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Research Article

Edible Cyanobacteria (*Nostochopsis* spp.) from Glass House, Queen Sirikit Botanical Garden, Thailand

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Abstract: *Nostochopsis* spp. are edible and rare cyanobacteria which form thick mucilaginous colonies, 0.1-8 cm in size, attached on the rocks or cobbles in transparent shallow streams or rivers. They are classified in the Order Nostocales, Family Hapalosiphonaceae. The objective of this study was to investigate the colonial growth, pigments and quality of water in the ponds at 3 glass houses in the Queen Sirikit Botanic Garden, Chiang Mai Province in which these cyanobacteria were growing during April-June 2012. The three glass houses were; the Aquatic house, the Bromeliad house and Orchids and Fern house. Ten colonies from each sampling site were measured once a week. The average increase in colonial size was found to be 0.17±0.06, 0.30±0.08 and 0.15±0.08 cm/week respectively. Chlorophyll, phycocyanin, allophycocyanin and carotenoid were highest in the samples from Aquatic house as 16.22±4.28, 11.95±8.55, 73.62±4.07 and 12.70±1.54 mg/g.dw, respectively. These cyanobacteria grew at 22-30°C, pH 6.17-8.75 and conductivity 112-171 μs/cm. The water quality was clean-moderate and in oligo-mesotrophic status.

Keywords: Cyanobacteria, edible algae, *Nostochopsis*, phycocyanin, pigments, water quality

INTRODUCTION

Cyanobacteria (Cyanophytes) are among the most fascinating organisms within the earth's biosphere (Komárek, 2006). They are found in most ecosystems. Many species such as *Spirulina* and *Nostoc* have been used as food and food supplements. *Nostochopsis* spp. are filamentous cyanobacteria that grow luxuriantly attached to the rock surface in the form of mucilaginous balls in fresh water lake and slow flowing stream and ponds (Pandey and Pandey, 2008a). They abundantly occur during the cool-dry season and the hot-dry season. In northern Thailand, especially in the Nan River of Nan Province, the local people use them to prepare local dishes in the form of salad with traditional seasoning (Peerapornpisal *et al*., 2006). The indigenous tribes in India use them as a dietary supplement (Pandey and Pandey, 2008a). *Nostochopsis* spp. contain high nutritional value such as protein, carbohydrate, lipid, high fiber, vitamin and mineral salt. In addition, they contain high amount of calcium as in the small fish. Besides, *Nostochopsis* spp. substantiated the nutraceutical potential, for example *N. lobatus* exhibited anti-gastric ulcer activity, as well as anti-inflammatory and antioxidant activity by 1,1-Diphenyl-2-Picrylhydrazyl (DPPH) radical scavenging activity assay (Thiamdao *et al*., 2011). Pandey and Pandey (2008b), reported its antioxidant activity of 140.50 μmole ascorbic acid equivalent capacity g/g fresh wt.

The Queen Sirikit Botanical Garden, Chiang Mai is Thailand's oldest and foremost botanical garden and a major center for scientific research and conservation of Thai flora. This garden holds collections and carries out research on rare, endemic and endangered species (The Botanical Garden Organization, 2012). So, this study aims to explore the ecology, biodiversity of *Nostochopsis* spp. based on the morphological characteristics and water quality in this area. Pigments e.g., chlorophyll, allophycocyanin, phycocyanin and charotenoid were also studied.

MATERIALS AND METHODS

The colonial growth, pigments and quality of water in which *Nostochopsis* spp. were growing during April-June 2012 (10 weeks) in the ponds at 3 glass houses in the Queen Sirikit Botanic Garden located at 18° 53′16.72′′ N 98° 51′ 42.57′′ E, Chiang Mai Province were investigated (Fig. 1). The three glass houses were; Aquatic house, Bromeliad house and the Orchids house. Ten colonies of *Nostochopsis* at each glass house were measured every week using quadrant size 26×26 cm to define the area of colonial study.

The water samples from each site were determined for temperature, conductivity, pH, DO, BOD, turbidity, nutrients (nitrate nitrogen, ammonium nitrogen and soluble reactive phosphorus) according to Eaton *et al.* (2005). The water quality was examined following,

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Fig. 1: Sampling sites at queen sirikit botanical garden chiang mai

Wetzel (2001) and Lorraine and Vollenweider (1981), the Guidelines of Standard Surface Water Quality of Thailand (Notification of the National Environmental Board, 1994) and AARL PC-Score (Peerapornpisal *et al*., 2004; Leelahakrjiengkrai and Peerapornpisal, 2011).

