International Scholars Journals

African Journal of Tropical Agriculture ISSN 2375-091X Vol. 6 (11), pp. 001-005, November, 2018. Available online at www.internationalscholarsjournals.org © International Scholars Journals

Full Length Research Paper

Indole acetic acid production and enhanced plant growth promotion by indigenous PSBs

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Accepted 18 September, 2018

Indigenous soil samples were tested for Phosphate solubilization. Efficient phosphate solubilizng bacteria were isolated. Effect of four different media on phosphate solubilization were determined. Auxin production by these bacteria were determined via bioassay and high performance liquid chromatography by the bacteria in liquid culture. Indole acetic acid and indole butyric acid were produced by these bacteria in varying concentration with and with out the addition of tryptophan. These bacteria showed stimulatory effects on the growth of root and shoot elongation of mung beans (*Vigna radiata*). Three promising isolates CMG854, CMG857 and CMG860 were investigated to establish the effect on plant growth. On a comparative basis isolate CMG860 was most promising in promoting plant growth.

Key words: Phosphate solubilizing bacteria, indole acetic acid, indole butyric acid, plant growth, phytohormones, vigna radiate.

INTRODUCTION

Plant Growth Promoting Rhizobacteria (PGPB) are considered to promote plant growth directly or indirectly. PGPB can exhibit a variety of characteristics responsible for influencing plant growth. The common traits include production of plant growth regulators (auxin, gibberellin, ethylene etc.), siderophores, HCN and antibiotics (Arshad and Frankenberger, 1992). Indole acetic acid (IAA) is one of the most physiologically active auxins. IAA is a com-mon product of L-tryptophan metabolism by several microorganisms including PGPR (Lynch, 1985; Frankenberger and Brunner, 1983). Microorganisms inhabiting rhizospheres of various plants are likely to synthesize and release auxin as secondary metabolites because of the rich supplies of substrates exuded from the roots compared with non rhizospheric soils (Kampert et al., 1975; Strzelczyk and Pokojska-Burdziej, 1984). Plant morphogenic effects may also be a result of differ-rent ratios of plant hormones produced by roots as well as by rhizosphere bacteria (Muller et al., 1989). Diverse soil microorganisms including bacteria (Muller et al., 1989), fungi (Stein et al., 1985) and algae (Finnie and Van Staden, 1985) are capable of producing physiologically active quantities of auxins, which may exert pronounced effects on plant growth and establishment. *Azotobacter paspali* secreted IAA into culture media and significantly increased the dry weight of leaves and roots of several plant species following root treatment (Barea and Brown, 1974). It was found that inoculation of wheat seedlings with Azospirillum brazilance increased the number and length of lateral roots (Barbieri et al., 1986). Inoculation of canola seeds with Pseudomonas putida GR12-2, which produces low levels of IAA, resulted in 2 - or - 3 fold increases in the length of seedling roots (Glick et al., 1986; Caron et al., 1995). Biosynthesis of IAA is not limited to higher plants. Organisms such as bacteria, fungi and algae are able to make physiologically active IAA that may have pronounced effects on plant growth and development. Many bacteria isolated from the rhizosphere have the capacity to synthesize IAA *in vitro* in the presence or absence of physiological precursors, mainly tryptophan (Trp) (Caron et al., 1995; Davies, 1995) . Microbial isolates from the rhizosphere of different crops appear to have a greater potential to synthesize and release IAA as secondary metabolites because of the relatively rich supply of substrates (Muller et al., 1989; Caron et al., 1995) . Production of IAA by microbial iso-lates varies greatly among different species and strains and depends on the availability of substrate(s). Different biosynthetic

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pathways for IAA production exist, some-times in parallel in the same organism (Davies, 1995). For many years it was assumed that Trp was the only precursor of IAA. However, work with tryptophan-auxotrophic mutants and isotope labeling has established that IAA biosynthesis can occur via a tryptophan-independent route (Normanly, 1997; Venis and Napier, 1991), although in the presence of Trp microbes release greater quantities of IAA and related compounds. There is firm evidence that indole-3-acetic acid (IAA) (Arshad and Frankenberger, 1991; Sarwar and Frankenberger, 1994; Barea and Brown, 1974; Brown, 1972; Brown and Burlingham, 1968; Lee et al., 1970; Scott, 1972), gibbe-rellins and cytokinins (Sarwar and Frankenberger, 1994; Barea and Brown, 1974), all produced by plants and essential to their growth and development, are produced also by various bacteria which live in association with plants. There is also evidence that the growth hormones produced by the bacteria can in some instances increase growth rates and improve yields of the host plants (Arshad and Frankenberger, 1991; Sarwar and Frankenberger, 1994; Barea and Brown, 1974). It is possible that bacteria capable of phosphate solubilization may improve plant productivity both by hormonal stimulation and by supplying phosphate.

