**Innovative Role of Endophytes in Management of Economically Important Diseases of Different Crops**

Ali Anwar, Effat Shahnaz, Asha Nabi, F. A. Bhat, Fazil Fayaz Wani, Shahid Mushtaq and T. A. Wani

Division of Plant Pathology, Faculty of Agriculture, SKUAST-K, Wadura, Sopore-193201, Jammu and Kashmir, India

Corresponding Author: zaman04@rediffmail.com

**Introduction**

Plant pathogens include fungi are the most visible threats to sustainable food production. 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, endophytes have 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 microorganismsthat 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 associatedwith 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). Endophytic 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 plantfrom attack by animals, insects or microbes (Yang *et al.,* 1994). Studies on microorganisms from plant species arerecently becoming more frequent, since these fungi and bacteria have been studied for biological control andproduction of compounds with pharmacological properties. They are different from phytopathogenic microorganismsbecause they are not detrimental, do not cause diseases to plants and are distinct from epiphytic microorganismswhich live on the surface of plant organs and tissues (Hallmann*et al*., 1997). Endophytic bacteria are able topenetrate and become systemically disseminated in the host plant, actively colonizing the apoplast (Quadt-Hallmann*etal*., 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 these endophytic bacteria can, therefore, act as biological control agents against pathogens (Hallmann*et al.,* 1997). In thissense, the suppression of plant diseases due to the action of endophytic microorganisms has been demonstrated inseveral pathosystems (Narisawa*et al*., 1998). Severalmechanisms may control this suppression, either directlyon the pathogen inside the plant by antibiosis (Sturz*et al.,* 1996) and competition for nutrients (Puentea, *et al.,* 2009), or indirectly by induction of plant resistance response (M'Piga*et al.,* 1997) and more recentlytheir potential for enhanced degradation of several pollutants has also been investigated (Doty, 2008). Thereare many reports demonstrating that many bioactivecompounds 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.

**Fungal Endophytes**

Endophytic fungi are taxonomically and biologically diverse but all share the character of colonizing internal plant tissues without causing apparent harm to their host (Wilson, 1995). The best understood of these are members of the Clavicipitaceae (Ascomycota), which are endophytes of some temperate grasses. In these systems, there is usually only one endophytic fungal species per host and these fungi appear to be highly coevolved with their host. Generally, these fungi are transmitted vertically (from mother to offspring through seeds, as reviewed by Clay and Schardl (2002); see also Saikkonen*et al.* (2004)). This transmission pattern is thought to promote beneficial relationships with the host plant (Herre*et al.,* 1999). Nonetheless, in grasses, the net effect of endophyte associations can range from parasitic (e.g., choke disease) to strongly mutualistic (Clay and Schardl, 2002). Beneficial effects for hosts include increased drought tolerance (Arechavaleta*et al.,* 1989), deterrence of insect herbivores (Breen, 1994; Rowan and Latch, 1994), protection against nematodes (Pedersen *et al.,* 1988; West *et al.,* 1988; Kimmons*et al.,* 1990), and resistance against fungal pathogens (Gwinn and Gavin, 1992; Bonos*et al.,* 2005; Clarke *et al.,* 2006). The last is also true for endophytes found in some tropical grasses (Kelemu*et al.,* 2001). Anti-pathogen protection mediated by endophytes has been observed also in nongramineous hosts. For example, endophytic fungi have been found to protect tomatoes (Hallman and Sikora, 1995) and bananas (Pocasangre*et al.,* 2001; Sikora*et al.,* 2008) from nematodes, and beans and barley (Boyle *et al.,* 2001) from fungal pathogens. However, even with the accumulating evidence that endophytic fungi can reduce pathogen damage in grasses and other host plants, little is known about the generality of this role in natural systems and whether it can be exploited as a biocontrol strategy in crop protection.

**How fungal endophytes can affect plant disease**

Evidence showing that endophytes have a role in the outcome of plant – pathogen interactions leading to disease has been increasing in recent years. Diverse mechanisms by which they may counteract pathogen development have been observed. For example, some endophytic species may induce plant defense mechanisms which counteract pathogen attack, others produce antibiotic substances which inhibit pathogen growth, competition for plant space and resources may also occur between resident endophytes and incoming pathogens; finally, some parasites of plant pathogens are known to behave as endophytes.

