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Impact of Endophytic Bacteria in Plants

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Abstract

Endophytic bacteria reside within the plant hosts without causing any visible disease symptoms. They are ubiquitously associated with almost every plant studied, where they colonize the internal tissues of their host plant and can form a range of different relationship such as symbiotic, mutualistic, commensalism and trophobiotic. Endophytic bacteria promote plant growth and yield, suppress pathogens. Endophytic bacteria can produce a wide range of natural products that could be harnessed for potential use in medicine, agriculture or industry. In addition endophytic bacteria also found to have some important role in nutrient cycling, biodegradation, and bioremediation and may play a role in soil fertility through nitrogen fixation and phosphate solubilisation. There is an increasing interest in developing the potential biotechnological applications of endophytic bacteria for improving phytoremediation and the sustainable production of non-food crops for biomass and biofuel production. This article has been attempted to provide a brief review on the different roles of endophytic bacteria in plants and their potentials.

Keywords: *Endophyticbacteria, biodegradation,phytoremediation, biotechnology*

Introduction

Endophytic bacteria are those bacteria that are living within the plant tissues and have no external sign of infections and any visible harmful effects on the plants (Schulz & Boyle, 2006). These bacteria enter the plant tissues via germinating radicals (Gagne et al., 1987), stomatas (Roos et al.,1983), secondary roots (Agarwal et al.,1987),or as a result of foliar damages (Leben et al.,1968). Endophytic organisms inside a plant may either become localized at the entry or spread throughout the plant (Hallmann et al., 1987) and can reside within cells (Jacobs. et al, 1985) intercellular spaces, (Patriquin et al., 1978) or in the vascular system (Bell et al., 1995). There are certain evidences that generally bacterial population larger in roots and gradually decreases in stems and leaves (Lamb et al, 1996). Plants constitute large and diverse niches for endophytic organisms. Many endophytic bacteria are isolated from monocot and dicot species (Table-1) but very few are reported from Pteridophytes. *Amphibacillus* and *Gracilibacillus* were reported as endophytes for the first time in the fern *Dicksonia sellowiana* (Irene et al., 2010). It is

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believed that the endophytic bacteria occur at lower population densities than the rhizospheric bacteria or bacterial pathogens (Rosenblueth et al., 2004). Hallmann et al., 1997 suggested that the population of endophytic bacteria could be better protected from biotic and abiotic stresses than the rhizospheric bacteria. The association of endophytic bacteria can be obligate or facultative and cause no harm to the host plants. They exhibit complex interactions like mutualism and antagonism with their hosts (Parker et al., 1999). These bacteria maintain a stable symbiosis and produce several compounds that promote plant growth and also help them adapt better to the environment (Das et al., 2009). Endophytic bacteria can also be beneficial by bringing us a variety of benefits, such as novel and effective bioactive compounds that cannot be synthesized by chemical reactions (Dhanya et al., 2014) and are used in medicine, agriculture or industry. Beside these roles, it has been investigated that they have the potential to remove soil contaminations by enhancing phytoremediation and may play the role in soil fertility through nitrogen fixation, phosphate solubilisation. Now a days there is an increasing interest in developing the potential biotechnological applications of endophytic bacteria for improving phytoremediation and the production of sustainable non-food crops for biomass and biofuel production (Robert et al., 2014).This article has been attempted to provide a brief review on the overview of the role of endophytic bacteria and their potential applications in sustainable agriculture and in phytoremediation.

Table-1: Examples of some reported bacterial endophytes and plants harbouring (Monica et al., 2006).

