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Endophytic Microbes: Potential Tools for Sustainable Crop Production

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Abstract
The use of endophytic microorganisms as potential tools to mitigate adverse environmental factors is a rapidly developing technology for sustainable crop production. These microbial symbionts colonize the host plant's intracellular and intercellular spaces without causing morphological changes or infections, whilst developing secondary active metabolites that protect their host plants from phytopathogens. The production of beneficial substances by these microbes not only aids plant growth and protect them in the face of biotic and abiotic stress, it positively impacts the goal to tackle world hunger that is as a result of increased global population. These microorganisms have recently been used in a number of biotechnological fields which include the development of biofertilizers to boost crop production, while reducing chemical inputs into the environment, biocontrol of plant pests and diseases. In this review, the diversity, population and mechanism of interaction of these endophytes with crop plants as well as their potential applications in sustainable crop production were highlighted.

Keywords: Endophytic microorganisms, crop production, biotic, abiotic, global, biocontrol, biofertilizers, intracellular, intercellular, phytopathogens

Introduction

The projections for global population increase have expectedly brought tremendous pressure on agricultural practices in a bid to cushion the inevitable effect on food security. In 2020, between 720 million and 811 million persons worldwide suffered acute hunger, and above 30% of the world's population were moderately or severely food-insecure, lacking regular access to adequate food (SDG, 2022). Factors such as the biotic (pests and pathogens) and abiotic (soil fertility, salinity, meteorological conditions such as drought, heat, flooding stresses) affect crop productivity adversely in terms of yield. This situation paved way for intensified focus on alternative ways to mitigate these challenges while providing sustainable and eco-friendly solutions. Several biotechnological tools have been employed to increase crop yield in such as molecular breeding techniques, application of chemical fertilizers and the use of pesticides and herbicides for pest and weed management; although they have been reported to degrade systems and produce food with high concentration of various contaminants (Tonial *et al.*, 2020). In contrast, use of endophytic microorganisms have also been reported to not only have potentials in creating sustainable solutions to problems related to food production and agronomic areas, but to have an inestimable potential for bioremediation of polluted environments (Sim *et al.*, 2019). Despite the success of a few well-known endophyte–plant relationships (Hardoim *et al.*, 2015), the use of endophytes to overcome threats to plant health is not common place in most conventional agriculture, and our reliance on agrochemicals continues to take precedence over alternative solutions. Currently, our widespread reliance on fungicides may incapacitate fungal biological agents (as well as the vectoring of bacterial agents by fungi), and high fertilizer levels reduce plant dependence on both fungal and bacterial endophytes, and other parts of the root microbiome (Le Cocq *et al.*, 2017). In view of this, this review intends to highlight the potentials of the endophytic symbionts and promote further research in understanding their extensive functional mechanisms.

Endophytes

Endophytes are omnipresent microorganisms that can be isolated from a wide variety of plants including; bryophytes, pteridophytes, gymnosperms, and angiosperms (Sun *et al.*, 2008; Bragina *et al.*, 2012; Zhikai *et al.*, 2017; Proença *et al.*, 2018). The word “endophyte” comes from the Greek words "endo/endon," which means "inside," and "phyte/phyton," which means "plant". De Bary (1866) coined the term "endophyte," which was once applied to all "organisms occurring within plant tissues" or "all organisms inhabiting plant organs that, at some point in their lives, can colonize interior plant tissues without inflicting obvious harm" (Petrini, 1991). They could be classified as beneficial, neutral and or detrimental depending on the kind of interaction with their host plant (Strobel and Daisy, 2003). These microbial symbionts can have profound effects on plant ecology, fitness and evolution (Petrini, 1991), shaping plant communities and manifesting strong effects on the biological activities of the plants (Felde, 2011). Recent reviews of bacterial and fungal endophytes suggest that the term endophyte should refer to 'habitat only, not function', and should include 'all microorganisms which, for all or part of their lifetime, colonize internal plant tissues', referring to the continuum of interaction between a host plant and the microbes that colonize it (Schulz and Boyle, 2005; Hardoim *et al*., 2015). However, Le Cocq *et al.* (2017) reviewed that Endophytes can be classified as "microbes which occur within plant tissue for at least part of their life cycle without causing disease under any known circumstances". They further explained that some microbes may be currently considered endophytic, but this designation may be changed if they are subsequently shown to be harmful to a plant host.

