Malaysian Journal of Microbiology
Published by Malaysian Society of Microbiology
(In SCOPUS since 2011)

Antimicrobial and enzymatic activities of endophytic bacteria isolated from *Mentha spicata* (MINT)

Akinsanya Mushafau Adewale[1 2], Goh Joo Kheng[1], Lim Siew Ping[1], and Ting Adeline Su Yien[1]*

[1]School of Science, Monash University Malaysia, Jalan Lagoon Selatan, 46150, Bandar Sunway, Selangor, Malaysia.
[2]Department of Medical Biochemistry, Faculty of Basic Medical Sciences, College of Medicine, Lagos State University, Lagos, Nigeria.
Email: adeline.ting@monash.edu

ABSTRACT

Aims: This study is to isolate and characterize endophytic bacteria for potential industrial enzymes and antimicrobial activities against some human pathogens.
Methodology and results: *Mentha spicata,* a local medicinal plant, was used to source for endophytes. The roots, stems and leaves of *M. spicata* were surface-sterilized to isolate the endophytic bacteria. The endophytic bacteria were subsequently characterized based on their 16S rRNA sequences. The endophytic bacteria were screened for both antimicrobial and enzymatic activities. We identified 15 isolates of 4 genus-*Pseudomonas* (7 species), *Bacillus* (3 species), *Enterobacter* (1 species) and *Comamonas* (1 species) with 97-100% similarity level. Isolates MSS-3 (*Pseudomonas putida*), MSR-4 (*Pseudomonas pictorum*), MSS-2 (*Bacillus thuringiensis*) and MSR-5 (*Pseudomonas straminea*) showed prominent antimicrobial activities against the pathogens tested with zones of inhibition between 6.3 to 15.3 ± 0.6 mm. All species examined have positive cellulase activities except *Comamonas guangdongensis* and only isolates of the *Bacillus* genus, *Pseudomonas pictorum* and *P. argentinensis* exhibited amylase activities.
Conclusion, significance and impact of study: Our findings revealed potential therapeutic uses of the bioactive compounds of these bacteria endophytes against pathogens. Their enzymatic potential can also be of use in various industries.

Keywords: Endophytic bacteria, diversity, antimicrobial, enzymatic, 16S rDNA

INTRODUCTION

Bacterial endophytes are found in the interstitial space of the host plant tissues, without harming the plants (Sturz *et al*., 1999; Kobayashi and Palumbo, 2000). The presence of the various Gram negative and Gram positive endophytic bacteria in numerous plants species is believed to be influenced by activities such as plant rotation, soil condition, and phytopathogen populations (Hallmann *et al*., 1997a; Kobayashi and Palumbo, 2000; Graner *et al*., 2003). Recent studies have focused on their roles within host plants in relation to plant growth (Chi *et al*., 2005), defense or stress responses (Cho *et al*., 2002) and as biological control agents (Dujiff *et al*., 1997; Nielsen *et al*., 2002). They have been found to possess novel metabolites exhibiting a variety of biological activities against different pathogens (Strobel *et al*., 2001). Many have antifungal activities against fungal pathogens such as *Fusarium oxysporum* and *Rhizoctonia solani* on cotton and potato (Strobel *et al*., 2001; Cho *et al*., 2002). Hence, they might act as potential biocontrol agents against vascular microbial pathogens (Chen *et al*., 1995; Nielsen *et al*., 2002). Some endophytes have also been found to express hydrolytic enzymes such as cellulases and pectinases (Verma *et al*., 2001). It has been hypothesized that endophytic bacteria enter into roots' interior of plants by hydrolyzing the cellulose cell wall, through water flow, and wound or at lateral root branching (Al-Mallah *et al*., 1987).

Mentha spicata (spear-mint) is a plant of great importance to Asian communities, used as herbal medicine for the treatment of various kinds of illness (e.g. gastrointestinal disorders), and as flavoring in the food industry (Alex, 2007). Its essential oil has antifungal and excellent antioxidant properties (Kanatt *et al*., 2007). The leaves have a strong spearmint flavor and are hence used as flavoring in salads or cooked foods. It has a very pleasant and refreshing taste of spearmint, leaving the mouth and digestive system feeling clean (Adam *et al*., 1998). Thus it can be hypothesized that endophytes from medicinal plants may harbour beneficial bioactive compounds due to their close relationship and co-evolution with the host plant.

