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Endophytic Bacteria: A beneficial organism

Sehrish Mushtaq*, Faiza Khan, Muhammad Shafiq, Mubasshir Hussain, Muhammad Saleem Haider

Institute of Agricultural Sciences, University of the Punjab, Quaid-e-Azam Campus, Lahore, Pakistan

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Abstract

Millions of microorganisms are associated with animals and plant species. Interaction between the microorganisms and plants lead beneficial effect on plant growth. Now a day due to this character they attain the keen interest of human. Wide variety of bioactive compounds are using in biotechnology are produce by these bacteria. They have ability to minimize the disease symptoms of plants. Interaction with host plant make remarkable changing in immunity of plant. Specific growth hormones produce by this interaction improve health of plant. Nutrient uptake and nitrogen fixing ability make their use as a bio fertilizer. Important metabolites and secondary metabolites are isolated from these bacteria which is useful in cancer and other chronic human disease. They play important role in degrading the heavy metals from the soil. In other words they play beneficial role in agriculture, medicine, biotechnology and in the field of food science.

* **Corresponding Author:** Sehrish Mushtaq ✉ sherry.a143@gmail.com

Introduction

Endophytes

Endophytes are organism which presents inside the living tissue and parts of plants like leaves, roots, stems and they are also present inside the soil. Endophytes form colony and remain concentrated at specific point. The organism that causes no any disease symptom in plant is termed as endophyte. Endophyte may be gram positive or gram negative. They are actually endosymbionts make mutualistic relation with plant. Approximately 300,000 plants existing on earth containing one or more endophytes. One plant contains more than one species of endophytes some are cultivable or some are non-cultivable. Molecular characterization and identification is very helpful for identification of bacteria. Entophytes colonized in plants without producing any disease symptom. Entophytes are suitable biocontrol agent for phytopathogens (Berg *et al.*, 2005). Endophytes have tendency to remove the contamination of soil (Ryan *et al.*, 2008).

In recent years, bacterial endophytes used as bio-fertilizers for improving crop production are gaining strong status among agronomists and environmentalists because they would significantly reduce chemical input into the environments(Ahemad and Kibret, 2014; Luo *et al.*, 2012). Thus, the exploitation of plant growth promoting endophytes (PGPEs) as one of the best options to increase biomass yield of the energy crops on marginal lands has become a hot research subject with more attention both from academia and industry (Weyens, 2009). For instance, *Bacillus* sp. SLS18 promoted the biomass production of sweet sorghum (Luo *et al.*, 2012).The growth of poplar tree was improved up to 60 % after inoculation with different endophytic strains (Taghavi, *et al.*,2009).So far, considerable number of PGPEs have been successfully isolated from a large diversity of plants and found to be beneficial for plant growth, yield and crop quality, including strains in the bacterial genera of *Acinetobacter, Alcaligenes*, *Arthrobacter, Azospirillium, Azotobacter, Azomonas, Bacillus, Beijerinckia, Burkholderia, Enterobacter, Erwinia, Flavobacterium, Klebsiella, Pseudomonas, Rhizobium and Serratia* (Rodriguez and Fraga 1999; Sturz *et al.*,2000; Sudhakar *et al.*, 2000; Berg, 2009). Due to their beneficial effects on growth and health for host plants, PGPEs have the potential for use in the friendly, sustainable and organic agriculture (Lucy *et al.*, 2004; Oconnell, 1992). Hence, diverse endophytic bacteria are now being used worldwide as bio-inoculants to promote plant growth and development under normal and various stresses like heavy metals, herbicides, insecticides, fungicides, salinity, and so forth (Ahemad and Kibret, 2014).

History of endophytes

Heinrich Friedrich Link used term endophytes first time in 1802. De Bary also use the term endophytes. Heinrich Anton de Bary in 1884 indicate endophytes presence in plant tissues (Hyde and Soytong, 2008). In 1898 Vogl identified the presence of endophytes.

Identification criteria of endophytes first time published by (Reinhold and Hurek, 1998). Bacteria and fungi are present in interior parts of vegetables plants first reported by Galippe. In 19th century and 20th century advantageous microorganism identified in plants. In 1888 a Dutch microbiologist Martinus Willem Beijerinck first time isolated the bacteria these bacteria are capable for nitrogen fixation. Endophytes have no pathogenic effect on plants.

