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Inside the plant: Bacterial endophytes and abiotic stress alleviation

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Abstract: Bacterial endophytes are the microbes internally associated with the plant, nourished in an isolated environment which is free from the external harsh and changeable ecological condition. They entered into the plant tissues and alleviate the biotic and abiotic stresses by producing numerous secondary metabolites. They are engaged with the *de novo* synthesis of structural compounds and stimulation of plant immunity. They are also involved in the process of exclusion of the pathogen by niche competition and actively take part in phenylpropanoid metabolism. Abiotic stresses in particular salinity problem, low pH, heavy metal toxicity and accumulation of recalcitrant complex compounds in the soil affecting the plant health are a major threat to the agriculture sector in crop production and stability of ecosystems. To cope with these problems agriculture productivity has been intensified by using synthetic chemicals and pesticides causes numerous problems worldwide. Endophytic bacteria are thus being utilized as a substitute to reduce the use of toxic chemicals and pesticides. They may be employed as a biological agent in the plant growth promotion and for the management of the global environment. There is a tremendous scope for the isolation and identification of new endophytic bacteria with excellent potential.

Keywords: Abiotic stress, Bioremediation, Endophyte, Endophytic bacteria, Salt stress

INTRODUCTION

Importance of microorganisms in human welfare have been ever realized from the ancient period of time in the dairy industry, fermentation industry, in biocontrol, production of antibiotics and up to more extent their utilization to produce novel bioactive compounds, secondary metabolites etc. (Bull, 2004). Their presence is noticed from various niches ranging from mountain to sea, from forest to desert and moreover, in cold and in hot spring also. In their various niches and unique places plants' inter-cellular space is one of them, which harbor vast variety and unnoticed group of microorganisms known as endophytes.

The term endophytic (endo- inside, phyte- plant) bacteria encompasses the internal association of bacteria with the plant (Hawksworth *et al.,* 1995; Suto *et al.,* 2002) and attributed various beneficial functions for the plant and the human welfare. They colonize the internal tissues of plants without any immediate negative or external symptoms. The study of endophytes may offer opportunities for discovering products and processes with potential applications in agriculture, industry, medicine, biotechnology and society. Endophytes can be found intracellularly either as obligate or in facultative association with lower and higher plants (Pandey *et al.,* 2013). History of exploitation of endophytes for their plentiful novel bioactive compounds were a century long, but very less progress was concerned towards bacterial endophytes which were found to be as a potential candidate as with other endophytic microorganisms like a fungus or more than them with their rapid multiplication, wide adaptability to variable environment, fast responses in various beneficial processes. Bacterial endophytes were reported to produce novel bioactive compounds which were not previously found to be reported naturally in plants such as insecticides (Ryan *et al.,* 2008), antimicrobials (Bacilio-JimeÂnez *et al.,* 2001; De Melo *et al.,* 2009; Wang *et al.,* 2009; Liu *et al.,* 2010) etc. responsible for adaptation in variable environment, induction of immune response and restriction of pathogens invasion in plant tissues (Sturz *et al.,* 2000; Lodewyckx *et al.,* 2002; Pandey *et al.,* 2015 and 2016), production of non- food crops for biomass and biofuel production (Rogers *et al.,* 2012).

The present world faces problems caused by abiotic factors which could generate an alarming condition to the world's biomes. Research based on the invention of new inocula of endophytic bacteria is a promising task to cope with the adverse effect of abiotic factors. There is a strong need for the production of new metabolites and to search for the alternative pathway by

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utilizing endophytic bacteria which are effective but cause less or no damage to the environment and replace the artificial chemicals and pesticides. There is a tremendous scope for the isolation and identification of new endophytic bacteria with excellent potential. The current review summarizes the significance of using endophytic bacteria to nullify the adverse effect of abiotic factors, especially the problems related to salinity, the heavy metal toxicity and bioremediation. A brief attempt was made towards their progressive evolution, host variability and biodiversity.