The cyanobacterial samples were taken off their substrates and preserved in 2.0% formaldehyde in plastic boxes at low temperatures (0-4°C). For morphological analysis, cell dimensions: length and width of the vegetative cells and diameter of heterocysts were measured. Type of branching was also determined. Samples were identified following the procedures indicated in the relevant books and publications (Bharadwaja, 1934; Desikachary, 1959; Anagnostidis and Komárek, 1988; Gugger and Hoffmann, 2004), Photographs of each species were taken using a Light Olympus Normaski Microscope. Chlorophyll, were determined from dried samples according to Becker (1994). For chlorophyll 10 mL 90% vlv methanol was added to 0.05 g of dried sample and incubated at 70°C for 5 min. The mixture was centrifuged at 3500 rpm for 15 min and the supernatant was measured at 650 and 665 nm. The chlorophyll (mg/g dw) was determined by the equation:

$$\text{Chlorophyll } a \text{ and } b \text{ (mg/g.dw)} = (4.0\times A_{665}) - (25.5\times A_{650})$$

Phycocyanin and allophycocyanin according to González-Delgadoa and Kafarov (2012) were determined by adding 5 mL 0.15 M NaCl to 0.05 g dried sample kept at 4°C for 24 h and thawed at room temperature. The mixture was ultra-sonicated for 1 min at 6 kHz (Sonic Vibra cellTM) and centrifuged at 3500 rpm for 15 min. The pigments were measured at 620 and 650 nm by the equation:

$$\text{Phycocyanin}\left(\frac{\frac{mg}{g}}{dw}\right) = \frac{(A_{620}-0.7)\times A_{650}}{7.38-10^{-3}}$$

$$\text{Allophycocyanin}\left(\frac{\frac{mg}{g}}{dw}\right) = \frac{(A_{650}-0.19)\times A_{620}}{5.65-10^{-3}}$$

Carotenoid was measured according to KMUTT (2001); by adding 10 mL of ethanol and 60% KOH to 15 mg of dried sample. The mixture was incubated at 50°C for 5 min and centrifuged at 3500 rpm for 10 min. The supernatant was added with 2 mL of ethanol and 20 mL of diethyl ether and 20 mL of 9% NaCl. The mixture was shaken and the green supernatant was discarded. The yellow supernatant was added with 20 mL of 9% NaCl and 0.05 mg of Na_2SO_4. Diethyl ether was then added to give a final volume of 25 mL. The amount of carotenoid was measured at 450 nm following the equation:

$$\text{Carotenoid}\ \frac{\text{mg}}{\text{g.dw}} = \frac{OD_{420} \times 25 \times 100}{260 \times \text{mg of dried Weight}}$$

RESULTS AND DISCUSSION

Colonies of *Nostochopsis* sp., were soft 0.2-3.5 cm in diameter (Fig. 2), blue-green, filaments with blue green branches (T-branching) which were found at the Aquatic house and *N. lobata* Wood ex Bornet et Flahault colonies were solid at young stage and hollow at the older stage, blue green or olive green, slightly brown. Blue-green filaments, commonly branched, (T-branching) were found at the Bromeliad House and Orchids and Fern House (Fig. 3). They were attached to rocks in the artificial ponds, which corresponded to the previous report by Desikachary (1959) who found *N. lobata* in standing or running water, attached or free floating, in paddy-fields, ponds, streams, on rocks in rivers in India and Tanuggyi Canals and in Royal lakes in Burma. Colonial growth of *Nostochopsis* sp., in the Aquatic house was 0.14+0.05 cm/week, *N. lobata* in the Bromeliad House and Orchids and Fern House was 0.25+0.03 and 0.14+0.03 cm/week respectively (Table 1). *N. lobata* in the Bromeliad House grew better than that in the Orchids and Fern House since the Bromeliad House was more transparent and got more sunshine than other glass houses (2,850 µmoles/m^2/s). Orchids and Fern house was shady (294.5 µmoles/m^2/s), Both glass houses were found to be non significantly ($p>0.05$) low in nutrient. Colonies of *N. lobata* grown for 8 weeks broke from the rock. Desikachary (1959) reported that *N. lobata* attached at first, later became free-floating with erect filaments surrounded with soft diffluent sheath. Moreover, mucilaginous colonies of *Nostochopsis* sp., at Aquatic houses the formed thick, 8 cm in diameter which were bigger than previously reported in Thailand (Peerapornpisal *et al*., 2006; Thiamdao *et al*., 2011). In addition, the water quality in all sampling sites was moderate. The pH varied from 6.17 to 8.75. The water temperature at the sampling sites ranged from 24-30.5°C. The water was clear (1-32 NTU) and had low conductivity at 112-171 µs/cm. The Bromeliad house showed the high level of Biochemical Oxygen Demand (BOD_5) at 2.2 mg/L with low nutrients at all sampling