Therefore, in our study, we source for endophytic bacteria with potentials to produce antimicrobial and hydrolytic enzymes, with potential uses in rapidly growing industries such as medical, biofuel, starch and paper.

***Corresponding author**

 ISSN (print): 1823-8262, ISSN (online): 2231-7538

MATERIALS AND METHODS

Isolation of endophytic bacteria

The herbal plants *M. spicata* were collected from Sungai Buloh, Selangor. The leaves, stem and roots were washed under running tap water and left for 10-15 min to drain. The tissues were cut into 2-3 cm pieces and rinsed in sterile distilled water with few drops of Tween-20. Surface sterilization was performed according to the methods described by Azevedo *et al*. (2000), with slight modifications to the duration for sterilization and ethanol concentration. Briefly, tissues were immersed in 75% ethanol (3 min), followed by sodium hypochlorite (3%) solution (2 min), and in 75% ethanol (3 min). The disinfected leaves, stems and roots were rinsed three times in sterile distilled water and drained. The stem and root tissues were cut longitudinally and placed on Nutrient Agar. The leaves, after surface sterilization, were homogenized with sterile 12.5 mM potassium phosphate buffer (pH 7.1) using sterile test-tube and glass rod. The homogenate (1 mL) was serially diluted up to 10^{-4} dilution using sterile 12.5 mM potassium phosphate buffer (pH 7.1) and then spread on nutrient agar plates. The inoculated plates were incubated for 36-48 h at 30±2 °C. The bacterial colonies were sub-cultured in sterile Nutrient Agar two to three times to establish pure cultures. In addition, the uncut surface-disinfected tissues, and the last rinsing water were also inoculated onto separate nutrient agar plates. This is to validate the effectiveness of the surface sterilization procedure (control) as bacterial growth in the control agar plates within 24 to 48 h of incubation (30±2 °C) indicates ineffective surface-sterilization.

Molecular characterization

The isolated endophytic bacteria were cultured in 5 mL of Nutrient broth for 15 to 18 h. Then, 2-3 mL of the culture suspension was centrifuged for 3 min at 6,000 rpm. The pellet was subjected to DNA extraction using GF-1 bacterial DNA extraction kit by Vivantis. The 16S rRNA gene was amplified using the forward primer [8F:5'-AGAGTTTGATCCTGGCTCAG-3'] and reverse primer [1492R: 5'-GGTTACCTTGTTACGACTT-3'] (Turner *et al*., 1999). The PCR conditions consist of initial temperature of 94 °C for 3 min, 30 amplification cycles of 94 °C for 30 sec (denaturation), 51 °C for 30 sec (annealing), and 72 °C for 50 sec (extension), and final extension of one cycle at 72°C for 5 min. The amplified PCR products were purified using NucleoSpin Gel and PCR Clean-up kit by Macherey-Nagel. The sequencing was performed by First-BASE Laboratories Pte. Ltd. with the forward and reverse DNA sequences edited using Bio-edit software to obtain the contigs. The isolates were identified based on hits analysis from megablast (highly similar sequences) output of the BLASTN program (http://ncbi.nlm.nih.gov). Sequences of the 16S rRNA gene were aligned using the multiple sequence alignment program (MUSCLE) (Tamura *et al*., 2011) and the phylogenetic analysis performed using Maximum Likelihood methods (MEGA6) (Tamura *et al*., 2011) with Bootstrap analysis performed using data resampled 1,000 times. The DNA sequences were deposited at NCBI and accession numbers obtained.

