHVGGGTATCTAATCC-3′) primers. An Illumina MiSeq platform was used for sequencing. Sequence data were analyzed by a commercial service (Macrogen, Ltd., Seoul, Republic of Korea). Alpha diversity was determined by analyzing OTUs, Chao1, Inverse Simpon, and Shannon indexes. Beta diversity was determined by principal coordinate analysis (PCoA) and weighted UniFrac distance matrix-based unweighted pair-group mean average (UPGMA) analysis. Venn diagram analysis was used to measure unique and shared species in different treatments.

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Microbial isolation and identification

To establish suitable probiotic strains, a total of 27 indigenous microbes within BFT water samples were isolated using different selective media procured from BD™ Difco™ (USA), namely Nutrient agar (NA) (pH 6.8±0.2), MRS (De Man, Rogosa and Sharpe) (pH 6.5±0.2), and R2A (Reasoner's 2A agar) (pH 7.2±0.2). All BFT water samples were obtained from AARC (NIFS, Changwon, Republic of Korea).

A suitable diluted water sample was spread onto each agar plate. Plates were incubated for 24-48 h. Single colonies with different morphologies were randomly picked, purified by streaking three times on agar plates, and stored in dimethyl sulfoxide (DMSO) (10%, v/v) at −80°C until use. The 27 isolated microbes were identified by 16S rDNA sequence using a commercial service (Macrogen Inc., Seoul, Republic of Korea). Genomic DNA was extracted from each isolate using an InstaGene™ Matrix (BIO-RAD, USA). The region of 16S rDNA gene from the extracted DNA was amplified using universal oligonucleotide 27F (AGAGTTTGATCMTGGCTCAG)/1492 R (TACGGYTACCTTGTTACGACTT) primers. Amplicons were purified and sequenced using 785F (GGATTAGATACCCTGGTA)/907R (CCGTCAATTCMTTTRAGTTT) primers and analyzed on an ABI PRISM 3730XL DNA analyzer (Applied Biosystem). Obtained sequences were then analyzed using GenBank Basic Local Alignment Search Tool (BLAST).

Enzymatic activities of the isolates

The 27 identified isolates were screened for enzymatic activities. They were grown on agar plates with corresponding media containing 1% (w/v) of different substrates, namely soluble starch, carboxymethyl cellulose (CMC), and skim milk for the detection of amylase, cellulase, and protease, respectively. Spirit blue agar medium was used for the detection of lipase. Amylase and cellulase activities were confirmed by Gram's iodine staining method (Kasana *et al.*, 2008). Lipase and protease-producing isolates were determined according to the clear zone size of hydrolysis on the respective media following 18-24 h of incubation.

Statistical analysis

Data regarding alpha diversity and the dominant phylum in the samples taken from BFT systems and fish growth were analyzed by one-way analysis of variance (ANOVA) with Tukey's HSD multiple range test at a significant level of $p<0.05$. Statistical analysis for the dominant genera in the samples taken of BFT systems relying on different culture temperatures, culture duration, and fish species was conducted by two-way ANOVA with Tukey's HSD multiple range test at a significant level of $p<0.05$. Correlation between the dominant genera and the BWG of fish was conducted by Pearson R analysis at a significant level of $p<0.05$. SPSS software with version 24 (SPSS Inc., Chicago, IL, USA) was used to conduct all statistical analyses. Figures were made with GraphPad Prism version 8 (California, USA). Data are expressed as means and standard error of means (mean±SEM).

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Results

Water quality

In the present study, we have measured the water quality parameters including the total ammonia nitrogen (TAN), NO_2-N, NO_3-N, and the biofloc formation before the fish culture period (Fig. 1). The TAN constantly declined along with the increase of NO_3-N concentration and biofloc forming volume, as well as the arched trend of NO_2-N concentration.

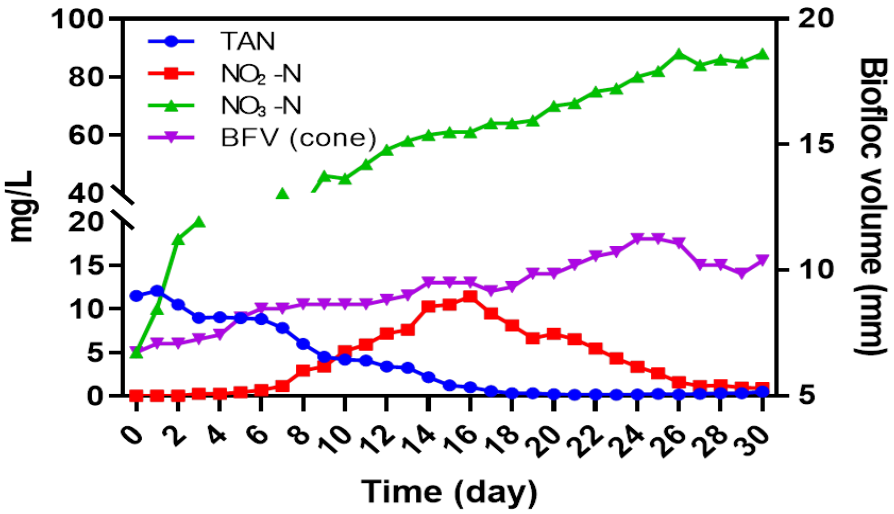

Figure 1: The changes of total ammonia nitrogen (TAN), nitrite (NO2-N), and nitrate (NO3-N) and the biofloc formation during both the fish culture period.

Fish growth

The growth of eel or catfish in biofloc-based tanks maintained at 20°C, 25°C, and 30°C was evaluated (Table 1). During 28 days of fish rearing in BFT tanks with nearly zero water exchange, no fish mortality was observed. Temperature-dependent increases in the final body weight, weight gain rate, and specific growth rate (SGR) of catfish were accompanied by a decrease in the feed/gain ratio. A higher weight gain rate, SGR, and feed/gain ratio were obtained in the 30°C culture temperature condition compared to the 20°C culture temperature condition.

Table 1: Growth performance of Eastern catfish (*Silurus asotus*) or Japanese eel (*Anguilla japonica*) at different culture temperatures.

Fish Species	Items	Culture temperature			*P*-value
		20°C	25°C	30°C	
Catfish	Initial body weight (g)	65.1±1.28	68.5±1.39	65.7±1.31	0.175
	Final body weight (g)	112.96±1.60^{a}	127.39±3.10^{b}	137.45±3.53^{c}	<0.001
	Weight gain rate (%)	69.92±0.37^{a}	91.65±0.73^{b}	106.77±0.62^{c}	<0.001
	Specific growth rate (%/day)	1.89±0.051^{a}	2.32±0.013^{b}	2.59±0.047^{c}	<0.001
	Feed/gain ratio	1.99±0.010^{a}	1.52±0.012^{b}	1.30±0.008^{c}	<0.001
Eel	Initial body weight (g)	107.0±2.03	103.0±1.50	102.1±1.93	0.158
	Final body weight (g)	130.05±2.75	134.55±1.40	136.13±2.60	0.197
	Weight gain rate (%)	25.04±0.69^{A}	28.08±1.40^{AB}	30.87±0.46^{B}	<0.001
	Specific growth rate (%/day)	0.80±0.042 ^{A}	0.92±0.013^{B}	0.96±0.029^{B}	0.003
	Feed/gain ratio	3.37±0.092^{A}	3.05±0.167^{AB}	2.73±0.041^{B}	0.002

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Bacterial diversity

Bacterial diversities in BFT tanks at the biofloc-preparation stage and fish-rearing stage were investigated. Indexes including species richness-based OTUs and Chao1 and evenness of abundance-based Shannon and Inverse Simpson were used to characterize bacterial diversity (Table 2). At the biofloc-preparation stage, the bacterial diversity enriched depending on culture duration. Higher OTU number was observed in 25°C-maintaining BFT tanks than in 0W- and 2W20°C-BFT tanks.

Table 2: Alpha-diversity indexes of microbiota in the biofloc technology (BFT) tanks.