Importance of endophytes

The potential of plant growth promoting bacterial endophytes (PGPBEs) to improve plant health has led to a great number of studies examining their applied use as inoculants, primarily in agricultural crops (Hallmann *et al.*, 1997; Kuklinsky-Sobral *et al.*, 2004). The potential for microbial inoculants to reduce the need for chemicals such as pesticides and fertilizers (Horrigan *et al.*, 2002) makes them important in the development of sustainable agricultural practices. In the following sections, we will review drivers that determine endophyte community structure and factors that will need to be considered for applied use of PGPBEs in a field setting.

Endophytes biodiversity and population

Endophytes bacteria isolated from vast variety of plants. Host specificity can be analyzed by molecular analysis of ribosomal genes. 15 bacterial species are also identified from the clover nodules. A large number of endophytes almost 689 characterized from the sorghum and maize crop and 47 endophytes bacteria were isolated from wheat and beans. From Grasses and legumes 27 species, 360 species from carrot were identified.

Easy culturable bacteria *Bacillus* and *Pseudomonas* are identified. Endophyte *Bacillus* genus reported from the kernel of corn crop (Rijavec, Lapanje, Dermastia, and Rupnik, 2007). *Bacillus* genus also reported from the potato, bananas, peas and beans. 264 bacterial endophytes from the radish. 72 endophytes characterized from the tomato (Deng *et al.*, 2011).

Types of endophytes

Streptomyces specie of endophytes source of many enzyme like cellulase, amylase etc. these endophytes reported from corn crop. *Piriformospora indica* fungal endophyte biotechnological application revealed that it promotes the plant development in wide range of host plant. Fungal endophytes promote plant growth in stress condition (Khan *et al.*, 2012). Bacterial endophytes classified as facultative endophytes and obligate endophytes. Obligate endophytes transmit through vector. Facultative endophytes are biphasic (Hardoim, van Overbeek, and van Elsas, 2008).

In vertical transmission endophytes transmitted through the vegetative part of plant like seeds. In horizontal transmission endophytes transmitted through soil. (Chi *et al.*, 2005) said that endophytes enter from soil into host plants and quickly cover the intracellular spaces of plants and cause infectious symptoms.

Endophytic bacteria colonization in plants

Bacteria enter into plant and form colonies this procedure is studied by advanced technological tool Auto fluorescent protein (AFP) method (Larrainzar and Morrissey, 2005). GFP green fluorescent**.** Protein technique also uses to detect the microbe into plant by producing the florescent. GFP is a marker technique Endophytes produce lytic enzyme which helped full in the penetration and colonization in plants. Endophytes bacteria colonization and infection was studied. Infection produce by endophytes proceeds by pectinolytic enzyme or cellulolytic enzyme. *Burkholderia* specie of endophytes produce infection in plant by producing these enzymes endogluconase and polygalacturonase, these enzymes are helpful in degrading the cell wall of plant cell (Compant *et al.*, 2005).

Azoarcus specie colonized in plant done with the help of pili structure. Pili provided helpful structure in adhering with the plant body. Plant host has great variety. Flavonoids stimulate the colonization of *Azorhizobium caulinodans* into wheat. Strain to strain variability also influences on colonization. Vast variety of endophytes is identified abled to make colonization and recolonization in tissue of plants instead the root (Romero *et al.*, 2001).

Nitrogen fixing ability of endophyte bacteria doesn't effect on colonization. Endophytes colonization of bacteria is influence by age of plant. Plant source and sampling time also has effect on colonization (Vendan and Rhee, 2010). *Pantoea* specie of endophytes aggressive for colonization they competed for colonization.

Position of endophytes bacteria into plant

Endophytes concentrated in below ground parts as compare to the aerial parts of plants (Rosenblueth and Martínez, 2004).Endophytes colonized into extracellular spaces as well as the conducting tissue of plants. Endophytes microorganism has ability to penetrate the plant tissue in xylem tissue or in the outer cortex of plant. Mid ribs of leaves conducting tissues and extra cellular spaces concentrated with endophytes as compare to other parts. Endophytes mostly are motile bacteria. Oligonucleotide probes used to identify the location and activity of endophytes in plants (James, 2000). Bacteria also enter into plant by vegetative propagation.

