Endophytes: An Indicator for Improved Phytoremediation of Industrial Waste

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ABSTRACT
Phytoremediation has been described as an efficient medium through which industrial wastes that can be identified by various classes of pollution could be removed from the soil, It however it suffers various limitations that has prevented the field application of the technique. Plants lack the metabolic enzymes required for full pollutant remediation and this often results in slowing the pace at which phytoremediation activity occurs. Such inherent limitation of plants for complete remediation of xenobiotic compounds calls for the idea to harness the effects of endophytic microbes in enhancing the degradation of toxic industrial waste. Various plants have been implicated in this new line of biotechnology. Whilst most of them defile the inherent limitations, others are affected by the challenges and therefore are unable to achieve the primary goal which is environmental waste management. This study provides an in-depth analysis of various endophytic-assisted phytoremediation studies on organic contaminated soil. It also highlighted the diversity of contaminant-resistant and degrading endophytes and the role of those microbes in maintaining a clean environment, providing explanations on how plant-endophyte relationship can be exploited for improved waste removal. Hence the study proffered better alternative plants for phytoremediation of organic chemical contaminants based on the type of contaminant and the intending remediation protocol to be followed.

Keywords: Endophytes, genetically modified plants, phytoremediation, xenobiotics, pollution, environmental management.

1. INTRODUCTION.

Human activities through increasing industrialization have lead to the release of large amounts of toxic waste into the soil. Lists of anthropogenic chemicals which include petroleum hydrocarbons, (PHC), polycyclic aromatic hydrocarbons (PAHs), polychlorinated biphenyls (PCBs), halogenated compounds and pesticides. These lists of pollutants cause threats to the environment as they accumulate in soil and are readily passed unto man and animals through the food chain (Danh et al., 2009). Most of the industrial wastes are grouped among the recalcitrant organic moieties regarded as Persistent Organic Pollutants (POPs) because of their persistence in the soil and the ability to bioaccumulate in the environment (Doty, 2008). These compounds are often viscous with reduced vapor pressure as a result soil is usually the sink since transportation over dispersed area is hindered. POPs are implicated in their carcinogenic, mutagenic and toxic properties hence environmental contamination by such compound is currently an environmental concern (Peuke and Rennenburg, 2005; Simarrow et al., 2013). Conventional systems used for the removal of these wastes from soil e.g. physical, chemical and microbiological methods were environmentally invasive, not cost effective and usually results in inefficient clean up. This led to the discovery of a new green technology known as phytoremediation; which uses plants to decontaminate soil, water and the environment (Shim et al., 2010).

Phytoremedition has been shown to be the most efficient and environmentally friendly means to restore original soil conditions when contaminated by industrial wastes (Aken et al., 2010). The technology is generally accepted as the proven technology for the removal of wastes due to its minimal energy requirement and the beauty of the technique (Cherian and Oliveira, 2005; Aken et al., 2010). However, POPs and high molecular weight pesticides with their recalcitrant, bioaccumulation and bioconcentration properties, generally defies conventional remediation practices and techniques. Hence in spite of the applaud given to phytoremediation practices, it is still marred by several limitations, and as such has affected the application of the technology in the field (Salt et al., 1998). Phytoremediation technique is only allowed for shallow contamination of moderately hydrophobic compounds that are susceptible to root absorption as a result plants with low yield and reduced root system do not support efficient remediation (Dietz, 2001; Hur et al., 2011). This led to the drawn inference that annual plants especially those with slow growth as well as low biomass production can only be committed for phytoremediation in the long term. The reason being because of the phytotoxicity inflicted on such plants by the contaminated environment resulting from industrial waste, which can however be ameliorated through endophyte-assisted phytoremediation either by means of natural colonization or by genetic manipulation of the plant (Doty, 2008; Ballach et al., 2003). Remediation in plant is usually slow and incomplete, this is often because plants lack the biochemical pathways that are necessary for it to achieve total mineralization of the recalcitrant POPs. This incomplete mineralization of the compounds could lead to the accumulation of toxic metabolites that can be released to the soil, bioaccumulate into the food chain or perhaps volatilize into the atmosphere (Pilon Smith, 2005; Eapen et al., 2007; Doty et al., 2007). Most importantly, plants sometimes take several years to reach its maturity and the toxicity of most of the POPs allows limited activity during plants dormant phases. These inherent limitation of plants in the remediation of recalcitrant compounds calls for the need to modify plants using plant-associated bacteria for a more potent environmental clean-up. Therefore, removal of toxic industrial waste from the soil can be enhanced by the use of endophytic bacteria.

