Endophytic microorganisms—promising applications in bioremediation of greenhouse gases

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Abstract Bioremediation is a technique that uses microbial metabolism to remove pollutants. Various techniques and strategies of bioremediation (e.g., phytoremediation enhanced by endophytic microorganisms, rhizoremediation) can mainly be used to remove hazardous waste from the biosphere. During the last decade, this specific technique has emerged as a potential cleanup tool only for metal pollutants. This situation has changed recently as a possibility has appeared for bioremediation of other pollutants, for instance, volatile organic compounds, crude oils, and radionuclides. The mechanisms of bioremediation depend on the mobility, solubility, degradability, and bioavailability of contaminants. Biodegradation of pollutions is associated with microbial growth and metabolism, i.e., factors that have an impact on the process. Moreover, these factors have a great influence on degradation. As a result, recognition of natural microbial processes is indispensable for understanding the mechanisms of effective bioremediation. In this review, we have emphasized the occurrence of endophytic microorganisms and colonization of plants by endophytes. In addition, the role of enhanced bioremediation by endophytic bacteria and especially of phytoremediation is presented.

Keywords Bioremediation · Greenhouse gases · Plant · Endophytes

Introduction

Many chemical compounds found in the Earth's atmosphere act as “greenhouse gases.” Greenhouse gases absorb infrared radiation and trap heat in the atmosphere, thereby enhancing the natural greenhouse effect defined as global warming. They may be caused by emissions associated with some human activities or occur naturally, and include a number of gases such as carbon dioxide, methane, nitrous oxide, and halogenated compounds. From year to year, the emission of these gases increases due to the changes in the economic output, extended energy consumption, increasing emission from landfills, livestock, rice farming, septic processes, and fertilizers as well as other factors. Nowadays, we look for modern, cheap, and promising solutions to decrease emission of greenhouse gases into the Earth's atmosphere. Therefore, techniques used for bioremediation of environmental contaminants are gaining considerable momentum.

One of the approaches is phytoremediation, in which living green plants in situ are used. They have the ability of decreasing and/or removing contaminants from soil, water, sediments, and air. In phytoremediation processes, selected or engineered microorganisms have been recently used in order to enhance phytoremediation. Numerous studies have demonstrated that endophytic microorganisms can accelerate these processes efficiently by interacting closely with their host plants (Khan and Dotty 2011; Li et al. 2012). These microorganisms reside inside both specific plant tissues and the root cortex or the xylem (Fig. 1). They also systematically colonize the plant by the vascular or apoplast system. Endophytes can also colonize dead and hollow hyaline cells of the plant genus Sphagnum (Fig. 1).

The huge variety of the metabolic pathways employed by endophytes makes them valuable tools for bioremediation, which can be used for assimilation of methane, fixation of nitrogen, bioremediation of pollutants (e.g., pesticides, herbicides, insecticides, petrochemicals, polychlorobiphenyls, phenols/chlorophenols), and biotransformation of organic substances, for example propylene to epoxypropane and production of chiral alcohols (Gai et al. 2009; Kim et al. 2012). On the other hand, endophytic microorganisms can produce...
secondary metabolites that may have an influence on antifungal and antibacterial properties, plant hormones, or their precursors such as plant growth factors, vitamins B12 (Ivanova et al. 2006) and B1 (Mercado-Blanco and Bakker 2007; Simons et al. 1997), and bioprotectants (Trotsenko and Khmelenina 2002).

The aim of this review was to present the potential use of the plant–endophyte system in bioremediation of greenhouse gas pollutions (particularly methane, carbon dioxide) as a method of mitigation of environmental problems without any need to excavate contaminated soil and dispose of it elsewhere. Furthermore, another aim of this paper was to emphasize the scope, magnitude, and complexity of endophytic activity in these studies.

