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Review Article

Endophyte: Understanding the Microbes and its Applications

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Abstract

The ability of endophytes to colonize every plant tissue has led to the opportunity of using the microorganism in a lot of biological applications. Endophytes are beneficial to their host cells as such its application is observed in every aspects of life. This study therefore endeavored to give an analysis of endophytes, what they were and what they had been used for till the present time. Sampling of several literature studies in endophytes was done in this study to enable a complete understanding of the mechanism of application of the actions of endophytes, so as to be able to do a thorough assessment of the current state in the knowledge of the microbes. From the complete analysis of the literature on the application and use of endophytes, in nutrient asquition and increase the stress tolerance in plants. This study provided a platform for further research gaps through the presentation of what endophytes were, what they had been used for till date, the mechanism of operation of the micro-organism and the type of interaction between them and their hosts. There are still ways to improve on the methods of application of endophytes as a type of biological organism. This will be done by adjusting to the current trends in biological studies using molecular mechanization, following an intensive further study on endophyte mechanisms.

Key words: Endophytes mechanism, host cells, pollutants, plant-microbes interactions, phytoremediation

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INTRODUCTION

It has been reported that all plants in their natural ecosystems appear to be symbiotic with endophytes[1]. This highly diverse group of microbes usually impact greatly on plant communities through the increasing of fitness. This increase of fitness in plants is achieved through the conferring of abiotic and biotic stress tolerance, increasing biomass, decreasing water consumption and/or decreasing of fitness by altering the allocation of resources from soil to the plants[2]. Although prolong research studies has ensued in this new line of biotechnology resulting in lots of studies, the ecological significance of these set of microorganisms and their interactions with plants have not been unraveled.

Endophytes are regarded as a micro-organism that lives in plant tissues partly or in all of their lifecycle. They could be classified as beneficial, neutral and or detrimental depending on the kind of interaction with their host plant[1]. Endophytes may not have been very much recognized however, all the well-known studied organisms could be regarded as endophytes. For example, *mycorrhizal* fungi and *rhizobia* are regarded as the beneficial ones, while *Fusarium* sp. that causes wilt disease in a banana are taken as the detrimental ones[3]. Nonetheless, endophytes are not directly detrimental to plants[1]. In the recent past, many studies have been focusing on the exposition of the modes of action of mutualistic endophytes. This has been clearly indicated by the reports of these studies, stating the ability of endophytes to activate their host plant's existing defense mechanisms before or after pest and pathogens are supplied into the system[1,2,4].

Right from the introduction of symbiosis as 'the living together of dissimilar organisms[5], an array of symbiotic lifestyles have been defined based on fitness benefits or impacts to macroscopic hosts and microscopic symbionts[6]. Collectively, literature has reported that most, if not all, plants in their natural ecosystems are symbiotic with microbial endophytes[1,7-8]. These microbial symbionts can have profound effects on plant ecology, fitness and evolution[8], shaping plant communities[9] and manifesting strong effects on the biological activities of the plants[3]. According to the fossil record, plants have been associated with bacterial endophytes and mycorrhizal fungi for >400 million years. 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[2,10-11].Unlike mycorrhizal fungi that colonize plant roots and grow into the rhizosphere, endophytes reside entirely within plant tissues and may grow within roots, stems and/or leaves, emerging to sporulate at plant or host-tissue senescence[12-16]. These bio-activities were made known by the mechanisms that resulted in the evolution of the concept of endophytism and its genetic basis, although much understanding has not been garnered[13].

Endophytes in its colonization ability, behave the same way as phytopathogens, as a result they can serve as a biocontrol agent[15]. According to literature, endophytes possess the ability to control the pathogens of plant, insects and nematodes[15-16]. Some of the organisms helps to in enhancing seedling emergence, promote plant establishment in adverse conditions and also enhance plant growth[3,15,17-18]. Other classes of endophytes have been shown to be involved in the synthesis of novel compounds and antifungal metabolites[15]. This biodiversity of strains of endophytes used for novel metabolites may assist in the identification of new drugs that could help in the effective treatment of diseases for humans, plants and animals[1,17,18]. In addition to the production of novel chemicals, some other endophytes are implicated in their natural capacity for xenobiotic degradation, which may also act as vectors that introduce traits for degradation purposes[19]. This overall importance of endophytes results from their exposure to diverse compounds in their interaction in plant-soil niche. At the moment, many investigations are underway as a way of improving the biocontrol capabilities of endophytes[15,20-23]. This review aimed to provide an understanding of endophytes and the current state of its application as a novel micro-organism.

