CHAPTER 5

Antimicrobial production

5.1 Introduction

Microorganisms have been shown to be attractive sources of natural compounds for the pharmaceutical, industrial and agricultural purposes. In pharmaceutical applications, microbial secondary metabolites, especially antimicrobial compounds, are used for human and animal therapy. However, pathogenic microorganisms'ability to develop resistance to multiple drugs has become a major problem in the treatment of infections. This antimicrobial resistance is presently an urgent focus of research and new bioactive compounds are necessary. About 70% of all known drugs have been isolated from actinomycetes bacteria of which 75% and 60% are used in medicine and agriculture, respectively [Miyadoh, 1993; Tanaka and Mura, 1993]. This fact has resulted in Streptomyces being the most frequently used bacteria in the fermentation manufacturing of active pharmaceutical compounds.

Bioactive molecules are secondary metabolites that are nonessential for growth and reproduction but presumably help form a defence mechanism enabling the producer microorganism to compete in nature. These active molecules are generally extra cellular and their isolation in the highest purity from the complex fermentation broth requires the application of a combination of various separation steps such as solvent extraction, chemical precipitation, ion exchange chromatography, HPLC purification, etc.

Actinomycetes are the most economically and biotechnologically valuable prokaryotes. They are responsible for the production of about half of the discovered bioactive secondary metabolites [Miyadoh, 1993], notably antibiotics [Miyadoh, 1993; Tanaka, and Mura 1993], antitumor agents [Williams, 1983], immunosuppressive agents [Kuster, 1964] and enzymes [Wipat, 1991, Lechevalier, 1970]. Because of the excellent track record of actinomycetes in this regard, a significant amount of effort has been

focused on the successful isolation of novel actinomycetes from terrestrial sources for drug screening programs over the past fifty years. However, the rate of discovery of new antimicrobial metabolites has been decreasing, so the discovery of actinomycetes acquired from several sources increases the chances of discovering new secondary metabolites [Hayakawa *et al.*, 2004]. Thus, it is crucial that new groups of actinomycetes from unexplored or under exploited habitats be pursued as potential sources of novel bioactive secondary metabolites.

For the purposes of screening antimicrobial production, the classical approach and the alternative approach were used. In the classical method, actinomycetes were cultivated on fermentation media and tested for antimicrobial activity but in the alternative method, actinomycete isolates were screened for biosynthetic genes which control the production of bioactive compounds. Modular polyketide synthases (PKS-I), polyketide synthases (PKS-II) [Shen, 2003] and non-ribosomal peptide synthetases (NRPS) [Schwarzer *et al.*, 2003] have extensively been described as being responsible for the synthesis of a broad range of structurally diverse secondary metabolites in actinomycetes. The specific primers targeted to PKS-I, PKS-II and NRPS actinomycete sequences have been previously designed and validated, supporting a quick detection of the biosynthetic enzymes [Ayuso-Sacido and Genilloud, 2005; Ostash *et al*., 2005].

In recent years, there has been a growing awareness of the potential value of freshwater habitats as a source of actinomycetes that produce secondary metabolites of clinical importance [Rifaat, 2003]. Aquatic microbes are particularly attractive because they have not been as extensively exploited as their terrestrial counterparts, and because there is a high potency required for bioactive compounds to be effective in the aquatic environment, due to the diluting effect of the water [Zhang, 2005]. A review of available literature has revealed that little is known concerning the actinomycetes exhibiting antimicrobial properties from this habitat. The list of novel actinomycetes and products derived from poorly explored areas of the world stresses the importance of investigating new habitats [Nolan, 1988]. Presently, some novel bioactive compounds were discovered from aquatic actinomyctes including riffamycin from *Micromonospora* [Jensen *et al*, 1991], the anticancer metabolite salinosporamide A from a *Salinispora*

strain [Fehling *et al.*, 2003], marinomycins from *Marinophilus* sp. [Jensen *et al.*, 2005], abyssomicin C from *Verrucosispora* sp. [Riedlinger *et al.*, 2004] and marinopyrroles from *Streptomyces* sp. [Hughes *et al.*, 2008].

Various novel compounds possessing antibiotic activity have been identified from alga-associated bacteria. These chemically diverse substances include new lipopeptides such as massetolide A, novel antibacterial lactones (macrolactines G-M), phanazines (i.e., pelagiomycin A) and korormicin, which exhibit a variety of activities against bacteria and fungi that are pathogenetic to man and plants, as well as to leukemic cells [Gerard *et al.*, 1997; Imamura *et al.*, 1997; Yoshikawa *et al.*, 1997; Tran *et al.*, 2007]. Recently, *Laminaria saccharina* represents a promising source in the isolation of new bacterial taxa and anti-microbially active bacteria [Wiese *et al.*, 2009]. In this chapter, we report on the antibiotic production potential of actinomycetes that have been isolated from freshwater algae in Thailand for new antimicrobial agents.

5.2 Materials and Methods

5.2.1 Preparation of actinomycetes in fermentation media

One loop of Actinomycete strains that were isolated from the algal samples, *Nostoc commune* Voucher ex Bornet & Flahault and *Nostochopsis* spp., were inoculated into 10 ml of 301 seed medium and were shook at 125 rpm at 25°C for 7 days. They were, then transferred into the M52 and Med.30 medium at 1% (Table 5.1), incubated at 25°C with shaking at 125 rpm for 7 days. After this incubation period, 10 ml of 95% ethanol were added and the samples were shook at 125 rpm for 30 minutes. The collected the supernatant (ethanol phase) was collected with the use of a centrifuge at 3,000 rpm (25°C) for 10 min.

