

CHAPTER 5

DISCUSSION

5.1 Diversity and taxonomic evaluation of cyanobacteria

Cyanobacteria are ancient microorganisms which can be found in all ecosystems from freshwater to terrestrial environments and also in extreme habitats such as thermal spring, hypersaline environments, etc. In hypersaline habitats, cyanobacteria are organisms successfully colonizing such habitats, because they have good mechanisms for their adaptations (Roussomoustakaki, 1996). They are usually found in form of microbial mats, sometimes mixed with other organisms. The majority components of other microorganisms are diatoms (Bauld, 1984; Stal, 2000), and sometimes green and purple sulfur bacteria also occurred in these mats (Nicholson *et al.*, 1987; Pierson *et al.*, 1987). In our localities, cyanobacteria formed their microbial mats with many species of diatoms and sulfur bacteria.

As it is well known, cyanobacteria can be found diverse in various habitats even in extreme environments. It can be found more than a hundred species in salinic conditions such as lakes, soils etc. However, as we know, the ecology (light, pH, temperature and also under extreme conditions) are the important factors which influence the morphology of cyanophytes, and it makes the identification and classification difficult also in a specific extreme habitat. For example, in several hypersaline habitats and also saltworks in Thailand (Petchaburi, Samut Songkhram

solar salterns and others) many unknown species were found, or still not clear up to now (Roussomoustakaki, 1996; Richert *et al.*, 2006; Chatchawan *et al.*, 2011). The taxonomy and position in phylogeny of many species must be solved in future by more precise methods. Therefore, in this study, many taxa are designated by marks “cf.” or “sp.” and most of them need intensive study in future.

From this study, cyanobacteria in Petchaburi and Samut Songkhram solar salterns are more diverse than in Chanthaburi (CP), Udon Thani (UT) and Sakon Nakhon (SN) salterns. The possible reason may be the pH in CP, UT and SN are in range of acidic but cyanobacteria are most abundant in neutral to slightly alkaline habitats (Steward, 1966).

Totally, thirty-two species of twenty-three genera of cyanobacteria were found. They belong to order Synechococcales, Pseudanabaenales, Oscillatoriales, Chroococcales and Nostocales. Only three Nostoclean species were found during our study. It may be due to it present relatively high sulfur content which is toxic to heterocytous forms (Nagasathya and Thajuddin, 2008; Howsley and Pearson, 1979). According to this study, *Coleofasciculus chthonoplastes*, *Oxynema thaianum* and *Spirulina subsalsa* should be considered as versatile species because of their being found throughout the whole study areas with high salinities ranging from 50-250 ppt. The number of cyanobacterial species in this study were mainly affected by salinity. It declined with higher salinities. This result correlates with the study of Nagasathya and Thajuddin (2008) in saltpans of the southeastern coast of India.

Numerous cyanobacteria found in this study are also reported from other hypersalinic habitats over the world (Hof and Frémy, 1933; Javor, 1989; Garcia-Pichel *et al.*, 2001; Abed *et al.*, 2002; Burns *et al.*, 2004; Richert *et al.*, 2006;). In the

Petchaburi and Samut Songkhram solar saltern, cyanobacteria often occurred in green to dark blue green microbial mats formed on the surface layer of wet soils (approximately up to 0.5-1 cm thick) or sometimes underneath the soil surface. These mats were mostly composed of *Microcoleus* (= *Coleofasciculus*) *chthonoplastes*, *Spirulina subsalsa*, *Oxynema thaianum* and *Leptolyngbya* spp., rarely of *Johannesbaptistia pellucida*, sometimes mixed with coccoid types including *Cyanosarcina* sp., *Aphanothece* cf. *halophytica*, *Halothece* sp. and *Chroococcus* spp. In comparison with other hypersalinic biotopes, these localities are slightly different in species composition of cyanobacteria. For the example, in the southern part of the Great Salt Lake (Utah, USA), cyanobacteria play a minor role in photosynthesis and form their mats in shallow sites. They are composed mostly of *Oscillatoria* sp. and *Aphanothece* sp. (Oren, 2002). Other filamentous genera, including *Phormidium*, *Microcoleus*, *Spirulina*, and *Nodularia* spp., were also found in this lake (Post, 1977; Oren and Seckbach, 2001). Cyanobacteria produced microbial mats up to 1 m thick on the bottom of Solar Lake, Sinai (Egypt). These were rich with biogenic carbonates and dominated by the unicellular *Aphanocapsa littoralis* and *Aphanothece halophytica*, and the filamentous *Microcoleus* sp. and *Oscillatoria* sp. (Jørgensen and Cohen, 1977). The planktonic, gas-vacuolated cyanobacterium “*Dactylococcopsis*” *salina* (= *Myxobactron salinum*) was also described from this locality (Walsby *et al.*, 1983). In many hypersaline environments, such as hypersaline lakes, cyanobacteria occur predominately as isolated unicellular types, in colonies and filamentous agglomerations. Caumette *et al.* (1994) reported the species compositions of microbial mats from a Mediterranean saltern in France during the warm season. The top brown layer was composed of the unicellular cyanobacterium *Aphanothece* spp.