**Interactions with plant pathogenic fungi**

Many endophytic species produce antibiotic substances (Strobel, 2002; Schulz and Boyle, 2005; Wang *et al*., 2007). Liquid extracts from endophyte cultureshave been found to inhibit the growth of several speciesof plant pathogenic fungi (Liu *et al*., 2001; Park *et al*., 2005; Inácio*et al*., 2006; Kim *et al*., 2007). If suchcompounds where produced by endophytes*in planta*, this could constitute a defense mechanism against fungalpathogens. Experiments where plant protection against pathogenic fungi is observed after the inoculation of plants with endophytes, as well as after the application of endophytic culture filtrates, suggest that the endophyte may produce an antifungal compound or a substance that induces plant defense mechanisms in the plant. This is the case with *Chaetomium*and *Phoma*endophytes of wheat, when these fungi were previously inoculated in plants, reduced severity of foliar disease caused by *Puccinia*and *Pyrenophora*spp. was observed and, the same protective effect was observed when only endophytic culture filtrates were applied to the plants(Dingle and McGee, 2003; Istifadah and McGee, 2006). In these experiments the effects of culture filtratesupon plant pathogens were not tested.When a mixture of six species of endophytes frequently isolated from cacao (*Theobroma cacao* L*.*) trees was used to inoculate leaves of endophyte-free seedlings of this plant species, the severity of a leaf disease caused by a *Phytophthora*sp. was significantly reduced in endophyte-inoculated leaves. A mechanism of induced plant resistance did not seem to be involved, because differences in disease severity were observed between endophyte-inoculated and non-inoculated leaves of the same plant. In this case, the protection against a pathogen could be the result of direct competition among endophytes already present in leaves and the pathogen (Arnold *et al*., 2003). For instance, most tissue available for infection may be already occupied, or endophytes may produce zones of inhibition restricting the entry of other fungi.Endophyte infection may alter plant biochemistry in a way that defense mechanisms against pathogens are induced. *Piriformosporaindica*Sav.Verma, Aj. Varma, Rexer, G. Kost and P. Franken is a root endophyte with a wide host range, including several species of cereals and *Arabidopsis*. Barley plants inoculated with this endophyte have shown resistance to a vascular[*Fusariumculmorum*(W.G. Sm.) Sacc.]and a leaf pathogen [*Blumeriagraminis*(DC.) Speer] in addition to an increase in yield and salt stress tolerance (Waller *et al*., 2005). The protection against the leaf pathogen appears to be mediated by a mechanism of induced resistance, because in the pathogen-inoculated plants there is a defense response involving the death of host cells. Some endophytes may be mycoparasites. *Acremoniumstrictum*W. Gams is an endophyte which has been frequently isolated from *Dactylisglomerata*L. and other grasses (Sánchez Márquez*et al*., 2007); recently it has been shown that this fungus is a mycoparasite of *Helminthosporiumsolani*Durieu and Mont., a potato pathogen (Rivera Varas*et al*., 2007). A significant increase in resistance to dollar spot disease, caused by *Sclerotiniahomoeocarpa*F.T. Benn., has been observed in *Festucarubra*L. cultivars infected by *Epichloëfestucae*Leuchtm.,Schardl and M.R. Siegel*.* (Clarke *et al*., 2006). Cultivars of several turfgrass species infected by *Epichloë*and *Neotyphodium*endophytes are commercially available at the present time. The efficient vertical transmission of these endophytes has allowed the production of infected seed at a commercial scale. Since *Neotyphodium*and *Epichloë* infected cultivars have shown increased resistance to herbivores, plant pathogens, and some conditions of abiotic stress, the use of such symbiotic cultivars can result in a reduction in the use of insecticides and fungicides in lawns (Brilman, 2005). Similar applications of other species of endophytes may be seen in the future. The above studies suggest that the outcome of some pathogen attacks may be dependent on the endophyticmycobiota associated to a host plant. Therefore, the endophytic assemblage of a given species may represent a source of organisms with potential applications for disease control in the same plant species. Out of the multiple species that can penetrate and infect a plant, only a relatively small subset, that of the pathogens, produces disease. This shows that part of the plant disease cycle is shared by pathogens of endophytes. Once a fungus enters a plant it can behave as an endophyte or as a pathogen, and it seems that a majority of plant associated fungi act as endophytes.What is the difference between infection processes caused by endophytes and pathogens, is a good question for plant pathologists. Some studies directed to answer such question point out to fungal as well as to host characteristics. For instance, a mutation in a single locus can convert a pathogen such as *Colletotrichummagna* Jenkins and Winstead in a mutualistic endophyte (Freeman and Rodríguez, 1993). However, some isolates of the above species may behave as a pathogen in cucurbits or as an endophyte in some species of other plant families (Redman *et al*., 2001).

