Polish Journal of Microbiology
2016, Vol. 65, No 3, 369–375

SHORT COMMUNICATION

Endophytic Detection in Selected European Herbal Plants

AGATA GORYLUK-SALMONOWICZ[1]*, MONIKA PIÓREK[1], HANNA REKOSZ-BURLAGA[1], MARCIN STUDNICKI[2] AND MIECZYSŁAW BŁASZCZYK[1]

[1]Department of Microbial Biology, Faculty of Agriculture and Biology, Warsaw University of Life Sciences, Poland
[2]Department of Experimental Design and Bioinformatics, Faculty of Agriculture and Biology
Warsaw University of Life Sciences, Poland

Submitted 17 June 2014, revised 30 April 2015, accepted 11 February 2016

Abstract

A total of 181 cultivable endophytic bacterial isolates were collected from stems of 13 species of herbs inhabiting Europe (Poland): *Chelidonium majus* L., *Elymus repens* L., *Erigeron annuus* L., *Euphrasia rostkoviana* Hayne, *Foeniculum vulgare* L., *Geranium pratense* L., *Humulus lupulus* L., *Matricaria chamomilla* L., *Mentha arvensis* L., *Papaver rhoeas* L., *Rosmarinus officinalis* L., *Solidago gigantea* L. and *Vinca minor* L. The isolates were screened for their antifungal activity and fifty three were found to inhibit fungal growth. Of these, five had strong antifungal properties. These selected isolates were identified as: *Pseudomonas azotoformans*, *P. cedrina*, *Bacillus subtilis* group and *Erwinia persicina*.

Key words: *Alternaria alternate*, antifungal activity, endophytic bacteria, herbs

It is widely believed that all plants are colonized by an endophytic microflora composed of microscopic fungi and bacteria that live inside plant tissues without causing them any harm. Endophytes have been isolated from above-ground parts of plants (stems, flowers, leaves and fruits), from roots and from seeds (Reinhold-Hurek and Hurek, 1998; Tan and Zou, 2001). Numerous studies confirmed that endophytes have a great applicable potential. They have found uses in three main fields connected with crop yield enhancement and growth promotion (1), industrial and medical applications (2) and environmental pollution control (3).

Numerous studies reported that endophytes control plant pathogens through synthesis of different antimicrobial compounds (1). Miller *et al*. (1998) described endophytic bacteria *Pseudomonas viridiflava* isolated from grasses and producing ecomycins B and C. It was investigated that these lipopeptides inhibit the human pathogens *Candida albicans*. Similar studies were conducted by Guan *et al*. (2005), who reported antimicrobial agent producing strain *Streptomyces griseus* inhabiting *Kandelia candel*. Most research has focused on the antifungal properties of isolated endophytes. Strobel *et al*. (2004) for example isolated oocydin A producing endophytes classified as *Serratia marcescens*. Antifungal compound producing endophytes (*Paenibacillus polymyxa*) were also reported by Beck *et al*. (2003). Endophytic-induced plant growth promotion is also achieved through fixation of atmospheric nitrogen, production of iron-chelating siderophores, solubilisation of minerals and production of phytohormones.

Endophytes can act as mini-factories and often produce novel compounds (2). Researchers reported endophytes producing novel antibiotics, anticancer and antiviral compounds, volatile organic compounds, insecticidal agents, immunosuppressive compounds and antioxidants (Strobel and Daisy, 2003; Owen and Hundley, 2004). Castillo *et al*. (2002) for example isolated from *Kennedia nigricans* endophytes classified as *Streptomyces* strain NRRL 30562 that produces antibiotic and antimalarial agents – munumbicins. Antimalarial compounds were also reported by Ezra *et al*. (2004), who obtained coronamycin producing *Streptomyces* sp. isolated from *Monstera* sp. Few studies have been published describing anticancer compounds producing endophytes. The first one was by Stierle *et al*. (1993), who reported taxol producing endophytic fungus *Taxomyces andreane*, isolated from *Taxus brevifolia*.

Some reports confirmed that endophytes enhance phytoremediation (3). These endophytes inhabit plants grown in xenobiotic contaminated soil and express different mechanisms necessary to degradation of contaminants (Germaine *et al*., 2004; 2006).

