Latin American Journal of Aquatic Research, 50(5): 681-691, 2022
DOI: 10.3856/vol50-issue5-fulltext-2932

Research Article

Effects of temperature, pH, and photoperiod on the performance of a freshwater cladoceran *Moina micrura* culture enriched with *Lysinibacillus fusiformis* and *Bacillus pocheonensis*

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ABSTRACT. The freshwater cladoceran *Moina micrura* has tremendous potential for mass culture as a live feed for larviculture. This study aimed to evaluate the efficacy of probiotics *Lysinibacillus fusiformis* A1 and *Bacillus pocheonensis* S2 in enhancing the population density, growth rate, and production of *M. micrura* under different environmental conditions. Four different temperatures (15, 20, 25, and 30°C), pH levels (4, 6, 8, and 10), and photoperiods (4L:20D, 6L:18D, 8L:16D, and 12L:12D) were set up. The daily number of individuals incubated under each environmental parameter was monitored for 12 days to determine the population density and growth rate of *M. micrura*. Meanwhile, the daily number of neonates from five females kept under respective environmental parameters was monitored until they died naturally. Probiotics were added individually at 5×10^4 CFU mL^{-1} twice during the first and sixth days of the experiment. *M. micrura* enriched with *B. pocheonensis* S2 at 30°C had the highest maximum population density (10 ± 0.2 ind mL^{-1}) and the number of neonates produced (132 ± 6.43 ind), whereas treatment at 20°C had the best growth rate (0.1863 ± 0.006 d^{-1}). *M. micrura* incubated with *B. pocheonensis* S2 at a normal photoperiod of 12L:12D had the highest maximum population density (10 ± 0.3 ind mL^{-1}) and the number of neonates produced (129 ± 4.58 ind) while incubation at 8L:16D had the best growth rate (0.2879 ± 0.0007 d^{-1}). *M. micrura* enriched with *L. fusiformis* A1 at pH 8 had the highest maximum population density (11 ± 0.8 ind mL^{-1}), growth rate (0.5508 ± 0.04 d^{-1}), and the number of neonates produced (129 ± 4.36 ind). Results recommend that a warmer temperature of 30°C, alkaline pH from 8 to 10, and a normal photoperiod of 12L:12D can be adopted for *M. micrura* enrichment with *B. pocheonensis* S2 to maximize its productivity for aquaculture use.

Keywords: *Moina micrura*; enrichment; probiotic; temperature; pH; photoperiod; aquaculture

Corresponding editor: Sandra Bravo

INTRODUCTION

Cladocerans are crustacean mesozooplankton commonly distributed in ponds and lakes (Taghavi et al. 2013). They are the major constituents of freshwater planktonic communities that consume phytoplankton and serve as food for many invertebrates and vertebrates (Azuraidi et al. 2013). *Moina* is one of Malaysia's most commonly found freshwater zooplankton (Habib et al. 2003). Major cladoceran species of the genus *Moina* display early reproduction, fast development, high population growth rates, and a tendency to produce resting eggs (Rojas et al. 2000, Halder et al. 2013). Despite having all these qualities, a sudden culture crash remains a reality.

Temperature is one of the most important environmental factors since it influences all biological processes (Engert et al. 2013) and the planktonic communities' structure, distribution, and biomass (Yvon-Durocher et al. 2011). This abiotic factor has gained increasing importance due to global warming (Pimm 2009). Lake stratification allows some zooplankton to migrate vertically through the water column to avoid high temperatures. However, puddle zooplanktons such as *Moina* spp. cannot drift into deeper layers of water (Engert et al. 2013).

The freshwater ecosystem is susceptible to acidification due to habitat alteration, water pollution, and eutrophication. This phenomenon negatively impacts the biodiversity of planktonic and benthic communities (Wærvågen & Nilssen 2003). Freshwater acidification depends largely on the quantity of calcium carbonate and the accumulation of rain (Halder et al. 2013). Lakes with higher pH are richer in zooplankton species. Approximately two to three times the number of genera are found in waters with a pH >5 (Halder et al. 2013). Since *Moina micrura* is sensitive to the alteration of pH, they are commonly used as bioindicators in water quality assessment (Parmar et al. 2016).

Light is one of the main stimuli regulating the activities of microcrustaceans, affecting their growth, maturation, reproduction, and feeding activities (Taghavi et al. 2013). The effects of photoperiod, either solely or combined with other environmental parameters, have been documented to influence the behavior, physiology, development, and reproduction of aquatic invertebrates (Zhang & Baer 2000, Savaş & Erdoğan 2006, Farhadian et al. 2013). The combination of reduced photoperiod and different algal diets were reported to influence the growth, population density, reproduction, and ephippia production of freshwater cladocerans in several zooplankton studies (Zhang & Baer 2000, Farhadian et al. 2013,Taghavi et al. 2013).

It is believed that organisms living in conditions near their environmental tolerance limits seem to be the most vulnerable. Thus, the impact of probiotics supplementation on the enhancement of the population density, growth, and reproduction of *M. micrura* when exposed to different environmental parameter levels will give some insight into the efficacy of probiotics as a tool to promote *M. micrura* tolerance and survival in extreme conditions. In addition, the present study also aimed to attain an optimal setting for probiotic enrichment of *M. micrura.*

Therefore, this study was designed to investigate the effects of increasing temperature, pH, and photoperiod on the production of probiotic-enriched *M. micrura*. A range of biological parameters are evaluated, including population density, population growth rate, and neonate production. The information generated from the present study would be essential in developing efficient culture techniques for this species.