Indole-3-acetic acid (IAA) is the main auxin in plants, controlling many important physiological processes including cell enlargement and division, tissue differentiation, and responses to light and gravity. Bacterial IAA producers (BIPs) have the potential to interfere with any of these processes by input of IAA into the plant's auxin pool. The consequence for the plant is usually a function of the amount of IAA that is produced. A root, for instance, is one of the plant's organs that is, most sensitive to fluctuations in IAA and its response to increasing amounts of exogenous IAA extends from elongation of the primary root, formation of lateral and adventitious roots, (Finnie and Van Staden, 1985).

It is now generally agreed that indole- 3- acetic acid (IAA) is the major and most abundant auxin in plants. IAA plays a key role in the regulation of plant growth and development (Moore, 1989; Lüthen et al., 1999; Davies, 1995). Over the last few years significant progress has been made in understanding the IAA-induced signal transduction pathway (Napier and Venis, 1995; Venis and Napier, 1991; Shahab and Ahmed, 2008). Although other auxins, such as indole-3-acetic acid) indole 3 butyiic acid (IBA) and phenyl acetic acid (PAA) have also been identified in plants (Normanly, 1997), little is known about their physiological function.

It is presumed that PGPB producing plant growth regulators play a critical role in plant growth promotion. To assess this hypothesis, local isolates of PSBs were screened for their intrinsic ability to produce IAA in the presence of L-tryptophan and their effect on root elongation of germinating seeds of test plants. Inoculations with PSBs have increased shoot length and root lenght of plants in both green house. In the experiments reported here we studied phytohormone production in PSBs in association to determine whether the bacteria might enhance plant growth by this mechanism.

MATERIALS AND METHODS Isolation,

purification and preservation

Soil samples for isolation of PSBs were taken from various localities of Karachi. PSBs were isolated by plating serial dilutions of this soil in the medium described by Shahab and Ahmed (2008). Bacteria were isolated and purified and screened for the phosphate solubilization activity in tris-minimal media in presence of glucose amended with zinc phosphate. Bacterial isolates producing clear halos around colonies showing large solubilization halos were selected and the persistence of their phosphate-solubilizing capacity checked by five successive subcultures in the same medium. The most efficient P solubilizing strains with the largest solubilization halos were selected for further studies, identification and preservation. They were identified by using API kits and were preserved in 20% glycerol.

Effect of various media on the efficiency of phosphate solubilization

Four different media were used to check the efficiency of solubilization. Media used in this study were:

1. Pikovskaya, (Pikovskaya, 1948).
2. Tris minimal salt medium, (Fasim et al., 2002).
3. NBRIP (National Botanical Research Institute's Phosphate growth medium) (Nautiyal, 1999).
4. MPYK (modified Pikovskya media) (Nautiyal, 1999).

Bio assay for IAA

IAA was determined in vitro by the method of (Salkowski Holt et al., 1994). All the test strains were screened for IAA production (Loper and Schroth, 1986). Briefly, test bacterial culture was inoculated in the nutrient broth with tryptophan (0.1 g/l) or without tryptophan incubated at 30°C. Cultures were centrifuged at 3000 rpm for 30 min. Two milliliters of the supernatant was mixed with 2 drops of orthophosphoric acid and 4 ml of reagent Salkowski (50 ml, 35% perchloric acid; 1 ml 0.5 FeCl3).

Determination of indole acetic and indole butyric acid via HPLC

HPLC chromatograms were produced by injecting 10 l of the filtered extracts onto a – (C18, 5 m 25 x 0.46 cm) in a chroma-tograph equipped with a differential ultraviolet detector absorbing at 280 nm. Mobile phase was methanol and water (80:20 [vol/vol]), flow rate was 1.5 ml/min, Retention times for peaks were compared to those of authentic standards added to the medium and extracted by the same procedures used with bacterial cultures. Quantification was done by comparison of peak heights.

Response to plant growth

Seeds of mung beans (vigina radiate) were surface sterilized with 95% ethanol and 2.5% sodium hypochlorite. Then washed with distilled water repeatedly up to 10 min. Similar-sized seeds of mung beans were selected and Seeds dressing were done via O/N cul-

Table 1. Basic Characterization of Phosphate Solubilizing Bacteria.

Strain code	Identification	Accersion no	Auxin production
CMG854	*Bacilllus thuringiensis*	EU697390	+
CMG857	*Bacilllus thuringiensis*	EU697391	+
CMG860	*Pseudomonas aeruginosa*	EU037096	+

cultures then seeds were put onto Petri plate having underlined soaked filter paper and incubated for 4 h at 37°C. After 4 h seeds, ten seeds per pot were sown at equal depth 1-cm^2 sections in to plastic pots having 200 g autoclaved soil. Soil in each treatment was moistened with an equal volume of autoclaved distilled water for daily watering. Lateral root and root hair formation was examined. Rroot and shoot length of the plants and controls were measured by using centimeter scale. Figures 2 and 3

RESULTS AND DISCUSSIONS

Isolation and selection of phosphate-solubilizing bacteria

A total of 28 isolates of phosphate solubilizing bacteria were isolated from soil and tentatively identified on the basis of API kit (Table 1). We selected the strain CMG860, CMG854 and CMG857 because, among all the other PSB isolated, they produced the largest halos, of (Figure 1) approximately 20 - 40 mm within 4 days of incubation. According to de Freitas et al. (1997), good phosphate-solubilizers produce halos around their colonies with diameters higher than 15 mm. Since it has been reported that some strains loose their phosphate-solubilizing capability after several cycles of inoculation, we corroborated the persistence of this trait in all three isolates by successive subcultures.