while the intermediate green layer was formed by *Phormidium* sp. In addition, the cyanobacterial community in Grande coastal lagoon, Lima, Peru was mainly composed of coccoid colonies and filamentous cyanobacteria, diatoms and some green algae (Montoya, 2009). In the Petchaburi solar saltern, cyanobacteria usually occurred in mats and were mostly dominated by filamentous cyanobacteria. Some species of diatoms, including *Amphora* sp. and *Nitzschia* spp., were mixed in communities of cyanobacteria. It follows from all of these findings that hypersaline habitats are only similar but not identical in cyanobacterial diversity (Kirkwood *et al.*, 2007). Cyanobacteria were found often mixed with diatoms and some green algae.

The probably cosmopolitan cyanobacterium “*Coleofasciculus chthonoplastes*” (= previous *Microcoleus chthonoplastes*) (Garcia-Pichel *et al.*, 1996; Siegesmund *et al.*, 2008) was reported from various hypersaline environments worldwide. In the Petchaburi solar saltern, *C. chthonoplastes* was usually found with other cyanoprokaryotes, including *Leptolyngbya* spp., *Spirulina subsalsa* and *Oxynema thaianum*. These findings are similar with the report of cyanobacterial taxa in the Grande coastal lagoon, where *C. chthonoplastes* (“*Microcoleus*”) was mainly associated with *Aphanothece stagnina*(?), *Spirulina subsalsa*, *Calothrix crustacea*(?), *Lyngbya aestuarii* and *Johannesbaptistia pellucida* (Montoya, 2009). Solar Lake, Sinai peninsula, was colonized by *Aphanothece halophytica*, *Microcoleus chthonoplastes*, *Spirulina subsalsa* and *Oscillatoria salina* (Campbell and Golubić, 1985).

This study provides information mainly about the appearance and diversity of cyanobacteria in saltern habitats in Thailand and also some ecophysiological features of some strains. The taxonomy of cyanobacterial flora in salinic localities from

Thailand was still problematic up to now, and therefore it needs more intensive study in future.

5.2 Isolation and cultivation of cyanobacteria

Isolation and cultivation are the basic methods for study cyanobacteria and other microorganisms in laboratories. As an idea, it would be very useful if all species found in each natural environment will be transferred into culture. However, in fact only few strains which are known only less than 1 % of occurring species in natural environments were isolated up to now (Embry and Stackebrandt, 1997; Ward *et al.*, 1990). In this study, the diversity of cyanobacterial flora in hypersaline localities from Thailand was found relatively low in diversity. However, most of them are interested and still problematic in their taxonomy and classification, and they need further studies. Therefore, the best way to solve this problem is tried to transfer all species found in nature to agar culture. According to this study, unfortunately, there are only 12 monospecific strains successfully grown in BG-11 agar medium. In the case of *Spirulina subsalsa* and *Coleofasciculus chthonoplastes* which found abundantly in this work but they were not successfully in culture. It may be because the salt concentrations that applied into the media is still low and unsuitable for their growth. However, this study is quite similar in cultivation aspect with the study of cyanobacterial populations that build “kopara” microbial mats in French Polynesia (Richert *et al.*, 2006), the study of other extreme environment such as hot springs in Thailand (Udomluk, 2006), and the taxonomic study of cyanobacteria microflora from alkaline marshes of northern Belize (Turicchia *et al.*, 2009), in which the transfer of all dominant species into the culture was also not successful. In the future,

the isolation and cultivation of halophilic flora should be developed and the suitable way to cultivate them must be studied.