* Corresponding author: A. Goryluk-Salmonowicz Department of Microbial Biology, Faculty of Agriculture and Biology, Warsaw University of Life Sciences, Poland; **e-mail: agatagoryluk@interia.pl**

In the past few years the search for endophytes inhabiting medicinal plants intensified. It is now recognized that herbs are a very rich source of microorganisms with different biochemical properties. Numerous recent studies have been devoted to the identification of endophytes colonizing herbs from Asian countries. One study isolated 18 endophytic bacteria from herbal plants in Indonesia, such as citrus, turmeric, *Andrographis paniculata* and *Piper crocatum* (Soka *et al*., 2012). Another study obtained 19 bacterial endophytes and 113 fungal endophytes from plants grown in India: *Digitalis lanata*, *Digitalis purpurea*, *Plantago ovata* and *Dioscorea bulbifera* (Ahmed *et al*., 2012). Indian herbs were also investigated by Amirita and colleagues, who managed to isolate 334 fungal strains inhabiting the internal tissues of *Adhatoda vasica*, *Costus igneus*, *Coleus aromaticus* and *Lawsonia* (Amirita *et al*., 2012). Another study of medicinal plants grown in Taiwan isolated 156 fungal endophytes from 20 species from the *Lauraceae* and *Rutaceae* family (Ho *et al*., 2012). In 2014 our team presented endophytic microflora of *Hypericum perforatum* (Rekosz-Burlaga *et al*., 2014). From stems and leaves of the tested plants four bacterial strains were isolated.

The aim of the present study was to describe the endophytic microflora of selected medicinal plants inhabiting European countries. Isolated bacteria were tested for their antifungal properties against a plant pathogenic fungi and strains displaying the greatest antifungal activity have been classified according to their morphological, physiological and molecular characteristics.

Plant samples were collected from two areas in central Poland, near Kozienice town (51.575°N, 21.750°E) and in Warsaw city (52.259°N, 21.020°E), during the vegetative seasons of 2007 and 2008. Bacterial endophytes were isolated from stems of 13 native growing herbal plant species: *Chelidonium majus* L., *Elymes repens* L., *Erigeron annuus* L., *Euphrasia rostkoviana* Hayne, *Foeniculum vulgare* L., *Geranium pratense* L., *Humulus lupulus* L., *Matricaria chamomilla* L., *Mentla arvensis* L., *Papaver rhoeas*L., *Rosmarinus officinalis* L. *Solidago gigantea* L. and *Vinca minor* L. Bacterial isolation from plant material was performed according to the procedure of Hung and Annapurna (2004). Five plants of each species were tested. Samples were first washed in distilled water for 5 minutes and the surfaces were sterilized by bathing in a mixture of 0,1% mercuric chloride ($HgCl_2$) and 70% ethanol. The samples were then rinsed four times in sterile distilled water and dried on sterile filter paper. To verify the efficiency of sterilization, samples were placed on nutrient agar. The water from the fourth rinse was also plated on nutrient agar to confirm sterilization. To isolate endophytes, the samples were cut longitudinally and placed cut side down on nonselective media, including water agar, nutrient agar, potato dextrose agar and tryptic soya agar. All plates were then incubated at 28°C for 2–7 days. The produced colonies were sub-cultured several times to obtain pure cultures. To confirm that the colonies originated from a single cell and were not mixed, their morphology was recorded and the bacteria were Gram-stained and observed under light microscope. Pure isolates were maintained at –20°C.

The antifungal properties of the bacterial isolates were assayed using an *in vitro* test with the following fungal strains: *Alternaria alternata* ES11, *Paecilomyces variotti* ES23, *Chaetomium* sp. E13, *Byssochlamys* sp. E9, *Aureobasidium* sp. E4 and *Fusarium* sp. E23. Fungal strains were obtained from Department of Microbial Biology, Warsaw University of Life Sciences in Poland. Fungal spores were spread onto the surface of potato-dextrose agar (PDA) and then three 20 µl drops of each bacterial culture (grown in nutrient broth at 30°C for 24 h with shaking at 180 rpm) were spotted onto the surface of the inoculated plate. All plates were then incubated for 7 days at room temperature (Goryluk *et al*., 2009). If bacteria inhibited fungal growth, a zone of inhibition appeared around the colonies. For the isolates with the strongest antifungal properties, the diameters of the hyaline inhibition zones were measured (mm). This test was repeated five times for each bacterial isolate. Data were analyzed using one-way analysis of variance. Homogenous groups of means were determined with the Tukey's procedure of multiple comparisons at the significance level 0.05. The analyses were performed using Statgraphics 4.1 statistical package. The isolates with the strongest antifungal activity were selected for further identification.