Stage	Treatment[1]	OTUs	Chao[1]	Shannon	Inverse Simpson
Before rearing	0W	267.0 ± 1.2^{ab}	332.6 ± 8.5^{abc}	4.9 ± 0.0^{ab}	0.9 ± 0.0^{a}
	2W20°C	185.3 ± 4.9^{c}	234.4 ± 7.0^{a}	2.6 ± 0.1^{c}	0.6 ± 0.0^{b}
	2W25°C	413.0 ± 2.5^{d}	449.7 ± 5.2^{de}	6.4 ± 0.0^{d}	1.0 ± 0.0^{a}
	2W30°C	392.3 ± 7.3^{de}	453.4 ± 17.9^{de}	5.7 ± 0.0^{e}	0.9 ± 0.0^{a}
	4W20°C	345.3 ± 1.2^{ef}	425.2 ± 18.6^{cde}	5.5 ± 0.1^{be}	0.9 ± 0.0^{a}
	4WB25°C	406.0 ± 7.1^{d}	498.5 ± 7.7^{e}	5.5 ± 0.2^{be}	0.9 ± 0.0^{ac}
	4W30°C	367.0 ± 4.9^{de}	421.6 ± 1.9^{cde}	5.6 ± 0.0^{e}	1.0 ± 0.0^{a}
After rearing	CATFISH20°C	191.0 ± 5.1^{c}	259.3 ± 16.3^{a}	3.3 ± 0.1^{f}	0.8 ± 0.0^{d}
	CATFISH25°C	222.0 ± 3.8^{ac}	277.8 ± 24.4^{ab}	4.1 ± 0.2^{g}	0.8 ± 0.0^{cde}
	CATFISH30°C	230.7 ± 5.5^{ac}	283.5 ± 15.4^{ab}	4.5 ± 0.1^{ag}	0.8 ± 0.0^{de}
	EEL20°C	288.3 ± 32.8^{bf}	333.0 ± 47.5^{abc}	4.6 ± 0.3^{ag}	0.9 ± 0.0^{ace}
	EEL25°C	299.3 ± 17.4^{bf}	363.2 ± 30.8^{bcd}	5.4 ± 0.2^{be}	0.9 ± 0.0^{a}
	EEL30°C	340.0 ± 3.1^{ef}	388.2 ± 7.8^{cd}	5.8 ± 0.1^{de}	1.0 ± 0.0^{a}
	P-value	<0.01	<0.01	<0.01	<0.01

[1]Treatment: 0W=control tank water sample; 2W20°C, 2W25°C, and 2W30°C=Water samples respectively collected from BFT tanks maintained at 20°C, 25°C, and 30°C for 2 weeks, respectively; 4W20°C, 4W25°C, and 4W30°C=Water samples collected from BFT tanks maintained at 20°C, 25°C, and 30°C for 4 weeks, respectively; CATFISH20°C, CATFISH25°C, and CATFISH30°C=Water samples collected from Eastern catfish-rearing BFT tanks maintained at 20°C, 25°C, and 30°C for 4 weeks, respectively; EEL20°C, EEL25°C, and EEL30°C=Water samples collected from Japanese eel-rearing BFT tanks maintained at 20°C, 25°C, and 30°C for 4 weeks, respectively. Data within a column with superscript small letters represent a significant difference.

In catfish-rearing BFT tanks, culture temperature did not significantly change bacterial diversity. A similar result was found in eel-rearing BFT tanks. Bacterial diversity before and after rearing fish was also analyzed which a decrease in bacterial diversity in catfish-rearing BFT tanks was observed, irrespective of the effect of culture temperature. This was only observed in eel-rearing BFT tanks at 25°C. The similarity of bacterial diversity in different conditions was presented by PCoA (Fig. 2A) and UPGMA-based (Fig. 2B) β-diversity analyses, which found differentially clustered bacterial diversity according to fish species, culture temperature, and culture duration in BFT tanks. Moreover, more closely clustered bacterial communities were observed in BFT tanks at 25°C and 30°C than that at 20°C, regardless of the effect of culture duration.

Venn diagram displayed unique and shared bacterial species in different culture conditions (Fig. 3). At the biofloc-preparation stage, we found 165 shared and 25, 77, and 58 unique bacterial species in 2W-maintained BFT tanks adjusted water temperature at 20°C, 25°C, and 30°C, respectively (Fig. 3A).

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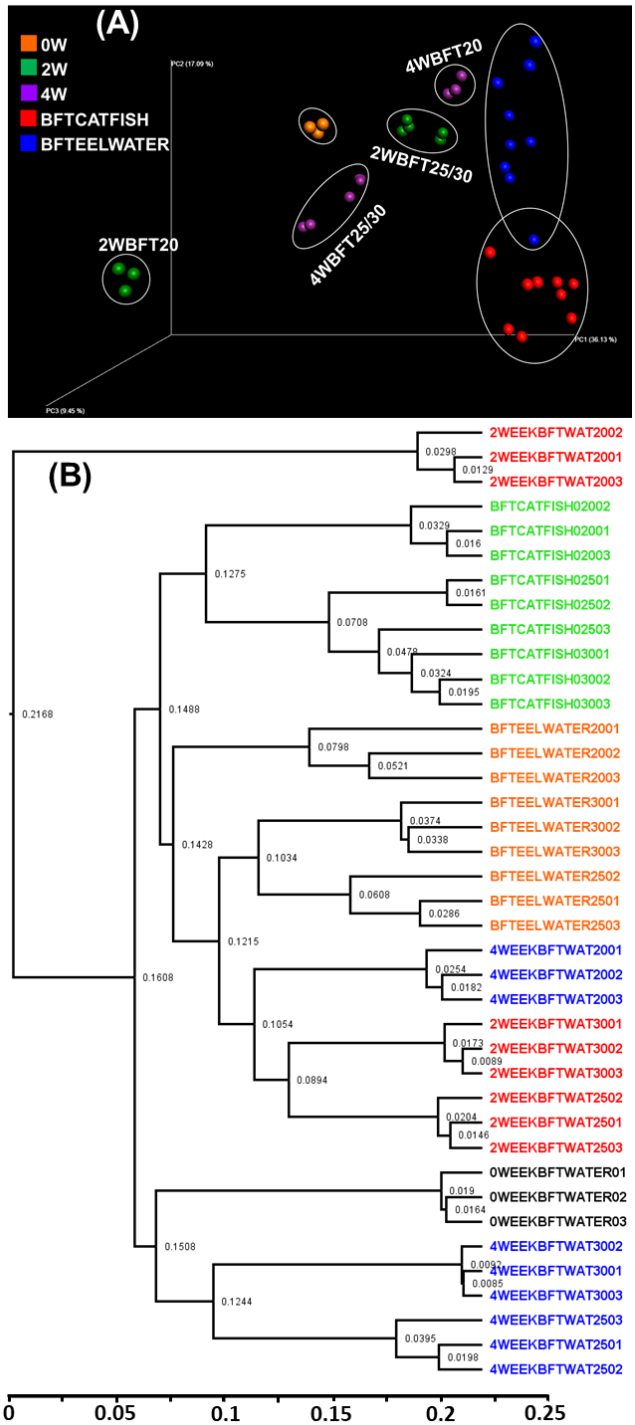

Figure 2: Similarity of the bacterial community in the biofloc technology (BFT) tanks. (A) Principal coordinate analysis (PCoA) plot based on weighted UniFrac distance matrix, (B) UPGMA clustering of samples based on the weighted UniFrac distance matrix. 0W=control tank water sample; 2W20°C, 2W25°C, and 2W30°C=Water samples respectively collected from BFT tanks maintained at 20°C, 25°C, and 30°C for 2 weeks, respectively; 4W20°C, 4W25°C, and 4W30°C=Water samples collected from BFT tanks maintained at 20°C, 25°C, and 30°C for 4 weeks, respectively; CATFISH20°C, CATFISH25°C, and CATFISH30°C=Water samples collected from Eastern catfish-rearing BFT tanks maintained at 20°C, 25°C, and 30°C for 4 weeks, respectively; EEL20°C, EEL25°C, and EEL30°C=Water samples collected from Japanese eel-rearing BFT tanks maintained at 20°C, 25°C, and 30°C for 4 weeks, respectively. Data within a column with superscript small letters represent a significant difference.

