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7. Endophytic Microorganisms: Important Role in Sustainable Environment & Agriculture - A Review

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Abstract

To meet the food demand of ever-growing global human population, agricultural practices are largely relied on the application of chemical fertilizers. Chemical fertilizers synthesis highly contributes in Global warming through greenhouse gases production. To feed the overgrowing human population as well as to maintain the global environmental & agricultural sustainability, understanding of soil complex is primarily important not only to supply sufficient food but also to maintain global environmental sustainability for upcoming generations. Soil microbial population has immense potential in attaining the agricultural sustainability in present environmental conditions Endophytic microorganisms are ubiquitous in most plant species, residing latently or actively colonizing plant tissues locally as well as systemically. Endophytes will be defined as those microorganisms that can be isolated from surface-disinfested plant tissue that do not visibly harm the plant. Historically, endophytic microorganisms have several beneficial effects on host plants such as plant growth promotion and increased resistance against plant pathogens and parasites. Phosphate solubilisation, biological N_2 fixation, production of siderophore & production of plant-growth substances used by endophytes for growth promotion of crops. Synthesis of antimicrobial compounds are the tools of endophytes to use for crop protection. Hence, it's high time to explore nonconventional resources for the development of sustainable crop production technologies without damaging the environmental sustainability.

Key Words: Chemical fertilizers, Agriculture Sustainability, Environmental Sustainability, Endophytic Microorganisms, Plant Growth Promoting Compounds.

1. Introduction

Today's world facing immense challenge in agriculture sector particularly for the production of environmentally sound and sustainable crops. Large amount of chemical fertilizers and pesticides are used for enhancing agricultural yield to fulfil the demands of ever-increasing population. Since this has placed a considerable burden on the agriculture, ecologically safe alternatives are required to improve productivity and sustainability in agriculture [1]. One of the options is the use of microorganisms, as they have huge potential, thereby reducing the consumption of chemical fertilizers [2].

Soil microorganisms provide several benefits to agriculture through improving plant nutrient availability, plant health, and soil quality [3]. Endophytes are defined as microorganisms, commonly bacteria and fungi which live the whole or some stage of their life cycle in inner plant cells without expressing any adverse effect. Owing to the potential role of endophytic bacteria in plant-growth promotion and disease management properties, endophytes can be used as bioinoculants in agriculture to increase crop productivity. Many reports are available with regard to the application of endophytic bacteria to enhance the plants resistance to disease and promote plant growth. Thus, employing endophytes in agricultural practices would result in better soil health and sustainable crop production [4].

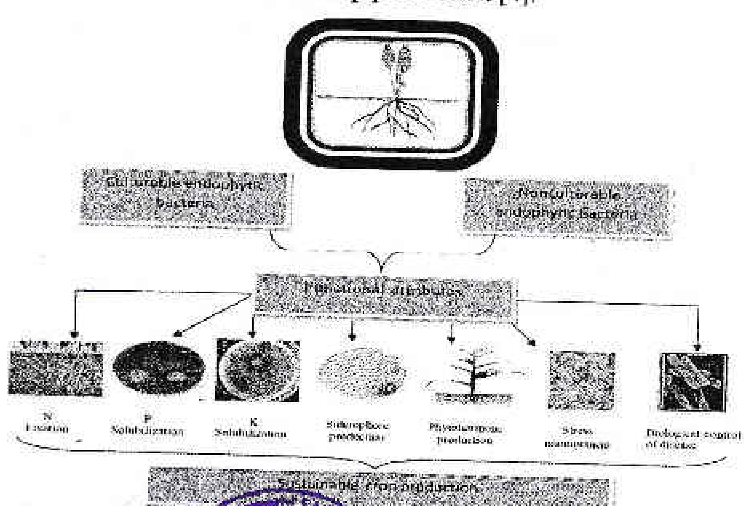

Fig: Role of Endophytic Bacteria for Sustainable Agriculture [5]

