

## Isolation and evaluation of antimicrobial activity of endophytic actinobacteria on May Chang tree (*Litsea cubeba*) against pathogenic bacteria causing diseases on common carp and tilapia

Nguyen Ngoc Tuan<sup>1,2,\*</sup>, Trinh Thi Trang<sup>1</sup>, Suphawadee Yaemkong<sup>2</sup>, Prapasiri Jaipong<sup>2</sup>, Phattanan Kotham<sup>2</sup>

<sup>1</sup>Faculty of Fisheries, Vietnam National University of Agriculture, Ha Noi, Viet Nam

<sup>2</sup>Faculty of Food and Agricultural Technology, Pibulsongkram Rajabhat University, Phitsanuloke, 65000 Thailand

\*Corresponding author: [mntuan245@gmail.com](mailto:mntuan245@gmail.com)

Received: 16 March 2017; Revised: 29 August 2017; Accepted: 12 September 2017; Available online: 1 December 2017

Paper selected from The 8<sup>th</sup> International Science, Social Sciences, Engineering and Energy Conference (I-SEEC 2017)

### Abstract

Tilapia and common carp are two main cultured species with high production annually in freshwater aquaculture in northern Vietnam, however, there are serious problems caused by bacterial infection. The use of antibiotics is not sufficient to mitigate the outbreaks due to antibiotic resistance rate are increasing. Therefore, to overcome the challenges of antibiotic resistance, antimicrobial compounds with a new mechanistic approach should be urgently sought. This study aimed isolate and evaluate antimicrobial activity of endophytic actinobacteria from May Chang tree (*Litsea cubeba*) against three pathogenic bacterial species *Aeromonas hydrophila* GL14, *Aeromonas caviae* HD60 and *S. agalactiae* HY10. The results showed that 9/32 (28.20%) endophytic actinobacteria isolates could inhibit at least one target pathogenic bacteria. Three isolates MTR711, MTR622 and MTL121 showed the highest antibacterial response with minimum inhibitory concentration (MIC) ranged from 93.30 to 300  $\mu\text{L mL}^{-1}$ , amongst these the lowest value is for MTR711 and MTR622 without significant difference. When combining three individual actinobacteria mentioned above for fractional inhibitory concentration (FIC) test, the synergistic effect was found for the pair of MTR711-MTR622 against three tested pathogenic bacteria chosen with  $\Sigma\text{FIC} \leq 0.50$ . The combination of two actinobacteria MTR711 and MTR622 improved bacterial inhibitory effect at least 4 times compared to individual treatment. The results are motivating enough to conduct further studies on use of endophytic actinobacteria for treating pathogenic bacteria in aquatic animals.

**Keywords:** May Chang; Actinobacteria, *Aeromonas*; Common carp; Tilapia

©2017 Sakon Nakhon Rajabhat University reserved

### 1. Introduction

In Vietnam, optimized use of geography and environment has improved aquaculture productivity significantly in recently years. In particular, production of freshwater fish has increased considerably, contributing significantly to

increase export and local consumption of fishery products [1]. There has been expansion in the fishing area and enhancement in the level of intensification. However, the aquaculture industry is facing serious problems such as environmental pollution and disease outbreaks. Several studies suggested that these antimicrobial effects have arisen due to massive use or misuse of antibiotics and has led to the phenomenon “antibiotic resistance” [2]. For example, antibiotic resistance has been reported against oxytetracycline, tetracycline, ampicillin, florfenicol [3, 4]. Therefore, many countries around the world have regulated the use of antibiotic in aquaculture [5].

The use of products from plant origin to replace antibiotics is being considered for both humans and animals in order to avoid antibiotic resistance. A lot of herbal plants contain antibacterial compounds such as tannin, phenol, citral, and quinone [6]. Numerous studies have shown that antimicrobial activity of the herbal plant is related to the beneficial actinobacteria as endophytic symbionts. They synthesize biological compounds which inhibit the bacteria and safe for human. Therefore, the selection of potential actinobacteria from herbal plants is a promising solution [7].