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We also found 205 shared and 50, 64, and 55 unique bacterial species in 4W-maintained BFT tanks adjusted water maintained at 20℃, 25℃ and 30℃ (Fig. 3B). After fish rearing, 129 shared and 46, 49, and 34 unique bacterial species, as well as 183 shared and 56, 36, and 71 unique bacterial species were found in the catfish or eel rearing BFT tanks adjusted water temperature at 20℃, 25℃ and 30℃, respectively (Figs. 3C and 3D). Altogether, the bacterial community within BFT tanks was more easily changed by the culture duration than by fish species and culture temperature.

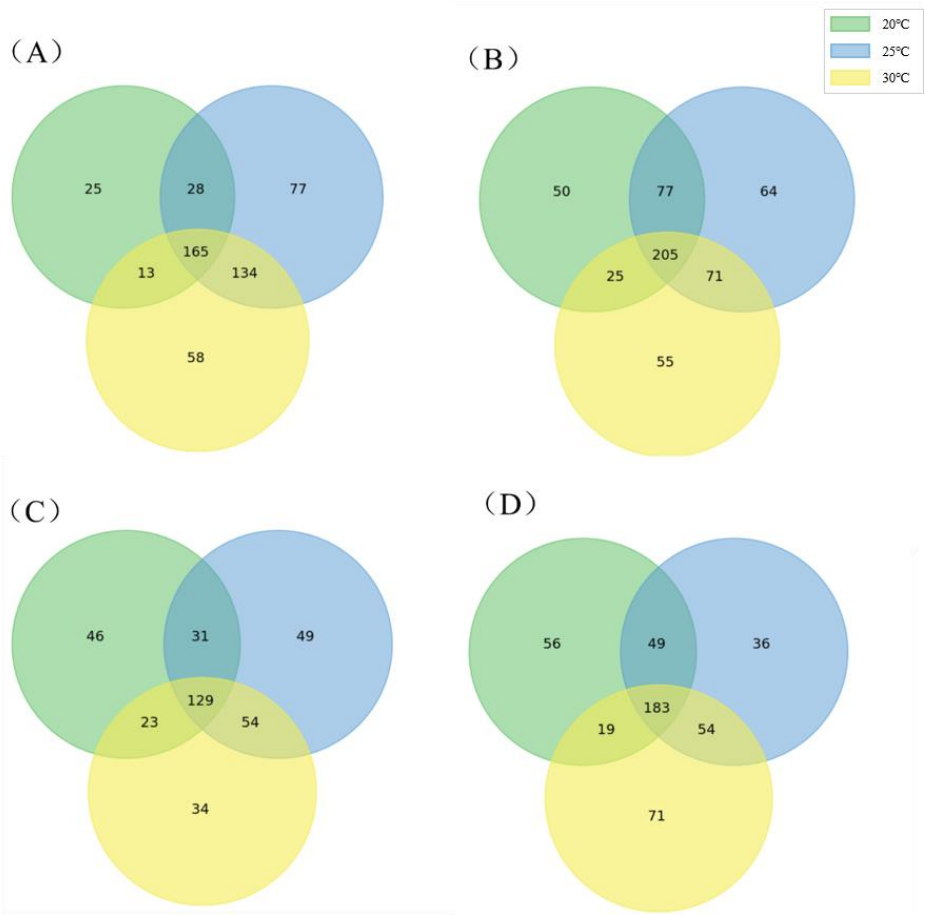

Figure 3: Shared and unique species in the biofloc technology (BFT) tanks displayed by Venn diagram. Culture duration of 2 weeks (A) and 4 weeks (B) at the biofloc-preparation stage, culture duration of 4 weeks for Eastern catfish (C) or Japanese eel (D). 20℃, 25℃, and 30℃=Water samples respectively collected from BFT tanks maintained at 20℃, 25℃, and 30℃.

Bacterial composition

Bacterial compositions in BFT tanks at the biofloc-preparation stage and fish-rearing stage were presented in Figure 4. Proteobacteria, Bacteroidetes, Verrucomicrobia, Firmicutes, and Fusobacteria were identified as the five most predominant phyla under all conditions. At the biofloc-preparation stage, the abundance of Proteobacteria exhibited a temperature-dependent increase while that of Bacteroidetes exhibited a temperature-dependent decrease in BFT tanks maintained for 2 weeks (Fig. 4A). In contrast, a reverse pattern of Proteobacteria and Bacteroidetes abundance was observed

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in BFT tanks maintained for 4 weeks (Fig. 4B). In catfish-rearing BFT tanks, 30°C culture temperature decreased the abundance of Proteobacteria and Bacteroidetes while increased the abundance of Fusobacteria when compared to those of 20°C culture temperature (Fig. 4C). In eel-rearing BFT tanks, the abundance of Proteobacteria was significantly decreased whereas that of Firmicutes was significantly increased at 30°C.The highest abundance of Fusobacteria was observed at 25°C (Fig. 4D).

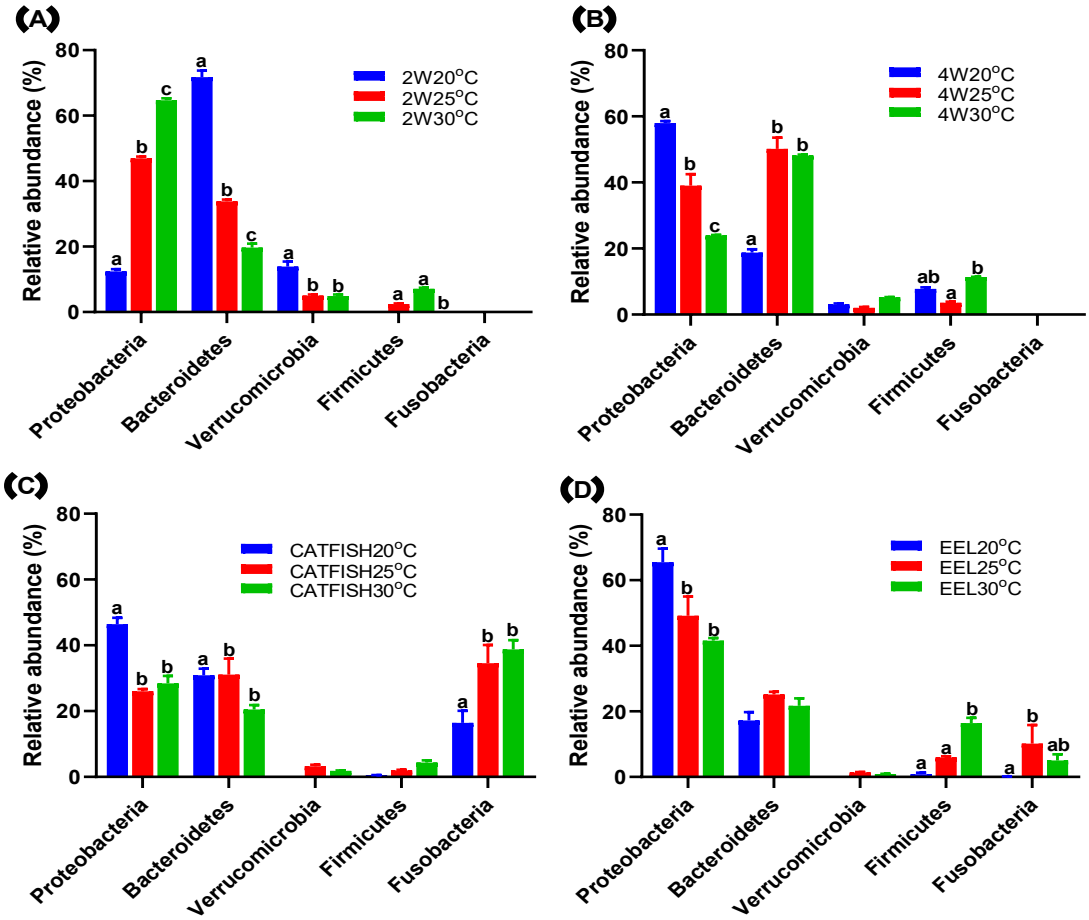

Figure 4: Relative abundance of predominant phyla in biofloc technology (BFT) tanks after 2 weeks (A) and 4 weeks (B) of maintaining at biofloc preparation stage or after 4 weeks of maintaining for rearing of Eastern catfish (C) or Japanese eel (D).