MATERIALS AND METHODS

Stock culture of *Moina micrura*

Moina micrura was obtained from the Aquatic Animal Health and Therapeutics Laboratory (AquaHealth), Institute of Bioscience (IBS), Universiti Putra Malaysia (UPM), Malaysia. *M. micrura* was maintained in 1 L plastic aquaria filled with sterile filtered pond water (0.45 µm Whatman fiberglass filters) and mild aeration. The water was exchanged once a week. *M. micrura* was fed ad libitum with *Chlorella vulgaris.*

Microalgal culture

The microalga *C. vulgaris* (Isolate number: UPMC-A0088) was obtained from the AquaHealth Laboratory, IBS, UPM, Malaysia. Cultures were enriched with bold's basal medium (BBM) and grown in sterile 1 L conical flasks under constant shaking on an orbital shaker (60 rpm) (Protech, Malaysia) and continuous illumination. The cell concentration of *C. vulgaris* was determined using an improved Neubauer counting chamber observed under a light microscope according to the following formula:

$$\text{Cellular density, d}\left(\text{cells mL}^{-1}\right) = \frac{(\text{number of cells counted} \times \text{dilution factor} \times 10{,}000)}{\text{number of squares counted}}$$

Culture of potential probiotic strains

Potential probiotics comprising *Lysinibacillus fusiformis* A1 and *Bacillus pocheonensis* S2 were isolated from microalgae, namely *Amphora* sp. and *Spirulina* sp. (Natasya-Ain 2018).

Potential probiotics, *L. fusiformis* A1 and *B. pocheonensis* S2, were aseptically inoculated into 20 mL trypticase soy broth (TSB) (Merck, Germany). The inoculation process was done individually for each probiotic. Cultures were incubated overnight with constant agitation on an orbital shaker (Biosan, Latvia) (30°C; 150 rpm). Both cultures were centrifuged (5000 g; 10 min) (Hermle, Germany) at room temperature for experimental use. The supernatants were discarded, and pellets were resuspended in sterile distilled water and washed once. The concentration of both cultures was determined using a spectrophotometer (Eppendorf, Germany) at 550 nm. The final concentrations of *L. fusiformis* A1 and *B. pocheonensis* S2 were adjusted to 5×10^4 CFU mL^{-1} in treatment tubes.

Experimental design

Individual effects of different temperatures (15, 20, 25, and 30°C), pH (4, 6, 8, and 10), and photoperiods (4L:20D, 6L:18D, 8L:16D, and 12L:12D) (hours of light:dark, L:D) on population density, growth rate, and production of *M. micrura* during enrichment with *L. fusiformis* A1 and *B. pocheonensis* S2 were evaluated. Eight different treatments (4 levels × 2 probiotics) were allocated for each environmental parameter with three replications. Additionally, a control treatment without probiotics was included for each parameter. Two experiments were run simultaneously under controlled laboratory conditions in a separate setup to minimize handling stress.

Experimental setup

Experiment I: population density and population growth rate

The experiment was run for 12 days. Ten one-day-old female *M. micrura* were allocated to a 50 mL Falcon tube filled with 30 mL filtered (through 0.45 µm Whatman filter paper) and autoclaved pond water. The tubes were kept in temperature-controlled water baths for the temperature experiment. The tubes were manually maintained under four different light treatments for the photoperiod experiment through light exposure and storage in a dark cabinet. For the pH experiment, hydrochloric acid (HCl) (Merck, Germany) and sodium hydroxide (NaOH) (Merck, Germany) at 1 N were used to adjust the pH level of the culture medium.

Probiotics (*L. fusiformis* A1 and *B. pocheonensis* S2) were added individually at 5×10^4 CFU mL^{-1} on the first and sixth days of the experiment. *M. micrura* was fed with *C. vulgaris* at 4×10^5 cells mL^{-1} once a day. The number of individuals from each tube was counted in a glass Petri dish every morning before feeding. The culture medium was partially exchanged daily after the count.

The following environmental conditions were used during each experiment except when the variable served as an independent variable. The temperature experiment fixed the pH and photoperiod at seven and 12L:12D, respectively. Meanwhile, the temperature and photoperiod were fixed at 25ºC and 12L:12D, respectively, for the pH experiment. As for the photoperiod experiment, the temperature and pH were fixed at 25ºC and 7, respectively. The temperature and pH were measured using a mercury thermometer (Brannan, UK) and a pH meter (Eutech, Singapore). The population growth rate was calculated according to the following equation (Azuraidi et al. 2013):

$$\text{Growth rate, } \mu = \frac{\ln(X_2) - \ln(X_1)}{t_2 - t_1}$$

where X_1: number of *M. micrura* at time t_1 (initial); X_2: number of *M. micrura* at time t_2 (final).

Experiment II: lifetime neonate production

Five one-day-old female *M. micrura* were reared in a 50 mL Falcon tube filled with 30 mL filtered and autoclaved (through 0.45 µm Whatman filter paper) pond water. All tubes were kept under similar conditions as in experiment I. Furthermore, probiotics and *C. vulgaris* were added similarly. The experiment was run until all *M. micrura* died naturally.

The number of neonates from each tube was counted in a glass Petri dish every morning before feeding. All neonates were discarded after the count. The culture medium was partially exchanged every day after counting.

Statistical analysis

All data were analyzed using one-way analysis of variance (ANOVA), and Tukey's test was carried out for pairwise comparisons of means. The significance level was declared at $P < 0.05$. All statistical tests were performed using GraphPad Prism 8 (GraphPad Inc., San Diego, CA, USA).

