าเทความวิจัย

# ผลของสาหร่ายสีเขียวแกมน้ำเงิน *Limnothrix* สายพันธุ์ LmTK01 ต่ออัตราการรอดของ กุ้งขาวแวนนาไมและปัจจัยที่มีผลต่อการเจริญเติบโตของ LmTK01

Effect of Cyanobacteria *Limnothrix* Strain LmTK01 on the Survival Rate of Shrimp *Litopenaeus vannamei* and Factors Effected on LmTK01 Growth

กนกวรรณ มาเอียด<sup>1,2</sup>, ธีญาภรณ์ แก้วทวี<sup>1</sup> และ มินตรา ศีลอุดม<sup>2</sup>

Kanokwan Maa-iad<sup>1,2</sup>, Teeyaporn Keawtawee<sup>1\*</sup> and Mintra Seel-audom<sup>2</sup>

<sup>1</sup> สาขาวิชาวาริชศาสตร์และนวัตกรรมการจัดการ คณะทรัพยากรธรรมชาติ มหาวิทยาลัยสงขลานครินทร์

<sup>2</sup> ภาควิชาสัตวศาสตร์และสัตว์น้ำ คณะเกษตรศาสตร์ มหาวิทยาลัยเชียงใหม่

<sup>1</sup> Division of Aquatic Science and Innovative Management, Faculty of Natural Resources, Prince of Songkla University

<sup>2</sup> Department of Animal and Aquatic Sciences, Faculty of Agriculture, Chiang Mai University

Received: 1 October 2022

Revised: 30 November 2022

Accepted: 9 December 2022

# าเทคัดย่อ

สาหร่ายสีเขียวแกมน้ำเงิน Limnothrix สายพันธุ์ LmTK01 เป็นหนึ่งในใชยาโนแบคทีเรียชนิดใหม่ที่พบการเจริญเติบโต ในบ่อเลี้ยงกุ้งขาวแวนนาไม (Litopenaeus vannamei) และเป็นสาเหตุทำให้ผลผลิตกุ้งมีปริมาณลดลง ซึ่งลักษณะทางสัณฐาน วิทยามีความคล้ายคลึงกับสาหร่ายสีเขียวแกมน้ำเงินสกุล Limnothrix ดังนั้นการศึกษาครั้งนี้มีวัตถุประสงค์เพื่อศึกษาผลของ สาหร่าย LmTK01 ต่ออัตราการรอดของกุ้งขาวแวนนาไม และปัจจัยของอุณหภูมิ พีเอช และความเค็มต่อการเจริญเติบโตของ สาหร่าย LmTK01 โดยคัดแยกสาหร่าย LmTK01 จากน้ำเลี้ยงกุ้งขาวในช่วงที่พบการบลูมในบ่อเลี้ยง แล้วนำมาเลี้ยงในอาหารเหลว BG-11 ที่ความเข้มแสง 2,000 - 3,000 ลักซ์ ให้แสงเป็นเวลา 12 ชั่วโมง หลังจากนั้นนำสาหร่าย LmTK01 ที่ความหนาแน่น 336.17, 703.70 และ 1,390.63 ไมโครกรัมต่อลิตร ไปเลี้ยงร่วมกับกุ้งขาวที่มีน้ำหนักเฉลี่ย 0.18 ± 0.05 กรัม ในน้ำทะเลปริมาตร 500 มิลลิลิตร เป็นเวลา 96 ชั่วโมง ผลการศึกษาพบว่า กุ้งขาวที่เลี้ยงร่วมกับสาหร่ายที่ความหนาแน่นต่างๆ มีการตายมากกว่า 50 เปอร์เซ็นต์ในทุกการทดลอง โดยพบว่าสาหร่าย LmTK01 จะส่งผลกระทบทั้งทางตรงและทางอ้อมต่อกุ้งขาว ซึ่งผลกระทบทางตรง คือพบเซลล์ของสาหร่ายติดอยู่บริเวณรยางค์ เหงือก และลำใส้ของกุ้ง ทำให้รบกวนกระบวนการหายใจและระบบการย่อยอาหาร ของกุ้งโดยตรง ขณะที่ผลกระทบทางอ่อมคือ ทำให้เกิดการเปลี่ยนแปลงของคุณภาพน้ำระหว่างเลี้ยง โดยพบการเพิ่มขึ้นของค่าพี เจชินน้ำ ผลกระทบดังกล่าวส่งผลให้กุ้งมีอาการเครียด อ่อนแอ และตายในที่สุด นอกจากนี้การศึกษาผลของปัจจัยต่อการ เจริญเติบโตของ LmTK01 พบว่า สาหร่ายเจริญเติบโตได้ดีที่อุณหภูมิ 28.00 องศาเซลเซียส pH 7 - 8 และความเค็ม 0 - 30 พีพีที ดังนั้นผลการศึกษาครั้งนี้แลดงให้เห็นว่าสาหร่ายสีเขียวแกมน้ำเงิน Limnothrix สายพันธุ์ LmTK01 ส่งผลเสียอย่างมากต่ออัตราการ รอดของกุ้งและเป็นสาเหตุลำคัญที่ทำให้เกิดการสูญเสียผลผลดิตของกุ้งขาวแวนนาไม