At the genus level, 45 predominant genera were identified in BFT tanks before and after fish-rearing (Tables 3 and 4). At the biofloc-preparation stage, the abundance of *Thermomonas*, *Pseudomonas*, *Bacillus*, *Citrobacter*, *Pseudoxanthomonas*, *Xanthobacter*, *Terrimonas*, *Bosea*, *Caulobacter*, and *Comamonas* was significantly increased, whereas the abundance of *Flavobacterium*, *Acidovorax*, *Prosthecobacter*, *Polynucleobacter*, and *Pedobacter* were significantly decreased with an increase of water temperature in BFT tanks maintained for 2 weeks. When BFT tanks were maintained for 4 weeks, the abundance of *Bacillus*, *Ferruginibacter*, *Mucilaginibacter*, *Terrimonas*, *Nitrospira*, *Sediminibacterium*, and *Clostridium* was

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significantly increased, while the abundance of *Acidovorax*, *Pseudomonas*, *Nannocystis*, *Terrimicrobium*, *Prevotella*, and *Devosia* was significantly decreased as an increase of culture temperature.

Table 3: Top 45 dominant genera in biofloc technology (BFT) tanks at the biofloc-preparation stage.

#	*Genus* (phylum)	0W		2W20°C		2W25°C		2W30°C		4W20°C		4W25°C		4W30°C		*P* value		
		mean	SEM	mean	SEM	mean	SEM	mean	SEM	mean	SEM	mean	SEM	mean	SEM	Temperature	Time	Temp.*time
		Relative abundance (%)																
1	*Flavobacterium* (B)	10.36	0.66	57.48	2.57	4.26	0.60	3.55	0.21	1.68	0.22	27.76	2.91	4.55	0.31	< 0.01	< 0.01	< 0.01
2	*Cetobacterium* (F1)	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.01	0.01	0.00	0.00	0.387	0.387	0.433
3	*Acidovorax* (P)	2.27	0.16	3.21	0.27	1.82	0.22	1.18	0.02	1.31	0.10	0.33	0.04	0.06	0.02	< 0.01	< 0.01	< 0.01
4	*Thermomonas* (P)	0.21	0.02	0.00	0.00	1.76	0.11	23.71	0.45	1.71	0.17	4.36	0.60	1.77	0.03	< 0.01	< 0.01	< 0.01
5	*Pseudomonas* (P)	4.72	0.34	0.74	0.23	3.85	0.12	4.10	0.32	21.09	1.56	3.42	0.37	3.05	0.10	< 0.01	< 0.01	< 0.01
6	*Bacillus* (F2)	0.80	0.05	0.04	0.01	1.46	0.15	5.64	0.29	1.67	0.23	2.58	0.30	9.66	0.08	< 0.01	< 0.01	< 0.01
7	*Prosthecobacter* (V)	14.77	1.25	10.90	1.64	0.50	0.12	0.12	0.04	0.04	0.01	0.10	0.01	0.09	0.01	< 0.01	< 0.01	< 0.01
8	*Flectobacillus* (B)	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.387	0.387	0.433
9	*Nannocystis* (P)	0.01	0.00	0.00	0.00	1.61	0.38	0.17	0.01	0.60	0.07	0.11	0.02	0.00	0.00	< 0.01	< 0.01	< 0.01
10	*Ferruginibacter* (B)	2.34	0.13	0.01	0.00	5.30	0.23	0.25	0.03	0.29	0.02	1.04	0.03	11.42	0.17	< 0.01	< 0.01	< 0.01
11	*Citrobacter* (P)	8.03	0.80	0.03	0.01	3.44	0.68	3.71	0.16	0.49	0.04	1.77	0.63	0.14	0.02	< 0.01	< 0.01	< 0.01
12	*Legionella* (P)	0.00	0.00	0.00	0.00	0.01	0.01	0.00	0.00	0.00	0.00	0.01	0.00	0.00	0.00	< 0.01	0.065	< 0.05
13	*Polynucleobacter* (P)	0.05	0.01	0.61	0.04	0.53	0.06	0.00	0.00	2.94	0.15	0.00	0.00	0.04	0.02	< 0.01	< 0.01	< 0.01
14	*Mucilaginibacter* (B)	0.05	0.00	0.00	0.00	0.03	0.01	0.00	0.00	0.00	0.00	0.01	0.01	0.38	0.02	< 0.01	< 0.01	< 0.01
15	*Acetobacteroides* (B)	3.64	0.36	3.86	0.18	3.45	0.20	4.38	0.28	0.21	0.03	0.20	0.11	0.05	0.01	0.448	< 0.01	0.266
16	*Pseudoxanthomonas* (P)	2.04	0.12	0.01	0.00	3.55	0.28	4.08	0.18	1.73	0.16	3.35	0.33	0.07	0.01	< 0.01	< 0.01	< 0.01
17	*Xanthobacter* (P)	0.44	0.08	0.02	0.00	0.95	0.07	6.97	1.31	2.51	0.19	3.96	0.56	0.13	0.01	< 0.01	< 0.01	< 0.01
18	*Ohtaekwangia* (B)	0.41	0.02	0.02	0.00	0.66	0.07	0.11	0.02	0.14	0.02	0.40	0.04	0.10	0.00	< 0.01	< 0.01	< 0.01
19	*Nemorincola* (B)	0.00	0.00	0.06	0.02	0.00	0.00	0.01	0.01	0.00	0.00	3.70	0.45	2.38	0.02	< 0.01	< 0.01	< 0.01
20	*Terrimonas* (B)	0.03	0.01	0.00	0.00	1.30	0.04	2.76	0.29	0.04	0.01	0.66	0.03	7.59	0.15	< 0.01	< 0.01	< 0.01
21	*Dyadobacter* (B)	2.08	0.13	0.22	0.05	3.54	0.31	0.20	0.02	1.81	0.12	2.27	0.55	0.13	0.02	< 0.01	< 0.01	< 0.01
22	*Variovorax* (P)	0.46	0.05	0.01	0.00	0.40	0.03	0.05	0.01	0.14	0.02	0.26	0.04	0.03	0.00	< 0.01	< 0.01	< 0.01
23	*Acinetobacter* (P)	5.56	0.60	0.20	0.03	1.08	0.20	0.37	0.03	1.85	0.15	0.87	0.24	3.01	0.15	0.237	< 0.01	< 0.05
24	*Hydrogenophaga* (P)	1.07	0.11	0.04	0.00	8.23	0.11	1.09	0.07	0.94	0.09	1.50	0.10	0.06	0.01	< 0.01	< 0.01	< 0.01
25	*Nordella* (P)	0.01	0.00	0.00	0.00	0.02	0.00	0.01	0.00	0.09	0.02	0.01	0.00	0.03	0.01	< 0.01	< 0.01	< 0.01
26	*Pedobacter* (B)	1.15	0.08	7.73	0.63	0.77	0.04	0.16	0.01	0.63	0.04	1.02	0.15	0.72	0.02	< 0.01	< 0.01	< 0.01
27	*Paucibacter* (P)	0.67	0.09	0.04	0.01	1.27	0.08	0.65	0.04	3.00	0.52	0.30	0.02	0.08	0.03	< 0.01	< 0.01	< 0.01
28	*Nitrospira* (N)	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.01	0.00	0.22	0.01	< 0.01	< 0.01	< 0.01
29	*Bosea* (P)	0.15	0.03	0.00	0.00	0.13	0.01	0.21	0.01	0.14	0.01	0.09	0.03	0.24	0.01	< 0.01	< 0.05	< 0.01
30	*Terrimicrobium* (V)	0.65	0.02	1.60	0.31	2.25	0.45	0.16	0.04	2.73	0.24	0.09	0.02	0.00	0.00	< 0.01	< 0.01	< 0.01
31	*Edaphobaculum* (B)	0.00	0.00	0.00	0.00	0.00	0.00	0.02	0.00	0.00	0.00	0.05	0.01	0.02	0.00	< 0.01	< 0.01	< 0.01
32	*Caulobacter* (P)	0.17	0.01	0.05	0.01	0.19	0.01	0.68	0.06	5.79	0.49	0.12	0.02	0.63	0.00	< 0.01	< 0.01	< 0.01
33	*Comamonas* (P)	0.82	0.08	0.09	0.01	0.41	0.03	0.77	0.05	2.62	0.38	0.46	0.07	0.80	0.11	< 0.01	< 0.01	< 0.01
34	*Paraclostridium* (F2)	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	-	-	-
35	*Sediminibacterium* (B)	0.00	0.00	0.00	0.00	6.97	0.24	0.44	0.01	0.00	0.00	0.05	0.01	0.12	0.02	< 0.01	< 0.01	< 0.01
36	*Novosphingobium* (P)	0.15	0.01	0.21	0.05	0.37	0.04	0.43	0.00	0.21	0.01	0.29	0.06	0.04	0.00	< 0.05	< 0.01	< 0.01
37	*Ideonella* (P)	0.05	0.01	0.00	0.00	1.79	0.01	0.77	0.02	0.03	0.00	2.05	0.17	0.02	0.01	< 0.01	< 0.01	< 0.01
38	*Rurimicrobium* (B)	0.02	0.01	0.00	0.00	0.00	0.00	0.01	0.00	0.00	0.00	2.89	0.18	0.00	0.00	< 0.01	< 0.01	< 0.01
39	*Prevotella* (B)	0.00	0.00	0.01	0.00	0.01	0.01	0.03	0.01	7.19	0.62	0.03	0.03	0.02	0.01	< 0.01	< 0.01	< 0.01
40	*Adhaeribacter* (B)	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.387	0.387	0.433
41	*Devosia* (P)	0.18	0.01	0.83	0.09	0.85	0.10	0.37	0.02	0.89	0.09	0.45	0.02	0.18	0.01	< 0.01	< 0.01	< 0.01
42	*Aeromonas* (P)	2.09	0.11	0.16	0.02	1.03	0.03	0.19	0.02	0.89	0.06	0.10	0.02	0.53	0.02	0.055	< 0.01	< 0.01
43	*Luteolibacter* (V)	0.52	0.07	0.02	0.00	0.29	0.05	0.53	0.04	0.08	0.01	0.39	0.06	4.57	0.14	< 0.01	< 0.01	< 0.01
44	*Rhodanobacter* (P)	0.00	0.00	0.00	0.00	0.00	0.00	0.01	0.00	0.00	0.00	0.24	0.09	0.04	0.01	< 0.05	< 0.01	< 0.01
45	*Clostridium* (F2)	0.07	0.01	0.06	0.00	0.39	0.06	1.14	0.06	0.35	0.06	0.40	0.06	1.17	0.08	< 0.01	< 0.01	< 0.01