Classes of Endophytes (Figure 1)

Plant-associated endophytes are either prokaryotic or eukaryotic. Bacteria and archaea are prokaryotic endophytes, while fungi, algae, and amoeba are eukaryotic endophytes. Actinomycetes and mycoplasmas are common bacterial endophytes found in plants (Figure 1). However, endophytes have been identified in 16 different bacterial phyla, with the majority of species belonging to the phyla *Actinobacteria, Firmicutes,* and *Proteobacteria* (Golinska *et al*., 2015). A number of algae grow as endophytes in seaweed; one of such is *Ulvella leptochaete*. This was recently discovered by a range of seaweed hosts from India (Anyasi and Atagana, 2019).

Plant-Endophyte Relationship

According to fossil records, plants have been associated with bacterial endophytes and mycorrhizal fungi for more than 400 million years (Anyasi and Atagana, 2019). This relationship starts the moment a land is colonized by plants, thus playing a long and important role in driving the evolution of life on land (Rodriguez *et al*., 2009). Endophytes live fully within plant tissues, growing in roots, stems, and/or leaves before sporulating when the plant or host tissue dies (Anyasi and Atagana, 2019).

According to Santoyo *et al.* (2016), some endophytes are known to increase nutrient uptake and to enhance the growth of the host plant. This group of endophytes can facilitate plant growth in agriculture and horticulture following mechanisms similar to those employed by rhizospheric plant growth-promoting bacteria. The authors stated that the ability of bacterial endophytes to promote the growth of their host plant can be either direct or indirect, whereby, direct promotion of growth occurs when a bacterium increases the level of plant growth hormones (auxin, cytokinin, or gibberelins) and facilitates the acquisition of essential nutrients (such as nitrogen or phosphorus); or indirectly, when the damage to host plants following infection by certain pathogens is decreased, basically through pathogen inhibition by plant growth promoting bacterial endophytes. The interaction between the host and the endophyte is thought to be complex and varied from host to host and microbe to microbe (Jalgaonwala and Maharanja, 2014). The relationship between an endophytic fungus and plant host is truly mutalistic because the fungus must obtain nourishment from the plant since it does not have contact with the soil, while the endophytic fungus enables the host plant in its nutrient uptake from the soil. Endophytes that inhabit foraging grasses e.g. rye grass, do not leave their plant host and can only reproduce by invading seed tissue of the plant (Stone *et al*., 2000). The mode of entry of microbial endophytes to the plant is equally an important factor to consider, as microbes can be transmitted vertically through seeds or can enter through stomata and be transferred horizontally from plant to plant (Hardoim *et al*., 2015).

Mechanism of Plant Protection by Endophytes (Figure 2)

Temperature, high light intensities, flooding, drought, wounding, radiation, predation, infections, nematodes, excessive salt concentrations, and the presence of toxic chemicals are just some of the biotic and abiotic variables that can limit plant growth and induce environmental stress (Sharma *et al*., 2020). Recent molecular research on endophytes has revealed that they not only boost plant development but also limit pathogen activities, aid in the solubilization of phosphate and other minerals, and assist plants assimilate nitrogen (Johnston-Monje and Raizada, 2011). Endophytic actinobacteria associate with their host at a very early stage of the plant development. Maximum numbers of endophytic actinobacteria have been recovered from roots followed by stems and least in leaves (Golinska *et al*., 2015). Recent studies carried out on endophytes have established their capacity to enhance host defense against diseases and reduce the damages attributed to pathogenic microorganism (Ganley *et al*., 2008; Mejía *et al*., 2008). Although some studies have presented some direct and indirect mechanisms used by endophytes in reducing the effects of pathogens, current knowledge about endophytes, pathogens, and plant defense regulations are still not fully understood (Ganley *et al*., 2008).

Endophytes and Biodiversity (Figure 3)

Among the many ecosystems on the planet, those with the most biodiversity also appear to have the most endophytes and the most biodiverse microorganisms (Strobel and Daisy, 2003). The most biologically diverse terrestrial environments on the planet are tropical and temperate rainforests. Even though the most endangered of these locations only span 1.44% of the land's surface, they are home to more than 60% of the world's terrestrial biodiversity (Mittermeier *et al*., 2011). Bills *et al.* (2002) reported a metabolic distinction between tropical and temperate endophytes based on statistical data comparing the quantity of bioactive natural products extracted from tropical endophytes to those isolated from temperate endophytes. Not only did they discover that tropical endophytes create more active natural products than temperate endophytes, but they also discovered that tropical endophytes produced much more active secondary metabolites than fungus from other tropical substrates.

