

RECENT ADVANCES IN THE ROLE OF BACTERIAL ENDOPHYTES IN PLANT DISEASE MANAGEMENT

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ABSTRACT

Since times immemorial people have feared plant diseases due to the widespread destruction they caused directly or indirectly. Renaissance ushered in a new era of questioning everything resulting in the germ theory of disease and subsequently disease management with chemicals. However, with the surge in the environment consciousness and global climate change, research in plant pathology has seen a shift in research focus from chemical to biological management. Endophytes have played their role well but research has been limited, with only five per cent of the endophytes been effectively screened for their disease management properties. Endophytic bacteria live within the tissues of the plant but unlike plant pathogens do not cause harm to the plants invaded. Roots are considered to be the points of invasion for potential endophytes from soil to the roots and generally higher populations of endophytes are present in the below ground parts as compared to the above ground parts. Endophytes can colonise the same tissues as phytopathogens and hence act as biocontrol agents. A number of mechanisms are employed by the endophytes for disease management. Various researchers have reported the use of endophytes for the management of wilts, rots and damping off, blights and leaf spots, powdery mildews, rusts, downy mildews, basal rots and various post harvest fungal diseases. Diseases caused by nematodes and plant pathogenic bacteria can also be managed by the use of endophytes. The present paper reviews the recent research in the field of endophytes for the benefit of researchers and scholars.

INTRODUCTION

Agricultural augmentation since the twentieth century has been greatly attained through the use of farm machineries, high-yielding varieties, vigorous tillage, irrigation, fertilizers and pesticides (Foley *et al.*, 2005). This is well illustrated by the global use of fertilizers that has increased from approximately 27 to 170 million nutrient tonnes over the past 50 years (Heffer, 2013). However, continuous use of fertilizers over a long period leads to deleterious effects on the soil. Accordingly, environmentally safe approaches have to be implemented to maintain sustainable agricultural production to overcome threats that lead to yield loss, including unfavourable environmental conditions to plants, as well as biotic stress induced by plant pathogens and pests. A viable alternative is the use of endophytic bacteria for the management of plant diseases (Jha *et al.*, 2013). Bacterial endophytes have been defined as bacteria that colonize the internal tissues without any deleterious effects on the host (Schulz and Boyle, 2006). Of the well over 3, 00,000 plant species that exist on the earth, very few endophytes have been described (Strobel *et al.*, 2004). Therefore, there are enormous prospects of upbringing beneficial endophytes from the diverse genera inhabiting different ecosystems.

Bacteria may live in soils, rhizosphere, rhizoplane or phyllosphere, and may establish symbiotic relations with plants (Smith and Goodman, 1999). Unlike phytopathogens, endophytic bacteria do not cause any symptoms on host plants, and their occurrence is not related to the morphological

changes that appear in plant tissues such as formation of root-nodule by symbionts. Endophytes colonize all plant parts, in between the spaces of the cell walls and vascular bundles of plant roots, stems and leaves, tissues or flowers, fruits and seeds (Trognitz, 2014). Population dynamics of endophyte bacteria may vary from 10^0 to 10^9 bacteria per gram of plant tissue (Chen *et al.*, 1995). Generally, higher endophytic populations are found in below ground parts as compared to above ground tissues (Reinhold-Hurek and Hurek, 2011). Further, roots are considered as point of invasion of the potential endophytes from soil to the host plant. Strong union amid host plant and endophytes is mediated through the action of secondary metabolites produced by the microorganisms and the host cells (Brader *et al.*, 2014). Bacterial endophytes may colonize the same plant tissue as plant pathogens, and consequently can act as biocontrol agents (Berg *et al.*, 2005). There are innumerable reports of endophytic bacteria having the capability to manage phytopathogens (Krishnamurthy and Gnanamanickam, 1997), insects (Azevedo *et al.*, 2000) and nematodes (Hallmann *et al.*, 1995).

