

Screening and Identification of Best Three Phosphate Solubilizing and IAA Producing PGPR Inhabiting the Rhizosphere of *Sesbania bispinosa*

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ABSTRACT: PGPR can be isolated from plant rhizospheres and used as inoculants for improving the growth and yield of agricultural crops. The present study was under taken for the screening and identification of best three indole-3-acetic acid producing phosphate solubilising rhizobacteria isolated from the rhizosphere soil of Dhaincha (*Sesbania bispinosa*). Twelve bacterial colonies with diverse growth features, designated as DD1 to DD12 were isolated from dhaincha rhizosphere and screened for their Phosphate solubilization ability. 7 showing positive results were further monitored for their Indole-3-acetic acid (IAA) production. All the seven PSBs were found to be producing IAA which was confirmed by colorimetric method. Isolate DD4 produced maximum amount of IAA (1260 ppm/Lit on 8th day of incubation) whereas isolates DD6 and DD3 produce 1087 ppm/Lit and 724 ppm/Lit respectively on 6th day incubation. Further investigations for optimization of Indole acetic acid production was carried out at different cultural conditions, such as pH, temperature and concentration of tryptophan. Maximum IAA production was recorded at pH 7, 37° C temperature and 1gm/Lit L-tryptophan concentration. The best 3 IAA producing isolates i.e. DD3, DD4 and DD6 were further identified by 16S rRNA partial gene sequencing as *Escherichia coli* DACG2 (GenBank Accession number JN858966) *Pseudomonas fluorescens* strain DACG3 (GenBank Accession No. KP641168) *Burkholderia sp.* DACG1 (GenBank Accession No. JN639877). These three isolates are needed to be further studied due to their potential to be developed as plant growth promoting rhizobacteria in the field application.

KEYWORDS: PGPR; Phosphate solubilising bacteria, Indole-3-acetic acid, *Escherichia coli* DACG2; *Pseudomonas fluorescens* DACG3; *Burkholderia sp* DACG1.; *Sesbania bispinosa*;

I. INTRODUCTION

Plant growth-promoting rhizobacteria (PGPR) are a heterogeneous group of bacteria found in rhizosphere in association with roots and root surfaces or free living in soil and influences plant growth directly or indirectly. A large number of bacteria including species of *Pseudomonas*, *Azospirillum*, *Azotobacter*, *Klebsiella*, *Enterobacter*, *Alcaligenes*, *Arthobacter*, *Burkholderia*, *Bacillus*, and *Serratia* have been reported to enhance plant growth [Kloepper (1989), Glick BR (1995)]. Plant growth promoting rhizobacteria play an imperative role in agriculture by promoting the exchange of plant nutrients and reducing the application of chemical fertilizers to a large extent. There are several mechanisms by which rhizosphere bacteria may stimulate plant growth that involves mechanisms like nitrogen fixation, solubilization of phosphorous and iron from the soil, and production of phytohormones.

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Phosphorus is an essential nutrient for plant growth and development consisting up to 0.2% plant dry weight (Harrison et al, 2002). Phosphorus is typically insoluble in soils. Although the average P content of soils is about 0.05% (W/W), only 0.1% of the total phosphorus exists in plant accessible form (Illmer and Schimmer, 1995). As a result large amounts of soluble forms of P fertilizers are applied to attain maximum crop production. However, the applied soluble forms of P fertilizers are easily precipitated into insoluble forms such as tricalcium phosphate [$\text{Ca}_3(\text{PO}_4)_2$], ferric phosphate (FePO_4) and aluminium phosphate (AlPO_4) (Achal et al, 2007). These insoluble forms are not efficiently taken up by the plants which again leads to an excess application of P fertilizer to crop fields (Khan et al, 2007). The unavailable phosphates built up in soils are enough to sustain maximum crop yield globally for about 100 years (Goldstein et al, 1993). Phosphate solubilizing PGPR solubilize sparingly soluble phosphate by releasing organic acids, chelation, and ion exchange reactions and make them available to plants (Kucey et al, 1989; Whitelaw, 2000; Vessey, 2003). The existence of microorganisms able to solubilize various forms of calcium phosphate has been reported frequently but relatively few studies investigated the solubilization of other phosphates such as AlPO_4 and FePO_4 . Keeping this in mind researchers tried to exploit phosphate solubilizing PGPR for crop improvement [Cattelan et al, (1999); Husen (2003); Orhan et al, (2006); Son et al (2006); Chandra et al, (2007); Zehra Ekin (2010)] in 1997, De Freitas et al demonstrated the potentiality of phosphate- solubilization by rhizobacteria isolated from field grown plants and observed that rhizobacteria enhanced growth and phosphorus uptake of canola (*Brassica napus* L., cv. Legend) in potted soil experiments in the growth chamber. Sundra et al (2002) studied the influence of phosphate solubilizing bacteria on the changes in soil available phosphorus and sugarcane and sugar yield. Singh et al (2010) studied the effect of phosphate solubilizing bacteria on plant growth promotion and nodulation in soybean (*Glycine max* (L.) Merr.). Taurian et al (2009) isolated phosphate solubilizing bacteria from peanut (*Arachis hypogaea* L.) rhizosphere and screened their plant growth promoting ability. Toro et al (1997), Seshadri et al (2000), Kundu et al (2002) studied different aspect of phosphate solubilization by PGPR.

