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Biotechnological Applications of *Piriformospora indica (Serendipita indica)* DSM 11827

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Abstract

Piriformospora indica (Hymenomycetes, Basidiomycota) is a cultivable endophyte that colonizes roots and has been extensively studied. *P. indica* has multifunctional activities like plant growth promoter, biofertilizer, immune-modulator, bioherbicide, phyto remediator, etc. Growth promotional characteristics of *P. indica* have been studied in enormous number of plants and majority of them have shown highly significant outcomes. Certain secondary metabolites produced by the intense interaction between the mycobiont and photobiont may be responsible for such promising outputs. *P. indica* has proved to be highly beneficial endophyte with high efficacy in field. This article is a review where this mycobiont has added value to these medicinal plant with special emphasis on *Curcuma longa L.* (Turmeric) and *Plantago ovata* (Isabgol) in the agricultural field.

Keywords: *Piriformospora indica*; Medicinal plants; Growth promoter

Introduction

Piriformospora indica, a model organism of the order Sebacinales, promotes growth as well as important active ingredients of several medicinal as well as economically important plants by forming root endophytic associations [1-7]. *P. indica* can be easily maintained and axenically cultured, it positions as an ideal models for beneficial fungus-plant interactions studies and has a promising perspective for application in sustainable horticulture and agriculture [8-12]. Exploiting these plant benefitting properties, a formulation of *P. indica* with magnesium sulphite was prepared where magnesium sulphite acts as a carrier. For this, 2% (w/w) of fungal biomass served as effective and stable formulation. On an average the colony forming unit (CFU) count was maintained as 109 and moisture content was 20%. Application of this formulation on plants presented enhanced overall growth and resistance to biotic and abiotic stress.

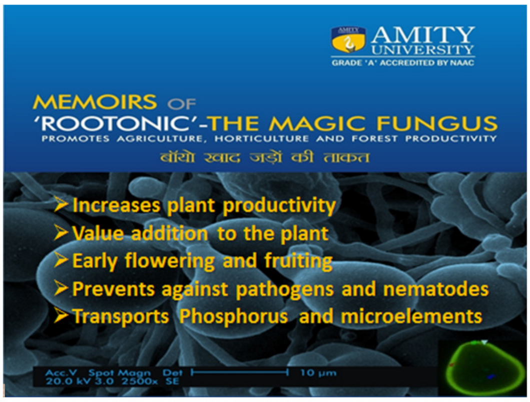

Figure 1: Functional Characteristics of *Piriformospora indica*.

The fungus has been established as bio protector [13], immuno regulator and agent for biological hardening of tissue culture raised plants [14]. Recently, *P. indica* was named as *Serendipita indica* [15] functions, in nutrient deficient soils, as a bio protector against biotic and abiotic stresses [16] including root and leaf pathogens and insect invaders, induces early flowering [17-19] and promotes growth [20] as shown in Figure 1. Entire genomic sequence of the fungus is available [21,22]. Decoding of *P. indica* genome has revealed its potential for application as bio agent and for targeted improvement of crop plants in biotechnological approaches [23]. In this communication, we report the applications of *P. indica* on medicinal plants with special emphasis on *Curcuma longa L.* (Turmeric) and *Plantago ovata* (Isabgol).

Cultivation and morphological characteristics

Simple morphological features of *P. indica* contain hyphae and pear shaped large spores. Expensive Hill and Kaefer medium is routinely used for the cultivation of fungus. The nutrient has been suitably modified and now we are growing on Jaggery as a sole nutrient energy source, a natural product from sugarcane (*Saccharum officinarum*). Mass cultivation of *P. indica* was done on Gur (Jaggery), the procedure used for extraction is highly simplified as shown in Figure 2. It is grown both in batch culture and fermenter using (4% w/v) Jaggery as shown in Figure 3. The optimum temperature and pH are 25°C±2, 6.8 respectively. Maximum biomass production was obtained after 7days incubation at120rpm [24].This innovation is patented [25] (Patent number: 944/DEL/2012 dt: 27.03.12)

Figure 2: Innovation : Jaggery (Gur)

