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ORIGINAL ARTICLE

Effects of high temperature on water quality, growth performance, enzyme activity and the gut bacterial community of shrimp (*Litopenaeus vannamei*)

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Abstract

Gut bacterial communities play a crucial role in shrimp growth and health. However, these communities are still vulnerable to pressures (such as high temperatures) that hinder their functions. Here, shrimps were cultured for 6 weeks at three different temperatures: a variable temperature (control group, falling from 23.7 to 20.8°C with ambient temperature) and two fixed temperatures (26 and 30°C) to study their effects on growth, enzyme activity and gut microbes of shrimp. The results indicated that increasing temperature significantly decreased the survival rate of shrimp but had a significant rise in shrimp growth performances with the higher phosphatase enzyme activities than control group. Although the high temperature did not change the alpha diversity indexes of the bacterial communities, their compositions were significantly different, compared with that of control group. The relative abundances of the top 10 families showed a discrepancy in the dominance of the bacterial community, represented by Vibrionaceae and Mycoplasmataceae at 26 and 30°C groups, and Rhodobacteraceae and Flavobacteriaceae in the control group. These results indicate that temperature changes mainly affected the compositions of bacterial community, which increased the susceptibility of shrimp to some pathogenic bacterial species, such as *Vibrio*, thus leading to a low survival rate of shrimp.

KEYWORDS
enzyme activities, growth performance, gut bacterial communities, *Litopenaeus vannamei*, temperature

1 | INTRODUCTION

Although shrimp farming has become one of the most crucial primaries in aquaculture industries because of its economic benefits, shrimp farming still faces many environmental pressures that lead to instability and invasion of pathogens, even shrimp death (Bachand et al., 2020; Ismail & Abdullah, 2013; Liao & Chien, 2011). Previous studies have extensively revealed that environmental changes such as temperature directly impact the physiological balance of aquatic animals and can impair physiological processes such as immune (Liang et al., 2014; Sun et al., 2016; Zhang et al., 2018). Except for the physiological effects, temperature change might also impact the gut microbial communities of the shrimp, which play an essential role in helping shrimp absorb nutrients, grow and resist disease (Hai, 2015; Hong et al., 2011; Nie et al., 2017).

It has been widely reported that gut microbiota is closely related to the host and is affected by changes such as the growth and survival of the host (Sha et al., 2016; Sommer & Bäckhed, 2013). Liu et al. (2019) reported that the gut bacterial communities of shrimp are in a dynamic balance state unless exposed to an external stimulus.

Moreover, several reports have stated that changes in the external and internal environments of the gut cause significant imbalances in gut microbial communities, thus leading to disease and death of animals (Li et al., 2017; Sha et al., 2016; Zhang et al., 2016; Zheng et al., 2016; Zhu et al., 2016). Water is a major transmission route for pathogens to the gut microbial community. Tang et al. (2014) revealed that the change in water temperature is an environmental factor that leads to the outbreak of waterborne diseases. The changes in water temperature may change the abundance of intestinal bacterial species or increase pathogenic species such as Vibrionaceae. However, little is known about environmental changes, especially temperature, on the gut microbial community and shrimp development.

Thus, this study aimed to discover the causes for cultured shrimp growth and survival under temperature changes from the perspectives of physiology and gut microbial ecology of shrimp. This study monitored the effects of three different temperatures (control, 26, and 30°C) on growth performance, enzymes activity and gut bacterial abundance of *L. vannamei*. To clarify the factors affecting bacterial communities, evaluate the presence of pathogenic bacteria and reveal the good indicators on shrimp growth. This study hypothesis that: (i) the differences in growth performance and survival of shrimp induced by temperature change; (ii) different water temperatures affect the bacterial community abundance and compositions; and (iii) the correlations of bacterial community and growth parameters were also analysed.

2 | MATERIALS AND METHODS

2.1 | Experimental design

This experiment was conducted on the 6th of October for 6 weeks at a local shrimp farm located at Zhanqi District of Ningbo City, China (29°32'N, 121°31'E). The experiment was split into three groups of varying temperatures: control group (ranging from 23.7 to 20.8 °C in decreasing order to the natural factor), medium temperature group (26°C) and high-temperature group 30 °C). Digital-controlled heaters kept the temperature of the two groups (26 and 30 °C) at the desired amount. The ponds (500 L) were uniformly filled, with 5% of the seawater being recycled every day for each pond. The 18 ponds were divided into three groups at random, each with six repetitions ($n = 6$). The shrimps were fed a commercial diet equal to 8% of their individual body weight three times per day (8:00, 12:00, and 17:00), and the commercial feed contains 7.04% nitrogen and 44.2% carbon (Alpha Feed Co., Ltd.). The weight of the shrimp and the scale of the feeding trays were measured and weighed weekly. Dead shrimps were counted, documented and removed on a regular basis. The uneaten feeds were collected in a mesh collector, dried and weighed on a regular basis to facilitate meticulous estimations of feed intake and feed conversion ratio (FCR). In each pond, 100 shrimps were deposited with the same weight (0.65±0.01 g) and average length (6.4±0.52 cm) at storage respectively. Seawater was purified and stored in container tanks using a sand filter. Water was not exchanged in all of the experimental ponds during the first week of the experiment, and then daily exchanged at a rate of 5% of the ponds were exchanged every day after that. Throughout the investigation, air stones were used to provide continual aeration in all experimental ponds, and the water lost due to natural evaporation was compensated on a regular basis. Except for the aforementioned changes, all experimental groups used the same fundamental management techniques.

2.2 | Environmental physical-chemical parameters analyses

Yellow Springs Instrument Co. multiprobe (YSI 550A, USA) was used to track the temperature, dissolved oxygen (DO), salinity and pH of the water ponds daily. The water samples were collected at four different time intervals (0, 10, 30 and 41 days) and filtered with a 100-μm polycarbonate film to remove organic debris, and filtered again through a 0.45-μm polycarbonate membrane to measure water quality parameters including NH_4^+ (mg/L), NO_2^- (mg/L), NO_3^- (mg/L), PO_4^{3-} (mg/L) and Chl-*a* (μg/L) according to the method (AQSIQ, 2007).

2.3 | Sample collection and growth performances

At the end of the experiment (6-week), all shrimps were deprived of feed for 24 hours before sample collection. To compute the survival rate, weight gain (WG g), specific growth rate (SGR % d^{-1}), FCR and the condition factor (CF g/cm^{-3}), shrimps were counted and estimate individually. At the same time, shrimp were dissected to capture the hepatopancreas for enzymatic activity and the gut for bacterial community analysis, which were held in 1.5-ml Eppendorf tubes and immediately frozen in liquid nitrogen before being shipped to the laboratory and stored at −80 °C until use.

2.4 | DNA Extraction

The identical technique was utilized for the shrimp gut samples in both the control and the treatment groups. The microbiome DNA was isolated by using a Power Soil® DNA isolation kit (MO BIO Laboratories, Carlsbad, CA, USA) according to the manufacturer's instructions for each sample. Gut microbiota DNA were extracted using a QIAamp DNA Stool Mini kit (Qiagen, GmbH, Hilden, Germany). After that, 1 μl of the collected DNA was analysed with a Spectrophotometer (NanoDrop Technologies ND-1000, Wilmington, DE, USA) and stored at −80 °C before amplification.