History, origin and evolution of endophytes: Vogl in 1898 reported the presence of an endophytic mycelium inside the grass seed *Lolium temulentum.* Freeman in 1904 reported an endophytic fungus in annual grass *Persian darnel*. Bacterial endophytes have been studied for more than 100 years (Anu Ranjan, 2012). The first endophytic bacterial presence within the plant tissues was reported in 1926 (Hallman *et al.*, 1997). Their relationship may have been thought to evolve during the hundred million years ago along with the higher plant appeared on the Earth (Anu Ranjan, 2012). It is found that certain microorganisms able to penetrate the plant tissues, either with the help of cell wall hydrolyzing enzymes like pectinease, cellulase or certain other mechanisms and reside inside the plant tissue and co-evolved. During co-evolution, they may be adapted towards the interior environment of the host plant involving the mechanisms of cross talk between the endophytes and the host plants (Pathak, 2011).

Host variability and biodiversity of the bacterial endophytes: Each individual plant on earth is host to one or several endophytes (Strobel *et al.,* 2004; Ryan *et al.,* 2008). Bacterial endophytes have been isolated from surface-sterilized plant tissues or extracted from internal plant tissues (James and Olivares, 1998). Strobel *et al.,* (2004) reported that the total plant species reported *i.e.* approximately 300000 present on the earth, each plant species carry one or more endophytes in their lifetime and nearly all vascular plant species (wheat, rice, sugarcane, pea, tea, banyan tree etc.) investigated to date were reported to harbor endophytic bacteria (Pathak, 2011; Pandey *et al.,* 2012; Bacilio-JimeÂnez *et al.,* 2001; James and Olivares, 1998; Elvira-Recuenco & Vuurde, 2000; Ratul *et al.,* 2013; Jha *et al.,* 2011). Their presence was also reported from different ecosystem such as from the copper mine wasteland, from agronomic and prairie plant's ecosystem, from deep water rice ecosystem, from grass ecosystem, from the pea field condition and from tropical grasses (Reinhold-Hurek & Hurek, 1998; Elvira-Recuenco & Vuurde, 2000; Verma *et al.,* 2001; Zinniel *et al.,* 2002; Phillips *et al.,* 2008; Sun *et al.,* 2010; Kelemu *et al.,* 2011).

Production of plant growth regulators (PGR): As we know hormones are organic compounds effective in very minute concentration, which after synthesis transported to another location where they interact with specific target tissue and regulate physiological functions such as division of cell, expansion of cell, differentiation, branching of shoot, cell death of the host plant and hence referred as plant growth regulators or Phyto-hormones (Auxins, Gibberellins, Ethylene, Cytokinins and Abscisic acid). Different endophytic bacterial groups like *Bacillus* species and *Klebsiella* species were reported to produce the indole-3-acetic acid, the most important auxin which regulates plant development such as cell expansion, division, differentiation, gene regulation and other tropic response (Ratul *et al.,* 2013; Ji *et al.,* 2014). It is found that the concentration and ratio of auxin to other phyto -hormones determine the physiological responses inside the plant and the microorganism which was able to produce IAA positively regulate the auxin levels (Patten and Glick, 1996; Lambrecht *et al.,* 2000). Cytokinins as another important phyto-hormone induce cell division of plant in combination with auxin. The ratio of high cytokinin to low auxin promotes shoot development, low cytokinin to high auxin ratio promotes root development and their equal concentration determines cell proliferation (callus formation) is the well known mechanism of action. Gibberellins are the phyto-hormone comprising terpenoids group consisting of up to 20 carbon atoms, but the GAs with 19 carbon atoms was found to be the most active. They are mainly involved in the cell division, cell elongation and participate in Internode elongation (Dodd *et al.,* 2010). Abcisic Acid is a phyto- hormone with 15 carbon atoms actively involved in the biotic and abiotic stress response of plant such as drought stress, salt stress and metal toxicity. It is responsible for negative regulation of seed germination, flowering and opening of stomata during stress conditions (Smyth, 2011). Ethylene is a kind of gaseous phyto-hormone affects several plant developmental processes such as root hair formation, root growth, flowering and well known for inducing fruit ripening, flower senescence (Dugardeyn and van der Straeten, 2008; Dodd *et al.,* 2010). They are also involved in breaking seed and bud dormancy. There are found to be synthesize in the response of various stress conditions such as in heat stress, in cold stress, in drought stress, in high levels of salt concentrations and in the case of excess heavy metals (Glick, 2005; Dodd *et al.,* 2010), hence also called as the stress hormone.