Endophytic microorganisms

Endophytes are defined as microorganisms (fungi, bacteria) that colonize living, internal tissues of plants without causing any immediate, negative effects. The term *endophyte* was first introduced in 1886 by De Bary for microorganisms (fungi, yeast, and bacteria) colonizing internal plant tissues (De Bary 1884). In 1887, Victor Gallipe postulated that soil microorganisms can penetrate healthy plant tissues; therefore, recognition of colonization mechanisms is so valuable (Galippe 1887). However, those early results were dismissed due to an overall belief that microorganisms discovered inside tissues constitute pollution resulting from the isolation process (Smith 1911). One hundred twenty years later, in 1986, Carrol changed his view of endophytic organisms. He postulated that fungi which cause asymptomatic infections entirely within the tissues of the host plants are endophytes (Carroll 1986). Petriini (1991) viewed them as all organisms living in plant organisms that can colonize tissues without any macroscopically visible symptoms. Hirsch and Braun (1992) described endobionts as a group of microorganisms colonizing tissues without any visible consequences of infection (latent pathogens). One of the latest definitions of endophytes was proposed by Posada and Vega (2005) who used this term to describe all organisms inhabiting different internal parts of plants, including seeds.

The first studies of the biology of *Agrostemma githago* L. endophytes started by Darnell in 1904 were focused on species richness and abundance, but not on their interactions (Tan and Zou 2001).

Between 1933 and 1989, intensive development of research on endophytes took place, focused particularly on identification of different grass species endophytes (Clay and Schardl 2002; Latch et al. 1985; Saha et al. 1987; Sampson 1938; White 1987). At the end of 1977–1983, great progress in the knowledge of coniferous trees was made (Carroll et al. 1977; Carroll and Carroll 1978; Carroll and Petriini 1983). A significant contribution to the research on endophytic microorganisms was made by Petriini, who examined deciduous trees such as willow and oak (Petriini and Petriini 1985; Petriini 1991, 1996).

In 1998, Schulz and coworkers introduced leaf imprint as a new method for checking the isolation protocols, aiming to eliminate epiphytic organisms (Schulz et al. 1998). Sánchez and Márquez (2008) used this approach as an excellent sterilization method for isolation of endophytes from one kind of grass (*Dactylis glomerata* L.). The method has been further developed (Arnold et al. 2001, 2007; Suryanarayanan and Kumaresan 2000).

Currently, a substantial body of research on endophytes is focused on the methods of isolation, biodiversity, secondary metabolites, and especially mechanisms of the interaction between the endophyte and the host.

Occurrence of endophytes

The isolation of endophytic organisms from almost all known plants is shown in a large number of literature reports. There are approximately 300,000 plant species living on the Earth, and each individual plant can be the host to one or even more kinds of endophytes (Petriini 1991; Strobel and Daisy 2003; Huang et al. 2007).

They may be isolated from roots, stems, leaves, and inflorescences of weeds, fruit plants, and important vegetables (Bulgari et al. 2012; Bhore et al. 2010; Munif et al. 2012). Endophytic bacteria have been isolated from monocotyledonous plants, e.g., Liliaceae, grass, zea, rice, and orchids (Gangwar and Kaur 2009; Kelemu et al. 2011; Lin et al. 2012; Miyamoto et al. 2004; Peng et al. 2006; Rogers et al. 2012), as well as dicotyledonous plants, for instance oak (Basha et al. 2012; Ma et al. 2013). Some endophytes have been characterized from different tree species, for example...
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phytes can be isolated from a single plant. It is said that the
wide host range. Therefore, several different species of endo-
organisms, classified as Bacillus sp., Enterobacter sp., and Sporosarcina aquimarina (Rylo sona Janarthine et al. 2011),
have been found in roots of some coastal mangrove pioneer plants (Avicennia marina).

Ting and his coworkers (2009) have performed an analysis
prevalence of Fusarium oxysporum fungi in terms of the
plant type, group, and their environmental setting. The aim of
determination of diversity was to present endophytes from
various beneficial plant species (fruit, ornamentals, weeds, medicinal plants). Antagonistic endophytes were shown to
be mainly fungal endophytes, and they were found primarily
in weed and medicinal plant samples. The highest rate of
occurrence of endophytes was observed in the medicinal
plants (three endophytic organisms per plant), while the weeds
were characterized by a lower rate of prevalence (2.4 endo-
phytes per plant) (Ting et al. 2009; Fig. 2).

Colonization of plants by endophytes

Endophytic bacteria show a tremendous diversity not only in
plant hosts, but also in bacterial taxa (Bacon and Hinton 2006;
Hardoim et al. 2008; Vendan et al. 2010). Some hosts are
reported to have several endophytes, and the latter may have a
wide host range. Therefore, several different species of endo-
phytes can be isolated from a single plant. It is said that the
diversity of endophytic communities in the endosphere is
regulated by stochastic events, which are influenced by deter-
ministic processes of colonization in turn (Battin et al. 2007).
It should be added that the microenvironment of soil has an
influence on the colonization of plant endophytes by diverse
bacteria and their community composition (Hardoim et al. 2008).

