The Role of Entophytic Microorganisms in Biocontrol of Plant Diseases

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Abstract: Endophytic microorganisms are to be found in virtually every plant on earth. Endophytic microorganisms exist within the living tissues of most plant species and do so in a variety of relationships, ranging from symbiotic to slightly pathogenic. Root endophytic microorganisms are seen as promising alternatives to replace chemical pesticides and fertilizers in sustainable and organic agriculture systems. The capability of colonizing internal host tissues has made endophytes valuable for agriculture as a tool to improve crop performance. This association is often mutualistic: endophytes provide the plant with antagonism against diseases. Once inside the plant, an endophyte occupies a niche with relatively low competition from other microorganisms, provided the endophyte gets there first. Novel endophytes usually have associated with them novel secondary natural products and/or processes. Identifying, understanding and utilizing endophytes or its products to control of plant diseases and to enhance crop production are integral parts of sustainable agriculture. In this review, we addressed the major topics concerning the control of plant diseases by entophytic microorganisms mediated by specific metabolites of microbial origin. [Life Science Journal 2010;7(2):102-107]. (ISSN: 1097-8135).

Keywords: Biological control, Entophytic microorganisms, pathogens

1. Introduction

Plant pathogens include fungi are the most visible threats to sustainable food production. Plant The decreasing efficacy of the fungicides as well as risks associated with fungicide residues on the leaves and fruit, have highlighted the need for a more effective and safer alternative control measures. In recent years, entophytes has received increasing attention as a promising supplement or alternative to chemical control. The strategic use of naturally occurring organisms to control pest populations and increase production of major crops represents a viable option to host-plant resistance and pesticide-based pest and pathogen control. Endophytic microorganisms, microorganisms that grow in the intercellular spaces of higher plants, are recognized as one of the most chemically promising groups of microorganisms in terms of diversity and pharmaceutical potential **(**Wagenaar and Clardy, 2001**).** Beneficial endophytic microorganisms comprise especially fungi and bacteria that colonize internal plant tissues without causing visible damage to their hosts (Petrini, 1991). Furthermore, the endophytic microorganisms are not considered as saprophytes since they are associated with living tissues, and may in some way contribute to the well being of the plant. Endophytes exist in a range of tissue types within a broad range of plants, colonizing the plant systemically with bacterial colonies and biofilms, residing latently in intercellular spaces, inside the vascular tissue or within cells (Ulrich *et al.* 2008). Endophytes, microorganisms that reside in the tissues of living plants, are relatively unstudied and potential sources of novel natural products for exploitation in agriculture. That is, the plant is thought to provide nutrients to the microbe, while the microbe may produce factors that protect the host plant from attack by animals, insects or microbes (Yang *et al.*, 1994). Studies on microorganisms from plant species are recently becoming more frequent, since these fungi and bacteria have been studied for biological control and production of compounds with pharmacological properties. They are different from phytopathogenic microorganisms because they are not detrimental, do not cause diseases to plants, and are distinct from epiphytic microorganisms which live on the surface of plant organs and tissues (Hallmann *et al*., 1997). Endophytic bacteria are able to penetrate and become systemically disseminated in the host plant, actively colonizing the apoplast (Quadt-Hallmann *et al.*, 1997b), conducting vessels (Hallmann *et al.,* 1997), and occasionally the intracellular spaces (Quadt-Hallmann *et al*., 1997a). This colonization presents an ecological niche, similar to that occupied by plant pathogens, and this endophytic bacteria can, therefore, act as biological control agents against pathogens (Hallmann *et al.,* 1997). In this sense, the suppression of plant diseases due to the action of endophytic microorganisms has been demonstrated in several pathosystems (Narisawa *et al*., 1998). Several mechanisms may control this suppression, either directly on the pathogen inside the plant by antibiosis (Sturz *et al.,* 1998) and competition for nutrients (Mari *et al.,* 1996 and Puentea, *et al.,* 2009), or indirectly by induction of plant resistance response (M'Piga *et al.,* 1997) and more recently, their potential for enhanced degradation of several pollutants has also been investigated (Doty 2008). There are many reports demonstrating that many bioactive compounds could be produced by endophytic microorganisms (Huang *et al*., 2001). At the same time, molecular markers provide gigantic sources of data that can assist scientists in developing tools to monitor the genetic and environmental fate of these agents.