May Chang tree (*Litsea cubeba*) is an herbal plant that grows in Asian countries including Vietnam. It contains many antimicrobial components [8]. Although *Litsea cubeba* oil is in use in daily life, but so far no studies have investigated the existence of the endophytic actinobacteria in May Chang tree. Also their antimicrobial activity against pathogenic bacteria causing diseases on fish in particular and on other aquatic animal in general is yet to be understood. This is reason that our research has focused on isolation and evaluation of endophytic actinobacteria on microbial resistance against *Aeromonas hydrophila*; *Aeromonas agalactiae* causing disease for tilapia and common carp.

## 2. Materials and methods

### Materials

#### *Pathogenic bacteria*

Tested isolates *Aeromonas hydrophila* GL14; *Aeromonas caviae* HD60 causing red spot disease on common carp and *S. agalactiae* HY10 causing pop eye disease on tilapia were provided from Environmental and fish pathology Department, Faculty of Fisheries, Vietnam National University of Agriculture.

#### *Medium*

Nutrient Agar (NA) and Nutrient Broth (NB) (Merck) were prepared in condition of 121 °C for 15 minutes. The composition of medium Gause I included starch powder - 20; K<sub>2</sub>HPO<sub>4</sub> - 0.5; MgSO<sub>4</sub>.H<sub>2</sub>O - 0.5; NaCl - 0.5; KNO<sub>3</sub> - 0.5; FeSO<sub>4</sub> - 0.01 (g L<sup>-1</sup>); pH = 7 - 7.4. The composition of the antibiotic producing medium A4-H includes Glucoza - 15; Soybean powder - 15; NaCl - 5; CaCO<sub>3</sub> - 1 (g L<sup>-1</sup>); pH = 7 - 7.4.

### Methods

#### *Endophytic actinobacteria (EA) isolation*

Roots, stems and leaves of May Chang tree were collected from Yen Bai, Son La, Ha Tay, Ninh Binh province, Vietnam. After collection, the surface of samples was disinfected following the process of Justin and Christopher [9] and then cultured on Gause I with complementing nalidixic acid (25 mg L<sup>-1</sup>), nystatin (50 mg L<sup>-1</sup>) and K<sub>2</sub>Cr<sub>2</sub>O<sub>7</sub> (50 mg L<sup>-1</sup>) to inhibit the growth of negative bacteria and fungi. After 4 days of incubation at 30 °C, EAs were

sub-cultured 3 times before screening antibacterial activity against tested pathogenic bacteria. Classification of EAs were based on system of color wheels of Tresner *et al.* [10].

#### *Screening of EAs antibacterial activity*

After isolation from May Chang tree, EAs were determined for antimicrobial activity with pathogenic bacteria *A. hydrophila* GL14, *A. caviae* HD60 and *S. agalactiae* HY10 by agar diffusion method [11]. In particular, EAs were inoculated in medium Gause I and incubated in condition of shaking 200 rpm, 28 °C, 7 days and after that centrifuged at 6,000 rpm in 10 minutes to get crude supernatant of each isolated EA strain. Tested bacteria were cultured on NB at 30 °C, 24h and then adjusted to  $10^8$  CFU mL<sup>-1</sup> by measuring at 600 nm wavelength with a spectrophotometer and confirmed by colony counting method on NA medium. Bacteria were spread and inoculated onto sterile NA medium in separate plates using sterile glass stick. Sterile paper discs (6mm) were placed on agar where bacteria have been placed. Crude supernatant of each EA strain (50 µl) was added separately into each disc and incubated at room temperature for 24 h, bacterial growth was observed and the zone of inhibition was measured [12].

#### *Determination of minimum inhibitory concentration (MIC) of EAs supernatant*

Isolated EAs indicating antimicrobial activity were selected for determination of MIC [13]. EAs were inoculated shakely in antimicrobial producing medium A4-H at 200 rpm, 30 °C. After 7 days of incubation, crude supernatant was separated by centrifuging at 6,000 rpm, 10 minutes and then serially 2 folders diluted. Briefly, 100 µl of EAs crude supernatant at diluted concentrations was separately added to 900 µl NB which have been mixed tested bacteria at  $10^8$  CFU mL<sup>-1</sup> and incubated at 30 °C, 24h before plating inoculum on NA plate and being examined after 24h. The MIC was defined as the lowest concentration of EAs crude supernatant preventing visible growth. All tests were performed in duplicate and analyzed by software SPSS 20 and assessed the differences by Turkey test.