2.5 | Enzyme activity assays

The reactive activity of the five enzymes: Total superoxide dismutase (SOD), Catalase (CAT), Acid Phosphatase (ACP), Alkaline Phosphatase (AKP) and Lysozyme (LZM) were triple-detected in each pond using a spectrophotometer at 550, 405, 520, 520 and 530 nm respectively.

According to the manufacturer's instructions, all the analyses of the enzyme activity levels were measured using the commercial assay kits (Nanjing Jiancheng Bioengineering Institute, Nanjing City, China). The test tubes of hepatopancreas samples stored previously at −80°C were taken out of the refrigerator and quickly lyophilized into powder in a vacuum freeze dryer for 48 hours (EYELA, Japan). The top of the tubes was closed with flexible plastic. A total of 0.5 mg tissue was weighted to new tubes and homogenized in 1 mL of saline phosphate buffer (PBS, pH = 7.5) with the electric grinding machine for 15 seconds. After homogenizing the samples again, they were centrifuged at 8000 rpm for 10 min. Under ice bath conditions of 4°C, the supernatant was quickly extracted for testing.

2.6 | Processing of Illumina sequencing data

Using the Quantitative Insights into Microbial Ecology software package (QIIME v1.9.1, http://qiime.org/index.html) toolkit (Caporaso et al., 2010). Raw sequences were accuracy filtered, and residual sequences were analysed for chimaeras using USEARCH v.6.1 (Edgar, 2010). The most abundant sequence from each taxon was identified as the representative sequence and matched with default settings in PyNAST (DeSantis et al., 2006). The *pick_open_reference_otus.py* script was used to test OTU selection, which was clustered into organizational taxonomic units (OTUs, 97% similarity) using the regular clustering algorithm UCLUST (Edgar, 2010). The SILVA 128 data sets were used to determine the taxonomic identity of each phylotype (DeSantis et al., 2006). Archaea, chloroplasts and mitochondria were omitted from the data set as OTUs that did not belong to bacteria and single units. OTUs accounting for less than 0.0001% of total bacterial sequences and were discarded to avoid potential sequencing errors. For each sample, the values of alpha (α-) diversity were rated, including richness, Shannon index, Pielou's evenness, and phylogenetic diversity (PD) and the Bray-Curtis distance for (β-) diversity was constructed using (QIIME v1.9.1).

2.7 | Statistical analyses

The variables that were determined were as follows:

$$\text{Weight gain (WG)} g = (W_t - W_0)/(W_0).$$

$$\text{Survival Rate (\%)} = (W_t)/(W_0) \times 100.$$

$$\text{Specific growth rate (SGR, } \%d^{-1}) = (\text{WG}, \%): = (\text{Ln}\, W_t - \text{Ln}\, W_0)/(\text{days}) \times 100.$$

Where W_t represents the final body weight, W_0 represents the initial body weight, while d represents the duration of the feeding experiment. One-way analysis of variance (ANOVA) was used to test the main effects of temperature manipulation in assays of growth performance, enzyme activity and water quality. When there were statistically significant differences ($p < 0.05$), the group means were compared further using Tukey-Kramer multiple range tests. All statistical analyses were performed using SPSS 20.0 (SPSS, IBM, USA).

QIIME v1.9.1 was used to compute estimates of alpha diversity and Bray-Curtis dissimilarity between samples. The OTU tables and files generated by QIIME were imported in R version 3.4.2 (R Development Core Team, 2010) for further processing and clarification of the results and were calculated using the 'vegan' R package. Principal coordinate analysis (PCoA) based on Bray-Curtis dissimilarity was applied to visualize samples and dissimilarities in gut bacterial community structure in the three groups. Analysis of similarity (ANOSIM) was used to explore the effects of temperature manipulation on the structures of the gut bacterial community using PRIMER-E V5 (Clarke & Gosrley, 2001). The 'pheatmap' package was used to approximate the most dominant taxa (top 20) for samples among the temperature levels. Pearson's correlation approximated the correlations between the OTUs of the most dominant taxa and shrimp survival rate, weight gain and enzyme variables.

2.8 | Ethics statement

There are no studies with human participants by any of the authors in this article. For the prevention of cruelty to animals, all applicable international, national and/or institutional standards were followed.

3 | RESULTS

3.1 | Effects of different temperatures on water quality parameters

Water samples were obtained during the cultivation period in four-time points to investigate the total ammonia nitrogen and chlorophyll-*a* concentration in each pond (Table 1). The results did not indicate any significant differences ($p > 0.05$) between the three groups, while the concentration of NH_4^+ showed a similar trend between control and treatments. The concentrations of NO_2^-, NO_3^- and PO_4^- were higher in the treatments than in control, especially at 30°C after 30 and 41 days, while the concentrations of chlorophyll-*a* trend opposite and showed the higher concentrations in control (Table 1).

Dissolved oxygen and pH did not differ significantly between control and treatments, except for a slight increase in salinity concentration at 30°C, 27.8 mg/L. In comparison, 26°C and the control group, the mean range was 24.6 mg/L. The dissolved oxygen was settled at 6.5 mg/L for all groups, and the pH was estimated at 7.4.

3.2 | Effects of different temperatures on survival, growth performance and enzyme activities

The survival rate, growth performance and morphological indices of the cultured shrimp *Litopenaeus vannamei* in different temperature levels were presented in Table 2. The health of

TABLE 1 Temporal dynamics of water physicochemical parameters over time

Index	Sampling days	Control	26 °C	30 °C
NH_4^+ (mg/L)	0 d	0.57 ± 0.22	0.2 ± 0.05	0.56 ± 0.38
	10 d	0.62 ± 0.49	0.09 ± 0.01	0.14 ± 0.03
	30 d	0.19 ± 0.04	2.8 ± 0.79	0.18 ± 0.02
	41 d	0.09 ± 0.01	0.12 ± 0.02	0.11 ± 0.02
NO_2^- (mg/L)	0 d	2.52 ± 0.85	12.52 ± 1.4	3.76 ± 1.14
	10 d	0.07 ± 0.03	8.49 ± 4.15	0.15 ± 0.02
	30 d	11.1 ± 2.58	1.1 ± 2.05	15.5 ± 1.49
	41 d	8.38 ± 3.86	19.6 ± 1.43	15.79 ± 8.87
NO_3^- (mg/L)	0 d	0.79 ± 1.38	9.78 ± 3.38	4.46 ± 1.09
	10 d	13.27 ± 1.87	15.54 ± 3.28	24.17 ± 3.96
	30 d	14.29 ± 4.43	0.43 ± 0.03	9.43 ± 1.44
	41 d	12.16 ± 3.95	0.89 ± 0.03	17.23 ± 2.76
PO_4^{3-} (mg/L)	0 d	0.43 ± 0.04	1.51 ± 0.21	0.48 ± 0.02
	10 d	0.85 ± 0.05	1.39 ± 0.42	0.82 ± 0.16
	30 d	1.59 ± 0.09	383.86 ± 223.23	1.42 ± 0.09
	41 d	1.41 ± 0.43	30.38 ± 14.92	1.17 ± 0.5
Chlorophyll-*a* (μg/L)	0 d	424.5 ± 154.32	81.66 ± 54.31	444.1 ± 275.58
	10 d	43.73 ± 28.02	16.71 ± 13.33	13.1 ± 4.61
	30 d	160.68 ± 49.54	0.2 ± 0.05	35.59 ± 26.35
	41 d	46.67 ± 38.27	0.09 ± 0.01	6.84 ± 5.68

Note: Effects of high temperature on water quality, growth performance, enzyme activity and the gut bacterial community of whiteleg shrimp (*Litopenaeus vannamei*).