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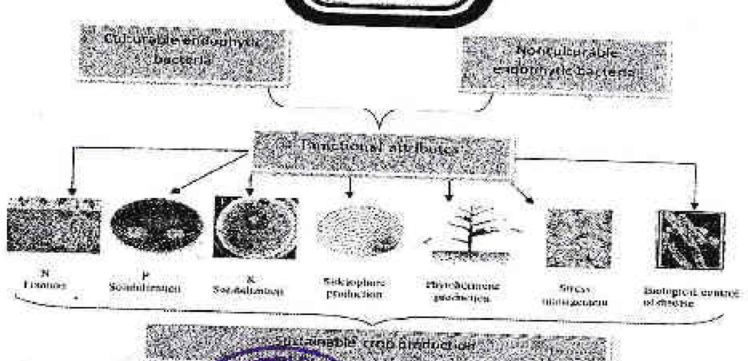

Fig: Role of Endophytic Bacteria for Sustainable Agriculture [5]

2. Diversity & Distribution of Plant Associated Endophytes

Sr.No	Endophytic Microorganism	Plant Species	Reference
1	*Pseudomonas putida, Bacillus pumilus, Aureobacteriumsaperdae, Burkholderia solanacearum,* and *Phyllobacterium rubiacearum*	Cotton	Chen et al., (1995)
2	*α-Proteobacteria: Erwinia sp., Agrobacterium sp. γ-Proteobacteria: Pseudomonas citronellolis, P. oryzihabitans, P.staminea, K. pneumoniae, K.oxytoca, Enterobacter sp., Pantoea sp., P. agglomerans Firmicutes,Bacillus fastidiosus*	Soybean	Zinniel et al. (2002), Kuklinsky-Sobral et al., (2004)
3	*Achromobacter xylosoxidans, Alcaligenes sp*	Sunflower	Forchetti et al., (2007)
4	*β-Proteobacteria: Burkholderia cepacia γ-Proteobacteria: Klebsiella sp. Firmicutes: Bacillus polymyxa Actinobacteria: Mycobacterium sp.*	Wheat	Balandreau et al., (2001),Zinniel et al., (2002), and Iniguez et al.,(2004)
5	*Bacillus and Sphingopyxis*	Strawberry	Dias et al., (2009)
6	*γ-Proteobacteria: Pseudomonas sp., P. syringae, P. aeruginosa, Escherichia coli,Firmicutes: Brevibacillus brevis*	Tomato	Pillay and Nowak (1997),Yang et al., (2011), and Patel et al., (2012)
7	*Trichoderma citrinoviride,Paecilomyces marquandii, Acremonium furcatum, Cylindrocarpon pauciseptatum, and Chaetomium globosum*	Actinidia macrospermа	Lu et al., (2011)
8	*Aspergillus niger, A. flavus, A. nidulans, Penicillium chrysogenum, P. citrinum, Phoma, Rhizopus, Colletotrichum, Cladosporium, and Curvularia*	Cannabis sativa	Gautam et al., (2013)
9	*Ramichloridium cerophilum*	Chinese cabbage	Xie et al., (2016)
10	*α-Proteobacteria: Rhizobium etli β-Proteobacteria Burkholderia pickettii, B. cepacia, Achromobacter, Herbaspirillum seropedicae γ-Proteobacteria: Erwinia sp., Enterobacter sp., E. cloacae, Stenotrophomonas sp., Klebsiella sp., K. terrigena, K. pneumoniae, K. variicola, Pseudomonas sp., P. aeruginosa, P. fluorescens Firmicutes: Bacillus sp., B. mojavensis, B. thuringiensis, B. megaterium, B. subtilis, B. pumilus, Lysinibacillus, Paenibacillus Actinobacteria: Corynebacterium sp., Arthrobacter globiformis, Microbacterium testaceum*	Maize	Fisher et al., (1992), McInroy and Kloepper (1995), Palus et al., (1996), Chelius and Triplett(2001), Zinniel et al., (2002), Rosenblueth and Martinez Romero (2004), and Rai et al., (2007)

Endophytes may enter the interior of the root through auxin-induced tumors, wounds, lateral branching sites by hydrolysing wall bound cellulose. Many plant species in the globe, each one hosts several to hundreds of endophytes creating an enormous biodiversity [6].

Distribution of endophytes depends on their ability to colonize and suitability of host plant resources, probably isolated endophytic bacteria from plants for the first time and till now, in 16 phyla more than 200 bacterial genera have been reported as endophytes [7].