The identification of selected isolates was made based on morphological observations (1) and the biochemical properties (2) of the bacteria. The results were analysed according to Bergey's Manual of Systematic Bacteriology (Brenner *et al*., 2005; Vos *et al*., 2009). To confirm the species identification, molecular analysis of 16S rRNA gene sequences (3 and 4) was performed.

1) For colony characterization of the selected strains, the nature of the colonies, their pigmentation and shape were recorded. To describe the properties of the bacterial cells, microscopic observations were made to determine their shape and size, their ability to move and form spores as well as their Gram-staining characteristics.
2) The biochemical properties of the bacterial isolates were tested following standard protocols. Seven different carbon compounds (lactose, saccharose, glucose, arabinose, mannitol, rahmnose, citrate) were used to check the ability to substrate utilization, provided as the sole carbon source. The ability to gelatin, starch, urea and arginine hydrolysis was

monitored. Mixed-acid fermentation test (Methyl-Red test) was performed. The activities of catalase, sulfhydrolase and lysine decarboxylase were determined. Production of fluorescent pigments, indole and acetoine was evaluated. The ability to grow at extreme temperatures (4°C and 55°C) was tested.

3) Molecular characterization
Genomic DNA was isolated from the selected strains as described by Hung and Annapurna (2004). Using these DNAs as templates and primers 8f (5'-AGAGTTTGATCCTGGCTCAG-3') and pH (5'-AAGGAGGTGATCCAGCCGCA-3') (Johri and Johri, 2004), fragments of the 16S rRNA genes were amplified by PCR. The amplicons were analyzed by electrophoresis on a 1% agarose gel, purified using a commercial kit (Clean up; A&A Biotechnology) and then sequenced using an automated DNA sequencer (454 GS FLX Titanium, Roche). The gene sequences were submitted to NCBI GenBank (accession numbers KJ130483-KJ130486). Bacterial strains were deposited in collection of Department of Microbial Biology, Warsaw University of Life Sciences in Poland.
4) Bioinformatics analysis
The 16S rRNA gene sequences from the bacterial isolates were compared with 16S ribosomal RNA sequences (Bacteria and Archaea) in the NCBI database using Standard Nucleotide BLAST with the default settings, to identify the most similar sequences. For each strain, 11 nucleotide sequences (unknown sequence and the 10 most similar) were aligned using CLUSTAL W2 (Larkin *et al*., 2007). The multiple sequence alignments were then used to create phylogenetic trees by the Neighbor Joining method with complete deletion of gaps, implemented in MEGA5 software (Saitou and Nei, 1987; Tamura *et al*., 2004; 2011).

To evaluate the antifungal properties of the selected bacterial isolates, their influence on the growth and development of *A. alternata* ES11 was assayed *in vitro*. A fungal spore suspension was prepared and added to 300 ml of PDB containing a suspension of bacterial cells. Control cultures were also prepared: PDB with fungal spores or PDB with bacteria. All cultures were incubated for 10 days at 25°C with shaking (1500 rpm). The numbers of bacteria and fungi were then evaluated using the plate count method and by microscopic analysis of the cultures. To prepare the fungal spore suspension *A. alternata* was cultured on PDA for one week at 25°C and the spores were harvested aseptically and suspended in sterile distilled water. To prepare the suspension of bacteria, the strains were cultured in nutrient broth for 12 h at 30°C with shaking (1500 rpm) and then 0.5 ml of these cultures were used to inoculate PDB. The plate count method was used to determine the concentration of fungal spores and bacterial cells.