Table 5.1 Composition of fermentation media for actinomycetes

medium	composition
301 seed medium	2.4% starch, 0.1% glucose, 0.3% peptone, 0.3% meat extract, 0.5% yeast extract, 0.4% $CaCO_3$ pH 7.0
M52	2% glycerol, 1%glucose, 0.5% corn steep powder (CSP), 1% soybean (defatted), 0.2% meat extract, 0.01% $MgSO_4.7H_2O$, 0.2% $CaCO_3$ pH7.0
Trace salt solution	1 g $FeSO_4.7H_2O$, 1 g $MnCl_2.4H_2O$, 1 g $ZnSO_4.7H2O$, 1 g $CuSO_4.5H_2O$, 1 g $CoCl_2.6H_2O$,1 L H_2O
Med. 30	301 seed medium + 5 $ml.L^{-1}$ trace salt solution

5.2.2 Antimicrobial activity

The seven pathogenic bacteria and fungi: *Aeromonas hydrophila***,** Methicillin resistant *Staphylococcus aureus* (MRSA), *Staphylococcus aureus***,** *Escherichia coli* O157**,** *Pseudomonas fluorescens*, *Vibrio parahaemolyticus* and *Candida albicans* ATCC 90028 were cultured in Mueller-Hinton broth for 18 – 24 hours and a the turbidity was adjusted with McFarland 0.5 standard. These pathogens were swabbed on the dried surface of a Mueller-Hinton agar plate.

The actinomycete supernatants were prepared as described above (5.2.1). The antimicrobial activity were tested by the agar well diffusion method [Odeyemi *et al.*, 2012] and incubated at 25°C for 7 days. Antimicrobial activities were defined by the formation of an inhibition zone. They were, then compared with the control (fermented media mixed with 95% ethanol, 1:1)

5.3 Results and Discussions

5.3.1 Effects of fermentation media

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 inoculated in 2 fermented types of media:, Med.30 and M52, were tested with 7 pathogenic bacteria by the agar well diffusion method. This showed that most of these isolates could inhibit the growth of the pathogens, while NTRHn7 could not inhibit *Staphylococcus aureus*. *Pseudomonas fluorescens* and *Staphylococcus aureus* were not inhibited by NTHn3 (Table 5.2-5.3).

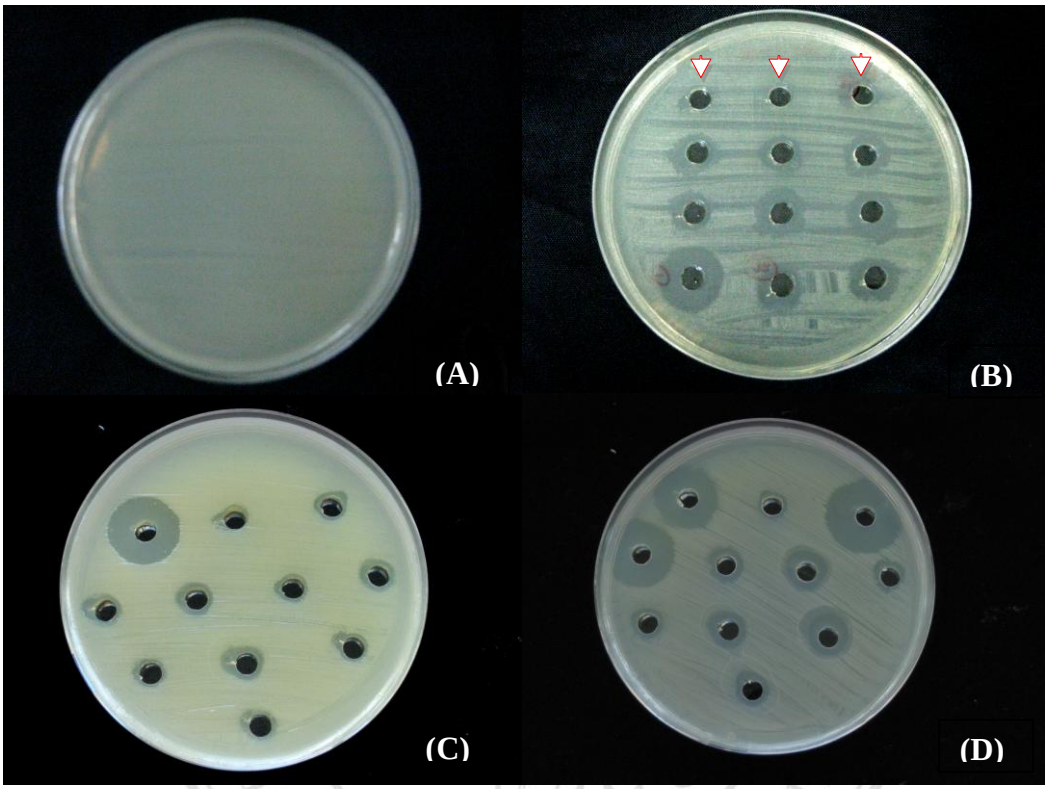

Figure 5.1 The screening of antimicrobial activity on Mueller-Hinton agar plate

(A) microbial plate

(B-D) positive results showing a clear zone around the well ; arrows indicate the control