TABLE 2 Growth performances, condition factor, and feed efficiency of *Litopenaeus vannamei* at different water temperatures for 6 weeks based on one-way ANOVA (means ±SD)

	Water temperature levels (°C)		
Index	Control (20 ±) °C	26 °C	30 °C
IBW (g)	0.65 ± 0.01	0.65 ± 0.01	0.65 ± 0.01
FBW (g)	1.75 ± 0.24^a	2.19 ± 0.21^a	3.4 ± 0.62^b
WG (g)	1.11 ± 0.24^a	1.54 ± 0.21^a	2.75 ± 0.62^b
SGR (%d^{-1})	2.37 ± 0.3^a	2.88 ± 0.24^b	3.89 ± 0.5^c
CF (g)	0.33 ± 0.04^a	0.36 ± 0.03^a	0.51 ± 0.05^b
FCR (g)	0.16 ± 0.01^c	0.14 ± 0.01^b	0.13 ± 0.01^a
Survival %	83.67 ± 9.48^b	62.17 ± 14.43^a	55.67 ± 16.12^a

Note: Different superscript letters indicate significant differences *($p < 0.05$) on the same line. Condition factor (CF g/cm^{-3}) to estimate the individual final length. ($n = 6$).

shrimp was significantly affected when water temperature increased during the culture time. The survival rate was significantly greater in the control group with the value of 83.7% than that in 26°C and 30°C groups, with survival rates of 62.2% and 55.7% respectively ($p < 0.05$). Final body weight, weight gain, specific growth rates and length gain (condition factor) were significantly greater at 30°C than at 26°C and control group ($p < 0.05$). The highest value of the weight gain rate was observed at 30°C, while the highest rate of feed conversion ratio was observed in the control group ($p < 0.05$).

Effects of different temperatures on enzyme activities in *L. vannamei* hepatopancreas were presented in Figures 1 and 2. The results indicated that SOD, CAT, LZM, ACP and AKP activities in hepatopancreas were significantly influenced by the different temperatures ($p < 0.05$). However, the response to the increase of temperature was different in each enzyme. The SOD and CAT activities were significantly ($p < 0.05$) decreased with the increase in temperature, compared with that in the control group (Figure 1 a and b), and the LZM activity at 30°C group was significantly ($p < 0.05$) higher than that in control and 26°C groups (Figure 1c). In addition, ACP and AKP activities showed an increasing trend with the increase in temperature, especially at the 30°C group, which were significantly ($p < 0.05$) higher than that in the control group (Figure 2).

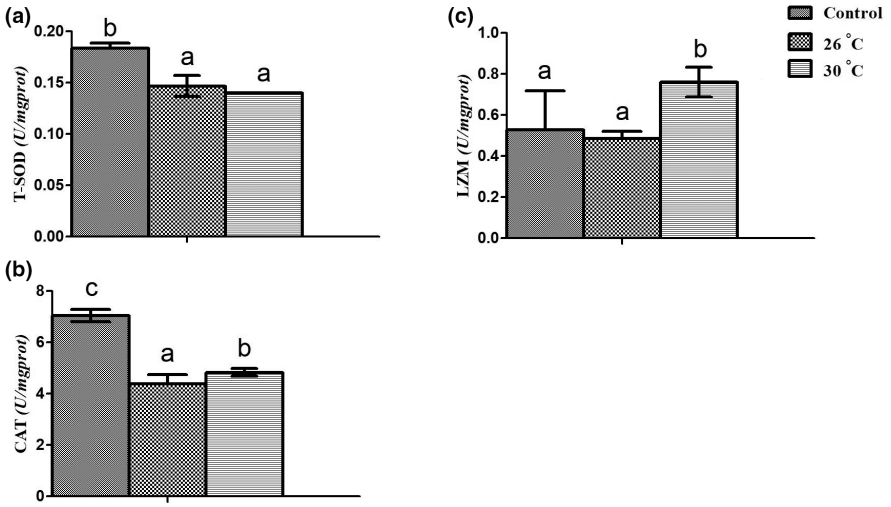

FIGURE 1 (a) Total superoxide dismutase (T-SOD) (*U/mgprot*), (b) Catalase (CAT) (*U/mgprot*), and (c) Lysozyme (LZM) (*U/mgprot*) indicated the dynamics of enzyme activity in the hepatopancreas of shrimp in the control and experimental groups during the experiment. Different letters are significantly different among treatments. Data present (means ±SD). (*n* = 6)

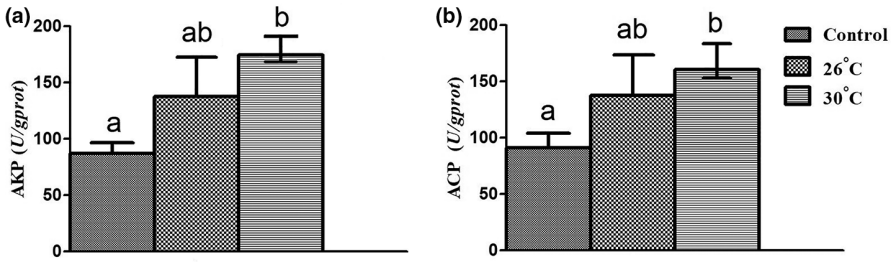

FIGURE 2 (a) Acid Phosphatase (ACP) (*U/gprot*) and (b), Alkaline Phosphatase (AKP) (*U/gprot*) indicated the dynamics of enzyme activity in the hepatopancreas of shrimp in the control and experimental groups during the experiment Different letters are significantly different among treatments. Data present (means ±SD). (*n* = 6)