It has been postulated that the early step in the colonization
of a plant may depend on absorption of soil aggregates,
biodiversity of plants and their physiology, as well as micro-
bial prevalence (Hardoim et al. 2008). The main factors that
may regulate microbial colonization include the plant geno-
type, the growth stage, the physiological status, the type of
plant tissues, some soil environmental conditions, as well as
some agricultural practices (Conrath et al. 2006; Singh et al.
2009). Moreover, the microbial metabolic pathways of colo-
nization may play an important role as determinants of endo-
phyte diversity. For example, the rate of motile bacteria iso-
lated from the interior part of roots was approximately fivefold
higher than that of bacteria in the soil tightly adhering to the
roots (Czaban et al. 2007). It has been proved that the ability of
soil bacteria to approach plant roots is induced by chemotaxis
and the efficiency in microcolony formation. These are the
key factors that determine the success of bacteria to become
endophytic (Bacilio-Jiménez et al. 2003). The process of plant
colonization by endophytic microorganisms is a complex
phenomenon. It includes recognition of the host, spor germi-
nation, penetration, colonization, and maintenance of endo-
phytes in the host cells (Van Antwerpen et al. 2002). Diverse
sources of endophytic microorganisms have been shown. They
can be contained in seeds and vegetative planting mate-
rial, since they originate from the surrounding natural envi-
nvironment such as the rhizosphere and phyllosphere. The
processes of colonization depend on several biotic and abiotic
factors. It has been shown that they include physical and
biological characteristics of the host plant, temperature, hu-
midity conditions, and seasonal fluctuations of other
cohabiting microorganisms (Quadt-Hallman et al. 1997).

Overall population densities of endophytic microorganisms
may be variable. It is said that the microbial population
densities are positively correlated with both the growth stage
and changes from the young phase to maturity in plants. For
example, the highest rate of endophytic colonization by
Solanum tuberosum L. cv. Bartina has been found in the
senescent stage, i.e., 6.93 colony forming units (CFU) g⁻¹ of
dry weight (DW) in comparison to young plants 4.67 CFU g⁻¹
of dry weight. There is substantial evidence showing that the
population density of endophytes depends on the host geno-
types (Singh et al. 2009). The highest population density of
Pseudomonas striata (133,334 CFU g⁻¹ DW) has been noted
in Zea mays L. cv. PRO 311, while the lowest density of the P.
striata community (50 CFU g⁻¹ DW) has been found in
another species of corn—zea Kiran. Likewise, the total pop-
ulation density of Piriformospora indica in the root of Z.
mays Mahikanchan, 247,334 CFU g⁻¹ DW, was higher than
that in Z. mays Seedtech which was 48,666 CFU g⁻¹ DW
(Singh et al. 2009). Seghers and coworkers (2004) have
shown that agricultural practices can affect the composition

Fig. 2 Endophyte recovery rate (endophyte/plant) for plants sampled from various plant types, landscapes, and plant groups. Bars indicate standard error of the means; modified from Ting et al. (2009)
of the endophyte community. Application of organic fertilizers resulted in increased total population density of endophytes, primarily of type I methanotrophs (Seghers et al. 2004).

The role of endophytic microorganisms in bioremediation

The collaboration between the plant and endophytes can play a key role in the degradation of hazardous contaminants in the rhizosphere. Recently, a promising area of exploitation of endophytic bacteria for phytoremediation of contaminated environments has been described. The advantages and disadvantages of removal of toxic metals, different organic and volatiles substances, greenhouse gases as well as mixed contaminants are listed in Table 1 (Doty 2008).

Bacterial endophytes might function more effectively than bacteria added to the soil because they participate in a process known as bioaugmentation (Newman and Reynol 2005). Large numbers of bacterial strains isolated from grapevine (Vitis vinifera L.) plants were resistant to lead, mercury, nickel, zinc, and manganese (Altalhi 2009). In their study, the authors Guo et al. (2010) showed that the endophytic bacterium Bacillus sp. reduced cadmium to approximately 94% in the presence of industrially used metalic inhibitors N,N′-dicyclohexylcarbodiimide (specific ATPase inhibitor, DCC) or 2,4-dinitrophenol (DNP). Similarly, inoculation with endophytic bacteria, Serratia nematodiphila LRE07, alleviated growth inhibition in Solanum nigrum L. in the presence of cadmium (Wan et al. 2012).