EXPLANATION OF THE CONCEPT

Since early 1866, there have been a lot of controversies surrounding the concept of endophyte and what could be taking as its definition, based on the notion put forward by De Barry[5]. However, Petrini[8] concluded that endophyte should be referred to as those micro-organisms that live in the plant organelles without causing diseases and this was widespread accepted. Furthermore, other schools of thought in trying to put meaning into the understanding of what an endophyte is described the phenomenon as when an organism colonized the internal tissue of a plant during its life-cycle formutualistic, commensalistic or sometimes parasitic relationship to the host[24]. In another word, endophyte: A concept of ecology is one natural part of plant-micro ecology system which can thoroughly be explained by the understanding of the ecological parameters. Endophytes exist in all plant, hence, are described to be extensive in nature[25-26]. However, literature reports estimated the existence of endophytes in microtubule of plant cells as well as in the intercellular spaces to several millionth species[27]. Various studies have reported on different methods that endophytes could be isolated from different plant species as such the organisms are found distributed in several plant cells; roots, stems, leaves, fruits and seeds[7,24,28]. The root of the plant is still the area with the highest density, which could get to as much as 106 colony forming units per gram (CFU g^{-1})[28].

Clearly, the type, numbers as well as the rate of growth of endophyte in plant is determined by the climatic and health condition of the plant[7]. Although a lot of techniques exist with which endophytes could be identified, the source of endophytes is still under debate as a result of the diversity of host's environmental conditions and the complexity of the relationship between them[29]. This helps to form the two hypotheses that are in existence at the moment: endogenous and exogenous system. While endogenous believes that endophytes evolve from the internal tissues: The mitochondrion and chloroplast of the plant cells, the reason being that they share similar genetic characters with the host. Exogenous system, however, is of the opinion that endophytes emanates from outside and enters the host through its surfaces[30-33]. With the help of biomarkers; key tools used to studying ecological processes, external colonization of plants by endophytes has been established[15,34-35].

ISOLATED MICROBIAL ENDOPHYTES

Based on the organism's classification protocols, endophytes are classified into bacterial, fungi and actinomyces or algae[31]. Following literature report, the first endophytic fungi (*Taxomyces andreanae*) were first isolated by a research study in 1993[1] using *Taxus brevifolia*: A medicinal plant[27,29]. The study of Huang *et al.*[36] reported also on endophytic fungi strains totaling 42, isolated from *Nerium oleander L. endophytic* fungi abounds in every ecosystem, but they seem to be most abundant in tropical hosts because of their high biodiversity[33-37]. 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 (Fig. 1). The ecological advantage of these algae to the host plant is that they serve in disease resistance[38]. Literature report indicated that this endophyte secretes anticancer metabolites known as Taxa; used in many cancer treatments[2]. Bacterial endophytes are another class of endophytes with abroad range of taxa, which include α, β and γ-proteobacteria, Firmicutes, Actinobacteria etc. These classes of endophytes are reported to be intracellular in roots and shoot cells of a variety of plants and are found to gain entrance through the cells of the meristem[31,35,36]. A list of some of the endophytes that have been isolated from different plants and used in different studies highlighted in Table 1.

Fig. 1: Micrograph image of green microalgae (*Ulvella leptochaete*)[38]

Table 1: List of endophytes isolated from different plants tissues[65]

Endophytes	Plants	Plant tissues	Sources
Staphylococcus, *Microbacterium*, *Pseudomonas*, *Curtobacterium*, *Bacillus*, *Arthrobacter*, *Paenibacillus*, *Leifsonia*	*Alyssum bertoloni*	Leaves, stems, roots	Barzanti *et al.*[89]
Bacillus sp.	*Alnus firma*	Roots	Shin *et al.*[90]
Pseudomonas fluorescens, *Microbacterium* sp.	*Brassica napus*	Roots	Sheng *et al.*[91]
Enterobacter sp., *Xanthomonadaceae*, *Pseudomonas* sp., *Pseudomonas fulva*, *Stenotrophomonas* sp., *Clostridium aminovalericum*, *Sanguibacter* sp.	*Nicotiana tabacum*	Seed	Mastretta *et al.*[92]
Microbacterium, *Bacillus*, *Arthrobacter*, *Flavobacterium*, *Chryseobacterium*, *Agrobacterium*, *Sphingomonas*, *Pseudomonas*, *Serratia*, *Curtobacterium*	*Solanum nigrum*	Leaves, stems, leaves	Luo *et al.*[93]
Phoma sp., *Alternaria* sp., *Peyronellaea* sp., *Steganosporium* sp., *Aspergillus* sp.*	*Arabis hirsute*, *Acacia decurrens*, *Symplocos paniculata*	Stems, leaves	Li *et al.*[46]
Methylobacterium oryzae, *Burkholderia* sp.	Rice	NM	Madhaiyan *et al.*[94]
Microsphaeropsis sp.*	*Solanum nigrum*	NM	Xiao *et al.*[95]
Pseudomonas putida	*Papulus trichocarpa*	Stem	Germaine *et al.*[24]
Burkholderia cepacia	*Zea mays*	NM	Wang and Guo[96]
Pseudomonas putida	Poplar	NM	Weyens *et al.*[97]
*Ulvella Leptochaete***	Green algae: *Cladophora glomerata*, red algae: *Laurencia obtusa*	NM	Bast *et al.*[38]