Determination of antimicrobial activity

The antimicrobial activities of bacterial endophytes were tested against bacteria and yeast pathogenic strains, which include-*Pseudomonas aeruginosa* (ATCC 10145), *Staphylococcus aureus* (ATCC 33591), *Bacillus cereus* (ATCC 14579), *Salmonella* typhi (ATCC 14028), *Proteus vulgaris* (clinical isolate), *Klebsiella pneumonia* (clinical isolate), *Escherichia coli* (ATCC 25922), *Streptococcus pyogenes* (clinical isolate) and *Candida albicans* (clinical isolate) using the Kirby-Bauer disc diffusion method (Bauer *et al*., 1966). The bacteria pathogens were pre-cultured overnight in Mueller Hilton broth at 35 ± 2 °C, 5 mL of the suspension culture were centrifuged at 6,000 × *g* for 5 min. The pellets were suspended in sterile distilled water and density adjusted to 0.5 McFarland standard. The suspension was seeded onto Mueller Hilton agar plates for antimicrobial testing. The endophytic bacteria were cultured in nutrient broth and incubated for 18 h at 35 ± 2 °C. The broth suspension cultures were centrifuged at 6,000 × *g* for 5 min. Twenty microliters of the crude metabolite in the supernatant obtained was impregnated onto sterile discs and placed on seeded agar plates. The plates were incubated at 35 ± 2 °C for 48 h and checked for zone of inhibition by measuring the diameter of annular clear zone. The experiment was performed in triplicates. Gentamicin (10 µg) standard antimicrobial discs were used as positive control.

Determination of enzymatic activity

To detect extracellular cellulase, xylanase, amylase and pectinase production from the endophytic bacteria isolates, the agar diffusion method was used (An *et al*., 2005). For cellulase activity assay, the isolates were grown on cellulase activity indicator medium [Nutrient Agar medium containing, 0.5 % (w/v) carboxylmethylcellulose, and 1.5 % agar (w/v)]. Spot dot inoculation was performed for the detection of activity. For xylanase activity assay, the isolates were grown on xylanase activity indicator medium [nutrient agar medium containing, 0.5 % (w/v) oat spelt xylan and 1.5% agar (w/v)]. To visualize the halos formed due to cellulase and xylanase activity, the plates were flooded with 0.5% Congo red solution for 30 min, rinsed with water, and then rinsed twice with 1M NaCl. Colonies positive for extracellular cellulase and xylanase activities were surrounded by a yellow halo against a red background. For amylase activity assay, the isolates were grown on amylase activity indicator medium (nutrient agar medium containing, 0.5% (w/v) starch powder and 1.5% agar (w/v), a clear zone surrounded by black coloration

 ISSN (print): 1823-8262, ISSN (online): 2231-7538

indicated the activity of amylase when rinsed with 0.1M Iodine solution. For detection of pectinase activity, cultures were grown on pectinase indicator medium [nutrient agar medium containing 0.7% (w/v) sodium polypectate and 1.5% agar (w/v)]. To visualize the halos formed due to pectinase activity, the plates were flooded with 10% of a saturated solution of copper acetate ($Cu(OAc)_2$) for 30 min. After excess stain was washed off, a halo against a blue background became visible (Park *et al*., 2000).

Statistical analysis

One-way ANOVA was used to analyze all data obtained. The analysis was carried out using the Statistical Package for Social Science (SPSS) version 16.0 and means compared using Tukey's Studentized Range Test ($HSD_{(0.05)}$).

RESULTS AND DISCUSSION

Fifteen endophytic bacteria were isolated from the medicinal plant *Mentha spicata*. The molecular characterization revealed 4 genera of *Pseudomonas* (9 isolates), *Bacillus* (3 isolates), *Comamonas* (2 isolates) and one isolate of *Enterobacter* (Table 1). The results also revealed that a higher diversity of bacterial endophytes were isolated from the stem tissues (7 species), as compared to the root (5 species) and leaf tissues (3 species). Analysis of the microbial communities of the endophytic bacteria in the roots, stem and leaf of *M. spicata* revealed that the tissues were dominated by *Pseudomonas* genus, in addition to the presence of three species of *Bacillus* and one each of *Enterobacter* and *Comamonas* species. Bacteria such as *Pseudomonas*, *Bacillus* and *Enterobacter* have previously been studied as frequently occurring endophytes in other plants such as cotton, ginseng root and cucumber root (Hallmann *et al*., 1997b; Cho *et al*., 2002). Siciliano and Germida (1999) identified 27 different endophytic bacterial genera among 1100 bacterial isolates from the roots of three canola cultivars. Therefore, this study further indicated the existence of these bacteria as endophytes in *M. spicata* plants.