คำสำคัญ: ไซยาโนแบคทีเรีย; กุ้งขาวแวนนาไม; คุณภาพน้ำ

#### Abstract

Cyanobacteria *Limnothrix* strain LmTK01 is a recently discovered cyanobacteria in Pacific white shrimp ponds (*Litopenaeus vannamei*), which causes shrimp production decrease. The morphology of LmTK01 is similar to cyanobacteria genus *Limnothrix*. The aims of this study were to investigate the effect of LmTK01 on the survival rate of shrimp (*L. vannamei*) and the factors of temperature, pH and salinity effected by LmTK01 growth. Algae LmTK01 were isolated from shrimp water culture during its blooming in the shrimp ponds. Algae were stocked in sterilization liquid BG-11 medium under light intensity 2,000 - 3,000 lux in a light: dark cycle, 12h: 12h. Different density of LmTK01 (336.17, 703.70 and 1,390.63 µg Chl-a/L) were inoculated with 0.18 ± 0.05 g of shrimp in the individual 500 mL of seawater for 96 hours. Water quality and survival rate of shrimp were measured every 6 hours. Results showed that shrimp died in over 50% of all treatments were inoculated with LmTK01. Algae LmTK01 showed the stress effect on shrimp directly and indirectly. The direct cause was algae clogging at the shrimp's gill and gastrointestinal tract, which interferes with the shrimp's breathing and digestion, while the indirect cause was changing in water quality, such as an increase in the pH value. These results caused the shrimp become to stressed, weak and eventually die. Moreover, it was found that LmTK01 could grow well in a temperature of 28.00 °C, pH 7- 8 and salinity 0 - 30 ppt. Therefore, these results indicated that LmTK01 showed a significantly bad effect on the survival rate of shrimp, resulting in loss of shrimp production.

**Keywords**: cyanobacteria; Pacific white shrimp; water quality

\*Corresponding author. E-mail: teeyaporn.k@psu.ac.th

#### Introduction

Algal blooms are a worldwide problem in water resources, including in aquaculture ponds. Cyanobacteria were found as the main group. The algae in this group such as *Microcystis*, *Anabaena*, *Nostoc* and *Aphanizomenon*, have a negative impact on water quality, soil quality, animal health, ecosystem and economic losses (Reddy & Mastan, 2011; Ruangrit *et al.*, 2011; Tayaban *et al.*, 2018). Cyanobacteria *Limnothrix* strain LmTK01 is one of the new findings that has occurred in the shrimp ponds with low shrimp productivity in Thailand. Its characteristics include filament and no branch. The cell length of the long side is greater than the width. The morphology was like the cyanobacteria genus *Limnothrix*, which can grow in a wide range of salinity, the ability to adjust the optimum pH for growth between 8.20 - 9.63 and grows more as the phosphorus content increases (Keawtawee, 2014).

Phytoplankton growth is regulated by several factors such as light, pH, water temperature and salinity (Xing et al., 2019). Light intensity influences the photosynthesis of the cyanobacteria (World Health Organization, 1999). The pH value affects the solubility of nutrients, the distribution of carbon dioxide in the water and affects the physical characteristics of algae (Rubban et al., 2014). The optimal pH range for photosynthesis of cyanobacteria is between 6 - 9. However, photosynthesis decreases when the pH is 10 or higher. When Microcystis aeruginosa bloom is the main species, the pH in the water ranges from 7.5 - 9.5 with a peak of 10.5 (Fang et al., 2018). In addition, Touloupakis et al. (2016) reported that dry weight, growth yield, phycocyanin and lipid content of Synechocystis were decreased when raised to pH 11. Temperature is an important factor in the growth and metabolic activity of algae. The high temperature of the water in the environment can increase the growth rate of cyanobacteria. The optimum temperature for cyanobacteria growth in the genus Anabaena, Oscillatoria and Microcystis are between 25 - 35 °C, which is higher than in green algae and diatoms (Sevrin & Pletikosic, 1990). Therefore, the population of cyanobacteria was found more than other species, especially in the summer (Davis et al., 2009). It was also found that temperature influenced the specific growth rate and fatty acid synthesis, which was higher than that at 25 °C (Xin et al., 2011). The highest phycocyanin production and growth rate of Arthrospira platensis were found at 28 °C with growth rate 0.095  $\mu_{max}$ /day and 27.22 mg/L/day cell mass productivity followed by 29 °C and 30 °C respectively (Mohite & Wakte, 2011). Many microalgae are able to tolerate a wide range of salinity, which is a biochemical mechanism that occurs in cells such in the production and accumulation of an efficient Na<sup>+</sup>/K<sup>+</sup> pump system or the formation of intracellular vacuoles at high salinity which were created to store ions, especially Na+ and Cl (Figler et al., 2019). For example, Chaetomorpha sp. can grow at 0 - 37 ppt (Jagadeesan et al., 2012). Salinity stress can reduce the chlorophyll and protein content of Spirulina platensis (Vonshak et al., 1996).