NM: Not mentioned, *Fungi, **Algae

According to literature reports, over 129 different endophytic bacteria have been isolated from a list of selected crop plants[37-39]. The species implicated included both the Gram-positives as well as the Gram-negatives, cutting across about 55 genera. The bacterial taxa isolated were reported to fall mostly into the *Pseudomonas* as well as the Enterobacteriaceae group[29,34]. Li *et al.*[39] reported on the isolation of about 98 non-symbiotic endophytic bacterial strains from a study involving 150 root nodules of soybean. Furthermore, colonization of endophytic bacteria was also reported in the literature to be found in naturally grown plants but in stressed conditions. This was exemplified in the isolation of several bacterial endophytes of various species found to be resistant to extreme pH conditions in the study of Kan and his group[36,37].

Hitherto, Streptomyces known to colonize both the under-ground and the above-ground tissues of the plant with a greater incident at the underground have been reported as the most isolated actinomyces[37-42]. Medicinal plants are important sources of this endophytic actinomyces as such plant is known to induce secondary metabolites[29,43], an important factor for biological activity in the drug-resistant activity according to the study of Castillo *et al.*[42]. Although the use of endophytes in pollution control and management has been demonstrated for a long time now[15], there seem to be relatively less research in its exploitation unlike in the medical science application[39-40]. The reason behind this is the inability to understand the mechanism of action of the endophytes in the environmental medium. This can only be unraveled through further practical applications[19].

DISTRIBUTION OF ENDOPHYTES IN PLANTS

Within the plants, endophytes are usually found in the intercellular space as well as in the vascular tissues. Most studies carried out on some selected food reported on this[15,17,41]. The ability of plants components to express its living condition is the factor that determines the colonization of endophytes[42]. This compartmentalization was made known by the study of Castillo *et al.*[42] and Bezerra *et al.*[43], when they failed to obtain the same isolates in adjacent plant compartment, even in the same soil counterpart. The study discovered that only *Pseudomonas* sp. was found in all the compartments of the plants, while *Bacillus* and *Arthrobacter* were found only in the roots and stems. Therefore, *Pseudomonas* was reported as the most abundant genus that was recovered from the plant's rhizosphere. This was as a result of the rhizode position of organic acids and aromatics like phenols[44]. The reported abundance of *Pseudomonas* sp. could be as result of its ability to degrade organic compounds used as a source of energy. The predominance of *Pseudomonas* sp. is evident in Table 1.

There are other studies that reported less compartmentalized isolates. For example, the study to find the incidences of microbes in a hybrid aspen plant, *Burkholderia*, *Methylobacterium* and other unidentified bacteria were cultured from every part of the plant[41]. *Arthrobacter*, *Pseudomonas* and another Microbacteriaceae were found in the root and leaf, while Microbacteriaceae, *Sphingomonas*, *Aerolata* and *Sphingomonas auranica* which are airborne related bacteria were isolated from the stem and leaves[45]. The greater number of *Sphingomonas* in the above-ground portion of the plant in this study explains the condition of life of the organism in that environment, establishing it's ability to withstand ultraviolet radiation[43]. These plant microbes that are often pigmented to enhance their condition on leaf surfaces have been reported by other schools of thought[44-46]. The discrepancies in compartmentalization of isolated endophytes in plants may have resulted from factors which perhaps might include the growth media used as well as the differences in plants physiology. This should, however, attract further studies to truly ascertain it causes[19].

There have been conflicts of reports on the frequency of colonization of endophytic fungi in trees. While most studies indicated the highest colonization in the stem, others reported on the leaves[39]. However, it was the study of Petrini[8] that diffused the confusion with the inference that different plant tissues and organs could represent distinct microhabitat. Therefore, depending on the host-plant age, associated vegetation, elevation of the vegetation and exposure to the environment, the general frequency of colonization of endophytes could be determined[47]. This means to say that the existence of endophytes is influenced by seasonal, environmental as well as host tissue's variations.

ISOLATION AND COLONIZATION

Methods of isolation of bacterial endophytes are constantly being reviewed[16]. The use of sodium hypochlorite in the disinfection of plant surfaces has been a common practice. But most of the residual sodium hypochlorite often affects the growth and induces mutagenesis as well as the death of the micro-organisms, thereby necessitating rinsing with a separate sodium thiosulphate to remove all the residual hypochlorite[48-51].