Another most commonly reported direct growth promotion mechanism by bacteria is the production of plant growth substances or hormones. Plant hormones are chemical messengers that affect a plant's ability to respond to its environment. Auxins, gibberellins, cytokinins, ethylenes are common plant hormones. The major and most abundant naturally occurring auxin is Indole-3-acetic acid (IAA) which has broad physiological effects on plants (Davies, 2010). It is now generally agreed that indole-3-acetic acid plays a key role in the regulation of plant growth and development (Moore, 1989; Lüthen et al., 1999; Davies, 1995). 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 Pokojaska-Burdziej, 1984). Many plant growth-promoting rhizobacteria (PGPR), including *Azospirillum*, *Azotobacter*, *Bacillus*, *Burkholderia*, *Pseudomonas* and *Rhizobium* produce IAA or related auxins (Dubeikovsky et al., 1993; Taghavi et al., 2009). Auxins have been implicated in signaling between microorganism and plant (Spaepen et al., 2007) leading to stimulation of cell division, initiation of lateral and adventitious roots (Malamy and Benfry, 1997), increase rate of seedling emergence (De Freitas and Germida, 1990) and results into elongation of stems and roots (Yang et al., 1993). It has been reported that IAA production by bacteria can vary among different species and strains, and it is also influenced by culture condition, growth stage and substrate availability (Matsukawa et al., 2007). 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). Tryptophan is believed to be the primary precursor for the formation of IAA in plants and rhizobacterium (Monteiro et al., 1988). 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), gibberellins 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).

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Now, Dhaincha (*Sesbania bispinosa*) is a leguminous crop generally cultivated for its nutritive value to soil. It is cultivated in monsoon season almost throughout India and grows well in loamy, clayey, black and sandy soils. It is an ideal green manure crop as it is fast growing, succulent, and easily decomposable with low moisture requirements. It forms both root and stem nodules in association with *Rhizobium sp.*, fixes more atmospheric N_2 and produces maximum amount of organic matter in the soil. It enriches concentration of Ca, P, S & micronutrients (Khan et al. 2010). It is a very important intercrop relayed with rice and other cereals. Nutrients derived from Dhaincha are very cheap as compared to those of chemical fertilizers. It increases physical and biological properties of soil, add organic matter to it and it gives long term residual effect to the following crops. However, till date, very few studies have been found on PGPR association of Dhaincha plants. Moreover, a better knowledge of PGPR habiting rhizospheric soil of Dhaincha and their implications on growth and yield of other crop might change traditional crop management practices. It is presumed that phosphate solubilizing bacteria producing plant growth regulators play a critical role in plant growth promotion and can improve plant productivity both by hormonal stimulation and by supplying phosphate. Keeping this in mind, the present investigation was an attempt to screen the phosphate solubilising bacteria isolated from dhaincha rhizosphere for their intrinsic ability to produce IAA and to optimize the various environmental factors such as pH, temperature and tryptophan concentration for IAA production and identification of the best three IAA producer.

II. MATERIALS AND METHODS

Isolation of bacteria:

Rhizosphere soil of *S. bispinosa* was collected from four different spots both from local farmer's field (around Kalyani area of Nadia district) and research plots of Horticultural faculty of Bidhan Chandra Krishi Viswavidyalay, State Agricultural University). Intact root system was plowed out and the rhizospheric soil samples at the vicinity of root were collected and bacterial strains were isolated by serial dilution plate count technique. Microbial strains were isolated by single colony isolation from the nutrient agar medium. The isolated colonies were sub-cultured in nutrient agar slants.

Determination of Phosphate solubilization:

Selected 12 isolates were plated on Pikovasky medium containing 5 g of tri-calcium phosphate [$Ca_3(PO_4)_2$] as sole phosphorus source for selectively screening of bacteria which have the ability to release inorganic phosphate from tri-calcium phosphate. After 3-days of incubation at 30°C, phosphate solubilizing bacteria developed clear zones around colonies. All the isolates having phosphate solubilizing activity were preserved in the laboratory as a pure culture for their further investigation.

Quantification of soluble Phosphate :

The phosphate solubilizing ability of each isolate was tested by using insoluble $Ca_3(PO_4)_2$ as sole P source in Pikovskaya's medium. One mL PSB were inoculated in 250 mL flask containing 100 mL Pikovskaya's medium (pH 7.0) and incubated at 30 °C on a rotary shaker at 160 rpm. The test medium without PSB served as control treatment. 5.0 mL of the cultures were taken aseptically on 1, 3, 5, 7 and 10 days, respectively, and used to determine pH, phosphorus solubilization activity and growth (O.D. at 600 nm). The culture supernatant obtained by centrifugation (4000 rpm, 15 min) was passed through a 0.45 M Millipore filter prior to analyzing the soluble phosphate concentration and pH by the molybdenum blue method (Murphy and Riley, 1962) and with a pH meter equipped with a glass electrode, respectively. The experiment was conducted in triplicate.

