Endophytic Fungi from Brazilian Tropical Hosts and Their Biotechnological Applications

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Abstract

Endophytic microorganisms are defined in different ways, and a recent definition considers them as all of the microorganisms culturable or not that inhabit the inner parts of plant tissues and cause no harm to their hosts. They can be divided into two groups: those that do not generate external structures from the host (group I) and those which are able to develop external structures such as the nodules of N_2-fixing bacteria and mycorrhizal fungi (group II). Endophytic microorganisms such as fungi and bacteria play important roles in their plant hosts. The first studies with endophytes were conducted in temperate regions but have recently also been studied in plants from tropical countries. This chapter provides selected data obtained in Brazil mainly for endophytic fungi and focuses on their agricultural applications including the biological control of diseases and insect pests, and the promotion of plant growth. The biotechnological potential of the endophytic fungi isolated from not yet fully explored Brazilian environments such as the Amazon and Atlantic rain forests and mangrove forests is also discussed.

Keywords

Endophytic fungi · Plant growth hormone · Biological control · Brazilian forest · Mangroves

2.1 Introduction

Endophytic microorganisms are defined by Carroll (1986) as asymptomatic microorganisms living inside plants whereas Petrini (1991) defined them as microorganisms that inhabit plant's inner tissues at least for one period of their life cycle, without causing any apparent harm to the host. Other definitions were also proposed to describe endophytic microorganisms (Wilson 1995; Hallmann et al. 1997). Using molecular techniques, it has been shown that bacteria and fungi that are not culturable on standard media and under normal condition can also be found inside plants. Thus, a modified version of the previous definitions was proposed by Azevedo and

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Araujo (2007) that considers that endophytes are all microorganisms that are culturable or not, inhabit the interior of plant tissues, cause no harm to their hosts, and do not develop external structures. This definition was modified by Mendes and Azevedo (2007) dividing the endophytes in two groups, the first group (group I) does not generate external structures from the host and the second group (group II) develops external structures from the host plant and includes symbiotic nitrogen-fixing bacteria and mycorrhizal fungi.

Endophytic microorganisms have important roles in providing protection to the plant host by acting against predators and pathogens including cattle and insect pests (Azevedo et al. 2000). Endophytes may also increase the resistance of plants against biotic and abiotic stresses and produce plant growth hormones, antibiotics, enzymes, and many other compounds of biotechnological interest.

Endophytic microorganisms have been isolated from practically all plants studied to date. They were found in plants growing in different environmental settings including forests, mangrove swamps, pastures, agricultural fields, etc. The first studies with endophytes were conducted with host plants from temperate regions; however, more recent research was dedicated to the endophytic fungi and bacteria inhabiting the plants from tropical countries such as Brazil and India (Pereira et al. 1993; Rodrigues 1994; Suryanarayanan and Vijaykrishina 2001; Mishra et al. 2012); indeed, examples of endophytic species of fungi from tropical plant hosts were recently reviewed (Azevedo and Araujo 2007). Endophytic microorganisms have been used for the biological control of insect pests and plant diseases and production of vitamins, enzymes, antibiotics, and anticancer drugs. In Brazil, several laboratories are engaged in research for the isolation of and using fungi and bacteria for biotechnological, applications. In this chapter, selected data obtained in Brazil are presented, mainly with regard to fungal endophytes and their useful roles in agriculture.

2.2 Endophytes Versus Pathogenic Microorganisms Isolated from Cultivated Plants

The endophytes isolated in our laboratory (Faculty of Agriculture, ESALQ, University of São Paulo, Brazil) from citrus plants indicated that the isolates from the genus *Guinardia* were morphologically very similar to the pathogenic *Guignardia citricarpa* (anamorph *Phyllosticta citricarpa*). *G. citricarpa* causes citrus black spot disease and this pathogenic fungus is subjected to phytosanitary legislation in the European Union and the USA. The distinction between the pathogenic and endophytic *Guignardia* isolated from citrus was investigated by amplified fragment length polymorphism (AFLP) analysis. The results have also shown that the pathogenic and endophytic isolates from citrus were similar though with slight differences, regarding the conidia sheaths and colony color on oat meal agar. The molecular analysis allowed the classification of one pathogenic group of isolates as *G. citricarpa* and the endophytes as *Guignardia mangiferae* (*Phyllosticta capitalensis*). The endophyte *G. mangiferae* occurs in the European Union and the USA on many host species including citrus and does not cause symptoms of citrus black spot, justifying its exclusion from quarantine measures (Baayen et al. 2002). The molecular differences allowed the construction of DNA primers for use in a diagnostic kit to distinguish the pathogenic from the endophytic species of *Guignardia*. To avoid spreading of the pathogen, this kit was used to safely export the healthy citrus from Brazil to other countries. More recently, new and efficient primers for the diagnosis of citrus black spot were developed by Stringari et al. (2009). The authors cloned exclusive random amplified polymorphic DNA (RAPD) markers of *G. citricarpa* that were used to obtain "sequence-characterized amplified regions " (SCARS) that allowed the development of specific primers for the identification of pathogenic strains. In addition, Romao et al. (2011) showed that *G. citricarpa* produces great-