Table 5.2 Qualitative antimicrobial activity of active actinomycetes associated with *Nostoc commune*, which were cultivated in two types of fermentation media

Isolates	Inhibition zone (mm)													
	Aeromonas		*Pseudomonas*		*Vibrio*		*Escherichia coli*		*Candida albicans*		*S. aureus*		*S. aureus* MRS	
	Med.30	M52	Med.30	M52	Med.30	M52	Med.30	M52	Med.30	M52	Med.30	M52	Med.30	M52
NTRHn01	7.66±0.58	5.00±0.00	6.00±0.00	5.33±0.58	6.00±0.00	6.00±0.00	5.00±0.00	5.00±0.00	5.00±0.00	5.66±0.58	5.33±0.58	6.00±0.00	5.33±0.58	5.66±0.58
NTRHn02	10.66±1.15	5.00±0.00	5.00±0.00	8.00±0.00	5.00±0.00	5.00±0.00	5.66±0.58	5.00±0.00	6.66±0.58	9.33±0.58	5.00±0.00	5.00±0.00	14.66±0.58	5.00±0.00
NTRHn06	16.00±3.61	7.00±0.00	5.33±0.58	5.33±0.58	6.00±0.00	7.33±0.58	12.00±1.00	10.66±1.15	13.66±1.53	7.33±0.58	5.00±0.00	5.33±0.58	14.33±2.52	6.00±0.00
NTRHn07	7.66±0.58	16.00±1.73	6.00±0.00	5.33±0.58	6.00±0.00	7.66±0.58	8.33±0.583	12.33±1.53	7.33±0.58	20.00±2.65	0.00±0.00	6.00±0.00	8.00±0.00	21.00±7.94
NTRHn08	7.66±0.58	7.66±0.58	8.66±0.58	8.33±0.58	5.33±0.58	6.00±0.00	6.00±0.00	6.00±0.00	6.00±0.00	6.66±0.58	5.33±0.58	5.33±0.58	9.33±0.58	6.00±0.00
NTRHn10	7.66±0.58	5.33±0.58	5.00±0.00	6.33±1.15	5.00±0.00	6.00±0.00	6.00±0.00	5.66±0.58	7.33±0.58	6.66±0.58	8.00±0.00	5.00±0.00	11.33±1.15	5.00±0.00
NTRHn11	8.00±0.00	5.00±0.00	5.00±0.00	5.66±0.58	5.00±0.00	5.33±0.58	6.00±0.00	6.00±0.00	5.00±0.00	6.00±0.00	5.00±0.00	5.00±0.00	8.66±0.58	5.00±0.00
NTRHn12	16.33±3.21	7.33±0.58	6.00±0.00	5.66±0.58	5.00±0.00	5.00±0.00	5.00±0.00	5.66±0.58	16.66±1.53	6.00±0.00	5.33±0.58	5.33±0.58	17.00±3.00	10.00±1.00
NTRHn16	7.00±0.00	9.00±0.00	6.00±0.00	6.00±0.00	8.33±0.58	6.00±0.00	6.00±0.00	6.00±0.00	5.00±0.00	8.33±0.58	6.00±0.00	6.00±0.00	7.66±0.58	5.00±0.00
NTRHn18	5.00±0.00	5.66±0.58	5.00±0.00	6.00±0.00	6.00±0.00	6.00±0.00	5.00±0.00	10.33±1.15	5.00±0.00	7.00±0.00	5.00±0.00	5.00±0.00	8.33±0.58	5.66±0.58
NTRH01	7.66±0.58	17.00±2.00	5.66±1.15	5.00±0.00	6.00±0.00	9.66±0.58	9.33±0.58	11.66±1.15	7.33±0.58	20.33±2.31	5.33±0.58	5.00±0.00	10.66±0.58	16.00±1.00
NTRH02	7.33±0.58	8.66±0.58	5.33±0.58	7.66±0.58	5.66±0.58	6.00±0.00	7.66±0.58	8.66±1.157	7.00±0.00	7.33±0.58	5.33±0.58	5.00±0.00	8.33±1.15	6.00±0.00
NTRH05	11.66±0.58	9.33±0.58	6.00±0.00	8.33±1.15	6.66±0.58	5.66±0.58	6.00±0.00	9.66±0.58	8.66±0.58	9.33±0.58	11.33±1.15	7.66±0.58	9.66±0.58	10.33±1.15
NTRH06	7.33±0.58	8.00±0.00	5.00±0.00	5.00±0.00	8.00±0.00	5.00±0.00	9.33±0.58	6.00±0.00	7.66±0.58	10.66±1.15	5.00±0.00	5.00±0.00	7.00±0.00	5.00±0.00
NTRH07	5.00±0.00	7.33±0.58	5.00±0.00	8.33±0.58	7.66±0.58	8.00±0.00	5.00±0.00	11.66±0.58	5.00±0.00	10.66±0.58	5.00±0.00	11.33±1.15	5.00±0.00	11.00±0.00
NTRH19	5.66±0.58	6.66±1.53	5.00±0.00	5.66±0.58	5.00±0.00	5.33±0.58	6.66±0.58	11.00±1.73	5.00±0.00	10.00±1.00	11.66±0.58	9.33±0.58	12.66±0.58	9.66±0.58

Table 5.2 (continued)