**Interactions with nematodes**

Inhibitory effects against some species of migratory and sedentary endoparasites occur in grasses infected by *Neotyphodium*endophytes (West *et al*., 1988; Kimmons*et al*., 1990). *Neotyphodium*species infect aerial tissues, not roots. Therefore, the inhibitory effects observed in infected plants were interpreted as the result of fungal alkaloids being translocated to roots. This was supported by the fact that some naturally occuring*Neotyphodium*strains deficient in the production of ergot alkaloids do not show protective effects as good against *Pratylenchus*sp. as those observed in ergot alkaloid producing strains (Timper*et al*., 2005). In contrast, other experiments showed that the amount of ergot alkaloids translocated to roots is very small, and experiments with *Neotyphodium*knockout mutants having their pathway of ergot alkaloid synthesis disrupted suggested that these alkaloids are not responsible for the inhibition of nematode populations in endophyte infected plants (Panaccione*et al*., 2006). Nevertheless, other types of alkaloids with antiherbivore activity are produced by *Neotyphodium*species, and chemical changes such as the production of phenolic compounds do occur in *Neotyphodium*-infected roots (Malinowski and Belesky, 2000). In conclusion, *Neotyphodium*endophytes provide host plants with protection against several nematode species, but the mechanism of action underlying this process is still unknown. Non pathogenic strains of *Fusariumoxysporum*E.F. Sm. and Swingle isolated from plant roots are other group of endophytes known to be implicated in antinematode activity. Culture filtrates of *F. oxysporum* have an inhibitory effect on *Meloidogyne incognita*Kofoid and White, suggesting that fungal toxins could be the mechanism of interaction (Hallmann and Sikora, 1996). However, the mechanism of *Fusarium*inhibition of nematodes appears to be more complex than a toxinoperated system. In an experimental setup where banana plants were grown in a split root system, the plantswere resistant to *Radopholussimilis*Cobb, Thorne in the root half which was not inoculated with a *Fusarium*endophyte. In this case, a phenomenon of systemic plant resistance induced by the endophyte appeared to be the mechanism of resistance to the nematode pathogen (Vu *et al*., 2006). Another type of plant protection mediated by endophytic fungi may come from nematophagous fungi which can inhabit plant roots as endophytes (Bordallo*et al*., 2002). In a similar fashion, some species of entomophagous fungi [e.g. *Beauveriabassiana*(Bals.-Criv.) Vuill.*,Torrubiellaconfragosa*Mains*, Metarhiziumanisopliae*(Metschn.) Sorokin] have been isolated from several host plants, and appears that part of their life cycle can be endophytic (Bills, 1996). In conclusion, it is very likely that fungal endophytes affect the outcome of nematode attacks in plants, and certain endophytes could be used for nematode protection in agriculture.

**Interactions with bacteria and viruses**

Tests of the influence of endophytes upon bacterial and viral pathogens are not as numerous as those made with other plant pathogens. Bactericidal effects of endophyte culture extracts have been demonstrated and do not seem to differ from those observed for fungi or nematodes (Wang *et al.,* 2007). In the case of viruses, the incidence of *Barley yellow dwarf virus* (BYDV) was lower in *Loliumpratense*infected by *Neotyphodium*than in endophyte free plants.Since BYDV is transmitted by means of aphid vectors,toxic fungal alkaloids may be the reason for this effect,in fact, aphid reproduction was lower in endophyte infected plants than in those free of endophyte (Lehtonen*et al*., 2006). A very interesting connection of a different kind exists among endophytes and viruses. A *Curvularia*endophyte of the plant *Dichanteliumlanuginosum*(Elliott) Gould was found to confer tolerance to high soil temperatures to the plant. Further observation of this system led to the discovery that a virus infecting the endophyte was an important factor contributing to the heat tolerance observed in the plants. Furthermore, the virus-infected endophyte could be used to confer heat tolerance to tomato plants (Márquez*et al*., 2007). *Epichloëfestucae* virus 1 (EfV1) is another virus which asymptomatically infects the grass endophyte*Epichloëfestucae*, in this case it is not known if the presence of the virus in the endophyte affects the plant host (Romo*et al*., 2007).

**Bacteria Endophytes**

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). Endophytic bacteria colonize an ecological niche similar to that of plant pathogens, especially vascular wilt pathogens, which might favor them as candidates for biocontrol agents. In addition.intensive work on rhizospherebiocontrol agents has recently shown that presence of six rhizobacteria, induced systemic resistance in cucumber and exhibited both external and internal root colonization (Kloepper*et al*., 1992b). Exploiting an additional microbial habitat for biocontrol purposes might enhance overall disease control and increase control consistency, since the control agent could avoid unfavorable conditions in one habitat by escaping into the other habitat.