Cyanobacteria blooming in shrimp ponds causes clogging of the gill and attachment to rostrums and mouth appendages (Massaut, 2003). This is a result of shrimp weakness, leading to susceptibility to pathogenic infection and causing off-flavor in shrimp that changes the taste of food (Newman, 2013). Several cyanobacteria produce toxins that are toxic to aquatic organisms and human health (Keawtawee *et al.*, 2012; Li *et al.*, 2016). Toxins are harmful to aquatic animals in many ways such as inhibiting the function of the nervous system that controls muscles, irregular gill movement, inability to swim, seizures and death from respiratory arrest. It also bioaccumulates in the muscle tissue of red claw crayfish and the visceral tissues of rainbow fish (Rodgers, 2008).

Moreover, it is also the cause of water quality problems due to dissolved oxygen reduction and pH stabilization (Wangwibulkit *et al.*, 2008). Photosynthesis is one of the reasons for pH increasing or decreasing that can cause stress or death in animals (Tucker & Abramo, 2008), and deoxygenation in the water is due to bacterial decomposition of organic material (You *et al.*, 2018). This is an important factor affecting the growth and health of Pacific white shrimp and other organisms in the pond (Supriatna *et al.*, 2017). Therefore, this study was interested in different algae densities on the survival rate of Pacific white shrimp and to study factors affecting the growth of cyanobacteria *Limnothrix* strain LmTK01 as a guideline for controlling this algae bloom in aquaculture ponds.

# Methods

## 1. Effects of Cyanobacteria Limnothrix strain LmTK01 at Different Densities on Pacific White Shrimp.

Pacific white shrimp  $0.18 \pm 0.05$  g were cultured with different densities of algae in 500 mL of plastic glass, under light intensity of 2,000 - 3,000 lux in a light: dark cycle at 12h: 12h, without adding aeration and feeding. The control and each treatment had 10 replications, one shrimp was used for each replication (10 shrimp in each treatment). Salinity 15 ppt was divided into three chlorophyll-a concentrations as 25% of algae (T1 =  $336.17 \mu g/L$ ), 50% of algae (T2 =  $703.70 \mu g/L$ ) and 100% of algae (T3 =  $1,390.63 \mu g/L$ ), respectively. The mortality characteristics of the shrimp were removed daily and observed under a stereo microscope. Water quality was measured four times daily. Dissolved oxygen was measured with DO meter YSI Pro 2030, pH using pH meter and chlorophyll-a concentration according to Boyd and Tucker method (1992). This was calculated using the equation 1:

Chlorophyll-a (
$$\mu$$
g/L) = 11.9 (A665 - A750) (V/L) (1,000/S) (1)

Where: A665 = the absorbance at 665, A750 the absorbance at 750, V = the acetone extract in mL, L = the length path in the spectrophotometer in cm, S = the volume in mL of sample filtered.



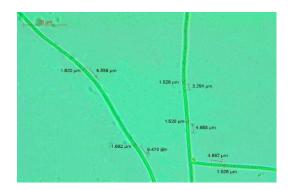


Figure 1 The characteristics and cell size of the cyanobacteria *Limnothrix* strain LmTK01 under a 1000x magnification microscope (Scale bar = 20 μm).

# 2. Growth of Cyanobacteria Limnothrix strain LmTK01 in Different Temperature, pH and Salinity.

Cyanobacteria *Limnothrix* strain LmTK01 were cultured in liquid BG-11 medium which autoclaved at 121  $^{\circ}$ C for 15 min under light intensity 2,000 - 3,000 lux in a light: dark cycle at 12h: 12h. The factors affecting temperature, pH and salinity on algal growth were conducted in the laboratory. LmTK01 was cultured at controlled temperature (28 ± 1  $^{\circ}$ C) and uncontrolled temperature (31.32  $^{\circ}$ C), at pH 6, 7, 8, 9, 10 and 11, respectively. The pH value in BG-11 medium was adjusted with hydrochloric acid and sodium hydroxide. The different salinities of water, which was enriched with BG-11 medium, was 0, 5, 10, 15, 20, 25 and 30 ppt, respectively. Salinity concentration was adjusted with sodium chloride and 20 mL of algae was added into 80 mL modified BG-11 medium. Algae growth in each treatment was measured at 680 nm wavelength using spectrophotometer (Yang *et al.*, 2012).