Endophytes	Plant species	Reference
α Proteobacteria		
Azorhizobium caulinodans	Rice	Engelhard et al., 2000
Azospirillum brasilense	Banana	Weber et al., 1999
Azospirillum amazonense	Banana, Pineapple	Weber et al., 1999
Bradyrhizobium japonicum	Rice	Chantreuil et al., 2000
Gluconacetobacter sp.	Sugarcane, Coffee	Jiménez-Salgado et al., 1997
Methylobacterium mesophilicum[a]	Citrus plants	Araujo et al., 2002
Methylobacterium extorquens	Scots pine, Citrus plants	Araujo et al., 2002; Pirttilä et al., 2004
Rhizobium leguminosarum	Rice	Yanni et al., 1997
Rhizobium (Agrobacterium) radiobacter	Carrot, Rice	Surette et al., 2003
Sinorhizobium meliloti	Sweet potato	Reiter et al., 2003
Sphingomonas paucimobilis[a]	Rice	Engelhard et al., 2000
β Proteobacteria		
Azoarcus sp	Kallar grass, rice	Engelhard et al., 2000
Burkholderia pickettii[a]	Maize	McInroy and Kloepper 1995
Burkholderia cepacia[b]	Yellow lupine, Citrus plants	Araujo et al., 2001; Barac et al., 2004
Burkholderia sp	Banana,Pineapple, Rice	Weber et al., 1999; Engelhard et al., 2000
Chromobacterium violaceum[a]	Rice	Phillips et al., 2000
Herbaspirillum seropedicae	Sugarcane, Rice, Maize, Sorghum, Banana	Olivares et al., 1996; Weber et al., 1999
Herbaspirillum rubrisulbalbicans	Sugarcane	Olivares et al., 1996
Enterobacter spp.	Maize	McInroy and Kloepper 1995
Citrobacter sp.	Banana	Martínez et al., 2003
Enterobacter sakazakiia	Soybean	Kuklinsky-Sobral et al., 2004
Enterobacter cloacaea	Citrus plants, maize	Araujo et al., 2002; Hinton et al., 1995
Enterobacter agglomerans[a]	Soybean	Kuklinsky-Sobral et al., 2004
Enterobacter asburiae	Sweet potato	Asis and Adachi 2003
Erwinia sp.	Soybean	Kuklinsky-Sobral et al., 2004
Escherichia coli[b]	Lettuce	Ingham et al., 2005
Klebsiella sp.	Wheat, sweet potato, Rice	Engelhard et al., 2000; Iniguez et al., 2004; Reiter et al., 2003
Klebsiella pneumoniae[b]	Soybean	Kuklinsky-Sobral et al., 2004
Klebsiella variicola[b]	Banana, Rice, Maize, Sugarcane	Rosenblueth et al., 2004.
Klebsiella terrigena[a]	Carrot	Surette et al., 2003
Klebsiella oxytoca[b]	Soybean	Kuklinsky-Sobral et al., 2004
Pantoea sp.	Rice, Soybean	Verma et al., 2004
Pantoeaagglomerans	Citrus plants, Sweetpotato	Araujo et al., 2001, Adachi et al., 2003
Pseudomonas chlororaphis	Marigold (*Targets* spp.), Carrot	Sturz and Kimpinski 2004; Curette et al., 2003
Pseudomonas putida[a]	Carrot	Curette et al., 2003
Pseudomonas fluorescens	Carrot	Curette et al., 2003
Pseudomonas citronellolis	Soybean	Kuklinsky-Sobral et al., 2004
Pseudomonas synxantha	Scots pine	Prittilä et al., 2004
Salmonella enterica[b]	Alfalfa, carrot, radish	Cooley et al., 2003;
Serratia sp.	Rice	Sandhiya et al., 2005
Serratiamarcescens[a]	Rice	Gyaneshwar et al., 2001
Stenotrophomonas[a]	Dune grasses (*Ammophilaarenaria and Elymusmollis*)	Dalton et al., 2004
Firmicutes		
Bacillus spp.	Citrus plants	Araujo et al., 2001, 2002
Bacillus megaterium	Maize, Carrot, Citrus plants	McInroy and Kloepper 1995; Surette et al. 2003.
Clostridium	Grass *Miscanthussinensis*	Miyamoto et al. 2004
Paenibacillus odorifer	Sweet potato	Reiter et al. 2003
Staphylococcus saprophyticus[b],	Carrot	Surette et al. 2003
Bacteroidetes		
Sphingobacteriumsp.[a]	Rice	Phillips et al. 2000
Actinobacteria		
Arthrobacter globiformis	Maize	Chelius and Triplett2000a
Curtobacterium flaccumfaciens	Citrus plants	Araujo et al. 2002
Kocuria varians	Marigold	Sturz and Kimpinski 2004
Microbacterium esteraromaticum	Marigold	Sturz and Kimpinski 2004
Microbacterium testaceum	Maize	Zinniel et al. 2002
Mycobacterium sp.[b]	Wheat, Scots pine	Prittilä et al. 2005
Nocardia sp[b]	Citrus plants	Araujo et al. 2002
Streptomyces	Wheat	Coombs and Franco 2003a

[a]Opportunistic human pathogenic bacteria.
[b] Common human pathogenic bacteria.

