CHAPTER 6

Conclusion

In this study, algae specimens were pretreated with methods which were modified from Bredholdt *et al*. [2007 and 2008] and Hong *et al*. [2009]. Twelve different types of media including Raffinose-Histidine (RH) agar, Gause No.2 (GNO2) agar, Starch casein (SC) agar, Hickey-Tresner (HT) agar, Proline-tap water (PTW) agar and Minimal medium (MM) agar, and all media that were supplemented with 10% algae water extract were used as the selective media for isolation. Fifty isolates of actinomycetes were recovered from *Nostoc commune*. Gause No.2 agar and Raffinose-Histidine added to algal extract showed the highest number of isolates. Thirty isolates were obtained from *Nostochopsis* spp., while Hickey-Tresner agar extract show the highest number of isolates.

Actinomyceres were not recovered from both algae when Proline-tap water agar and Proline-tap water agar added to the algal extract were used. Diverse groups of actinobacteria were associated with edible freshwater algae. The diversity of the actinobacteria could be a potential source of novel taxa for bioprospecting. In addition, other edible algae in Thailand, such as *Spirogyra* spp., and *Cladophora* spp. were investigated. Actinomycetes associated with these edible algae were considered desirable.

The diversity of actinobacteria was assessed by the cloning and sequencing of actinobacterial-derived partial 16S rRNA gene. In total, 70 clones were obtained from *N. commune*. The majority of the clones (45 clones, 64.3%) were found to be actinobacteria. The isolation of *Gordonia terrae* - like isolates raises a concern for the safety as this alga that is currently being consumed by a fair number of Thai local people. *Gordonia terrae* has been described as a human pathogen in immuno compromised patients or as a health-care associated pathogen [Blanc *et al*. 2007]. Five cases of catheter-related bacteremia caused by *Gordonia terrae* have been reported

[Pham *et al*. 2003]. In this study, six genera were isolated from cultivation technique and compared to 18 genera that were revealed by molecular technique. Only one genus, *Arthrobacter* was detected by both approaches. No clone sequence was fond to be similar to those isolated strains. *Streptomyces* was not found in the clone library, though it represented over 80% of the isolated actinobacteria. These results were similar to those of Sun *et al.* [2010] who reported on the cultivation of *Salinispora* from marine sponges but this species was not detected in a culture-independent method. It is widely accepted that culture-independent and culture-dependent approaches can lead to very different actinobacterial diversity [Zhang *et al*. 2006; Xin *et al*. 2008]. Several possible explanations suggest that this exists because of the differences between the diversity obtained from the molecular and cultivation based studies. The bias from the molecular technique is one well- recognized reason [Tamaki *et al*. 2005]. Secondly, our clone library was not large enough to cover all actinobacteria in the alga. The isolation method in this study may not be optimum in obtaining all associated actinobacteria. All of these explanations could lead to an underestimation of actinobacterial diversity from *Nostoc commune*. The development of the next generation sequencing, such as pyrosequencing might provide a more powerful tool to access greater diversity and overcome the limitations of the clone library technique as exemplified in a previous study on the bacterial diversity in Antarctic terrestrial and aquatic microbial mats [Tytgat *et al*. 2014]. Culture-independent approaches revealed that *Arthrobacter, Microbacterium* and *Pseudonocardia* were dominant actinobacteria associated with *Nostoc commune*. However, *Arthrobacter* represented only a small proportion of cultured actinobaceria while *Microbacterium* and *Pseudonocardia* were not found at all on the isolation plates. Seventeen genera namely *Actinomyces, Amycolatopsis, Corynebacterium, Demetria, Dermacoccus, Fodinibacter, Janibacter, Kytococcus, Leucobacter, Microbacterium, Micrococcus, Micromonospora, Mycobacterium, Ornithinimicrobium, Pseudonocardia, Solirubrobacter* and *Yimella,* were detected by the molecular method but were not isolated by cultivation in this study. Apparently, this observation reflects the limitations of the cultivation method which is in urgent need of improvement to better our understand the species of diversity and to provide a valuable source for potential exploitation. It is therefore essential to apply both

culture-independent and culture-dependent approaches to describe the diversity of actinobacteria. The information from the culture-independent study could be used to improve cultivation technique to reduce the gaps between the two approaches.

The antimicrobial activities of 83 strains of actinomycetes, of which 51 strains were isolated from *Nostoc commune* Voucher ex Bornet&Flahault and 32 strains were isolated from *Nostochopsis* spp., and were investigated with 7 pathogenic bacteria:, *Aeromonas hydrophila***,** Methicillin resistant *Staphylococcus aureus* (MRSA), *Staphylococcus aureus***,** *Escherichia coli* O157**,** *Pseudomonas fluorescens, Vibrio parahaemolyticus* and *Candida albicans* ATCC 90028. The results of the fermention media indicated that 73 isolates from Med.30 and 61 isolates from M52 exhibited antimicrobial activities against at least one of the pathogenic bacteria. NCMn07 and NTRHn08 displayed activities against all 7 pathogens in this study. These 2 strains were identified through a comparison with the data in the Eztaxon database. The 16S rDNA sequence of NCMn07 revealed an 81% rate of similarity to *Streptomyces atrovirens* NRRL B-16357$^{(T)}$, while the isolate NTRHn08 revealed a 97% rate of similarity to *Streptomyces griseoflavus* LMG 19344$^{(T)}$.

Additionally, the isolates from *Nostochopsis* spp. are likely to be candidates for the control bacteria aquaculture, e.g. *Aeromonas hydrophila* and *Pseudomonas fluorescens.* These isolates might potentially be new sources of anti-pathogens, especially with regard to aquaculture industries in the future.

This study makes up the first report on the diversity of actinobacteria associated with the freshwater macroalgae, *Nostoc commune* Voucher ex Bornet&Flahault and *Nostochopsis* spp. Our results showed that diverse groups of actinobacteria were associated with this macroalgae and some of them were likely to be new taxa. At least 18 genera of actinobacteria were presented in *N. commune*. The high diversity range of actinobacteria that was observed could lead to potential sources of novel taxa for bioprospecting. The data obtained from this study provides important background information which extends our understanding on actinobacterial diversity in freshwater macroalgae.