Table 1: Similarity values of 16S rRNA gene sequences obtained from the endophytic bacteria from *Mentha spicata* plants.

Isolates (**accession No.)	Nearest relatives* (accession number)	Similarity (%)
MSS-1 (KM280647)	*Bacillus anthracis* (NR074453)	98%
MSS-2 (KM280648)	*Bacillus thuringiensis* (NR102506)	99%
MSS-3 (KM280649)	*Pseudomonas putida* (NR074596)	97%
MSS-4 (KM280650)	*Enterobacter ludwigii* (NR042349)	99%
MSS-5 (KM280651)	*Pseudomonas resinovorans* (NR103921)	99%
MSS-10 (KM280652)	*Pseudomonas argentinensis* (NR043115)	99%
MSS-11 (KM280653)	*Comamonas guangdongensis* (NR108203)	99%
MSL-4 (KM280656)	*Pseudomonas putida* (NR074739)	98%
MSL-5 (KM280657)	*Comamonas guangdongensis* (NR108203)	98%
MSL-6 (KM280658)	*Pseudomonas entomophila* (NR102854)	100%
MSL-2 (KM280654)	*Pseudomonas putida* (NR074739)	97%
MSL-3 (KM280655)	*Pseudomonas cichorii* (NR026532)	99%
MSR-3 (KM280659)	*Bacillus toyonensis* (NR121761)	99%
MSR-4 (KM280660)	*Pseudomonas pictorum* (NR041957)	99%
MSR-5 (KM280661)	*Pseudomonas straminea* (NR113859)	100%

MSL, MSS and MSR represent isolates from root, stem and leaf respectively.
*Closest relative species in the 16S rRNA gene sequence database.
**Accession numbers of isolates' 16S rRNA gene sequences deposited to NCBI.

 ISSN (print): 1823-8262, ISSN (online): 2231-7538

Table 2: Enzymatic activities of bacterial endophytes isolated from *M. spicata*.

Isolates	Cellulase	Xylanase	Amylase	Pectinase
MSL-2	+	-	-	+
MSL-3	+	+	-	-
MSL-4	+	+	-	+
MSL-5	-	+	-	-
MSL-6	+	+	-	-
MSS-1	+	++	+	+
MSS-2	+	++	+	-
MSS-3	+	-	-	+
MSS-4	+	-	-	-
MSS-5	+	+	-	-
MSS-10	+	-	-	+
MSS-11	-	-	-	-
MSR-3	+	+	+	++
MSR-4	+	-	+	+
MSR-5	+	-	+	+

(++) strong positive activity, (+) weak positive activity, (-) negative activity

The enzyme production of the endophytic bacteria was investigated and results revealed that two isolates (MSS-1) *Bacillus anthracis* and (MSR-3) *Bacillus toyonensis* produced all enzymes assayed (xylanase, cellulase, pectinase and amylase), four isolates (MSL-4) *Pseudomonas putida*, (MSS-2) *Bacillus thuringiensis*, (MSR-4) *Pseudomonas pictorum* and (MSR-5) *Pseudomonas straminea* produced 3 enzymes (cellulase and two other enzymes), six isolates (MSL-2) *P. putida*, (MLS-3) *Pseudomonas cichorii*, (MSL-6) *Pseudomonas entomophila*, (MSS-3) *P. putida*, (MSS-10) *Pseudomonas argentinensis* and (MSS-5) *Pseudomonas resinovorans* produced 2 enzymes (cellulase and one other enzyme), two isolates (MSL-5) *Comamonas guangdongensis*, and (MSS-4) *Enterobacter ludwigii* were only capable of producing one enzyme each (cellulase or xylanase) and one isolate (MSS-11) *C. guangdongensis* had no enzyme activity (Table 1). However, all species examined (13 out 15 isolates) have positive cellulase activities except *C. guangdongensis*. It is also important to note that all the endophytic bacteria isolated from the root tissues showed (*P*. *pictorum*, *P*. *argentinensis* and *B*. *anthracis*) cellulase, amylase and pectinase activities.