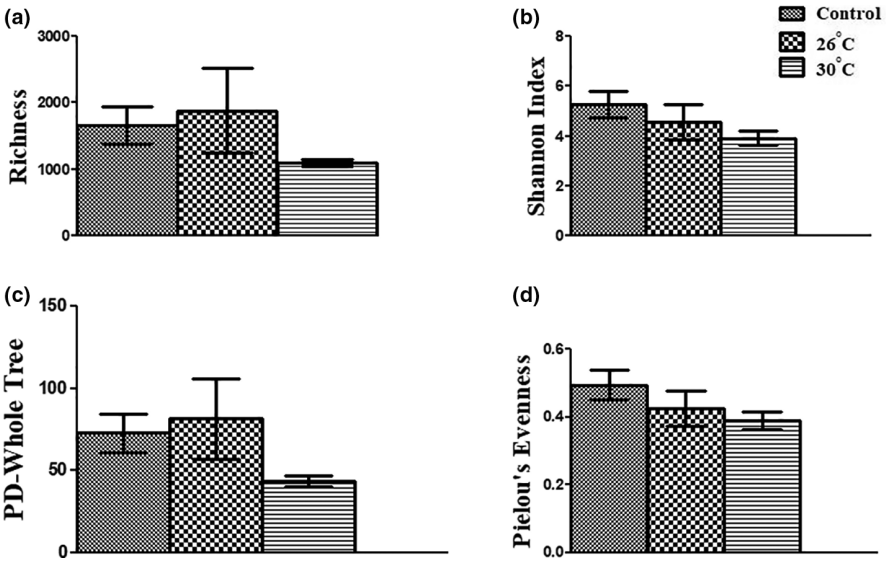

FIGURE 3 (a) Richness, (b) Shannon, (c) PD-Whole Tree, and (d) Pielou's Evenness. Data were analyzed utilizing one-way ANOVA and Tukey-Kramer post hoc comparisons and showed no significant differences among control and treatment groups ($p > 0.05$). PD - Whole tree = phylogenetic diversity

3.3 | Effect of different temperatures on the gut bacterial community of shrimp

Based on the data of α-alpha diversity indexes shown in Figure 3, a slightly lower number of bacteria were detected in the gut of the high-temperature group 30°C. However, in the 26°C and control groups, shrimp had higher richness, evenness, Shannon index and phylogenetic diversity than the high-temperature group 30°C. One-way ANOVA indicated that there was no significant difference between them ($p > 0.05$). The Principal Component Analysis (PCoA)

showed that the samples from the same group were agglomerated together. A significant difference ($p < 0.05$) of the bacterial community structure in the gut among the three groups based on Bray-Curtis dissimilarity (indicated by ANOSIM) (Figure 4). Permutational multivariate analysis of variance (PERMANOVA) revealed that the bacterial communities were significantly different between high temperature and control groups, but there was no significant difference between 26°C and 30°C (Table 3).

The relative abundances of the dominant phylum/class (relative abundance >1% at least in one group) were significantly different among the three groups (Figure 5a and b). The main dominant phylum at 30°C were Proteobacteria (63.87%), Tenericutes (23.04%), Bacteroidetes (7.83%) and Firmicutes (2.74%). At 26°C, there were Tenericutes (40.78%), Proteobacteria (30.6%), Bacteroidetes (22.21%) and Firmicutes (1.29%). While the most dominant of the control group was Tenericutes (19.47%), Proteobacteria (18.66%), Bacteroidetes (17.33%) and Firmicutes (4.97%) (Figure 5a). At the class level, the top dominant bacteria of the three groups were significantly different ($p < 0.05$). At 30°C, they were Gammaproteobacteria (53.62%), Mollicutes (20%), Clostridia (17.21%), Alphaproteobacteria (13.40%) and Bacteroidia (5.6%). The main dominants at 26°C were Mollicutes (34.42%), Bacteroidia (19.66%), Gammaproteobacteria (15%) and Alphaproteobacteria (5.6%). The main dominants of the control group were Alphaproteobacteria (26.42%), Gammaproteobacteria (18.93%) and Flavobacteriia (16.19%) (Figure 5b). At the family level, the main dominant families in the 30°C group were Vibrionaceae (46.8%), Mycoplasmataceae (15.1%) and Clostridiales_Family XII (13.2%). Likewise, the main dominants at 26°C were Vibrionaceae (30.7%), Mycoplasmataceae (30.2%) and Rhodobacteraceae (6.5%), while the main dominants in the control group were Flavobacteriaceae (19.9%), Rhodobacteraceae (17.8%) and Alteromonadaceae (17.6%) (Figure 6). In another way to illustrate the dominance at the OTU level along the temperature gradient, the heat-map examined the top 20 abundant OTUs (Figure 7). The abundance of dominance differed significantly between the three levels, as it was noted that the dominant appearance at (26 and 30°C) was OTU11737 (*Candidatus* bacilloplasma), OTU15823 (*Vibrio diabolicus*), OTU5315 (*Fusibacter*), and OTU15825 (*Photobacterium*), with a significant absence in the control group. Furthermore, the dominant taxa in the control group were OTU16351 (Rhodobacteraceae), OTU2292 (Flavobacteriaceae), OTU5024 (*Shewanella*), OTU13757 (*Aliagarivorans*) and OTU15456 (*Ruegeria*) (Figure 7).

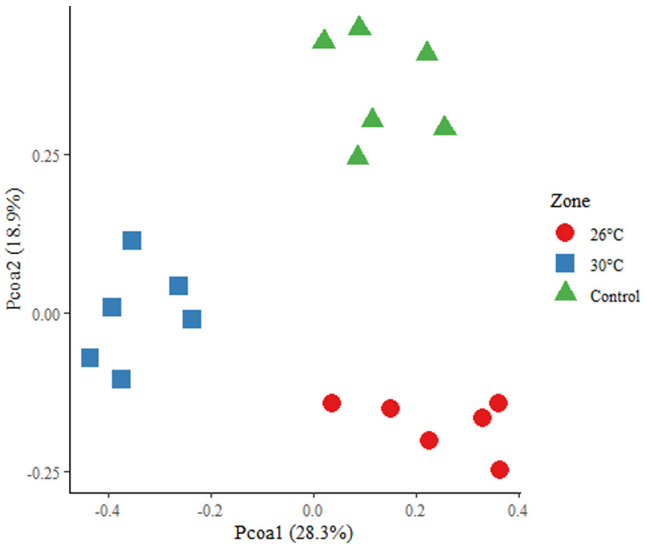

FIGURE 4 Principal coordinate analysis (PCoA) plot based on Bray-Curtis distance indicates variation pattern and compositions in shrimp gut bacterial communities structures in the different temperatures after 6 weeks ($n = 6$). Shapes in green triangles represent the control group, shapes in red circles represent the 26°C group, and shapes in blue squares represent the 30°C group. The *P* value indicates differences in community structure between the three groups. $p < 0.05$ indicates significant differences in community structure between the three groups

TABLE 3 Analysis of similarity (ANOSIM) based on Bray-Curtis dissimilarity for comparison on the bacterial community composition between control and experimental groups in the gut of shrimp at each sampling day

Groups	*R*	*p*-values
30°C Vs. Control	0.293	0.019
26°C Vs. Control	0.061	0.034
30°C Vs. 26°C	0.202	0.43

Note: All *P*-values were two-sided; a $^{*}p < 0.01$, $^{**}p < 0.001$. ($n = 6$).