Endophytic bacterial are those non-pathogenic bacteria that occur naturally in the internal tissues of plants and are beneficial to their host plants through: plant growth promotion, synthesizes range of natural products that contributes in enhancing remediation of soil contaminant (McGuinness and Dowling, 2009). Almost all plant species have been reported to have at least one endophytic species, this means that its accessibility is varied. Phytoremediation has been reported to, amongst other advantages, have the ability to be used in combination with other techniques (phytostabilization, phytovolatilization, rhizofiltration etc), therefore the use of endophytic bacteria capable of degrading toxic industrial waste from soil in addition to the specific plants could offer an efficient, economic and sustainable remediation technology. It is therefore imperative that an improved method of remediating such environmental condition be invented. In the recent past, concerns relating to threats to the health and ecology of humans as caused by soil pollution most especially the POPs triggered an intensive research into the new and improved remediation technologies.

With plant-endophyte partnership in phytoremediation, provisions for the limitation to phytoremediation could be enhanced by introducing microorganisms known to be involved in metabolism of pollutants on its own or with other organisms into plants of interest for improved waste removal (Hur et al., 2011). Genetic transformation of the endophytic microbes (Transgenic preparation) could also be done by incorporating into plant endophyte those natural microbes ability to conjugate with each other by means of movable DNA elements (vectors) between a microbial population (Khan and Doty, 2011). 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 was improved (Taghavi et al., 2005).

Table 1: Overview of endophytes able to degrade organic contaminants (Khan and Doty, 2011)

Host plants	**Plant tissues**	**Endophytes**	**Degrading organic contaminants**	**References**
Allium macrostemon	stem	*Enterobacter* sp.	Pyrene	Sheng et al.*2008a*
Lolium multiflorum and*Lotus corniculatus*	root, shoot	*Sphingopyxis* sp., *Pseudomonas* sp.	Alkane	Yousaf et al.*2010*
Lolium multiflorum	root	*Pseudomonas* sp.	alkanes	Andria et al.*2009*
Perennial rye grass, alfalfa, tall wheat grass, Altai wild rye, and Nuttal's salt meadow grass	root	*Pseudomonas* spp. , *Brevundimonas* and *Pseudomonas rhodesiae*	*Alkane,* PAH	Phillips et al.*2008*
Phragmites australis, Ipomoea aquatica, and*Vetiveria zizanioides*	NM	*Achromobacter xylosoxidans* etc.	catechol and phenol	Ho et al.*2009*
Populus cv.*Hoogvorst*	stem	*Burkholderia macroides*	BTEX, TCE	Moore et al.*2006*
Populus trichocarpa ×*deltoides* cv. Hoogvorst	stem	*Pseudomonas putida*	2,4-dichlorophenoxyacetic acid	Germaine et al. *2009*
Zea mays	NM	*Burkholderia cepacia*	Phenol, toluene	Wang et al.*2010*
Populus cv. *Hazendans*	root, stem	*Pseudomonas tolaasii, Ps. Jessenii, Ps. Rhodesiae, Ps. Plecoglossicida, Ps. veronii, Ps. Fulva, Ps. Oryzihabitans, Acinetobacter lwoffi*, *A. nicotianae, Bacillus megaterium, Paenibacillus amylolyticus*	BTEX, TCE	Moore et al.*2006*
Poplar	NM	*Pseudomonas putida*	TCE	Weyens et al.*2010*