Abiotic stress alleviation by bacterial endophytes: According to Food and Agriculture Organization, (2012) world's population will be 2.3 billion by 2050 result to rise in food production demand, which is possible only by increasing agricultural farmland and productivity, which is limited by several restrictions such as urbanization, water scarcity, phytopathogens, adverse effect and high cost value of synthetic fertilizers and pesticides. Hence, the alternatives for sustainable and secure agriculture have been in demand globally

with grand promise in the improvement of agricultural yields. Sustainable agriculture contributes in national income as a major export earnings in today's developing countries as offering food security and employment to meet our future needs which conventional agriculture will not able to do.

According to an estimate more than 20% of agricultural soil faces increase in salinity problems (Zhu, 2000) and nearby 50% of the agriculturally important land will be affected by salinity stress by the year 2050 (Munns and Tester, 2008). Rising of salt concentrations in soil and irrigation water creates a key threat to agricultural scenario, which could be may manage by bacterial endophytes by osmotic adjustments, stomatal regulation, root morphology modification, increase in uptake of minerals and by reducing the toxic effects of Na^+ and Cl^- (Sairam and Tyagi, 2004; Pandey *et al.,* 2012).

Essential nutrients taken from plants by absorption of soluble salts stored in soil, but excessive accumulation of salts retarded the growth of plants (Patel *et al.,* 2011). Problem of salinity is recognized as the major threats to environmental resources and human health affecting about 1 billion ha globally. According to an estimate in India about 9.38 million ha land occupied by saline soil (Metternicht and Zinck, 2003; Yensen, 2008; Patel *et al.,* 2011; Misra and Dave, 2013). Efficient resource management and crop improvement can help to overcome salinity stress, but such approach being cost intensive and time taking. Hence, the use of microorganisms for exploitation of their unique properties such as synthesis of compatible solutes, tolerance to salinity and production of plant growth promoting hormones may be utilized for sustainable agriculture (Shrivastava and Kumar, 2015).

Heavy Metal toxicity is identified as one of the main abiotic stress factors that causes about 25–80% yield losses in various crop plants grown on soils (Singh *et al.,* 2011). Acidic soil engaged with poor crop productivity and low soil fertility due to the combined effect of Heavy Metal (Al^{+3} and Mn^{+2}) toxicities coupled with nutrient deficiencies (P, Ca, Mg and K) (Singh *et al.,* 2011). It is reported to highly toxic to plant roots (Kinraide, 1991) results in poor development of the root system (Foy, 1988; Singh *et al.,* 2011). Heavy Metal toxicity became a serious problem which limits crop productivity on acidic soils. Endophytic bacteria are recognized as to take part in the mobilization and immobilization of metal cations, thereby, influences their availability to plants.

Endophytic bacteria found to be more active in inducing strong defense responses against stresses than the other rhizospheric or soil microbes (Andrews, 1992, Pandey *et al.,* 2012). An endophytic bacterium *Pseudomonas pseudoalcaligenes* combined with a rhizospheric *Bacillus pumilus* in paddy were reported to protect the paddy plant from abiotic stress by induction of osmoprotectant and antioxidant proteins. At lower concentrations of salinity levels endophytic bacterium *P. pseudoalcaligenes* inoculated to the plants showed a considerable concentration of glycine betaine - like quaternary compounds and increase in shoot biomass. While a mixture of both *P. pseudoalcaligenes* and *B. pumilus* at higher salinity levels showed improved response against the adverse effects of salinity in the condition of Anand, Gujarat (Jha *et al.,* 2011). Bacteria that produce exopolysaccharates (EPS) when inoculated with wheat seedlings influence the uptake of sodium and plant growth promotion in high stress of salinity (Grover *et al.,* 2010).