3.4 | Linking of the top 20 abundant OTUs with shrimp growth parameters and enzyme activities

The Pearson correlation analysis demonstrated that 14 OTUs taxa of the top 20 abundant extensively correlated with the weight gain, survival rate and enzyme activities under the effects of different temperatures (Figure 8). Moreover, the distribution of these correlated taxa was entirely different among the groups (control, 26 and 30°C), and the proportion of the total negative correlations at (26 and 30°C) were higher than that of control (Figure 8). In detail, the correlations in the control group consisted of six OTUs taxa OTU16351 (Rhodobacteraceae), OTU15456 (Rhodobacteraceae *Ruegeria*), OTU2292 (Flavobacteracea), OTU5024 (Shewanellaceae *Shewanella*), OTU13757 (Alteromonadaceae *Aliagarivorans*) and OTU15010 (Flavobacteriaceae *Formosa*) respectively. Except for OTU5024 (Shewanellaceae *Shewanella*), all OTUs taxa in the control group correlated positively with survival rate. In contrast, all OTUs taxa were negatively correlated with growth rate. Similarly, the correlations with enzymes were positive to all OTUs taxa in CAT, and SOD, and negatively OTU5024 (Shewanellaceae *Shewanella*), OTU13757 (Alteromonadaceae Aliagarivorans), and OTU15010 (Flavobacteracea *Formosa*) correlated in LZM, and OTU16351 (Rhodobacteracea), OTU15456 (Rhodobacteracea

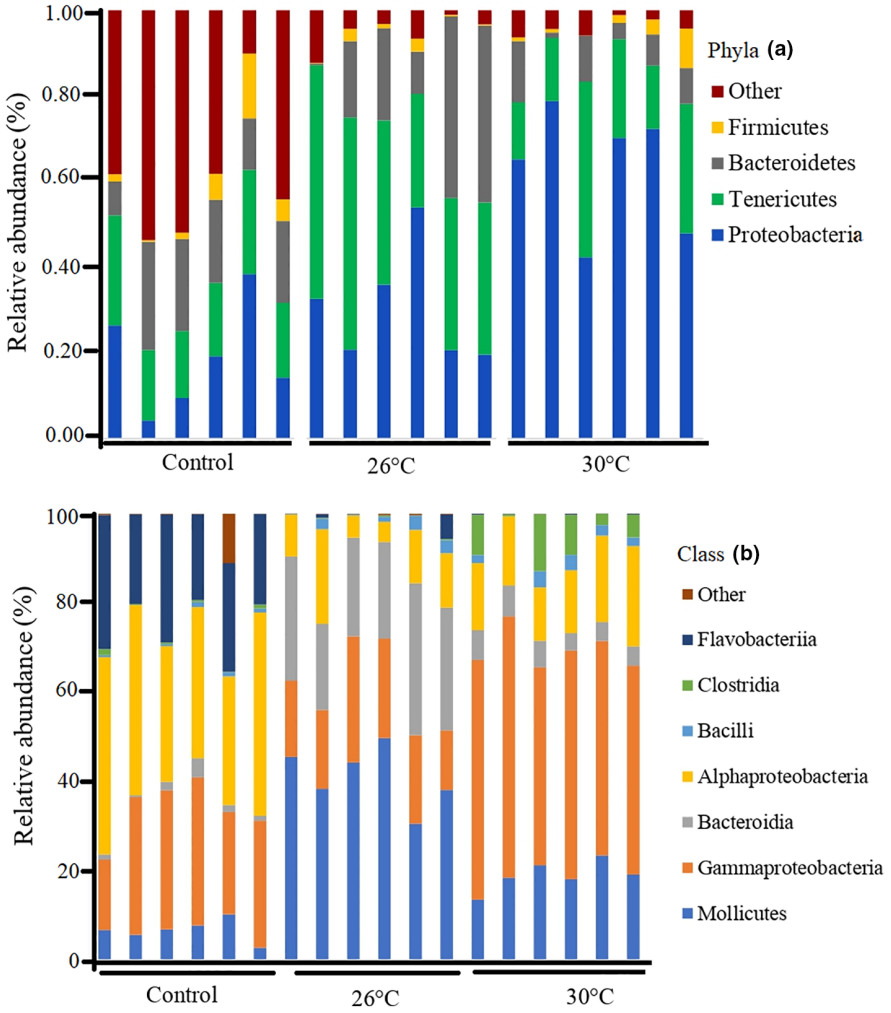

FIGURE 5 Average relative abundances of the dominant gut bacterial Phyla/Class (> 1% in at least one sample) bacterial taxa at (a) phylum and (b) class in different temperatures after 6 weeks. The taxa of less than 0.01% of the total abundance were combined together as a single group termed as others. ($n = 6$). All *P*-values were two-sided; a '*'($p < 0.05$), '**'($p < 0.001$)

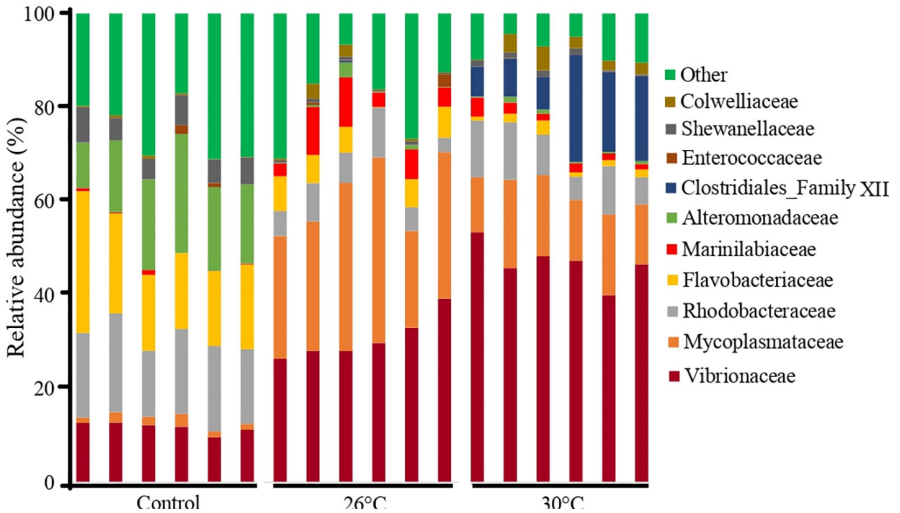

FIGURE 6 Relative abundances of the dominant families (> 1% in at least one sample) in gut bacterial samples after 6 weeks. The taxa of less than 0.01% of the total abundance were combined together as a single group termed as others. All *P*-values were two-sided; a '*'($p < 0.05$), '**'($p < 0.001$)

Ruegeria), OTU5024 (Shewanellaceae *Shewanella*), OTU13757 (Alteromonadaceae *Aliagarivorans*) in ACP.

