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Potentials of endophytic bacteria in sustainable agriculture and the use of genomic approach in plant-endophytic interactions

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Abstract

Bacterial endophytes are ubiquitous colonizers of the inner plant tissues where they do not normally cause any substantial morphological changes and disease symptoms. These microorganisms represent a potential source of novel natural products for medicinal, agricultural and industrial uses, such as antibiotics, anticancer agents, biological control agents and other bioactive compounds (Li *et al.*, 2008). Endophytic bacteria can promote growth and increase the yield of the plant and also protect their host against phytopathogens. Genomic analysis can give a deeper insight into the capabilities of endophytes and their possible role in plant growth and health and use of available post genomic tools in understanding the functionality of endophytic bacteria in plants. This will help achieving eco-friendly approach for increasing the growth and yield of plants in a sustainable manner.

Keywords: Biocontrol, bioactive compounds, endophytes, genomic tools, plant-endophytic interactions, etc.

Introduction

In a natural ecosystem, all the healthy and asymptomatic plants host a diverse group of the microbial community including bacteria, fungi, viruses and protista collectively, known as plant microbiota (Hiruma et al., 2016) ^[15]. Among the plant-associated microorganisms, endophytes are the bacterial and fungal population colonizing within a plant tissue for a part of its life cycle without showing any apparent pathogenesis (Tan and Zou, 2001)^[11]. Endophytes are ubiquitous with rich biodiversity, which have been found in every plant species examined to date. It is noteworthy that, of the nearly 3,00,000 plant species that exist on the earth, each individual plant is the host to one or more endophytes. In this view of the special colonization in certain hosts, it is estimated that there may be as many as 1 million different endophyte species. However, only a handful of them have been described. Culture-dependent and independent community profiling revealed their active association virtually with all the tissues of a host plant, including the intercellular spaces of the cell walls, vascular bundles, and in reproductive organs of plants, e.g. flowers, fruits, and seeds. Their association was even logged from aseptically regenerated tissues of micro propagated plants (Dias et al., 2009) ^[13]. Environmental parameter including soil nutrients and different abiotic stresses influence the diversification of the endophytic entity in a plant may play a significant role in the natural fitness in particular environment (Bulgarelli et al., 2013)^[12]. In this mutualistic relationship, the plant provides primary nutritive components and a protective niche for the endophytic organisms, whereas the endophytes produce useful metabolites and systemic signals (Rosenblueth and Martínez-Romero, 2006) ^[10]. This diversity of endophytes ranges from Gram positive to Gram negative bacteria which include genera like Achromobacter, Acinetobacter, Agrobacterium, Bacillus, Brevibacterium, Burkholderia, Chromobacterium, Curtobacterium, Enterobacter, Kocuria, Lysinibacillus, Methylobacterium, Microbacterium, Paneibacillus, Pantoea, Phyllobacterium, Pseudomonas, Rahnella, Rhodanobacter, Stenotrophomonas, Streptomyces, Xanthomonas etc which have been isolated from diverse plant species including maize, potato, tomato, sugarcane, and cucumber (Bacon and Hinton, 2007). The diversity of endophytic bacteria can be studied by cultivation-dependent and cultivation independent techniques (metagenomic analysis). Cultivation-based techniques help in the recovery and testing of isolates, whereas cultivation-independent techniques help to screen the variations in the total endophytic communities.

Colonization and diversity of endophytic bacteria

It is found that during the course of evolution certain microbes were able to enter the plant tissues, either with the help of synthesis of cell wall hydrolyzing enzymes like pectinase,

cellulase or by developing some 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 (Pandey *et al.*, 2016). They colonize internal tissues of the plants either as obligate or in facultative manner without showing any immediate negative or external symptoms and reported to display the beneficial effects, put forward opportunities for discovering products and processes with potential applications in agriculture (Pandey *et al.*, 2017).

Bacterial entry

The preferable sites of bacterial attachment and subsequent entry are the apical root zone with the thin-walled surface root layer such as the cell elongation and the root hair zone (zone of active penetration), and the basal root zone with small cracks caused by the emergence of lateral roots (zone of passive penetration). At these sites bacteria are often arranged in micro colonies comprising several hundreds of cells (Zachow et al., 2010). Bacterial endophytes benefit from inhabiting the plant's interior because it is a protected niche in which there is relatively little competition from other microorganisms for a constant and reliable source of nutrition. The internal colonization protects endophytes from exposure to extreme environmental conditions, such as temperature, osmotic potentials and ultraviolet radiation. Thus the internal colonization by endophytes provides an added ecological advantage to them over epiphytes.

Plant Growth Promoting (PGP) activities of endophytic bacteria

Bacterial endophytes stimulate plant growth, directly or indirectly thereby increasing their yield and several parameters utilized by living things for their life prospects (Pandey *et al.*, 2016). They offer an extensive range of benefits to the host plant against biotic and abiotic stresses (Hurek and Hurek, 2011). In return, the bacterial endophytes may be benefited by the various secondary metabolites and the growth regulators produced by the host plants (Schulz and Boyle, 2006)^[7].

Due to their residence inside the host plants, their products can have significant effect on physiology and metabolism of the plants in which they reside. The microbial production of auxins is known to trigger increase in cell elongation, cell division and differentiation in various plants (Thibodeaux and Liu 2011) ^[5]. Microbial population also performs phosphate solubilization by the secretion of organic acids which convert the insoluble phosphates into soluble monobasic and dibasic ions and thereby making it available to plants (Taurian *et al.* 2009). ACC deaminase production by plant associated bacteria promotes plant growth by regulating the synthesis of ethylene and thereby reducing its harmful effects (Glick 2014). Endophytic bacteria, which produce Siderophore, can restrict the growth of plant pathogens because of their strong affinity towards Fe (III) (Yang *et al.* 2009).