From literature, a dilution-plate method of isolation seems to be the most widely used method owing to its less complication in operation. This method entails a stepwise process which must be followed thoroughly. The protocols to be followed include collection and washing of samples, followed by surface-sterilization with disinfectant and finally the incubation in a petri dish with growth medium[52]. If the sterilization time goes for too long, the plant cell would be broken so that no endophytes will be isolated[26]. List of compounds mostly used as a disinfectant for surface-sterilization includes $HgCl_2$, ethanol, H_2O_2, NaOCl and DMSO. It is usually important to confirm the success of surface-sterilization by inoculating the last rinse of distilled water after sterilization in a Petri dish containing a medium[53]. The plant cell is considered clean when no bacterial colony is found on the Petri dish after inoculation. The grounding of a little quantity of the plant cell with sterile water is required before proper inoculation is done. Depending on the type of endophytic microbe in question, it most often requires the addition of fungicides to prevent extraneous organisms like fungi from covering the colony[53-54]. Endophyte isolated from one plant could equally be inserted successfully into the tissues of other plants. A successful demonstration of such was reported by Lopez-Fernandez *et al.*[55] and Liu *et al.*[56] in their study using bacteria endophytes. Also using endophytic *fungic Acremonium* of *F. arundinacea*/*L. perenne* and *M. acuminata* to confer resistance to plants demonstrated the phenomenon[53]. With the use of confocal electron microscope, an Excalibur plant-*Streptomyces* sp., observed could indicate the presence of green fluorescent protein infected in the seed of the plant. This is an evidence of pure colonization of endophytes[56]. The use of biomarker system of Auto fluorescent protein (AFB) known as green fluorescent protein (GFP) is currently being used to establish colonization of endophytes[15].

PLANT-ENDOPHYTE RELATIONSHIP

In spite the fact that pollutants mostly organics are toxic to plants as well as micro-organisms, several plants that are found growing in the polluted soil tend to host a lot of bacteria that shows ability in the degradation of such pollutant[57,58]. Endophytes, however, are known to exhibit their symbiotic responsibilities to its host in most of the occasion, this they do by the various physiological interactions between them and the plants viz: Plant supplies nutrients to endophyte, while the microbes, in turn, release active metabolites as a result of their activities[15,30]. The released active metabolites help the plants in its root development having greater access to nutrients[35]. It also helps to protect the plant from desiccation and from insects as well as parasitic fungi[47]. This symbiotic activity enables endophyte to promote host plant growth and the supply of resistance mechanisms as explained in Fig. 2. Wide spread phenomenon has been

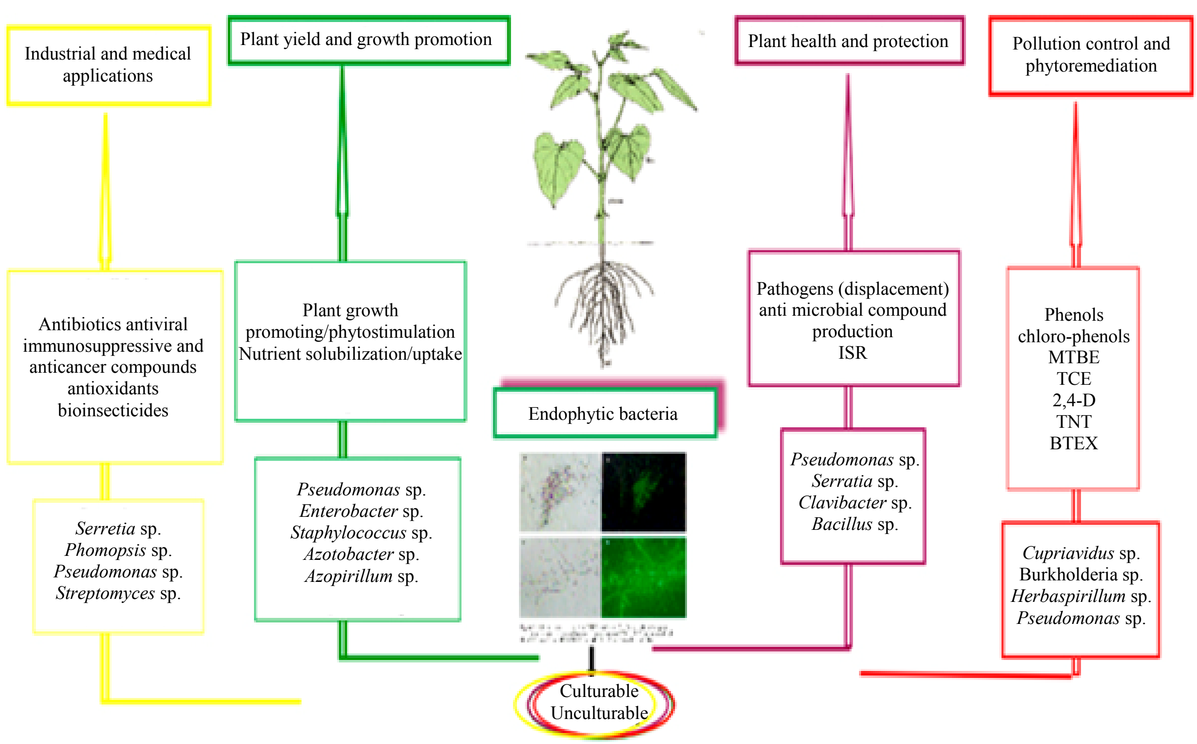

Fig. 2: Schematic diagram of the different plant–bacterial endophyte interactions that[16]