RESULTS

Population density

The effect of temperature

The effect of different temperatures on *M. micrura* population density during enrichment with *L. fusiformis* A1 and *B. pocheonensis* S2 throughout the culture period was shown (Fig. 1). With each probiotic at all

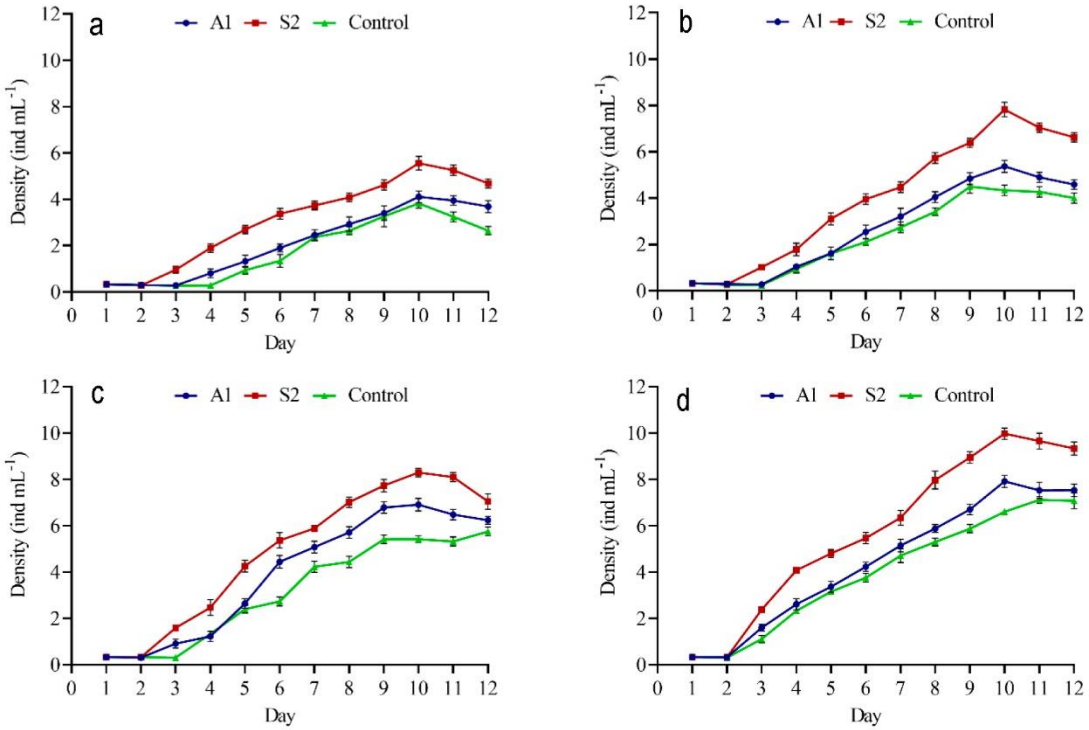

Figure 1. Population densities of *Moina micrura* enriched with *Lysinibacillus fusiformis* A1 and *Bacillus pocheonensis* S2 at a) 15°C, b) 20°C, c) 25°C, and d) 30°C. Vertical bars indicate the standard error of the mean (n = 3).

temperatures, the enrichment was able to enhance its population density compared to the control. The population densities of *M. micrura* enriched with each probiotic displayed an increasing trend until day 10 of culture and declined after that in all treatments (Fig. 1). *M. micrura*, enriched with *B. pocheonensis* S2 at 30ºC, had the highest maximum population density (10 ± 0.2 ind mL^{-1}) on day 10 of culture. Significantly higher ($P < 0.05$) than those enriched at 15ºC (6 ± 0.3 ind mL^{-1}), 20ºC (8 ± 0.3 ind mL^{-1}), and 25ºC (8 ± 0.2 ind mL^{-1}) (Fig. 4a). Furthermore, the highest maximum population density achieved when *M. micrura* was enriched with *B. pocheonensis* S2 was significantly higher ($P < 0.05$) than the control of the same treatment regardless of the temperature (Fig. 4a).

The effect of pH

The impact of different pH levels on the population density of *M. micrura* during enrichment with *L. fusiformis* A1 and *B. pocheonensis* S2 throughout the culture period was shown (Fig. 2). The population densities of *M. micrura* enriched with each probiotic in acidic conditions (pH 4 and 6) were lower than the control until day 9 during the culture period (Figs. 2a-b). Meanwhile, at pH 8, the enrichment of *M. micrura* with *L. fusiformis* A1 yielded the best outcome. From day 9 onwards, the population density was higher than in those enriched with *B. pocheonensis* S2 and the control (Fig. 2c). Additionally, on day 12 of culture, *M. micrura* enriched with *L. fusiformis* A1 at pH 8 had the highest maximum population density (11 ± 0.9 ind mL^{-1}). Significantly higher ($P < 0.05$) than those enriched at pH 4 (1 ± 0.1 ind mL^{-1}), 6 (4 ± 0.3 ind mL^{-1}), and 10 (9 ± 0.5 ind mL^{-1}) (Fig. 4b). Moreover, the highest maximum population density of *M. micrura* enriched with *L. fusiformis* A1 at pH 8 was significantly higher ($P < 0.05$) than the control (10 ± 0.06 ind mL^{-1}) of the same pH (Fig. 4b).