Culturing of Endophytes (Figure 4)

A great diversity of endophytic bacteria/fungi have been isolated from various plant tissues. Almost 70% of the endophytes that have been currently isolated belong to the fungi kingdom and the rest 30% are bacteria, based on the sequences deposited in the NCBI nucleotide database (Manias *et al*., 2020). Until recently, only 1% of microbes present in bulk soil have been amenable to culture. However, the development of isolation chip, the 'ichip' (Nichols *et al*., 2010), has resulted in the culture of up to 50% of the microbes present in soil. The preliminary steps for isolation and identification of endophytes from collection of samples to surface sterilization and fragmentation of plant tissue (Manias *et al*., 2020). Following the selection of a plant for study, the plant is identified and its location plotted using a global positioning device. After removing excess moisture from the plant, small stem sections are cut and stored in sealed plastic bags. The materials are kept at 4°C as much as possible until isolation operations begin (Strobel and Daisy, 2003). Rebotiloe *et al.* (2018) reported the protocol for isolating bacterial endophytes from *Crinum macowanii* bulb surface sterilization procedure. They described how they first outer layer of the bulb (covered with heavy soil) was peeled off and the bulb washed several times with tap water to remove soil on the second layer. The bulb was treated with sufficient volume covering the whole bulb, of Tween 80 (surfactant) with vigorous shaking for 10 min. This was followed by several washes with sterile distilled water, after which the bulb was immersed in70% ethanol for 1 min with shaking. The ethanol was rinsed off with sterile distilled water and the bulb further sterilized with 1% sodium hypochlorite (NaOCl) for 10 minutes. The sample was finally rinsed with sterilized distilled water 3 times. The last distilled water rinse was plated on nutrient agar plates as control. There are numerous unculturable endophytic microorganisms (especially bacterial) and their identification greatly depends on molecular techniques. The first step of molecular-based identification involves the extraction of DNA from the samples using either the CTAB (cetyl trimethylammonium bromide) method or, commercially available DNA kits (Allen *et al*., 2006; Utturkar *et al*., 2016).

Application of Endophytes to Agriculture

La Cocq *et al.* (2017) reported that the most obvious approach for the application of endophytes in agricultural systems is to add inoculants to the soil or as seed dressings. Silva *et al* (2012) corroborated this approach in the study involving sugarcane production where they evaluated the shelf life and the colonization efficiency of novel liquid and gel-based inoculant formulations for sugarcane. The different inoculant formulations were all composed of a mixture of five strains of diazotrophic bacteria (*Gluconacetobacter diazotrophicus, Herbaspirillum seropedicae*, *H. rubrisubalbicans*, *Azospirillum amazonense* and *Burkholderia tropica*). However, the use of inoculation is often unsuccessful on a field scale because of problems associated with the establishment of the biological agent (O'Callaghan, 2016). Robinson *et al*. (2016) highlighted the challenge of the best time for inoculation in their report on wheat, where it was determined that vertical transmission did not occur when surface-sterile excised embryos were inoculated with potential endophytes. They further reported on the higher likelihood of the seed-adhering microbes being able to colonize the endosphere after germination, supporting the application of potential endophytes as seed dressings. Other approaches include; soil amendment to encourage the indigenous microbial community to respond and aid host plant growth and defense (La Cocq *et al*., 2017).

Challenges/Knowledge gaps

At present, there is little understanding of how the genomes of bacterial and fungal endophytes and plant pathogens differ. It seems that altered gene regulation and gene disruption, rather than deletion, are important in the development of a non-pathogenic relationship with the plant host (Xu *et al*., 2014; Hacquard *et al*., 2016) although the best strategy is not yet known. Despite the success of a few well-known endophyte–plant relationships (Hardoim *et al*., 2015), the use of endophytes to overcome threats to plant health is not commonplace in most conventional agriculture, and our reliance on agrochemicals continues to take precedence over alternative solutions. Indeed, any bacterial and fungal endophytes that suppress herbivory or plant diseases must be rigorously tested for toxin production for human and animal safety.