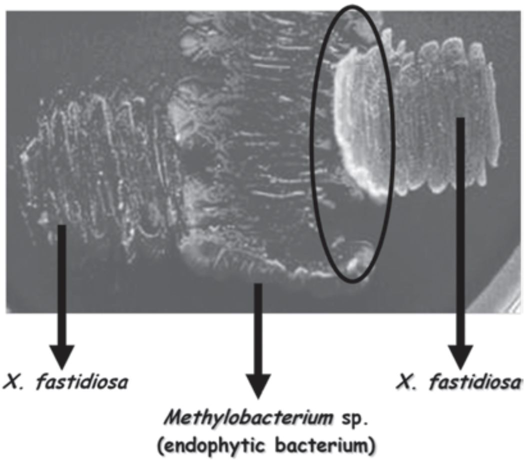

Fig. 2.1 Increasing growth of *Xylella fastidiosa* in the presence of the endophyte *Methylobacterium*. (Lacava 2000)

er amounts of certain enzymes such as amylases, endoglucanases, and pectinases compared to *G. mangiferae,* suggesting that these enzymes could be the key in the development of citrus black spot, mainly pectin lyases, which makes the pathogenic strains more effective for pectin degradation. Although not involving fungi, we also studied bacterial endophytes from healthy plants and plants with symptoms of citrus variegated chlorosis (CVC), a disease caused by the bacterium *Xylella fastidiosa*. We observed a relationship between the symptoms of CVC and the frequency of isolation of species from the genus *Methylobacterium*, which were frequently isolated from symptomatic plants. In contrast, *Curtobacterium flaccumfaciens* was more frequently isolated from asymptomatic plants (Araujo et al. 2001). This and other findings (Araujo et al. 2002) permitted us to conclude that *X. fastidiosa* could in fact be an endophyte that with the assistance of certain *Methylobacterium* isolates, changes its state from endophytic to pathogenic and returns to the endophytic state via interference of *Curtobacterium* endophytic isolates (Fig. 2.1).

2.3 Endophytes and Plant Growth Hormone Production

Endophytic microorganisms affect plant growth directly or indirectly and can provide the hosts with compounds that are produced by the fungi for facilitating the uptake of nutrients from the environment. Endophytes can also act by decreasing or preventing the deleterious effect of pathogens. Varma et al. (1999) demonstrated that the fungus *Piriformospora indica* increases the growth of various hosts suggesting that it may be useful for the promotion of plant growth.

Experiments conducted at our university have shown that two varieties of soybean (*Glycine max*) are colonized by several genera of endophytic fungi (Mendes et al. 2001; Mendes and Azevedo 2007). The endophytic fungi were isolated from the leaf, stem, and root tissues and some of the endophytic fungi were able to increase the growth of plantlets (Fig. 2.2). Similar results were obtained by Pimentel et al. (2006) and by Kuklinsky–Sobral et al. (2004) using soybean seeds treated with endophytic bacteria and fungi.

Some endophytes isolated from *Eucalyptus* were also able to promote the growth of seedlings thereby preventing diseases in the early stages of plant development (Procopio 2004). The inoculation of endophytes in *Eucalyptus* is being successfully employed, thus promoting better growth of the plantlets. Indeed, such endophytes have been used by cellulose and paper companies in Brazil to increase the viability and growth of *Eucalyptus* plantlets.

2.4 Endophytes and Biological Control of Plant Pathogens, Insects, and Ticks

Endophytic microorganisms colonize an ecological niche similar to that of phytopathogens which might favor endophytes as candidates for use as biocontrol agents. Several studies demonstrate the ability of endophytes to control pests and