Detection of IAA production by the selected isolates:

The P- solubilising rhizobacteria were then selected for their phytohormone (Auxin) production ability. Test bacterial cultures inoculated in Nutrient Broth medium (Jensen's Media) containing L-tryptophan (5gm/ Lit), and incubated for 96 hour. Cultures were centrifuged at 10,000 rpm for 10 minutes. 2ml of supernatant was taken. 2 drops of Orthophosphoric acid and 4ml of Solawaski's reagent were added to it. (Solawaski's reagent – 50ml of 35% Perchloric acid and 1ml of 0.5 (M) $FeCl_3$). Then it was kept for 20 min in dark room temperature. Salkowski reagent was used for detecting the presence of IAA. The reagent gives reaction with IAA and does not interact with L-tryptophan This reagent is an important option for qualitative and semiquantitative determination that assure the presence of the hormone

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in the supernatant of bacterial cultures or liquid formulations of biological inoculants based on the change of colour from yellow to pink, the presence of Indole Acetic Acid was confirmed. The values of the various strains along with Salkowski reagent was checked using UV-VIS spectrophotometer and their values were noted down in terms of Optical density. O.D. was taken at 540 nm. The produced IAA level in each culture medium was estimated by comparison with a standard IAA graph. The measurements were taken in triplicate. The IAA producing bacterial strain was screened for further study.

Optimization of different environmental conditions for IAA production

Indole acetic acid (IAA) production is influenced by different environmental factors. The isolated rhizobacterial strains produce surprisingly a huge amount of plant growth promoting hormone IAA. For that reason, it should be necessary to optimize different environmental factors for maximum IAA production. The parameters studied were incubation period, pH of the media, temperature and concentration of tryptophan. The incubation period was studied by inoculating into 100 ml/ L - tryptophan supplemented production medium and incubated for 96 hrs at 28 °C in a rotary shaker at 120 rpm. Samples were withdrawn every 48 hrs and growth and indole acetic acid concentration were determined. The pH was adjusted to different values ranging from 4 to 9 and the effect of varying pH on IAA production was studied. Effect of temperature on IAA production was examined by incubating the bacterial cultures for 96 hours in different temperature range from 20°C to 50°C. Effect of tryptophan on the IAA production was determined using production medium supplemented without or with tryptophan in varying concentrations (0.2, 0.4, 0.6, 0.8, 1.0 and 1.2 gm/lit).

Identification of best three IsAA producing strains

The best three IAA producing strains were identified using Molecular method of identification by 16S ribosomal DNA gene sequencing. Genomic DNA was isolated from the pure culture pellet. The ~1.4kb rDNA fragment was amplified using high fidelity PCR polymerase. The PCR product was sequenced bi-directionally using the forward, reverse and internal primer. The sequence data was aligned and analyzed to identify the bacterium and its closest neighbors. The partial sequences were submitted to NCBI GenBank. The phylogenetic tree was made using Neighbor joining method.

Statistical analysis

Statistical analysis was conducted by using Analysis of Variance (ANOVA) The data were analyzed with Statistical Package for Social Sciences (SPSS) version 21, and Sigma Plot (ver. 11) Software.

II. EXPERIMENTAL RESULT

Isolation of bacteria

Total 12 bacterial colonies with different growth characteristics were successfully isolated from the rhizosphere soil samples of *S. bispinosa* field from four different areas of Nadia district (three bacterial colonies from each of the soil sample) They were designated as DD1, DD2, DD3, DD4, DD5, DD6, DD7, DD8, DD9, DD10, DD11 AND DD12.

Determination of Phosphate solubilization:

Phosphate solubilizing capability of the 12 isolated bacterial strains were ascertained by their phosphate solubilizing efficiency, which were visualized by the clear zone developed around the colonies after 3 days of incubation. Among them, 7 isolates (DD3, DD4, DD6, DD9, DD10, DD11 AND DD12) showed prominent phosphate solubilization zones. Maximum zone observed by the isolate DD6 (24mm) followed by DD4, DD10 and DD3. To further analyze the phosphate solubilizing activity of the isolates, the soluble phosphate content in liquid cultures was evaluated by the Mo-blue method. The amount of phosphate solubilization by isolates varied from 236 to 640 mg L⁻¹ (Fig 1). Four isolates solubilized more than 400 mg L⁻¹ P from insoluble Ca₃(PO₄)₂. DD6 showing the highest P solubilization.

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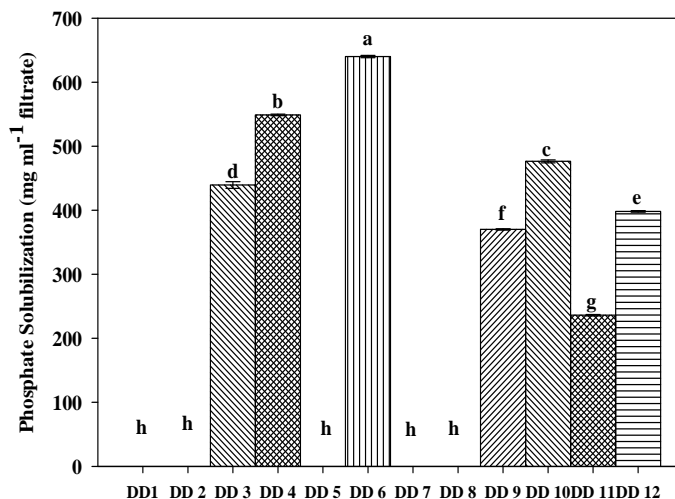