The endophytic bacteria contribute in plant disease management by (i) assisting in nutrient availability and uptake (ii) enhancing stress tolerance and (iii) providing disease resistance (Ryan *et al.*, 2008; Hamilton *et al.*, 2012). They are correlated with the enhanced plant growth by the production of hormones that increase accessibility of nutrients, such as nitrogen, potassium and phosphorus (Glick *et al.*, 2012). Induced disease resistance activities are allied with the abilities to produce secondary metabolites, such as antibiotics or

chitinase enzymes, which can inhibit growth of plant pathogens (Wang *et al.*, 2014; Pieterse *et al.*, 2014). Endophytic bacteria can also promote seedling emergence and stimulate plant growth (Chanway, 1997) under stress conditions (Bent and Chanway 1998).

The recent research on the role of endophytes in the management of different diseases is reviewed under the following heads:

Fusarium wilts

Nandhini *et al.* (2012) tested endophytic bacteria isolated from root, stem, leaves and fruits for their antagonistic activity against *Fusarium* wilt disease in tomato. All the isolates belonged to four bacterial genera viz., *Bacillus*, *Pseudomonas*, *Klebsiella* and *Citrobacter*. The results revealed that only 50% of the isolates exhibited strong antagonistic activity against tomato wilt pathogen. Sundaramoorthy *et al.* (2012) found that a consortium of rhizospheric and phyllospheric bacterial strains (*P. fluorescens* (Pf1) and *B. subtilis* (EPCO16 and EPC5) strains) reduced *Fusarium* wilt incidence in chilli by 17-30% compared to control. Six endophytic and four rhizospheric bacterial isolates obtained from root and corm tissues of banana plants reduced the incidence of *Fusarium* wilt of banana (Thangavelu and Muthukathan, 2015).

Rots and damping-off

Of the seventy one endophytic bacterial strains isolated from cotton, 40 strains protected cotton plants from *R. solani* infection (Chen *et al.*, 1995). Benhamou *et al.* (1998) revealed seed bacterization (*Serratia plymuthica*) of cucumber protected the seedlings from infection by damping-off. Cucumber seeds treated with endophytic bacterium *S. plymuthica* reduced the incidence of damping-off (Benhamou *et al.*, 2000). Bhowmik *et al.* (2002) reported that seed treatment with endophytic bacterium (PR 8) reduced the incidence of damping-off disease of cotton. On the other hand, Anith *et al.*, (2003) isolated a strain PN-026 from underground shoot portions of rooted cuttings of black pepper and tested it against foot rot of pepper. The results revealed that strain PN-026 was efficient in reducing *Phytophthora capsici*, the causal agent of severe infestation of foot rot disease. Muthukumar (2008) tested nine endophytic bacterial isolates obtained from chilli plants, some of them (isolated from stem and root) exhibited high inhibition of *P. aphanidermatum* (51.4, 41.7 and 40.0%) causing chilli damping-off. The maximum inhibition on the mycelial growth of *R. bataticola* was in chickpea by *P. fluorescens* strains PFBC-25 and 26 (Khan and Gangopadhyay, 2008). Bacterial endophytes (46 strains) were obtained from amaranthus and tested against *R. solani* by dual culture technique. Among these, six bacteria exhibited highest mycelial growth inhibition of *R. solani* (Uppala *et al.*, 2009). About 67 bacterial endophytes were isolated from cassava; they were subjected to 16S rRNA sequencing and FAME analysis. The bacterial profile revealed that 25% of endophytic isolates belonged to the genus *Bacillus*. Among these, the isolate *B. pumilus* MA11M4a showed a strong inhibitory activity against *R. solani*, *P. aphanidermatum* and *S. rolfsii* (Pereira de Melo *et al.*, 2009). In Kashmir root and collar rots of apple are frequently surfacing feature in orchards owing to the shift of paddy land into apple orchards. Use of endophytes can prove to be promising

approach to manage them but till now no bacterial endophyte has been evaluated against it. However, mycorrhizal evaluation has revealed the presence of some potential antagonist against *Dematophora* sp. and *Pythium* sp. causing root rot and collar rot of apple in Kashmir. (Banday *et al.*, 2008a; Banday *et al.*, 2008b and Dar *et al.*, 2009).