Endophytes bacterial interaction with host plants species

Endophytes interact with plant species for the ecological benefits as well as evolutionary benefits (Compant *et al.*, 2005). (De Weert *et al.*, 2002) said that the mobility of endophytes make interaction with host plant by chemotaxis. Carbohydrates and organic acid play important role in interaction with host plant in below ground parts. Endophytes attached with root bacteria and increase the plant growth and other beneficial mechanism in plants (Elbeltagy *et al.*, 2000). Cracks formed by herbivores and damage provide by pathogen create the way of entering for endophytes bacteria. Endophytes produce endoglucanases enzyme, this enzyme is also helpful in bacterial entrance (Reinhold *et al.*, 2006). Another enzyme endo polygalacturonidases produce the way of entrance for endophytes bacteria (Elbeltagy *et al.*, 2000). Host plant growing stage important for the population of endophytes it varies from growing stage.

Table 1. List of different entophytic bacteria along with their sources.

Bacterial species	Source	Reference
Enterobacter specie	Corn	(Kuklinsky Sobral *et al.*, 2004)
Bacillus megaterium	Carrot	(Araujo *et al.*, 2001)
Enterobacter sakazakiia	Soya bean	(Kuklinsky Sobral *et al.*, 2004)
Klebsiella specie	Wheat ,corn	(Conn and Franco, 2004)
Paenibacillus odorifer	Sweet potato	(Garbeva ,Van Overbeek, Van Vuurde, and Van Elsas, 2001)
Mycobacterium sp	Scots	(Conn and Franco, 2004)
Bacillus specie	Citrus	(Araujo *et al.*, 2001)
Gluconacetobacter diazotrophicus	Sugar cane	(Idogawa, Amamoto, Murata, and Kawai, 2014)
Pseudomonas citronellolis	Soya bean	(Kuklinsky Sobral *et al.*, 2004)
Escherichia coli	Lettuce	(Pirttila, Joensuu, Pospiech, Jalonen, and Hohtola, 2004)
Enterobacter agglomeran	Soya bean	(Kuklinsky Sobral *et al.*, 2004)
Stenotrophomonasa	Dune grasses	(Simpson *et al.*, 2014)
Kocuria varians	Marigold	(Sturz and Kimpinski, 2004)
Arthrobacter sp.	Pepper	(Sziderics, Rasche, Trognitz, Sessitsch, and Wilhelm, 2007)
Pseudomonas synxantha	Scots pine	(Pirttila *et al.*, 2004)
Bacillus polymyxa	Wheat	(Lodewyckx *et al.*, 2002)
Rhizobium etli	Maize	(Romero, Carrion, and Rico Gray, 2001)
Herbaspirillium seropedicae	Rice	(Zinniel *et al.*, 2002)
Erwinia sp	Soya bean	(Kuklinsky Sobral *et al.*, 2004)
Rhizobium leguminosarum	Rice	(Araujo *et al.*, 2002)
Nocardia sp	Citrus	(Araujo *et al.*, 2002)
Azospirillum brasilense	Banana	(Pirttila *et al.*, 2004)
Klebsiella sp	Rice , citrus	(Zinniel *et al.*, 2002)
Pseudomonas spp	Mustard plant, Rice	(Ma *et al.*, 2011)
Methylobacterium mesophilicuma	Citrus	(Araujo *et al.*, 2002)
Enterobacter	Citrus	(Araujo *et al.*, 2002)
Stenotrophomonas spp	Mustard plant	(Alstrom, 2001)
Rhizobium radiobacter	Mustard plant	(Pirttila *et al.*, 2004; Poonguzhali, Madhaiyan, and Sa, 2006)
Gluconacetobacter diazotrophicus	Coffee	(Pirttila *et al.*, 2004; Poonguzhali *et al.*, 2006)
Burkholderia cepacia	Oil palm	(Sapak, Meon, Ahmad, and Abidin, 2008)
Aeromonas ichthiosmia	Mustard plant	(Pirttila *et al.*, 2004; Poonguzhali *et al.*, 2006)
Gluconacetobacter diazotrophicus	Arabidopsis	(Cocking, Stone, and Davey, 2006)
Azospirillum amazonense	Pine apple	(Pirttila *et al.*, 2004)

Isolation and identification of endophytic bacteria

Molecular identification and characterization first time reviewed by (Ryan *et al.*, 2008) Endophytes population intensity minimum as compare to the pathogenic bacteria (Rosenblueth and Martínez, 2004).