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Endophytic bacteria as a biocontrol agent

Nowadays endophytic bacteria are used as an effective biocontrol agent rather than the chemical control. Endophytic bacteria are able to prevent the deleterious effects of certain pathogenic organisms. According to Compant.et al.2005, the beneficial effects of bacterial endophytes on their host plants are similar to the mechanisms for the rhizosphere associated bacteria. In vitro studies of the endophytic bacteria *Bacillus subtilis* isolated from *Speranskia tuberculata* (Bge) Bail was found to be strongly antagonistic to the pathogen *B. cinerea* (Wang et al,2009). It has been demonstrated that certain bacterial endophytes trigger a phenomenon known as induced systemic resistance (ISR), which is phenotypically similar to the systemic acquired resistance (SAR). The development of SAR occurs when a plant successfully activate their defence mechanism in response to the primary infection by a pathogen, especially when the latter induces a hypersensitive reaction through which it becomes limited in a local necrotic lesion of brown desiccated tissue (Van Loon et al., 1998; Robert et al., 2007). In ISR which is effective against different types of pathogen but differs from SAR is that the inducing bacterium does not cause visible symptoms on the host plant (Van Loon et al., 1998; Robert et al., 2007). A recent review on bacterial endophytes and their role in ISR have been done by Kloepper et al., 2006.Bacterial endophytes have the capacity to suppress nematode proliferation and this may benefit other crops in rotation with the host plants (Sturz et al.,2004). *Curtobacterium flaccumfaciens* frequently isolated from a symptomic citrus plants infected with the pathogen *Xyllela fastidiosa* suggested that the endophytic bacteria may help citrus plants to better resist the pathogenic infection (Araujo et al., 2002). It is believed that the endophytic bacteria from patato plants showed antagonistic activity against fungi (Berg et al. 2005). Some of these endophytic bacteria produced antibiotics and siderophores in vitro (Sessitsch et al., 2004). It has been shown that the endophytic actinobacteria are effective antagonists of the pathogenic fungus *Gaeumannomyces graminis* in wheat (Coombs et al. 2004). The most of the assays to the test antagonism in vitro is cost effective and it remains to be established if this correlates to effects in nature. Nowadays not only naturally occurring endophytes are used as biocontrol agents but also they are genetically engineered to express antipest proteins like lectins for insect control (Fahey et al., 1991). The endophytes, *Herbaspirillum seropedicae* and *Clavibacter xylii* have been genetically modified to produce and excrete the δ-endotoxin of *Bacillus thuringensis* to control insect pests (Downing et al. 2000). Recently the use of genetically engineered endophytic bacteria with biological control potential in agricultural crops has been one of the important studies.

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Natural metabolites from endophytic bacteria

Organism	Plant Association	Active Agent	Activity	Reference
Taxomycesandreanae	Taxusbrevifolia	Taxol	Anticancer	Strobel et al. (1993)
Pseudomonas viridiflava	Grass	Ecomycins B and C	Antimicrobial	Miller et al. (1998)
Streptomyces griseus	Kandeliacandel	p-Aminoacetophenonic acids	Antimicrobial	Guan et al. (2005)
Streptomyces NRRL 30562	Kennedianigriscans	Munumbicins Munumbicin D	Antibiotic Antimalarial	Castillo et al. (2002)
Streptomyces NRRL 30566 s	Grevilleapteridifolia	Kakadumycin	Antibiotic	Castillo et al. (2003)
Serratiamarcescens	Rhyncholacispenicillata	Oocydin A	Antifungal	Strobel et al. (2004)
Cytonaema sp.	Quercus sp. 103	Cytonic acids A and D	Antiviral	Guo et al. (2000)
Streptomyces sp.	Monstera sp.	Coronamycin	Antimalarial antifungal	Ezra et al. (2004)

Endophytic bacteria produce some specific metabolites which needed for the interaction with the host. Several endophytic bacterial genera like *Pseudomonas*, *Burkholderia* and *Bacillus* are the member of soil bacteria (Lodewyckx et al., 2002). These genera have diverse range of secondary metabolic products including antibiotics, antifungal, antiviral, anticancer compounds, volatile organic compounds, insecticidal and immunosuppressant agents. The natural products isolated from the endophytic bacteria (Table-2) which is active at low concentrations against a range of human, animal and plant pathogens. *Pseudomonas viridiflava*, an enophytes which has been isolated on and within the tissues of many grass species (Miller et al., 1998), was found to produce two novel antimicrobial compounds called ecomycins. Ecomycins represent a family of novel lipopeptides which made up of some unusual amino acids including homoserine and β-hydroxy aspartic acid. It was found that these compounds have the ability to inhibit the human pathogens *Cryptococcus neoformans* and *Candida albicans* (Miller et al., 1998). With the advent of time bioplastics are receiving increasing commercial interest. In the year 1926 Lemoigne first described a bioplastic, poly-3-hydroxybutyrate (PHB) produced by *Bacillus megaterium*. They are polyesters and

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are produced by a range of microorganisms cultured under different nutrient and environmental conditions. P-3-hydroxyalkanoate (PHA) and PHB are the most widely produced microbial bioplastic. Kalia et al., 2003 suggest that genomic analysis indicates that many species of bacteria have the potential to produce bioplastics.