reported to exist on the symbiosis of endophytes and plants. But the report indicated that the relationship switches from mutualistic to parasitic[1]. Nonetheless, an alteration of any particular gene has the ability to change the entire endophytic mechanism. This was demonstrated by *E. festucae*: A wild-type fungus found to grow in the intercellular spaces, which was reported not only to be responsible for the promotion of plant growth but also enhanced the resistance of plant in a contaminated environment[48]. This study demonstrated the loss of apical dominance that eventually leads to the death of the plant the moment a plasmid of an enzyme was inserted into the coding region of the endophyte[48]. This, therefore, means that infection of same endophytes into different hosts results in diverse activities. The study of Xu *et al.* [53] described this phenomenon by reporting that *Colletotrichum magna* could cause a certain disease of the plant when inoculated to cucurbit plants, but when in non-cucurbit species it grows asymptomatically[54]. The endophytes-plant relationship could either be in parasitism or mutualism. Symbiosis (mutualism), leads to the distinctions of their effects into bio-control, growth promotion and in bioremediation.

BIOLOGICAL CONTROL OF ENDOPHYTES

According to literature reports, fungi were the first endophytic organisms to be isolated. Hence the notion that fungi are the most developed of the endophytes[1,8,31]. These organisms have found its application in plants growth promotion and protection. Although endophytes are known to be taxonomically diverse, their fungal classifications are broadly based on ecological categories or functional classes[2].

Pests and diseases have been the factors threatening the development of agriculture because of the immense loss it has inflicted on agricultural practices and its products. The development of chemical pesticides was adjudged to be the solution to the menace of pests and pathogens, it has however added in worsening the health of man and the environment[17]. The effect of pesticides on the emboldening soil and water has tremendously impacted the ecological status of the environment[54]. Certain bacterial endophytes have the ability to lessen harmful effects of some of these pests and its diseases[15,58]. For example, entomopathogenic micro-organisms had been reported as an alternative means of controlling the effects of pests as to enable the control and reduction of the use of chemicals in agriculture[18,51]. According to the study undertaken by Elmi *et al.*[59], they were a reduction in the number of root-knot entomopathogenic microbes (nematodes) found in tall fescue after infection with fungal endophytes for 35 days, without affecting the root dry weight. Other studies have also reported on the effects of plant colonized endophytic bacterial in preventing the infection of diseases[4,6]. This means that colonization of entomopathogenic microbes in plants following inoculation enhances plants microbial diseaseresistance[59]. Some other endophytic bacterial are implicated in the prevention of the effects of the pathogen in plants[26]. The various applications of endophyte are explained in a nutshell in Fig. 2.

Although different symbiotic lifestyles occur among endophytes, depending on the species involved, however, the two possible modes of action of endophytes as was reported are the parasitic and/or mutualistic relationships between the hosts. It is from these two possible modes of actions that other modes draw its string from. This is explained in Fig. 3 and it flows from there. For this reason, the interaction between endophytes and plants has been described as acontinuum[51].

In parasitism, *Fusarium oxysporum*, for example, is being reported to parasitize the egg of the banana weevil in the laboratory. This has been the mode of parasitic endophytes in attacking the pest or the causal agent of diseases. In competition, there are two things that particularly drive the endophytes[49], Competition for nutrients and that of the infection sites. *Pseudomonas* sp., for example, produces siderophores that chelate iron, thus depriving pathogens of this essential nutrient. This means that the organism will be competing for iron nutrient centers in the body of its host[37]. The production of secondary metabolites is undoubtedly the major mode of action amongst endophytes[35]. So many secondary metabolites have been identified in endophytes. Isolated endophytes of *F. oxysporum* from tomatoes were found to cause juvenile mortality among nematodes by

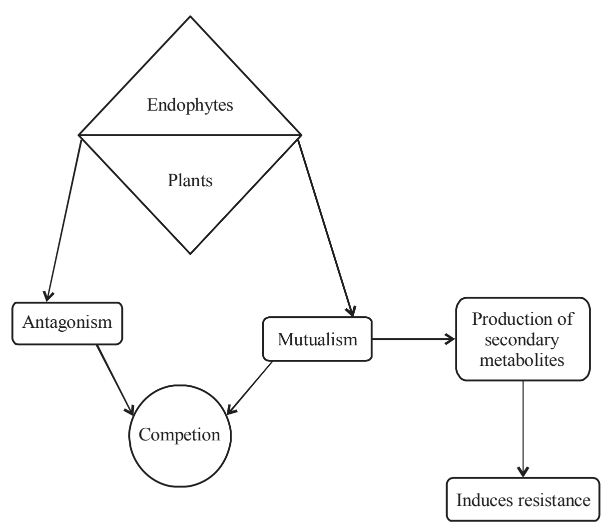

Fig. 3: Schematic representation of endophyte-plant interaction and mode of action