Recent studies have related the enzymatic activities of endophytic bacteria to the mechanism used by bacteria to gain entry and colonize the interior tissues of plants (Timmusk *et al*., 2005; Cho *et al*., 2007). In this study, we found over 85% of bacterial endophytes isolated have positive cellulase activity, and 50% has either xylanase or pectinase activity. Hallmann *et al*. (1997a) demonstrated hydrolysis of wall-bound cellulose in the vicinity of bacterial cells. Suto *et al*. (2002) reported that 52 bacteria produced xylanase from 14 plants in total. Hence, it has been suggested that hydrolytic enzymes might be produced by endophytes during the early invasion phase and not after residing in the plant tissue. It has also been noted that this may play important roles in plant-microbe interactions and intercellular colonization of roots, but not all endophytic bacteria express these activities and so those endophytes might have gained entry into root interior by another mechanism(s).

The antimicrobial assay revealed all isolates except both isolates of *C. guangdongensis* (MSL-5 and MSS-11) have antimicrobial activities against at least one pathogen tested (Table 3). Activities were prominent in five isolates; MSS-2 (*B. thuringiensis*), MSS-3 (*P. putida*), MSR-4 (*P. pictorum*), MSR-5 (*P. straminea*) and MSS-5 (*P. resinovorans*) inhibiting 6 to 9 pathogens with zone of inhibition ranging from 6.3 to 15.3 ± 0.6 mm. This was followed by MSS-4 (*E. ludwigii*) and MSS-1 (*B. anthracis*) inhibiting 4 to 5 pathogens with zone of inhibition ranging from 6.6 to 10.6 ± 0.6 mm. Isolates MSL-2 (*P. putida*), MSL-3 (*P. cichorii*), MSL-4 (*P. putida*), MSL-6 (*P. entomophila*), MSS-10 (*P. argentinensis*), MSS-11 (*C. guangdongensis*), MSR-3 (*B. toyonensis*) were the least effective isolates inhibiting 1 to 3 pathogens with zone of inhibition ranging from 6.7 to 11.3 ± 0.6 mm. The Gram negative (*S. typhi*, *P. vulgaris*, *K. pneumonia*), Gram positive (*S. aureus*, *B. cereus*) pathogens and *Candida albicans* were more vulnerable to the antimicrobial compounds from the endophytic bacteria as they were strongly inhibited by 6 to 11 isolates. *Escherichia coli* was more resistant to the antimicrobial compounds from the bacteria endophytes as it was only inhibited by 3 of the 14

 ISSN (print): 1823-8262, ISSN (online): 2231-7538

Table 3: Antimicrobial activity of endophytic bacteria from *M. spicata* plant.