Our results presented in this paper revealed that 12 tested herbs – *Chelidonium majus* L., *Elymus repens* L., *Erigeron annuus* L., *Euphrasia rostkoviana* Hayne, *Foeniculum vulgare* L., *Geranium pratense* L., *Humulus lupulus* L., *Matricaria chamomilla* L., *Mentha arvensis* L., *Rosmarinus officinalis* L., *Solidago gigantea* L. and *Vinca minor* L. are inhabited by bacterial endophytes. This is the first examination of endophytic microflora of the tested herbs, except *C. majus* L. which was tested for the first time by our team in 2007 (Goryluk *et al*., 2009). From the tested plants 181 bacterial isolates were obtained. The highest number of bacterial isolates came from *C. majus* (48 isolates) and from *G. pratense* (30 isolates) and the smallest number (6 isolates) from: *F. vulgare*, *S. gigantea* and *Matricaria chamomilla*. No isolates were obtained from *Papaver rhoeas*. This is not the first time, that a studied plant has apparently lacked endophytes. Soka *et al.* (2012) failed to isolate any endophytes from a specimen of the herb *Nothopanax scuttelarium*. It is not known whether the inability to isolate endophytic microorganisms from some species of plants is because they are naturally sterile, or if there is some methodological problem. All of the plants examined in our work share some common properties: they are very expansive, they grow in poor environments and have medicinal properties like anti-inflammatory, antibacterial, antifungal or anticancer activities. Plant extracts have frequently been used to treat medical conditions, and of the plants studied here, *C. majus* L. has been the source of numerous medicines: Ukrain (anti-cancer activity), Chelifungin (anti-fungal properties) and Di-Sancor (anti-HIV properties). In addition, infusions of *C. majus* have been shown to have a positive influence on the nervous and digestive systems (Ożarowski, 1976). This herb contains a number of active substances such as alkaloids. One outstanding issue is the contribution made by endophytic microorganisms to the medicinal properties of such plants. There are some examples where endophytes produce biologically active substances. One report presented in 1993 showed that *Taxomyces andreanae*, an endophytic fungus isolated from the yew tree *Taxus brevifolia*, produced paclitaxel (Stierle *et al*., 1993). This compound is used as an anticancer medicine (Taxol®) and previously had to be isolated from yew trees by a process that was long and costly due to low yield. The discovery that the endophyte is responsible for synthesizing paclitaxel has made the production of this medicine much easier. Another example is the endophytic fungus *Chaetomium globosum*, which produces hypericin isolated from the herb *Hypericum perforatum* (Kusari *et al*., 2008).

Endophytic bacteria isolated from tested plants were screened for antifungal properties against plant pathogenic fungi – *A. alternata*, *Paecilomyces variotti*,

Chaetomium sp., *Byssochlamys* sp., *Aureobasidium* sp. and *Fusarium* sp. These facultative fungal pathogens causes diseases of different plants all over the world, including various vegetables, fruits and cereal crops. Some of them, for example *A. alternata*, produces mycotoxins, which can be transferred from the infected plant to the tissues used as food. *Fusarium* species also produce mycotoxins, like zearalenone or fumonisins. *Fusarium* spp. they are important pathogens of many agricultural plants, like corn, wheat or soybean (Muthomi and Mutitu, 2003). Antifungal activity of bacteria has been detected in many genera and one of the significance of this kind of researches is the possibility to use of these bacteria as plant protection agents. Of the 181 endophytic isolates tested in our study, 53 displayed antifungal properties (29%). The highest number of bacteria with such activity was obtained from *C. majus* (22 isolates, 45%). 10 out of 18 isolates from *Erigeron annuus* L. (55%) and almost half of the isolates from *Mentha arvensis* L. (6 out of 13, 46%) had antifungal properties. Only one isolate with antifungal properties was obtained from *G. pratense* L. (3%), *Humulus lupulus* L. (12%), *S. gigantea* L. (16%) and *R. officinalis* L. (12%). Among the fungal species tested, *A. alternata* was the most sensitive to bacterial influence. The growth of this fungus was inhibited by 39 of the bacterial isolates. On the other hand, the least sensitive was *Fusarium* sp., which was inhibited by only one isolate: 30B. The growth of *Byssochlamys* species was inhibited by 7 isolates. The other fungi tested, *P. variotti*, *Chaetomium* sp. and *Aureobasidium* sp., were inhibited by 25, 26 and 27 of the bacterial isolates, respectively. The obtained results are in accordance with those achieved by our team earlier (Goryluk *et al.*, 2009) when isolates obtained from *C. majus* exhibited antifungal activity against *A. alternata*, *Chaetomium* sp. and *P. variotti*. In another study, Sgroy *et al.* (2009) used *A. alternata* to show that two endophytic bacteria (*Brevibacterium halotolerans*, *Bacillus pumilus*) isolated from *Prosopis strombulifera*, can inhibit fungal growth. Hui *et al.* (2012) isolated one endophytic species (*Bacillus subtilis*) from *Prunus mume* with antifungal properties against *A. alternata* and *Fusarium* sp.

Endophytic bacteria with the strongest antifungal properties were selected for further researches – 2–5b and 30B isolate from *C. majus*, P2 and P3 from *E. repens* and N2-1a from *S. gigantea*. First, taxonomic classification was conducted based on morphological, physiological and molecular characteristics. Afterwards, the diameter of fungal growth inhibition zones produced by these isolates and their influence on mycelium development of the fungus *A. alternata* was examined.