In the present review we will focus on examples of associations between endophytic microorganisms and plants, especially those that result in diseases control. The intent of this review is to provide insights into the presence of endophytes in nature, the products that they make, and how some of these organisms are beginning to show some potential for control of plant pests and diseases.

2. What is an Endophyte?

The term endophyte refers to interior colonization of plants by bacterial or fungal microorganisms. Endophytic microorganisms, microorganisms that grow in the intercellular spaces of higher plants, are recognized as one of the most chemically promising groups of microorganisms in terms of diversity and pharmaceutical potential (Wagenaar and Clardy, 2001). Furthermore, the endophytic microorganisms are not considered as saprophytes since they are associated with living tissues, and may in some way contribute to the well being of the plant. It seems that other microbial forms, e.g., mycoplasmas and archaebacteria, most certainly exist in plants as endophytes, but no evidence for them has yet been presented. The most frequently isolated endophytes are the fungi. Endophytic bacteria colonize an ecological niche similar to that colonized by plant pathogens but do not cause damage to their hosts. It turns out that the vast majority of plants have not been studied for their endophytes. Thus, enormous opportunities exist for the recovery of novel fungal forms, taxa, and biotypes. Hawksworth and Rossman estimated there may be as many as 1 million different fungal species, yet only about 100,000 have been described (Hawksworth, 1991). As more evidence accumulates, estimates keep rising as to the actual number of fungal species. It seems obvious that endophytes are a rich and reliable source of genetic diversity and novel, undescribed species. The endophytes that we are most concerned with are the ones growing inside a turfgrass plant. Finally, in our experience, novel microbes usually have associated with them novel natural products. This fact alone helps eliminate the problems of dereplication in compound discovery.

3. Effects of entophytic microorganisms towards pathogens

Indeed, intensive work has shown that endophytic microorganisms can have the capacity to control pathogens (Duijff**,** *et al*., 1997 and Sturz, and Matheson. 1996), and nematodes (Hallmann *et al.,* 1998). The first record of an endophyte affecting a plant disease was that by Shimanuki (1987) who showed that timothy (*Phleum pratense*) plants infected with the choke fungus, *Epichloe typhina*, were resistant to the fungus *Cladosporium phlei*.

In some cases, they can also accelerate seedling emergence and promote plant establishment under adverse conditions and enhance plant growth and development (Lazarovits, and Nowak. 1997, and Pillay, and Nowak. 1997). Furthermore, several antagonistic entophytes bacterial species have been isolated from the xylem of lemon roots (*Citrus jambhiri*), including *Achromobacter* spp., *Acinetobacter baumannii*, *A. lwoffii*, *Alcaligenes-Moraxella* spp., *Alcaligenes* sp., *Arthrobacter* spp., *Bacillus* spp., *Burkholderia cepacia*, *Citrobacter freundii*, *Corynebacterium* spp., *Curtobacterium flaccumfaciens*, *Enterobacter cloacae*, *E. aerogenes*, *Methylobacterium extorquens*, *Pantoea agglomerans*, *Pseudomonas aeruginosa*, and *Pseudomonas* spp. against root pathogens (Araújo ***et al***., 2001, and Lima ***et al.***, 1994). Several bacterial endophytes have been reported to support growth and improve the health of plants (Hallmann *et al.*, 1997, Stoltzfus ***et al.***, 1998) and therefore may be important sources of biocontrol agents. *Erwinia carotovora*, for example, is inhibited by numerous endophytic bacteria, including several strains of *Pseudomonas* sp. ,*Curtobacterium luteum*, and *Pantoea agglomerans* (Sturz ***et al.***, 1999). Furthermore, Wilhelm ***et al***. (1997) demonstrated that *Bacillus subtilis* strains isolated from the xylem sap of healthy chestnut trees exhibit antifungal effects against *Cryphonectria parasitica* causing chestnut blight. Endophytic bacteria have the ability to promote growth and inhibit plant disease, and as they are in intimate contact with the plant they are an attractive choice as biological control agents. For example, Sturz *et al*. (1999) found that 61 of 192 endophytic bacterial isolates from potato stem tissues were effective biocontrol agents against *Clavibacter michiganensis* subsp. *sepedonicus*. In oak, endophytic bacteria biologically active against the oak wilt pathogen *Ceratocystis fagacearum* have been isolated (Brooks ***et al.***, 1994). A number of the biologically active endophytes and root-colonizing microorganisms that have been isolated or detected belong to the actinobacterial phylum, specifically the genus *Streptomyces* (Coombs, and Franco. 2003, Sessitsch ***et al***., 2001, Xaio ***et al***., 2002). The first actinobacterial endophyte isolated, belonging to the genus *Frankia*, is a nitrogen-fixing actinobacterium that forms actinorhizae with eight families of angiosperms (Provorov ***et al***., 2002). A number of endophytic actinobacteria were previously isolated by culture-dependent methods, with the major genera being *Streptomyces*, *Microbispora*, *Micromonospora*, and *Nocardioides* (Coombs and. Franco. 2003). A number of these isolates were capable of suppressing fungal pathogens of wheat in vitro and in planta, including *Rhizoctonia solani*, *Pythium* spp., and *Gaeumannomyces graminis* var *tritici*, indicating their potential use as biocontrol agents (Coombs ***et al.***, 2003).