Endophytic niche provide protection to those bacterial population that colonized into plant and present in intercellular spaces mid ribs of leaves roots and in conducting tissue. Endophytes can be isolated from monocotyledonous plants and dicotyledonous plants, herbaceous plants and woody trees.

Isolation of endophytes is done by disinfect the sample with sodium hypochlorite.(Lodewyckx *et al.*, 2002) told the method of isolation of endophytes. 1 ml sodium hypochlorite in 99 ml water used for sterilization for 5 mints than sample wash with autoclave distilled water for 5 mints. Surface sterilized leaf dry on the autoclave filter paper than these 1 cm sample placed on the media plates.

Table 2. Types of endophytes.

Type	Uses
Fungal endophytes	Source of antibiotics
Actinomycetes	Source of Agrochemical
Bacterial endophytes	Sources of secondary metabolites

These plates incubate at temperature between the 28°C to 37°C for 24 hrs. to 1 week. 16s RNA technique used for molecular identification (Zinniel *et al.*, 2002). GFP used to detect the endophytes bacteria. Finger printing and clone analysis techniques used to study the composition of endophytes bacteria (Garbeva *et al.*, 2001). Genome analysis of endophytic bacteria tells us basis of transcriptome and proteome.

Use of endophytes as bio control agent

Endophytes bacteria and fungi used as a bio control agent for many pathogens. Benefits on plant occur through a closely related mechanism (Gray and Smith, 2005). Infection and disease with virus bacteria and or other disease causing agent like nematode and insect can be treating by inoculation of endophytes (Berg and Hallmann, 2006). Endophytes produce induced systemic resistance (ISR), response in plants while the plants produce SAR systemic-acquired resistance mechanism as a defense response because of primary infection cause by a pathogen. ISR mechanism used but ISR system totally different from SAR role of ISR by endophytes bacterium reviewed by (Kloepper and Ryu, 2006). (Harish and Samiyappan , 2009) reported that endophytes bacteria isolated from the root corm of banana plant used as a biocontrol agent for banana bunchy top disease.(Gao and Liu, 2010) said that endophytes made plant resist against the stress. (Zabalgogeazcoa, 2008) told that endophytic bacteria provided resistant against the nematodes to plant. Biocontrol procedure of endophytes includes competition for an ecological niche production of compounds these compounds inhibit effect on pathogen, induce systemic resistance (ISR) in plants for broad spectrum of pathogens, and minimized abiotic stresses.

Table 3. List of secondary metabolites produced by endophytes.

Taxol	Benzopyranones,	Steroids,
Ergo flavin	Chinones	Tetralones,
Methyl eugenol	Phenolic acids	Xanthones
Alkaloids	Terpenoids,	Flavonoids,

Shimanuki (1987) first report the effect of endophytes on phytopathogen. Many endophytes isolated from citrus and resistant against root pathogens (Araujo *et al.*, 2001). Black rot in cabbage controlled by colonization of endophytic bacteria *Xanthomonas campestris* enhances the plant health. Endophytes bacteria active in oak plant they provide protection against the oak wilt pathogen. Pathogen growth decrease in plants as endophytes colonized into host plant.

Endophytes Pseudomonas *putida* and *Serratia marcescens* control the disease of Cucumber Mosaic Virus, *Fusarium* wilt and anthracnose in cucumber. 61 endophytes isolated from potato stem used as a biocontrol agent against *Clavibacter michiganensis* (Bargabus and Jacobsen, 2002) Endophytes *Bacillus pumilis* control disease of *Cercospora* leaf spot (Reiter and Sessitsch, 2003) in sugar beet crop. Endophytes bacteria stimulate the resistant

mechanism in citrus plants against the disease pathogen (Araujo *et al.*, 2002; Waweru and Dubois, 2014) told that endophytic bacteria resisted against the banana nematode.