NM not mentioned

While metals and metalloids are not degradable hence could stay in the soil for centuries if no intervention is employed to control or remedy it, industrial wastes for example PCBs, PAHs and pesticides on the other hand can be degraded in the soil by several means (Reeves and Baker, 2000; Martinez et al., 2006). PCBs and PAHs can be degraded by plants with the help of various agents in the soil such as enzymes and microorganisms, such agents could be natural or through the use of biotechnology so as to enable the remediation of soil containing such chemicals (Pillon-Smits, 2005). Meanwhile, endophyte offers some other benefits that include amongst others phytohormone production. This could equally be used to enhance plant growth and other environmental waste removal prone-activities as an enablement for increased phytoremediation. Table 1 above highlights on some of the organic contaminants that has been phytoremediated by the use of plant-endophyte interaction. This study therefore will aim to explore the effects of plant-associated endophytic bacteria in the remediation of soil contaminated by industrial waste that constitute a complex contaminated soil system and the interaction between those compounds.

Since early 1886, 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 (1866). However, Petrini (1991) concluded that endophyte should be referred to as those microorganisms that live in the plant organelles without causing diseases. And this was widely accepted. Sikora (2010) expanded the definition of endophyte, as an organism that colonized the internal tissue of a plant during its life-cycle no matter whether it was beneficial, detrimental or neutral to its host. In other words, endophyte which is a concept of ecology is systematically one natural part of plant-micro ecology system. Endophytes are thought to be present in all plants, hence are widespread in nature. But according to estimation from past research studies, there are millions of endophytic microbes existing in special circumstance of microtubule plant cells and intercellular space (Li, 2005). These endophytes have been isolated through various means by various studies from different plants, example tomato, pepper, Eucommia ulmoides and Taxus yunnanensis as there are found distributed in roots, stems, leaves, fruits and seeds of such plants (Petrini et al., 1991; Backman and Sikora, 2008; Cornick et al., 2005). Most of these reports demonstrated that plants root still maintains the area of high density, with as much as about 104-106 Colony Forming Units/gram (CFU/g).

Clearly the type and numbers of endophyte in plant of tropics and subtropics outnumbers those of cold and dry climate, even the rate of its growth is different. Meanwhile healthy plant tends to colonise more endophyte than when plants are grown in an unfavourable condition. 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 (Wen et al., 2004). This helps to form the two hypotheses that are in existence at the moment: endogenous and exogenous. While endogenous believes that endophytes evolves from mitochondria and chloroplast in the plant cells, hence possesses similar genetic backgrounds to the host. Exogenous on the other hand is of the opinion that endophytes emanates from outside and enter the host through its surfaces (Zhenhua et al., 2012).

Based on the organism classification protocols, endophytes are classified into bacterial, fungi and actinomyces (Tan and Zou, 2001). Following literature report, the first endophytic fungi (Taxomyces andreanae) was first isolated by Strobel (1993) using Taxus brevifolia: a medicinal plant (Li, 2005). The study of Huang et al. (2007) reported on endophytic fungi strains totalling 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 (Azevedo et al., 2000). Osterhage isolated an endophytic and obligate marine fungus Ascochyta salicorniae from the green alga Ulva sp. Hence, fungi are reported to be the earliest, most isolated and most studied of the endophytes (Osterhage et al., 2000). These have found its application in plants growth promotion and protection.