Only one of the isolates produced spores and was Gram-positive (30B). This isolate, in contrast to the remaining isolates, produced bigger cells (2.5×1.2 μm), formed chains and was not able to move. Gram-negative isolates formed smaller single cells (1.6×1.1 μm) and were able to move but very weakly. Some of the four Gram-negative isolates could be differentiated by the nature of their colonies. Isolate 2–5b produced round pink colonies with a regular shape, and a glossy and smooth appearance. Colonies of isolates P2 and P3 were similar in shape and appearance to those of 2–5b, but they produced fluorescent pigments. Isolate N2-1a also produced similar colonies, but without any pigmentation. In contrast, colonies of isolate 30B had an irregular crater-like shape and matt appearance.

All of the isolates could utilize lactose, sucrose, glucose, arabinose and mannitol, they were indole negative and catalase positive (Table I). Based on the

Table I
Biochemical characteristics of selected endophytic isolates.

Substrate utilization	Isolate				
	2–5b	30B	P2	P3	N2-1a
Lactose/saccharose	+/+	+/+	+/+	+/+	+/+
Glucose/arabinose	+/+	+/+	+/+	+/+	+/+
Mannitol/ rhamnose	+/+	+/–	+/–	+/–	+/+
Citrate/gelatin	+/–	–/+	+/+	+/+	+/–
Starch/urea	–/–	+/–	–/–	–/–	–/–
Arginine/lysine	–/–	+/–	+/+	+/+	–/+
Methyl-Red test	–	–	–	–	–
Metabolite production					
Fluoresceine/Pyocyanine	–/–	–/–	+/–	+/–	–/–
Hydrogen sulfide	–	+	–	–	+
Indole/catalase	–/+	–/+	–/+	–/+	–/+
Acetoine	+	+	–	–	+
Growth temperature 4°C/55°C	–/–	–/–	+/–	+/–	–/–

Table II
Phylogenetic affiliation of isolates based on the analysis of 16S rDNA fragments.

Isolate[a] (GenBank acc. No.)	Best match with database[b] (GenBank acc. No.)	Microbial affiliation
2–5b (KJ130483)	*E. persicina* (NR026049)	*E. persicina*
P2 (KJ130484)	*P. azotoformans* (NR037092)	*P. azotoformans*
P3 (KJ130485)	*P. cedrina* (NR042147)	
N2-1a (KJ130486)	*P. cedrina* (NR024912)	*P. cedrina*

[a] The sequence of isolate
[b] The sequences of isolates were compared with nucleotide sequences from database (similitude in 99%) and the phylogenetic tree was constructed; the best match was selected as the closest sequence from the phylogenetic tree

morphological and biochemical properties of the isolates, they were identified as *Bacillus* sp. (30B), *Erwinia* sp. (2–5b) and *Pseudomonas* spp. (P2, P3, N2-1a). The PCR amplification, sequencing and bioinformatic analysis of 16S rRNA gene sequences from each of the isolates enabled the identification of four of them to species level (Table II). Their phylogenetic affiliation was estimated based on the phylogenetic tree constructed to visualize the relationship between the sequences of isolates and related organisms from the GenBank database. Isolate 2–5b was determined as *Erwinia persicina* (GenBank accession number KJ130483), isolate P2 and P3 as *Pseudomonas azotoformans* (GenBank accession numbers KJ130484 and KJ130485, respectively) and isolate N2-1a as *Pseudomonas cedrina* (GenBank accession number KJ130486). Isolate 30B was placed within the *Bacillus subtilis* group, but further investigations are required to verify this species identification.