The effect of photoperiod

Meanwhile, the effect of different photoperiods on *M. micrura* population density enriched with *L. fusiformis* A1 and *B. pocheonensis* S2 throughout 12 days of culture was shown (Fig. 3). Similar to the temperature experiment, the enrichment with each probiotic under all photoperiod regimes improved the population densities of *M. micrura* compared to the control. In general, the population densities of *M. micrura* enriched with each probiotic peaked on day 10. Thereon they declined for all treatments (Fig. 3). Also,

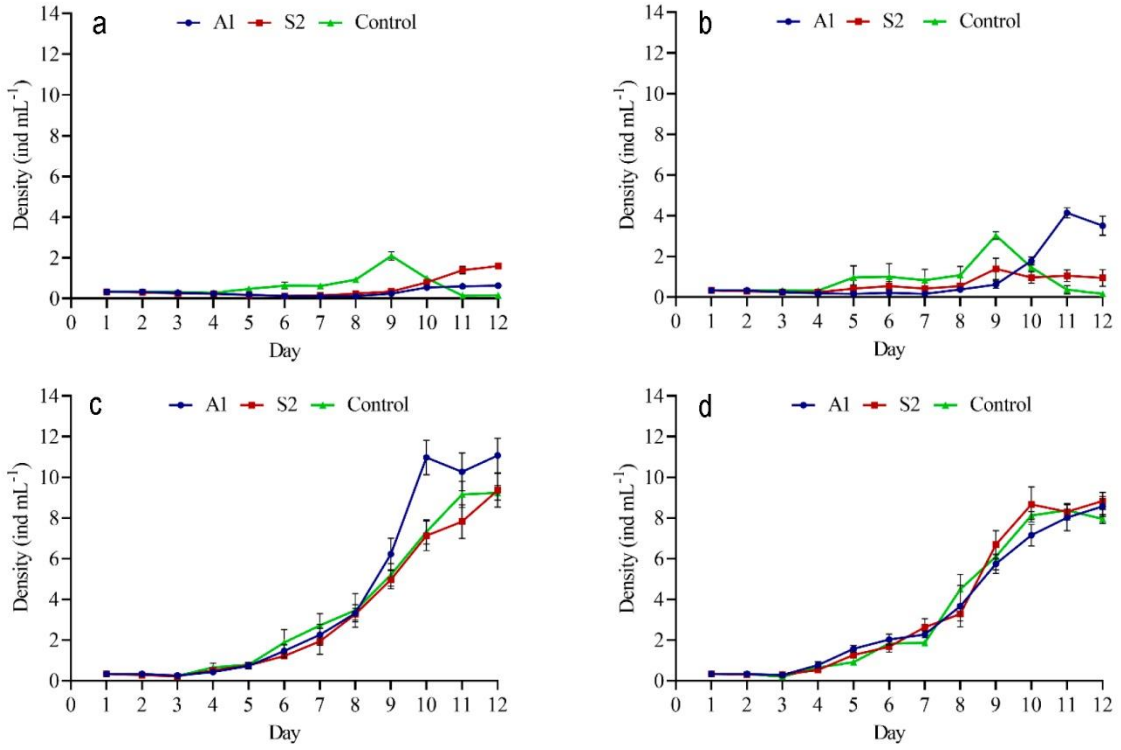

Figure 2. Population densities of *Moina micrura* enriched with *Lysinibacillus fusiformis* A1 and *Bacillus pocheonensis* S2 at a) pH 4, b) pH 6, c) pH 8, and d) pH 10. Vertical bars indicate the standard error of the mean (n = 3).

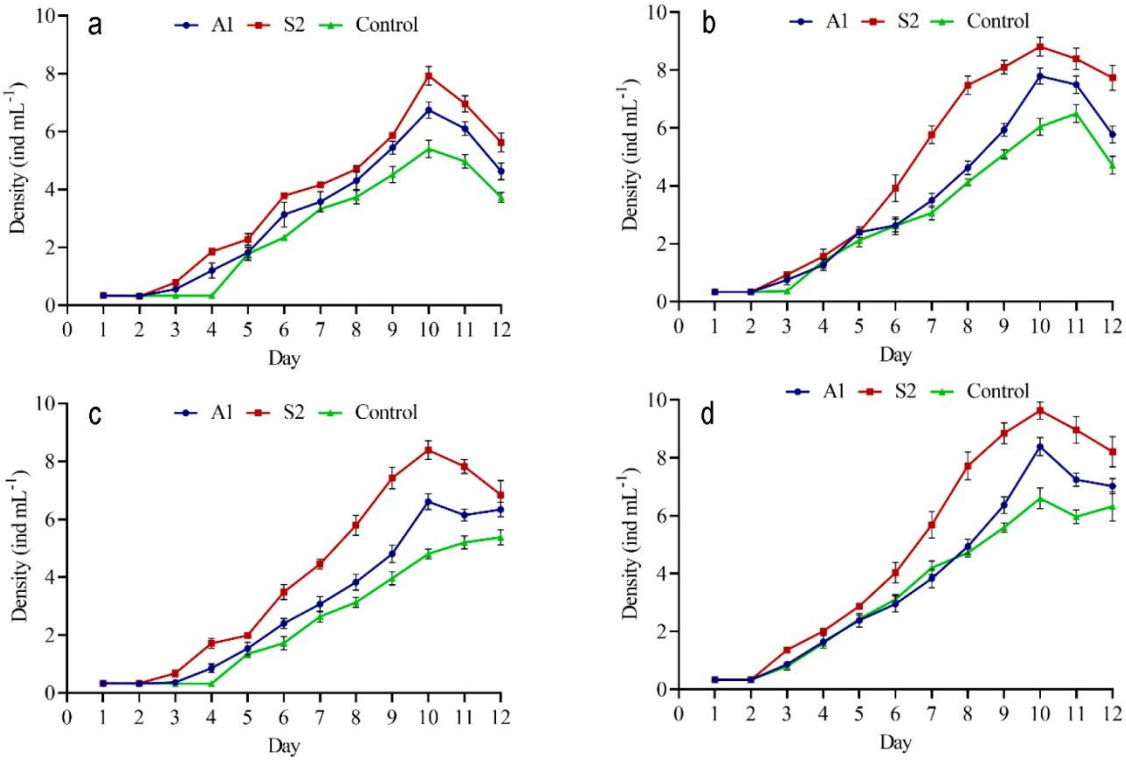

Figure 3. Population densities of *Moina micrura* enriched with *Lysinibacillus fusiformis* A1 and *Bacillus pocheonensis* S2 at a) 4L:20D, b) 6L:18D, c) 8L:16D, and d) 12L:12D photoperiod regimes. Vertical bars indicate the standard error of the mean (n = 3).