Isolates	Inhibition zone (mm)													
	Aeromonas		*Pseudomonas*		*Vibrio*		*Escherichia coli*		*Candida albicans*		*S. aureus*		*S. aureus* MRS	
	Med.30	M52	Med.30	M52	Med.30	M52	Med.30	M52	Med.30	M52	Med.30	M52	Med.30	Med.30
NTMn03	6.66±0.58	6.66±0.58	6.00±0.00	5.00±0.00	7.66±0.58	6.66±0.58	7.66±0.58	10.00±1.00	7.00±0.00	11.66±0.58	5.00±0.00	5.00±0.00	12.33±1.15	11.33±1.53
NTM03	10.33±0.58	11.33±1.15	6.33±0.58	5.33±0.58	7.33±0.58	7.33±0.58	8.66±0.58	6.00±0.00	7.33±0.58	7.33±0.58	5.00±0.00	6.00±0.00	6.33±1.15	5.00±0.00
NTHn01	5.33±0.58	5.33±0.58	5.33±0.58	10.00±0.00	5.00±0.00	5.00±0.00	5.66±0.58	5.00±0.00	5.00±0.00	12.66±0.58	5.33±0.58	5.33±0.58	5.33±0.58	6.00±0.00
NTHn02	7.66±0.58	7.33±0.58	5.00±0.00	5.33±0.58	5.66±0.58	6.00±0.00	6.33±1.15	5.00±0.00	6.33±0.58	5.66±0.58	5.33±0.58	5.66±0.58	5.00±0.00	5.00±0.00
NTHn03	11.00±1.00	7.66±0.58	0.00±0.00	5.00±0.00	9.66±0.58	6.66±0.58	5.66±0.58	5.00±0.00	6.00±0.58	7.33±1.15	0.00±0.00	5.00±0.00	6.00±0.00	6.00±0.00
NTGn01	5.00±0.00	7.66±0.58	5.00±0.00	8.66±0.58	5.00±0.00	5.33±0.58	5.00±0.00	6.00±0.00	5.00±0.00	6.00±0.00	5.00±0.00	5.33±0.58	5.00±0.00	9.66±0.58
NTGn05	9.66±0.58	9.33±0.58	5.00±0.00	5.66±0.58	6.33±0.58	6.33±0.58	5.00±0.00	5.00±0.00	6.66±0.58	6.00±0.00	5.00±0.00	5.00±0.00	6.00±0.00	6.00±0.00
NTGn08	12.33±0.58	9.66±0.58	5.66±0.58	6.00±0.00	10.33±0.58	12.00±1.73	13.66±1.53	10.33±0.58	7.33±0.58	11.66±1.53	5.00±0.00	5.00±0.00	9.33±0.58	6.00±0.00
NTG01	5.00±0.00	8.66±0.58	5.00±0.00	10.00±1.00	5.00±0.00	5.33±0.58	5.00±0.00	10.66±0.58	5.00±0.00	9.66±0.58	5.00±0.00	5.00±0.00	5.00±0.00	10.33±0.58
NTG02	8.00±0.00	9.66±0.58	6.00±0.00	6.00±0.00	5.00±0.00	5.00±0.00	5.00±0.00	5.00±0.00	5.00±0.00	6.00±0.00	5.00±0.00	5.33±0.58	5.66±0.58	5.33±0.58
NTG03	11.33±1.15	8.66±0.58	5.00±0.00	5.00±0.00	7.66±0.58	7.33±0.58	6.33±1.15	5.00±0.00	7.33±0.58	7.33±0.58	5.33±0.58	5.66±0.58	5.66±0.58	5.00±0.00
NTG04	18.00±3.61	19.66±1.15	5.33±0.58	6.00±0.00	11.66±1.15	9.33±0.58	11.66±1.53	10.66±1.15	18.66±3.51	20.00±2.00	5.66±0.58	5.00±0.00	17.66±4.62	16.33±1.53
NTG05	7.33±0.58	8.66±0.58	5.33±0.58	5.66±0.58	6.00±0.00	6.00±0.00	11.00±1.00	7.00±0.00	6.00±0.00	6.33±0.58	5.00±0.00	5.00±0.00	8.00±0.00	7.66±0.58
NTG07	7.33±0.58	7.33±0.58	5.33±0.58	5.33±0.58	5.66±0.58	5.00±0.00	7.66±0.58	6.00±0.00	7.00±0.00	6.33±0.58	5.33±0.58	5.66±0.58	10.33±0.58	11.66±1.15
NTG08	5.00±0.00	7.33±0.58	5.00±0.00	9.33±0.58	5.00±0.00	5.00±0.00	5.00±0.00	7.00±0.00	5.00±0.00	5.00±0.00	5.00±0.00	5.00±0.00	5.00±0.00	11.00±1.00
NTG09	11.66±1.53	7.33±0.58	5.66±0.58	5.33±0.58	8.33±0.58	8.33±0.58	5.66±0.58	5.00±0.00	4.00±3.46	9.66±1.15	5.33±0.58	5.00±0.00	5.66±0.58	5.33±0.58
NTG10	5.66±0.58	8.66±1.15	5.33±0.58	7.66±0.58	5.33±0.58	5.33±0.58	5.00±0.00	5.00±0.00	5.00±0.00	10.66±0.58	5.00±0.00	5.00±0.00	5.33±0.58	5.66±0.58
NTG11	7.00±0.00	8.33±0.58	5.00±0.00	8.33±0.58	5.00±0.00	5.00±0.00	5.00±0.00	6.66±0.58	5.00±0.00	5.00±0.00	5.00±0.00	5.00±0.00	5.00±0.00	10.66±1.15