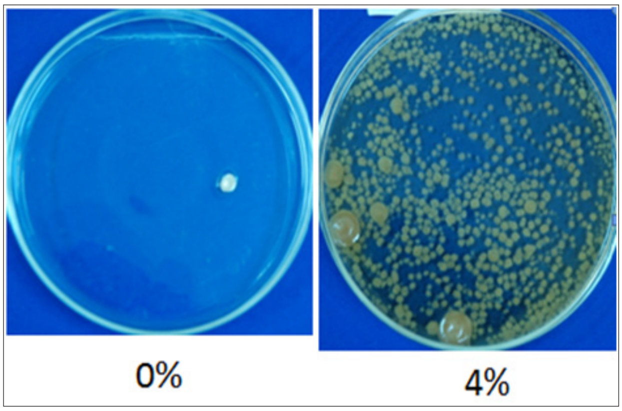

Figure 3: Cultivation of *P. indica* on Jaggery. Optimum concentration was 4% (w/v).

Jaggery composition

A. The Jaggery contains approximately:

a. 60-85% Sucrose

b. 5-15% Glucose

c. Variable-Fructose

d. 0.4% of protein

e. 0.1g of fat

f. 0.6 to 1.0g of minerals (8mg -calcium, 4mg - phosphorus and 11.4mg - iron)

g. Traces of vitamins

h. Amino acids

B. 100g of Jaggery gives 382 Kcal of energy

Functional characteristics of *P. indica*

3.3.1.Plant growth promotion: *P. indica* promotes growth of plants of forestry, horticulture and agriculture importance. Numerous plants have been tested for the effect of *P. indica* on their growth and interestingly majority of them have shown beneficial effect. Few results of its effect on sugarcane, *Pinus* and potato are also reported. It is important to note that in addition to enhancement of plant growth, the fungus also helps in enhancement of active ingredients in plants. In the case of Ratoon crop of sugarcane it was seen that plants not associated with *P. indica* turned yellow due to iron deficiency, whereas plants subjected to *P. indica* treatment remained green, indicating that the fungus also helps in iron transport. Almost 39% enhancement in iron content and 16% increase in sugar content were recorded in *P. indica* treated plants. Noticeable increase in plant size and tuber size was observed in the case of *Pinus* and potato, respectively.

Value addition in plants of medicinal importance

Effect of *P. indica* has been studied on large number of spices and plants of medicinal importance. To name few are *Curcuma longa, Spilanthus calva, Artemisia annua, Anacyclus pyrethrum, Cyamopsis tetragonoloba, Tridax procumbens, Aloe vera, Abrus precatoriu, Bacopa monnieri, Coleus forskohlii, Ocimum tenuiflorum, Brassica rapa, Adhatoda vasica, Helianthus annuus, Abrus precatorius, Withania somnifera, Chenopodium album, Chlorophytum tuberosum, Foeniculum vulgare, Linum album, Stevia rebaudiana, Podophyllum sp*, etc [26]. The organism has shown significant increase in concentration of active ingredients like curcumin, artemisnin, podophyllo- toxin and bacoside leading to value addition to the plant. In this communication, out of 24 plants tested we are giving elaborated on the following:

Turmeric *(Curcuma longa L.)*

Turmeric (*Curcuma longa L.*) is a medicinal plant belonging to the family of Zingiberaceae and is a medicinal plant. Its modified underground stem (rhizome) is extensively used as alternative

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medicines (Ayurveda, Unani and Siddha) and has a long history and is known to exhibit a variety of pharmacological effects including anti-inflammatory, anti-tumor, anti-HIV and anti-infectious activities. It is taken as the blood purifier and is very useful in the common cold, leprosy, intermittent, affections of the liver, dropsy, inflammation and wound healing. The rhizome of the turmeric plant is highly aromatic and antiseptic. It is even used for contraception, swelling, insect stings, wounds, whooping cough, inflammation, internal injuries, pimples, injuries, as a skin tonic. Sweetened milk boiled with the turmeric is the popular remedy for cold and cough. It is given in liver ailments and jaundice [27-31].

The secondary metabolite curcum in scavenges active oxygen species including superoxide, hydroxyl radical and nitric oxide [32]. Furthermore, the yellow powder and raw rhizome is widely used as flavoring and coloring agent in the Asian diet. Curcumin (diferuloylmethane) comprises of Curcumin I (curcumin), Curcumin II (demethoxycurcumin) and Curcumin III (bisdemethoxycurcumin), which are found to be natural antioxidants [33]. Its yellow color is imparted by curcumin (diferuloylmethane), a polyphenolicpigment [34]. The powered rhizome of this plant is used as an condiment and as an yellow dye. It is used to colour and flavour the foodstuff. It is used in the preparation of medicinal oils, ointments and poultice. It is even used in the cosmetics.