#### Statistical analysis

Statistical analysis was performed using the analysis of variance (ANOVA). The Tukey's HSD Test was used to determine the significant differences between the means at P<0.05. All statistical evaluations were carried out using R studio program.

#### Results

## 1. Effects of Cyanobacteria Limnothrix strain LmTK01 at Different Densities on Pacific White Shrimp.

Results showed that, the survival rate of shrimp was 100% at control (without algae) after 96 hours of rearing. While shrimp survival rate in treatment 3 (100% of algae) remained 20% and all shrimp died at treatment 1 and 2 (25% and 50% of algae) after 78 hours of rearing (Figure 2-a). Results of water qualities showed that



chlorophyll-a concentration was the highest in treatment 3, in which shrimp were cultured with 100% of algae (Figure 2-b). In addition, pH rang was 7.50 - 8.94 and there were no differences between treatments (Figure 2-c). However, dissolved oxygen (DO) values were not stable in all treatments (Figure 2-d). Results of shrimp cultured with cyanobacteria *Limnothrix* strain LmTK01 showed the shrimp molting but were not found in the control group (without algae). The characteristics of the dead shrimp included the filament of cyanobacteria clogging on the gill and attached to pereopod also found in stomach, intestine and feces (Figure 3). In addition, average dissolve oxygen in treatments 1, 2 and 3 were  $7.96 \pm 0.14$ ,  $7.95 \pm 0.14$  and  $8.00 \pm 0.14$  mg/L, respectively. These were significantly different from the control at  $7.69 \pm 0.06$  mg/L.

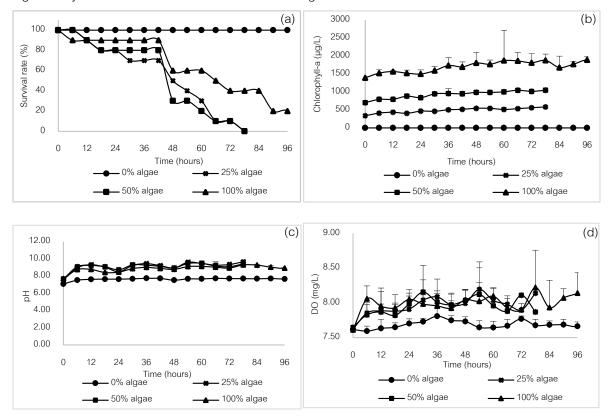


Figure 2 Survival rate of Pacific white shrimp (a) and water qualities; chlorophyll-a (b), pH (c) and dissolved oxygen (DO) (d) cultured with cyanobacteria Limnothrix strain LmTK01 at different density.



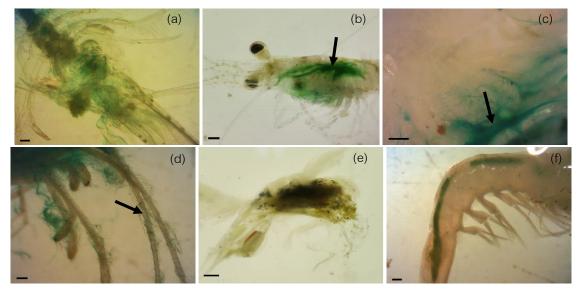


Figure 3 Mortality characteristics of pacific white shrimp cultured with cyanobacteria *Limnothrix* strain LmTK01 at different density; molting of shrimp (a), filament of algae clogging on gill of shrimp (b-c), attached to pereopod (d), stomach (e) and intestine (f). Black arrowheads indicate the filament of cyanobacteria *Limnothrix* strain LmTK01 (Scale bar = 20,000 μm).

#### 2. Growth of Cyanobacteria Limnothrix strain LmTK01 in Different Temperature, pH and Salinity.

Results of the growth of cyanobacteria Limnothrix strain LmTK01 showed the maximum density of LmTK01 was  $0.611 \pm 0.02$  at controlled-temperature treatment ( $28.00 \pm 1$  °C) on day 5 of culture (Figure 4-a). This was significantly different (p<0.05) when compared to the uncontrolled-temperature treatment under an average room temperature at 31.32 °C, which was  $0.089 \pm 0.02$ . The result of growth showed that the maximum density of OD was  $0.950 \pm 0.13$  at pH 8 on day 9 of culture followed by pH 7, respectively. It was found that the growth of this algae was low at high pH (Figure 4-b). In addition, the pH of algae tests at pH 7 - 11 was adjusted to the range of 8.46 - 9.41, while the algae test at pH 6 had an average of 6.50 throughout the experiment. Growth of algae at different salinity is shown in figure 4. The algae were grown at all salinities, but the maximum density of OD was  $1.246 \pm 0.04$  at 15 ppt on day 10 of culture (Figure 4-c).