Isolates	Annular zone of inhibition (mm)								
	P. aeruginosa	*S. typhi*	*P. vulgaris*	*K. pneumoniae*	*E. coli*	*S. aureus*	*S. pyogenes*	*B. cereus*	*C. albicans*
MSL-2	-	-	-	-	-	-	7.3±0.6^{a}	-	-
MSL-3	-	8.6±0.6^{a}	7.3±0.12^{ab}	-	-	-	11.6±0.6^{c}	-	-
MSL-4	-	-	10.3±0.1^{e}	-	-	-	9.3±0.6^{b}	-	7.6±0.6^{abc}
MSL-6	-	-	-	-	-	8.6±0.6^{bc}	-	-	8.3±0.6^{bcd}
MSS-1	6.6±0.6^{a}	-	-	-	-	8.6±0.6^{bc}	-	8.6±0.6^{ab}	7.6±0.6^{abc}
MSS-2	8.6±0.6^{b}	8.6±0.6^{a}	6.6±0.1^{a}	7.3±1.0^{a}	7.0±0.1^{a}	10±0.0^{cd}	-	9.0±0.6^{ab}	7.3±0.6^{ab}
MSS-3	8.0±0.0^{ab}	11±0.0^{b}	10±0.1^{de}	14.3±1.0^{c}	15.3±1.0^{b}	10.6±0.6^{d}	7.6±0.6^{a}	9.6±0.6^{bc}	11.6±0.6^{e}
MSS-4	-	8.3±0.6^{a}	-	7.3±1.0^{a}	-	8.3±0.6^{bc}	-	10.6±0.6^{c}	9.6±0.6^{d}
MSS-5	-	8.3±0.6^{a}	8±0.12^{abc}	9±0.7^{b}	-	8.6±0.6^{bc}	-	8.6±0.6^{ab}	8.3±0.6^{bcd}
MSS-10	-	-	9.3±0.1^{cde}	-	-	8.3±0.6^{bc}	-	-	9.0±0.0^{cd}
MSS-11	-	7.6±0.6^{a}	8.7±0.1^{bcd}	-	-	11±1.0^{d}	-	-	-
MRS-3	-	-	6.7±0.1^{a}	-	-	8±0.0^{ab}	-	-	9.0±0.0^{cd}
MSR-4	8.6±0.6^{b}	11.6±0.6^{b}	8.3±0.1^{bc}	9.7±0.7^{b}	8.0±0.1^{a}	9.6±0.6^{bcd}	10±0.0^{b}	10.6±0.6^{c}	9.0±0.0^{cd}
MSR-5	8.3±0.6^{b}	10.3±0.6^{b}	7.3±0.1^{ab}	9.3±0.7^{b}	-	6.3±0.6^{a}	-	8.0±0.6^{a}	-
Gentamicin (10 µg)	18.3±0.6^{c}	20.3±0.6^{c}	25.3±1.0^{f}	22.3±1.0^{d}	20.3±1.0^{c}	20.3±0.6^{e}	30.6±0.6^{d}	22.6±0.6^{d}	27.6±0.6^{f}

Data are mean ±SD values. One way ANOVA was used to analyse data using Tukey's studentized range test. Values are statistically significant when $p>0.05$.

bacteria endophytes. However, *S. aureus* appeared to be the most susceptible to the isolates, as 11 of the 14 isolates were active against the pathogen. In addition, MSS-3 (*P. putida*) and MSR-4 (*P. pictorum*) appeared to be most active against all pathogens tested followed by MSS-2 (*B. thuringiensis*) and MSR-5 (*P. straminea*) (Table 3).

Bio-prospecting for antimicrobial in endophytic bacteria has been investigated in *M. spicata*. We found MSS-3 (*P. putida*) and MSR-4 (*P. pictorum*) to have broad spectrum antimicrobial activities against the pathogens tested. There is some evidence that endophytic bacteria such as *Pseudomonas* and *Bacillus* can have antimicrobial effect on crops such as cotton, tomato and potato (Dujiff *et al*., 1997; Cho *et al*., 2007). In addition, *Paenibacillus* sp. is known to produce antimicrobial and biosurfactant substances such as surfactin, iturin, and fengysin (Lin *et al*., 1999; Yao *et al*., 2003). In this study, over 90% of all strains from *M. spicata* were found to exude antimicrobial substances towards human pathogens when tested *in vitro* (Table 3). Isolates MSS-3 (*P. putida*), MSR-4 (*P. pictorum*), MSS-2 (*B. thuringiensis*) and MSR-5 (*P. straminea*) showed prominent antimicrobial activities against the pathogens tested with zones of inhibition between 6.3 to 15.3 ± 0.6 mm.

CONCLUSION

This study revealed that the endophytic communities in *M. spicata* were mostly *Bacillus* sp. and *Pseudomonas* sp. and these isolates have good antimicrobial and some enzymatic activities. Four isolates, (MSS-3) *P. putida*, (MSR-4) *P. pictorum*, (MSS-2) *B. thuringiensis* and (MSR-5) *P. straminea* have the most potential for future explorations to derive antimicrobial and enzymes that may be of beneficial use in medicine and some agro-allied industries.