Effect of media

Tris minimal media (Shahab and Ahmed, unpublished data) was found the most efficient phosphate solubilizing medium followed by NBRIP medium. Hence Tris minimal media was employed for solid, liquid culture, containing the carbon source, usually D-glucose 10 g l - l (BDH) or specified .All chemicals are used either of Oxoid or specified.

Indole acetic acid and indole butyric acid production

These bacterial isolates were screened for their ability to produce plant growth regulator, IAA. Varying levels of IAA production were recorded. The range of IAA production in PSBs isolates with tryptophan was 57 - 288 g/ml. while indole butyric acid was in range of 22 – 34 g/ml.

Our findings of IAA production in PSBS isolates are in agreement with those of other researchers. These isolates varied greatly in their intrinsic ability to produce IAA.

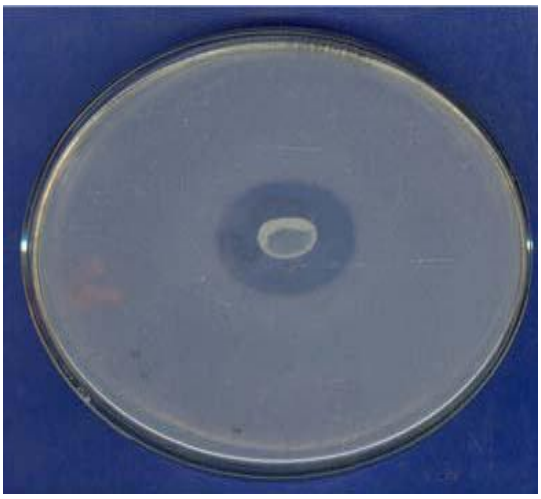

Figure 1. Halos formation around the colony of PSB.

Figure 2. Growth promotion of mung beans by PSBS.

Productions of IAA and phosphate solubilization by the PSBs were examined as possible contributing factors of mung beans growth promotion. Tests for production of the auxin IAA were positive for all test stimulant strains, suggesting a potential mechanism whereby these bacteria may regulate plant growth. This interpretation is in line with the well-known characteristic of certain phytohormones (e.g., auxin, ethylene). Induction of longer roots with increased number of root hairs and root laterals is a growth response attributed to IAA production by other rhizobacteria,

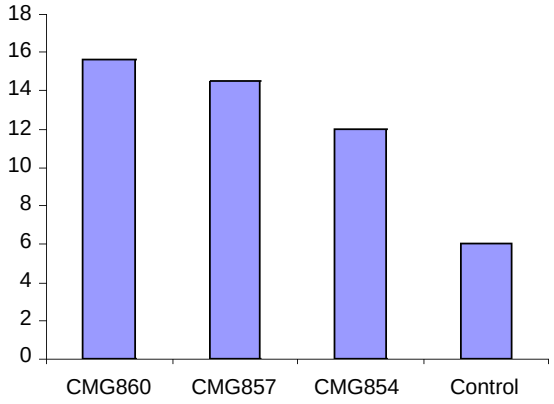

Figure 3. Effect of PSBs on shoot length of Mung beans.

which improves their nutrient uptake efficiency.

Response to plant growth

The symbiotic performance of three isolates (CMG854, CMG857 and CMG 860) with the mung beans shoot and root was evaluated in greenhouse experiments. At shoot length of plants inoculated with strain 860 ranked the highest and it was greater than that of the un inoculated plants the root elongation of germinating seeds of Vigna radiata was highest with isolate CMG860, followed by CMG857 and CMG854 compared to the control, CMG860 showed significant root and shoot elongation. (Shahab and Ahmed, unpublished data) Almost all lateral roots were densely covered by root hairs whereas very few or none developed on un inoculated control plants.

The findings of the present investigation highlighted that IAA producing bacteria from local soil could be easily isolated and may be exploited after strain improvement for local use. However, further studies using IAA mutant strains of these isolates are needed to explore the exact contribution of IAA production in the promotion of plant growth as well as the contribution of other PGP traits. There are numerous soil microflora involved in the synthesis of auxins in pure culture and soil (Barazani and Friedman, 1999). Different plant seedlings respond differrently to variable auxin concentrations (Sarwar and Frankenberger, 1994) and type of microorganisms. Substances produced by bacteria are released continuously and especially when they are produced on the surfaces or within the plant tissue since the bacteria grow there. It seems probable that plant growth substances produced by PSBs improve plant growth by their direct effects on metabolic processes. However, since they induce proliferation of lateral roots and root hairs and thus increase nutrient absorbing surfaces, this may lead to greater rates of nutrient absorption. This in turn would be expec - ted to significantly increased the shoot and brootb length

length of the plants.

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