At 26°C, which contains four OTUs taxa, OTU10140 (Flavobacteriaceae Spongiimonas), OTU11737 (Maycoplasmataceae *Candidatus* Bacilloplasma), OTU19633 (Marinilabiaceae *Saccharicrinis*) and OTU11260 (Pseudoalteromonadaceae *Pseudoalteromonas*), there were no significant correlations between taxa and growth parameters or survival rate, except a single positive correlation for OTU10140 (Flavobacteriaceae *Spongiimonas*) with survival. In enzymes, the negative correlations were found in CAT with (Maycoplasmataceae *Candidatus* Bacilloplasma) and OTU19633 (Marinilabiaceae *Saccharicrinis*) and SOD with (Marinilabiaceae *Saccharicrinis*). In contrast, positive correlations

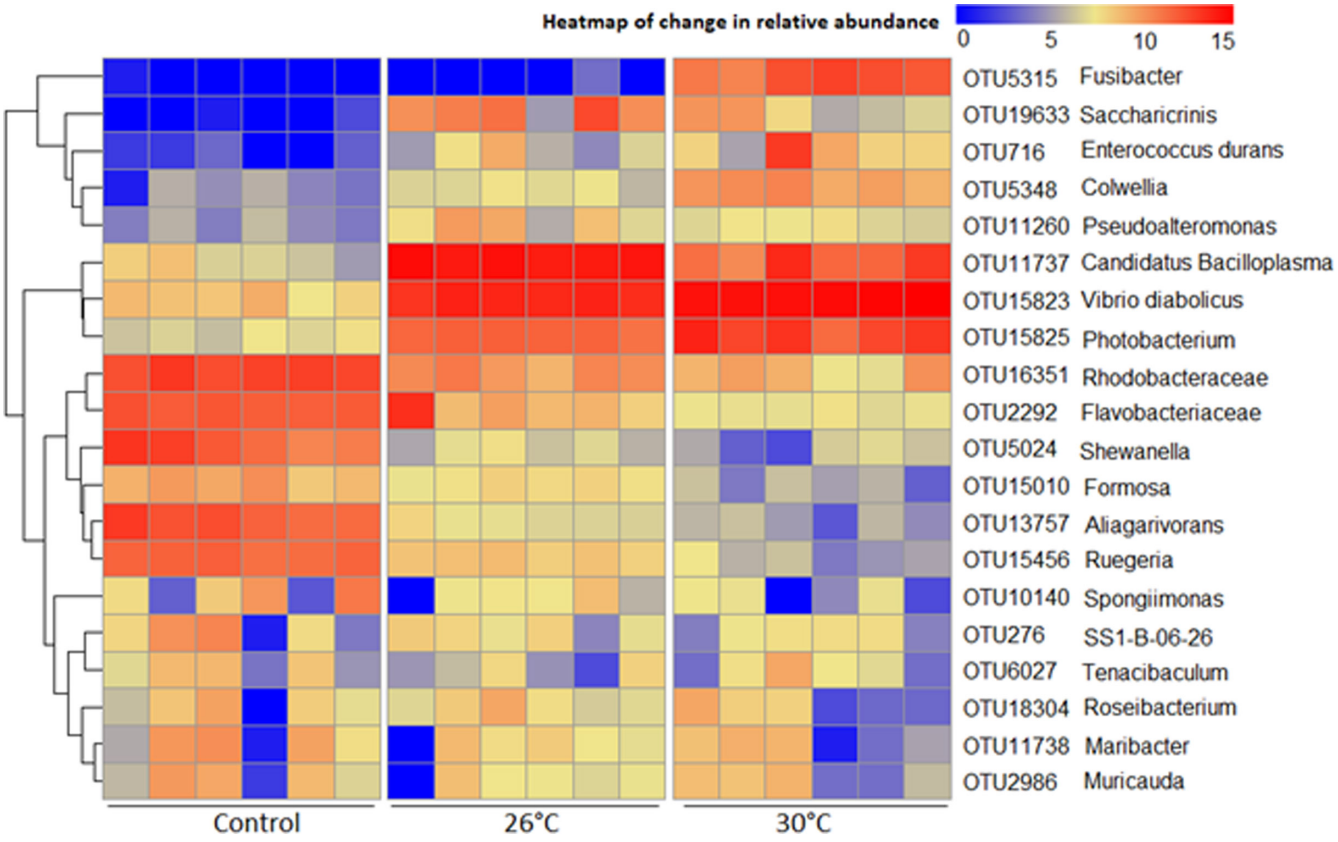

FIGURE 7 Heat map characterizes the most abundance genera (top 20 OTUs) specific to the control, 26°C, and 30°C groups in the shrimp guts. The abundances of the most dominant genera were log10 (Abundance +1) transformed. ($n = 6$)

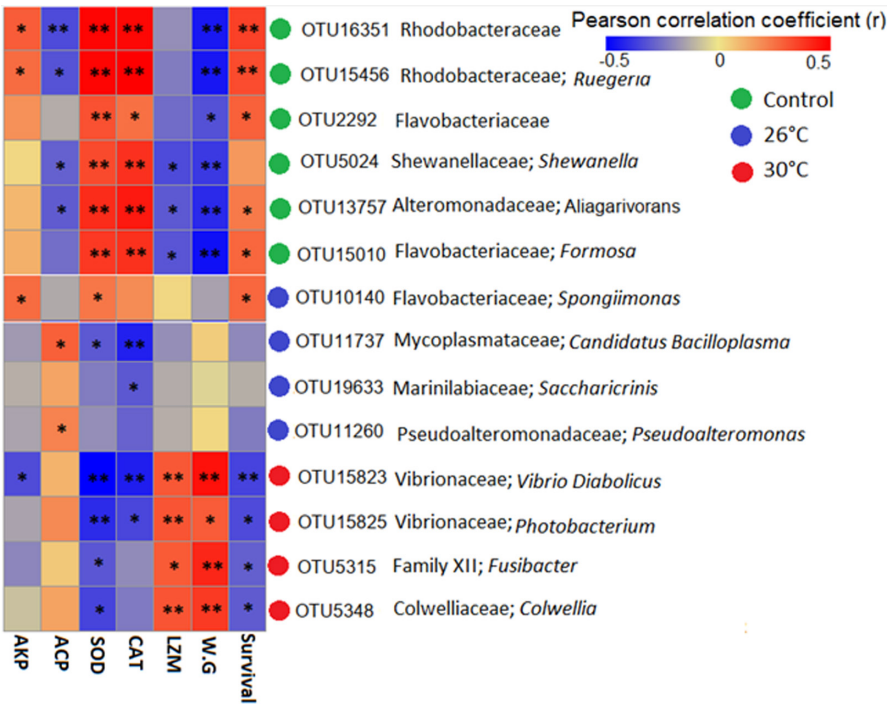

FIGURE 8 Pearson correlation between the most abundant intestinal OTUs and survival rate, weight gain rate, and enzyme activity. Green circles represent the most abundant OTUs in the control group, blue circles represent the most abundant OTUs at 26°C group, and red circles represent the most abundant OTUs at 30°C group. Correlation coefficients are indicated by colour intensity ranging from dark blue to red. '**' $p < 0.01$; '*' $p < 0.05$

were found in SOD with (Flavobacteriaceae *Spongiimonas*), ACP with OTU11737 (Maycoplasmataceae *Candidatus* Bacilloplasma) and OTU11260 (Pseudoalteromonadaceae *Pseudoalteromonas*), and AKP with OTU10140 (Flavobacteriaceae *Spongiimonas*).