Endophytes classified as *Bacillus* and *Pseudomonas* species are very often isolated by researchers (Goryluk *et al*., 2009; Lodewyckx *et al*., 2001; Narayan *et al*., 2013; Rekosz-Burlaga *et al*., 2014). Liu *et al*. (2014) for example isolated *Bacillus* sp. strain and *P. azotoformans* strain as an endophytes from xerophilous moss *Grimmia montana*. *P. cedrina* was isolated by Behrendt *et al*. (2003) as plant associated bacteria inhabiting phyllosphere of *Solanum tuberosum* L. *Bacillus* and *Pseudomonas* genera are well-known for its production of diverse secondary metabolic products. Our results are in accordance with this statement because the strongest antifungal activity against *A. alternata* was reported for *Bacillus* sp. strain 30B (Table III). Fungal growth inhibition zone had 24 mm. Smaller inhibition zones were observed for *E. persicina* strain 2–5b and *P. cedrina* strain N2-1a, while the narrowest inhibition zones were produced by *P. azotoformans* strains P2 and P3. In contrast, these strains had the strongest activity against *Chaetomium* sp. while other strains produced smaller inhibition zones. Only one strain, *Bacillus* sp. 30B, inhibit the growth of all tested fungi, even *Fusarium* sp. strain. Similar results were obtained by Narayan *et al*. (2013) who isolated *B. tequilensis* endophytic strains with strong and broad spectrum of antifungal activity against all tested pathogenic fungi, like *Alternaria panax* and *Fusarium oxysporum*. This strain produced fungal inhibition zones wider than 8 mm. Other researchers, Tschen and Tseng (1989) isolated *Bacillus* sp. strains active against *Fusarium* sp. and *Paecilomyces* sp. which produced bacereutin. Axelrood *et al*. (1996) in turn, obtained *B. amyloliquefaciens* strains with antifungal activity against *Fusarium oxysporum*. *B. amyloliquefaciens strains* with strong activity against *Fusarium* sp.

Table III
Antifungal activities of bacterial endophytes (named 2–5b, 30B, P2, P3, N2-1a) against six fungal strains.

Isolates	Fungal growth inhibition zone (mm)					
	A. alternata	*Chaetomium* sp.	*P. variotti*	*Byssochlamys* sp.	*Aureobasidium* sp.	*Fusarium* sp.
2–5b	18.6 (b)	10.6 (a)	n	n	n	n
30B	24.0 (c)	11.2 (a)	19.0	6.2	7.0	14.4
P2	8.4 (a)	16.4 (bc)	n	n	n	n
P3	N9.4 (a)	18.0 (c)	n	n	n	n
N2-1a	15.2 (b)	12.4 (ab)	n	n	n	n

n – no inhibition zone
Letters in parenthesis indicate homogenous groups of means, which do not differ significantly at $\alpha = 0.05$. Analysis were prepared separately for each fungal strain.

were also isolated by Cuijuan *et al*. (2014). To confirm the antifungal activity against *A. alternata*, dual-culture experiment was performed. After 10 days of incubation of fungal spores with the bacteria, no fungal colonies were detected in any of the cultures, while the bacterial number were slightly reduced (*E. persicina* strain 2–5b and *Bacillus* sp. strain 30B) or unchanged (*Pseudomonas* spp. strains P2, P3 and N2-1a). Microscopic observations showed that fungal spores incubated in presence of bacteria were deformed and unable to grow and form mycelium.

Presented researches revealed that tested herbs: *C. majus* L., *E. repens* L., *E. annuus* L., *Euphrasia rostkoviana* Hayne, *F. vulgare* L., *G. pratense* L., *H. lupulus* L., *Matricaria chamomilla* L., *Mentha arvensis* L., *Rosmarinus officinalis* L., *Solidago gigantea* L. and *Vinca minor* L. are inhabited by endophytic bacteria. 29% of the isolates displayed antifungal properties against plant pathogens. The highest number of endophytes with antifungal activity was obtained from *C. majus* L., *E. annuus* L. and *Mentha arvensis* L. (45%, 55% and 46%, respectively). The highest antifungal activity was recorded for *Bacillus* sp. strain 30B which was isolated from *C. majus* L.

Literature

Ahmed M., M. Hussain, M. Dhar and S. Kaul. 2012. Isolation of microbial endophytes from some ethnomedicinal plants of Jammu and Kashmir. *J. Nat. Prod. Plant Resour.* 2: 215–220.

Amirita A., P. Sindhu, J. Swetha, N. Vasanthi and K. Kannan. 2012. Enumeration of endophytic fungi from medicinal plants and screeening of extracellular enzymes. *World J. Sci. Tech.* 2: 13–19.

Axelrood P.E., A.M. Clarke, R. Radley and S.J. Zemcov. 1996. Douglas fir root-associated microorganisms with inhibitory activity towards fungal plant pathogens and human bacterial pathogens. *Can. J. Microbiol.* 42: 690–700.

Beck H.C., A.M. Hansen and F.R. Lauritsen. 2003. Novel pyrazine metabolites found in polymyxin biosynthesis by *Paenibacillus polymyxa*. *FEMS Microbiol. Lett.* 220: 67–73.