4. Mechanisms of diseases control displayed by endophytic

In this sense, the suppression of plant diseases due to the action of endophytic microorganisms has been demonstrated in several pathosystems (Narisawa ***et al.***, 1998). Several mechanisms may control this suppression, either directly on the pathogen inside the plant by antibiosis and competition for nutrients , or indirectly by induction of plant resistance response

(M'Piga *et al*., 1997). Endophytes usually occur in above-ground plant tissues, but also occasionally in roots (for example, dark septate endophytic fungi have been isolated from various plants), and are different from mycorrhizae by lacking external hyphae (Mandyam and Jumpponen, 2005 and Leho Tedersoo *et al*., 2009). Although some root endophytic fungus requires host cell death for proliferation during forming mutualistic symbiosis with plant (Deshmukh *et al.*, 2006), it is universally hypothe-sized that endophyte-host interactions involve a balance of antagonism and exhibit great phenotypic plasticity compared to plant pathogens (Schulz and Boyle, 2005). Only few documents refer to the plant secondary metabolism mediated by the fungal endophytes. Currently, endophytes are viewed as an outstanding source of bioactive natural products because there are so many of them occupying literally millions of unique biological niches (higher plants) growing in so many unusual environments. Thus, it appears that these biotypical factors can be important in plant selection, since they may govern the novelty and biological activity of the products associated with endophytic microbes. Peppermint growth and terpene production of *in vitro* generated plants (*Mentha piperita*) in response to inoculation with a leaf fungal endophyte indicate variation of the essential oil profile by fungal infection. The other study showed that the weight of roots, seedlings and terpenoid production of *Euphorbia pekinensis* increased after they were inoculated with an extensive host range endophytic *Phomopsis* sp. Meanwhile, microbial elicitor derived from some fungal endophytes also promotes biomass and induces the terpenoids (artemisinin) biosynthesis and production in plant suspension cells (Wang et al., 2006). It seems likely that both mycorrhizal fungi and fungal endophytes infection might result in specific-enhancement of the MEP pathway metabolic flux in plants. The red resin of *Dracaena cochinchinensis* is commonly used in traditional Chinese medicine for the treatment of traumatic and visceral hemorrhages. Chemical studies have revealed that the resin contains various flavonoids (Zheng *et al*., 2004). In addition, endophytic actinomycetes may also affect plant growth either by nutrient assimilation or enhanced secondary metabolites (anthocyanin) synthesis (Hasegawa *et al*., 2006). Furthermore, the production of antimicrobial substances, such as antibiotics or HCN, is an important mechanism to fight phytopathogens (Blumer, and Haas. 2000). Koshino *et al*. (1989) have described compounds, toxic to some fungi, which include sesquiterpenes, chokols, hydroxyl-unsaturated fats, phenolic glycerides and an aromatic sterol which are produced in the mycelial-choked heads of timothy. Endophytes effectively inhibits and kills certain other fungi and bacteria by producing a mixture of volatile compounds (Strobel *et al.*, 2001). The majority of these compounds have been identified by gas chromatography-mass spectrometry, synthesized or acquired, and then ultimately made into an artificial mixture. This mixture mimicked the antibiotic effects of the volatile compounds produced by the fungus. The newly described *Muscodor roseus* was twice obtained from tree species growing in the Northern Territory of Australia. This fungus is just as effective in causing inhibition and death of test microbes in the laboratory as *Muscodor albus* (Worapong *et al*., 2002). Another endophytic s*treptomycete* (NRRL 30566), from a fern-leaved *Grevillea* tree (*Grevillea pteridifolia*) growing in the Northern Territory of Australia, produces, in culture, novel antibiotics called kakadumycins **(**Castillo *et al.*, 2003). Each of these antibiotics contains, by virtue of their amino acid compositions, alanine, serine, and an unknown amino acid. Colletotric acid, a metabolite of *Colletotrichum gloeosporioides*, an endophytic fungus in *Artemisia mongolica*, displays antimicrobial activity against bacteria as well as against the fungus *Helminthsporium sativum* (Zou *et al*., 2000). Another *Colletotrichum* sp., isolated from *Artemisia annua*, produces bioactive metabolites that showed varied antimicrobial activity as well. Yue *et al.* (2000) have identified a number of compounds produced by cultures of *Epichloe* and *Neotyphodium* species that have antifungal activity against the chestnut blight fungus *Cryphonectria parasitica* and suggest that they may play a similar role against other pathogens, the compounds in this study which showed the greatest antifungal activity were the indole derivatives indole-3-acetic acid and indole-3-ethanol, a sesquiterpene and a diacetamide. Indirect disease control is achieved by mechanisms modulating the plant immune response, including the induction of systemic acquired resistance (van Wees *et al*. 1999).