Fig. 2: Different colonies of *Nostochopsis* spp

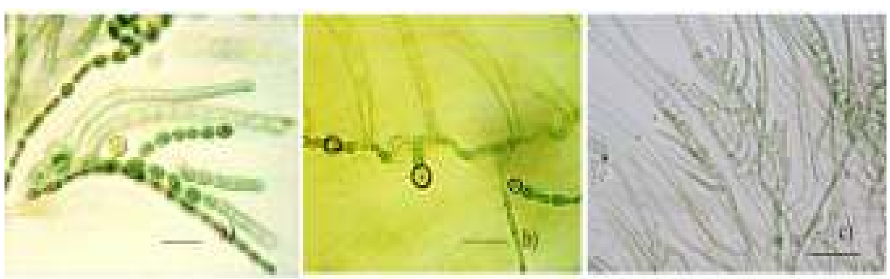

Fig. 3: Filaments of *Nostochopsis* spp. in the ponds of three glass houses, (a) *Nostochopsis* sp., at aquatic house, (b) *N. lobata* wood ex bornet et flahault at bromeliad house, (c) *N. lobata* wood ex bornet et flahault at orchids and fern house; scale bar = 20 µm

Table 1: Growth of *Nostochopsis* spp. colonies (mm) in each week

Observation sites/time	21 April -28 April	28 April -5 May	5 May -12 May	12 May -19 May	19 May -26 May	26 May -2 June	2 June -9 June	9 June -16 June	16 June -23 June	23 June -30 June
Aquatic house Mean 0.14±0.05	0.21±0.01	0.28±0.04	0.02±0.06	0.06±0.02	0.13±0.03	0.16±0.09	0.17±0.08	0.10±0.03	0.18±0.07	0.13±0.02
Bromeliad house Mean 0.25±0.03	0.30±0.03	0.30±0.02	0.05±0.02	0.09±0.02	0.38±0.02	0.20±0.04	0.23±0.04	0.26±0.05	0.38±0.03	0.35±0.02
Orchids and fern house Mean 0.14±0.03	0.30±0.02	0.12±0.04	0.04±0.02	0.07±0.03	0.07±0.01	0.20±0.04	0.20±0.03	0.14±0.07	0.14±0.01	0.14±0.01

