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Endophytic microorganisms of tropical tuber crops: Potential and perspectives

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Abstract: Endophytic microorganisms which include both bacteria and fungi colonise almost every plant species. In order to colonize the plant and compete with other microorganisms, they produce a plethora of secondary metabolites, including toxins, enzymes, antibiotics, anti-cancer, anti-inflammatory and antifungal compounds. Endophytic fungi can have profound impacts on plant communities which include abiotic and biotic stress tolerance, increase of biomass, decrease of water consumption and alteration of resource allocation, nitrogen fixation, increased drought resistance, thermal protection, survival under osmotic stress and degradation of pollutants. Though tuber crops are the second most important group of crop plants providing food energy to humans after cereals, less attention has been paid to the these traditional crops in general. Investigations regarding the association of endophytes with the tuber crops have been sparsely studied though in some tuber crops like cassava, sweet potato and yams, presence of endophytes have been reported. Hence from the scarcely available literature, in the current review an attempt was made to put light on the various beneficial activities of endophytes on tuber crops. These reports glorified many symbiotically associated endophytes to have antagonistic properties against many plant pathogens like *Rhizoctonia solani*, *Pythium aphanidermatum* and *Sclerotium rolfsii*. Species like *Rahnella* was resilient to cold shock, UV irradiation and antibiotics. Many diazotropihic and non-diazotropihic endophytic bacteria were involved in nitrogen fixation. Actinomycetes endophytes were novel sources of industrially important thermostable amylolytic enzymes. However, inspite of all these profound beneficial effects endophytic associations are still to be studied in many tuber crops like taro, elephant foot yam, greater yam etc. So this review put forward the urge to carry out comprehensive research on these important microbes on such important crops.

Keywords: Cassava, Endophytes, Sweet potato, Tuber crops, Yam, Yam bean

INTRODUCTION

The term endophyte was first coined in 1886 by German Botanist and father of plant pathology Anton de Bary who described endophytes as microorganisms that colonize internal tissues of stems and leaves of plants. Infection caused by endophytes was further clarified to be asymptomatic i.e. without causing any apparent harm to the host (Hallmann *et al*., 1997). They can colonise roots as well as shoots and may not remain as endophyte throughout their life cycle (Wilson, 1995; Porras-Alfaro and Bayman, 2011). Endophytic microorganisms which include both bacteria and fungi colonise almost every plant species. Perhaps ninety species in a single tropical tree leaf and more than fifty different genera of endophytes are associated with roots of an arid grassland species (Porras-Alfaro and Bayman, 2011). In order to colonize the plant and compete with other microorganisms, they produce many enzymes, toxins and a plethora of secondary metabolites which includes enzymes, antibiotics, anticancer, anti-inflammatory and antifungal compounds (DeMelo *et al*., 2009). Fungal endophytes appear to be associated symbiotically with most, if not all, plants in natural ecosystem and constitute important components of plant micro-ecosystems. These fungal symbionts can have profound effects on plant ecology, fitness, and evolution, shaping plant communities and manifesting strong effects on the community structure and diversity of associated organisms (e.g. bacteria, nematodes and insects). Endophytic fungi can have profound impacts on plant communities. They may increase fitness by conferring abiotic and biotic stress tolerance, increasing biomass and decreasing water consumption, or may decrease fitness by altering resource allocation (Rodriguez *et al.,* 2008). Fungal endophytes were also reported to produce‘gold’ bioactive compound paclitaxel (taxol) and many other bioactive molecules like alkaloids, terpenoids, steroids, quinones, lignans, phenols and lactones (Zhao *et al.,* 2010). Besides fungi, endophytic bacteria have been detected inside the endorhiza, in stems, leaves as well as inside plant reproductive organs of different host plants. However, they occur at lower population densities than rhizospheric bacteria or bacterial pathogens (Rosenblueth and Martinez-Romero, 2006). Several bacterial endophytes provide phytohormones, low