ACKNOWLEDGEMENT

The authors gratefully acknowledge the sponsorship for Higher Degree for Research (HDR) scholarship of Monash University Malaysia for this project. The authors report no conflict of interest and are responsible for the content and writing of the manuscript.

REFERENCES

Adam, K., Sivropoulou, A., Kokkini, S., Lanaras, T. and Arsenakis, M. (1998). Antifungal activities of *Origanum vulgare* subsp. *hirtum*, *Mentha spicata*, *Lavandula angustifolia*, and *Salvia fruticosa* essential oils against human pathogenic fungi. *Journal of Agricultural and Food Chemistry* **46, 1739-1745.**

Al-Mallah, M. K., Davey, M. R. and Cooking, E. C. (1987). Enzymatic treatment of clover root hairs removes a barrier to Rhizobium-host specificity. *Biotechnology* **5, 1319-1322.**

An, J. M., Kim, Y. K., Lim, W. J., Hong, S. Y., An, C. L., Shin, E. C., Cho, K. M., Choi, B. R., Kang, J. M., Lee, S. M., Kim, H. and Yun, H. D. (2005). Evaluation of a novel bifunctional xylanase and cellulase constructed by gene fusion. *Enzyme and Microbial Technol*ogy **36, 989-995.**

Azevedo, J. L., Maccheroni, Jr W., Pereira, J. O. and De Araújo, W. L. (2000). Endophytic microorganisms: A review on insect control and recent advances on tropical plants. *Electronic Journal of Biotechnology* **3, 15-16.**

Bauer, A. W., Kirby, W. M. M., Sherris, J. C. and Turck, M. (1966). Antibiotic susceptibility testing by a standardized single disk method. *American Journal of Clinical Pathol*ogy **36, 493-496.**

Chen, C., Bauske, E. M., Mussion, G., Rodriquez-Kabana, R. and Kloepper, J. W. (1995). Biological control on *Fusarium* wilt on cotton by use of endophytic bacteria. *Biological Control* **5, 83-91.**

Chi, F., Shen, S. H., Cheng, H. P., Jing, Y. X., Yanni, Y. G. and Dazzo, F. B. (2005). Ascending migration of endophytic rhizobia from roots to leaves, inside rice plants and assessment of benefits to rice growth physiology. *Applied and Environmental Microbiology* **71, 7271-7278.**

Cho, S. J., Park, S. R., Kim, M. K., Lim, W. J., Ryu, S. K., An, C. L., Hong S. Y., Lee, Y. H., Jeong, S. G., Cho, Y. U. and Yun, H. D. (2002). Endophytic *Bacillus* sp. isolated from the interior of ballon flower root. *Bioscience, Biotechnology, and Biochemistry* **66, 1270-1275.**

Cho, K. M., Hong, S. Y., Lee, S. M., Kim, Y. H., Kahng, G. G., Lim, Y. P., Kim, H. and Yun, H. D. (2007). Endophytic bacterial communities in Ginseng and their antifungal activity against pathogen. *Microbial Ecology* **54, 341-351.**

Dujiff, B. J., Gianinazzi-Pearson, V. and Lemanceau, P. (1997). Involvement of the outer membrane lipopolysaccharides in the endophytic colonization of tomato roots by biocontrol *Pseudomonas fluorescens* strain WCS417r. *New Phytologist* **135, 325-334.**

Frampton, A. (2007). The Complete illustration book of herbs. The reader's digest association, London. **pp. 32.**

Graner, G., Persson, P., Meijer, J. and Alstrom, S. (2003). A study on microbial diversity in different cultivars of *Brassica napusin* relation to its wilt pathogen, *Verticillium longisporum*. *FEMS Microbiology Letters* **224, 269-276.**

Hallmann, J., Hallmann, Q. A., Mahaffee, W. F. and Kloepper, J. W. (1997a). Bacterial endophytes in agricultural crops. *Canadian Journal of Microbiology* **43, 895-914.**