Fig 1 : A bar diagram showing the Phosphate solubilizing ability of the isolates

Detection of IAA production by the selected isolates:

Seven isolates which showed phosphate solubilization ability were further selected for their phytohormone (IAA) production ability. IAA productions by the seven isolates were estimated by their OD value taken in 540 nm, which ranges from 1260 ppm/lit to 180 ppm/lit in 8 days study (Table 1). All the seven isolates were positive for IAA production. Among them isolate DD4 shows highest (1260 ppm) production of IAA followed by DD6 (1087 ppm) and DD3 (724 ppm) whereas the isolate DD11 shows lowest (180 ppm) production.

Table 1 : IAA production by the isolates

Isolates	Duration				
	Day 2	Day 4	Day 6	Day 8	Day 10
DD3	280 ± 17.4	599 ± 23.7	724 ± 53.3	332 ± 21.5	90 ± 6.3
DD4	490 ± 23.5	850 ± 56.2	1123 ± 67.7	1260 ± 34.2	582 ± 34.2
DD6	376 ± 15.6	678 ± 32.4	1087 ± 75.2	868 ± 45.8	404 ± 24.5
DD9	73 ± 2.1	122 ± 9.6	213 ± 12.4	98 ± 4.3	0 ± 0
DD10	110 ± 6.8	283 ± 12.3	590 ± 34.3	259 ± 13.2	64 ± 3.8
DD11	92 ± 4.3	180 ± 9.4	104 ± 6.4	72 ± 3.4	0 ± 0
DD12	108 ± 25.2	318 ± 23.6	198 ± 10.2	110 ± 6.6	32 ± 2.3

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Optimization of environmental parameters for production of IAA by the PSBs :

IAA production by bacteria can vary among different species and strains, and it is also influenced by different culture condition. The production of this strain was then enhanced by using various media parameters such as incubation period, pH, temperature, concentration of tryptophan etc.

From the experiments it was found that Indole acetic acid production by the isolates started at 2nd day of incubation and reached maximum at 6th and 8th day when the bacteria reached stationary phase of growth, and then decreased slowly (Fig 2).

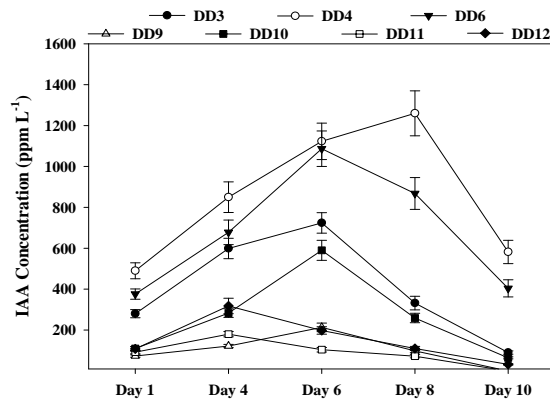


Fig.-2 : Effect of incubation period on IAA production

It was also observed that the acidic or high alkaline condition was not suitable for IAA production. All the isolates produce maximum IAA either at pH 7 (DD4, DD6, DD10) or pH 7.5 (DD3, DD9, DD11, DD12) (Fig 3).

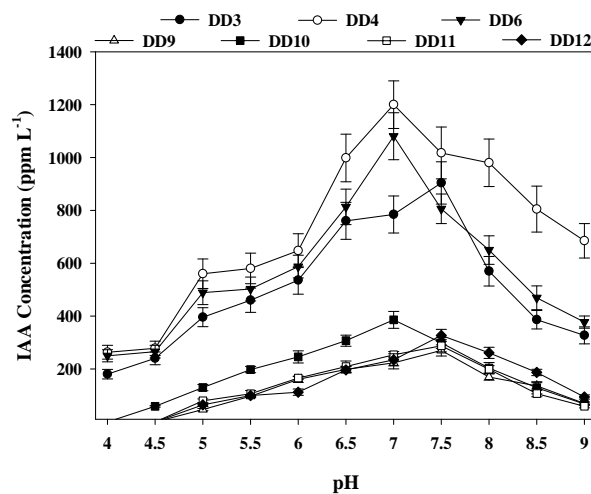


Fig-3 : Effect of pH on IAA production

The range of IAA production by the isolates in different temperatures vary from 26- 1300 ppm. IAA concentration was increased significantly when the temperature was increased from 20-37° C, and then declined. Thus

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37° C was best temperature for IAA production by DD4, DD6, DD3 and DD11 whereas, DD10, Dd12 and DD9 produce their maximum at 35° C temperature. higher temperature exerted the adverse effects on IAA production (Fig 4).