B: Bacteroidetes, F1: Fusobacteria, P: Proteobacteria, F2: Firmicutes, V: Verrucomicrobia, N: Nitrospirae.

Most of the predominant genera were highly affected by culture temperature or duration and their interactive effects in BFT tanks. After fish-rearing, the abundance of *Cetobacterium*, *Bacillus*, *Prosthecobacter*, *Xanthobacter*, *Nitrospira*, *Paraclostridium*, *Rurimicrobium*, *Adhaeribacter*, *Devosia*, and *Clostridium* were significantly increased, while the abundance of *Flavobacterium*, *Flectobacillus*, *Nannocystis*, *Polynucleobacter*, *Ohtaekwangia*, *Pedobacter*, *Paucibacter*, *Sediminibacterium*, and *Ideonella* were significantly decreased as an increase of culture temperature in catfish-rearing BFT tanks. The abundance of *Bacillus*, *Prosthecobacter*, *Acetobacteroides*, *Ohtaekwangia*, *Hydrogenophaga*,

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Paucibacter, *Nitrospira*, *Comamonas*, and *Novosphingobium* were significantly increased, while the abundance of *Flavobacterium*, *Flectobacillus*, *Nannocystis*, *Polynucleobacter*, *Mucilaginibacter*, *Dyadobacter*, and *Sediminibacterium* were significantly decreased as an increase of culture temperature in eel-rearing BFT tanks.

Table 4: Top 45 dominant genera in biofloc technology (BFT) tanks at fish-rearing stage.

#	*Genus* (phylum)	CATFISH20°C		CATFISH25°C		CATFISH30°C		EEL20°C		EEL25°C		EEL30°C		*P* value		
		mean	SEM	mean	SEM	mean	SEM	mean	SEM	mean	SEM	mean	SEM	Temperature	Fish type	Temp.* fish type
		Relative abundance (%)														
1	*Flavobacterium* (B)	7.55	0.57	0.06	0.03	0.00	0.00	1.05	0.53	0.14	0.04	0.01	0.00	< 0.01	< 0.01	< 0.01
2	*Cetobacterium* (F1)	16.45	3.64	34.56	5.55	38.75	2.84	0.15	0.03	10.20	5.66	5.02	1.87	< 0.01	< 0.01	0.115
3	*Acidovorax* (P)	36.16	1.95	1.69	0.02	5.05	0.85	3.63	1.04	1.34	0.57	2.17	0.06	< 0.01	< 0.01	< 0.01
4	*Thermomonas* (P)	0.00	0.00	0.75	0.08	0.53	0.08	0.40	0.12	8.77	0.29	1.12	0.26	< 0.01	< 0.01	< 0.01
5	*Pseudomonas* (P)	0.04	0.02	0.03	0.01	0.07	0.02	0.17	0.04	0.15	0.03	0.47	0.09	< 0.01	< 0.01	< 0.05
6	*Bacillus* (F2)	0.01	0.00	0.02	0.00	0.04	0.01	0.04	0.03	1.37	0.20	13.29	1.20	< 0.01	< 0.01	< 0.01
7	*Prosthecobacter* (V)	0.00	0.00	1.41	0.28	1.49	0.20	0.00	0.00	0.00	0.00	0.05	0.01	< 0.01	< 0.01	< 0.01
8	*Flectobacillus* (B)	15.11	1.01	11.06	2.26	0.16	0.02	0.05	0.04	0.00	0.00	0.00	0.00	< 0.01	< 0.01	< 0.01
9	*Nannocystis* (P)	0.41	0.03	0.18	0.02	0.03	0.02	22.33	3.81	0.30	0.13	0.00	0.00	< 0.01	< 0.01	< 0.01
10	*Ferruginibacter* (B)	0.01	0.00	0.01	0.00	1.10	0.17	2.76	1.42	0.14	0.03	0.02	0.00	0.119	0.235	< 0.05
11	*Citrobacter* (P)	0.00	0.00	0.11	0.01	0.08	0.02	0.08	0.01	1.71	1.15	0.55	0.15	0.213	0.089	0.282
12	*Legionella* (P)	0.28	0.03	4.31	0.70	1.99	0.62	10.58	3.92	0.44	0.08	0.53	0.20	0.066	0.242	< 0.01
13	*Polynucleobacter* (P)	0.19	0.01	0.13	0.02	0.00	0.00	12.65	4.25	0.26	0.09	0.00	0.00	< 0.01	< 0.05	< 0.01
14	*Mucilaginibacter* (B)	0.01	0.00	11.65	1.74	4.51	1.02	0.02	0.01	0.00	0.00	0.00	0.00	< 0.01	< 0.01	< 0.01
15	*Acetobacteroides* (B)	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.01	0.00	< 0.05	< 0.01	< 0.05
16	*Pseudoxanthomonas* (P)	0.05	0.01	0.00	0.00	0.00	0.00	0.41	0.28	0.01	0.01	0.06	0.02	0.137	0.157	0.285
17	*Xanthobacter* (P)	0.00	0.00	0.02	0.01	0.07	0.02	0.00	0.00	0.00	0.00	0.00	0.00	< 0.05	< 0.01	< 0.05
18	*Ohtaekwangia* (B)	0.05	0.01	0.00	0.00	0.00	0.00	0.17	0.11	0.33	0.11	12.36	2.99	< 0.01	< 0.01	< 0.01
19	*Nemorincola* (B)	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	8.43	1.30	0.01	0.00	< 0.01	< 0.01	< 0.01
20	*Terrimonas* (B)	0.02	0.00	0.00	0.00	0.00	0.00	1.13	0.71	0.17	0.08	0.84	0.18	0.288	< 0.05	0.307
21	*Dyadobacter* (B)	0.09	0.02	1.06	0.10	0.00	0.00	2.46	0.78	0.04	0.00	0.02	0.01	< 0.01	0.108	< 0.01
22	*Variovorax* (P)	0.10	0.01	2.10	0.10	0.30	0.01	0.05	0.02	7.08	1.31	2.35	0.27	< 0.01	< 0.01	< 0.01
23	*Acinetobacter* (P)	0.00	0.00	0.01	0.00	0.02	0.00	0.01	0.01	0.28	0.16	0.03	0.01	0.1	0.101	0.114
24	*Hydrogenophaga* (P)	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.02	0.00	0.05	0.02	< 0.05	< 0.01	< 0.01
25	*Nordella* (P)	1.08	0.20	2.61	0.25	1.30	0.15	2.55	0.58	3.42	0.27	1.27	0.05	< 0.01	< 0.05	0.081
26	*Pedobacter* (B)	0.09	0.01	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	< 0.01	< 0.01	< 0.01
27	*Paucibacter* (P)	0.32	0.04	0.30	0.07	0.01	0.00	1.12	0.18	1.23	0.16	2.08	0.10	< 0.05	< 0.01	< 0.01
28	*Nitrospira* (N)	0.02	0.01	0.20	0.02	1.37	0.27	0.73	0.40	0.89	0.16	5.80	0.47	< 0.01	< 0.01	< 0.01
29	*Bosea* (P)	0.15	0.02	3.55	0.67	0.78	0.14	0.06	0.01	1.87	0.24	1.60	0.11	< 0.01	0.215	< 0.01
30	*Terrimicrobium* (V)	0.00	0.00	1.44	0.21	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	< 0.01	< 0.01	< 0.01
31	*Edaphobaculum* (B)	5.67	0.29	0.15	0.01	2.85	0.28	0.00	0.00	0.01	0.00	0.00	0.00	< 0.01	< 0.01	< 0.01
32	*Caulobacter* (P)	0.14	0.02	0.13	0.01	0.23	0.03	0.13	0.05	0.34	0.04	0.15	0.02	< 0.05	0.176	< 0.01
33	*Comamonas* (P)	0.02	0.00	0.27	0.02	0.03	0.01	0.20	0.07	0.62	0.35	1.58	0.13	< 0.01	< 0.01	< 0.01
34	*Paraclostridium* (F2)	0.40	0.01	0.58	0.05	2.26	0.21	0.31	0.17	2.98	0.23	2.03	0.32	< 0.01	< 0.01	< 0.01
35	*Sediminibacterium* (B)	0.25	0.03	0.01	0.00	0.00	0.00	0.33	0.08	0.17	0.05	0.00	0.00	< 0.01	< 0.05	0.195
36	*Novosphingobium* (P)	0.33	0.01	0.87	0.04	0.80	0.02	0.10	0.03	2.09	0.50	2.28	0.04	< 0.01	< 0.01	< 0.01
37	*Ideonella* (P)	0.02	0.00	0.02	0.00	0.01	0.00	0.36	0.15	0.28	0.00	2.75	0.41	< 0.01	< 0.01	< 0.01
38	*Rurimicrobium* (B)	0.01	0.01	0.42	0.04	1.50	0.16	0.01	0.01	3.24	0.49	0.00	0.00	< 0.01	< 0.05	< 0.01
39	*Prevotella* (B)	0.00	0.00	0.08	0.06	0.01	0.01	0.08	0.05	0.28	0.06	0.03	0.02	< 0.01	< 0.01	0.084
40	*Adhaeribacter* (B)	0.00	0.00	0.00	0.00	1.65	0.18	0.00	0.00	5.69	0.45	0.33	0.01	< 0.01	< 0.01	< 0.01
41	*Devosia* (P)	0.60	0.06	1.32	0.02	1.88	0.14	0.03	0.00	0.04	0.00	0.03	0.01	< 0.01	< 0.01	< 0.01
42	*Aeromonas* (P)	0.03	0.01	0.05	0.00	0.20	0.04	0.07	0.01	1.87	1.72	0.06	0.01	0.386	0.338	0.337
43	*Luteolibacter* (V)	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.53	0.07	0.04	0.01	< 0.01	< 0.01	< 0.01
44	*Rhodanobacter* (P)	0.00	0.00	0.43	0.02	0.32	0.03	0.00	0.00	5.92	0.15	0.00	0.00	< 0.01	< 0.01	< 0.01
45	*Clostridium* (F2)	0.11	0.02	0.30	0.03	0.33	0.03	0.40	0.21	1.32	0.12	0.87	0.10	< 0.01	< 0.01	< 0.05