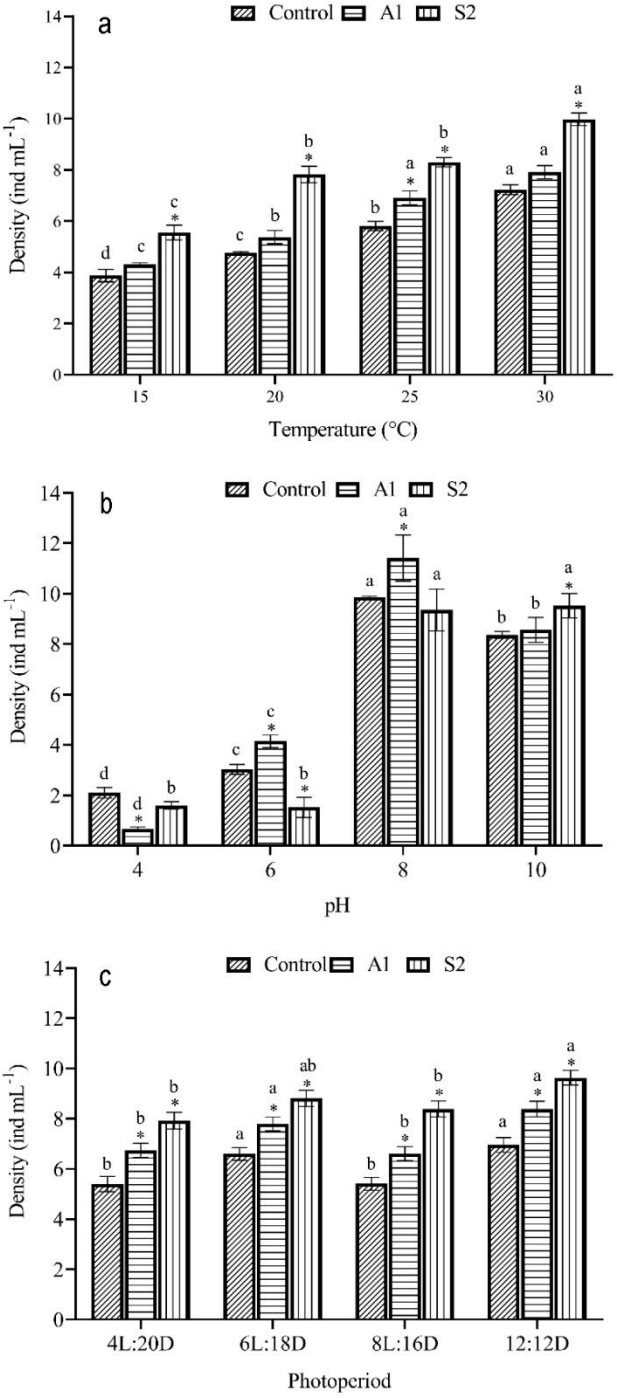

Figure 4. Effects of increasing a) temperature, b) pH, and c) photoperiod on the highest maximum population density achieved when *Moina micrura* was enriched with *Lysinibacillus fusiformis* A1 and *Bacillus pocheonensis* S2. Each value is the mean ± mean standard error (n = 3). *Significantly different from the control of the same treatment group at $P < 0.05$. Different superscripts indicate significant differences ($P < 0.05$) between groups (comparing columns with identical patterns).