**Fungal Disease Suppression by endophytes**

**Wilt diseases**

Studies using endophytic bacteria as biocontrol agents are conducted mostly on wilt diseasesof different commercial and food crops. Endophytic bacteria isolated from potato tubers showed *in vitro* antibiosis against *F. avenaciarum, F. sambucinum*and *F. oxysporum*. The antibiosis of the isolates decreased progressively with depth of their site of isolation from the tuber surface. Possibly, the plants might have adopted bacteria as part of a disease suppressive response to pathogenic attack (Sturz*et al*., 1999.). Endophytic bacteria isolated from live oak stems, showed *in vitro* antagonism against oak wilt pathogen, *C.fagacearum*(Brooks *et al*., 1994). A pre-inoculation with endophytic isolates *P. denitrificans* and *P. putida*significantly reduced crown loss. *Bacillus* spp. injected into Spanish oak stems, enabled more efficient colonization of the plants by *Pseudomonas spp*. (Brooks *et al*., 1994). Endophytic bacteria isolated from rape and tomato plants, inhibited mycelial growth and reduced the incidence and severity of diseases caused by *V. dahliae*and *F. oxysporum*f sp. *lycopersici*in these crops by more than 75% and increased the plant height and shoot dry weight (Nejad and Johnson, 2000). These strains produce volatile metabolites other than hydrogen cyanide. *Bacillus* spp., present in xylem vessels, reduced the percentage of silver maple stem colonization by *V. dahliae*(Hall *et al*., 1986.).

**Rots and Damping-off Diseases**

Endophytic bacteria isolated from rice seeds, when applied as seed treatment, colonized thestellar region of the root and exhibited strong anti-fungal activity against *R. solani, Pythiummyrotylum, Guamanomycesgraminis*and *Heterobasidiumannosum* (Mukhopadhyay*et al*., 1996). Two endophytic bacterial strains isolated from cotton, which were antagonistic to *R. solani in vitro*, reduced infection of cotton plants from *R. solani* infection by 60% (Qui *et al*., 1990.), Of the 170 endophytic bacterial strains isolated from cotton, 40 strains protected cotton plants from *R. solani*infection (Chen *et al*., 1995.). Endophytic bacteria from potato tubers were inhibitory to *Phytophthorainfestans*(Sturz*etal*., 1999). Endophytic*Bacillus* and *Pseudomonas* spp. isolated from seeds and plants of different crops were tested for their antagonistic potential against various pathogenic fungi including *P. ultimum, R. solani and S. rolfsii*(Pleban*et al*., 1995). *P. fluorescens*, isolated from cotton, *B. cereus* strain 65 from Sinapis. *B. cerells*from cauliflower.*B. pumulis*from sunflower, *B. subtilis*from onion were found antagonistic to one or more of the fungal pathogens tested. Radioactive labelling showed that *B. cereus* strain 65, when introduced into cotton, was present at the root stem junction for 16 days. The bacteria were found up to 72 days after their introduction into the root and stem atlevels of 2.8X 105 and 5Xl04 CFU g-1 fresh weight of root and stem tissue, respectively. Various endophytic bacterial strains tested,including *B. cereus* strain 65, when introduced into the plant during the seed germination period, protected cotton and bean seedlings from infection with *R. solani*and *S. rolfsii*by more than 50%. Strain 65 produced a 36 kDachitinolytic enzyme characterized as chitobiosidase and the crude preparation of extracellular proteins significantly decreased spore germination of *F. oxysporum*f.sp. *meloni*(Pleban*et al*., 1997). *P. fluorescens*, isolated from the internal tissues of micropropagated apple plantlets, effectively colonized the root tips of beans. This strain was cloned to express the chiA gene coding for the major chitinase of *Serratiamarcescens*, and the recombinant strain when introduced into bean seedlings provided effective protection from *R. solani*. Addition of this bacterium to soil did not confer protection indicating that internal colonization by *P. fluorescens*was essential for disease control (Downing and Thomson, 2000).

**Galls and Abnormal Growth**

Lima *et al*. (1994) isolated 160 bacterial strains from the woody tissues of lemon and sour orange, 55 of which were antagonistic to *Phomatracheiphila*the casual agent of citrus malsecco disease. Nine of the most effective antagonistic strains were tested for disease control by inoculating them into the stem of sour orange seedlings 15 days before pathogen inoculation. Three isolates of *B. subtilis*and one isolate of *P. fluorescens*significantly lowered the disease symptoms and maintained higher populations in the internal tissues of the host plant (Lima *et al*., 1994.)