reducing the rate of hatching. *Colletotrichum* sp., *Pseudomonas* sp. and *actinobacteria* are good candidates for the production of lists of antibacterial, antifungal and antiviral metabolites[49]. Induced resistance is a phenomenon whereby the defense mechanism of plants is activated when it comes in contact with either biotic or abiotic elicitors. This actions usually result in reduced pest or pathogen damage[48,51,60]. Before the inception of the molecular methods for the identification of fungi in endophyte research, the real number of endophytic species in a sample has always been underestimated. The reason is that in this technique, obligate endophytes: Mostly fungi not growing well in the selected medium is usually not isolated[51,56]. But with the introduction of rDNA sequencing, the number of fungal species identified per host plant species increased substantially[61]. This is because an important proportion of fungal isolates may be sterile in laboratory cultures. Genotypic identification methods have helped in the identification and distinction of sterile cultures[12]. Therefore, the introduction of modern biotechnological techniques has really aided the study of endophytes and its biocontrol activities.

PROMOTION OF GROWTH BY ENDOPHYTES

The ability of endophytes for plant growth promotion differs from that of the strains for biocontrol because they do not counteract the activities of pathogens rather they necessitate the increase in growth from the cycling of nutrients[15]. Biological activities of endophytes have been reported to enhance the synthesis of phytohormones that plays a part in root elongation and growth promotion in plants[62-64]. Various metabolites have been implicated in these systemic activities of endophytes and they include indole acetic acid, gibberellin, indole acetonitrile, cytokinine and all these are involved in plant growth promotion. There are other beneficial effects of endophytes on the growth of plants; they include adjustment of osmotic balance, regulation of stomata, root morphology modification, mineral uptake enhancement and alteration of metabolism[15]. Literature has it that certain specific hormones were extracted from an endophytic fungus of medicinal plants[30] and when those hormones were incorporated into an orchid pant it enhanced growth improvement of the plant. Endophytes have also been tested in their seed germination enhancing abilities and positive inferences have been derived with a report of reduction in disease symptoms that would have resulted from such practices[57]. The plant growth-promoting bacterial endophytes and its effect are currently been used in forest regeneration and phytoremediation activities[23]. Endophytes are known to improve plant's adaptations as well as growth using their means of growth-promoting activities[19]. This they do through a series of mechanisms: Through phosphate solubilisation by so doing improving resistance to environmental stress of the host; through enhancement of growth hormone production; promotion of siderophore production; increased synthesis of biological compounds in plants for example vitamins; aiding of osmotic regulation; stomatal adjustment; promotion of uptake of minerals; helps in the alteration of nitrogen accumulation as well as plant metabolism of compounds[15].

BIOREMEDIATION

There is strong adaptation exhibited by plants growing in an extreme environment. This phenomenon results in a specialized physiological mechanism, much of which is related to the strengthening of defense mechanisms of plants by expressing genes responsible for that[23]. Hence, it is easy to find unique growth-promoting hormones from the strains of the endophytes with great potentials[19,25]. For this reason, the approach can be employed in the remediation of polluted environment especially when such a plant is colonized by an endophyte isolated from plants growing in a polluted environment. A demonstration of this principle was made when pyrene-degrading endophyte of an *Enterobacter* strain was isolated from a plant growing in soil contaminated by petroleum aromatic hydrocarbon (PAH), which on analysis was able to synthesize various metabolites with greater pyrene removal ability than the control experiment without the endophytes[65]. Furthermore, Germain *et al.*[24] studied the degradation of herbicides with bacterial endophytes (*Pseudomonas* sp.), this study reported that there was no sign of accumulation of the herbicide in the plant tissue and no sign of phytotoxicity, unlike the uninoculated plant. Although some few studies have been able to report on the potential of some fungi associated with grass to be effective in soil remediation xenobiotics[63], however, such approach has really not been demonstrated in the field[59]. Therefore, there is a need for interests in the creation of awareness on endophytic fungi been found in plants from the environment so that it could be employed in the field on this subject[64,65]. The importance of endophytic bioremediation ranges from the ability of the strain to reach larger population size because of reduced competition to their ability to degrade any absorbed xenobiotics in plant a in such a way as to reduce phytotoxic effects[15]. There are two different types of bioremediation (*in situ* and *ex-situ* bioremediation), this is understood based on whether the treatment is done on-site or out of site[66]. *In situ* bioremediation which is recognized as the on-site remediation are also divided into different types based on the processes involved. Phytoremediation is a typical example of *in situ* bioremediation which uses plants to decontaminate soil, water and the environment using energy from sunlight[23]. For some time now, there have been shifts in endophytic applications focusing on the exploitation of the use of endophytes in curbing the constraints of phytoremediation[21]. Endophytic bioaugmentation has proven to offer a lot of benefits over traditional bioaugmentation where bacteria in the former case experience less competition from the surrounding microbes and the plants in return provides the nutrient required to support growth and establishment[23].