Blights and leaf spot

Foliar application with *Pseudomonas* spp. induced disease resistance in rice against sheath blight pathogen. In spite of the absence of this bacterium on plant surfaces, its presence in the internal stem led to suppression of disease (Krishnamurthy and Gnanamanickam 1997). Endobacterium *B. subtilis*, isolated from xylem fluid of chestnuts, suppressed the growth of chestnut blight pathogen, *Cryphonectria parasitica* under *in vitro* conditions. The same bacterium reduced the lesion areas on stems, when applied three days prior to challenge inoculation (Wilhelm *et al.*, 1998). The endophytes viz., *Bacillus circulance* and *Serratia marcescense* supplemented with chitin inhibited the conidial germination of early and late tikka leaf spot in groundnut (Kishore *et al.*, 2005). Four endophytic bacteria (OS-9, OS-10, OS-11 and OS-12) were isolated from healthy leaves of *Ocimum sanctum* and tested against five plant pathogens namely *R. solani*, *S. rolfsii*, *F. solani*, *A. solani* and *C. lindemuthianum*. Of these, the bacterial strain OS-9 was highly inhibitory to the growth of *R. solani*, *A. solani*, *F. solani* and *C. lindemuthianum* while OS-11 alone was found antagonistic to *A. solani* (Kalra *et al.*, 2010). The culture filtrate of endophytic bacteria CE-6 exhibited the highest inhibition on the mycelial growth of *Cercospora* (61.3%) *in vitro* (Hima *et al.*, 2013).

Powdery mildew

Recently (Gao *et al.*, 2015), isolated 14 endophytic bacterial strains from wheat leaves and tested them against *Blumeria graminis* f.sp. *tritici* causing wheat powdery mildew disease. The results revealed that *B. subtilis* strain (E1R-j) significantly reduced per cent disease index by 90.97% in pot culture under greenhouse conditions.

Rust

The endophytic bacteria were isolated from leaves and branches of *Coffea arabica* and *Coffea robusta* and tested against leaf rust pathogen *Hemileia vastatrix* by detached leaf and leaf disc method. The bacterial isolates TG4-la (*Bacillus lentimorbus* Dutky) and TF9-la (*Bacillus cereus* Frank & Frank) exhibited highest growth inhibition against coffee rust pathogen (Shiomi *et al.*, 2006). Endophytic bacteria E1R-j, isolated from wheat leaves, showed strong inhibitory effect on wheat stripe rust in both greenhouse and field conditions (Li *et al.*, 2013).

Downy mildew

Sixty different endophytic bacterial isolates belonging to different genera were isolated from root and stem tissues of five medicinal plants (*Cymbopogon citratus*, *Azadirachta indica*, *Phyllanthus emblica*, *Boerhaavia diffusa* and *Boerhaavia repens*) and two agricultural crops (*Pisum sativum* and *Sorghum bicolor*) and one weed plant (*Parthenium hysterophorus*) and were tested against pearl millet downy mildew disease. The pearl millet seeds treated with endophytic bacteria *P. fluorescens* ISR 34 and *Bacillus* sp. ISR 37 recorded greater control of downy mildew disease by 68 and 63%,

respectively. The endophytic bacterial strains not only reduced the disease incidence but also increased the plant growth by induced systemic resistance (Chandrashekhara *et al.*, 2007).

