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Full Length Research Article

Plant Growth Promotion by Cellulolytic Actinomycetes (EGAS) from the gut of Earthworm, *Eisenia Foetida*

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ABSTRACT

The metabolic functions of earthworm intestinal microorganisms must be rigorously defined for better understanding of the role of earthworms in nature and their potential usage in bioconversion of unavailable carbon sources. However, little attention has been paid on this topic to learn the contributions of earthworm and its products to soil health. With the worldwide perspective, to help the environmentally safe soil ecosystem and vowing to the importance of earthworms and their effective role in degradation of cellulose. Actinomycete appears to play a great role in cellulose decomposition. The actinomycetes strains used in our laboratory have been isolated and maintained primarily by enrichment from gut of earthworm, *Eisenia foetida*. Locally available carbon sources as cellulosic forms were tested for the degradation, so that inoculants of actinomycete can be developed and supplemented as biofertilizers to the farmyard manure or directly added to the farm soil. Plant growth promotion directly by employing organism or indirectly using the enzyme has been evaluated to establish the influence of the organism on seed germination and plant growth.

Key words: Actinomycetes, cellulolytic actinomycetes, EGAS (Earthworm Gut Actinomycetes), Earthworm, Eisenia foetida.

INTRODUCTION

Growth in plant cells depends in part on the mechanical properties of the cell wall, where a complex material comprising fibrous cellulose microfibrils is embedded in an amorphous matrix of polysaccharide and protein (Yong Woo Park, *et al.,* 2003). Streptomyces microorganisms play an important role in the decomposition of organic matter and nutrient mineralization, which promote plant growth. They can also act as biocontrol agents on cellulose cell wall bearing (17-25%) microorganisms such as *Phytophthora* and *Pythium* (Lima, 1998). All Streptomycete isolates colonized the root system of in vitro tomato seedlings. The constant exsudation of compounds, such as glutens, lecithins, flavonoids, and polysaccharides by the root cells and microorganisms, is responsible for the recognition between microorganisms and plants (Kijine *et al.,* 1998) and the rhizosphere effect which culminates in the root colonization by microorganisms (Kortemaa *et al*., 1994). These compounds act on the outside of the cellular membrane, capturing iron molecules in solution, and binding then specifically to receptors of the complex localized in the membrane, through which they are absorbed, thereby making it available for plant growth (Neilands and Leong 1986).

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These compounds act as growth promoters due to their ability to inhibit the proliferation of plant pathogens in the rizosphere, by depriving pathogens from this essential nutrient (Wei *et al*., 1996). The production of growth promoting substances such as plant hormones is part of the metabolism of various bacteria associated with plants causing modifications in the morphology of roots, influencing nutrient and water absorption, and consequently promoting plant growth (Bashan and Holgium, 1997). All studied streptomycetes produced indole-acetic acid. Among the known biosynthetetic pathways of indole-acetic acid are the ones depending on tryptophan and the pathways independent of this amino acid, which have as precursors, 3-indole-acetamine, 3-indole-pyruvate acid, and 3-indole-acetonitrile (Patten and Glick, 1996). By producing plant hormones, microorganisms can stimulate plant growth in order to increase production of plant metabolites which can be utilized for microbial growth (Oliveira *et al*., 2003).

MATERIALS AND METHODS

Experiments were conducted to determine the plant growth by taking 100% sterile soil (Control), 60% sterile soil + 40% cellulosic material (Sample 1) and 60% sterile soil + 40% cellulosic material + EGAS1 (CFU 10^8/ml) (Sample 2) and kept for degradation for 2 weeks and they were tested for plant growth promotion.

Pot Assay Technique

Groundnut seeds of cultivar JL-24 were chosen as a test sample, a common cultivar of Chittoor District. The seeds were surface sterilized in 0.01% mercuric chloride solution, washed thrice with sterile distilled water and then sown in pots of Control, Sample 1 and Sample 2. Percent of seed germination was recorded. Each treatment was replicated four times and pots were monitored the growth. The pots were watered daily to keep the moisture content constantly at 70%; plants were examined after 21 days. Height and weight of the plants were recorded for a period of 21 days. The total length of root and shoot of the seedlings was measured to calculate the Seedling Vigour Index (SVI) (Abdul-Baki and Anderson, 1973).

Seedling Vigour Index (SVI) = Total length of seedling X Germination percentage
(Root + Shoot)

RESULTS

Plant Growth Promotion by Egas1

There was a positive effect on over all height (root and shoot) of groundnut plants. An 100% seed germination was observed with 60% sterile soil + 40% cellulosic material + EGAS1 (CFU 10^8/ml) (Sample 2), 80% seed germination was observed with 60% sterile soil + 40% cellulosic material (Sample 1) when compared to the control (40%). The seeds sown in sample 1 and sample 2 pots have shown distinct improved germination and had grown more rapidly than the control.

Table 1. Effect of sample-1 and sample-2 on percent of seed germination, root length, shoot length and seedling vigor index of groundnut cultivar JL-24

S.No	Seed treatment	% Seed germination	Root length (cm)	Shoot length (cm)	Height Root + Shoot (cm)	Weight (gms)	Seedling index
1	100% sterile soil (Control),	40	17	26	43	6.90	1720
2	60% sterile soil + 40% cellulosic material (Sample 1)	80	15	25	40	6.50	3200
3	60% sterile soil + 40% cellulosic material + EGAS1 (CFU 10^8/ml) (Sample 2)	100	19	28	47	7.25	4700

The results of effect of root length, shoot length and Seedling Vigor Index (SVI) were recorded. An increase in root length (19) in sample 2 and sample 1(15) was observed when compared to the control (17). The sample 2 had shown highest increase in root length. Similarly an increase in shoot length was observed when compared to the control. An increase in SVI i.e., 3200 in sample 1 and 4700 in sample 2 was observed when compared to the control 1720 Table 1.