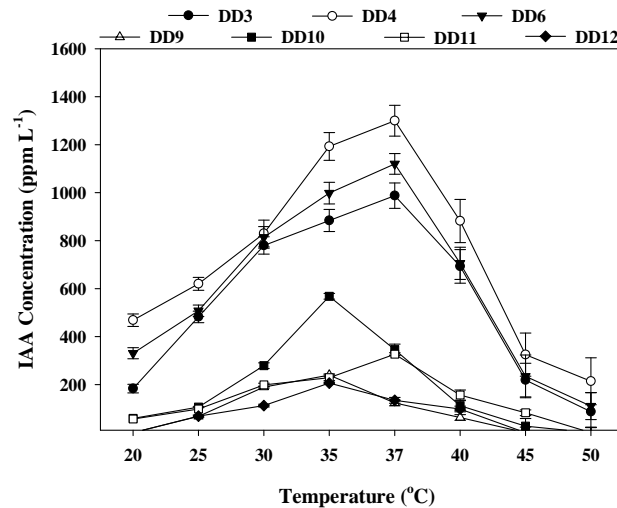


Fig-4 : Effect of temperature on IAA production

DD4 produce maximum amount of IAA i.e. 1300 mg/lit IAA in 1 gm/lit L-tryptophan concentration followed by DD6 and DD3. The range of IAA production by the isolates without tryptophan varies from 45.3 – 204.6 ppm. (Fig 5).

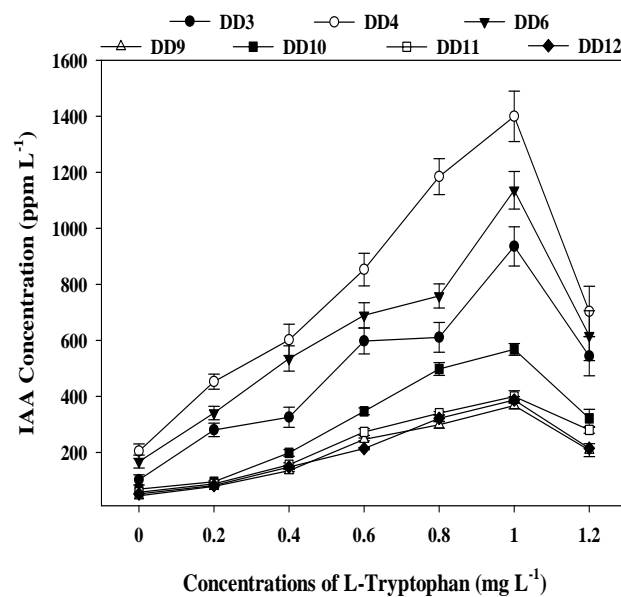


Fig-5 : Effect of tryptophan concentration on IAA production

IAA concentration was increased significantly when the concentration of tryptophan in the medium was increased from 0.2 to 1 gm/lit, and then declined when it was from 1.2 gm/lit. Thus, L-tryptophan at a concentration of 0.8 to 1.2 were best for IAA production where higher concentration of tryptophan exerted the adverse effects on IAA production. DD4 produce maximum amount of IAA i.e. 1399.8 mg/lit IAA in 1 gm/lit L-tryptophan concentration

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followed by DD6 and DD3. It is also clear that L-tryptophan was more active for IAA production, though the isolates were able to produce IAA in absence of tryptophan. In presence of tryptophan, the microbes release greater quantities of IAA and related compounds. As the concentration of tryptophan in the medium increases, the amount of IAA produced increased [Karnwal , A. 2009]. Thus, the optimum conditions for maximum production of IAA by the isolates were found to be the LGI medium supplemented with tryptophan (1 g/l), and pH 7 at 37°C on shaker at 200 rpm in dark after 6 days of incubation.

From the result, it was clear that the best three isolates that show both of the phosphate solubilizing and IAA producing ability were DD4, DD6 and DD3. Though DD10 is a good phosphate solubilizer, but did not possess good IAA producing ability.

3.5.Molecular identification of the three best IAA producing PSB

For the molecular identification, these three isolates were identified by 16 S rDNA sequencing. 16 S rDNA gene sequences compared with the available sequences in the databank with help of BLAST homology search. DD3 was identified as *Escherichia coli* DACG2 and deposited it in the GeneBank database with Accession number JN858966. The phylogenetic tree made by neighbour joining method (Fig-6).