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molecular weight compounds, enzymes, antimicrobial substances and side rophores which supported plant growth and increased nutrient uptake. Some endophytes have been identified as ideal candidates for biological control those could offer increased resistance to pathogens. Endophytes are also found to help plants in nitrogen fixation, increased drought resistance, thermal protection and survival under osmotic stress. In recent studies several pollutants has also been found to be degraded by this group of microbes (Khan and Doty, 2009; Dotty, 2008). The most commonly isolated genera include *Bacillus*, *Burkholderia*, *Enterobacter*, *Erwinia*, *Pseudomonas* and *Xanthomonas* (Hallmann *et al*., 1997; Teixeira *et al.,* 2007). Endophytes are indirectly associated with the induction of secondary metabolites production by plants as observed in aromatic and medicinal plants. Some metabolites are produced by a plant in combination with associated endophytic bacteria where the plant is unable to produce them alone (Brader *et al*., 2014). Similarly many novel compounds and antifungal metabolites synthesised *de novo* mediated by bacterial endophytes endophytes have been shown to prevent disease development in plants (Ryan *et al*., 2008).

Endophytic microorganisms have frequently been reported to be associated with crop plants, including wheat (*Triticum aestivum*), wild barley (*Hordeum brevisubulatum* and *Hordeum bogdanii*), soya bean (*Glycine max*), and maize (*Zea mays*). Some of the endophytic fungi in these crops conferred resistance of the plant to insect or fungal pathogens (Yuan *et al*., 2010). However, the frequent occurrence of endophytic microbes in many other important agricultural crops such as tuber crops and subsequent relevance to crop production systems is yet to be explored widely (Loeffler *et al*., 1986; Krebs *et al.,* 1998; DeMelo *et al*., 2009).

Roots and tubers are some of the most important consumed crops and have a major place in our diet and include extremely important crops worldwide. These crops contribute significantly to income generation, sustainable development and household food security especially in low income countries which are mainly located in the tropical regions. Tuber crops are the second most important group of crop plants which provide 5.4 % of food energy to humans towards global food security after cereals which provide 49 % (Nayar, 2014; FAO, 2009). More importantly, tropical root and tuber crops which include cassava, sweet potato, yams and aroids, are essential as staple foods and are utmost important for world food security. These are major sources of energy in developing countries with fast population growth and high urbanisation rates. With a contribution of 3.9 % of human energy and an average consumption of 28.6 kg per capita per year (76 kcals capita-1 day-1) tropical tuber crops (TTCs) like cassava, sweet potato and yam stand among the top 15 crop plants of the world when cultivation area is concerned (Lebot, 2009; Nayar, 2014). Other tuber crops include elephant foot yam, yam bean and edible aroids like taro. Despite such importance, less attention have been paid to these traditional crops towards their improved production and association with microorganisms. Though the presence of endophytes has been explored in some of these crops, sufficient information is lagging regarding the importance and utilisation of these important microbes in these important crops. In the current review, isolation of a wide range of bacterial as well as fungal endophytes and subsequently their bioactive potential has been discussed. The aim of the review is to put forward the need of intensified research in this direction.

Endophytes of cassava: Cassava (*Manihot esculenta* Crantz ssp. *esculenta*) is a perennial woody shrub in the Euphorbiaceae (spurge) family native to South America but now grown in tropical and subtropical areas worldwide, for the edible starchy roots (tubers), which are an major food source in the developing world, including Africa, South America and Oceania. Many ethno varieties are being cultivated by Brazilian Amazon Indian tribes. Cassava roots contain about 25-35 % starch and serve as a primary staple food over 800 million people of the world majority of them belongs to poorest population of humid tropical countries (Lebot, 2009; Chipeta and Bokosi, 2013). Cassava is yet to receive major attention in crop research programs despite being a globally important as a subsistence crop (DeMelo *et al.,* 2009). The existence of endophytes in cassava was suspected long before due to the following reasons: (a) Plants having no symptoms of disease growing in the same field frequently showed a wide range of variation in root yield.(b) Root yield of low-yielding, virus free plants of traditional clones could be increased by meristem culture (c) the performance (i.e., Root yield) of meristem culture-derived plants decreased sharply and uniformly under field conditions and (d) the long growing cycle of cassava and its vegetative propagation allowed infection and dissemination of these parasites. Because of these, several fungal species have been isolated from symptomless plant parts of cassava. The fungal species included *Septoria nodurum*, *Fusarium oxysporum*, *Colletotrichum gloeosporioides*, *C. graminicola*, *Alternaria termissima*, *Trichoderma* sp., *Botrytis sp*., *Torula sp*., *Nigrospora sp*., and others (Rivera *et al.* 1993; CIAT, 1992).