Table-2: Natural products that have been derived or produced from various endophytic bacteria(Robert et al., 2008)

Role of endophytic bacteria in phytoremediation

The term phytoremediation refers to an emerging technology using plants and their associated microbes to remove, transfer, stabilize, decrease and decompose pollutant in the environment. Besides some higher plants there are many pteridophytes (Table: 3) which are identified as heavy metals cleaners from soil and water. Among them most works have been done with different species of *Pteris sp*. Komar et al. in 1998 discovered first the arsenic hyperaccumulator *Pteris vittata* L. (Chinese brake fern). The capacity of this plant in hyperaccumulation was also reported by several other scientists (Chen et al., 2002; Ma et al., 2001).

Table-3: List of heavy metal hyper accumulating pteridophytes

Ferns	**Heavy metals**	**References**
Pteris vittata L	As	Komar et al., 1998
Pteris cretica L	As	Zhao et al., 2002
Pteris longifolia L	As	Zhao et al., 2000
Pteris umbrosa R Br	As	Zhao et al., 2000
Pteris biaurita L	As	Srivastava et al.,2005
Pteris quadriaurita Retz	As	Srivastava et al.,2005
Pteris ryukyuensis Tagawa	As	Srivastava et al.,2005
Athyrium yokoscense (Fr & Sav) C Ch	Pb,Cd,Cu	Nishizono et al., 1987
Azolla filiculoides Lam	Pb,Cd, Cr, Ni, Zn	Benaroya et al.,2004
Salvinia minima Baker	Pb, cd	Olguin et al.,2002
Maesilea minuta L	Cd	Das etal.,2013

Several studies showed that the endophytic bacteria have the capacity to reduce the heavy metal contamination by accumulating the heavy metals. Plants grown in soil contaminated with xenobiotics naturally recruited endophytes with the necessary contaminant- degrading genes (Siciliano et al., 2001). The phyto-symbiotic strain of

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Methylobacterium, which was isolated from hybrid Poplar trees (*Populus deltoids x nigra*), was capable of biodegrading numerous nitro-aromatic compounds such as 2, 4, 6-trinitrotoluene (Van Aken et al., 2004). Siciliano et al. (2001) showed that the bacteria degrading recalcitrant compounds are more abundant among endophytic populations, which could mean that endophytes have a role in metabolizing these substances.

Reynold et al., (2005) discussed the advantages and obstacles to use bioengineered endophytes and also demonstrated that a major advantage being where genetic engineering of a xenobiotic degradation pathway is required, bacteria are easier to manipulate than plants. Genetically engineered endophytes of yellow lupin shows resistance to nickels and were able to increase the nickel accumulation and tolerance of inoculated plants (Lodewyck et al., 2001). It is demonstrated that genetically engineered endophytic *Burkholderia cepacia* strains improved phytoremediation and promoted plant tolerance to toluene ((Barac et al. 2004). Inoculation of *Pseudomonas* endophytes capable of degrading the organochlorine herbicide, 2,4-dichlorophenoxyacetic acid (2,4-D). When inoculated plants were exposed to 2,4-D, they showed experienced little or no signs of phytotoxicity, whereas uninoculated plants showed significant accumulation of 2,4-D and displayed signs of toxicity including a reduction in biomass, leaf abscission and callus development on their roots. Large rhizospheric populations were also observed during these experiments, which were responsible for enhanced degradation of 2,4-D in soil (Germaine et al.,2006).

Table-4: A non-exhaustive list of pollutants that have been associated with bacterial

Compound	**Plant association**	**Organism**	**Reference**
Mono-and dichlorinated benzoic acids	Wild rye (*Elymusdauricus)*	*Pseudomonas aeruginosa strain* R75 and *Pseudomonas savastanoi strain* CB35	Siciliano et al. (1998)
2,4-D Methane	Poplar (*Populus*) and willow (Salix) Poplar tissues(Populusdeltoidesnigra DN34)	*P. putida* VM1450 *Methylobacteriumpopuli* BJ001	Germaine et al. (2006) Van Aken et al. (2004)
TNT, RDX, HMX	Poplar tissues (*Populusdeltoidesnigra)*	*Methylobacteriumpopuli* BJ001	(Van Aken et al. (2004)
MTBE, BTEX, TCE	*Populus* cv. *Hazendans* and cv. *Hoogvorst*	*Pseudomonas sp*	Germaine et al. (2004), Porteous-Moore et al. (2006)
Toluene	Poplar (*Populus*)	*B. cepacia* Bu61(pTOM-Bu61)	Taghavi et al. (2005)
TCP and PCB	Wheat	*Herbaspirillum* sp. K1	Mannisto et al. (2001)
Volatile organic compounds and toluene	Yellow lupine (*Lupinusluteus* L.)	*Burkholderiacepacia* G4	Barac et al. (2004)