5.3 Ecophysiological studies

5.3.1 Growth dependence under salinity gradients

The adaptation of various cyanobacterial populations to salinic and hypersalinic biotopes is still currently studied. In this study, the growth dependence on salt concentrations in several studied strains was found. The maximal growth in small salt concentrations, and the decreasing growth intensity was detected when salinity increasing in majority of strains. From this study, the dependence was characteristic for each species. The growth under salt gradients confirms that majority of strains were halotolerant rather than halophilic. The only exception from all studied strains were *Oxynema thaianum*. The *Nostoc* sp.2 was isolated only from soils and never observed in natural samples. It grows in experiments only in very low salinity and dies in concentrations over 5 %. It is probable that it does not belong to typical halophilic microflora and that its diaspores were transported in saltworks, which are open to the influence from environmental regions. For *Oxynema thaianum*, this strain is rather a halophilic species with optimum growth at 10-20 % of salinity. According to this experiment, it is quite in agreements to the results from other literature (Golubić, 1980; Borowitzka, 1981; Komárek and Lukavský, 1988).

5.3.2 Crossed gradients experiments

In general, many environmental factors can regulate the growth of algae and also cyanobacteria, but the primary important factors are light and temperature (Siver, 1983; Rai and Gaur, 2001; Kvíderová and Lukavský, 2005). The effects of these factors have become increasingly important for understanding the ecophysiology of

algae (Siver, 1983). The unit of crossed gradients of light and temperature is equipment that helps to understand the ecology and physiology of algae (Halldal and French, 1958). In this study, only two species of *Nostoc* (*Nostoc* sp.1 and *Nostoc* sp.2) were performed on this unit. Both of them showed their growth rates slightly similar with each other. They grew very well under temperature range from 20-24 °C (Figure 62-63). In contrary, at lower and higher temperature more than 20-24 °C, both strains of *Nostoc* grew very slowly. It exist some data report also from Manual on the production and use of live food for agriculture reported that optimum temperature for cultivation most algal species in liquid media should be ranged from 16-27 °C (Lavens and Sorgelous, 1996). From this study, it was surprising that both *Nostoc* spp. grew in relatively low temperature. Even all samples were collected from tropical region, however all of strains were isolated and cultivated under temperate conditions (20 °C). Therefore, it is possible that during the cultivation periods, all strain were adapted themselves to live under such environment already. It means similar experiments should be realized with material collected from nature, without a long “adaptation” under changed culture conditions.

5.4 *Oxynema thaianum*

From this study, there were occurred several interesting species from halophilic environments, to which belong, e.g. *Oxynema thaianum* (= *Phormidium lloydianum*). *Oxynema thaianum* were found quite common especially in Petchaburi solar salterns (and also in Samut Songkhram salterns) in salinities ranged from 50-250 ppt (Chatchawan *et al.*, 2011, 2012). It may be widely distributed in such similar habitats in Thailand. This cyanophyte species was firstly reported and isolated from Petchaburi saltfields (and also from Samut Songkham salterns). It is a typical

halophilic species while many other strains isolated from hypersaline biotopes are rather halotolerant to higher salt concentration (Hof and Frémy, 1933; Komárek and Lukavský, 1988; Chatchawan *et al.*, 2011).