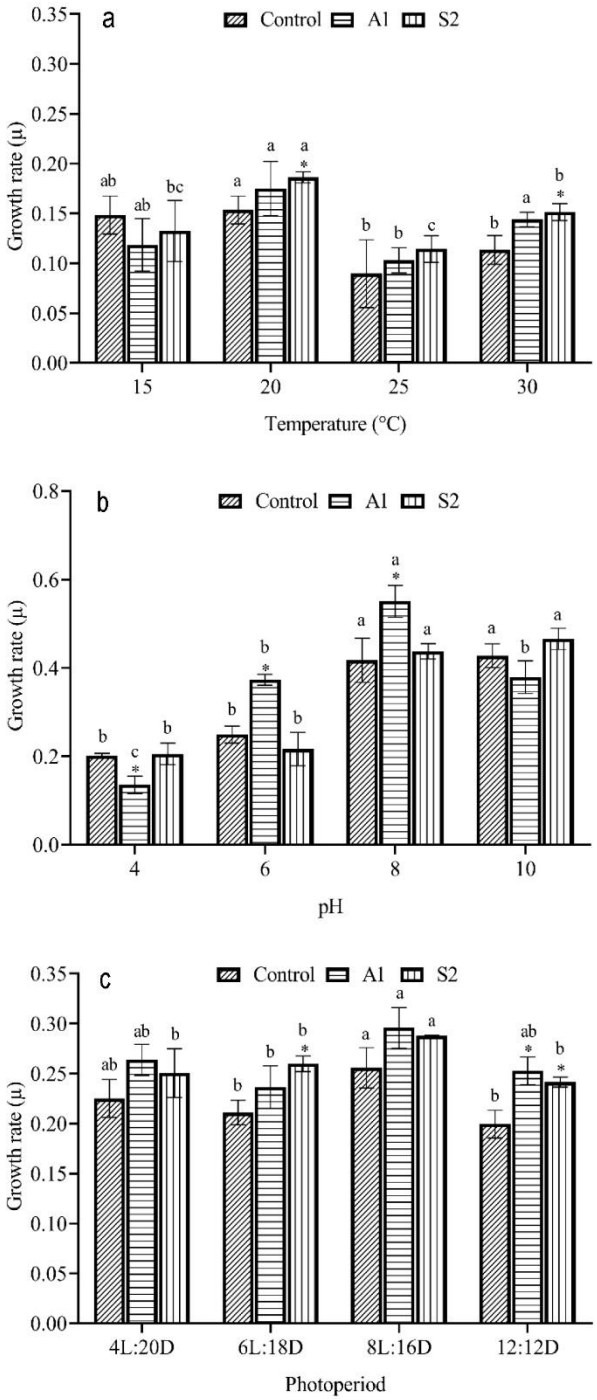

Figure 5. Effects of increasing a) temperature, b) pH, and c) photoperiod on the growth rate of *Moina micrura* enriched with *Lysinibacillus fusiformis* A1 and *Bacillus pocheonensis* S2. Each value is the mean ± standard error (n = 3). *Significantly different from the control of the same treatment group at $P < 0.05$. Different superscripts indicate significant differences ($P < 0.05$) between groups (comparing columns with identical patterns).

the population densities of *M. micrura* enriched with *B. pocheonensis* S2 were higher than those enriched with *L. fusiformis* A1 and the control from day 3 onwards, irrespective of photoperiod regimes (Fig. 3). *M. micrura* enriched with *B. pocheonensis* S2 under 12L:12D photoperiod had the highest maximum population density (10 ± 0.3 ind mL^{-1}) on day 10 of culture. Significantly higher ($P < 0.05$) than those enriched under 4L:20D (8 ± 0.3 ind mL^{-1}) and 8L:16D photoperiods (8 ± 0.3 ind mL^{-1}) (Fig. 4c). Furthermore, the highest maximum population density achieved when *M. micrura* was enriched with *B. pocheonensis* S2 was significantly higher ($P < 0.05$) than the control of the same treatment regardless of the photoperiod (Fig. 4c).

Population growth rate

Results of individual effects of different temperatures, pH levels, and photoperiods on the population growth rate of *M. micrura* enriched with *L. fusiformis* A1 and *B. pocheonensis* S2 were shown (Fig. 5). Both probiotics, *L. fusiformis* A1 and *B. pocheonensis* S2, effectively stimulated the population growth of *M. micrura* at 20, 25, and 30°C compared to the control (Fig. 5a). Regardless of temperature, *M. micrura* enriched with *B. pocheonensis* S2 had better growth rates than those treated with *L. fusiformis* A1. *M. micrura* enriched with *B. pocheonensis* S2 at 20°C had highest growth rate (0.1863 ± 0.006 d^{-1}), significantly higher ($P < 0.05$) than those enriched at 15°C (0.1325 ± 0.03 d^{-1}), 25°C (0.1144 ± 0.01 d^{-1}), and 30°C (0.1515 ± 0.008 d^{-1}). Additionally, the growth rate of *M. micrura* enriched with *B. pocheonensis* S2 at 20°C was significantly higher ($P < 0.05$) than the control (0.1536 ± 0.01 d^{-1}). pH-wise, *L. fusiformis* A1 was most effective at enhancing the growth rate of *M. micrura* at pH 8, while *B. pocheonensis* S2 worked best at pH 10 (Fig. 5b). The enrichment of *M. micrura* with *L. fusiformis* A1 at pH 8 showed highest growth rate (0.5508 ± 0.04 d^{-1}), significantly higher ($P < 0.05$) from other treatments at pH 4 (0.1358 ± 0.02 d^{-1}), pH 6 (0.3733 ± 0.01 d^{-1}), and pH 10 (0.3793 ± 0.04 d^{-1}). Moreover, the growth rate of *M. micrura* enriched with *L. fusiformis* A1 at pH 8 was significantly higher ($P < 0.05$) than the control (0.4175 ± 0.05 d^{-1}). For the photoperiod experiment, *M. micrura* enriched with *L. fusiformis* A1 at 8L:16D had the highest growth rate (0.2958 ± 0.02 d^{-1}), significantly higher ($P < 0.05$) than those incubated under 6L:18D (0.2364 ± 0.02 d^{-1}) photoperiod (Fig. 5c). However, the growth rate of *M. micrura* enriched with *L. fusiformis* A1 at 8L:16D was not significant ($P > 0.05$) to the control (0.2558 ± 0.02 d^{-1}).

Lifetime neonate production

Individual effects of different temperatures, pH levels, and photoperiods on the lifetime neonate production of *M. micrura* enriched with *L. fusiformis* A1 and *B. pocheonensis* S2 were presented (Fig. 6). Temperature-wise, *M. micrura* enriched with *B. pocheonensis* S2 at 30°C produced the highest number of neonates (132 ± 6.43 ind), significantly higher ($P < 0.05$) than those enriched at 15°C (49 ± 4.98 ind) and 20°C (67 ± 4.16 ind) (Fig 6a). Moreover, the number of neonates produced after enrichment with *B. pocheonensis* S2 at 30°C was significantly higher ($P < 0.05$) than the control (112 ± 3.51 ind). As for pH, the highest number of neonates was produced after enrichment with *L. fusiformis* A1 at pH 8 (129 ± 4.36 ind). Significantly higher ($P < 0.05$) than those enriched at pH 4 (16 ± 2.33 ind), pH 6 (42 ± 3.28 ind), and pH 10 (86 ± 10.35 ind) (Fig. 6b). However, the number of neonates produced after enrichment with *L. fusiformis* A1 at pH 8 was not significant ($P > 0.05$) to the control (118 ± 4.41 ind). Regarding photoperiod, the enrichment with *B. pocheonensis* S2 at 12L:12D showed the highest number of neonates produced (129 ± 4.58 ind), significantly higher ($P < 0.05$) than those enriched at 8L:16D (115 ± 2.33 ind) (Fig. 6c). Additionally, the number of neonates produced after enrichment with *B. pocheonensis* S2 at 12L:12D was significantly higher ($P < 0.05$) than the control (106 ± 4.10 ind).