**Leaf spots and leaf blights**

Limited attempts have been made to use endophytic bacteria as biocontrol agents of leaf spot and leaf blight diseases. *Bacillus subtilis*, isolated from xylem fluid of chestnuts, suppressed the growth of chestnut blight pathogen, *Cryphonectriaparasitica*and reduced the lesion areas on stems, when applied 3 days prior to fungal challenge, in an *in vitro* bioassay (Wilhelm *etal*., 1998). Also.*B. subtilis*induced the synthesis of acidic chitinase and β- 1,3-glucanase in chestnut (Wilhelm *et al*., 1998). Krishna Murthy and Gnanamanickam (1997) reported that *Psuedomonas*spp. induced systemic resistance in rice against sheath blight diseases caused by *R. solani*. Inspite of the absence of this bacterium on plant surfaces, its presence in the internal stem led to suppression of disease. In a survey of antagonistic microorganisms, 8 bacteria and 24 fungi were found antagonistic to *P. infestans*in the phyllosphere,rhizosphere and endoshpere of tomato (Garita*et al*., 1988) indicated the endophytic nature of antagonistic organisms. Crown gall caused by *Agrobacterium tumefaciens*is one of the most extensively worked out system for biological control using rhizosphere isolates (Kerr, 1980). Endophytic bacteria isolated from xylem sap of grapevine plants were screened for their antagonistic activity against a range of tumerogenic*A. tumefaciens*biovar 3 strains (Bell *et al*., 1995). Despite the variabilities in *in vitro* antibiosis, 24 of the 851 strains were strongly inhibitory to *A. vitis*, which produces galls in grapevine. These bacteria were identified as *E. agglomerans,Rahnellaaquatilis*and *Pseudomonas* spp. An isolate of *P. corrugata*effectively reduced the populations of *Agrobacterium* strains *in situ*, In further trials with grape cultivar, chardonnay, where the incidence of galled vines was moderate, three endophytic bacterial strains gave significant protection against the disease. But these strains were ineffective in protecting gewurztraminer vines. In which the incidence of galls was severe (Bell *et al*., 1995). These studies also suggest that the performance of endophytic bacteria as biocontrol agents is largely dependent on the host genotype.

**Nematode diseases**

Endophytic bacteria have an additional advantage in contro1 of nematodes since the wounds produced by nematodes favour bacterial colonization of the root surface and their introduction into the root tissue (Bookbinder *et al*., 1982; Khan, 1993). Inoculation of cotton and cucumber plants with *Meloidogyne incognita* resulted in a higher density and diversity of total endophytic bacteria in these plants. To determine, the interaction between Meloidogyne and endophytic bacteria *E. asburiae*Strain JM22 was used as a model system (Hallmann*et al*., 1998). A large number of JM22 were observed on the surface of nematode galls, especially where the root epidermis had disrupted due to gall expansion. Accumulation of bacterial cells around necrotic plant cells in the vicinity of galls was observed by electron microphotographs (Hallmann*et al*., 1998). Soil amendment with chitin (1 % w/w), protected cotton from the attack of plant-parasitic nematodes (Hallmann*et al*., 1999).When the soil bacteria and endophytic microbial communities in chitin-amended and non-amended soils were compared.*B. cepacia*populations were found to be same in both the soils, but it effectively colonized the internal tissues of cotton only in chitin-amended soil (Hallmann*et al*., 1999). Seven endophytic bacteria, *Aerococcusviridans, B. megaterium, B. subtilis,P. chlororaphis, P. vesicularis, S. marcescens*and *Sphingomonaspaucimobilis*isolated from cotton and cucumber plants, on seed bacterization, significantly protected cucumber seedlings from *M. incognata*infection (Hallmann*et al*., 1995).

**Effects of endophytic microorganisms towardspathogens**

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 (*Phleumpratense*) plantsinfected with the choke fungus, *Epichloetyphina*, were resistant to the fungus *Cladosporiumphlei*. 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., *Acinetobacterbaumannii*, *A. lwoffii*, *Alcaligenes-Moraxella* spp., *Alcaligenes*sp., *Arthrobacter*spp., *Bacillus* spp., *Burkholderiacepacia*, *Citrobacterfreundii*, *Corynebacterium*spp., *Curtobacteriumflaccumfaciens*, *Enterobacter cloacae*, *E. aerogenes*, *Methylobacteriumextorquens*, *Pantoeaagglomerans*, *Pseudomonasaeruginosa*, 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. *Erwiniacarotovora*, for example, is inhibited by numerous endophytic bacteria, including several strains of *Pseudomonas* sp. *,Curtobacteriumluteum*, and *Pantoeaagglomerans*(Sturz*et al.,* 1999). Furthermore, Wilhelm *et al*. (1997)demonstrated that *Bacillus subtilis*strains isolated from thexylem sap of healthy chestnut trees exhibit antifungaleffects against *Cryphonectriaparasitica*causing chestnutblight. Endophytic bacteria have the ability to promotegrowth and inhibit plant disease, and as they are in intimatecontact with the plant they are an attractive choice asbiological control agents. For example, Sturz*et al*. (1999)found that 61 of 192 endophytic bacterial isolates frompotato stem tissues were effective biocontrol agents against*Clavibactermichiganensis*subsp. *sepedonicus*. In oak,endophytic bacteria biologically active against the oak wiltpathogen *Ceratocystisfagacearum*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 actinobacterialphylum, specifically the genus *Streptomyces* (Coombs, and

Franco. 2003, Sessitsch*et al*., 2001, Xaio*et al*., 2002). The first actinobacterialendophyte 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 endophyticactinobacteria were previously isolated by culturedependentmethods, 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 invitro and in planta, including *Rhizoctoniasolani*, *Pythium*spp., and *Gaeumannomycesgraminis*var*tritici*, indicating their potential use as biocontrol agents (Coombs *et al.,* 2003).