Behrendt U., A. Ulrich and P. Schumann. 2003. Fluorescent pseudomonads associated with the phyllosphere of grasses; *Pseudomonas trivialis* sp. *nov*., *Pseudomonas poae* sp. *nov*. and *Pseudomonas congelans* sp. *nov*. *Int. J. Syst. Evol. Microbiol.* 53: 1461–1469.

Brenner Don J., N.R. Krieg and J.R. Staley. 2005. Volume 2: The Proteobacteria, Part B: The Gammaproteobacteria. In: Bergey's Manual of Systematic Bacteriology. 2nd ed., Springer-Verlag, New York, NY.

Castillo U.F., G.A. Strobel, E.J. Ford, W.M. Hess, H. Porter, J.B. Jensen, H. Albert, R. Robison, M.A. Condron, D.B. Teplow and others. 2002. Munumbicins, wide-spectrum antibiotics produced by *Streptomyces* NRRL 30562, endophytic on *Kennedia nigriscans*. *Microbiology* 148: 2675–2685.

Cuijuan S., P. Yan, J. Li, H. Wu, Q. Li and S. Guan. 2014. Biocontrol of *Fusarium graminearum* growth and deoxynivalenol production in wheat kernels with bacterial antagonists. *Int. J. Environ. Res. Public Health.* 11: 1094–1105.

Ezra D., U.F. Castillo, G.A. Strobel, W.M. Hess, H. Porter, J.B. Jensen, M.A. Condron, D.B. Teplow, J. Sears, M. Maranta and others. 2004. Coronamycins, peptide antibiotics produced by a verticillate *Streptomyces* sp. (MSU-2110) endophytic on *Monstera* sp. *Microbiology* 150: 785–793.

Germaine K., E. Keogh, G. Garcia-Cabellos, B. Borremans, D. Lelie, T. Barac, L. Oeyen, J. Vangronsveld, F.P. Moore, E.R. Moore and others. 2004. Colonisation of poplar trees by gfp expressing bacterial endophytes. *FEMS Microbiol. Ecol.* 48: 109–118.

Germaine K., X. Liu, G. Cabellos, J. Hogan, D. Ryan and D.N. Dowling. 2006. Bacterial endophyte-enhanced phyto-remediation of the organochlorine herbicide 2,4 dichlorophenoxyacetic acid. *FEMS Microbiol. Ecol.* 57: 302–310.

Goryluk A., H. Rekosz-Burlaga and M. Błaszczyk. 2009. Isolation and characterization of bacterial endophytes of *Chelidonium majus* L. *Pol. J. Microbiol.* 58: 355–361.

Guan S.H., I. Sattler, W.H. Lin, D.A. Guo and S. Grabley. 2005. p-Aminoacetophenonic acids produced by a mangrove endophyte: *Streptomyces griseus* subspecies. *J. Nat. Prod.* 68: 1198–1200.

Ho M-Y., W-Ch. Chung, H-Ch. Huang and W-H. Chung. 2012. Identification of endophytic fungi of medicinal herbs of Lauraceae and Rutaceae with antimicrobial property. *Taiwania* 57: 229–241.

Hui L., W. Xiaoxian, H. Meizhe, Z. Zhenzhen, W. Minqian, T. Qin, L. Changhong, K. Brian, G. Yucheng, S. Jinglei and others. 2012. Endophytic *Bacillus subtilis* ZZ120 and its potential application in control of replant diseases. *Afr. J. Biotechnol.* 11: 231–242.

Hung P.Q. and K. Annapurna. 2004. Isolation and characterization of endophytic bacteria in soybean (*Glycine* sp.). *Omonrice* 12: 92–101.

Johri P. and B. Johri. 2004. Phylogenetic analysis of bacterial endophytes showing antagonism against Rhizoctonia solani. *Curr. Sci.* 87: 687–692.

Kusari S., M. Lamshoft, S. Zuhkle and M. Spiteller. 2008. An endophytic fungus from *Hypericum perforatum* that produces hypericin. *J. Nat. Prod.* 71(2): 159–162.

Larkin M., G. Blackshields, N. Brown, R. Chenna, P. McGettigan, H. McWilliam, F. Valentin, I. Wallace, A. Wilm, R. Lopez and others. 2007. ClustalW and ClustalX version 2. *Bioinformatics* 23(21): 2947–2948.