B: Bacteroidetes, F1: Fusobacteria, P: Proteobacteria, F2: Firmicutes, V: Verrucomicrobia, N: Nitrospirae

Overall, the abundance of *Nitrospira*, *Bacillus*, *Prosthecobacter*, *Flavobacterium*, *Flectobacillus*, *Nannocystis*, *Polynucleobacter*, and *Sediminibacterium* were mainly changed by culture temperature rather than by fish species.

Correlation between the dominant genera and fish growth

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Next, we analyzed the mutual relationship between the predominant genera *per se* and their relationship with fish growth (Fig. 5). The abundance of *Cetobacterium*, *Terrimicrobium*, *Prosthecobacter*, *Ferruginibacter*, *Nitrospira*, *Paraclostridium*, *Novosphingobium*, *Rurimicrobium*, *Adhaeribacter,* and *Devosia* genera showed a positive correlation with catfish growth, whereas the abundance of *Flavobacterium*, *Acidovorax,* and *Flectobacillus* showed a negative correlation with catfish growth (Fig. 5A). Notably, the abundance of *Devosia* showed a significantly positive effect on the BWG of catfish. Furthermore, Flavobacterium-Prosthecobacter and Rurimicrobium-Flectobacillus showed significantly negative relationships whereas Ferruginibacter-Paraclostridium, Legionella-Mucilaginibacter, Variovorax-Bosea, Variovorax-Nordella, Paraclostridium-Nitrospira, Terrimicrobium-Dyadobacter, and Paraclostridium-Adhaeribacter showed significantly positive relationships. The correlation between the predominant genera and eel growth was also analyzed (Fig. 5B).

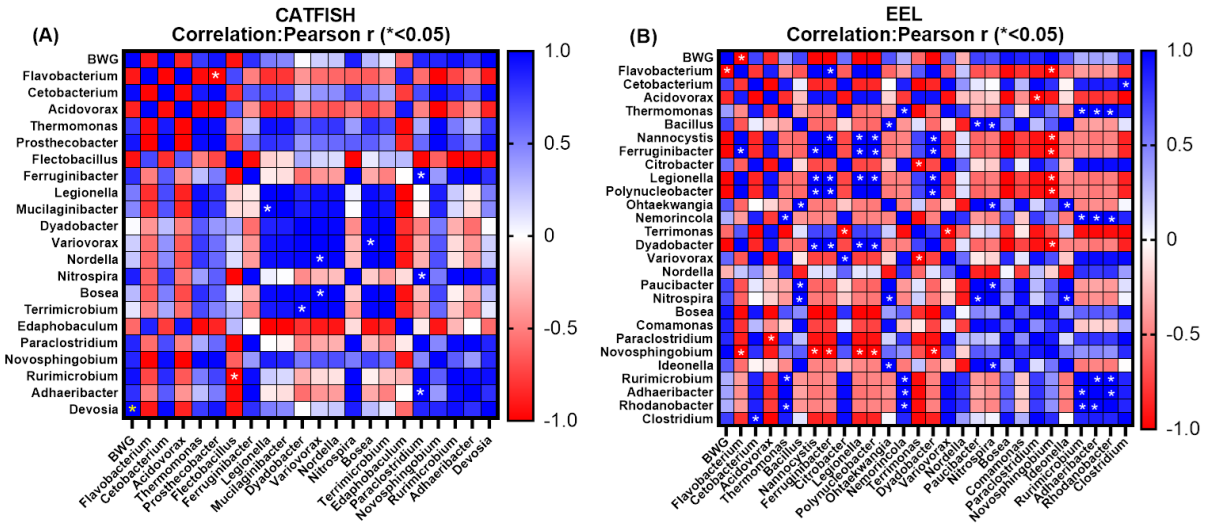

Figure 5: Correlation between the top 21 dominant genera and body weight gain rate (BWG) of the Eastern catfish (A) or Japanese eel (B) in BFT tanks.