Table 5.2 (continued)

Isolates	Inhibition zone (mm)													
	Aeromonas		*Pseudomonas*		*Vibrio*		*Escherichia coli*		*Candida albicans*		*S. aureus*		*S. aureus* MRS	
	Med.30	M52	Med.30	M52	Med.30	M52	Med.30	M52	Med.30	M52	Med.30	M52	Med.30	M52
NTSn04	9.66±0.58	9.33±1.15	5.00±0.00	5.33±0.58	6.33±0.58	6.66±0.58	6.00±0.00	6.00±0.00	7.66±0.58	7.33±0.58	5.00±0.00	5.00±0.00	6.66±0.58	6.00±0.00
NTSn05	7.66±0.58	10.33±0.58	5.33±0.58	5.00±0.00	6.00±0.00	9.33±0.58	5.00±0.00	6.00±0.00	6.66±0.58	6.33±0.58	5.33±0.58	5.00±0.00	6.33±0.58	6.66±0.58
NTSn06	7.66±0.58	12.33±1.15	6.00±0.00	6.00±0.00	7.00±0.00	9.00±1.73	5.66±0.58	6.00±0.00	6.33±0.58	6.66±0.58	5.33±0.58	5.66±0.58	6.00±0.00	6.00±0.00
NTSn07	7.33±0.58	7.33±0.58	6.00±0.00	5.33±0.58	5.00±0.00	6.00±0.00	5.66±0.58	5.00±0.00	6.00±0.00	6.00±0.00	5.00±0.00	5.33±0.58	5.33±0.58	5.00±0.00
NTSn08	9.33±1.15	5.00±0.00	5.00±0.00	7.00±0.00	5.00±0.00	5.00±0.00	8.33±0.58	11.00±3.00	11.33±1.15	6.66±0.58	11.33±1.15	5.00±0.00	12.00±1.00	5.00±0.00
NTSn09	5.00±0.00	5.33±0.58	5.00±0.00	5.00±0.00	7.66±0.58	5.00±0.00	5.00±0.00	8.66±0.58	5.00±0.00	5.00±0.00	9.66±0.58	5.00±0.00	11.33±0.58	5.00±0.00
NTSn10	9.33±1.15	5.00±0.00	5.33±0.58	6.66±0.58	7.33±1.15	9.33±0.58	5.00±0.00	10.66±2.89	6.00±0.00	7.00±0.00	11.33±1.15	5.33±0.58	12.66±0.58	5.00±0.00
NTSn11	5.00±0.00	8.33±0.58	5.33±0.58	5.33±0.58	6.66±0.58	7.66±1.15	6.00±0.00	7.66±0.58	7.33±0.58	7.66±0.58	5.00±0.00	5.00±0.00	6.00±0.00	6.00±0.00
NTSn12	10.66±0.58	5.66±0.58	5.00±0.00	5.33±0.58	8.00±0.00	7.33±0.58	7.33±0.58	11.33±4.16	9.33±0.58	9.33±0.58	12.66±0.58	5.33±0.58	11.66±2.89	7.33±1.15
NTS01	8.66±0.58	9.00±0.00	9.00±0.00	8.33±0.58	7.33±1.15	6.00±0.00	6.00±0.00	5.00±0.00	6.66±0.58	7.33±0.58	12.66±1.15	7.00±0.00	6.00±0.00	6.00±0.00
NTS02	11.66±1.53	8.33±0.58	5.00±0.00	5.00±0.00	6.33±0.58	6.33±0.58	6.00±0.00	6.00±0.00	6.66±0.58	7.33±0.58	5.00±0.00	5.00±0.00	7.00±0.00	6.66±0.58
NTS03	7.33±0.58	5.00±0.00	5.00±0.00	5.33±0.58	5.00±0.00	5.00±0.00	5.00±0.00	8.66±0.58	5.00±0.00	5.66±0.58	6.00±0.00	5.00±0.00	11.66±1.15	5.33±0.58
NTS04	10.00±0.00	5.33±0.58	5.00±0.00	8.00±0.00	6.33±1.15	5.66±0.58	8.33±0.58	10.33±0.58	5.00±0.00	5.00±0.00	5.00±0.00	5.33±0.58	12.00±1.73	5.00±0.00
NTS05	5.00±0.00	5.00±0.00	5.00±0.00	5.00±0.00	7.66±0.58	7.66±0.58	5.00±0.00	5.00±0.00	5.00±0.00	5.00±0.00	5.00±0.00	5.00±0.00	5.00±0.00	5.00±0.00
NTS07	8.66±0.58	5.00±0.00	5.00±0.00	5.33±0.58	7.00±0.00	6.00±0.00	5.00±0.00	7.00±0.00	6.66±0.58	5.33±0.58	6.00±0.00	5.00±0.00	5.00±0.00	5.66±0.58
NT07	5.66±0.58	8.00±0.00	5.00±0.00	6.00±0.00	6.00±0.00	5.00±0.00	5.33±0.58	5.00±0.00	5.00±0.00	5.66±0.58	5.00±0.00	5.00±0.00	5.66±0.58	6.00±0.00
NT08	5.00±0.00	7.33±0.58	5.33±0.58	5.33±0.58	5.33±0.58	5.66±0.58	5.00±0.00	5.00±0.00	5.00±0.00	6.33±0.58	5.33±0.58	5.33±0.58	5.00±0.00	5.66±0.58