**Mechanisms of diseases control displayed byEndophytes**

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 septateendophytic fungi have been isolated from various plants), and are different from mycorrhizae by lacking external hyphae (LehoTedersoo*et al*., 2009). Although some root endophyticfungus requires host cell death for proliferation during forming mutualistic symbiosis with plant (Deshmukh*et al.,* 2006), it is universally hypothe-sized that endophyte-hostinteractions 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 becausethere 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(*Menthapiperita*) in response to inoculation with a leaf fungal endophyte indicate variation of the essential oil profile by fungal infection. The other study showed that theweight 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 endophytesinfection 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, endophyticactinomycetes may also affect plant growth either by nutrient assimilation or enhancedsecondary metabolites (anthocyanin) synthesis. Furthermore, the production of antimicrobialsubstances, 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 inhibit and killcertain other fungi and bacteria by producing a mixture ofvolatile 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 *Muscodorroseus*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 *Muscodoralbus*(Worapong*et al*., 2002). Another endophytics*treptomycete*(NRRL 30566), from a fern-leaved *Grevillea*tree (*Grevilleapteridifolia*) 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 *Colletotrichumgloeosporioides*, an endophytic fungus in *Artemisiamongolica*, displays antimicrobial activity against bacteria as well as against the fungus *Helminthsporiumsativum*(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 *Cryphonectriaparasitica*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).

**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 phylogeneticmarker (Amann*et al.,* 1995) provide a powerful approachto 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 plantendophyticassociation (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*inplanta*, the assessment of endophyte diversity in a globallydistributedpool 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 nonculturableendophytes in potato.

**References**

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.

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.

Arechavaleta, M., Bacon, C.W., Hoveland, C.S., Radcliffe, D., 1989.Effect of the tall fescue endophyte on plant response to environmental stress.*Agronomy Journal***81**: 83–90.

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.

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.

Blumer, C., and D. Haas. 2000. Mechanism, regulation, and ecological role of bacterial cyanide biosynthesis. *Arch. Microbiol.***173**:170-177.

Bonos, S.A., Wilson, M.M., Meyer, W.A., Reed Funk, C., 2005.Suppression of red thread in fine fescues through endophyte-mediated resistance.Applied Turfgrass Science.doi:10.1094/ATS-2005-0725-01-RS.

Boyle, C., Gotz, M., Dammann-Tugend, U., Schultz, B., 2001.Endophyte– host interaction III.Local vs. Systemic colonization.*Symbiosis***31**: 259–281.

Breen, J.P., 1994. Acremoniumendophyte interactions with enhanced plant resistance to insects.*Annual Review of Entomology***39**: 401–423.

Brilman, L.A., 2005. Endophytes in turfgrass cultivars. In: *Neotyphodium*in cool season grasses (Roberts C.A., West C.P., Spiers D.E., eds). Blackwell Publishing, Iowa, USA. pp. 341-349.

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.

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 Grevilleapteridifolia. *FEMS Lett.***224**:183-190.

Clarke B.B., White J.F., Hurley H., Torres M.S., Sun S., Huff D.R., 2006. Endophyte-mediated suppression of dollar spot disease in fine fescues.*Plant Dis***90**: 994-998.

Clarke, B.B., White Jr., J.F., Hurley, R.H., Torres, M.S., Sun, S., Huff, D.R., 2006. Endophyte-mediated suppression of dollar spot disease in fine fescues. Plant Disease 90, 994–998.

Clay, K., Schardl, C., 2002. Evolutionary origins and ecological consequences of endophyte symbiosis with grasses.*The American Naturalist***160**: 99–127.

Clay, K., Schardl, C., 2002. Evolutionary origins and ecological consequences of endophyte symbiosis with grasses.*The American Naturalist***160**: 99–127.

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

Coombs, J. T., P. P. Michelsen, and C. M. M. Franco. 2003. Evaluation of endophyticactinobacteria as antagonists of *Gaeumannomycesgraminis var. tritici*in wheat. *Biol. Control***29**:359-366.

Deshmukh, S., Hückelhoven, R., Schäfer, P., Imani, J., Sharma, M., l. Weiss, M., Waller, F., Kogel, K.H. 2006. The root endophytic fungus *Piriformos-poraindica*requires host cell death for proliferation during mutualistic symbiosis with barley. *Proc. Natl. Acad. Sci.***103**: 18450–18457.