PHYTOREMEDIATION

Phytoremediation has been shown to be the most efficient and environmentally friendly means to restore original soil conditions when contaminated by various environmental contaminants[62,67]. The technology is generally accepted as the proven technology for the removal of environmental contaminants due to its minimal energy requirement and the beauty of the technique[22,68]. However, heavy metals, POPs and high molecular weight pesticides with their recalcitrant, bioaccumulation and bioconcentration properties, generally defy conventional remediation practices and techniques[69,70]. The limitation of phytoremediation is often as a result of the toxicity of these chemicals or their toxic end products in plants[65-66]. This can, however, be ameliorated through endophyte-assisted phytoremediation either by means of natural colonization or by genetic manipulation of the plant as this has recently drawn attention[22,71]. Various studies have reported that plants sometimes take several years to reach its maturity and the toxicity of most of the heavy chemicals allows limited activity during plants dormant phases. As a result modification of plants using plant-associated bacteria for a more potent environmental clean-up could become a remedy for such shortcoming[72-74]. Therefore, removal of toxic compounds from the soil can be enhanced by the use of endophyte-assisted phytoremediation; this is because endophytes assist their host plants through various means as elucidated in current application[65]. Since all plant species is reported to have at least one endophytic species means that its accessibility is varied[7]. Phytoremediation has been reported to amongst other advantages to having the ability to be used in combination with other techniques (phytostabilization, phytovolatilization, rhizofiltration etc.), therefore, the use of endophytes capable of degrading environmental contaminants in addition to the specific plants could offer an efficient, economic and sustainable remediation technology[75-78]. Literature also reported the ability for genetic transformation of the endophytic microbes (Transgenic preparation) for enhanced remediation activity[7,79]. This could be done by incorporating into plant endophyte those natural microbes ability to conjugate with each other by means of movable DNA elements (vectors) between microbial populations[23]. For example, by introducing bacterial genes pTOM-Bu61 involved in the metabolism of toluene and TCE, the tolerance and uptake of such compounds known to be phytotoxic to conventional phytoremediation were improved[22].

FURTHER USES OF ENDOPHYTES

Endophytes are implicated in some other application for example as decomposition agents of the aging plant of salt marshes. Some other endophytes have demonstrated their ability to enhance osmoregulation in plant tissues, a boost to such plant's resistance to extreme condition, for example, drought[59]. Most of these endophytic strains have been employed commercially in turf grasses to aid their effects in resisting both biotic and abiotic conditions. Some endophytes in their way of plant growth promotion indirectly compete with pathogens and reduce their activity in the process[57]. In genetic studies, endophytes serve as a molecular tool used as a pool for gene carrier[78,79], for this reason, it is possible to genetically engineer endophytes which serve as protection for the host. The first successful demonstration of exogenous gene expression was demonstrated by Fahey[74,80]. In the past 2 decades, greater attention has been focused on the use of endophytes in the phytoremediation of various environmental contaminants. In the soil, bacteria gene *Burkholderia cepacia* L. S. 2. About 4 genetically modified by the introduction of a Ptom toluene degradation plasmid of *B. cepacia* G4, a natural endophyte of yellow lupine were used for phytoremediation of toluene[19,20]. This resulted in the inducement of strong degradation of toluene by the recombinant strain. Germaine *et al.*[24] also described inoculation of pea plant (*Pisumsativum*) with genetically modified bacterial endophytes that naturally possessed the ability to degrade 2,4-dichlorophenoxyacetic acid. According to the results, there was higher degradation capacity of the compound from the soil by plants inoculated with *Pseudomonas putida*[21]. In another subsequent study, there was an improvement of toxic metal phytoremediation from the environment using engineered endophytes to ensure better clean-up of the environment[19]. Such study has indicated the potential of microbe-plant relationship in the remediation of polluted sites and could be useful in the development of efficient environmental contaminant removal systems. Hence, these interactions between plants and micro-organisms require further understanding for recognition in order to be employed in the field.