Basal stem rot/ganoderma wilt/thanjavur wilt

An endophytic bacterium *Pseudomonas cepacia* (B3) and *Pseudomonas aeruginosa* (P3) isolated from root tissues of oil palm exhibited strong inhibition on the growth of *G. boninense* causing ganoderma wilt (Dikin *et al.*, 2003). Histological studies revealed that bacterial endophytes were confined to the vascular bundles of the roots taken from symptomless palms (Zaiton *et al.*, 2006). Total of 581 endophytic bacteria were isolated from root tissues of oil palm and tested against *Ganoderma lucidum*, the wilt pathogen. Among these, three endophytic bacteria namely *Pseudomonas aeruginosa* GanoEB1, *Burkholderiacepacia* GanoEB2, and *Pseudomonas syringae* GanoEB3 were highly effective in inhibiting the mycelial growth of test pathogen. The results revealed that the isolate *P. aeruginosa* GanoEB1 was highly effective in controlling disease incidence of 13.3-26.7% compared to control (60%). (Ramli *et al.*, 2016)

Post harvest fungal diseases

A number of efforts have been made for using endophytic bacteria for the control of storage diseases. Endophytic bacteria were tested to control stone fruit rot pathogens *Monilinia laxa* and *Rhizopus stolonifer*. Twenty two bacterial strains were isolated from different fruits including red pepper, tomato, white plum, egg plant and zucchini. Of these 20 strains were able to control *M. laxa* in apricot and plum fruits. *R. stolonifer* was less susceptible to antagonistic bacteria than *M. laxa* and only one strain effectively controlled *R. stolonifer* (Pratella *et al.*, 1993). Endophytic bacteria (*B. subtilis*) isolated from stored apples have been used in the biocontrol of post harvest diseases of apple (Sholberg *et al.*, 1995). Further, an inhibitory compound acidic peptide produced by *B. subtilis*, was responsible for the inhibition of *Botrytis cinerea* but not to *Penicillium expansum* (Bechard *et al.*, 1998). The acidic peptide had a wide spectrum activity against Gram-negative bacteria. The endophytic bacterium *Bacillus thuringiensis* is capable of releasing volatile substances that lead to the inhibition of *Fusarium sambucinum* in potato tubers (Sadfi *et al.*, 2001). Two hundred and fifty eight endophytic bacteria were isolated from chilli leaves and screened against chilli fruit rot pathogen *Colletotrichum capsici* by fruit bioassay method. Of the endophytes tested, *B. megaterium* (ENB-86) recorded the highest suppression of lesion development in chilli fruits (59.66%). (Ramanujam *et al.*, 2012)

Nematode diseases

Endophytic bacteria have an additional advantage in control of phytoparasitic nematodes since the injuries produced by nematodes favour the entry of bacteria and colonize the root surface leading to their introduction into the root tissue (Khan, 1993). In cotton and tomato root knot nematode infection, peanut root knot and reniform nematode infection can be effectively controlled by using *B. subtilis* (Sikora, 1988). Seven endophytic bacteria *Aerococcus viridans*, *B. megaterium*, *B. subtilis*, *P. chlororaphis*, *P. vesicularis*, *S. marcescens* and *Sphingomonas paucimobilis* from cotton and cucumber plants were tested against cucumber root knot nematode. Seed

bacterization with endophytic bacteria completely protected cucumber seedlings from *M. incognata* infection (Hallmann *et al.*, 1995). Culture filtrate of *P. fluorescens* strains CHA0 or CHA0/PME3424 were tested against tomato root knot nematode. The results revealed that the inoculum levels of 10^7 , 10^8 , 10^9 cfu/g showed greater disease control under glasshouse conditions (Siddiqui and Shaukat, 2003). In another treatment, plants treated with culture filtrates of *B. subtilis*, *B. cereus* and *Arthrobotrys cladodes* reduced the soil population of *M. incognata* (Vetrivelkalai *et al.*, 2009).