At 30°C, which contains four OTUs taxa, OTU15823 (Vibrionaceae *Vibrio Diabolicus*), OTU15825 (Vibrionaceae *Photobacterium*), OTU5315 (Family XII *Fusibacter*) and OTU5348 (Colwelliaceae *Colwellia*), the correlations of all 4 OTUs taxa have

been found positive with weight gain and LZM enzyme, and negative correlations with survival and SOD. In addition, another negative correlation was found in CAT with OTU15823 (Vibrionaceae *Vibrio Diabolicus*) and OTU15825 (Vibrionaceae *Photobacterium*) and AKP with OTU15823 (Vibrionaceae *vibrio Diabolicus*) (Figure 8). The results indicated that the correlations have a criterion determining the OTUs distribution, which may respond to the temperature effect.

4 | DISCUSSION

4.1 | Growth performance and water quality

Farmed shrimp are still exposed to many environmental obstacles that represent a stumbling block in the development of this industry despite all attempts have developed to improve shrimp growth to resist the environmental changes that occur (Liang et al., 2014; Sun et al., 2016; Zhang et al., 2018). Shrimp growth requires a healthy aquatic environment (Dauda et al., 2018). However, an increase in temperature leads to a significant increase in the amounts of toxic nitrogen (Hostins et al., 2015; Souza et al., 2014). This may cause a deterioration in water quality and a deterioration in the nutrient dynamics of aquatic systems (Shen et al., 2017) and eventually lead to shrimp mortality (Zhu et al., 2021). In this study, although the results did not indicate any significant differences ($p > 0.05$) when the temperature increased, it was noticeable that the concentration of NO_2^- increased at 26 °C on day 41 and at 30°C on day 30 and 41. Likewise, the same increase in NO_3^- concentrations was observed with rising temperatures (Table 1), indicating that the increase in NO_2^- concentration may cause a decrease in the survival rate of shrimp. Similar results reported the relation of decreased survival rate of shrimp *Penaeus vannamei* when NO_2^- concentrations were increased due to the temperature change (Ponce-Palafox et al., 2019). In addition, temperature is a major factor that contributes to controlling the metabolic activities of shrimp growth and development (Santos-Romero et al., 2017; Ponce-Palafox et al., 1997). In the current study, the best growth of *Litopenaeus vannamei* was observed at high temperatures of 30 and 26°C after six weeks of culture respectively ($p < 0.05$). This was consistent with the results of a previous study on the white leg shrimp *Penaeus vannamei* that had better growth rates were found in the temperature range (28 to 30 °C) (Ponce-Palafox et al., 1997). The increase was also noticeable for other growth parameters, SGR, CF, and WG (Table 2). In similar investigations of several sources, the results confirmed the improvement of growth of aquatic animals at temperatures ranging from 26 to 30°C. Wang et al. (2006) indicated that the SGR rate in juvenile shrimp *Macrobrachium nipponense* was higher at 22 to 32°C than at 16 to 20°C, European seabass found that FBW and SGR increased when the temperature was climbed up to 25 °C (Ruyet et al., 2004), Atlantic salmon (Handeland et al., 2008), *P. vannamei postlarvae* (Ponce-Palafox et al., 2019) and *Polyprion oxygeneios* (Khan et al., 2014). Indicating that the temperature between 26 and 30°C promoted digestion and utilisation of food and enhanced the growth of shrimp *L. vannamei* (Table 2). However, some studies have found that a rapid or excessive increase in temperature causes mortality for *L. vannamei* (Souza et al., 2016; Yan et al., 2007). A similar conclusion was found in our current results, where the lowest survival rate was observed at 30, followed by 26°C. Although the interpretation of the crucial condition of shrimp stability is still challenging because of inconsistencies with good growth, some earlier results revealed that survival rate decreased to 30°C and (Ponce-Palafox et al., 1997; Souza et al., 2016; Wyban et al., 1995; Yan et al., 2007). Souza et al. (2016) attributed the reduced survival rate of shrimp in high heat to oxidative damage caused by high lipid peroxidation levels in the haemolymph and pancreas. Suggesting that the improvement of growth does not necessarily mean that it is a good enough indicator of the stability of shrimp to perform their physiological and immune processes (Ponce-Palafox et al., 2019; Souza et al., 2014). Moreover, studies have revealed that temperature change is an environmental factor facilitating waterborne disease outbreaks (Tang et al., 2014). Gut bacterial communities are closely related to host stability; thus, outbreaks of waterborne diseases may disrupt intestinal microbial diversity, impair host stability or promote disease (Hai, 2015; Hong et al., 2011; Nie et al., 2017; Xiong et al., 2015). To understand the mechanism behind the reduced survival rate from the perspective of the internal homeostasis of the gut microbiota. The current study found the highest relative abundance in the shrimp gut at 30°C for species such as *Vibrio*, *Candidatus* bacilloplasma, and *Photobacterium*, which were classified as potential opportunistic pathogens for *L. vannamei* (Rungrassamee et al., 2016). This subtraction was consistent with the results of a previous study that 30°C is the optimum temperature for bacterial community growth in soil (Wu et al., 2015). Among the results reported by (Thompson et al., 2004), *Vibrio* bacterium could grow in various conditions and tolerate changes. This study suggests that the high abundance of Vibrionaceae in the gut might have been responsible for the death of shrimp.

4.2 | Enzymes activities

An increase or decrease in temperature affects antioxidant responses in aquatic animals, including shrimp (Lushchak & Bagnyukova, 2006; Madeira et al., 2013; Parihar et al., 1997; Souza et al., 2016; Xian et al., 2011). SOD and CAT are two critical enzymes involved in antioxidant enzymes that respond to oxidative damage (Campa-Córdova et al., 2002; Liu et al., 2011). In this study, the highest activity of SOD and CAT enzymes was found in the control group (Figure 1a and b), which indicates that low temperatures in the control group were a stress factor that led to the accumulation of high-level free radicals. In agreement with a study that showed that increased activity of CAT responds to eliminating free radical accumulation caused by low temperature (Souza et al., 2014). Moreover, the results of (Wang et al., 2006) found the highest antioxidant activity to counteract the ROS overproduction in shrimp was observed at 15 and 33°C. Therefore, this study indicates that the effect of shrimp survival rates at 26 and 30°C might be related to different causes

such as pathogens (Figures 6 and 7). The LZM enzyme is considered one of the most primary strains connected to the immunological defence in aquatic species (Sieroslawska et al., 2012). AKP and ACP enzymes exert similar regulatory features across phosphorylation and dephosphorylation, permeability, cell differentiation and absorption (Cajaraville et al., 2000; Xiong et al., 2018). In addition, they contribute to the destruction of pathogens and diseased cells (Hong et al., 2019). In this study, the ACP, AKP and LZM enzymes showed an upward trend with the increase in the temperature gradient (Figures 1c, 2a and b), where the maximum activity was observed at 30°C compared with the control group. Assuming that an increase led to the outbreak of waterborne diseases, and consequently, disturbance of the internal balance of the gut microbiota, which led to a rise in the activity of acid phosphatase in the shrimp hepatopancreas. This is consistent with a study that considered increased ACP activity a possible indicator of microbicidal activity in cells (Cheng & Thomas, 1989). Liu et al. (2012) considered AKP an essential cellular enzyme that improves the automatic response to disease resistance in crustaceans and fish. Likewise, LZM is an N-acetylmuramide glycanohydrlase involved in amino acid and molecular biology and might be protective against pathogens (Shahkar et al., 2018). In the current study, improved ACP, ACP and LZM activities promoted the immunological function and antipathogen activity of shrimp hepatopancreas after stimulation with increased temperature.