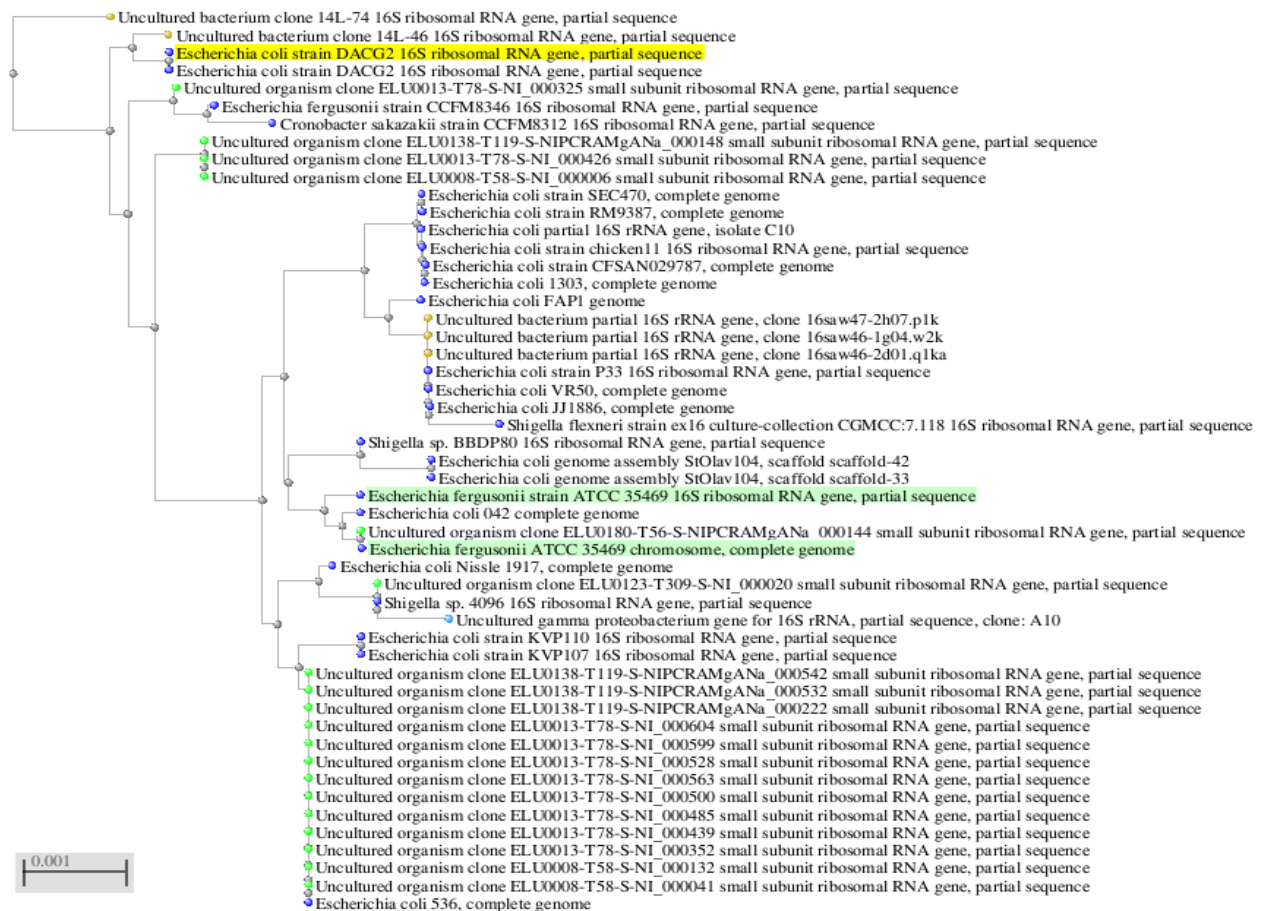


Fig 6: Phylogenetic tree of *Escherichia coli* DACG2 based on 16S rDNA sequences drawn using the neighbour joining method. The scale bar indicates 0.001 substitutions per nucleotide position.

The nearest homolog of DD3 was found to be *Escherichia coli* 12 (NCBI Accession No.-HM007573).

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DD4 was identified as *Pseudomonas fluorescens* strain DACG3 (GenBank Accession No. KP641168). The phylogenetic tree was constructed by neighbour joining method (Fig-7). The strain shows similarity with *Pseudomonas fluorescens* strain CCM 2115.