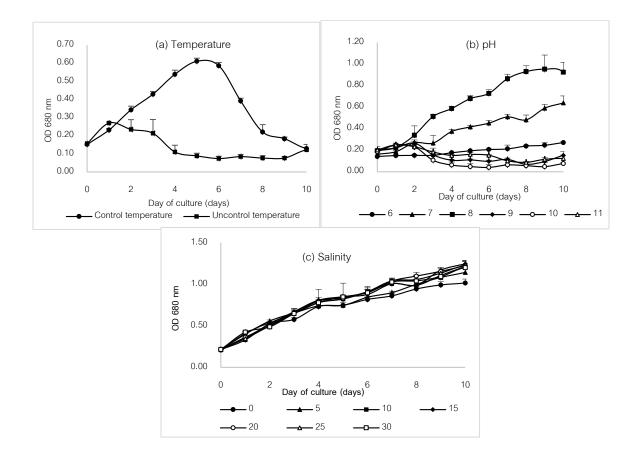


Figure 4 The growth of cyanobacteria *Limnothrix* strain LmTK01; temperatures at 28 ± 1 °C and room temperature (a), pH at 6, 7, 8, 9, 10 and 11 (b) and salinity at 0, 5, 10, 15, 20, 25 and 30 ppt (c), respectively.

#### Discussion

# 1. Effects of Cyanobacteria Limnothrix strain LmTK01 at Different Densities on Pacific White Shrimp.

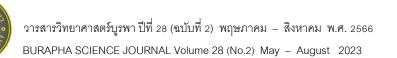
Shrimp culturing with algae LmTK01 showed a decreased survival rate of shrimp, found the filamentous of algae attached to the appendages, gills and digestive tract of shrimp and change in water quality. This problem can cause disturbances in the respiratory and digestive systems. This results in shrimp weakness and increases the risk of bacterial infection. In addition, it was reported that shrimp ponds where the predominant cyanobacteria were found resulted in shrimp mortality, accumulation of microcystin- LR and nodularin and swelling in hepatopancreatic tissue (Massaut, 2003; Galanti et al., 2013; Zimba et al., 2006). The optimal range of pH for culture Pacific white shrimp is from 7.5 - 8.5 (Wangwibulkit et al., 2008). High pH is a common problem found at



the surface of aquaculture ponds and filamentous algae are the main algae species. The abundance of algae results in more  $CO_2$  eliminated by photosynthesis during the day than the  $CO_2$  released by plant respiration at night (Tucker & Abramo, 2008). Moreover, high pH increases the toxicity of ammonia, hydrogen sulfide, dissolved metal and affects the growth of aquatic animals (Tucker & Abramo, 2008; Wurts, 2003). The amount of DO in surface waters could be related to the amount of chlorophyll-a (Sriyasak *et al.*, 2015). Dissolved oxygen in Pacific white shrimp farms was  $4.84 \pm 0.41$  mg/L with a range of 3.48 - 6.90 mg/L, these optimum DO for Pacific white shrimp culture were more than 3 mg/L. DO below 3 mg/L would cause shrimp stress, leading to susceptibility to disease, low food consumption and slow shrimp growth (Supriatna *et al.*, 2017). Furthermore, the population of phytoplankton affects the balance of microbial populations. The proliferation of pathogens and the reduction of beneficial microorganisms in the pond, which negatively affects the shrimp (Ding *et al.*, 2021).