2. DISTRIBUTION OF ENDOPHYTES IN PLANTS

Inside the plant tissues endophyte bacteria are usually found in the intercellular space as well as in the vascular tissues, this has been reported by the many studies that indulge in the isolation of the bacteria from various plants, which include cotton, sweet corn cultivars and pea cultivars (Cho *et al*., 2007). Therefore endophyte colonization tends to be selective in the plant compartments mostly in the areas with plants growth diversity (Yrjala *et al*., 2010). This compartment selectivity by endophytes in plants was reported by Moore et al. (2006), in their comparative to ascertain the possibility of obtaining the same isolates from the same plant species from an adjacent soil. According to the study, only *Pseudomanas sp.* was found in all the compartments of the plants while *Bacillus* and *Arthrobacter* were located only in the roots and stems (Kamath *et al*., 2004). This reported abundance of *pseudomonas sp.* could be as result of its ability to degrade phenols and aromatics in contaminants resulting from industrial wastes. Other isolates are therefore less compartmentalized (Yrjala *et al*., 2010). The discrepancies in compartmentalization of isolated endophytic microbes in plants accordingp to Yrjala et al. (2010) may have resulted from both the internal and external environmental factors. This should however attract further studies to truly ascertain its cause.

3. ISOLATED BACTERIAL ENDOPHYTES

According to literature reports, over 129 different endophytic bacteria have been isolated mostly from different crop plants. They included both the Gram-positive and Gram-negative species which represent over 54 genera. Majority of the isolated bacterial taxa belong to the earlier *Pseudomonas* group (*Pseudomonas*, *Burkholderia*, Phyllobacterium) and *Enterobacteriaceae* (*Enterobacter*, *Erwinia*, *Klebsiella*) (Pullen *et al*., 2002). Li *et al*. (2008) reported on the isolation of about 98 non-symbiotic endophytic bacterial strains from a study involving 150 root nodules of soybean. Furthermore, some plants grown in natural stressed conditions also were found to be colonized by endophytic bacteria. For example, Kan (2007) isolated 61 endophytic bacteria from Vicia, Oxytropis, Medicago, Melilotus and Onobrychis species grown in Qinghai-Tibet plateau and Loess plateau, some of which can be resistant to high alkaline (pH 11) and high salinity (3-5%, w/v) (Kan *et al*., 2007).

Hitherto, the most endophytic actinomyces isolated is Streptomyces, which usually colonizes the roots, stems and leaves of plant with highest incident in root (Wang et al., 2008). It has been reported that medicinal plant is one of the important resource for the isolation of endophytic actinomyces. This endophyte can induce secondary metabolite of very important value. Isolation of Streptomyces NRRL 3052 from a native medicinal plant snakevine (Kennedia nigriscans) in Australia, showed a release of *munumbicins B* which usually counteracts the effect of methicillin-resistant strain of Staphylococcus aureus, as well as demonstrates biological activity against Bacillus anthracis and multidrug-resistant Mycobacterium tuberculosis (Castillo *et el*., 2002). Although there seem to be relatively less research in this branch of endophytic study, it has however played a very important role in its application in medical science.

4. PLANT-ENDOPHYTE RELATIONSHIP

In spite the fact that most industrial wastes are phytotoxic to plants as well as microorganisms, plants growing in industrial waste polluted soil usually habours a lot of bacteria that shows ability in the degradation of such pollutant (Afzal *et al*., 2014). 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 endophyte will itself release active metabolites through their metabolic activities (Zhenhua *et al.,* 2012). The release of active metabolites helps the plants in its root development having greater access to nutrients (Li *et al*., 2008). It also helps to protect the plant from desiccation, and from insects as well as parasitic fungi (Dutta *et al*., 2014). The symbiotic activities enable endophytes to promote host plant growth and the supply of resistance mechanisms. Wide spread phenomenon have been reported to exist on the symbiosis of endophytes and plants. But the report indicated that the relationship switches between mutualistic and parasitic (Strobel and Daisy, 2003). Nonetheless, a single gene alteration can change the mechanism of endophyte. For example, *Epichloe festucae* which is a wild-type fungus that grows systemically in intercellular spaces, has been reported to promote the growth of plants and also enhances the resistance of plant in a contaminated environment. When a single-copy plasmid of a NADPH oxidase gene, noxA, was inserted in the coding region of the microbe, the plant loses its apical dominance, hence becoming severely stunted, as it advanced in growth and eventually died (Tanaka *et al*., 2006). These therefore mean that when same endophytes are colonized in different host, it can react in the different lifestyles. *Colletotrichum magna* could cause anthracnose when inoculated in cucurbita plants (Plant species which include pumpkin), but in non-cucurbit species grows asymptomatically (Zhenhua *et al*., 2012).