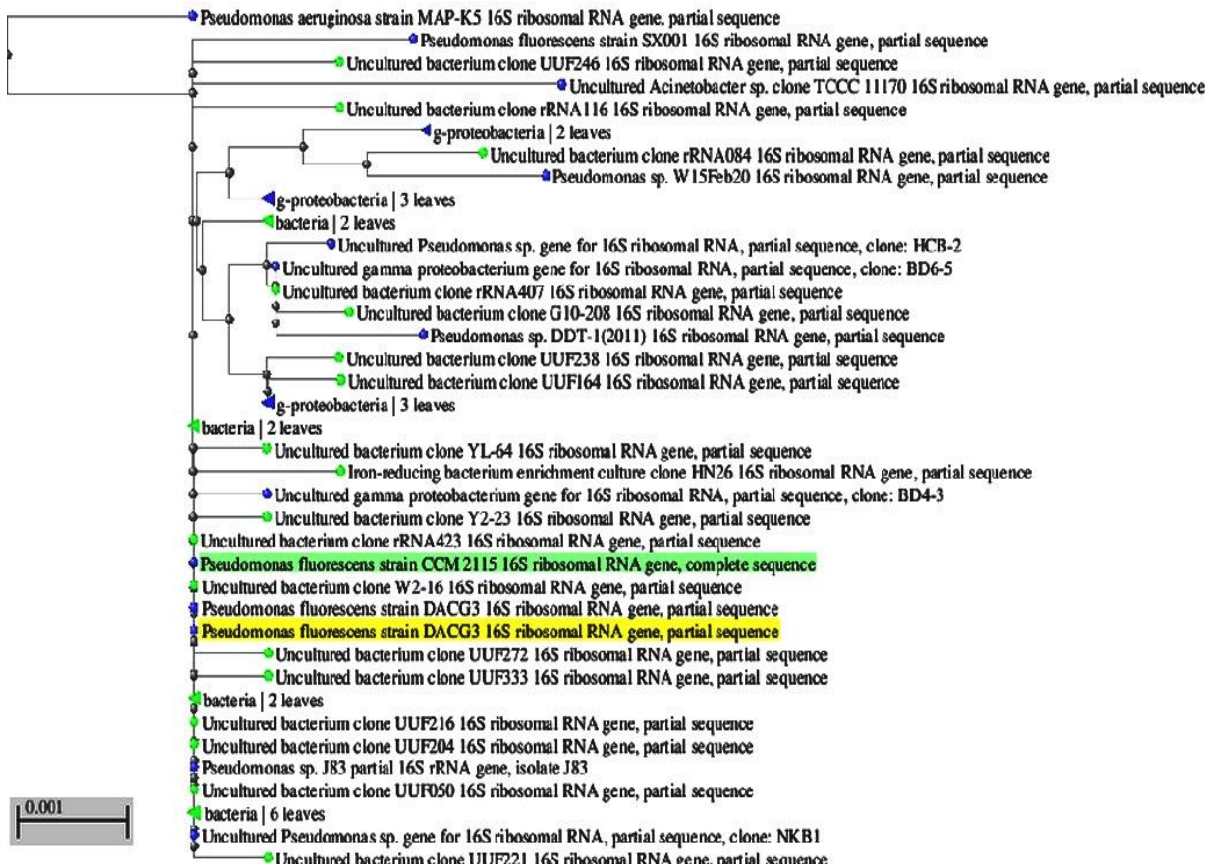


Fig-7: Phylogenetic tree of *Pseudomonas fluorescens* DACG3 (GenBank Accession No. KP641168) The scale bar indicates 0.001 substitutions per nucleotide position

Based on nucleotide homology and phylogenetic analysis, DD6 was identified as *Burkholderia sp.* DACG1. The sequence was submitted to GenBank with Accession No. JN639877. Nearest homolog was found to be *Burkholderia sp.* (GenBank Accession No.: HO728578). The phylogenetic tree was constructed using the same method as the previous two, i.e. using the neighbour joining method (Fig-8).

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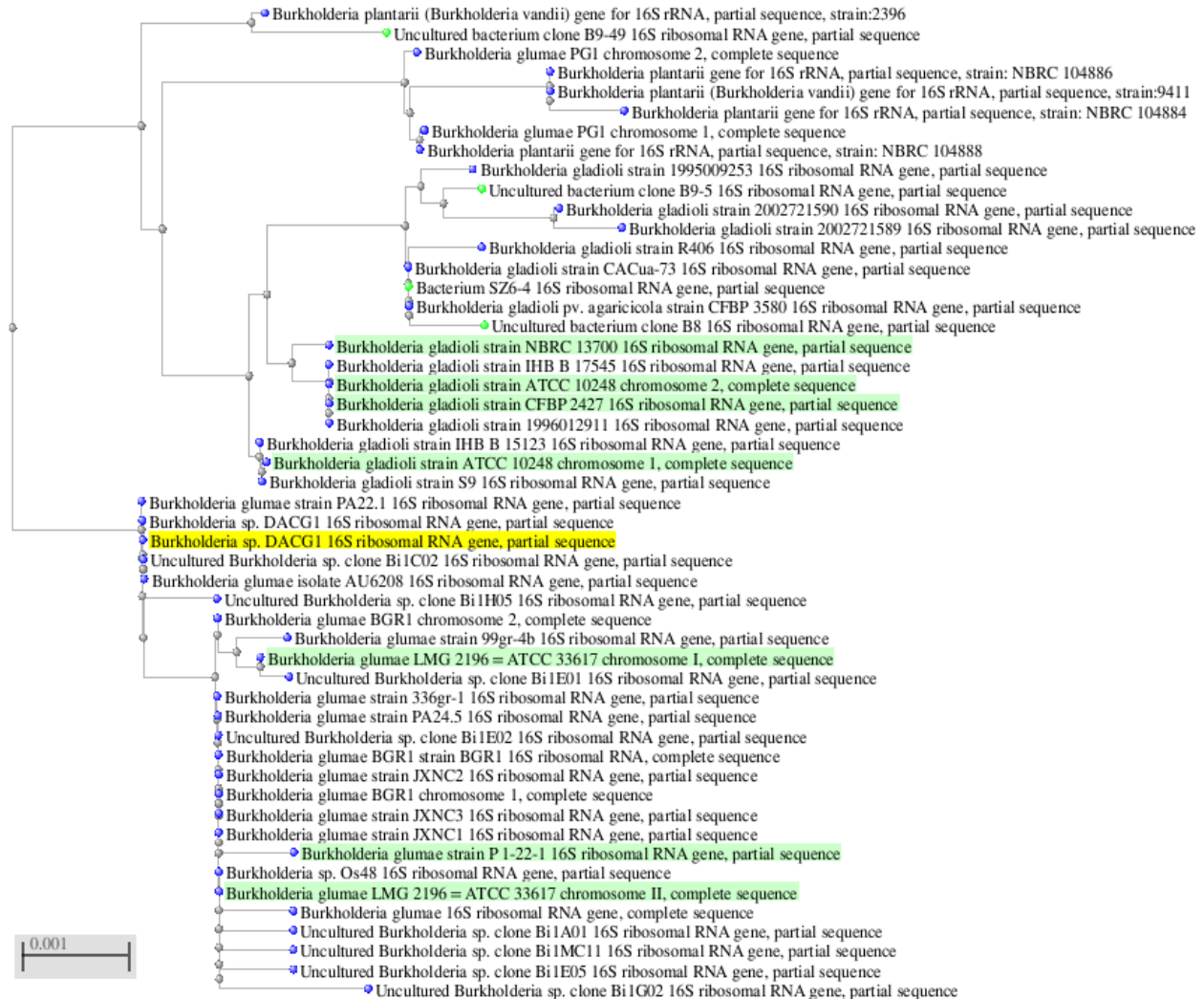