# 2. Growth of Cyanobacteria Limnothrix strain LmTK01 in Different Temperature, pH and Salinity.

The studied of factors on the growth of algae showed that the factors affect the growth of algae. Factors affecting the proliferation of cyanobacteria include nutrients, dissolved oxygen, salinity, temperature, light and pH (Thipbharos, 2010; Wangwibulkit et al., 2008). According to Xin et al. (2011) the microalgae cultured at different temperatures showed the density of microalgae at 20, 25 and 30 °C were significantly higher than 10 °C. Cyanobacteria Limnothrix sp. 37-2-1 estimated the phycocyanin content and found the highest phycocyanin at 28 °C and significantly the lowest at 30 °C (Patel et al., 2016). Moreover, the temperature at 25 °C or greater also affects the processes of photosynthesis, specific respiration rate and growth rate of cyanobacteria (Robarts & Zohary, 1987). The temperature influences the buoyancy and growth rate of algae. For example, M. aeruginosa had the highest dispersion and growth rate at 28 and 27.5 °C, respectively (You et al., 2018), while the optimal temperature for shrimp growth is 28 - 30 ° C. This was significantly the optimal range for survival rate and the highest growth in Pacific white shrimp (Washim et al., 2020). However, the optimum temperature for the growth of cyanobacteria was 20 - 30 °C (Konopka & Brock, 1978). The photosynthesis was inhibited when the pH is below 6 and higher than 9. The optimum pH in water for the growth of cyanobacteria was between 7.5 - 9.0 (Wangwibulkit et al., 2008). During the experiment, the pH was adjusted. This is consistent with the results of Keawtawee (2014), algae changed the pH during the experiment to the range of 8.20 - 9.63. The algae have strong buffering capacity for pH due to carbon dioxide and nutrient metabolism (Xing et al., 2019). Cyanobacteria have processes to maintain pH homeostasis of intracellular depending on their natural habitat and the ability of the cells internally (Buck & Smith, 1995; Golda, 2017). Cyanobacteria can be both halotolerant and halophilic forms, such as Pseudanabaena lonchoides, Synechocystis aquatilis and Lyngbya contorta. Hence, the cyanobacteria could not be clearly classified



on the salinity level (Bano & Siddiqui, 2004). However, the salinity affects the physiological characteristics of algae. For instance, the filament of *Oscillatoria* sp. were aggregated at a salinity higher than 15 ppt. This also affects the disturbance of ion balance and photosynthesis of cyanobacteria (Wangwibulkit *et al.*, 2008). Rafiqul *et al.* (2003) reported that *Spirulina fusiformis* can grow in a wide range of salinities, which depends on the physical mechanism, including the amount of inorganic and organic osmoregulation. The reduction of biomass and the specific growth rate due to the process of photosynthesis and the respiratory system were inhibited, which was caused by the rapid diffusion of sodium into cells. This is the result of the detachment of phycobillisomes from the thylakoid membranes. The effect of different salinities on algae growth in this experiment was similar to the salinity range found in Pacific white shrimp culture, which can grow in salinity from 1 - 40 ppt (Su *et al.*, 2010).

#### Conclusions

Results indicated that, cyanobacteria *Limnothrix* strain LmTK01 showed a negative direct and indirect effect on shrimp survival rate and production. It was found that shrimp died due to the molting of shrimp, and the filament of algae attached to the appendages, gills and digestive tract. The pH value increased gradually if cyanobacteria was blooming. This resulted in shrimp stress, weakness and eventual death. In addition, cyanobacteria *Limnothrix* strain LmTK01 grew well in a wide range of pH and salinity. However, pH and temperature were related to algae growth. The results showed that the optimal pH and temperature for the growth of algae were at 7 - 8 and 28 °C. Therefore, results from this study can be applied as a guideline for prevention of cyanobacteria bloom in shrimp culture in the future.

# Acknowledgements

We would like to express our sincere gratitude to Participatory and Integrative Support for Agricultural Initiative (PISAI) Project ERASMUS +-Capacity Building in Higher Education Program of the European Union for their support in the mobility of the double Master's program activities.

We would like to thank the Discipline of Excellence in Sustainable Aquaculture, Prince of Songkla University for financial support (TAMDOE60040) and the Center of Excellence in Agricultural and Natural Resources Biotechnology (CoE-ANRB): phase3, Faculty of Natural Resources, Prince of Songkla University.

#### References

- Bano, A. & Siddiqui, P. (2004). Characterization of five cyanobacterial species with respect to their requirement for pH and salinity. *Pakistan Journal of Botany*, 36(1), 133-143.
- Boyd, C.E. & Tucker, C.S. (1992). Water quality and pond soil analyses for aquaculture. Alabama: Alabama Agricultural Experiment Station, Auburn University.
- Buck, D.P. & Smith, G.D. (1995). Evidence for a Na<sup>+</sup>/ H<sup>+</sup> electrogenic antiporter in an alkaliphilic cyanobacterium Synechocystis. FEMS Microbiology Letter, 128(3), 315-320.
- Davis, T.W., Berry D.L., Boyer, G.L. & Gobler, C.J. (2009). The effects of temperature and nutrients on the growth and dynamics of toxic and non-toxic strains of *Microcystis* during cyanobacteria blooms. Harmful Algae, 8(5), 715-725.
- Ding, Y., Song, X., Cao, X., He, L. & Yu, Z. (2021). Healthier communities of phytoplankton and bacteria achieved via the application of modified clay in shrimp aquaculture ponds. *International Journal of Environmental Research and Public Health*, 18(21), 11569.
- Fang, F., Gao, Y., Gan, L., He, X. & Yang, L.J. (2018). Effects of different initial pH and irradiance levels on cyanobacterial colonies from Lake Taihu, China. *Journal of Applied Phycology*, 30(3), 1777-1793.
- Figler, A., Viktória, B., Dobronoki, D., Márton, K., Nagy, A.S. & Bácsi, I. (2019). Salt Tolerance and Desalination Abilities of Nine Common Green Microalgae Isolates. *Water*, 11(12), 2527.
- Galanti, N.L., Amé, V.M. & Wunderlin, A.D. (2013). Accumulation and detoxification dynamic of cyanotoxins in the freshwater shrimp *Palaemonetes argentinus*. *Harmful Algae*, 27, 88-97.
- Golda, L.R. (2017). Assessing the influence of environmental pH on algal physiology using a novel culture system. United States: Oregon Health and Science University.