#### *Evaluation of interaction between endophytic actinobacteria (ΣFIC)*

From MICs EAs supernatant, the interaction between EA metabolites was evaluated by determining the fractional inhibitory concentration (ΣFIC) based on the method of Gutierrez *et al.* [14]. The test was carried out on 96 plates with 270 µL of each tested bacteria suspension containing  $10^8$  CFU mL<sup>-1</sup> and 15 µL crude supernatants of each EA. After that, the plates were incubated at 30 °C for 24 hours before plating on NA to check the growth of bacteria. A combination of crude supernatant of two EAs at different concentration was presented in Table 1.

**Table 1** Combination of EAs crude supernatant at different concentration of MICs

ΣFIC		EA 1						
		2 MIC	1.5 MIC	1 MIC	1/2 MIC	1/4 MIC	1/8 MIC	1/16 MIC
EA 2	2 MIC	4	3.50	3	2.50	2.25	2.13	2.06
	1.5 MIC	3.50	3	2.50	2	1.75	1.63	1.56
	1 MIC	3	2.50	2	1.50	1.25	1.13	1.06
	1/2 MIC	2.50	2	1.50	1	0.75	0.63	0.56
	1/4 MIC	2.25	1.75	1.25	0.75	0.50	0.38	0.31
	1/8 MIC	2.13	1.63	1.13	0.63	0.38	0.25	0.19
	1/16 MIC	2.06	1.56	1.06	0.56	0.31	0.19	0.13

$\Sigma$ FIC was determined as a minimum combination of two EAs crude supernatant which can inhibit the growth of bacteria. So,  $\Sigma$ FIC was calculated as  $FIC_{EA1} + FIC_{EA2}$ ; whereas  $FIC_{EA1} = MIC_{EA1 \text{ in combination}} / MIC_{EA1 \text{ in single}}$  and  $FIC_{EA2} = MIC_{EA2 \text{ in combination}} / MIC_{EA2 \text{ in single}}$ . The result was interpreted the combination of EA1 and EA2 as: synergy with  $\Sigma FIC \leq 0.5$ ; addition with  $0.5 < \Sigma FIC \leq 1$ , indifference with  $1 < \Sigma FIC \leq 4$ , antagonism with  $\Sigma FIC > 4$ . The test was carried out in triplicate.

### 3. Results and Discussion

#### *Isolation of endophytic actinobacteria (EA)*

**Table 2** Color classification and antimicrobial activity of endophytic actinobacteria

Color group of EAs	Number of EAs	Percentage of EAs (%)	Antimicrobial activity	
			Number of EAs	Percentage of EAs (%)
White (Albus)	13	40.60	4	12.50
Grey (Griseus)	8	25	2	6.30
Pink (Roseus)	3	9.30	1	3.10
Grey (Chromogenes)	9	28.10	2	6.30
Total	33	100	9	28.20

There were 32 EA strains being isolated (Table 2). Based on system of color wheels of Tresner and Buckus [15] and the colour of sporulating aerial mycelium, EAs were classified into 4 color groups as White, Grey, Pink and Brown. Within 32 EA strains, White group stand the biggest position with 13 strains (40.60%), and followed by Grey group (28.10%) and Pink group (9.30%). This result was in agreement with the study of Le Thi Hien *et al.* [16] which showed 37.10% of total 43 EA strains from soil belongs to White group. Apart from that, the isolation of EA in herbs was carried out by many previous studies. In particular, Gangwar *et al.* [17] was isolated 40 EA strains from roots, stems and leaves of three medicinal plants viz. *Aloe vera*, *Mentha* and *Ocimum sanctum*.