Research interests involving the resistance of host by endophytes are lacking and the reasons behind it may not be far-fetched. One of the reasons could be drawn from the complex relationship between endophytes and hosts; while the other has to do with the fact that it is virtually difficult to understand the mechanisms of life and conditions of endophytes to be able to duplicate it. But according to Xu *et al.*[53], one could be able to build on such understanding using the points below:

- Considering the plant promotion as well as the pathogen/insect resistant ability of the endophytes might have a link with the toxicant which they synthesis in the process. These toxicants mostly inhibit the activities of such pathogens[81,82]. Therefore, endophytes growth promotion and pathogen resistant ability is relative to the toxicant it produces
- Also, the secondary metabolites produced by endophytes could also be responsible for its resistant ability to various unfavorable conditions which enhance the competitiveness of their host; this has been supported by literature[83,84]. However, the demonstration of this claim by literature has not doused the controversies that are growing on this subject. While literature has shown the direct relationship between pathogens and the mechanism of endophytes, others researchers still maintain that it is an indirect relationship to the systems of insects. For instance, the inhibition of insect gall provides the hosts of endophytes resistance to the effect of such insect[16]. The above phenomenon has generated many discussions in the recent past regarding the effect of endophytes in harnessing disease control in insects[75,81,84]. The study of Zabalgogeazcoa[86] highlighted on the mechanisms of operation of the endophytes and their interactions with plant pathogen. The study indicated that some of the endophytes are specialists in their infections while others are generalist[85,86]. However, in resistance induction to disease facilitation, Adame-Alvarez *et al.*[87] reported that the order of arrival of endophytes in plants tissues affects endophytes-pathogen interactions which could lead to facilitation (positive inducement of disease pathogen), negatively enhance resistance by the host or causing no effect[83,88]. These require further studies for more confirmation

FUTURE PROSPECTS

The importance of endophytes in managing the natural environment as well as its exploitation cannot be overemphasized (Fig. 4). However, they have been limitations to the complete understanding of these taxa. Such limitation

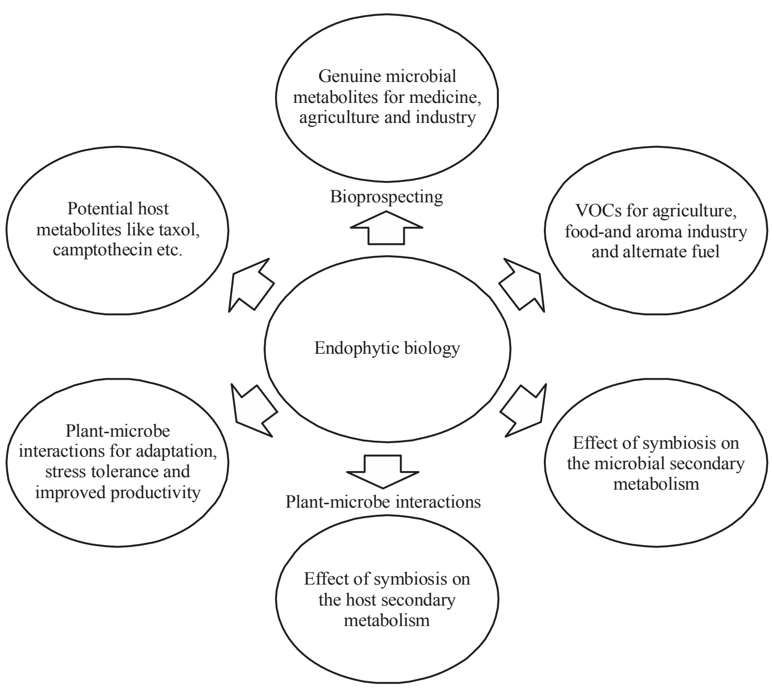

Fig. 4: Current interest in the study of endophytic biology[80]

includes the fact that the method of isolation of endophyte relies on culture-dependent, while many micro-organisms are there which can not be cultured[15]. This, therefore, presents nonculture-dependent technology as the limiting factor for the study of endophytes. Exploitation of plant-endophyte interaction can also play a key role in promoting sustainable agriculture[16]. There is this claim that the amount of secondary metabolites synthesized by endophytes is insignificant to the host, which disproof's the antagonistic effect between endophytes and pathogens. To douse or accept this claim requires many evidential studies. Therefore, in order for an improvement in the applicability of endophyte-assisted-biological applications most especially in the field, intensive future studies are required to demonstrate a better understanding of the organism in its host. Therefore, the endophytes mechanism is an important topic for future research.

CONCLUSION

The limitation to the complete understanding of the interaction of endophytes and their host with regards to their application in biological activities was inferred to depend on the continued trust in the use of generic method in their processing. Most organisms cannot be identified using those generic methods as they tend to forgo them due to transformations. Therefore, by employing sophisticated molecular methods in their processing will bring about such better understanding and will support the practice of endophytic application in the field. With this, the application of endophytes in agriculture/food processing, medicine and environmental management will be improved on, while new applications are also developed.

SIGNIFICANCE STATEMENT

This study was able to discover that in as much as endophyte has been applied in the following biological means: they help in nutrient acquisition through fixing of nitrogen in the root nodules, they aid growth and development using such mechanisms such as phytohormone production, they help to increase stress tolerance in plants, increase the ability of plants for disease resistance, assists in the degradation of various contaminants, production of important bioactive metabolites, assists in the translocation of and accumulation of metals in plants and helps in the production of enzymes that are likely involved in contaminant degradation. There are gaps in understanding the interaction between the organisms and its host so as to engage them in optimum application.

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