- Jagadeesan, K., Raja, S. & Sampath, K. (2012). Studies on effect of salinity on the culture of algal forms (Oscillatoria spp. and Chaetomorpha sp.) and their impact on shrimp (Penaeus indicus) culture. New Vistas in Indian Aquaculture Book of Abstracts, 1045, 85.
- Keawtawee T. (2014). Using the specific bacteria for controlling the blue green algae blooms strain Osci-TK01 in a shrimp farm, Prince of Songkla University, Thailand, 2014. (in Thai)
- Keawtawee, T., Fukami, K., Songsangjinda, P. & Muangyao, P. (2012). Use of a *Noctiluca*-killing bacterium *Marinobacter salsuginis* strain BS2 to reduce shrimp mortality caused by *Noctiluca scintillans*. *Fisheries Science*, 78(3), 641-646.
- Konopka, A. & Brock, D.T. (1978). Effect of temperature on blue-green algae (cyanobacteria) in Lake Mendota. Applied and Environmental Microbiology, 36(4), 572-576.
- Li, Y., Liu, L., Xu, Y., Li, P., Zhang, K. Jiang, X., Zheng, T. & Wang, H. (2016). Stress of algicidal substances from a bacterium *Exiguobacterium* sp. h10 on *Microcystis aeruginosa*. *Letters in Applied Microbiology*, 64(1), 57-65.
- Massaut, L. (2003). Cyanobacteria toxicity tested in shrimp larvae *Oscillatoria brevis* reduces *P. vannamei* survival. *Global Aquaculture Advocate*, 90-92.
- Mohite, S.Y. & Wakte, S.P. (2011). Assessment of Factors Influencing Growth and C-Phycocyanin Production of *Arthrospira platensis* from Meteoritic Crater Lake. *Journal of Algal Biomass Utilization*, 2(2), 53-68.
- Newman, S.G. (2013). Risks of toxins from blue green algae to aquaculture. *Infofish International*, 5, 24-28.
- Patel, V., Berthold, E.D., Dhandayuthapani, S., Rathinavelu, A. & Gantar, M. (2016). Optimization of C-phycocyanin production by *Limnothrix* sp. 37-2-1. *Journal of Algal Biomass Utilization*, 7(1), 104-111.

- Rafiqul, M.I., Hassan, A., Sulebele, G., Orosco, A.C., Roustaian, P. & Jalal, A.C.K. (2003). Salt stress culture of blue-green algae Spirulina Fusiformis. Pakistan Journal of Biological Sciences, 6(7), 648-650.
- Reddy, M. R. K. & Mastan, S. A. (2011). Algal toxins and their impact on human health. *Biomedical & Pharmacology Journal*, 4(1), 129-134.
- Robarts, D.R. & Zohary, T. (1987). Temperature effects on photosynthetic capacity, respiration, and growth rates of bloom forming cyanobacteria. *New Zealand Journal of Marine and Freshwater Research*, 21(3), 391-399.
- Rodgers, J.H. (2008). Algal toxins in pond aquaculture. *Southern Regional Aquaculture Center*, Publication No. 4605.
- Ruangrit, K., Whangchai, N., Pekkoh, J., Ruangyuttikarn, W. & Peerapornpisal, Y. (2011). First Report on Microcystins Contamination in Giant Freshwater Prawn (*Macrobrachium rosenbergii*) and Nile Tilapia (*Tilapia nilotica*) Cultured in Earthen Ponds. *International Journal of Agriculture and Biology*, 13(6), 1025-1028.
- Rubban, K., Sughan, V., Wong, L.S. & Meekin, C. (2014). The effects of pH and cell density to the responses of immobilized cyanobacteria for copper detection. *Journal of Life Sciences and Technologies*, 2(2), 78-81.
- Sevrin, R.J. & Pletikosic, M. (1990). Cyanobacteria in fish ponds. Aquaculture, 88(1), 1-20.
- Sriyasak, P., Chitmanat, C., Whangchai, N., Promya, J. & Lebel, L. (2015). Effect of water de-stratification on dissolved oxygen and ammonia in tilapia ponds in Northern Thailand. *Proceedings of 53rd Kasetsart University Annual Conference: Plants, Animal, Veterinary Medicine, Fisheries, Agricultural Extension and Home Economics*, 3-6 February 2015 Bangkok Thailand.