The related species “*Oxynema lloydianum*” was firstly described from Bretagne, France by Gomont (1899) as *Oscillatoria* and was re-classified to genus *Phormidium* by Anagnostidis and Komárek (1988) and Komárek and Anagnostidis (2005), in respect to its trichome morphology. Presently, many strains of *Phormidium* in each group were studied, re-classified and transferred to others genera such as part of members in previous *Phormidium* group IV were transferred to genus “*Phormidesmis*” (Komárek *et al.*, 2009) because their morphology was characteristics and different from general *Phormidium* and also molecular analysis (16S rRNA) showed their form very separated cluster from typical *Phormidium* members. Another part of the group IV was re-classified to the genus *Wilmottia* (Strunecky *et al.*, 2011). The group VII (type of *Ph. autumnale*) must be transfer to the genus *Microcoleus* (Siegesmund *et al.*, 2008). In the case of our strain (*Oxynema thaianum* CCALA 960) from the Petchaburi saltworks, which enabled molecular 16S rRNA gene sequencing, elucidated the phylogenetic position of the *Oxynema* morphotype in relation to both mentioned genera (*Oscillatoria* and *Phormidium*)(figure 63 and 64, table 7). It follows from our analyses (Figure 66) that our strain, which is also distinctly characterized morphologically, represents a separate clade (separated from the nearest clades by less than 93% of genetic similarity), together with two other strains, which have similar characteristic morphological features and were isolated also from similar extreme habitats (saline localities and thermal springs). These types correspond more or less to the species *Phormidium lloydianum* and *Ph. acuminatum*.

This whole group of cyanobacteria was classified in the review of Komárek & Anagnostidis (2005) as “*Phormidium* group I” (not containing the type species of the genus *Phormidium* = *Phormidium lucidum*). This large group of species is more or less unique ecologically and has a very similar and specific morphology. The main characteristic features are the cylindrical trichomes growing often in fasciculated mats, narrowed ends with bent, elongated and distinctly pointed terminal cells, without calyptra. The sheaths are lacking or facultative and diffluent.

In respect to all previous data, the “*Phormidium* cluster I” sensu Komárek & Anagnostidis (2005) is therefore must be transferred into a new genus *Oxynema*, with the type species *Oxynema thaianum* spec. nova. In consequence of these results, the morphological similar species *Phormidium lloydianum* and *Phormidium acuminatum*, which were controlled according to exsiccation (from herbarium; Figure 65) and which were morphologically related also to the same phylogenetic cluster and were included in the same group I [cf. Figure 66, strain EF 444714 or CENA 135(HQ730084)] should be reclassified into the same generic unit. They differ only slightly from *O. thaianum* both morphologically (width of trichomes, morphology of cells, facultative presence of distinct sheaths) and, in the case of *Ph. acuminatum* also ecologically (thermal springs) (Figure 64; cf. Anagnostidis, 1961), which justify the designation of specific species within the same generic clade:

Oxynema lloydianum (Gomont) comb. nova [basionym: *Oscillatoria lloydiana* Gomont, 1899, Bull. Soc. Bot. France 46: 39, syn. = *Phormidium lloydianum* (Gomont) Anagnostidis et Komárek 1988, Algolog. Stud. 50-53: 405. Descriptions:

Gomont, 1899, p. 39; Geitler, 1932, p. 977; Komárek & Anagnostidis, 2005, p. 402 and 578].

Oxynema acuminatum (Gomont) comb. nova [basonym: *Oscillatoria acuminata* Gomont 1892, Ann. Sci. Nat. VII. Bot. 16: 227; *Phormidium acuminatum* (Gomont) Anagnostidis et Komárek 1988, Algolog. Stud. 50-53: 404].

In consequence of the results of this study, the other species which occurred in such hypersaline habitats including *Oscillatoria salina* Biswas, *Oscillatoria brevis* sensu Claus 1961, *Oscillatoria janthiphora* (Fior.-Mazz.) Gomont, *Oscillatoria acuminata* sensu Rino 1972, *Oscillatoria paulseniana* Boye-Petersen, *Oscillatoria sonorensis* Drouet, *Oscillatoris animalis* sensu Borge 1933, *Oscillatoria rostrata* Borge, *Oscillatoria animalis* sensu Anagnostidis, *Oscillatoria karakalpanensis* Muzafarov, *Oscillatoria acuminata* var. *longe-attenuata* Geitler et Ruttner, *Phormidium anabaenoides* Drouet and *Phormidium richardsii* Drouet should be probably re-evaluated and determined whether they belong to the genus *Oxynema* or in others cluster. However, up to now, of these species, only the population described in this study was transformed in culture.