#### *Screening of antimicrobial activity of EA strains in May Chang tree*

All 32 EA strains were tested for antimicrobial activity against 3 isolates of pathogenic bacteria *A. hydrophila* GL14; *A. caviae* HD60; *S. agalactiae* HY10 causing diseased on common carp and tilapia. From table 2, the result was displayed that nine in the 32 strains (28.20%) exhibited inhibitory activity against at least one of the pathogenic microorganisms tested. While as, six out of the nine strains exhibited antimicrobial activity with both three bacteria at different level (Table 3). The result showed that two strains MTR711 and MTR622 have inhibitory zone ranged from 26.20 to 25.60 mm with 3 pathogenic bacteria (Fig. 1). In following, the value of MTL121 fluctuated from 10.90 to 15.40 mm. The inhibitory activities of these strains against a variety of pathogens suggested that these endophytic actinobacteria may be potential candidates for the production of bioactive compounds. Although six other EA strains showed antimicrobial capacity, inhibitory zone was small and unstable, only 3 stains MTR711;

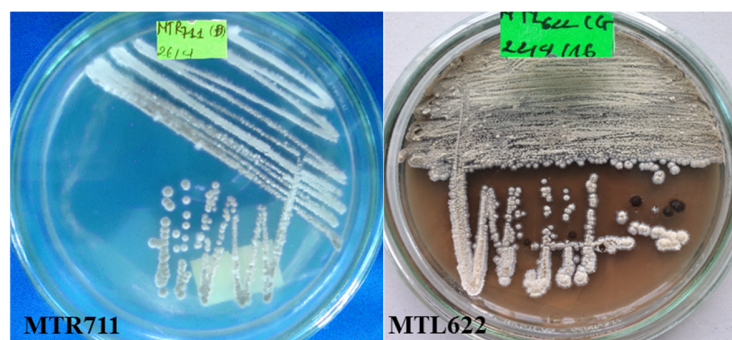
MTR611 and MTL121 were selected for further tests. In table 3, MTT723 and MTR101 did not show inhibitory activity to *A. hydrophila* GL14 and *A. caviae* HD60 and that was the same to MTT112 with *S. agalactiae*. It could be explained that *A. hydrophila* GL14 and *A. caviae* HD60 were different with *S. agalactiae* in type of Gram. *A. hydrophila* and *A. caviae* were negative Gram with have 2 basic layers of membrane. In contrast *S. agalactiae* was positive Gram which have 3 basic layers of membrane. The result was interpreted that MTT723 only inhibit negative gram bacteria, while as MTT112 was sensitive with positive gram ones.

**Table 3** Antimicrobial activity of endophytic actinobacteria (EA) in May Chang tree

EA strains	Inhibitory zone (mm)		
	<i>A. hydrophila</i> GL14	<i>A. caviae</i> HD60	<i>S. agalactiae</i> HY10
MTR711	29.60 ± 2.50	22.60 ± 0.90	23.20 ± 1.30
MTL721	6.50 ± 0.80	5.20 ± 1.30	7.80 ± 1.90
MTL214	4.30 ± 1.40	6.30 ± 2.30	8.30 ± 1.30
MTR622	23.0 ± 2.10	25.60 ± 0.90	26.20 ± 0.80
MTT723	-	-	7.10 ± 1.00
MTL121	15.40 ± 0.70	12.50 ± 0.90	10.90 ± 0.50
MTR611	12.20 ± 1.80	1.80 ± 1.30	1.60 ± 1.60
MTR101	-	-	7.50 ± 0.80
MTT112	5.40 ± 3.30	4.0 ± 4.20	-

(-) None of antimicrobial activity

In nature, there are many endophytic actinobacteria being capable of production of bioactive compounds against pathogenic micro-organisms such as fungi, bacteria. Therefore, many of them have been used as materials for extraction, synthetic of drug and chemical to mitigate disease for human and animals. Many studies proofed antimicrobial activity of EAs. Zhao *et al.* [18] reported that there were 26 out of total 560 EA strains being isolated from 26 medical plants in Panxi, China exhibiting inhibitory activity with at least (10.70%). Similarly, Li *et al.* [19] isolated 41 EAs belonging to *Streptomyces*, including 65.90% and 24.40% of total EAs against *E. coli* and *Staphylococcus aureus*, respectively. In spite of many researches on antimicrobial activity of EAs on human pathogenic microorganisms, there is a lack of studies carrying out on aquatic animals.