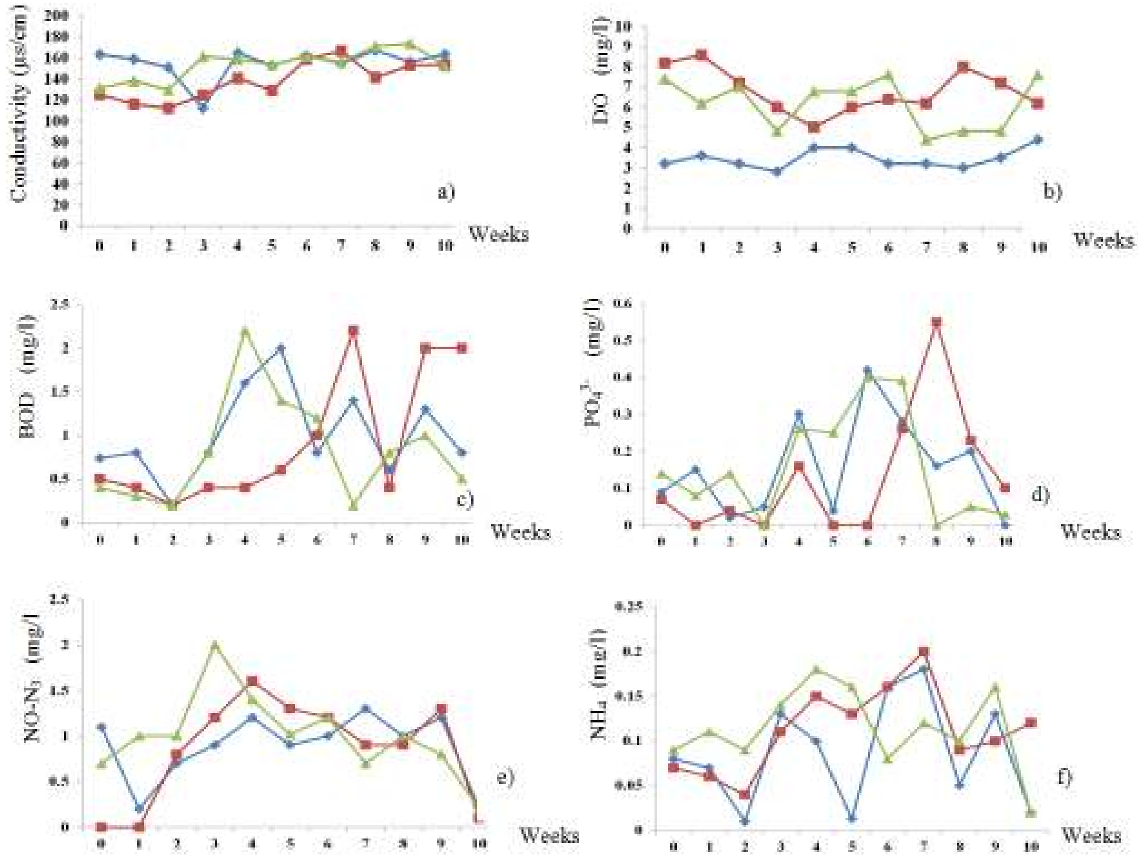

Fig. 4: Physical-chemical factors of water in the three glass houses, (a) conductivity; (b) dissolved oxygen; (c) biochemical oxygen demand; (d) soluble reactive phosphorus, (e) nitrate-nitrogen, (f) ammonium-nitrogen
: Aquatic house; : Bromeliad house; : The orchids and fern house

Table 2: Pigments of *Nostochopsis* spp. in ponds of three glass house

Pigments/samples	Aquatic house (mg/g.dw)	Bromeliad house (mg/g.dw)	Orchids and fern house (mg/g.dw)
Chlorophyll	16.22±4.28[a]	12.04±5.42[ab]	5.24±1.44[b]
Phycocyanin	11.95±8.55[a]	10.07±8.33[a]	5.15±1.97[a]
Allophycocyanin	73.62±4.07[ac]	53.74±2.07[b]	46.32±6.17[b]
Carotenoid	12.70±1.54[a]	12.20±0.31[a]	5.35±1.00[b]

Data are expressed as the mean±Standard Deviation (S.D.) of three replicates; different letters represent the statistical comparisons between groups in each column by using ANOVA and post hoc Tukey's b test ($p<0.05$)

sites and all the time (Fig. 4). Similarly, water quality was moderate at the Nan River (Peerapornpisal *et al*., 2006) and *N. lobata* grew in non-polluted creeks, rivers and streams (Komárek and Hauer, 2013). *Nostochopsis* sp., at Aquatic house showed significantly ($p<0.05$) highest allophycocyanin at 73.62±4.07 mg/g.dw but not difference significant in chlorophyll and carotenoid from *N. lobata*. Phycocyanin was not significant different between *Nostochopsis* sp. and *N. lobata.* (Table 2). Pandey and Pandey (2008b) reported that, *N. lobata* showed potentially high production of biomass, chlorophyll and carotenoids with significant improvement under immobilized cell culture when 10 mg/L K_2HPO_4 was added. Moreover, when 3 mg/L $FeNH_4$ citrate was supplemented phycocyanin, phycoerythrin, nutritive value and antioxidant capacity were also higher.

CONCLUSION

Two species of the edible cyanobacteria; *Nostochopsis* sp. and *N. lobata* were found at the glasshouses of Queen Sirikit Botanical Garden, attached on rocks and free floating in standing water with slowly flowing clean-moderate water quality. These species showed potential production of pigments; chlorophyll, phycocyanin, allophycocyanin and carotenoid which have nutritional value.

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