3. Important Role of Endophytes in Sustainable Agriculture

Endophytic bacteria play a major role in increasing plant growth through beneficial effects on host plant. These bacteria enhance plant growth through increase in germination percentage, leaf area, chlorophyll content, biomass production, root & shoot ratio, nitrogen concentration, protein content, hydraulic activity and stresses tolerance against drought, flood, salinity, etc. These bacteria also enhance plant-growth actively by increasing plant nutrient availability, reduction in ethylene production, and passively by developing tolerance against myriads of plant pathogens [8].

4. Biological Nitrogen Fixation

Nitrogen is the most important nutrient for plant growth [9].,78% of the N_2 gas present in the environment but it is unavailable to most of the plants and animals. Plants absorb nitrogen in the form of nitrate and ammonium ions. Conversion of gaseous nitrogen into ammonium ion through bacterial activity is called as biological nitrogen fixation (BNF). The Nitrogenase enzyme is a key enzyme in this Biological Nitrogen fixation.

Table: Biological Nitrogen Fixation by Endophytes (Name and associated Plants) [10].

Endophytes	Associated Plants
1. *Rhizobium leguminosarum bv. Trifolii*	Rice
2. *Burkholderia*	Rice
3. *Azospirillum*	Rice
4. *G. diazotrophicus, H. seropedicae, H. rubrisubalbicans, A. amazonense and Burkholderia sp.*	Sugarcane

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5. Phosphorus Solubilisation

The use of endophytic microorganisms in agriculture has increased in recent years. Such microbes promote the growth of plants and facilitate the control of biological pests and phytopathogens, as well as the production of metabolites of pharmaceutical interest [11]. Several soil microorganisms, including bacteria and fungi are able to mineralize organic phosphates and solubilize inorganic phosphates. Phosphate solubilization might be achieved via several mechanisms such as hydrolysis or processes involving enzymes like phosphatases. Phosphatases produce organic and inorganic acids through pH reduction, carbon dioxide formation and the enzymatic reduction of metals [12]. Among the bacteria able to solubilize phosphate, the genera *Rhizobium* [13] *Agrobacterium, Pseudomonas, Burkholderia, Erwin, Paenibacillus, Bacillus and Lysinibacillus sp.* [14].

Table: Mechanisms in Microorganisms for P-Solubilisation [15]

Sr.No	Microorganisms	Features	References
1	*Serratia marcesence*	Produce gluconic acid and solubilizes P	Krishnaraj and Goldstein (2001)
2	*Rahnella aquatilis*	Solubilize P and produce gluconic acid in E. coli DH5a	Kim et al., (1998)
3	*Enterobacter agglomerans*	Solubilize P in E. coli 109, does not lower pH	Kim et al., (1997)
4	*Pseudomonas cepacia*	Solubilize P and produce gluconic acid in E. coli JM 109	Babu-Khan et al., (1995)
5	*Erwinia herbicola*	Solubilize P and produce gluconic acid in E. coli HB 101, probably involve in synthesis of PQQ	Goldstein and Liu (1987)
6	*Bacillus subtilis CB8A*	Solubilise P and produce gluconic acid	Mehta et al., (2013)

6. Potassium Solubilisation

K solubilisation is done by a wide range of saprophytic bacteria, fungal strains and actinomycete [16]. There are strong evidences that soil bacteria are capable of transforming soil K to the forms available to plant effectively. There is considerable population of KSB in soil and in plant rhizosphere. These include both aerobic and anaerobic isolates that the most frequently KSB in soil are aerobic. A considerably higher concentration of KSB is commonly found in the rhizosphere in comparison with non-rhizosphere soil. Solubilization of K by KSB from insoluble and fixed forms is an import aspect regarding K availability in soils. The ability to solubilize the silicate rocks by *B. mucilaginous, B. circulanscan, B. edaphicus, Burkholderia, A. ferrooxidans,* *Arthrobacter sp., Enterobacter hormaechei, Paenibacillus mucilaginosus, P. frequentans, Cladosporium, Aminobacter, Sphingomonas, Burkholderia, and Paenibacillus glucanolyticus* has been reported. Among the soil bacterial communities *B. mucilaginosus, B. edaphicus and B. circulanscan* have been described as effective K solubilizers [17].