5. Genetic and environmental modifications influencing diseases control by endophytes

Identification of endophytes has relied mainly upon cultivation-based methods (Bell *et al.*, 1995). Molecular techniques based on the rRNA gene as a phylogenetic marker (Amann *et al.,* 1995) provide a powerful approach to circumvent drawbacks related to cultivation . Molecular markers provide the means to assess genetic variation in endophytes and host plants, providing an insight into the relationship between variation in endophyte and host plants and the variability of agronomic traits (Gamper *et al*., 2008). Researchers have endeavored to elucidate the molecular mechanisms during the establishment of plant-endophytic association (Bailey *et al.,* 2006). Techniques such as terminal restriction fragment length polymorphism (T-RFLP) analysis or denaturing gradient gel electrophoresis (Smalla, *et al*., 2001) in combination with sequence analysis of rRNA genes allow rapid characterization of microbial communities. Comparison with data from amplified fragment length polymorphism (AFLP) data demonstrated that the SSR markers are informative for assessing genetic variation within and between endophyte species. Following the development of these markers for the sensitive detection of endophytes *in planta*, the assessment of endophyte diversity in a globally-distributed pool of perennial ryegrass germplasm are reported. Recently, Garbeva *et al.* (2001) monitored endophytic populations of potato by PCR-denaturing gradient gel electrophoresis, which revealed the occurrence of a range of organisms falling into several distinct phylogenetic groups. Their results also suggested the presence of nonculturable endophytes in potato.

6. Concluding

Endophytes are a poorly investigated group of microorganisms that represent an abundant and dependable source of bioactive and chemically novel compounds with potential for exploitation in a wide variety of medical, agricultural, and industrial arenas. The mechanisms through which endophytes exist and respond to their surroundings must be better understood in order to be more predictive about which higher plants to seek, study, and spend time isolating microfloral components. This may facilitate the product discovery processes. The results presented in this review show that, as expected, great diversity has been found among endophytes isolated from plant hosts. They play important roles for protecting plants against diseases. Certainly, one of the major problems facing the future of endophyte biology and natural-product discovery is the rapid diminishment of rainforests, which hold the greatest possible resource for acquiring novel microorganisms and their products. The role of endophyes protecting plants against diseases has been quite well studied. However, Countries need to establish information bases of their biodiversity and at the same time begin to make national collections of microorganisms that live in these areas.