Ma et al. (2011) isolated Ni-resistant endophytic bacteria from tissues of Alyssum serpyllifolium growing in serpentine soils in Braganca in the northeast part of Portugal. Inoculation of Brassica juncea seeds with this strain significantly increased the plant biomass. Bioremediation of heavy metals involving endophytic bacteria L14 (EB L14) isolated from a cadmium hyperaccumulator Solanum nigrum L. has been described by Chen et al. (2012). The endophytic microbial community may also assist in phytoremediation of petroleum. Preference for petroleum-degrading bacteria in the root interior has been illustrated with an example of plants growing in petroleum-contaminated soil (Siciliano et al. 2001). van Aken and coworkers (2004) have indicated that Methylobacterium populum sp. nov. strain BJ001 isolated from poplar trees is able to degrade energetic compounds such as 2,4,6-trinitrotoluene (TNT), hexahydro-1,3,5-trinitro-1,3,5-triazine (HMX), and hexahydro-3,5-trinitro-1,3,5-triazine (RDX). Mineralization of about 60% of RDX to carbon dioxide was observed within 2 months' time. The bioremediation potential during degradation of xenobiotic compounds by three strains of Pseudomonas sp. isolated from xylem sap of poplar trees was tested by Germaine et al. (2004). Recently, Oliveira et al. (2012) have isolated three strains from Cerrado plants exhibiting the capacity for degradation of different fractions of petroleum, diesel oil, and gasoline.

Over the recent years, much more attention has been focused on the application of endophytic bacteria for phytoremediation. Burkholderia cepacia L.S.2.4 bacteria genetically modified by introduction of a pTOM toluene-degradation plasmid of B. cepacia G4, a natural endohyde of yellow lupine, were used for phytoremediation of toluene (Barac et al. 2004). The recombinant strain induced strong (up to 50–70%) degradation of toluene. Germaine and colleagues (2009) described inoculation of the pea (Pisum sativum) with a genetically modified bacterial endohyde that naturally possessed the ability to degrade 2,4-dichlorophenoxyacetic acid.

The results showed that the plants inoculated with Pseudomonas putida VM1441(pNAH7) had a higher degradation capacity of up to 40% for 2,4-dichlorophenoxyacetic acid from the soil (Germaine et al. 2009). The first in situ inoculation of poplar trees growing on a trichloroethylene (TCE)-contaminated site with TCE-degrading strain P. putida W619-TCE was done by (Weyens et al. 2009). This kind of inoculation resulted in a 90% reduction of TCE evapotranspiration under the field conditions. This promising result was obtained after introduction of P. putida W619-TCE to poplar trees, as a root endohyde. Probably, the TCE metabolic activity in the members of the poplar's endogenous endophytic population was obtained by further horizontal gene transfer (Weyens et al. 2009). In subsequent studies, Weyens et al. (2010) used engineered endohydes for improving phytoremediation of environments contaminated by organic pollutants and toxic metals. The yellow lupine was inoculated with B. cepacia VM1468 possessing (a) the pTOM-Bu61 plasmid coding for constitutive trichloroethylene degradation and (b) the ncc-nre Ni resistance/sequestration. Inoculation with B. cepacia M1468 into plants resulted in a decrease in Ni and TCE phytotoxicity, which was reflected by a 30% increase in root biomass and up to a 50% decrease in the activities of enzymes involved in antioxidative defense in