DISCUSSION

Cladocerans are a representative group of freshwater zooplankton and the most effective filter feeders in almost all freshwater ecosystems (Yoon et al. 2000). Temperature, pH, and light are the main abiotic parameters affecting zooplankton growth, reproduction, and behavior (Rojas et al. 2000).

The temperature has the most definite effects on zooplankton species living in shallow-water ecosystems, whereby temperature changes are not buffered by a large water mass (Mooji et al. 2007). In addition, microcrustacean plankton is an excellent bioindicator of toxicity as their abundance, population density, growth, and biological activity are negatively altered under extreme pH conditions (Halder et al., 2013). Furthermore, photoperiods have been documented to directly affect cladocerans' behavior, physiology, development, and reproduction (Ismail et al. 2011a, Gust et al. 2019).

In previous studies, *L. fusiformis* A1 and *B. pocheonensis* S2 displayed strong antibacterial activity against two marine pathogens, mainly *Vibrio harveyi*

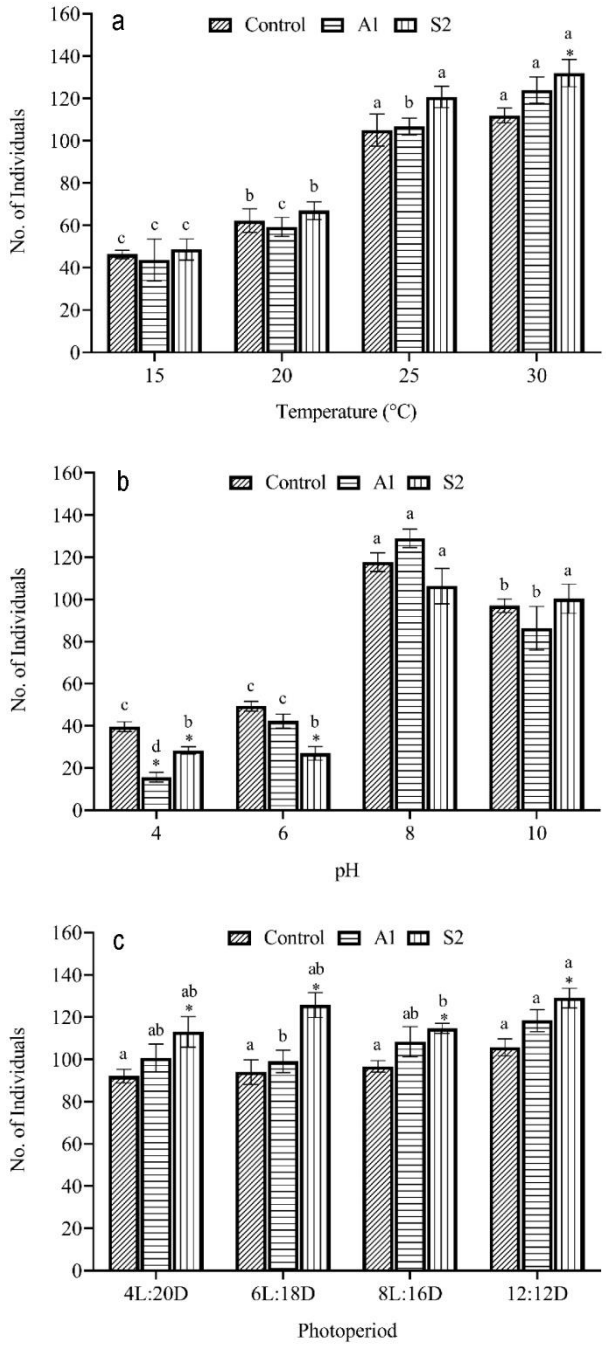

Figure 6. Effects of increasing a) temperature, b) pH, and c) photoperiod on the lifetime neonate production of five female *Moina micrura* enriched with *Lysinibacillus fusiformis* A1 and *Bacillus pocheonensis* S2. Each value is the mean ± mean standard error (n = 3). *Significantly different from the control of the same treatment group at $P < 0.05$. Different superscripts indicate significant differences ($P < 0.05$) between groups (comparing columns with identical patterns).

and *Vibrio parahaemolyticus* (Rosland et al. 2021), and two freshwater pathogens, including *Aeromonas hydrophila* and *Streptococcus agalactiae* (Samat et al. 2021). Thus, the present study aimed to evaluate the potential of these strains in aiding *M. micrura* performance at different levels of environmental parameters. In this study, the highest maximum population density and neonate production of *M. micrura* were recorded when enriched with *B. pocheonensis* S2 at a warmer temperature of 30°C. Meanwhile, the enrichment at 20°C showed the highest growth rate. In general, temperatures ranging from 20 to 25°C are considered optimal for the survival and reproduction of *Moina* sp. (Benider et al. 2002).

In some cases, *Moina* sp. continues to thrive at a temperature above 32°C (Rottmann et al. 2017). The present study showed a similar outcome in which the neonate production of *M. micrura* correlated positively to temperature. *M. micrura* seems to be adapted with a maximum reproduction at a higher temperature of 30°C. Meanwhile, the maximum reproductive output of *Moina mongolica* occurs at 20°C (He et al. 2001). The difference between these species is that *M. mongolica* is naturally distributed in temperate environments while *M. micrura* is adapted to warm tropical environments (Ismail et al. 2011b). Besides that, Benider et al. (2002) also agreed that temperature and longevity of *Moina* sp. are inversely correlated. Similarly, *M. micrura* in the present study had a shorter lifespan at 30°C despite producing more offspring. These findings confirmed the hypothesis proposed by Engert et al. (2013), whereby as temperature increases, the growth rate of *Moina* sp. will increase while longevity will decrease.