Both detrimental and beneficial effects of endophytes have been observed in plants and in the case of cassava it depended on the method of inoculation. For example *Curvularia sp*. was found to be detrimental when spray -inoculated, but beneficial when inoculated by immersion or puncturing on plantlets and callus tissues of cassava variety M Col 2215. This phenomenon may be due to the pathogenic nature of some strains of

Curvularia towards many plants. Some endophytes e.g. *Rhizoctonia* behaved as endophyte in a particular tissue (leaf and stem) but it induced necrosis in roots. Varietal interactions were also observed to vary for endophytic activity in case of cassava (Rivera *et al.,* 1993; CIAT, 1992). Several bacterial endophytes having multiple bioactivities were also found to be associated symbiotically with cassava plant. In many cases these bacteria were with profound antagonistic effect against many devastating plant pathogens. Many cassava cultivars were found to have a strong association with bacterial endophyte *Hyphomicrobium*. The bacteria appeared on culture media when explants carrying an axillary bud of cassava cultivar TME204 from the greenhouse and field, was introduced for ebryogenesis (Chauhan *et al*., 2013).

DeMelo *et al.* (2009) isolated sixty seven endophytic bacteria from freshly collected healthy roots, stems and leaves of six cassava varieties grown by Indian tribes located in the Autazes region, Amazonas State of Brazil. About 25 % of the cultures belonged to *Bacillus* species of which bacterial strain *Bacillus pumilus* MAIIIM4A produced an antifungal compound pumilacidin, hence exhibited strong antagonistic activity against plant pathogenic fungi like *Rhizoctonia solani*, *Pythium aphanidermatum* and *Sclerotium rolfsii*. Other antagonistic bacteria such as *Enterobacter cancerogenus* MAIVM2a, *Bacillus anthracis* MAIVM3a, *B. anthracis* MAIIIM2b, *Kluyvera cryocrescens* MAIIR2b, *Bradyrhizobium japonicum* MAIIIR3a, *B. pumilus* MAIIIM4a, *B. cereus* MAIF4b, *Clavibacter michiganensis isidiosum* MAIF6b, *B. cereus* MAIVM1b and *Burkholderia cepacia* were also isolated along with *Bacillus pumilus* MAIIIM4A. Similarly, twenty endophytic bacteria were isolated from surface sterilised cassava stems of which *Pseudomonas* species exhibited *in vitro* bacteriostatic activity against cassava bacterial blight pathogen *Xanthomonas campestris pv. manihotis*. The size of inhibiting or clear zone was more than 30 mm (Purnawati and Nirwanto, 2013). Teixeira *et al.* (2007) isolated 482 endophytic microorganisms from cassava land races and commercial areas in three Brazilian states such as Sao Paulo, Amazonas and Bahia. After cultural and morphological groupings" of endophytes from all three states, 47 different species belonging to 27 genera were identified and the most frequently isolated were *Bacillus*, *Burkholderia*, *Enterobacter*, *Escherichia*, *Salmonella*, *Stenotrophomonas* and *Serratia*. These genera represented approximately 71 % of isolates identified. Among bacterial isolates 60 % belonged to the group *B. cereus* (including the species *B. cereus*, *B. anthracis* , *B. mycoides* , and *B. thuringiensis*), 16.3 % of the species were *B. pumilus* and 9 % of the species were *B. megaterium*. The other 18.4 % belonged to species such as *B. lentimorbus*, *B. subtilis*, *B. sphaericus and B. Atrophaeus*. Other cassava endophytic bacteria like *Paenibacillus* sp. IIRAC-30 which was isolated from cassava suppressed the plant pathogen *Rhizoctonia solani*. In addition to that *Paenibacillus* sp. IIRAC-30 also produced ethyl acetate in potato dextrose medium which indicated presence of C15- lipopeptide belonging to surfactin series (Canova *et al.,* 2010; Menpara and Chanda, 2013).

Endophytes of sweet potato: Sweet potato (*Ipomoea batatas* (L.) Lam) is the second most important root tuber and the seventh most important food crop of the world, an important root crop grown for its sweet testing starchy, tuberous roots (Mitra, 2012). This carbohydrate rich root crop is used as subsidiary food, the vine tips used as vegetables and vines along with leaves serves as fodder. Among the world's major food crops, sweet potato produces the highest amount of edible energy per hectare per day (Horton and Fano, 1985). It has also shown potential to tolerate and absorb heavy metal pollutants like lead, iron and cadmium, as well as mixed pollutants contained in landfill leachate (DeAraujo *et al.,* 2004).