**Fig. 1** EA strains MTR711 and MTL622 in medium Gause I

*Minimum inhibitory concentration (MIC) of EA strains in May Chang tree*

From the result above, three EAs strains MTR711, MTR622 and MTL121 showing the biggest inhibitory zone were chosen for MIC determination. The result from Table 4 presented that MIC of MTR711 and MTR622 ranged from 93.30 to 143.30  $\mu\text{L mL}^{-1}$  and was not significantly different ( $p \leq 0.05$ ) against *A. hydrophila* GL14 and *A. caviae* HD60. The MIC of strain MTL121 showed the highest value with the range of 240.0 – 300.0  $\mu\text{L mL}^{-1}$ . The result proved that antimicrobial effect of the strains MTR711 and MTR622 were higher than that of MTL121. Our result was in agreement with Nguyen Hai Van *et al.* [20] which MIC of endophytic actinobacteria named MPT28 in May Chang tree arranged 50 – 333  $\mu\text{L mL}^{-1}$  against human pathogenic bacteria.

**Table 4** Minimum inhibitory concentration of endophytic actinobacteria (EA) in May Chang tree

EA strains	Minimum inhibitory concentration (MIC) ( $\mu\text{L mL}^{-1}$ )		
	<i>A. hydrophila</i> GL14	<i>A. caviae</i> HD60	<i>S. agalactiae</i> HY10
MTR711	126.70 <sup>a</sup> $\pm$ 3.30	126.70 <sup>a</sup> $\pm$ 8.80	93.30 <sup>a</sup> $\pm$ 3.30
MTR622	143.30 <sup>a</sup> $\pm$ 3.30	100 <sup>a</sup> $\pm$ 5.70	130 <sup>b</sup> $\pm$ 5.70
MTL121	240 <sup>b</sup> $\pm$ 5.70	283.30 <sup>b</sup> $\pm$ 6.70	300 <sup>c</sup> $\pm$ 10

Note: Values followed by different letters within a column are significantly different Turkey ( $p \leq 0.05$ )

*Interaction effect of EA strains ( $\Sigma\text{FIC}$ ) on antimicrobial activity***Table 5** Interaction effect of EA strains on antimicrobial activity

Combination of EA strains	Pathogenic bacteria	$\Sigma\text{FIC}$	Interaction*
MTR711-MTR622	<i>A. hydrophila</i>	0.30	Synergy
	<i>A. caviae</i>	0.50	Synergy
	<i>A. agalactiae</i>	0.50	Synergy
MTR711- MTL121	<i>A. hydrophila</i>	0.70	Addition
	<i>A. caviae</i>	1.00	Addition
	<i>A. agalactiae</i>	1.00	Addition
MTR622-MTL121	<i>A. hydrophila</i>	1.60	Indifference
	<i>A. caviae</i>	1.10	Indifference
	<i>A. agalactiae</i>	1.80	Indifference

\*Synergy ( $\Sigma\text{FIC} \leq 0.50$ ); Addition ( $0.50 < \Sigma\text{FIC} \leq 1$ ); Indifference  $1 < \Sigma\text{FIC} \leq 4$ ; Antagonism ( $\Sigma\text{FIC} > 4$ )

The interaction effect of 3 EA strains in pair combination was presented in Table 5. The result indicated that the combination of MTR711 and MTR622 showed synergy effect of antimicrobial activity against all three tested bacteria ( $\Sigma\text{FIC} \leq 0.50$ ). The combination of MTR711 and MTL121 has resulted in addition effect with  $\Sigma\text{FIC}$  in range of

0.50 – 1. Indifference effect of MTR622 and MTL121 was observed with  $\Sigma\text{FIC} > 1$ . Therefore, the combination of MTR711 and MTR622 could decrease concentration at least 4 times comparing single treatment.