Furthermore, the effectiveness of probiotic bacteria can be affected by temperature. In the current study, *M. micrura* enriched with *B. pocheonensis* S2 at 30°C had the highest population density and number of neonates produced. *Bacillus* spp. is manipulatable for producing extracellular enzymes with high enzymatic activity in various temperatures (Dash et al. 2015). Extracellular enzymes aid in the digestion of food constituents to ensure maximum feed utilization (Midhun et al. 2017). Besides, to ensure maximum effectiveness of the added probiotics, Ibrahim et al. (2004) recommended adjusting probiotic dosages by water temperature, thus further suggesting that temperature influences probiotics' ability to confer benefits. The incubation temperature variation could also influence probiotics' adhesive ability on aquatic animals' skin and intestinal mucus (Ibrahim et al. 2004).

Although pH has been reported to influence the survival and reproduction of *M. micrura*, the mechanism of action is still poorly understood. The current study revealed that the enrichment of *M. micrura* with *L. fusiformis* A1 at pH 8 resulted in the highest maximum population density, growth rate, and number of neonates produced. At pH 8 and 10, in terms of time and efficiency, *M. micrura* treated with *L. fusiformis* A1 and *B. pocheonensis* S2 started to reproduce on day 4, and population density continued to increase until the final day of the experiment. The former suggested that *M. micrura* enriched with each probiotic strain can be harvested at any period from day 4, allowing more cultivation batches per cycle. A speedy and efficient production is important for commercial live feed producers. Meanwhile, the enrichment with each probiotic strain in acidic pHs of 4 and 6 may have negatively affected the production of *M. micrura*.

The efficiencies of the widely utilized *Bacillus* spp. as probiotics greatly depend on their high tolerance to acidity and alkalinity. Midhun et al. (2017) revealed that the growth of *Bacillus* sp. was not affected even after 24 h of incubation in a slightly alkaline bile juice. Furthermore, the ability of bacteria to survive in bile juice may influence their adherence to the gastrointestinal tract of aquatic animals (Merrifield et al. 2010). The present study also revealed that the enrichment with both strains in acidic environments negatively affected the population growth and reproduction of *M. micrura*. Locke & Sprules (2000) revealed that low food quality due to acidic conditions reduced the abundance of the *Daphnia pulex* and *Bosmina longirostris*. The study suggested that algal food quality may be affected by water chemistry. Therefore, the algae growing in an acidic environment provided poorer nutrients for the zooplankton because the chemical composition of algae, such as the N:P ratio, varies with fluctuations in ambient water chemistry. However, it should be noted that some acidophilic *Chlorella* spp. are capable of inhabiting strongly acidic environments (Ňancucheo & Johnson 2012). Declines in food availability are one of the factors contributing to the reduction of body sizes of offspring, population growth, reproduction, and development rates (Betini et al. 2019).

Both unenriched and probiotic-enriched *M. micrura* started to reproduce earlier on day 3 of culture when exposed to a 12L:12D photoperiod regime. *M. micrura* enriched with *B. pocheonensis* S2 had the highest maximum population density and number of neonates produced at 12L:12D photoperiod, while incubation at 8L:16D photoperiod showed the highest growth rate. These results suggest that *M. micrura* cultivated on *B. pocheonensis* at 12L:12D matured earlier and thus can be harvested earlier. While different photoperiod regimes may not have a noticeable impact on the activity and effectiveness of the added probiotic bacteria, they may influence microalgae's growth and nutritional composition (Wahidin et al. 2013). *Chlorella vulgaris*, which was used as a food source for *M. micrura*, is a photosynthetic microorganism whose biochemical content is highly regulated by light quality and quantity (Khoyi et al. 2009).

CONCLUSIONS

The influence of abiotic parameters on potential probiotics enrichment of *M. micrura* was determined in this study. For temperature, the highest maximum population density and number of neonates produced was attained at 30°C as *M. micrura* reproduced much earlier at a higher temperature. However, the enrichment at 20°C showed the highest growth rate. Meanwhile, *L. fusiformis* A1 at pH 8 was most effective at enhancing the population density, growth rate, and neonate production of *M. micrura*, while *B. pocheonensis* S2 worked best at pH 10.

This study expands the knowledge on the biological response of *M. micrura* to different temperatures, pHs, and photoperiod regimes when supplemented with potential probiotics. Furthermore, the enrichment of *M. micrura* with potential probiotics was not significantly affected by different photoperiod regimes. Nevertheless, the enrichment with *B. pocheonensis* S2 at a normal 12L:12D photoperiod showed the highest maximum population density and number of neonates produced. In contrast, the incubation at 8L:16D photoperiod showed the highest growth rate. Thus, *M. micrura* can be enriched best with B. pocheonensis S2 at a warmer temperature of 30°C, in alkaline pH ranging from 8 to 10, and in a normal 12L:12D photoperiod regime. Moreover, the study provided grounds to improve the culture of this highly nutritious live feed for application in aquaculture.

ACKNOWLEDGMENTS

This research was funded by the Ministry of Higher Education Malaysia (MOHE) under the Fundamental Research Grant Scheme FRGS/1/2020/WAB04/UPM/02/9. Special thanks to MOHE for the Malaysia-Japan SATREPS-COSMOS (JPMJSA 1509) matching grant and Higher Institution Centre of Excellence (HiCoE)

grant of Innovative Vaccine and Therapeutics, Vote N°6369100 for providing facilities at the Institute of Bioscience and Faculty of Agriculture, UPM.

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Received: May 20, 2022; Accepted: September 9, 2022

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