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endophytephytoremediation strategies (Robert et al.,2008)

TNT, 2,4,6-trinitrotoluene; 2,4-D, 2,4-dichlorophenoxyacetic acid; TNT, 2,4,6trinitrotoluene; RDX, hexahydro-1,3,5-trinitro-1,3,5-triazene; HMX, octahydro-1,3,5,7-tetranitro-1,3,5-tetrazocine; NDAB, aliphatic nitramine 4-nitro-2,4-diazabutanal; BTEX, benzene, toluene, ethylbenzene, and xylene; TCP, 2,3,4,6-tetrachlorophenol; PCB, polychlorinated biphenyl.

Most of the important advantage of using endophytic bacteria as pollutant degraders is that any toxic xenobiotics taken up by the plant may be degraded in planta, thereby reducing phytotoxic effects and eliminating any toxic effects on herbivorous fauna residing on or near contaminated sites. Robert et al.,2008 demonstrated that endophytic bacteria efficiently expressing the necessary catabolic genes can promote the degradation of xenobiotic compounds or their metabolites as they are accumulated or while being translocated in the vascular tissues of the host plant. Now a day phytoremediation playing an ever-increasing role in the clean-up of contaminated land and water, it is clear that endophytes will play a major role in enhancing both the range of contaminants that can be remediated and the rate of their degradation. This review shows the Pteridophytes and the endophytic bacteria have a unique role to accumulate and reduce the concentration of heavy metals. Very few endophytic bacteria were reported from pteridophytes. Further research needed to explore the endophytic bacteria and what role they do play in heavy metal remediation in association with pteridophytes.

Role of endophytes as plant growth

Endophytic bacteria produce a wide range of phytohormones, such as auxins, cytokinins, and gibberellic acids. It is evidence that *Burkholderia vietnamiensis*, a diazotrophic endophytic bacterium isolated from wild cottonwood (*Populustrichocarpa*), produced indole aceticacid (IAA), which promotes the growth of the plant (Xin et al., 2009). Endophytic bacteria also promote plant growth by a number of similar mechanisms of Rhizospheric bacteria. These include indoleacetic acid production (Lee et al., 2004), phosphate solubilisation activity (Wakelin et al., 2004), and the production of a siderophore (Costa et al., 1994). Endophytic bacteria also supply essential vitamins to plants (Pirttila et al., 2004). Addition to these a number of other beneficial effects on plant growth have been attributed to endophyteic bacteria and include osmotic adjustment, stomatal regulation, modification of root morphology, enhanced uptake of minerals and alteration of nitrogen accumulation and metabolism (Compant et al., 2005). With the advent of time these plant growth-promoting bacterial endophytes are being used are in the developing areas of

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forest regeneration and phytoremediation of contaminated soils.

Conclusion

The endophytic bacteria performing a major role to increase food and non-food crop yields, remove contaminants, inhibit pathogens, and produce fixed nitrogen or novel substances**.** They also produce natural metabolites like bioplastics, and others are including hormones which may use for beneficial purpose for human beings.

A very few work is done on endophytic bacteria in association with Pteridophytes. Irene et al., 2010 reported association of endophytic bacteria in *Dicksonia sellowiana.* They isolated bacterial species including *Bacillus* spp. (*B. cereus, B. megaterium, B. pumilus*and *B. subtilis*), *Paenibacillus* sp., *Amphibacillus* sp., *Gracilibacillus* sp. *Micrococcus* sp. and *Stenotrophomonas* spp. (*S. Maltophilia* and *S. nitroreducens*). *B. Pumilus* was the most frequently isolated bacterial species. *Amphibacillus* and *Gracilibacillus* were reported as endophytes for the first time. Endophytic bacteria that displayed antagonistic properties against different microorganisms were also detected, but no obvious correlation was found between their frequencies with plant tissues or with plants from different growth regimes. It will be a promising area of research for future studies in the diversity of endophytes and their specific roles in plant and environment as well.

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