The interaction effect of antimicrobial compounds has conducted by some studies. Cai *et al.* (2007) reported that MIC of allicin alone was  $512 \mu\text{g mL}^{-1}$ , but it facilitated antibacterial activity of all three  $\beta$ -lactams tested at sub-inhibitory concentrations [21]. In particular,  $\Sigma\text{FI}$  của cefazolin was 0.5 ( $1/4\text{MIC}_{\text{allicin alone}}$  và  $1/4\text{MIC}_{\text{cefazolin}}$ ),  $\Sigma\text{FIC}$  of oxacillin was 0.375 ( $1/8\text{MIC}_{\text{allicin alone}}$  và  $1/4\text{MIC}_{\text{oxacillin}}$ ). The study of Zafar Ahmed *et al.* (2013) showed that Amoxicillin and Cefadroxil have synergy effect against 47 isolates *Staphylococcus aureus* with value  $\Sigma\text{FIC}$  ranged in 0.14 – 0.50, while as, Streptomycin and Cefadroxil synergized on antimicrobial activity against 44 isolates *S. aureus* ( $\Sigma\text{FIC}_{\text{min}}$  0.03 – 0.50). The study of Nguyen Hai Van *et al.* [22] on interaction between EAs and May Chang oil indicated that synergy effect of the oil and EA strain named MPT28 against 4 isolates of human pathogenic bacteria.

#### 4. Conclusion

There were 9 out of total 32 EA strain in May Chang tree exhibiting antimicrobial effect with three pathogenic bacteria *A. hydrophila* GL14, *A. caviae* HD60 and *S. agalactiae* HY10 causing diseases on common carp and tilapia. Three EA strains MTR711, MTR622 và MTL121 have wide inhibitory zones ranged from 10.90 to 29.60 mm. MICs of 2 strains MTR711 and MTR622 displayed no significant difference in a range from 93.30 to  $143.30 \mu\text{L mL}^{-1}$  against all of three tested bacteria. The combination of MTR711 and MTR622 showed synergistic effect against 3 tested bacteria to enhance antimicrobial activity at least 4 times comparing with single test. This result could be a potential and promising application for sustainable therapy in aquaculture.

#### 5. Acknowledgement

The research was a part in the project named “Study on the prevention of bacterial infectious diseases for common carp and tilapia from May Chang (*Litsea cubeba*) and metabolites of endophytic actinobacteria” (code 03VB2016) being funded by Vietnam National University of Agriculture – French Speaking Universities Council (CUD-Belgium).

#### 6. References

- [1] V.H. Nguyen, S.V. Ngo, Freshwater fish in Vietnam - Cyprinidae family, Agriculture Press, Vietnam, 2001, pp. 237 – 252.
- [2] F.C. Cabello, H.P. Godfrey, A. Tomova, L. Ivanova, H. Dölz, Antimicrobial use in aquaculture re-examined: its relevance to antimicrobial resistance and to animal and human health, *Environ Microbiol.* 15 (2013) 1917 – 1942.
- [3] H.C. Su, G.G. Ying, R. Tao, R.Q. Zhang, L.R. Fogarty, Occurrence of antibiotic resistance and characterization of resistance genes and integrons in Enterobacteriaceae isolated from integrated fish farms in South China, *J Environ Monit.* 13 (2011) 3229 – 3236.
- [4] C.D. Miranda, R. Rojas, Occurrence of florfenicol resistance in bacteria associated with two Chilean salmon farms with different history of antibacterial usage, *Aquaculture.* 266 (2007) 39 – 46.
- [5] F.C. Cabello, Heavy use of prophylactic antibiotics in aquaculture: a growing problem for human and animal health and for the environment, *Environ. Microbiol.* 8 (2006) 1137 – 1144.
- [6] M. Reverter, N. Bontemps, D. Lecchini, B. Banaigs, P. Sasal, Use of plant extracts in fish aquaculture as an alternative to chemotherapy: Current status and future perspectives, *Aquaculture.* 433 (2014) 50 – 61.