Hallmann, Q., Benhamou, N. K and loepper, J. W. (1997b). Bacterial endophytes in Cotton: Mechanisms of entering the plant. *Canadian Journal of Microbiology* **43, 577-582.**

Kanatt, S. R., Chander, R., and Sharma, A. (2007). Antioxdant potential of Mint (*Mentha spicata*) in radiation processed Lamb meat. *Food Chemistry* **100, 451-458.**

Kobayashi, D. Y. and Palumbo, J. D. (2000). Bacterial endophytes and their effects on plants and uses in agriculture. *In:* Microbial Endophytes. Bacon, C. W. and White, JF. Jr. (eds.). Marcel Dekker, New York. **pp. 189.**

Lin, T. S., Chen, C. L., Chang, L. K., Tschen, J. S. and Liu, S. T. (1999). Functional and transcriptional analyses of a fengycin synthetase gene fenC, *Bacillus subtilis*. *Journal of Bacteriology* **181, 5060-5067.**

Nielsen, T. H., Sorensen, D., Tobiasen, C., Andersen, J. B., Chrssistophersen, C., Givskov, M. and Sørensen, J. (2002). Antibiotic and biosurfactant properties of cyclic lipopeptides produced by fluorescent *Pseudomonas* spp. from the sugar beet rhizosphere. *Applied and Environmental Microbiology* **68, 3414-3423.**

Park, S. R., Kim, M. K., Kim, J. O., Bae, D. W., Cho, S. J., Cho, Y. U. and Yun, H. D. (2000). Characterization of *Erwinia chrysanthemi* PY35 *cel* and *pel gene* existing in tandem and rapid identification of their gene products. *Biochemical and Biophysical Research Communications* **268, 420-425.**

Siciliano, S. D. and Germida, J. J. (1999). Taxonomic diversity of bacteria associated with the roots of field-grown transgenic *Brassica napus* cv. Quest, compared to the non-transgenic *B. napus* cv. Excel and *B. rapa* cv. Parkland. *FEMS Microbiology Ecology* **29, 263-272.**

Strobel, G. A., Dirksie, E., Sears, J., Markworth, C. (2001). Volatile antimicrobials from a Novel endophytic fungus. *Microbiology* **147, 2943-2950.**

Sturz, A. V., Chrssistie, B. R., Matheson, B. G., Arsenault, W. J. and Buchanan, N. A. (1999). Endophytic bacterial communities in the periderm of potato tubers and their potential to improve resistance to soil-borne pathogens. *Plant Pathology* **48, 360-369.**

Suto, M., Takebayashi, M., Saito, K., Tanaka, M., Yokota, A., Tomita, F. (2002). Endophytes as producers of xylanase. *Journal of Bioscience and Bioengineering* **93, 88-90.**

Tamura, K., Peterson, D., Peterson, N., Stecher, G., Nei, M. and Kumar, S. (2011). MEGA6: Molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. *Molecular Biology and Evolution* **28, 2731-2739.**

Timmusk, S., Grantcharova, N., Wagner, E. G. H. (2005). *Paenibacillus polymyxa* invades plant roots and forms biofilms. *Applied and Environmental Microbiology* **71, 7292-7300.**

Turner, S., Pryer, K. M., Miao, V. P. W. and Palmer, J. D. (1999). Investigating deep phylogenetic relationships among cyanobacteria and plastids by small subunit rRNA sequence analysis. *Journal of Eukaryotic Microbiology* **46, 327-338.**

 ISSN (print): 1823-8262, ISSN (online): 2231-7538

Verma, S. C., Ladha, J. K. and Tripathi, A. K. (2001). Evaluation of plant growth promoting and colonization ability of endophytic diazotrophs from deep water rice. *Journal of Biotechnology* **81, 127-141.**

Yao, S., Gao, X., Fuchsbauer, N., Hillen, W., Vater, J. and Wang, J. (2003). Cloning, sequencing, and characterization of the genetic region relevant to biosynthesis of the lipopeptides itulin A and surfactin in *Bacillus subtilis*. *Current Microbiology* **47, 272–277.**

 ISSN (print): 1823-8262, ISSN (online): 2231-7538