International Journal of Current Microbiology and Applied Sciences
ISSN: 2319-7706 **Volume 3 Number 2 (2014) pp. 568-574**
http://www.ijcmas.com

Original Research Article

Isolation of indole acetic acid (IAA) producing rhizobacteria *of Pseudomonas fluorescens* and *Bacillus subtilis* and enhance growth of onion *(Allim cepa*.L*)*

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A B S T R A C T

The present study was under taken for isolation of *Pseudomonas fluorescens* and *Bacillus subtilis* from rhizosphere of Onion and analysis of these bacteria for *invitro* indole acetic acid production and studying the effect of these bacteria on plant growth of onion Plant. The soil samples were collected from rhizosphere of onion plants from Botanical garden, Department of Botany, Annamalai University. These two microorganisms are isolated by using KB and NB medium. Effect of IAA producing *Pseudomonas fluorescens* and *bacillus subtilis* bacteria on plant growth was studied by pot culture experiments by using sterilized air dried soil and viable onion seeds . Both bacteria demonstrated increase in root length, shoot length, root and shoot fresh and dry weight, on bacterial inoculated onion seeds over control.

Keywords

Pseudomonas fluorescens; Bacillus subtilis; IAA; PGPR

Introduction

The rhizosphere, representing the thin layer of soil surrounding plant roots and the soil occupied by the roots, supports large active groups of bacteria (Villacieros *et al.*, 2003) known as plant growth promoting rhizobacteria (PGPR) (Kloepper et al.,1980). Plant growth promoting rhizobacteria are known to rapidly colonize the rhizosphere and suppress soilborne pathogens at the root surface (Rangajaran et al., 2003). These organisms can also be beneficial to the plant by stimulating growth (Bloemberg and Lugtenberg, 2001; Moeinzadeh et al., 2010). Among these organisms, Fluorescent *Pseudomonads* are considered to be the most promising group of plant growth promoting rhizobacteria involved in biocontrol of plant diseases (Gardner et al., 1984; Moeinzadeh et al., 2010). They produce secondary metabolites such as antibiotics (Keel et al., 1992), phytohormones (Keel et al., 1992), volatile compound Hydrogen cyanide (HCN) (Defago and Haas 1990), and siderophores (Neil and JB, 1995). Plant growth-promoting ability of these bacteria is mainly because of the production of

indole-3- acetic acid (IAA); (Patten and Glick 2002), siderophores (Schippers et al., 1987) and antibiotics (Colyer and Mount1984).Rhizosphere is a rich niche of microbes and should be explored or obtaining potential plant growth promoting rhizobacteria (PGPR) which can be useful in developing bio-inoculants for enhancement of growth and yield of crop plant. Bacteria predominates the rhizosphere and take nutritional substances (amino acid, vitamins and other nutrients) released from plant tissues for growth .The products of microbial metabolism that are released into the soil also influence the growth of plant. Interaction between plant and microbes is well known for beneficial effect and such free-living soil bacteria isolated from the rhizosphere of plants are known as plant growth promoting rhizobacteria (Kloepper et al 1980). Some bacteria support plant growth indirectly by producing antagonistic substances or by inducing systemic resistance against plant pathogen (Tilak et al 2005).

Plant growth promoting rhizobacteria are free living, soil-borne bacteria, which enhance the growth of the plant either directly or indirectly .The direct mechanism involve nitrogen fixation. Phosphorus solubilisation, HCN production, production of phytohormone such as auxin, cytokinin and gibberellins and lowering of ethylene concentration (Kloepper et al., 1989; Glick, 1995; Glick et al., 1999).In wheat *Azospirillum brasilense* of wheat seedlings increase the number and length of lateral roots (Barbieri et al., 1986). Importance of IAA production by PGPR has been widely acknowledged (Kennady et al., 2004; Rosech et al., 2007 Ashrafuzzaman et al., 2009). IAA selected by stimulating plant cell elongation or cell division or indirectly by influencing bacterial 1-aminocyclopropane-1carboxylic acid (ACC) deaminase activity (pedraza et al., 2004) similarly role of phosphate solubilising bacteria in plant growth and development has been well documented De.Freital et al ., 1997: Richardson et al., 1999.

Materials and Methods

All glass ware first soaked in chromic acid cleaning solution containing potassium dichromate in 25% sulphuric acid or 3 hrs and washed thoroughly in tap water. After a second was in detergent solution. They were again washed thoroughly in tap water and finally rinsed in distilled water and dried in oven.