Table 5.3 Qualitative antimicrobial activity of active actinomycetes associated with *Nostochopsis* spp., which were cultivated in two types of fermentation media

Isolates	Inhibition zone (mm)													
	Aeromonas		*Pseudomonas*		*Vibrio*		*Escherichia coli*		*Candida albicans*		*S. aureus*		*S. aureus* MRS	
	Med.30	M52	Med.30	M52	Med.30	M52	Med.30	M52	Med.30	M52	Med.30	M52	Med.30	M52
NCM01	5.00+0.00	5.00±0.00	5.00±0.00	5.00±0.00	7.66±0.58	7.66±0.58	5.00±0.00	5.00±0.00	5.00±0.00	5.00±0.00	5.00±0.00	5.00±0.00	5.00±0.00	5.00±0.00
NCM04	8.00+0.00	5.00±0.00	5.00±0.00	5.00±0.00	6.33±0.58	5.66±0.58	5.00±0.00	5.00±0.00	6.66±1.15	5.00±0.00	6.66±0.58	5.33±0.58	18.66±0.58	12.33±0.58
NCMn01	7.00+2.00	12.00±1.00	5.00±0.00	7.66±0.58	5.00±0.00	5.33±0.58	5.66±1.15	7.66±0.58	6.66±1.53	11.66±1.15	5.00±0.00	5.33±0.58	12.00±3.00	15.66±2.08
NCMn02	8.33±1.15	5.00±0.00	5.00±0.00	5.330.58	5.00±0.00	8.66±1.53	5.00±0.00	5.00±0.00	5.66±0.58	5.00±0.00	7.33±0.58	5.00±0.00	12.66±2.52	10.00±1.00
NCMn03	12.66±2.08	10.66±0.57	5.00±0.00	7.33±0.58	5.00±0.00	5.00±0.00	5.00±0.00	5.00±0.00	5.66±0.58	5.00±0.00	5.00±0.00	7.66±0.58	5.66±0.58	5.00±0.00
NCMn07	7.66±0.58	7.00±0.00	6.00±0.00	6.00±1.00	6.00±0.00	5.00±0.00	8.33±0.58	7.33±0.58	7.33±0.58	7.33±0.58	7.33±0.58	7.33±0.58	8.00±0.00	5.00±0.00
NCMn09	9.66±0.58	9.66±0.58	7.00±0.00	7.66±0.58	5.00±0.00	5.00±0.00	5.00±0.00	5.00±0.00	7.66±0.58	5.00±0.00	5.00±0.00	6.00±0.00	7.33±0.58	5.00±0.00
NCMn10	10±1.00	9.66±0.58	5.00±0.00	6.00±0.00	5.00±0.00	5.00±0.00	6.00±0.00	5.00±0.00	5.00±0.00	5.00±0.00	5.00±0.00	5.00±0.00	5.66±0.58	5.00±0.00
NCR01	11.66±1.53	11.33±0.58	7.33±1.15	8.00±0.00	5.66±0.58	6.00±0.00	5.00±0.00	5.00±0.00	6.00±0.00	5.00±0.00	6.00±1.73	7.66±0.58	5.33±0.58	5.00±0.00
NCR02	10.66±1.15	5.00±0.00	7.33±0.58	8.66±0.58	6.00±0.00	7.00±0.00	5.33±0.58	6.33±0.58	6.00±0.00	6.00±0.00	6.00±0.00	12.33±1.53	8.00±0.00	6.00±0.00
NCR03	9.66±0.58	8.33±1.15	5.66±1.15	6.00±1.00	6.33±1.15	5.33±0.58	6.00±0.00	5.33±0.58	6.00±0.00	5.66±0.58	5.66±0.58	11.33±1.53	6.33±1.15	5.00±0.00
NCR04	6.00±1.00	15.00±2.00	5.33±0.58	5.33±0.58	6.33±1.15	7.33±0.58	5.00±0.00	9.00±1.00	5.33±0.58	11.66±1.15	5.33±0.58	5.00±0.00	23.00±1.73	14.00±1.00
NCR05	9.66±0.58	9.66±0.58	11.66±0.58	7.66±0.58	5.00±0.00	5.00±0.00	5.00±0.00	5.00±0.00	9.33±0.58	6.00±0.00	7.00±0.00	6.00±0.00	7.33±0.58	5.00±0.00
NCR06	12.66±1.15	5.00±0.00	7.00±0.00	9.66±0.58	6.00±0.00	6.00±0.00	5.00±0.00	5.00±0.00	6.00±0.00	6.00±0.00	8.33±0.58	8.66±0.58	7.33±1.15	6.00±0.00

Table 5.3 (continued)