Growth and stress tolerance inducing endophytes: Several bacterial endophytes from sweet potato have been isolated, identified and their biological significance in crop production, protection and improvement has been emphasised. The population levels of endophytic bacteria ranged from 10^2 to 10^4 g^{-1} fresh weight of surface sterilised stem of sweet potato (Adachi *et al.,* 2002). Eleven culturable bacterial endophyte strains belonging to the genera, *Enterobacter*, *Rahnella*, *Rhodanobacter*, *Pseudomonas*, *Stenotrophomonas*, *Xanthomonas* and *Phyllobacterium*, have been isolated from sweet potato stems. Among these endophytes *Pseudomonas, Rahnella* and *Enterobacter* produced higher amount of Indole acetic acid (IAA) which proved to have plant growth promoting effect. *Rahnella sp.* also resilient to stresses like cold shock, UV irradiation and antibiotics (Khan and Doty, 2009). Plant growth promoting (PGP) activity was also observed in case of fungal endophytes of sweet potato. Thirty out of thirty six fungal endophytes, isolated from six healthy looking leaves, stems and roots of sweet potato from Baguio City of Philippines exhibited plant growth promoting effect on rice. Two such isolates viz. *Fusarium oxysporum* Isolate UOA/HCPF and *Emericella nidulans* Strain FH5 produced secondary metabolites having significant PGP effect which increased significantly rice plant length for the seedlings treated with the culture filtrates without causing external manifestations of infection (Hipol, 2012).

Diazotrophic and non-diazotrophic endophytic bacteria: The diazotrophic nature of some bacterial endophytes also has been verified by growth in nitrogen free media and the presence of *nifH* sequences has been detected. Many Japanese sweet potato cultivars were found to be associated with diazotrophic endophytic bacteria. Four diazotrophic *Pantoea* spp. and five *Klebsiella* spp along with three non-diazotropihic *Enterobacter* spp. have been isolated from Japanese sweet potato stems by stem pieceincubation and acetylene reduction activity (SPI-ARA) method using a semi-solid (1.8 grams agar/L) modified Rennie (MR) medium (Elbeltagy *et al*., 2001; Rennie, 1991). The acetylene reduction activity of diazotrophic strain *P. agglomerans* was found to be increased when co-cultured with non-diazotrophic strain *E. asburiae* (Asis jr and Adachi, 2005). Another endophytic diazotroph *Klebsiela oxytoca* was isolated from Japanese sweet potato cultivar Beniotome (Adachi *et al.,* 2002). Potential nitrogen fixing endophytes were isolated from sweet potato varieties collected in Uganda and Kenya. The *nifH* gene sequences had high homologies to the nitrogenise reductases of known nitrogen fixing bacteria (Reiter *et al.,* 2003). The occurrence of these endophytic nitrogen fixers apparently supports the findings of Yoneyama *et al.* (1998) on the possible contribution of biological nitrogen fixation in sweet potato.

Endophytes associated with *Dioscorea* species: Plants under the genus *Dioscorea* are commonly known as yam, tuber crop belonging to the family Dioscoreaceae which are mainly cultivated for the consumption of their starchy tubers in Africa, Asia, Latin America, the Caribbean and Oceania. Yams are among the oldest food crops recorded and are defined as an economically useful plant belongs to the botanical genus *Dioscorea*. Speciesof *Dioscorea* are also important source of sapogenins, alkaloids, steroid derivatives and phenolic compounds which are used in the pharmaceutical industry and diosgenin, which is used in the manufacture of oral contraceptives and sex hormones (Maggirwar *et al*., 2013; Ravi *et al.,* 1996). Association of microbial endophytes with *Dioscorea* species has been reported mainly in yam crop where a poor diversity of endophytes was observed. Bacterial strains belonging to *Erwinia* and *Bacillus* species e.g. *Erwinia pyrifoliae* and *Erwinia-Pantoea* complex have been isolated from yam rhizomes or tubers. Zhang *et al*. (2010) and Omoregie *et al*. (1999) isolated 14 and 10 numbers of endophytes from various yam tubers in two separate studies, respectively. In some cases co-occurrence of Dark Septate Endophyte (DSE) and Arbuscular Mycorrhizae Fungi (AMF) which are good symbionts have also been observed in Yam (Maggirwar *et al*. 2013). However, this endophytic colonisation is being a constraint in commercial propagation of yams by tissue culture technique and eventually difficulties arise to be used these in formal seed system. To eliminate such endophytic bacteria like *Burkholderia* spp., *Bacilluscereus* and *Luteibacter rhizovicinus* antibiotics like rifampicin are being used. Similarly, endophytic fungi like *Cladosporium* sp, *Verticillium* sp and *Amerosporium* sp are eliminated by a fungicide mixture of lambda-cyhalothrin andmancozeb/carbendazim (IITA; Wakil and Mbah *et al.,* 2012).