Table- Classification of potassium-solubilizing microorganisms (KSM) [18]

Sr.No	Isolation Source	Closes related Species	References
1	Weathered materials of denatured rock mountain in Vietnam	*A. tumefaciens*	Diep and Hieu (2013)
2	Soil in India	*B. metallica*	Saiyad et al., (2015)
3	Wheat in India	*A. piechaudii*	Verma et al., (2015)
4	Tea soil in India	*P. putida*	Bagyalakshmi et al., (2012a, b)
5	Agricultural soils in India	*F. aurantia*	Ramarethinam and Chandra (2006)
6	Mica mine in India	*B. amyloliquefaciens*	Gundala et al., (2013)
7	Soil in India	*B. mucilaginosus*	Sukumaran and Janarthanam (2007)

7. Antimicrobial Activity

Application of endophytic bacteria for suppression of diseases (biological control) can be an eco-friendly approach in sustainable agricultural practices [19]. Application of endophytes and their metabolites were found to have promising potential in control of plant & human pathogens and diseases.

Table- Examples of the endophytic activities against microbes [20] [21]

Sr. No	Endophytic isolates	Plants	Pathogenic fungi/bacteria
1	*Phomopsis sp*	*Excoecaria agallocha*	*Candida albicans* and *Fusarium oxysporum*
2	*Penicillium sp.*	*Acrostichum aureurm*	*Staphylococcus aureus, Candida albicans*
3	*Nodulisporium sp*	*Juniperus cedre*	*Bacillus megaterium, Microbotryum violaceum, Septoria tritici, Chlorella fusca*
4	*Bacillus pumilus*	*Pea*	*F. oxysporum f. sp. Pisi*
5	*Bacillus cereus*	*A. thaliana*	*Pseudomonas syringae*
6	*P. fluorescens*	*Tomato*	*F. oxysporum f. sp. radicislycopersici*

8. Conclusion

Large use of chemical pesticides and fertilizers for increasing agriculture productivity has disturbed the ecological balance which has led to the buildup of pesticide resistance among pathogens. The application of endophytic bacteria for sustainable agriculture is an economically sound, attractive, and eco-friendly approach. These bacteria have shown many beneficial impacts on their host plant and contribute significantly to maintain sustainable agriculture. They have been documented for promoting plant growth through several functional attributes viz., increase in nutrient availability to the plants through fixation and solubilization of nutrients in soil and by producing plant-growth regulators. The major impact regarding application of endophytic bacteria in the agriculture is the significant reduction in the indiscriminate application of agrochemicals like pesticides, inorganic fertilizers, other artificial chemicals, etc. Successful utilization of endophytes would make crop production eco-friendlier and sustainable. In the future, researchers would be able to engineer microbial endophytes for increasing their potential to be used as microbial inoculants, after fully understanding their function.

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PHB Production from Dairy Industry Soil Isolates using Whey as Carbon Source

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Abstract: *PHB producing bacterium was isolated from dairy industry soil. Identification was performed by Sudan black B and Nile blue A staining. PHB production was performed by using whey as sole carbon source with minimal medium. Comparative production using pure sugars as carbon source was also carried out. Molecular characterization of most efficient producer was done by 16S rRNA sequencing. The strain was identified as Bacillus cereus (NCBI Accession number- MZ605040). Effect of different parameters on production was also carried out and it was found that maximum production (54%) takes place with 3% of whey at pH 7 and temperature 35°C after 48 hours. On comparison with pure sugars, efficient production was observed with whey. The formed PHB was initially confirmed by UV-VIS spectrophotometry with maximum absorbance at 235nm confirmed by FTIR, GCMS, LCMS, HPLC, DSC, 13C- NMR, 1H-NMR. Biodegradation studies of produced polymer were also carried out and the polymer was found to be completely biodegradable in both in vivo and in vitro conditions. The present investigation aims to isolate and identify potent PHB producers on a cheap and easily available carbon source that is whey.*

Keywords: PHB, 16S rRNA Sequencing, Bacillus cereus, FTIR, GCMS, LCMS, DSC, ^{13}C- NMR, ^{1}H-NMR.