Soil sampling

Soil sample were collected from the rhizosphere of onion in the Botanical garden Department of Botany Annamalai University. Intact root system was dugout and the rhizosphere soil samples were collected in plastic bags.

Isolation of bacteria from rhizosphere soils

The plant growth promoting rhizobacteria were isolated from the rhizosphere soil sample by serial dilution plate methods. Appropriate dilution was spread on NB and KB agar plates. Plates were incubated at 28±2˚c for 24 to 48 hrs. Colonies were picked from these plates and maintained as pure culture in respective media with periodic transfer to fresh media and stocked for further use

Production of indole acetic acid

The selected antagonistic bacterial strains were grown in 5ml of nutrient broth

medium in test tube or 24 hrs. After incubation period, bacterial culture were harvested and centrifuged at 100000xg for 15min at 4˚c. Two drops of orthophosphoric acid were added to 2ml of cell free supernatant and the development of colour was observed the presence of a pink colour indicate positive reaction for indole acetic acid and yellow colour indicate negative reaction.

Method of seed treatments

Bio control agents were grown in respective medium on rotating shaker (150rpm) for 2 days and centrifuged at 10,000 rpm for 5min the pellet was mixed with sterile carboxyl methyl cellulose (CMC) suspension .Onion seeds surface sterilized with sodium hypochlorite solution were placed in CMC cell suspension and air dried inside laminar air flow chamber (jagadeesh, 2000).The bio coated seeds were transplanted into the field .

Shoot length and root length (cm)

Three plants were randomly selected for recording the root length and shoot length of onion plants. They were measured by using centimeter scale.

Fresh and dry weight (mg/g dry wt.)

Three plant samples were randomly selected at experimental pot. They were separated into root and shoot. Their fresh weight was taken by using an electrical single pan balance. The fresh plant materials were kept in a hot air oven at 80°C for 24 hr and then their dry weight were also determined

Results and Discussion

In the present studies were carried out to isolate the bacterial culture from the rhizosphere of onion plant. The isolates of *Psedomonas fluorescens* and *Bacillus subtilis* were screened for their plant growth promoting activities of Indole acetic acid (IAA) production from the rhizosphere of onion plant.

Indole acetic acid production (IAA) (µg/ml)

The results originated from both qualitative and quantitative assays of IAA reflected the ability of two tested microorganisms to produce indole compounds. The two tested microorganisms exhibited a pink to red colour with a little variation in intensity. In the quantitative measurements, the highest value of IAA production was obtained by *P. fluorescens* followed by *B. subtilis*, as they produced (15.38±0.537) and (12.67±0.325) respectively.

Root length (cm)

Data presented in Table 2 showed that the effect of different biocontrol applications on the root length of Onion. The highest root length was recorded in *Pseudomonas fluorescens (*7.500 ± 0.100) treated plants when compared to *Bacillus subtilis* (7.333 ± 0.152) and control (5.666 ± 0.404).

Shoot length (cm)

Significantly higher shoot length was produced in *Pseudomonas fluorescens* (27.41 ± 0.102) compared to *Bacillus subtilis* (25.30 ± 0.173) and control (25.10 ± 0.34).

Shoot Fresh weight of plant (g)

The data pertaining to the effect of biocontrol agents on shoot fresh weight of onion plants are depicted in Table 1. The fresh shoot weight of onion plants was significantly high in *Pseudomonas Fluorescens (*3.590 ± 3.777), compare to *Bscillus subtilis (*2.830 ± 0.060). The least weight of whole plant was observed in the uninoculated control (2.556 ± 0.297).

Root Fresh weight of plant (g)

The data pertaining to the effect of biocontrol agents on Root fresh weight of onion plants are depicted in (Table 1). The fresh root weight of onion plants was significantly high in *Pseudomonas fluorescens* (0.953 ± 0.047), compare to *Bacillus subtilis* (0.556 ± 0.030). The least weight of whole plant was observed in the uninoculated control (0.380 ± 0.057).

Shoot Dry weight of plant (g)

The results of the dry weight of shoot onion under two different biological control on recorded in (Table 2). Shoot dry weight of plant was highest in the *Pseudomonas fluorescens (*0.260 ± 0.100), *and Bacillus. Subtilis (*0.233 ± 0.005).The least dry weight was seen in the control plants (0.226 ± 0.025).