- Su, Y., Ma, S. & Feng, C. (2010). Effects of Salinity Fluctuation on the Growth and Energy Budget of Juvenile *Litopenaeus Vannamei* at Different Temperatures. *Journal of Crustacean Biology*, *30*(3), 430-434.
- Supriatna, S., Koesman, M., Hariati, A. & Mahmudi, M. (2017). Dissolved oxygen models in intensive culture of white leg shrimp, *Litopenaeus vannamei*, in East Java, Indonesia. *Aquaculture, Aquarium, Conservation & Legislation International Journal of the Bioflux Society*, 10(4), 768-778.
- Tayaban, M.M.K., Pintor, L.K. & Vital, G.P. (2018). Detection of potential harmful algal bloom-causing microalgae from freshwater prawn farms in Central Luzon, Philippines, for bloom monitoring and prediction. *Environment Development and Sustainability*, 20(12), 1311-1328.
- Thipbharos, V. (2010). Prevention and decreasing management of geosmin flavor in processed fishery products. *Suthiparithat*, *24*, 103-109. (in Thai)
- Touloupakis, E., Cicchi, B., Benavides, S.M.A. & Torzillo, G. (2016). Effect of high pH on growth of *Synechocystis* sp. PCC 6803 cultures and their contamination by golden algae (*Poterioochromonas* sp.). *Applied Microbiology and Biotechnology*, 100(3), 1333-1341.
- Tucker, C.S. & Abramo, L.R.D. (2008). Managing High pH in Freshwater Ponds. *Southern Regional Aquaculture Center*, Publication No. 4604.
- Vonshak, A., Kancharaksa, N., Bunnag, B. & Tanticharoen, M. (1996). Role of light and photosynthesis on the acclimation process of the cyanobacterium *Spirulina platensis* to salinity stress. *Journal of Applied Phycology*, 8, 119-124.
- Wangwibulkit, S., Limsuwan, C. & Chuchird, N. (2008). Effects of salinity and pH on the growth of blue-green algae, *Oscillatoria* sp. and *Microcystis* sp., isolated from pacific white shrimp (*Litopenaeus vannamei*) ponds. *Kasetsart University Fisheries Research Bulletin*, 32(1), 1-9.

- Washim R. M. M., Siddiky, M. S. N. M. & Ahmmed, S. (2020). Improved extensive shrimp farming uplifted yield of Coastal Ghers in Southwest Bangladesh. *International Journal of Sciences: Basic and Applied Research*, 52(1), 78-87.
- World Health Organization. (1999). *Toxic cyanobacteria in water: A guide to their public health consequences, monitoring and management.* London: E & FN Spon.
- Wurts, A.W. (2003). Daily pH cycle and ammonia toxicity. World Aquaculture, 34(2), 20-21.
- Xin, L., Hu, H.Y. & Zhang, Y.P. (2011). Growth and lipid accumulation properties of a freshwater microalga Scenedesmus sp. under different cultivation temperature. Bioresource Technology, 102(3), 3098-3102.
- Xing, R., Ma, W., Shao, Y., Cao, X., Chen, L. & Jiang, A. (2019). Factors that affect the growth and photosynthesis of the filamentous green algae, *Chaetomorpha valida*, in static sea cucumber aquaculture ponds with high salinity and high pH. *PeerJ*, 7(4), e6468.
- Yang, L., Maeda, H., Yoshikawa, T. & Zhou, G. (2012). Algicidal effect of bacterial isolates of *Pedobacter* sp. against cyanobacterium *Microcystis aeruginosa*. *Water Science and Engineering*, 5(4), 375-382.
- You, J., Mallery, K., Hong, J. & Hondzo, M. (2018). Temperature effects on growth and buoyancy of *Microcystis aeruginosa. Journal of Plankton Research*, 40(1), 16-28.
- Zimba, V.P., Camus, A., Allen, H.E. & Burkholder, M.J. (2006). Co-occurrence of white shrimp, *Litopenaeus vannamei*, mortalities and microcystin toxin in a southeastern USA shrimp facility. *Aquaculture*, 261(3),1048-1055.