Several endophytic bacterial strains have been shown to have beneficial effects on their host plants by production of plant growth enhancing chemicals such as indole acetic acid or cytokinins and protection against biotic and abiotic stresses. Beneficial effects on plant growth may also be achieved by improved nutrient acquisition including nitrogen fixation. As such, these properties have attracted agronomic interest. Similar to the Rhizobium-legume symbiosis, endophytes fix atmospheric nitrogen by means of the enzyme nitrogenase which is oxygen sensitive. In the case of endophytes, it is not certain how the nitrogenase is protected from oxygen. In the case of the sugarcane endophyte, the rapid respiration from metabolism of high levels of sucrose in the stems resulted in a micro aerobic environment needed for the nitrogenase enzyme. Others have shown that endophytes may use physical barriers including Exopolysaccharides, internal vesicles or biochemical methods to exclude the oxygen. Most of these endophytes were associated with disease suppression, stress tolerance, growth promotion, or providing fixed nitrogen to the plants.

Biocontrol of phytopathogens by endophytic bacteria

Currently, several possible mechanisms are suggested for the inhibition of phytopathogens by endophytic bacteria:

- 1. Competition with pathogens for the ecological niche/substrate (siderophores) in the rhizosphere;
- Production of antibiotics (cyclic lipopeptides, iturin, fengycin) and antifungal substances (2, 4diacetylphloroglucinol);
- 3. Production of extracellular chitinase and laminarinase to lyse fungal cells and degrade fusaric acid produced by fungal pathogens; and
- 4. Production of volatile organic compounds (such as 2,3butanediol and acetoin, which act as signaling molecules to mediate plant–microbe interactions), which could strongly inhibit pathogen growth on different hosts54and elicit plant growth by induced systemic resistance (ISR).

Biocontrol of phytopathogens can be based on several mechanisms which include antibiosis, CNN (competition for nutrients and niches) and ISR (induced systemic resistance). Colonization of plants by biocontrol endophytes induces several cell-wall modifications, such as deposition of callose, pectin, cellulose and phenolic compounds leading to the formation of a structural barrier at the site of potential attack by phytopathogens (Benhamou *et al.*, 2000) ^[2]. Another common response of bacterized plants challenged with a pathogen is an induction of defense-related proteins such as peroxidase, chitinases and β -1,3-glucanases (Fishal *et al.*, 2010) ^[3]. These reactions result in a substantial reduction of pathogen spreading in a plant.

Most likely, a combination of several mechanisms is exhibited by many biocontrol endophytic bacteria. This notion is supported by the fact that some antimicrobial compounds are involved in both antibiosis and triggering ISR (Ongena *et al.*, 2007) ^[6]. The presence of other mechanisms such as competition for iron and for colonization sites is proposed for some endophytes based on the analysis of their genomes.

Genomic and post genomic view of plant-endophyte interactions

In recent years a number of genomes of endophytic bacteria have been sequenced. The beneficial traits of bacteria wise, N fixation, IAA, ACC deaminase, *etc* are reflected in their genomes. Moreover, analysis of their genomes also revealed the existence of a high number of genes involved in iron uptake and metabolism (Taghavi *et al.*, 2010)^[8]. Endophytic bacteria are well-equipped to survive in a low-iron environment and can efficiently compete for this element with other microorganisms, including phytopathogens. In addition to the above-mentioned plant beneficial traits, a number of genes involved in QS (quorum sensing) have been identified in the endophytic genomes (Liu *et al.*, 2011)^[5].

Applying post genomic approaches, such as metaproteomics, metaproteogenomics and metatranscriptomics, can link the genomic potential with function and therefore give a deeper insight into plant-endophyte interactions. These tools deal with global expression of proteins (metaproteomics) or mRNA (metatranscriptomics) from microbial communities. Metaproteogenomics links the proteome and the genome of the environmental sample. This allows identification of more proteins (functions) than proteomics alone. Recently, a Metaproteogenomics approach was used to study microbial communities in the phyllosphere and rhizosphere of rice (Knief *et al.*, 2011)^[4]. If such an approach could be applied to study the endosphere, more significant data regarding the endophyte functionality can be collected.

Future line of work/ Future prospects

There are reports that endophytes can become parasites under certain conditions and vice versa. Hence, host-microbe interactions can range from mutualism through commensalism to parasitism in a continuous manner. As disease is the exception in plant-microbe interactions, it can potentially be regarded as an unbalanced status of a symbiosis. The molecular and biochemical basis for the switch from endophyte to parasite is still to be elucidated, but recent findings in studies of compatible plant-microbe interactions have enhanced our understanding of what factors determine endophytic and parasitic lifestyles. Decoding the switches that lead to mutualistic symbiosis or disease will reveal new targets and strategies for human intervention into these processes; for example, by formulating inocula with endophytes, generating improved plant germplasm or developing small molecules that interfere with plant-microbe interactions.

Conclusions

Endophytic microorganisms have even been reported to possess the ability to produce plant specific metabolites due to the possible shared or co-evolved biosynthetic machinery (Rosenblueth and Martínez-Romero 2006) ^[10]. Moreover, their diverse metabolic activities contribute significantly to the health, growth and development of plants. Thus biosynthetic and enzymatic basis of endophytes and its impact on plants provide more opportunities to understand plant-microbe interactions, which can have immense applications in sustainable agriculture.

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