4.3 | Gut bacterial communities

The gut microbial communities are closely related in maintaining host health (Hou et al., 2018; Nie et al., 2017), but environmental factors cause dynamic instability of the microbial community, which may impair host stability (Huyben et al., 2018; Miller et al., 2011; Ptacnik et al., 2008). Temperature changes are one of the factors that cause outbreaks of waterborne diseases (Tang et al., 2014). However, the effect of temperature change on the intestinal bacterial community in shrimp still needs further investigation. In the current study, the difference of intestinal bacterial communities was evident ($p < 0.05$) between the control group and the experimental groups (Figure 4). This difference was also observed when analysing the similarity (ANOSIM) of bacterial community composition between the control group and (26 and 30°C), which indicates that the temperature had a significant effect ($p < 0.05$) on the gut bacterial community composition in the shrimp (Table 3). It has been reported that the environmental factors can change the structures and function of water microbial communities in shrimp cultural (Hou et al., 2017). The increase in bacterial diversity is a favourable sign of health and that a lack of diversity promotes host instability (Costello et al., 2010; Shanahan, 2010). Nonetheless, other research has discovered that high diversity does not always imply that the bacterial community is stable (Shade, 2017). In this study, although the diversity index was not significant ($p > 0.05$), the bacterial diversity decreases with temperature increased was evident, especially at 30°C (Figure 3). Hou et al. (2018) observed the high alpha diversity was related to healthy shrimp compared to shrimp with white faecal syndrome. Moreover, a decrease in Shannon index diversity was observed concurrently in shrimp suffering from acute hepatopancreatic necrosis disease (AHPND) (Chen et al., 2017). The conclusions indicate that the low diversity of the microbial community in the gut is an indicator of the instability in shrimp, which is consistent with what we found in (Figure 3), indicating that the temperature caused a disturbance in the normal microbial balance, and transformed the bacterial community into a community dominated by some families.

In this study, the gut bacterial communities mainly consisted of Proteobacteria, Bacteroidetes, Tenerictes and Firmicutes (Figure 5a) in all of the control and experimental groups, and Previous studies have found that these bacterial communities represent the dominant majority in shrimp farming water (Cardona et al., 2016; Zhang et al., 2014). The relative abundance of the most dominating families was consistent in both groups (26 and 30°C) as the temperature rose. It was found that the increase in temperature significantly enhanced the rate of evolution of the majority of some bacterial taxa (Vibrionaceae, Mycoplasmatacea, Marinilabiaceae and Clostridiales_Family XII (Figure 6), indicating an unbalanced pattern and high variance between groups for a limited number of families. In contrast with the control group, in which families such as Rhodobacteraceae, Flavobacteraceae and Alteromonadaceae appeared to be more prominent. The high relative abundance of some bacterial species may be beneficial for the growth and survival of aquatic organisms. Xue et al. (2017) found the Rhodobacteraceae abundance in healthy grouper *Epinephelus* sp higher than in diseased fish, which is possible for its ability to co-exist with the host (Pujalte et al., 2014). Moreover, Flavobacteraceae and Alteromonadaceae families include various genes that may degrade the organic molecules and produce secondary metabolites, potentially inhibiting pathogen development (López-Pérez & Rodriguez, 2014; Tully et al., 2014). Meanwhile, shrimp diseases and mortality have been linked to the increased abundance of Vibrionaceae and Mycoplasmataceae (Guo et al., 2020; Liu et al., 2004; Xiong et al., 2015; Zhang et al., 2014). These findings were consistent with the result in (Figure 6). This difference was also confirmed using the Heat map analysis, which indicate the most abundance genera (top 20 OTUs) (Figure 7), the increase in temperature was a contributing factor to the variation in the relative abundance and the disruption of the normal balance of microbial communities. Consequently, another analysis was made to investigate the correlations between these abundant taxa with shrimp performance. The findings showed Rhodobacteraceae, Flavobacteraceae and Alteromonadaceae positively associated with survival rate when shrimp grew in normal temperature (Figure 8). However, determining the possible links between dominant families and shrimp health, development, or stress tolerance remains a challenge. We speculate that temperature may have increased the dominance of families connected to shrimp suffering, while limiting the abundance of bacterial families such as Rhodobacteraceae that could have hindered the dominance of pathogenic families, but the underlying mechanism

still unclear. Differences in the shrimp survival in response to rising temperature can also be reflected by conditionally dominant bacterial families. Rhodobacteraceae, Flavobacteraceae and Alteromonadaceae could hydrolyze organic molecules to produce secondary metabolites and inhibit the development of pathogens (Alonso & Pernthaler, 2006; Alonso-Sáez & Gasol, 2007; López-Pérez & Rodriguez, 2014; Tully et al., 2014). Pujalte et al. (2014) showed that Rhodobacteraceae is a significant strain coexists with host and highly engaged in carbon/sulphur cycles in the gut. It has also been confirmed the ability of most Rhodobacteraceae members to produce shrimp development requirement as vitamin B12 (Sañudo-Wilhelmy et al., 2014). As a result, it is possible that rising temperatures resulted to a drop in the relative abundance of Rhodobacteraceae in the gut, which may have contributed to the host's incapacity to benefit.

5 | CONCLUSIONS

In this study, we discovered that the growth rate of shrimp was better at the high-temperature groups (26 and 30°C) than that in the control group, but the survival rate was decreased overtime, especially at 30°C. Furthermore, the high temperature reduced the enzyme activities of SOD and CAT but significantly increased the activities of AKP and ACP. In responding to temperature fluctuations, the gut bacterial community compositions were changed significantly. The relative abundance of some OTUs belonging to Rhodobacteraceae showed a significant positive association with survival rate and enzyme activities. Future research will focus on delving further into the dynamics of the gut bacterial system to develop more practical recommendations for achieving stable bacterial communities.

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CONFLICT OF INTEREST

There are no potential conflicts of interests for the authors to disclose.

AUTHORS' CONTRIBUTIONS

The study conception and design were contributed by all of the authors. Zaher A. Al-Masqari and Haipeng Guo handled Materials preparation. Feeding trial and laboratory works performed by Zaher A. Al-Masqari, Ruoyu Wang and Pengsheng Dong. Data analysis was performed by Zaher A. Al-Masqari, Huizhen Yan and Haipeng Guo. Manuscript wrote by Zaher A. Al-Masqari and Haipeng Guo. Modification comments and guidance were provided by Haipeng Guo and Demin Zhang. The manuscript was reviewed and approved by all authors.

DATA AVAILABILITY STATEMENT

The sequencing data can be available at the NCBI BioProject https://www.ncbi.nlm.nih.gov/sra/ PRJNA765533 under accession number PRJNA765533.

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