DISCUSSION

In the present study there is a positive effect on over all height (root and shoot) of ground nut plants. An increase in present seed germination was observed with test sample 1 and 2. The changes in the physicochemical and microbial properties of the soil after substitution of 40% cellulosic material to the sterile soil and 40% cellulosic material + *Streptomyces albaduncus* to the sterile soil, could explain some of the plant growth response. Shiralipour *et al.* (1992) *Streptomyces albaduncus* to the sterile soil, could explain some of the plant growth response. Shiralipour *et al.* (1992) reported similar plant responses in container media substituted with composted urban waste. The increased growth of groundnut seedlings was probably due to nutritional / or physical factors.

According to Frakenberger and Arshad (1995) and Igarashi , 2003 microorganisms not only mineralize complex substances into plant available nutrients but can also synthesize a whole series of biologically active substances including plant growth regulators. In the present study *Streptomyces albaduncus* presented cellulolytic activity. Cellulose is the most abundant polysaccharide (20-50%) in the vegetable biomass, is formed by glucose chains forming a glycosidic or β-1,4,-bond with C-4 of glucose, and may be degraded by various microbial enzymes such as cellulase (Murasimha *et al.,* 2002; Lynd *et al.,* 2002). Streptomyces isolates play an important role in the decomposition of organic matter and nutrient mineralization, which promote plant growth.

They can also act as biocontrol agents on cellulose cell wall bearing (17-25%) microorganisms such as *Phytophthora* and *Pythium* (Lima, 1998). The production of growth promoting substances such as plant hormones is part of the metabolism of various bacteria associated with plants causing modifications in the morphology of roots, influencing nutrient and water absorption, and consequently promoting plant growth (Bashan and Holgium, 1997).

Patten and Glick, 1996 reported that all the streptomycetes sps are producing indole-acetic acid a plant growth hormone. By producing plant hormones, microorganisms can stimulate plant growth in order to increase production of plant metabolites which can be utilized for microbial growth (Oliveira *et al.,* 2003).

Besides acting as organic matter decomposers, these microorganisms have great potential as agents for control of plant pathogens (Hoster *et al.,* 2005; Thirup *et al.,* 2001) and/or for plant growth promotion (Nassar *et al.,* 2003). This is due to their capacity to produce antibiotics, siderophores, enzymes that have antimicrobial activity, substances that promote plant growth, solubilization of phosphates and competition with plant pathogens for substratum and nutrients (Cattelan and Hartel, 2000).

The presence of the strain EGAS1 is producing higher cellulase activity and plant growth promotion. This is an indication of useful bacterial flora in the gut of earthworm which is naturally protecting the nutritive values of soil by their diversified activities. A wide array of beneficial microorganisms has been categorized as Plant Growth Promoting Bacteria (PGPB) including mainly diazotrophs, bacilli, pseudomonads, actinomycetes and rhizobia (Antoun & Prévost, 2006). PGPB may induce plant growth promotion through different direct or indirect modes of action (Glick *et al.,* 1999; Antoun & Prévost, 2006). Direct mechanisms include improvement of plant nutrient status (liberation of phosphates and micronutrients from insoluble sources; non-symbiotic nitrogen fixation), iron sequestration by

siderophores, the production of bacterial volatiles and phytohormones and lowering of the ethylene level in the plant. The indirect effects can be exerted by antibiotic production, depletion of iron from the rhizosphere, induced systemic resistance, synthesis of antifungal metabolites, production of fungal cell wall lysing enzymes, competition for sites on the root, stimulation of other beneficial symbioses and degradation of xenobiotics in inhibitor-contaminated soils. Somers *et al.* (2004) have classified PGPB into the following functional groups depending on their inherent activities as: i) biofertilizers (increasing the availability of nutrients to the plant), ii) phytostimulators (plant growth promoting, usually by the production of phytohormones: auxin, cytokinin, gibberelin), iii) rhizoremediators (degrading organic pollutants), and iv) biopesticides (controlling diseases, mainly by the production of antibiotics and antifungal metabolites). Pathogen suppression by plant growth promoting microorganisms can result from one or more mechanisms depending on the particular antagonist involved (Barea *et al.,* 2005). Earlier reports showed that microorganisms capable of hydrolyzing the fungal cell wall are the most efficient in controlling the population of fungal pathogens (Mitchell *et al.,* 1961; Shapira *et al.,* 1989; Sivan and Chet 1989; Chet *et al.,* 1990; Haran *et al.,* 1993; Tanaka and Watanabe, 1995; Bapat and Shah, 2000; Wang *et al.,* 2002). Many *Pseudomonas* and *Bacillus* species are capable of producing hydrolytic enzymes. For example, *Pseudomonas stutzeri* produces extracellular chitinase which lyse the pathogen *Fusarium* sp., *Clostridium werneckii* and *Bacillus cepacia* hydrolyze fusaric acid produced by *Fusarium* (Bashan & de-Bashan, 2010).

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