Referance

1. Amann, R. I., W. Ludwig, and K. H. Schleifer. 1995. Phylogenetic identification and in situ detection of individual microbial cells without cultivation. Microbiol. Rev. 59:143-169.
2. Araújo, W. L., H. O. Saridakis, P. A. V. Barroso, C. I. Aguilar-Vildoso, and J. L. Azevedo. 2001. Variability and interactions between endophytic bacteria and fungi isolated from leaf tissues of citrus rootstocks. Can. J. Microbiol. 47:229-236.
3. Bailey, B.A., Bae, H., Strem, M.D., Robert, D.P., Thomas, S.E., Crozier, J., Samuels, G.J., Choi, I.k.-Young, Holmes, K.A. 2006. Fungal and plant gene expression during the colonization of cacao seedlings by endophytic isolates of four *Trichoderma* species. Planta. 224: 1449–1464.
4. Bell, C. R., G. A. Dickie, W. L. G. Harvey, and J. W. Y. F. Chan. 1995. Endophytic bacteria in grapevine. Can. J. Microbiol. 41:46-53.
5. Blumer, C., and D. Haas. 2000. Mechanism, regulation, and ecological role of bacterial cyanide biosynthesis. Arch. Microbiol. 173:170-177.
6. Brooks, D. S., C. F. Gonzalez, D. N. Appel, and T. J. Filer. 1994. Evaluation of endophytic bacteria as potential biological control agents for oak wilt. Biol. Control 4:373-381.
7. Castillo, U., J. K. Harper, G. A. Strobel, J. Sears, K. Alesi, E. Ford, J. Lin, M. Hunter, M. Maranta, H. Ge, D. Yaver, J. B. Jensen, H. Porter, R. Robison, D. Millar, W. M. Hess, M. Condron, and D. Teplow. 2003. Kakadumycins, novel antibiotics from Streptomyces sp. NRRL 30566, an endophyte of Grevillea pteridifolia. FEMS Lett. 224:183-190.
8. Coombs, J. T., and C. M. M. Franco. 2003. Isolation and identification of actinobacteria isolated from surface-sterilized wheat roots. Appl. Environ. Microbiol. 69:5303-5308.
9. Coombs, J. T., P. P. Michelsen, and C. M. M. Franco. 2003. Evaluation of endophytic actinobacteria as antagonists of *Gaeumannomyces graminis var. tritici* in wheat. Biol. Control 29:359-366.
10. Deshmukh, S., Hückelhoven, R., Schäfer, P., Imani, J., Sharma, M., l. Weiss, M., Waller, F., Kogel, K.H. 2006. The root endophytic fungus *Piriformos-pora indica* requires host cell death for proliferation during mutualistic symbiosis with barley. Proc. Natl. Acad. Sci. 103: 18450–18457.
11. Doty S.L. 2008. Tansley review: enhancing phytoremediation through the use of transgenics and endophytes. New Phytol 179:318–333
12. Duijff, B. J., V. Gianinazzi-Pearsonand, and P. Lemanceau. 1997. Involvement of the outer membrane lipopolysaccharides in the endophytic colonization of tomato roots by biocontrol *Pseudomonas fluorescens* strain WCS417r. New Phytol. 135:325-334.
13. Hallmann, J., A. Quadt-Hallmann, R. Rodríguez-Kábana, and J. W. Kloepper. 1998. Interactions between *Meloidogyne incognita* and endophytic bacteria in cotton and cucumber. Soil Biol. Biochem. 30:925-937.
14. Hallmann, J., A. Quadt-Hallmann, W. F. Mahaffee, and J. W. Kloepper. 1997. Bacterial endophytes in agricultural crops. Can. J. Microbiol. 43:895-914.
15. Hasegawa, S., Meguro, A., Shimizu, M., Nishimura, T., Kunoh, H. 2006. Endophytic actinomycetes and their interactions with host plants. Actinomycetologica. 20: 72–81.
16. Hawkswo, D.L. 1991. The fungal dimension of biodiversity: magnitude, significance, and conservation. Mycol. Res., 95, s. 641-655.
17. Huang Y., Wang J., L.I. G., Zheng Z., Su W. 2001. Antitumor and antifungal activities in endophytic fungi isolated from pharmaceutical plants Taxus mairei, Cephalotaxus fortunei and Torreya grandis. FEMS Immunol. Med. Microbiol., 34, s. 163-167.
18. Gamper H.A., Young J.P.W., Jones D.L., Hodge A. 2008. Real-time PCR and microscopy: are the two methods measuring the same unit of arbuscular mycorrhizal fungal abundance?. *Fungal Genetics and Biology* 45: 581–596.
19. Garbeva, P., L. S. van Overbeek, J. W. L. van Vuurde, and J. D. van Elsas. 2001. Analysis of endophytic bacterial communities of potato by plating and denaturing gradient gel electrophoresis (DGGE) of 16S rDNA based PCR fragments. Microb. Ecol. 41:369-383.
20. Koshino, H., Yoshihara, T. , Sakamura, Y. , Shimanuki, S., Sato, T. and Tajimi. A. 1989. A