Table 1: Results showing effect of *P. indica* on *Curcuma longa* (Turmeric) by studying various parameters.

Parameters	Percent Increase Over Control
Volatile Oil	21.09
Curcumin	19.00
Yield in quintal/Kanal	12.69

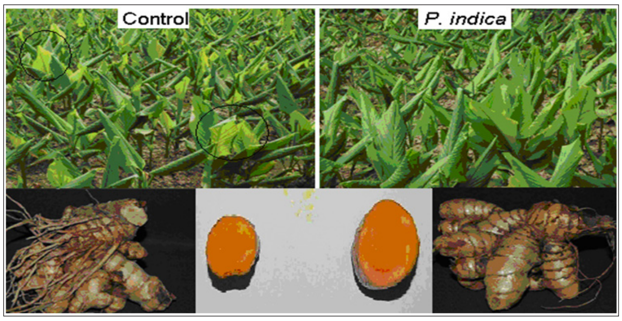

Figure 4 : Interaction of *P. indica* with *Curcuma longa* (Turmeric).

Interactionof P.indica with *C. longa* resulted in approximately 21, 19 and 13% increase in essential oil, Curcumin and rhizome yield as shown in Table 1. Field trials showed that increase in rhizome yield after treatment with *P. indica* would benefit a farmer with Rs.16,000/(US$280.00) per hectare of land as shown in Figure 4 . It is also probable that healthy and shiny rhizomes would fetch better price. *P. indica* promotes growth and development of turmeric, similar to reports for other plant species. This has agricultural implications, because the increase growth rate and better performance of the plant is associated with higher levels of curcumin, an important spice in Asia, structure is described in Figure 5.

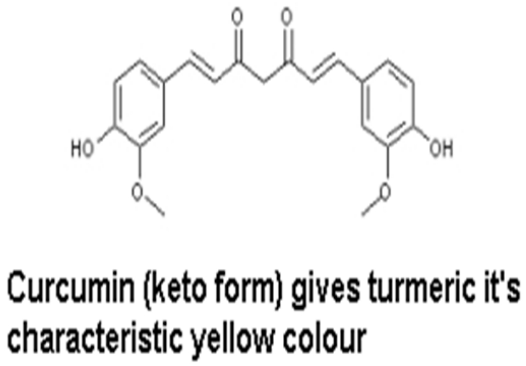

Figure 5 : Chemical structure of an active ingredient Curcumin in Curcumin longa (Turmeric).

Application of *Piriformospora indica* on *Plantago ovata* (Isabgol)

Isabgol (*Plantagoovata*) are annual plant species that majorly grow in the arid and semi-arid regions and are extensively used in conventional and modern pharmacology. The seeds of blond psyllium are mainly valued for mucilaginous rosy white husk. The *mucilage* comprises of reserve carbohydrates mainly pantosans [35]. The husk is commonly used for getting relief from constipation as per being a dietary fiber supplement acting as a bulk-forming laxative. It releases constipation through mechanically stimulating the intestinal peristalsis.

The seeds of Isabgol (*Plantago ovata*) were treated with formulation of the AM fungi *Piriformospora indica* to study the effect on the growth and development of plant species. Nursery trails were conducted based on the season in the month of November. On application of *Piriformospora indica*, it was observed that the overall growth of the plant was promoted. Table 2 shows the mean yield in Isabgol husk, increased to 33% in P.indica treated seeds. There also was observed an early flowering in case of *P. indica* treated seeds as shown in Figure 6. Psylliumor ispaghula is the common name used for several members of the plant genus Plantago whose seeds are used commercially for the production of *mucilage*. Psyllium is mainly used as a dietary fiber to relieve symptoms of both constipation and mild diarrhea and occasionally as a food thickener. Research has also shown benefits in reducing cholesterol levels.

Table 2: Effect on Isabgol crop yield on interaction with *P. indica*.