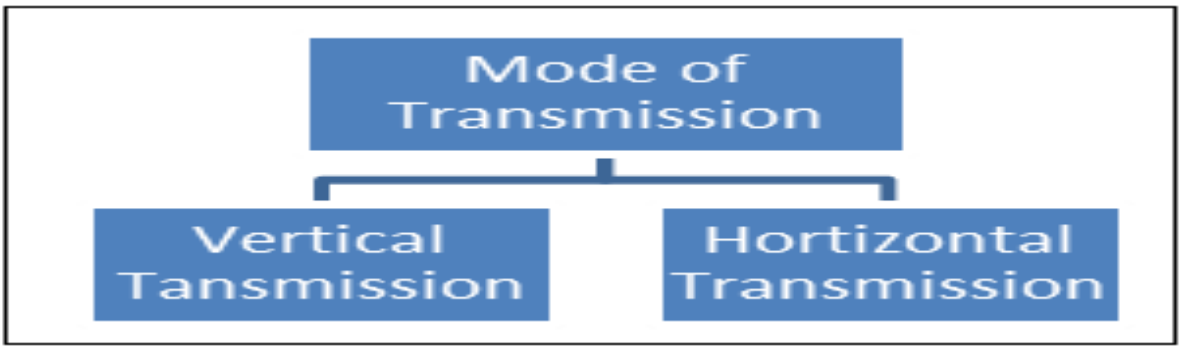

Fig. 1. Mode of transmission.

Endophytes bacteria also produce antibiotics (Sessitsch and Berg, 2004). Endophytes bacteria also control the fungal pathogen in wheat. (Aravind and Ramana, 2009) reported bacteria against the *Phytophthora capsici* a disease of black pepper. Endotoxin is produced by *Bacillus thuringensis;* it is helpful in controlling the insect pest. (Chernin and Chet, 2002) said that these bacteria also produce hyper parasitic activity when attack on pathogen, they secrete cell wall hydrolase enzyme during this activity. Endophytes have importance for biocontrol of soil borne diseases and antifungal activity (Yuliar and Rahmansyah, 2013) . (Lacava and Azevedo, 2004) told there is no any studies which revealed the interactive relation between the endophytes and phytopathogen.

Endophytes as a source of allelochemicals

Endophytes produce allelochemicals like lytic enzyme detoxifyication enzymes and antibiotics. Biocidal volatiles are also produce by endophytes.

Endophytic influence on plant growth

Rhizobacteria don't effect on pathogen but they increase the growth of plant. Endophytes bacteria increase the efficiency of plant for nutrient uptake. Endophytes bacteria have ability to fixed nitrogen and present in root nodules of plant (Yuliar *et al.*, 2013). Some endophytes have ability to solubilize the phosphate, phosphate solubilizing bacteria they increase the growth of plant (Wakelin and Ryder, 2004). Rhizobacteria soil bacteria that influence on the host plant growth (Compant *et al.*, 2005). Endophytes also source of indole acetic acid and siderophore (Pirttila *et al.*, 2004) told that endophytes source of many vitamins which play important role in growth of plants. Endophytes also influence on the physiology of plant like osmotic adjustment stomata opening and closing, root uptake and mineral or nutrient uptake.

Very important thing is metabolism of plant. Endophytes are plant growth promoting bacteria (PGPB) and these used in regrowth of forest and phytoremediation degradation of heavy metals. Endophytes improve nitrogen availability for plants. Fungal endophytes are very important for the growth of plant. Fungal endophytes source of some important enzymes cellulase, gelatinase, amylase, pectinase, tyrosinase (Fouda and Ewais, 2015). Endophytic bacteria secrete IAA; indole acetic acid enhances the growth of plants parts like shoot and root, endophytes also influence on cell division cell elongation and cell enlargement (Patten and Glick, 2002).

Endophytes as a degrading agent

Plant growth promoting endophytes decrease the metal toxicity in plants (Chen *et al.*, 2010). Recently endophytes involved in biodegradation of soil pollutants. Endophytes used as phytoremediation (Ryan *et al.*, 2008). Waste of industry and automobile industry cause severe air and soil Pollution of heavy metals it is directly threat to the life because heavy metals are carcinogens and cause cancer and mutagenicity but phytoremediation of soil solving this problem by degrading it.