- [7] H. Wang, Y. Liu, Chemical Composition and Antibacterial Activity of Essential Oils from Different Parts of *Litsea cubeba*, *Chem. Biodiversity*. 7 (2010) 229 – 235.
- [8] A.K. Saikia, D. Chetia, M. D'Arrigo, A. Smeriglio, T. Strano, G. Ruberto, Screening of fruit and leaf essential oils of *Litsea cubeba* Pers. from north-east India-chemical composition and antimicrobial activity, *J. Essent. Oil Res.* 25 (2013) 330 – 338.
- [9] T.C. Justin, M.M. Christopher Franco, Isolation and Identification of Actinobacteria from Surface-Sterilized Wheat Roots, *Appl. Environ. Microbiol.* 69 (2003) 5603 – 5608.
- [10] H.D. Tresner, J.A. Hayes, E.J. Backns, Differential tolerance of *Streptomyces* to sodium chloride as a taxonomic aid, *Applied Microbiol.* 16 (1968) 1134 – 1136.
- [11] D. Dhanasekaran, N. Thajuddin, A. Panneerselvam, Applications of Actinobacterial Fungicides in Agriculture and Medicine. Chapter 2, In: *Fungicides for Plant and Animal Diseases*, INTECH Open Access Publisher, Croatia, 2012, pp. 29 – 54.
- [12] A. Kafur, A.B. Khan, Isolation of endophytic actinomycetes from *Catharanthes roseus* (L.) G. Don leaves and their antimicrobial activity, *I. J. Biotech.* 9 (2011) 302 – 306.
- [13] M.P. Dore, M.S. Osato, G. Realdi, I. Mura, D.Y. Graham, A.R. Sepulveda, Amoxycillin tolerance in *Helicobacter pylori*, *J. Chemother.* 43 (1999) 47 – 54.
- [14] J. Gutierrez, C. Barry-Ryan, P. Bourke, Antimicrobial activity of plant essential oils using food model media: Efficacy, synergistic potential and interactions with food components, *Food Microbiol.* 26 (2009) 142 – 150.
- [15] H.D. Tremer, E.J. Buckus, System of color wheels for *Streptomyces* taxonomy, *Appl. Microbiol.* 11 (1963) 335 – 338.
- [16] T.H. Le, V.L. Dinh, T.V. Vu, V.G. Nguyen, Isolation and Screening *Streptomyces* spp. against Plant Pathogenic Fungi, *J. Sci. and Devel.* 12 (2014) 656 – 664.
- [17] M. Gangwar, S. Dogra, N. Sharma, Antagonistic bioactivity of endophytic actinomycetes isolated from medicinal plants, *JALRP.* 2 (2011) 154 – 157.
- [18] K. Zhao, P. Penttinen, Q. Chen, T. Guan, K. Lindström. X. Ao, L. Zhang, X. Zhang, The rhizospheres of traditional medicinal plants in Panxi, China, host a diverse selection of actinobacteria with antimicrobial properties, *Appl. Microbiol. Biotechnol.* 94 (2012) 1321 – 1335.
- [19] P. Xu, W.J. Li, S.K. Tang, Y.Q. Zhang, G.Z. Chen, H.H. Chen, *Naxibacter alkalitolerans* gen. nov., sp. nov., a novel member of the family 'Oxalobacteraceae' isolated from China, *Int. J. Syst. Evol. Microbiol.* 55 (2005) 1149 – 1153.
- [20] H.V. Nguyen, T.H.N. Vu, T.T. Vu, Q.T. Phi, T.N. Khieu, S. Sarter, K.S. Chu, Antimicrobial activities and interaction effects of Vietnamese *Litsea cubeba* (Lour.) Pers essential oil and its endophytic actinobacteria, *J. of sci. and tech.* 54 (2016) 234 – 241.
- [21] Y. Cai, R. Wang, F. Pei, B. Liang, Antibacterial activity of Allicin alone and in combination with b-lactams against *Staphylococcus* spp. and *Pseudomonas aeruginosa*, *J. Antibiot.* 60 (2007) 335 – 338.
- [22] H.V. Nguyen, D. Caruso, M. Lebrun, N.T. Nguyen, T.T. Trinh, J.C. Meile, S. Chu-Ky, S. Sarter, Antibacterial activity of *Litsea cubeba* (Lauraceae, May Chang) and its effects on the biological response of common carp *Cyprinus carpio* challenged with *Aeromonas hydrophila*, *J Appl Microbiol.* 121 (2016) 341 – 351.