Treatment	Average Yield	% increase
Control	283.3	
P. indica	375.0	32.3

The soluble fiber in psyllium is arabino xylan, a hemicellulose. Psyllium is produced mainly for its *mucilage* content. The term *mucilage* describes a group of clear, colorless, gelling agents

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derived from plants. The *mucilage* obtained from psyllium comes from the seed coat. *Mucilage* is obtained by mechanical milling (i.e. grinding) of the outer layer of the seed. *Mucilage* yield amounts to about 25% (by weight) of the total seed yield. Plant ago-seed *mucilage* is often referred to as husk, or psyllium husk. The milled seed *mucilage* is a white fibrous material that is hydrophilic, meaning that its molecular structure causes it to attract and bind to water. Upon absorbing water, the clear, colorless, mucilaginous gel that forms increases in volume by tenfold or more.

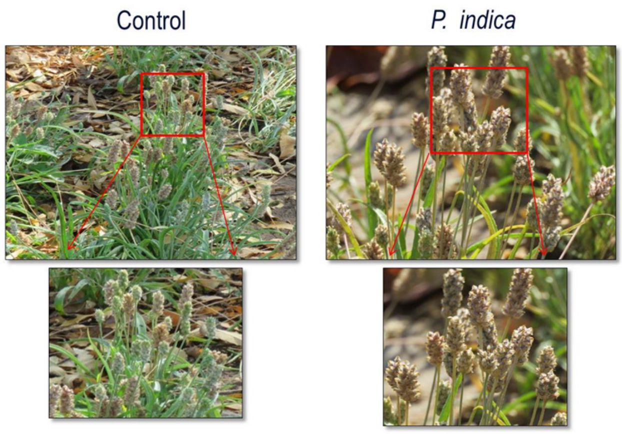

Figure 6: Early flowering and maturation observed in *P. indica* treated *Plantago ovata* (Isabgol) plants.

Mechanisms behind the unique action of *P. indica*

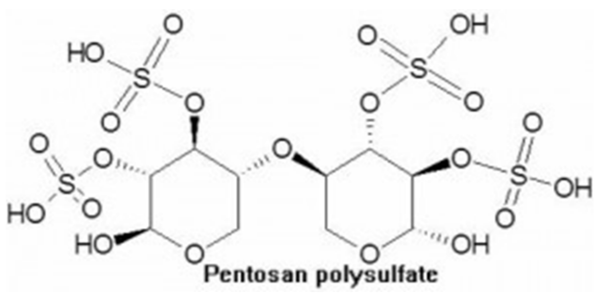

Figure 7: Chemical structure of an active ingredient in *Plantagoovata* (Isabgol).

The fungal interactions are characterized by increase in efficiency of nutrient uptake from soil due to better hyphal penetration as compared to thicker root hairs. Plants deliver phosphorus assimilates to fungus and during mycorrhizal associations; plants acquire phosphates from extensive network of extra radical hyphae. Interaction of *P. indica* with plant alters pathway for nitrogen metabolism, thereby helping plants to absorb more nitrogen. This phenol-menon gives higher resistance to water deficiency and makes plants drought tolerant. Enhanced growth of plants under mycorrhizal condition amplifies its starch requirement. This starch is obtained from deposition in root amyloplasts. Thus, it is interpreted that one of the major starch degrading enzymes, the glucan-water dikinase is activated by *P. indica* [36]. Uptake and transportation of important macronutrients like iron, zinc, manganese, copper, etc. are also regulated by the fungus. Along with this, beneficial phyto hormones are synthesized by plants associated with P. indica. The cumulative effect of macro-micro-nutrients and phyto-hormones regulates plant metabolism leading to value addition, early flowering, plant growth promotion, etc. Massive proliferation of useful rhizospheric micro-organisms sustains soil fertility (Figure 7).

Conclusion

P. indica is a rewarding organism with its huge and distinguished properties. Colonization by *P. indica* increases nutrient uptake, allows plants to survive in drought, salt-stress and temperature stress. Excellent plant growth promotion, growth at extremes of climate and bio-protecting capability of the organisms has paved way for its varied field applications. Large field trials at various locations in India showed beneficial effects of *P. indica* on plant growth and development. Promising outputs of field trials showed that it should be used at large scale so that common farmers are benefited and finally countries economy is at profit. Increase in productivity of certain crop upon interaction with *P. indica* will increase total land usage. Enhanced field usage of the microorganisms requires its mass production. Field trials of the same are done by formulating biomass with powder and inoculating the mixture into root of plants. The formulation is termed "Rootonic". The journey from *P. indica* to Rootonic is exciting and very fulfilling. Large scale production and application of the product is still under process and we are looking forward to its commercialization soon.

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