ring B aromatic sterol from stromata of*Epichloe typhina*.Phytochemistry 28:771-772

21. Leho Tedersoo, Pärtel, K., Jairus, T., Gates, G., Põldmaa, K. and Tamm , H. .2209. Ascomycetes associated wit ectomycorrhizas: molecular diversity and ecology with particular reference to the ***Helotiales*** Environmental Microbiology .11(12), 3166–3178
22. Lazarovits, G., and J. Nowak. 1997. Rhizobacteria for improvement of plant growth and establishment. Hortscience 32:188-192.
23. Lima, G., A. Ippolito, F. Nigro, and M. Salerno. 1994. Attempts in the biological control of citrus mal secco (*Phoma tracheiphila*) using endophytic bacteria. Dif. Piante 17:43-49.
24. M'piga, P., Bélanger, R.R., Paulitz, T.C. and Benhamou, N. 1997. Increased resistance to *Fusarium oxysporum* f. sp. *radicis-lycopersici* in tomato plants treated with the endophytic bacterium *Pseudomonas fluorescens* strain 63-28. Physiological and Molecular Plant Pathology, v.50, p.301-320.
25. Narisawa, K., Tokumasu, S.and Hashiba, T. 1998. Suppression of clubroot formation in chinese cabbage by the root endophytic fungus, *Heteroconium chaetospira*. Plant Pathology, v.47, p.206-210.
26. Pillay, V. J., and J. Nowak. 1997. Inoculum density, temperature and genotype effects on in vitro growth promotion and epiphytic and endophytic colonization of tomato (*Lycopersicum esculentum* L.) seedlings inoculated with a pseudomonad bacterium. Can. J. Microbiol. 43:354-361.
27. Provorov, N. A., A. Y. Borisov, and I. A. Tikhonovich. 2002. Developmental genetics and evolution of symbiotic structures in nitrogen-fixing nodules and arbuscular mycorrhiza. J. Theor. Biol. 214:215-232.
28. Puentea, M., Ching Y. Li b,1, Yoav Bashana,C. 2009. Endophytic bacteria in cacti seeds can improve the development of cactus Seedlings. Environmental and Experimental Botany 66 (2009) 402–408
29. Schulz, B., Boyle, C. 2005. The endophytic continuum.Mycol. Res. 109: 661–686.
30. Sherameti, I., Shahollari ,B., Venus, Y., Altschmied, L., Varma, A., Oelmuller, R. 2005. The endophytic fungus *Piriformospora indica* stimulates the expression of nitrate reductase and the starch-degrading enzyme glucan-water dikinase in tobacco and *Arabidopsis* roots through a homeodomain transcription factor that binds to a conserved motif in their promoters. J. Biol. Chem. 280: 26241–26247.
31. Shimanuki, T. 1987. Studies on the mechanisms of the infection of timothy with purple spot disease caused by *Cladosporium* (Gregory) de Vries. *In* Res. Bull. 148 Hokkaido Natl. Agric. Exp. Sta. pp 1-56.
32. Smalla, K., G. Wieland, A. Buchner, A. Zock, J. Parzy, S. Kaiser, N. Roskot, H. Heuer, and G. Berg. 2001. Bulk and rhizosphere soil bacterial communities studied by denaturing gradient gel electrophoresis: plant-dependent enrichment and seasonal shifts revealed. Appl. Environ. Microbiol. 67:4742-4751.
33. Strobel, G. A., Dirksie, E., Sears, J. and. Markworth, C. 2001. Volatile antimicrobials from a novel endophytic fungus. Microbiology 147:2943-2950
34. Sturz, A. V., and Matheson. B. G. 1996. Populations of endophytic bacteria which influence host-resistance to ***Erwinia***-induced bacterial soft rot in potato tubers. Plant Soil 184:265-271.