Doty S.L. 2008. Tansley review: enhancing phytoremediation through the use of transgenics and endophytes. *New Phytol***179**:318–333.

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.

Freeman S., Rodríguez R.J., 1993. Genetic conversion of a fungal plant pathogen to a non-pathogenic, endophyticmutualist.*Science***260**: 75-78.

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 arbuscularmycorrhizal fungal abundance?*Fungal Genetics andBiology* **45**: 581–596.

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 anddenaturing gradient gel electrophoresis (DGGE) of 16S rDNA based PCR fragments. *Microb. Ecol.***41**:369-383.

Gwinn, K.D., Gavin, A.M., 1992. Relationship between endophyte infestation level of tall fescue seed lots and Rhizoctoniazeaeseedling disease.*Plant Disease***76**:911–914.

Hallman, J., Sikora, R., 1995. Influence of Fusariumoxysporum, a mutualistic fungal endophyte, on Meloidogyne incognita infection of tomato. *Journal of Plant Disease and Protection***101:** 475–481.

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.

Hallmann, J., A. Quadt-Hallmann, W. F. Mahaffee, and J. W. Kloepper. 1997. Bacterial endophytes in agricultural crops. *Can. J. Microbiol.* 43:895-914.

Hawksworth, D.L. 1991. The fungal dimension of biodiversity: magnitude, significance, and conservation. *Mycol. Res.,***95**: 641-655.

Herre, E.A., Knowlton, N., Muller, U., Rehner, S., 1999. The evolution of mutualisms: exploring the paths between conflict and cooperation. *Trends in Ecology & Evolution***14**: 49–53.

Huang Y., Wang J., L.I. G., Zheng Z., Su W. 2001. Antitumor and antifungal activities in endophytic fungi isolated from pharmaceutical plants Taxusmairei, Cephalotaxusfortunei and Torreyagrandis. *FEMS Immunol. Med. Microbiol.,***34**:163-167.

Kelemu, S., White, J.F., Mun˜oz, F., Takayama, Y., 2001. An endophyte of tropical forage grass Brachiariabrizantha. Isolating, identifying and characterizing the fungus and determining its antimycotic properties. *Canadian Journal of Microbiology***47**:55–62.

Kim, H.Y., Choi G.J., Lee H.B., Lee S.W., Kim H.K., Jang K.S., Son S.W., Lee S.O., Cho K.Y., Sung N.D., Kim J.C., 2007. Some fungal endophytes from vegetable crops and their anti-oomycete activities against tomato late blight.*LettApplMicrobiol***44**:332-337.

Kimmons, C.A., Gwinn, K.D., Bernard, E.C., 1990.Nematode reproduction on endophyte-infected and endophyte-free tall fescue.*Plant Disease***74**: 757–761.

Koshino, H., Yoshihara, T. ,Sakamura, Y. , Shimanuki, S., Sato, T. and Tajimi. A. 1989. A ring B aromatic sterol from stromata of*Epichloetyphina.Phytochemistry***28**:771-772

Lazarovits, G., and J. Nowak. 1997. Rhizobacteria for improvement of plant growth and establishment. *Hortscience***32**:188-192.

LehoTedersoo, Pärtel, K., Jairus, T., Gates, G., Põldmaa, K. and Tamm , H. .2209. Ascomycetes associated witectomycorrhizas: molecular diversity and ecology with particular reference to the *Helotiales*. .Environmental Microbiology .11(12), 3166–3178 20

Lima, G., A. Ippolito, F. Nigro, and M. Salerno. 1994. Attempts in the biological control of citrus mal secco (*Phomatracheiphila*) using endophytic bacteria. Dif. Piante 17:43-49.

LIU C.H., ZOU W.X., LU H., TAN R.X., 2001. Antifungal activity of *Artemisia annua*endophyte cultures against phytopathogenic fungi. J Biotech 88, 277-282.

M'piga, P., Bélanger, R.R., Paulitz, T.C. and Benhamou, N. 1997. Increased resistance to *Fusariumoxysporum*f. sp. *radicis-lycopersici*in tomato plants treated with the endophytic bacterium *Pseudomonasfluorescens*strain 63-28. Physiological and Molecular Plant Pathology, v.50, p.301-320.

Narisawa, K., Tokumasu, S.andHashiba, T. 1998. Suppression of clubroot formation in chinese cabbage by the root endophytic fungus, *Heteroconiumchaetospira*. Plant Pathology, v.47, p.206-210.

nov.anendophyte from *Grevilleapteridifolia*. Mycotaxon 81:463-475.

PARK J.H., CHOI G.J., LEE H.B., KIM K.M., JUNG H.S., LEE S.W., JANG K.S., CHO K.Y., 2005. Griseofulvin

pathogenic fungi. J Microbiol Biotech 15, 112-117.