Bosea, *Comamonas*, *Paraclostridium*, *Novosphingobium*, and *Clostridium* genera all showed positive effects on the BWG of eel, whereas *Flavobacterium*, *Acidovorax*, *Nannocystis*, *Ferruginibacter*, *Legionella*, *Polynucleobacter*, and *Dyadobacter* genera all showed negative effects on the growth of eel. Among these genera, *Flavobacterium* had a significantly negative correlation with the BWG of eel. Positive correlations were observed among Cetobacterium-Clostridium, Thermomonas-Rhodanobacter, Thermomonas-Rurimicrobium, Thermomonas-Nemorincola, Bacillus-Nitrospira, Bacillus-Paucibacter, Bacillus-Ohtaekwangia, Nannocystis-Dyadobacter, Nannocystis-Polynucleobacter, Nannocystis-Legionella, Ferruginibacter-Dyadobacter, Ferruginibacter-Polynucleobacter, Ferruginibacter-Legionella, Ferruginibacter-

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Flavobacterium, Ferruginibacter-Nannocystis, Citrobacter-Variovorax, Legionella-Dyadobacter, Polynucleobacter-Dyadobacter, Ohtaegwangia-Nitrospira, Ohtaegwangia-Ideonella, Nemorincola-Rurimicrobium, Nemorincola-Adhaeribacter, Nemorincola-Rhodanobacter, Paucibacter-Nitrospira, Nitrospira-Ideonella, Rurimicrobium-Adhaeribacter, Rurimicrobium-Rhodanobacter, and Adhaeribacter-Rhodanobacter genera, whereas negative correlations were observed among Novosphingobium-Flavobacterium, Novosphingobium-Nannocystis, Novosphingobium-Ferruginibacter, Novosphingobium-Legionella, Novosphingobium-Polynucleobacter, Novosphingobium-Dyadobacter, Terrimonas-Variovorax, Terrimonas-Citrobacter, and Acidovorax-Paraclostridium genera. Correlation between fish growth and the predominant genera and the relationship among themselves could guide researchers to manipulate bacterial community and improve the growth performance of reared fish.

Enzymatic activities of the isolates

To further track biofloc-forming bacteria in BFT tanks, we isolated and identified 27 bacteria and evaluated their enzymatic activities (Table 5).

Table 5: Identification and characterization of enzymatic activity of the isolates.

Stock #	Isolation	Identification			Enzymatic activity				BFT Water samples
	Media	Description	Query coverage (%)	Identity (%)	A	C	P	L	
SK4897	NA	*Caulobacter segnis*	96	99	-	-	-	+	
SK4898	NA	*Microbacterium saccharophilum*	98	98	-	-	-	-	
SK4899	NA	*Luteibacter yeojuensis*	99	99	-	-	+	+	
SK4900	NA	*Brevundimonas nasdae*	98	99	-	-	-	-	0w
SK4903	R2A	*Brevundimonas vesicularis*	98	99	-	-	+	-	
SK4905	R2A	*Rhodotorula dairenensis*	81	99	+	+++	+	+	
SK4906	R2A	*Sphingobium rhizovicinum*	97	99	-	-	-	-	
SK4907	R2A	*Variovorax paradoxus*	100	99	-	-	-	+	
SK4908	NA	*Flavobacterium cauense*	100	96	-	-	+	+	
SK4910	MRS	*Staphylococcus warneri*	100	99	-	+	-	-	
SK4912	MRS	*Enterococcus gallinarum*	99	99	-	-	-	-	2W20ºC
SK4913	R2A	*Exiguobacterium acetylicum*	96	99	+	+++	+++	-	
SK4914	NA	*Aeromonas media*	98	99	-	+	+++	+	
SK4915	NA	*Microbacterium oxydans*	98	99	-	+	+	+	
SK4918	NA	*Exiguobacterium indicum*	99	99	+	+++	+++	-	
SK4919	NA	*Flavobacterium terrae*	100	98	-	+	+	-	
SK4920	MRS	*Lactococcus taiwanensis*	98	99	-	-	-	-	
SK4921	MRS	*Lactococcus garvieae*	99	99	-	-	-	-	2W25ºC
SK4923	R2A	*Acinetobacter johnsonii*	96	99	-	-	-	+	
SK4924	R2A	*Paenarthrobacter nicotinovorans*	98	99	-	+	+	+	
SK4925	R2A	*Citrobacter freundii*	80	99	-	-	-	+	
SK4926	R2A	*Bacillus cereus*	97	99	+	+++	+++	+	
SK4929	NA	*Bacillus nealsonii*	98	99	-	+	-	-	2W30ºC

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Table 5 (continued):

Stock #	Isolation	Identification			Enzymatic activity				BFT Water samples
	Media	Description	Query coverage (%)	Identity (%)	A	C	P	L	
SK4930	NA	*Pseudomonas alcaligenes*	100	99	-	-	+	+	
SK4932	MRS	*Lactococcus lactis*	98	99	-	-	-	-	
SK4935	R2A	*Microbacterium flavescens*	98	99	-	+	-	-	
SK4936	R2A	*Thermomonas koreensis*	100	99	-	-	+	+	

-, not detected; +, normal ability; ++, good ability, +++ excellent ability. A: Amylase activity; C: cellulase activity; P: proteinase activity; L: lipase activity.

In original BFT water samples (0W), eight bacteria were identified. Among them, SK4905 was identified as *Rhodotorula dairenensis* showing diverse enzymatic activities. In 2W20°C water samples, *Exiguobacterium acetylicum* SK4913 exhibited the highest cellulase and protease activities among the four identified isolates. In 2W25°C water samples, *Aeromonas media* SK4914, *Exiguobacterium indicum* SK4918, and *Bacillus cereus* SK4926 showed amylase, cellulase, and protease activities among the 10 identified bacteria. In 2W30°C water samples, the isolates showed weak enzymatic activities. For these identified bacteria, the NGS sequencing data were further analyzed to determine the abundance of the corresponding species (Table 6). Only *Flavobacterium cauense*, *Flavobacterium terrae*, *Exiguobacterium acetylicum*, *Lactococcus lactis*, *Variovorax paradoxus*, *Citrobacter freundii*, *Acinetobacter johnsonii*, *Pseudomonas alcaligenes*, and *Thermomonas koreensis* were detected. Among these species, *C. freundii*, *A. johnsonii*, and *T. koreensis* were highly dominant in BFT tanks maintained at 30°C.

Table 6: Relative abundance of the identified isolates.

Genus	species	0W			2W20°C			2W25°C			2W30°C			4W20°C			4W25°C			4W30°C		
		Relative abundance (%)																				
Flavobacterium (B)	*F. cauense*	0.02	±	0.00	0.02	±	0.01	0.01	±	0.00	0.21	±	0.02	0.29	±	0.04	0.00	±	0.00	0.02	±	0.01
Flavobacterium (B)	*F. terrae*	0.00	±	0.00	0.00	±	0.00	0.00	±	0.00	0.00	±	0.00	0.00	±	0.00	0.00	±	0.00	0.00	±	0.00

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Table 6 (continued):

Genus	species	0W	2W20°C	2W25°C	2W30°C	4W20°C	4W25°C	4W30°C
		Relative abundance (%)						
xiguobacterium (F1)	*E. acetylicum*	0.16 ± 0.03	0.00 ± 0.00	0.14 ± 0.02	0.06 ± 0.02	0.13 ± 0.04	0.09 ± 0.01	0.00 ± 0.00
Lactococcus (F2)	*L. lactis*	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.02 ± 0.00	0.01 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Variovorax (P)	*V. paradoxus*	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Citrobacter (P)	*C. freundii*	8.03 ± 0.80	0.03 ± 0.01	3.44 ± 0.68	3.71 ± 0.16	0.49 ± 0.04	1.77 ± 0.63	0.14 ± 0.02
Acinetobacter (P)	*A. johnsonii*	5.38 ± 0.58	0.05 ± 0.00	1.03 ± 0.19	0.20 ± 0.01	0.76 ± 0.08	0.84 ± 0.24	3.00 ± 0.15
Pseudomonas (P)	*P. alcaligenes*	0.02 ± 0.01	0.02 ± 0.00	0.33 ± 0.05	0.43 ± 0.03	0.33 ± 0.05	0.05 ± 0.00	1.07 ± 0.03
Thermomonas (P)	*T. koreensis*	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	19.33 ± 0.39	0.00 ± 0.00	2.05 ± 0.25	0.60 ± 0.06

B: Bacteroidetes, F1: Fusobacteria, F2: Firmicutes, P: Proteobacteria

Discussion

It has been reported earlier that the optimal temperature for culturing eel is 29°C in an intensive pond aquaculture system (Masuda *et al.*, 2013). The optimal growth temperature of yellow catfish *Pelteobagrus fulvidraco* (Richardson, 1846) has been reported to be at 27°C (Wongkiew *et al.*, 2017). Similarly, faster growth of catfish or eel was observed at higher culture temperatures (25°C and 30°C) in the present study. However, the optimal culture temperature for each fish species needs to be further investigated to ensure no side effects.