35. Sessitsch, A., B. Reiter, U. Pfeifer, and E. Wilhelm. 2001. Cultivation-independent population analysis of bacterial endophytes in three potato varieties based on eubacterial and Actinomycetes-specific PCR of 16S rRNA genes. FEMS Microbiol. Ecol. 1305:1-10.
36. Stoltzfus, J. R., R. So, P. P. Malarvithi, J. K. Ladha, and F. J. de Bruijn. 1998. Isolation of endophytic bacteria from rice and assessment of their potential for supplying rice with biologically fixed nitrogen. Plant Soil 194:25-36.
37. Sturz, A. V., B. R. Christie, B. G. Matheson, W. J. Arsenault, and N. A. Buchanan. 1999. Endophytic bacterial communities in the periderm of potato tubers and their potential to improve resistance to soil-borne plant pathogens. Plant Pathol. 48:360-369.
38. Ulrich, K., Ulrich A. and Ewald, D. 2008 . Diversity of endophytic bacterial communities in poplar grown under field conditions. FEMS Microbiol Ecol 63:169–180
39. Worapong, J., G. A. Strobel, B. Daisy, U. Castillo, G. Baird, and W. M. Hess. 2002. *Muscodor roseus* anna. nov. an endophyte from *Grevillea pteridifolia*. Mycotaxon 81:463-475.
40. van Wees, S. C., M. Luijendijk, I. Smoorenburg, L. C. van Loon, and C. M. Pieterse. 1999. Rhizobacteria-mediated induced systemic resistance (ISR) in *Arabidopsis* is not associated with a direct effect on expression of known defense-related genes but stimulates the expression of the jasmonate-inducible gene *Atvsp* upon challenge. Plant Mol. Biol. 41:537-549.
41. Wagenaar, M.M.and Clardy, J. 2001. Dicerandrols, new antibiotics and cytotoxic dimmers produced by the fungus *Phomopsis longicolla* isolated from an endangered mint. J. Nat. Prod., 64, s. 1006-1009.
42. Wang, J.W., Zheng, L.P.and Tan, R.X. 2006. The Preparation of an elicitor from a fungal endophyte to enhance artemisinin production in hairy root Cultures of *Artemisia annua* L. Chin. J. Biotechnol. 22: 829–834.
43. Wilhelm, E., W. Arthofer, and Schafleitner, R. 1997. *Bacillus subtilis*, an endophyte of chestnut (*Castanea sativa*), as antagonist against chestnut blight (*Cryphonectria parasitica*), p. 331-337. *In* A. C. Cassells (ed.), Pathogen and microbial contamination management in micropropagation. Kluwer Academic Publishers, Dortrecht, The Netherlands.

44. Xaio, K., L. L. Kinkel, and D. A. Samac. (2002). Biological control of *Phytophthora* root rots on alfalfa and soybean with *Streptomyces*. Biol. Control 23:285-295.
45. Yang, X., Strobel, G., Stierle, A., Hess, W.M., Lee, J. and Clardy, J. 1994. A fungal endophyte-tree relationship: Phoma sp. in Taxus wallachiana. Plant Sci., 102, , s. 1-9.
46. Yue, Q., Miller, C. J. ,White, J. F. and Richardson. M. D. 2000. Isolation and characterization of fungal inhibitors from *Epichloe festucae.* J. Agric. Food Chem. 48:4687-4692.
47. Zheng, Q.A., Li, H.Z., Zhang, Y.J., Yang, C.R. (2004). Flavonoids from the resin of *Dracaena cochinchinensis*. Helvetica Chim. Acta.87: 1167–1171.
48. Zou, W. X., J. C. Meng, H. Lu, G. X. Chen, G. X. Shi, T. Y. Zhang, and R. X. Tan. 2000. Metabolites of *Colletotrichum gloeosporioides*, an endophytic fungus in *Artemisia mongolica*. J. Nat. Prod. 63:1529-1530.

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