I. INTRODUCTION

PHA is polyesters that are naturally or artificially accumulated as water insoluble granules within a variety of microorganisms, subject to specific conditions. PHAs are regarded as a renewable resources-based alternative to petrochemical polymers. The advantageous character of PHAs lies in its environmental biodegradability and bio compatibility. PHAs cover a large scale of biological polyesters having properties in the range from thermoplastic to elastomers. The first identified and still most investigated PHA is poly-3-hydroxy butyrate (PHB) (Kovalcik et al. 2019, Devi et al. 2015 and Mohapatra et al. 2015).

Poly-L-hydroxybutyrate (PHB) is thermoplastic polyester. It is biocompatible and biodegradable, and therefore, of industrial interest. In the cell, PHB is an intracellular storage material synthesized during unbalanced growth conditions. All bacteria which are capable of PHB synthesis accumulate PHB during the stationary phase of growth when the cells become limited for an essential nutrient but have an excess for carbon sources (Aslim et al. 1998, Ghate et al. 2011 and Reddy & Thirumala 2012).

II. MATERIALS AND METHODS

2.1: Collection of Samples

The soil samples were collected from Nanded Dairy situated in the MIDC area.

2.2: Isolation of Bacteria

The samples were serially diluted and used as inoculum for streaking on nutrient agar plates. The colonies showing positive results for Sudan black B staining and Nile blue staining were taken forward for PHB production and identification by microbiological tests according to Bergey's Manual ((Abdel Kareem et al. 2017).

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2.3: Screening for PHB Production

After incubation, the colonies were subjected to Sudan black B and Nile blue A staining. The colonies showing the presence of accumulation of granules were used to proceed further (Bhuwal et al. 2013, Bhuwal et al. 2014, Reddy & Thirumala 2012).

2.4: PHB Production with Pure Sugars

For pure sugars, the strains were inoculated in minimal media supplemented with 2% lactose and for crude source, medium was supplemented with 2% whey. The flasks were incubated for 24hrs, 48hrs, and 72hrs at 37°C.

2.5: PHB extraction & Confirmation

PHB extraction was carried out by Abdel Kareem et al. 2008 and Aguirre et al. 2017 method.
The cells were lysed with NaOH digestion method and centrifuged at 6000rpm for 20min.
The extracted PHB was confirmed by UV-VIS Spectrophotometer. In this method, the PHB was acidified with concentrated sulphuric acid to form crotonic acid which was measured at 235nm.

2.6: Molecular Characterization

Molecular characterization was performed by using techniques FTIR, ^{1}H-NMR and ^{13}C-NMR, HPLC, GCMS, LCMS and DSC (Bhuwal et al. 2013, Bhuwal et al. 2014).

2.7: Biodegradation studies

A. In-vitro biodegradation of PHB

The PHB degrading ability of fungal and bacterial cultures in solid medium was determined by over layer plate assay, and in liquid medium was determined by measuring turbidity in growing culture having PHB as sole carbon source.

B. In-vivo biodegradation of PHB

Biodegradation of PHB biofilms: PHB biofilms will be prepared using chloroform as solvent as per the method described by Nadia Altaee et al and subjected to biodegradation.
(Gangurde et al. 2017, Ramchander Merugu 2012, Kumaravel et al. 2010, Altaee et al. 2016, Singh et al. 2013, Tansengco & Dogma 2004 and Rech et al. 2020).

III. RESULTS

3.1: Identification of PHB Producing Bacteria

In Sudan black B staining, Purple to black granules were observed intracellular with pink background in PHB accumulating bacteria. The PHA-accumulating colonies, after Nile blue A staining, showed strong bright fluorescence on irradiation with UV light.

3.2: PHB Production

The bacterial strains that showed positive results for Sudan black B and Nile blue A staining were selected for PHB production. The production media were supplemented with crude carbon sources and a comparative set with pure sugars was also set up.

- Effect of substrate concentration on PHB production by the isolates from dairy industry soil

	Production (%) with Whey			Production (%) with Lactose		
	1%	2%	3%	1%	2%	3%
Bacillus (D1)	23	40	54	10	12	11
Lactobacillus (D2)	11	27	35.8	6	11	8