It is slow process but effective and ecological friendly (Rajkumar and Freitas, 2011). Some methods of physiochemical remediation are not constructive for ecosystem. Endophytic bacteria have many functions intracellular polymers, adsorption, precipitation for minimizing the toxicity of heavy metals ion (Rajkumar and Freitas, 2013).

The endophyte *Kluyvera ascorbata* is resistant against heavy metal, Nikel in canola and tomato (Magnani *et al.*, 2010). Cadmium is the mobile and toxic heavy metal in the soil and it cause plant death slow up the growth and greensickness in plant. 2, 4, 6-trinitrotoluene. Toxic chemical also degraded by endophytes. Bio degrade ability of bacteria can be enhanced by genetic, modification bacteria (Lodewyckx *et al.*, 2001).

Endophytes increase the solubility of this metal in soil as compare to non-inoculated soil. (Sandalio and Del Rio, 2001). Study of endophytes with relation to the soil heavy metals remediation has a broad scope (Weyens and Vangronsveld, 2009). *Burkholderia cepacia* has ability increase through the genetic engineering plant become more tolerant against toluene and decrease the transpiration of toluene to the atmosphere (Barac *et al.*, 2004). Pseudomonas has ability to degrade the organochlorine herbicide, 2, 4-dichlorophenoxyacetic acid (2, 4-D).

Role of endophytes in sustainable agriculture

Economical used of endophytic bacteria in sustainable agriculture production of non-food crops for production of biofuel and biomass. To overcome the energy crisis in the world biofuel cheap and free from air pollutants. Bio mass used as organic fertilizer and in other industry like paper industry and food industry. Endophytes used directly to treat the seeds (Ryan *et al.*, 2008).

Endophytes source of metabolites

Endophytes have importance for these secondary metabolites production with unique structural character volatile organic compounds, antifungal, antiviral, insecticidal and immunosuppressant Agents. Endophytes metabolites resisted against the pathogen.

Isolation of endophyte producing–taxol cheaper than fermentation. A Unique anticancer agent Ergo flavin isolated from endophytes, it has a great invention for human health (Pimentel, Molina, Dionísio, Marostica Junior, and Pastore, 2010). (El-Deeb, Bazaid, Gherbawy, and Elhariry, 2012) said that Endophytes existed in plants great source of antibacterial substances. Research focus on endophytic bacteria to isolate the noval antimycobacterial compounds (Alvin, Miller, and Neilan, 2014). Bacteria Alternaria present in cultivated and non-cultivated *Rosa damascaena* (Abrahao and Pastore, 2013).

Conclusion

The study involved in this article mostly deals with the beneficial used of endophytic bacteria. Identification and characterization of endophytic bacteria and their evaluation against fungus and pathogenicity of endophytes play a very important role in sustainability of agriculture, promoting plant growth and development and rich source of antibiotics metabolites and anticancer treatment. Recent use of endophytes such as a bio fertilizer biocontrol agent and phytoremediation has great scope in future.The enormous importance of studies on the endophytic system is related to the connection between the specific metabolic abilities and the use of innovative microbial sources which are valuable in biotechnology nowadays. For instance, endophytic microorganisms can synthesize bioactive metabolites in different diseases, ensuring biological control of induced systemic resistance (ISR) and systemic-acquired resistance (SAR) factors, which may reduce plant pathogens. Endophytic microorganisms may accelerate phytoremediation or bioremediation processes. The best way to extend the knowledge is to conduct research in the following areas:

The practical application of bioremediation, particularly phytoremediation techniques.

A better understanding of plant–endophyte interactions and the dynamics of endophytic microorganisms (growth population and biodiversity).

The possibility of exploitation of woody plants for phytoremediation.

Determination of the biodegradation rate of contaminants.

Stress tolerance in plants.

Focus on endophytic microorganisms degrading multiple metal or organic contaminants by phytoremediation.

Construction of wetlands for remediation and using microbes to enhance native plants for restoration.

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