Bioflocs comprise diverse autotrophic and heterotrophic microorganisms in aquatic ecology. Exploring the dynamic

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change of microbial communities in response to different environmental parameters will help us to establish better BFT aquaculture systems. Environmental conditions such as habitats, culture time, and water temperature all could affect the microbial community in situ. A recent study has determined effects of carbon source and culture period on bacterial community in a BFT-based *Litopenaeus vannamei* aquaculture system and reported that bacterial diversity and richness show changes similar to the present results (Kim *et al.*, 2021). In addition, other studies have confirmed that the microbial diversity and abundance in genus and species levels are increased at high water temperatures (≥30°C) compared to those at low water temperatures (≤20°C) (Tang *et al.*, 2014). Microbial abundance could also be affected by fish species reared in a BFT system. A previous study has reported that Proteobacteria, Bacteroidetes, Firmicutes, Verrucomicrobia, Nitrospirae, and Fusobacteria are the dominant phyla, and Hydrogenophaga and Bacillus are the most abundant genera in BFT-based tilapia aquaculture (Meenakshisundaram *et al.*, 2021). In another study, Proteobacteria, Bacteroidetes, and Verrucomicrobia phyla were found dominantly in BFT-based shrimp culture (Addo *et al.*, 2021). Similarly, in the present study high abundance of Proteobacteria, Bacteroidetes, Verrucomicrobia, Firmicutes, and Fusobacteria phyla were observed in all treatments, indicating their wide distribution in a BFT system. Indigenous microbiota of Proteobacteria and Bacteroidetes might have come from the original water used in the tanks (Wei *et al.*, 2016; Liu *et al.*, 2019). Moreover, herein, the abundance of Fusobacteria was highly increased in the presence of catfish or eel, suggesting that indigenous microbiota within the fish *per se* could also influence microbial diversity and composition in a BFT system.

In the present study, the abundance of *Flavobacterium*, *Flectobacillus*, *Nannocystis*, *Polynucleobacter*, and *Sediminibacterium* were significantly decreased with an increase in the culture temperature before and after fish rearing. Among them, *Flavobacterium* and *Flectobacillus* are known to be dominant genera on the skin and gills of fish (Lowrey *et al.*, 2015; Colston and Jackson, 2016). However, some species in these genera could act as pathogens in fish, especially *Flavobacterium columnare* which can cause columnaris and *Flectobacillus roseus* which can cause flectobacillosis in freshwater fish species (Shoemaker *et al.*, 2008; Adikesavalu *et al.*, 2015). Nannocystis is one of the most widely distributed myxobacteria in both terrestrial and aquatic environments (Moradi *et al.*, 2022). It can produce geosmin which is associated with off-flavors that cause undesirable taste and flavor of fish (Auffret *et al.*, 2013; Azaria and van Rijn, 2018).

On the other hand, the relative abundance of *Nitrospira*, *Bacillus*, and *Prosthecobacter* were significantly increased with an increase of water temperature regardless of fish species. *Bacillus* can survive in a wide range of environments. It is versatile to produce diverse active sources such as digestive enzymes and antimicrobial substances. It can also improve water quality by

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decomposing organic matter and reducing nitrogen wastes when it is added to an aquaculture system as a probiotic (James *et al.*, 2021). The comammox *Nitrospira* has been discovered to exist in diverse surroundings such as aquifers, drinking water systems, wastewater treatment plants, and recirculating aquaculture systems (Heise *et al.*, 2021). This genus could coexist in equal abundances in an aquaculture system and contribute to more effective utilization of nitrite and oxygen as well as complete oxidation of ammonia to nitrate (Preena *et al.*, 2021). *Cetobacterium* was enriched in both catfish and eel-rearing water in the present study. It has been reported as a component of the intestinal microbiota of carp (van Kessel *et al.*, 2011). *Cetobacterium* can produce vitamin B12 to support the requirements of farmed animals (Tsuchiya *et al.*, 2008). *Acidovorax* is a common genus found in freshwater environments with a biofilm-forming ability (Bahar *et al.*, 2010). This genus was detected in water samples at different water temperatures before and after fish rearing in the present study.

The genus *Bosea* is one of the predominant gut microbes in fish (Mohd Nosi *et al.*, 2018; Yu *et al.*, 2021). Eel known as one of carnivorous fish species is likely to contain specific intestinal microbial communities such as *Cetobacterium*, *Clostridium*, and *Fusobacteria* (Hsu *et al.*, 2018; Chen *et al.*, 2019). *Cetobacterium* plays an important role in the growth and development of the host because it can synthesize fats, proteins, and carbohydrates for use by the host. As a result, it was confirmed that an increment in the water temperature could positively contribute to changes in bacterial abundance, growth of fish, and improvement of water quality in a BFT system.

Besides microbial diversity and compositions which are of great importance in a BFT system, enzymatic features of specific microbes are also very important. Indigenous bacteria were isolated from BFT water samples and characterized for their enzymatic activities. Among these isolates, *R. dairenensis* SK4905 displayed good enzymatic activities. However, it was not detected by NGS sequencing, implying it might be less abundant. *E. acetylicum* displayed high cellulase and protease activities. It was detected by both culture-dependent and NGS-dependent methods. *E. acetylicum* can degrade shrimp shell wastes with a broad potential to be an environment-friendly agent to extract chitin (Sorokulova *et al.*, 2009). *E. indicum* and *B. cereus* also showed high enzymatic capabilities, although they were not detected by the NGS sequencing method. *C. freundii* and *A. johnsonii* presented higher abundance among the identified microbes, although they showed very weak enzymatic abilities. Herein, metagenomic data in combination with characteristics of the identified isolates suggest that exogenous supplementation of *R. dairenensis* and *Exiguobacterium* spp. in freshwater tank could be good for biofloc forming.

In the present study, we monitored dynamic changes in bacterial community and fish growth performance in biofloc-based eel or catfish cultures at different water temperatures. A culture temperature-dependent growth of catfish or eel was observed. Herein, our results suggest the

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water temperature could be maintained at 20°C during biofloc preparation and at a range of 25-30°C during fish growth considering fish production and system maintaining costs. The bacterial community in BFT tanks is quite susceptive to rearing conditions. Proteobacteria and Bacteroidetes were identified as the most dominant phyla. Forty-five dominant genera were also identified. The abundance of *Flavobacterium* genus could negatively affect eel growth, while *Devosia* abundance could positively affect catfish growth. Both *Flavobacterium* and *Devosia* are temperature-sensitive, indicating a possible manner to improve fish production by modulating the culture temperature. In addition, the isolate *E. acetylicum* SK4913 showed probiotic potential to be used in biofloc-based aquaculture owing to its multi-enzymatic activities.

Conclusions

The present study firstly profiled bacterial community in a biofloc-based culture for Eastern catfish (*Silurus asotus*) or Japanese eel (*Anguilla japonica*) and their relationship with fish growth. We found a temperature-dependent higher growth performance in Eastern catfish (*S. asotus*) or Japanese eel (*A. japonica*). In addition, *Devosia* abundance is likely to promote the growth of catfish while the *Flavobacterium* abundance may have a negative effect on eel growth. Moreover, *Exiguobacterium acetylicum* SK4913 exhibited multi-enzymatic activities showing probiotic potential in BFT-based aquaculture. These data could help researchers and fish farmers upgrade the existing BFT systems. Moreover, further exploration of the probiotic properties of the isolated bacteria is helpful to establish suitable probiotics used in eel or catfish culture.

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Conflicts of interest

The authors declare that no conflicts of interest among us.

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