Conclusion

A key consideration for the introduction of endophytes in crop production is their behaviour under a range of conditions, and it is critical to understand their full life cycles and genome plasticity in order to assess their risk of becoming pathogenic, either through a shift in abiotic conditions or adaptation to an alternative host (Rodriguez *et al*., 2010). A novel approach would be to modify the root exudation chemistry of crops to select a more beneficial microbiome – this may also be one of the factors determining cultivar responses to drought, starvation and disease. We need a much better understanding of the interactions between the host and the soil microbiome in order to exploit it and recruit beneficial endophytes, as well as the interactions that take place between microorganisms in this system. This is likely to be achieved through a better understanding of signaling between the host plant and the microbiome, and, ultimately, the manipulation of root exudation profiles to recruit a more beneficial root microbiome, of which the endosphere is an integral part.

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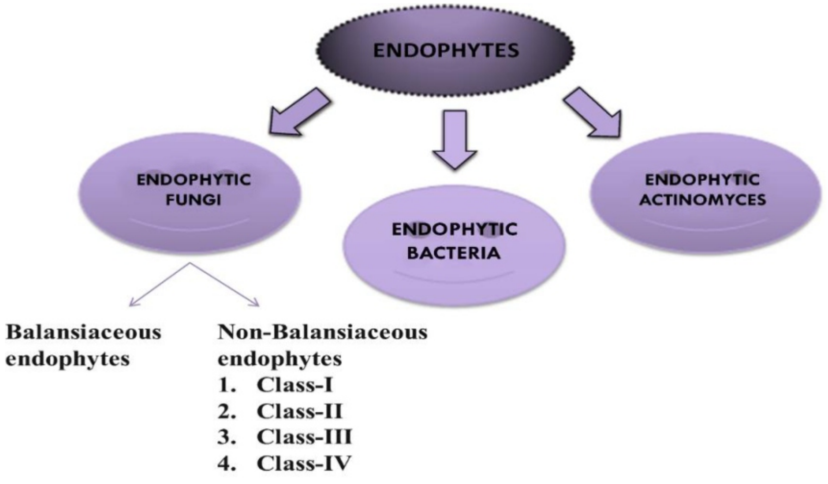

Figure 1: General classification of endophytes (Kumar *et al.*, 2017)