Pedersen, J.F., Rodriguez-Kabana, R., Shelby, R.A., 1988. Ryegrass cultivars and endophyte in tall fescue affect nematodes in grass and succeeding soybean. Agronomy Journal 80, 811–814.

PFENNING L.H., ARAUJO A.R., 2006.Antifungal metabolites from *Colletotrichumgloeosporioides*, an endophytic fungus in *Cryptocaryamandioccana*Nees (Lauraceae).BiochSystEcol 34, 822-824.

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 (*Lycopersicumesculentum*L.) seedlings inoculated with a pseudomonad bacterium. *Can. J. Microbiol*.**43**:354-361.

Pocasangre, L., Sikora, R.A., Vilich, V., Schuster, R.P., 2001. Survey of banana endophytic fungi from Central America and screening for biological control of burrowing nematode (*Rhadopholussimilis*).*ActaHorticulturae***531**: 283–290.

Provorov, N. A., A. Y. Borisov, and I. A. Tikhonovich. 2002. Developmental genetics and evolution of symbiotic structures in nitrogen-fixing nodules and arbuscularmycorrhiza. J. Theor. Biol. 214:215-232.

Puentea, M., Ching Y. Li b,1, YoavBashana,C. 2009. Endophytic bacteria in cacti seeds can improve the development of cactus Seedlings. *Environmental and Experimental Botany***66:** 402–408.

RiveraVaras V.V, Freeman T.A., Gusmestad N.C., SecorG.A., 2007.Mycoparasitism of *Helminthosporiumsolani*by *Acremoniumstrictum*.*Phytopathology***97**: 1331-1337.

Rowan, D.D., Latch, G.C.M., 1994. Utilization of endophyte-infected perennial ryegrasses for increased insect resistance. In: Bacon, C.W., White, J.F., Jr. (Eds.), Biotechnology of Endophytic Fungi of Grasses. CRS Press, Boca Raton, pp. 169–183.

Saikkonen, K., Wali, P., Helander, M., Faeth, S.H., 2004.Evolution of endophyte–plant symbioses.*Trends in Plant Science***9**: 275–280.

Sánchez Márquez S., Bills G.F., Zabalgogeazcoa, I., 2007. The endophyticmycobiota of the grass *Dactylisglomerata*.*Fung Divers***27**: 171-195.

schulz B., Boyle C., 2005. The endophytic continuum.*Mycol Res***109**:661-686.

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.

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.

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.

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.

Strobel G.A., 2002. Rainforest endophytes and bioactive products.*Crit Rev Biotech***22**: 315-333.

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

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.

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.

Ulrich, K., Ulrich A. and Ewald, D. 2008 .Diversity of endophytic bacterial communities in poplar grown under field conditions.*FEMS MicrobiolEcol***63**:169– 180

Wagenaar, M.M.andClardy, J. 2001. Dicerandrols, new antibiotics and cytotoxic dimmers produced by the fungus *Phomopsislongicolla*isolated from an endangered mint. *J. Nat. Prod.,***64**: 1006-1009

Waller F., Achatz B., Baltruschat H., Fodor j., Becker K., Fischer M., Heier T., Hückelhoven Wang F.W, Jiao R.H., Cheng A.B., Tan S.H., Song Y.C. 2007. Antimicrobial potentials of endophytic fungi residing in *quercusvariabilis*and brefeldina obtained from *cladosporium*sp. *World j microbiol biotech***23:**79-83.

West, C.P., Izekor, P., Robbins, R.T., Oosterhuis, D.M., Robbins, R.T., 1988. The effect of *Acremoniumcoenophialum*on the growth and nematode infestation of tall fescue.*Plant and Soil* **112:** 3–6.

Wilson, D., 1995. Endophyte—The evolution of a term and clarification of its use and definition. *Oikos***73**: 274–276.

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.

Yang, X., Strobel, G., Stierle, A., Hess, W.M., Lee, J. and Clardy, J. 1994. A fungal endophyte-tree relationship: Phoma sp. in Taxuswallachiana. *Plant Sci.,***102** : 1-9.

Yue, Q., Miller, C. J. ,White, J. F. and Richardson. M. D. 2000. Isolation and characterization of fungal inhibitors from *Epichloefestucae.J. Agric. Food Chem.***48**:4687-4692.

Zheng, Q.A., Li, H.Z., Zhang, Y.J., Yang, C.R. (2004). Flavonoids from the resin of *Dracaenacochinchinensis*.*Helvetica Chim. Acta.***87**: 1167–1171.

Zou, W. X., J. C. Meng, H. Lu, G. X. Chen, G. X. Shi, T. Y. Zhang, and R. X. Tan. 2000. Metabolites of *Colletotrichumgloeosporioides*, an endophytic fungus in *Artemisia mongolica*. *J. Nat. Prod.***63**:1529-1530.