Root dry weight of plant (g)

The results of the Root dry weight of onion under two different biological control on recorded in (Table 2). Root dry weight of plant was highest in the *Pseudomonas fluorescens (*0.600 ± 0.010), *and Bacillus Subtilis (*0.500 ± 0.010).The least dry weight was seen in the control plants (0.433 ± 0.057).

Pseudomonas and Bacillus bacteria posses multiple plant growth promoting activity. Compare to these two bacteria, *Pseudomonas fluorescence* have potential to produce high amount of IAA production to *Bacillus subtilis* and control. The result shows that these isolates possessed PGP activities the range of percentage of IAA production of these bacterial activities are varied greatly. Mahalakshmi and Reetha (2009), (Saitou N and Nei M 1987) reported similar results in the assessment of PGP activities of bacterial isolates from the rhizosphere of tomato. The rhizosphere microorganism especially fluorescent pseudomonas, have exceptional ability to promote the growth of host plant by various mechanism to suppress plant diseases including production of powerful sideraphores (O'sullivan and O'Gara 1992): Has and Defago 2005).

The property of synthesizing indole acetic acid is considered as an effective tool for screening beneficial microorganism as there have been reports suggesting that IAA producing bacteria having profound effect on plant growth(Kuklinsky et al 1999),Ali B and Hashin S 2007) The improved plant growth by PGPR is due to its ability to produce phytohormones such as indole acetic acid (IAA) (Pattern and Glick, 2002), gibberellic acid (Mahmoud *et al.* 1984), cytokinin (Tien *et al.*, 1979) and its ability to produce ACC-deaminase to reduce the ethylene in the roots of the developing plants thereby increasing the root length and growth (Penrose and Glick, 2001). *B. subtilis* isolates improved the germination, emergence; increased root growth and nodulation in groundnut along with consistent colonization were reported by Turner and Backman (1991). Tsavkelova *et al.* (2006) stated that *Bacillus* involves the modulation of plant development through the production of phytohormones. Thus, several *Bacillus*

Table.1 Effect of PGPR inoculation on Growth parameters of onion plant

Treatment	Shoot length	Root length	Shoot fresh weight	Root fresh weight
Control	25.10 ± 0.34	5.666 ± 0.404	2.556 ± 0.297	0.380 ± 0.057
Pseudomonas fluorescence	27.41 ± 0.102	7.500 ± 0.100	3.590 ± 3.777	0.953 ± 0.047
Bacillus subtilis	25.30 ± 0.173	7.333 ± 0.152	2.830 ± 0.060	0.556 ± 0.030

The values are mean ± SD for three samples in each group

Table.2 Evaluation of the ability of the microorganisms to exhibit IAA Production in *in-vitro* condition

Treatment	Shoot dry weight	Root dry weight	IAA Production Colour intensity µg/ml	
Control	0.226 ± 0.025	0.433 ± 0.057	-	-
Pseudomonas fluorescence	0.260 ± 0.100	0.600 ± 0.010	+++	15.38±0.537
Bacillus subtilis	0.233 ± 0.005	0.500 ± 0.010	++	12.67±0.325

The values are mean ± SD for three samples in each group

species are capable of producing auxin that might stimulate root proliferation and nutrient uptake (Spaepen *et al.,* 2007). Khan *et al.* (2003) described that soil application of *Aspergillus awamori* or *P. fluorescens* increased the dry weight of shoot by 19.0% and 9.5% respectively, compared to uninoculated control. Combined application of a *P. fluorescens* along with *Bradyrhizobium* in groundnut significantly enhanced groundnut root and shoot dry weight, nodule number, nodule dry weight, and per cent nitrogen content of shoot (Vikram *et al.,* 2007). In the quantitative measurements, the highest value of auxin production was obtained *P. fluorescens* followed by *B. subtilis* and *T. harzianum*. This is in consonance with the work of (and Verma *et al.* (2010) who found that high proportion of rhizo-microorganisms are able to produce plant growth hormone, *i.e.,* indole acetic acid, which acts to stimulate plant growth and provides it with more branching and larger surface area. Thus these IAA producing bacteria like *pseudomonas fluorescens and bacillus subtilis* were further studied or their effect on plant growth under controlled conditions. Data obtained from the pot experiments demonstrated positive effect on root elongation of treated plants over the control .This indicate both bacteria have the efficiency to improve the root and shoot length of development of the plant and these can be considered as plant growth promoters.

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