Isolates	Inhibition zone (mm)													
	Aeromonas		*Pseudomonas*		*Vibrio*		*Escherichia coli*		*Candida albicans*		*S. aureus*		*S. aureus* MRS	
	Med.30	M52	Med.30	M52	Med.30	M52	Med.30	M52	Med.30	M52	Med.30	M52	Med.30	M52
NCRHn01	8.66±0.58	9.00±0.00	8.00±0.00	8.33±0.58	6.33±0.58	7.66±0.58	5.00±0.00	6.00±0.00	7.33±0.58	7.33±0.58	7.00±0.00	12.00±1.73	6.00±0.00	6.00±0.00
NCRHn04	11.33±1.53	5.00±0.00	7.66±0.58	8.66±0.58	6.00±0.00	9.33±0.58	6.00±0.00	6.66±0.58	7.33±0.58	7.00±0.00	5.66±0.58	13.33±0.58	7.00±0.00	7.00±0.00
NCRHn05	7.33±0.58	5.00±0.00	5.00±0.00	7.66±0.58	8.00±0.00	5.66±0.58	9.33±0.58	8.00±0.00	7.66±0.58	5.00±0.00	5.00±0.00	6.00±0.00	7.00±0.00	8.00±0.00
NCRHn08	6.33±1.15	5.00±0.00	5.00±0.00	5.00±0.00	5.00±0.00	5.66±0.58	5.66±1.15	5.00±0.00	5.00±0.00	5.00±0.00	5.33±0.58	5.00±0.00	15.002.00	17.66±2.52
NCG04	15.33±1.53	7.00±0.00	7.66±0.58	5.66±0.58	5.00±0.00	5.00±0.00	5.00±0.00	5.00±0.00	5.33±0.58	5.00±0.00	5.00±0.00	5.00±0.00	5.00±0.00	5.00±0.00
NCGn01	10.66±1.15	13.00±1.73	5.66±0.58	9.00±1.73	5.00±0.00	7.00±1.00	5.00±0.00	5.00±0.00	6.66±2.08	12.00±1.00	5.66±1.15	7.66±0.58	11.00±1.00	13.33±2.31
NCGn02	7.33±0.58	7.00±0.00	5.00±0.00	7.33±0.58	5.00±0.00	5.00±0.00	5.00±0.00	5.00±0.00	5.00±0.00	5.00±0.00	5.00±0.00	5.00±0.00	5.00±0.00	5.00±0.00
NCGn04	7.66±0.58	12.66±1.15	5.00±0.00	5.33±0.58	5.00±0.00	7.33±1.53	5.00±0.00	9.33±0.58	6.66±2.08	12.33±1.15	5.66±1.15	5.33±0.58	10.00±1.00	16.00±1.00
NCGn07	7.66±2.52	5.00±0.00	5.00±0.00	5.00±0.00	5.00±0.00	5.00±0.00	5.00±0.00	5.66±1.15	7.00±2.00	5.00±0.00	5.66±0.58	5.66±1.15	14.66±0.58	14.33±0.58
NCH01	8.66±0.58	9.00±0.00	5.00±0.00	7.66±0.58	6.33±±1.15	5.00±0.00	5.00±0.00	5.00±0.00	5.00±0.00	6.00±0.00	5.66±0.58	9.66±1.53	5.66±0.58	5.00±0.00
NCH03	9.66±0.58	5.00±0.00	7.00±0.00	7.33±0.58	5.66±0.58	5.00±0.00	5.66±0.58	5.00±0.00	6.00±0.00	5.00±0.00	7.33±0.58	7.66±0.58	5.66±0.58	5.00±0.00
NCH05	7.66±0.58	7.33±0.58	6.33±0.58	5.00±0.00	5.00±0.00	8.00±1.00	5.33±0.58	6.00±0.00	5.00±0.00	7.33±0.58	5.00±0.00	6.00±0.00	5.66±0.58	5.00±0.00
NCH06	7.33±0.58	90.00±0.00	5.33±0.58	5.33±0.583	5.66±0.58	6.66±0.58	7.66±0.58	6.66±0.58	7.00±0.00	7.66±0.58	5.33±0.58	6.00±0.00	7.66±0.58	5.00±0.00
NCHn03	8.66±1.15	5.00±0.00	5.00±0.00	5.00±0.00	5.00±0.00	5.00±0.00	5.00±0.00	5.66±1.15	6.33±1.53	5.00±0.00	5.66±0.58	5.00±0.00	15.66±1.53	21.66±2.08
NCHn05	7.66±0.58	5.00±0.00	0.00	0.00±0.00	5.00±0.00	5.00±0.00	5.00±0.00	5.00±0.00	5.66±1.15	5.00±0.00	5.33±0.58	5.00±0.00	10.33±1.53	12.00±1.00
NCS03	7.33±0.58	8.00±0.00	5.33±0.58	7.00±1.00	6.00±0.00	7.66±1.15	5.00±0.00	5.00±0.00	5.66±0.58	8.33±0.58	5.66±0.58	5.33±0.58	5.00±0.00	6.00±0.00
NCS05	12.33±1.15	8.66±0.58	10.66±0.58	9.33±0.58	5.00±0.00	5.00±0.00	5.00±0.00	5.00±0.00	7.66±0.58	5.00±0.00	5.00±0.00	7.00±0.00	7.66±0.58	5.00±0.00
NCSn01	8.66±0.58	13±1.73	8.00±0.00	6.00±0.00	5.00±0.00	6.00±0.00	5.00±0.00	5.00±0.00	6.66±0.58	6.00±0.00	6.00±0.00	9.33±1.15	7.33±0.58	6.00±0.00

From a comparison of the inhibited zone with the control (Table 5.4), 29 actinomycete strains isolated from *Nostoc commune* were inoculated in M30 medium and showed an inhibitory effect against Methicillin-resistant *Staphylococcus aureus*. In M52 medium, *Escherichia coli* O157 was inhibited by 21 isolates. However, *Staphylococcus aureus* was not affected by any isolates from *N. commune*. On the other hand, the isolates from *Nostochopsis* spp., 20 isolated which were incubated in Med.30 and 18 isolates incubated in M52 showed an inhibitory effect against *Aeromonas hydrophila*.