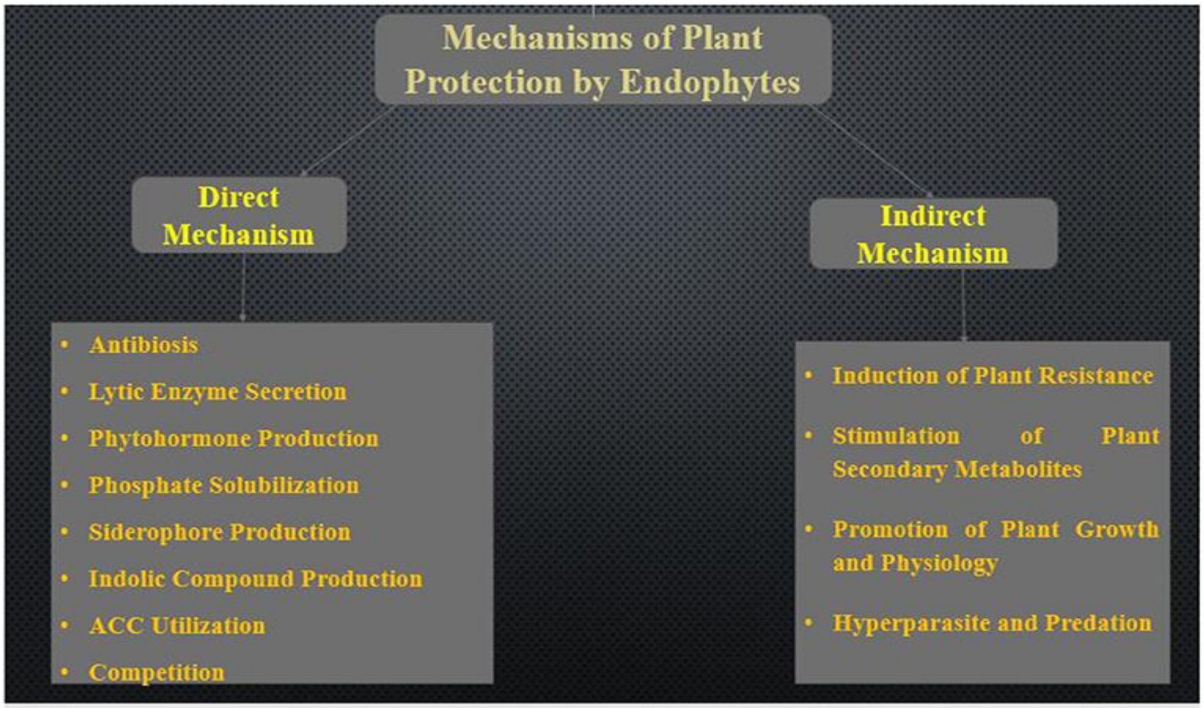

Figure 2: Mechanism of plant protection by endophytes (Fadiji and Babalola, 2020)

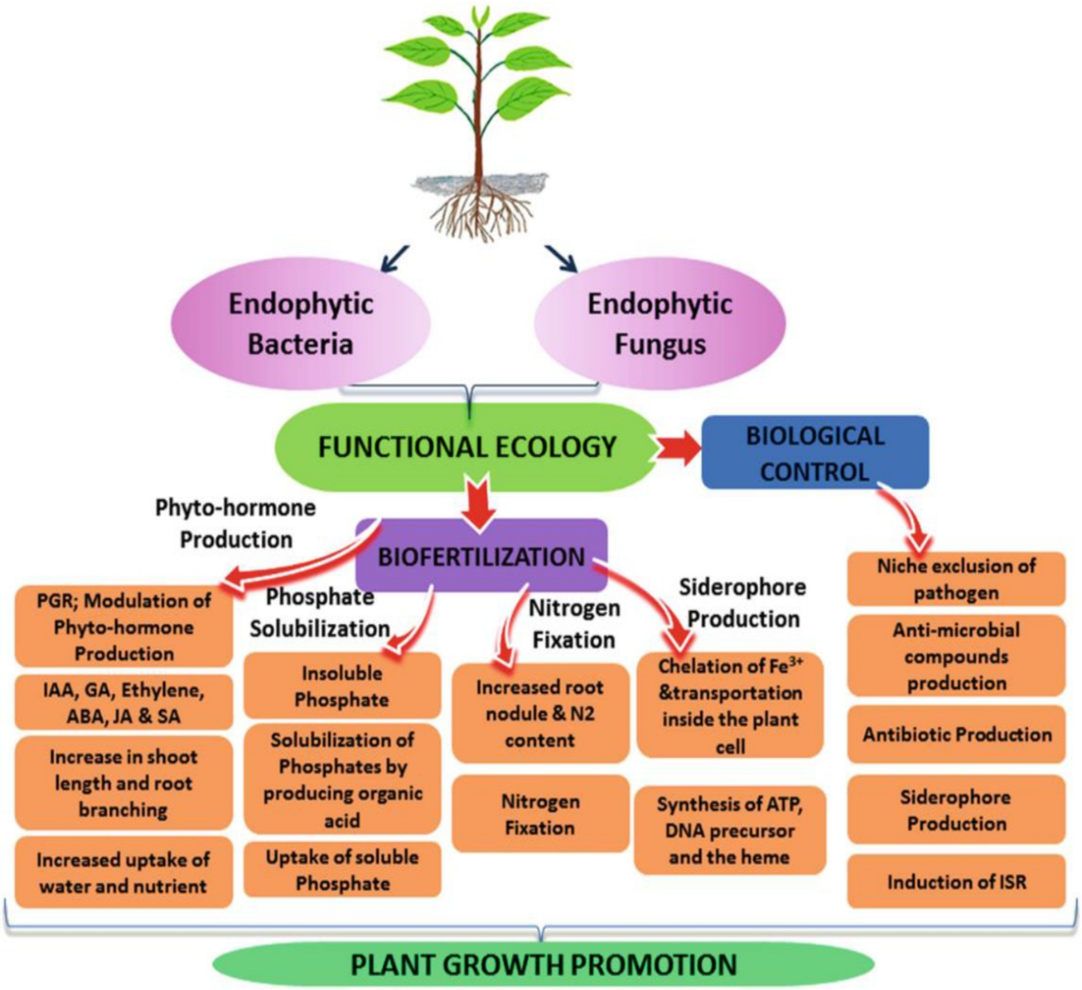

Figure 3: Application of endophytic bioactive compounds in crop production (Fadiji and Babalola, 2020)