5. ISOLATION AND COLONIZATION

The most popular way of isolating endophytes is the dilution-plate method because of ease in operation. It follows three protocols. Firstly, collection of simples and washing it clean with water. Secondly, surface sterilization of the simples with disinfectant; this is the most important step. Endophytes get contaminated if the samples are not properly sterilized. And if the sterilization time goes for too long, the plant cell would be broken so that no endophytes will be isolated. A typical list of compounds mostly used as sterilization include: $HgCl_2$, ethanol, H_2O_2, NaOCl and DMSO. Thirdly, incubate the plant tissues on the plate. Plant tissue is grinded with normal saline under aseptic condition, followed with the spreading of a certain amount of grinding fluid on the plant with media. When the plant tissue become a little hard, partition the sample into small fragments and then put on the media. When endophytic actinomyces is involved, fungicide will be added so as to prevent the fungi covering the growth of such endophytic actinomyces. Endophyte isolated from a plant could also be colonized successfully in the other plants. This was successfully demonstrated by the study undertaken by Koga *et al.* (1997) and Pereira *et al*. (1999) using endophytic fungic Acremonium of F. arundinacea/L. perenne, and M. acuminata to confer resistance to the plants (Zhenhua *et al*., 2012). Using 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 (Coombs and Franco, 2003).

6. APPLICATIONS

Endophytes are known to improve plant's adaptations as well as growth using their means of growth-promoting activities. Hence they can improve host resistance to environmental stress, alter the host physiological characteristics, induce Plant hormone and synthetize other biological compounds. All these offer great potential application in controlling phytopathogen and promoting the plant growth. In biological control, entomopathogenic microorganism can be used to control pests and diseases in agriculture (Elmi et al., 2000); synthesis of metabolins can help in the control and promotion of growth (Zhang et al., 2012); some endophytic fungi can assist in osmoregulation in plants; they could also be used as molecular tool for gene transfer; and mostly important as it pertains to this study is the use of endophytes for the removal of waste from the environment. Sheng (2008) isolated one pyrene-degrading endophytic bacteria, Enterobacter sp. 12J1, from the plant grown in the PAH contaminated soils. This strain of endophytic bacteria was able to produce indole acetic acid (IAA), siderophore and solubilize inorganic phosphate, which are important factors necessary for plant growth and development hence good for phytoremediation. However, most of the plants that have been used to demonstrate the ability of endophytes to remove industrial wastes from the soil are in the crop family hence the need to shift attention towards other plants that has the same characteristic like those that have been used.

7. FUTURE PROSPECTS OF ENDOPHYTES

The importance of endophytes in managing the natural environment as well as its exploitation cannot be over emphasized. However, there have been limitations in complete understanding of these taxa. Such limitation includes the fact that the method of isolation of endophyte relies on culture-dependent methods, while lots of microorganisms are there which cannot be cultured (Ryan et al., 2008) This therefore presents non culture-dependent technology as the limiting factor for the study of endophytes. Also exploitation of plant endophyte interaction can play a key role in promoting sustainable agriculture (Khan and Doty, 2011). There are claims that the amount of secondary metabolites synthesized by endophytes is insignificant to the host and that disprove the antagonistic effect between endophytes and pathogens. There is therefore need for further studies to enable the establishment of such fact. However, several studies have demonstrated the beneficial effects of endophytes in enhancing remediation of pollutants (Doty et al., 2007). Therefore, to enhance improvement in the applicability of endophyte-assisted phytoremediation as well as in other 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.

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