Endophytes of yam bean: Yam bean (*Pachyrhizus erosus* L. Urban) is a tuber legume, native to Central Mexico and the Northern Amazon Region. Its tubers are used as a source of starch for various applications, particularly in the food industry (Stamford *et al.,* 2007). Till date some actinomycetes were reported to occur endophytically with yam bean tubers. These actinomycetes were found to be novel sources of industrially important thermostable amylolytic enzymes. Thermo stability upto 70 ^{0}C of α-amylase enzyme from actinomycete *Nocardiopsis* sp isolated from yam bean was observed. The α-amylase was effective in a wide range of pH and was having high residual activity (Stamford *et al*., 2001). Similarly another endophytic actinomycete isolated from tubers of yam beam was classified as a novel species and nominated as *Kitasatospora recifensis* based in phenotypic and genotypic analysis. This strain produced simultaneously two amylases (α-amylase and amyloglucosidase) that showed thermostable properties. These desirable enzyme characteristics indicate that this strain has great potential for use in agricultural and biotechnological applications for starch hydrolyzation (Stamford *et al.,* 2007).

Tuber crops from which endophytes still to be reported: Despite the above explorations, many important tuber crops are still to be looked for endophytic associations. Reports regarding such mutualism is lacking in the database. However, in a recent study by Nayak *et al.* (2016), endophytic colonisation in tuber crops like taro, greater yam and elephant foot yam was explored. Taro [*Colocasia esculenta* (L.) Schott] is a root crop cultivated mainly for the edible corms. It is grown in nearly all parts of the humid tropics in more than 65 countries worldwide and serves as an important staple food and as a source of carbohydrate for inhabitants in some subtropical and virtually all tropical regions (Tsedalu *et al*., 2014). Taro is known to be a good source of carbohydrate, fibre, minerals especially potassium and vitamins (vitamin A, C and B complex) which is more than that found in whole milk. Considering this importance, an International Network for Edible Aroids (*INEA*) has been started in April, 2011 involving 22 countries. Central Tuber Crops Research Institute Regional Centre (CTCRI RC), Bhubaneswar, India is one of the experimental stations. Three putative fungal endophytes have been isolated from leaf cuttings of three *INEA* taro lines viz. BL/SM/158, BL/SM/132 and CE/IND/10 which are being identified and evaluated at CTCRI RC along with two isolates from greater yam genotype BBSR-1 and one isolate from elephant foot yam genotype NDA-4. Studies regarding isolation, identification and utility of endophytes from other lines and varieties are being carried out at this centre along with studies for the establishment of mutualistic relationship of endophytes with these crops.

Conclusion

The potential of endophytic microorganism for a sustainable agricultural production is enormous. Though breeding programmes and cultivar genotyping solve many problems in agriculture, trapping of these ecologically important microbes may also complement such efforts. Beneficial endophytic fungi and bacteria can be used as inoculant in roots and other plant tissues for many tuber crop plants to enjoy the benefits of these mutualists which they confer to their original plant hosts. Many growth promoting endophytes may be applied as potential bio-fertilizers in tuber crops with minimal environmental risks. The non-pathogenic existence of endophytes in their host (like yam bean) and their genetic element could be explored for possible exploitation as vectors in the genetic engineering. Investigation of biodiversity of endophytes may explore new possibilities for biological control for many plant diseases. However, the colonization and establishment pathway of endophytes into the plants as well as the extent and mechanism of the contribution of the biologically fixed Nitrogen by the diazotrophic endophytes to the host plant remain to be studied. Hence, a consolidated effort is required to further intensify studies regarding isolation and utilisation of more numbers of endophytic microbes from tuber crops.

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