Bacterial diseases

B. subtilis, isolated from healthy chestnut trees showed strong antagonistic activity against *Cryphonectria parasitica*, the cause of chestnut blight (Wilhelm *et al.*, 1998). Five strains of *Pseudomonas* inhibited the growth of *X. axonopodis* sp. *malvacearum* and also increased cotton seed germination and seedling growth by 12.8% and 22.4 %, respectively (Mondal, 1999). The endophytic bacteria *B. amyloliquefaciens*, *B. subtilis* and *B. pumilus* produce several antibiotics (surfactin, iturin, bacillomycin, azalomycin F, surfactin, arthrobactin, surfactin, amphomycin, arthrobactin and valinomycin) which are highly inhibitory to the growth of *X. campestris* sp. *Campestris*, the cause of black rot of crucifers (Wulff *et al.*, 2002). The cotton seeds treated with the endophytic bacterium (Endo PR8) reducing cotyledonary infection with black arm of cotton (*X. campestris* sp. *malvacearum*) (Bhowmik *et al.*, 2002). The shoots dipped with endophytic bacterium before planting grapevine produced highest fresh weight of the shoots and roots, and quick growth with more lignin deposits (Barka *et al.*, 2002). Similarly, cotton seeds treated with bacterial endophyte (EPCO 102) showed increased plant vigour under *in vivo* conditions (Rajendran *et al.*, 2006). Foliar spraying and seed soaking with bacterial antagonist *Delftia suluensis* (strain HR4), isolated from root region of rice plants showed reduced bacterial blight infection in range of 7-32% (Han *et al.*, 2005). Under greenhouse conditions, endophytic *B. subtilis* strain Lu144 remarkably protected mulberry plants against *Ralstonia solanacearum* causing bacterial wilt disease (Ji *et al.*, 2008). Bacterial endophytes such as *Pantoea agglomerans*, *Pseudomonas* sp. and *Curtobacterium luteum* reduced the growth of *Erwinia carotovora* (Figueiredo *et al.*, 2009). Ninety three isolates of rhizobacteria were tested against *Xanthomonas axonopodis* sp. *malvacearum*. Of these, *B. subtilis* B49 recorded highest inhibition on the growth of pathogen *in vitro* and was highly effective in controlling bacterial blight of cotton under greenhouse and field conditions (Salaheddin *et al.*, 2010). Bacterial endophytes (*B. amyloliquefaciens* Bg-C31) isolated from *Bruguiera gymnorhiza* was effective in controlling bacterial wilt of chilli under pot and field condition (Hu *et al.*, 2010). Endophytic bacterium, *B. subtilis* applied as seedling dip, soil and foliar application resulted in reduced the bacterial blight infection in rice under laboratory and field condition and it was found to increase the plant growth and yield (Nagendran *et al.*, 2013). *P. fluorescens* strain (PDY7) was highly effective in reducing the incidence of bacterial blight of rice under glass house and field condition (58.83 and 51.88%, respectively). This is mainly due to the production of antibiotics 2, 4-diacetylphloroglucinol (DAPG) (Velusamy *et al.*, 2013).

Twenty six bacterial strains were isolated from leaf, root and stem region of mangrove plant (*Rhizophoramucronata*). Among these, highest number of bacterial isolates were from leaf (38.5%) followed by root (34.5%) and stem (26.9%). Of these, five bacterial strains namely *Serratia*, *Bacillus*, *Pseudomonas*, *Micrococcus* and *Enterobacter* exhibited broad-spectrum of antagonistic activity against fungal and bacterial pathogen (Jose and Christy, 2013). Among the bacterial strains tested, strain MB04 and MB08 were highly inhibitory to the growth of *X. campestris* pv. *oryzae* causing rice bacterial blight (Yuliar, 2014). The endophytic bacteria isolated from tomato plants were tested against bacterial wilt pathogen. Of the isolates tested, only Ps1 and Ps8 could inhibit *R. solanacearum* in vitro using seed coat method. In *in vivo* test, 30 days old tomato seedlings, soaked with endophytic bacteria showed 8.07-9.19% disease suppression within 15-16 days incubation period (Purnawati *et al.*, 2014). Cotton seeds treated with endophytic bacteria strains *B. subtilis* UFLA285 recorded the lowest bacteria blight incidence of 26% (de Medeiros *et al.*, 2015). Four endophytic bacteria were isolated from potato stem tissue and tested against the growth of *Streptomyces scabies* in agar plate method. The results revealed that all the isolates were highly inhibitory to the growth of test pathogen (Flatley *et al.*, 2015).

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