Liu X.L., S.L. Liu, M. Liu, B.H. Kong, L. Liu and Y.H. Li. 2014. A primary assessment of the endophytic bacterial community in a xerophilous moss (*Grimmia montana*) using molecular method and cultivated isolates. *Braz. J. Microbiol.* 45: 163–174.

Lodewyckx C., S. Taghavi, M. Mergeay, J. Vangronsveld, H. Clijsters and D. van der Lelie. 2001. The effect of recombinant heavy metal resistant endophytic bacteria in heavy metal uptake by their host plant. *Int. J. Phytoremediation* 3: 173–187.

Miller C.M., R.V. Miller, D. Garton-Kenny, B. Redgrave, J. Sears, M.M. Condron, D.B. Teplow DB and G. Strobel. 1998. Ecomycins, unique antimycotics from *Pseudomonas viridiflava*. *J. Appl. Microbiol.* 84: 937–944.

Muthomi J.W. and E.W. Mutitu. 2003. Occurrence of mycotoxin producing *Fusarium* species and other fungi on wheat kernels harvested in selected districts of Kenya. *African Crop Science Conference Proceedings* 6: 290–294.

Narayan Ch.P., S.H. Ji, J.X. Deng and S.H. Yu. 2013. Assemblages of endophytic bacteria in chili pepper (*Capsicum annuum* L.) and their antifungal activity against phytopathogens *in vitro*. *Plant Omics. J.* 6: 441–448.

Owen N. and N. Hundley. 2004. Endophytes- the chemical synthesizers inside plants. *Sci. Prog.* 87: 79–99.

Ożarowski A. 1976. Herbal handbook for doctors. (in Polish). National Institute of Medical Publications, Warsaw Poland

Reinhold-Hurek B. and T. Hurek. 1998. Life in grasses: diazotrophic endophytes. *Trends in Microbiology* 6: 139–144.

Rekosz-Burlaga H., M. Borys and A. Goryluk-Salmonowicz. 2014. Cultivable microorganisms inhabiting the aerial parts of *Hypericum*

perforatum. Acta Scientarum Polonarum Hortorum Cultus 13(5): 117–129.

Saitou N. and M. Nei. 1987. The neighbor-joining method: A new method for reconstructing phylogenetic trees. *Mol. Biol. Evol.* 4: 406–425.

Sgroy V., F. Cassán, O. Masciarelli, M. Florencia Del Papa, A. Lagares and V. Luna. 2009. Isolation and characterization of endophytic plant growth-promoting (PGPB) or stress homeostasis-regulating (PSHB) bacteria associated to the halophyte *Prosopis strombulifera*. *Appl. Microbiol. Biotechnol.* 85: 371–381.

Soka Y., S. Magdalena and D. Rachelia. 2012. The genetic diversity of endophytic and phyllosphere bacteria from several Indonesian herbal plants. *Makara J. Sci.* 16: 39–45.

Stierle A., G. Strobel and D. Stierle. 1993. Taxol and taxane production by T*axomyces andreanae*, an endophytic fungus of pacific yew. *Science* 260: 214–216.

Strobel G. and B. Daisy. 2003. Bioprospecting for microbial endophytes and their natural products. *Microb Mol Biol R* 67: 491–501.

Strobel G., B. Daisy, U. Castillo and J. Harper. 2004. Natural products from endophytic microorganisms. *J. Nat. Prod.* 67: 257–268.

Tamura K., M. Nei and S. Kumar. 2004. Prospects for inferring very large phylogenies by using the neighbor-joining method. *Proc. Natl. Acad. Sci. USA* 101: 11030-11035.

Tamura K., D. Peterson, N. Peterson, G. Stecher, M. Nei and S. Kumar. 2011. MEGA5: molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. *Mol. Biol. Evol.* 10:2731–2739.

Tan R.X. and W.X. Zou. 2001. Endophytes: a rich source of functional metabolites. *Nat. Prod. Rep.* 18: 448–459.

Tschen J.S. and C.N. Tseng. 1989. Bacereutin, an antifungal antibiotic isolated from metabolites of *Bacillus cereus* CHU 130. *Proc. Natl. Sci. Council*, *PR China-Part B. Life Sciences* 13: 258–61.

Vos P., G. Garrity, D. Jones, N. Krieg, W. Ludwig, F. Rainey, K-H. Schleifer and W. Whitman (eds.). 2009. Volume 3: The Firmicutes. In: Bergey's Manual of Systematic Bacteriology. 2nd ed., Springer-Verlag, New York, NY.