A wheat endophytic bacterium (*Pseudomonas aeruginosa* PW09) was evaluated for its ability to alleviate abiotic stress in cucumber. PW09 culture was inoculated to cucumber seeds, and the seedlings were subjected to the high salt concentration (NaCl 150 mm). Their role was evaluated in alleviating the stress by assessing plant biomass accumulation under NaCl stress as well as at the physiological level through phenylpropanoid metabolism, antioxidant activities and proline accumulation. The endophyte increased biomass accumulation significantly up to 18% under NaCl stress compared with endophyte-untreated seedlings. Application of PW09 also induced higher accumulation of proline (1.4-fold) and total phenolics (1.1-fold) and activities of polyphenol oxidase (1.5-fold), phenylalanine ammonia lyase (1.27-fold) and superoxide dismutase (1.39-fold) under NaCl stress, which shows their ability in alleviating abiotic stress in cucumber in the pot experiments conducted at the Institute of Agricultural Sciences, Banaras Hindu University, Varanasi, India (Pandey *et al.,* 2012). Leite *et al.* (2014) isolated endophytic and rhizospheric bacteria from the sugarcane's root and rhizosphere respectively in the culture media supplemented with NaCl (5%) and without NaCl at Pernambuco, Brazil. They investigated the level of salinity tolerance along with the other plant growth promoting traits such as biological nitrogen fixation (BNF), production of indole acetic acid (IAA), inorganic phosphate solubilization, quorum sensing molecule and genetic diversity of isolated endophytic bacteria (Leite *et al.,* 2014).

The accumulation of salts near the root's surface causes toxicity to plant and alter water absorption (Freire & Freire, 2007). Generally stresses have lethal effects on plant development and growth. Report's of Pandey *et al.* (2012) suggest that microbial inoculation may recover plant health in stress condition via stimulating plants latent environmental stress response (Pandey *et al.,* 2012).

Bioremediation by bacterial endophyte: Now-a-days, excess use of herbicides, insecticides and other synthetic chemicals leads to severe large-scale pollution. With increasing environmental awareness, developing biological strategies using endophytic bacteria are new alternatives for solving such problems. Biore-

mediation is the use of biological agents to remediate contaminants in the environment or in the other word use of microorganisms to degrade toxins. Phytoremediation is a rapidly expanding field in which plants are used to remediate environmentally toxic compounds. Rhizodegradation, which is a branch of phytoremediation, is the use of plants to stimulate the microbial community near the root–soil interface to enhance the degradation of recalcitrant compounds in the soil. Plants can enhance the bioavailability of soil contaminants by releasing the low molecular weight organic acids, they release carbon and nitrogen containing compounds to nurture root associated microbes and secreted exudates which can enhance the degradation of soil contaminants through inducing biochemical pathways inside the endophytic bacteria (Leigh *et al.,* 2002; White *et al.,* 2003; Newman and Reynolds, 2005).

Endophytic bacteria *Methylobacterium populum* sp. nov., strain BJ001 is involved in the degradation of 2,4,6 trinitrotoluene (TNT), hexahydro-1,3,5-trinitro-1,3,5-triazine (HMX) and hexahydro-1,3,5-trinitro-1,3,5-triazine (RDX) (Van Aken *et al.,* 2004a; 2004b). Toluene is hazardous and affects the physiological process. When inhaled at high levels can cause unconsciousness or even death in humans. It is also harmful to plant (Newman and Reynolds, 2005). Van der Lelie's (2005) group has developed a method for genetically endowing a plant endophyte with the ability to degrade toluene (Barac *et al.,* 2004; Newman and Reynolds, 2005). *Burkholderia cepacia* is having ability to grow in the presence of elevated levels of toluene and degrade it. The natural ability of endophyte to degrade xenobiotics is being investigated with regard to improving phytoremediation (Siciliano *et al.,* 2001; Barac *et al.,* 2004; Germaine *et al.,* 2004; 2006; Porteous-Moore *et al.,* 2006; Ryan *et al*., 2008).

Conclusion

It is concluded that the endophytic bacteria must be used to ameliorate the abiotic stresses arising due to variable environmental conditions, physiological alteration inside the plants and adverse effect of synthetic fertilizers and pesticides. The endophytic bacteria have ability to produce several kinds of novel bioactive compounds and metabolites which can be able to positively regulate the physiological disorder inside the plants. Their efficiency has been realized as they have a rapid multiplication cycle, have wider adaptability to variable and changing environment, have fast responses in induction of host immune defense. They provide an insight towards the alternatives for the sustainable and protected cultivation in progressive agriculture. Bioremediation by employing endophytic bacteria to remediate toxic recalcitrant residues of herbicides, insecticides and synthetic chemicals along with excess heavy metals which is toxic to plants is an emerging field of research. Further, studies of endophytic bacteria will offer a better understanding of their association with the host plant and provides a new finding in the endophytic research.

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