### Table 1: Advantages and disadvantages of phytoremediation (Doty 2008)

| Advantages | Disadvantages |
|------------|---------------|
| Low cost   | Inhibition of plant growth by poor soil quality |
| In situ    | Contaminant, phytotoxicity |
| Solar-powered technology | Unknown effects of biodegradation products |
| Maintains in top soil | A slower method |
| Large social acceptance | Lack of the metabolic capacity of the plant to deal with |
| Nondestructive to the soil structure | High levels of these contaminants |
the roots. In addition, the decreasing trend in TCE evapotranspiration showed about a fivefold higher Ni uptake observed after inoculation of plants (Weyens et al. 2010). Bioaugmentation of two grass species (Festuca arundinacea Schreb. and Festuca pratensis Huds) with endophytic fungi Neotyphodium coenophialum and Neotyphodium uncinatum resulted in PAH and TPH removal from the plant rhizosphere of 80–84 and 64–72 %, respectively, compared with 56 and 31 % in control plants (Soleimani et al. 2010). At the same time, Chen and coworkers (2012) demonstrated that culturable endophytes in aquatic plants have the potential to enhance in situ phytoremediation. This was one of the first studies aimed at isolation and comparison of culturable endophytic bacteria among different aquatic plants showing great diversity of microorganisms dominated by Gammaproteobacteria. Ho et al. (2012) isolated endophytic bacteria tolerating aromatic compounds from plants predominantly occurring in constructed wetlands, including reed (Phragmites australis) and water spinach (Ipomoea aquatica). Achromobacter xylosoxidans strain F3B was chosen for in planta studies using Arabidopsis thaliana as a model plant. It promoted removal of catechol or phenol pollutants (Ho et al. 2012). Kang and colleagues (2012) reported a novel endophyte from the hybrid poplar (Populus deltoides × P. nigra). This unique endophyte, identified as Enterobacter sp. PDN3, showed high tolerance to TCE up to 55.3 μM (Kang et al. 2012). This strategy is promising for improvement of the efficiency of phytoremediation of volatile organic contaminants. Furthermore, recombinant endophytic bacteria are easier in application than genetic plants because their strains can successfully colonize multiple plants. In addition, other benefits to plants such as nitrogen fixation, phosphate solubilization, and stress tolerance have been observed (Dimkpa et al. 2009; Doty et al. 2009; Gai et al. 2009; Jing et al. 2007; Li et al. 2012).

Besides bioremediation of volatile organic compounds, a lot of research has been focused on greenhouse gas emissions (particularly methane and carbon dioxide) depending on the composition of vegetation (Chen and Murrell 2010; Parmentier et al. 2011; Goraj et al. 2013; López et al. 2013). Methane and carbon dioxide are the main greenhouse gases (IPCC 2007). Thus, at the time of the global warming effect, Methane and carbon dioxide are the main greenhouse gases belonging to the genus endophytic methanotrophic bacteria found in moss tissues. One of the most recent discoveries has shown that the endophytic methanotrophic bacteria found in moss tissues belong to the genus Sphagnum (Raghoebarsing et al. 2005). It has been demonstrated that methanotrophs inhabiting Sphagnum spp., e.g., Methylocella palustris and Methylocapsa acidiphila, oxidize methane to carbon dioxide, which is later used by Sphagnum plants in the process of photosynthesis (Raghoebarsing et al. 2005; Stepnienska et al. 2013; Fig. 1). This discovery substantially changed the description of the carbon cycle in peat ecosystems and at the same time the global carbon cycle. In this way, methanotrophic endophytes inhabiting Sphagnum spp. can act as a natural methane filter that can reduce CH4 and CO2 emission from peatlands by up to 50 % (Kip et al. 2012; Fig. 3). Other field studies have shown the potential ability of the plant–methanotrophic bacteria systems to reduce methane emission up to 77 %, depending on the season and the host plant (Goraj et al. 2013). Furthermore, isolated endophytes from Sphagnum spp. could colonize crops and promote their growth. Molecular genetic analysis has shown that the dominant endophytic groups belong to the genera Burkholderia, Pseudomonas, Flavobacterium, Serratia, and Collimonas. Sheherbakov and colleagues (2013) have suggested that the isolated strain can be a promising object for the development of effective growth-promoting and protective microbiological preparations to be used in agriculture. Furthermore, the endophytes inhabiting Sphagnum spp. can be used for the inoculation of plants inhabiting an artificial wetland system used to treat mixed contaminations (for example heavy metals, different organic contaminations, and greenhouse gases). A majority of artificial wetland systems use the common reed Phragmites sp., cattail Typha sp., and willow Salix sp. that can be components of indigenous peatland flora (Moshiri 1993).

Recent studies have indicated the big potential of plants in the remediation of polluted sites. The excellence of adaptation abilities and promising remediation efficiencies strongly imply the superiority of endophytes in the bioremediation of mixed contamination at their low concentrations. It could be useful for developing an efficient metal removal system (Li et al. 2012). On the other hand, the adaptation abilities and the remediation efficiencies of endophytic microorganisms still
need further understanding and recognition for practical applications.

Concluding remarks and future perspective

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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