Additionally, the pathogens, which displayed a disturbance in the aquaculture such as *Aeromonas hydrophila* and *Pseudomonas fluorescens*, were identified by actinomycetes isolated from *Nostochopsis* spp., a freshwater type of algae. These isolates might be the potential new sources of anti-pathogens especially with regard to the aquaculture industries in the future.

Table 5.4 Antibacterial activities of actinomycetes associated with *Nostochopsis* spp. on Med.30 and M52 with a wider inhibitory zone compared to the control

Bacterial Pathogen	**No. of actinomycetes in fermentation media (Isolates)**			
	Med. 30		**M52**	
	Nostoc commune	*Nostochopsis* spp.	*Nostoc commune*	*Nostochopsis* spp.
Aeromonas hydrophila	15	20	13	18
Pseudomonas fluorescens	2	14	4	12
Vibrio parahaemolyticus	25	4	14	4
Escherichia coli O157	18	3	21	5
Candida albicans	19	6	13	9
Staphylococcus aureus Methicillin-resistant	9	2	0	8
Staphylococcus aureus	29	19	11	11

However, there are only 2 isolates, NCMn07 and NTRHn08, which showed the activities against all 7 pathogens. These 2 strains were identified through the process of comparing them with the data in the NCBI database and the results are shown in Table 5.5. Moreover, the 16S rDNA sequence of NCMn07 has revealed 81% similarity to *Streptomyces atrovirens* NRRL B-16357$^{(T)}$, while the isolate NTRHn08 has revealed 97% similarity to *Streptomyces griseoflavus* LMG 19344$^{(T)}$.

Table 5.5 Similarity percentages of sequences of the isolates which showed activities against 7 pathogens compared with other reference sequences in the Eztaxon database

Isolate	**Most closely related hit**	**Accession number**	**Similarity (%)**
NCMn07	*Streptomyces atrovirens* NRRL B-16357$^{(T)}$	DQ026672	81%
NTRHn08	*Streptomyces griseoflavus* LMG 19344$^{(T)}$	AJ781322	97%

Streptomyces atrovirens from the rhizosphere of *Undaria pinnatifida* displayed potent antimicrobial activity against bacterial fish pathogens. From this activity, two active compounds were isolated:, 2-hydroxy-5-(3-methylbut-2-enyl)benzaldehyde (B1) and 2-hepta-1,5-dienyl-3,6-dihydroxy-5-(3-methylbut-2-enyl) benzaldehyde (B2). B1 compound is a new benzaldehyde derivative. This is the first time that either of these compounds have been reported in the genus *Streptomyces*. [Cho and Kim, 2012]. On the other hand, Aouiche *et al.* [2014] reported that *Streptomyces griseoflavus* LMG 19344$^{(T)}$ produced 2 bioactive compounds, named P44 and P40. The P40 showed a strong activity against *Candida albicans*, *Bacillus subtilis*, and *Staphylococcus aureus*, while P44 was identified as chaetoglobosin A, and is known for its antimicrobial, anticancer, and cytotoxic effects.

According to the findings of the BLAST procedure, the database indicated that most of the isolates in this study displayed a high percentage of similarities with the genus *Streptomyces*, which make up a significant population of the actinomycetes. Within the Actinomycetales, the genus *Streptomyces* represents the most frequent producers of antibiotic agents. Examples are tetracyclin (*Streptomyces viridifaciens*), vancomycin (*Streptomyces orientalis*), fosfomycin (*Streptomyces fradiae*), streptomycin (*Streptomyces griseus*), and the macrolide erythromycin (*Streptomyces erythreus*) [Cheng *et al*., 1999; Zheng *et al*., 2000; Watve *et al*., 2001]. Recently, the actinomycete strains that possess antimicrobial activities that have been isolated from the algae, *Laminaria saccharina*, were reported by Wiese *et al*. [2009]. Most of the actinobacteria in the study of Wiese were identified as belonging to the *Streptomyces* species and these isolates showed significant activities against pathogenic bacteria that consisted of gram positive bacteria, gram negative bacteria and fungi, as well.

The effect of fermentation media for antimicrobial production was studied. It was found that active isolates produced antimicrobial metabolites in both Med.30 and M52. These 2 media are composed by different carbon and nitrogen-sources and also have revealed certain differences in being inhibitory as a result of the study. Theobald *et al*. [2000] reported that different combinations of C-source and N-sources in the fermentation media can promote actinomycete to produce different types of antibiotics. It is possibly that the chemical component in this medium can promote antimicrobial production on actinomycetes. From this study, it is clear that associated bacteria with algae can provide a rich source of antimicrobial metabolites-producing actinomycetes.

However, the interactions between members of actinobacteria communities and relationships between these communities are not fully under stood. It is assumed that the bacterial communities in part are especially associated, but also include opportunistic commensal as well as algae-degrading microorgaisms [Staufenberger *et al*., 2008]. Furthermore, a proportion of isolates might be able to produce secondary metabolites against the human pathogenic *E. coli* strain and/or against members of clinically relevant *Candida* species, like *Candida albicans*, or others bacteria which likely may become more serious pathogens.