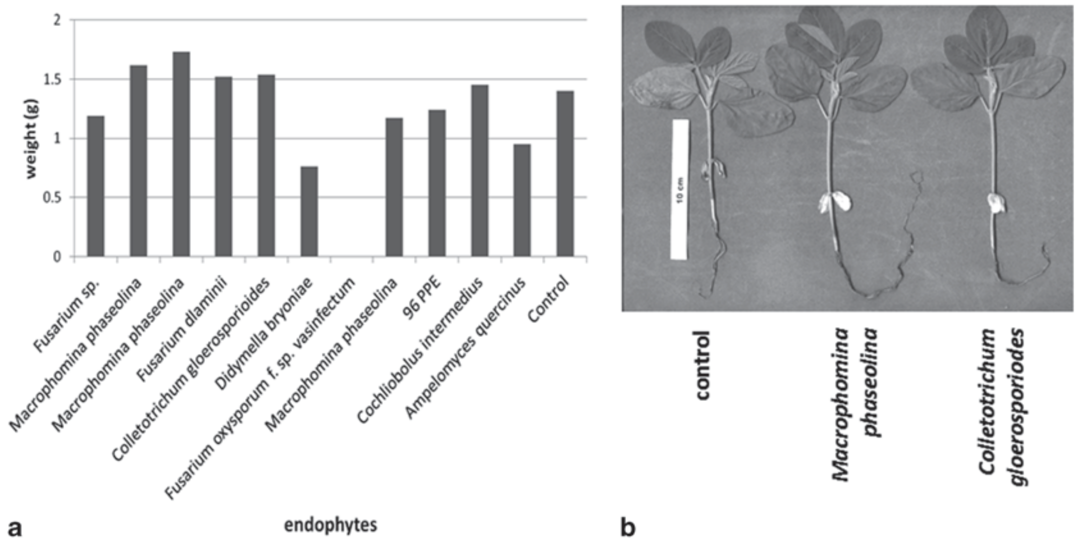

Fig. 2.2 **a** Wet weight (in g) using several endophytic fungi from soybean. **b** Photo showing growth promotion of soybean (*Glycine max*) plantlets that were treated with two endophytic fungi. (Mendes and Azevedo 2007)

diseases vectors (Carroll 1986; Azevedo et al. 2000). In Brazil, the *Basidiomycete Moniliophtora perniciosa,* the causal agent of witches' broom disease of cacao (*Theobroma cacao*) is one of the main limiting factors for cacao production and is considered the most important pathogen of this crop (Griffith and Hedger 1994). The endophytic fungal communities of infected and healthy Brazilian *T. cacao* plants were isolated and evaluated both in vitro and in vivo by their ability to inhibit *M. perniciosa*. Among these isolates, some were identified as potential antagonists and the fungus *Gliocladium catenulatum,* reduced the incidence of the disease in cacao seedlings to 70 % (Rubini et al. 2005). Among the isolated fungi from cacao, *M. perniciosa* was found colonizing healthy parenchymatic tissues showing for the first time that this fungus may also behave as an endophyte (Lana et al. 2011).

Another important disease that occurs in Brazil is the leaf anthracnose of guarana (*Paullinia cupana*) caused by *Colletotrichum gloeosporioides* and related species of this genus. This crop is an important Amazon plant used in the production of soft drinks and several medicinal products that are used in Brazil and exported to other countries mainly Europe and the USA. This crop is cultivated by small farmers in the Maués region of the Amazon, and the disease is causing severe economic and social losses. In collaboration with a research group from the Federal University of Amazonas, we isolated fungi and bacteria from the host plant. The initial results (data not yet published) indicate that some endophytic fungi and bacteria have the potential to control the pathogen.

It is known that some entomopathogenic fungi behave as endophytes. Bing and Lewis (1991, 1992) isolated *Beauveria bassiana* from maize (*Zea mays*) and the fungus was used to control the European corn borer (*Ostrinia nubilalis*). In Brazil, some laboratories isolated endophytic fungi that are known as insect and nematode controllers; these isolates were obtained from plant hosts including, among others, sugar cane (*Saccharum* sp.), maize, and soybean (Pimentel 2001; Pimentel et al. 2006; Stuart et al. 2010). Some of the *Beauveria* strains isolated from maize were used against the insect pest *Spodoptera frugiperda* and the results showed that these endophytes are as good or even better biocontrol agents than the commercial entomopathogenic strains used in Brazil. These endophytic *Beauveria,* belonging to the *Beauveria bassiana* species and *Beauveria amorpha*, are also able to control the bovine tick *Rhipicephalus microplus*, an ectoparasite that causes substantial economic losses due to the reduced productivity caused by anemia, toxicity, and the transmission of various diseases to their hosts. The *Beauveria* strains were effective in laboratory bioassays and under field test conditions. (Campos et al. 2010) It was also shown that both chitin and tick cuticle, induced fungal chitinase production. A scanning electron microscopy (SEM) analysis of the endophytic *Beauveria* infecting *R. microplus* showed appressorium formation during the penetration on cattle tick's cuticle (Campos et al. 2005).

2.5 Endophytic Fungi Isolated from Not Yet Fully Explored Environments Such as Brazilian Forests and Mangroves

Several laboratories in Brazil have been searching for endophytes in plants of not yet fully explored environments such as mangrove plants and plants from the Amazon and Atlantic rain forests. Recently some endophytic fungi producing apparently new antimicrobials were isolated from mangroves (Sebastianes et al. 2012). Additionally, some endophytes from petrol-contaminated mangroves were found to be able to reduce oil contamination. Endophytes from host plants growing in the Brazilian Amazon region and Atlantic forest were also isolated (Cassa-Barbosa 2001; Costa-Neto 2002; Souza et al. 2004), and more recently not yet published results demonstrate the biotechnological potential of endophytes from unexplored Brazilian plant hosts and they may result in new valuable products for agricultural, medical, and other applications.

2.6 Final Considerations

Brazil is one of the few countries in the world that still retains a large animal, plant, and microbial diversity. Approximately 20% from 300,000 plant species in our planet are found in Brazil. Endophytes inhabiting these hosts are poorly studied and, as source of important compounds of biotechnological value, remain to be discovered. A major problem is the rapid reduction of forests and mangroves in tropical areas of Brazil, a situation that could result in the extinction of many fungi and other endophytic microorganisms with the loss of potentially important products for use in agricultural, pharmaceutical, environmental, and other fields of interest.

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