Fig 3: Phylogenetic tree of *Burkholderia* sp. DACG1 (Gene Bank Accession No. JN639877) based on 16S rDNA sequences drawn using the neighbour joining method. The scale bar indicates 0.001 substitutions per nucleotide position.

III. DISCUSSION

Phosphite solubilisation and IAA production by the bacteria present in the rhizosphere is an important property contributing to plant growth. Thus, the purpose of this research was to investigate IAA producing Phosphate solubilising plant growth promoting rhizobacteria from the rhizosphere of *Sesbania bispinosa* (Dhaincha).

Phosphorus is one of the most important nutrients for plant growth and development. In both acidic and alkaline soils, a large fraction of phosphorus is insoluble and relatively unavailable to plants. Phosphate-solubilizing bacteria (PSB) have the capacity to increase the availability of phosphorus (Bhattacharyya and Jha, 2012). In addition to phosphate solubilization, the plant growth promoting bacteria (PGPR) may promote plant growth indirectly by suppressing the growth of phytopathogens, and directly through synthesizing phytohormones (Bhattacharyya and Jha, 2012). Twelve strains were isolated from Dhaincha rhizosphere, of which seven had ability to solubilize insoluble inorganic phosphates in liquid medium and four of them showed promising results. The most efficient isolates were identified as

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Burkholderia sp. DACG1 (DD6), which solubilize 640 g/ml phosphate followed by Pseudomonas fluorescens DACG3 (DD4) and Escherichia coli DACG2 (DD3). This observation came in a good agreement with those reported by other researcher. Several research works supports Burkholderia, Pseudomonas and E.coli as good phosphate solubilizer. According to Ok-Ryul Song et al, 2008; J Stephen - K Zhao - 20112014 J Stephen, 2015, Burkholderia is a potential *phosphate solubilizing bacteria* (PSB). K.-H. Park et al., 2009; K Parani, 2012 showed that Pseudomonas fluorescens having a high ability in solubilizing insoluble phosphate. Escherichia coli isolated and characterized from endorhizosphere of sugarcane (Saccharum sp.) and rye grass (Lolium perenne) is found to solubilise phosphate and thus is found to help in the growth of the plants [Gangwar M, Kaur G, 2009].

In line with the previous investigations, Sattar and Gaur, 2013, studied that Phosphorus solubilization ranged from 4.01 to 7.34 mg / L by the isolates from the rhizosphere of dhaincha. Comparing to these and the other other phosphate solubilizing isolates (Azziz et al., 2012; Fernández et al., 2012), the amount of phosphate solubilized by the isolates of our research were higher. Four isolates solubilized more than 400 mg mL⁻¹ phosphate, and could be considered as potential biofertilizer strains. (Azziz et al., 2012; Fernández et al., 2012). Solubilization of phosphate is commonly accompanied by a remarkable drop in pH (Kloepper et al., 1999; Azziz et al., 2012; Fernández et al., 2012), as was the case for all our isolates.

In addition to P solubilization, the selected bacteria showed many traits associated with plant growth promotion. The isolates can improve plant growth by production of IAA. IAA is the member of the group of phytohormone and is generally considered the most important native auxin [Kloepper JW, Reddy SM, 2004] and is the product of common tryptophan metabolism by several microorganisms (Gravel *et al.*, 2007). Among these selected seven PSBs, IAA production was shown in all (Table 1). In the quantitative measurements, the highest value of auxin production was obtained from Pseudomonas fluorescens DACG3 followed by Burkholderia sp. DACG1 and Escherichia coli DACG2. Pseudomonas produce remarkably huge amount of IAA.

Compare to these three bacteria, *Pseudomonas fluorescens* have potential to produce high amount of IAA production to *Burkholderia sp* and *Escherichia coli*. 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. In our experiment, *Pseudomonas fluorescens* produce 1300 ppm IAA. This is in consonance with the work of Ahmad, F., I. Ahmad and M.S. Khan. 2005 who found that production of high levels of IAA by *Pseudomonas* is a general characteristic. *Pseudomonas* isolates varied greatly in their intrinsic ability to produce IAA.

The indole acetic acid production was very much influenced by the incubation period. The decrease in indole acetic acid production after 72 hours might be due to release of indole acetic acid degrading enzymes such as indole acetic acid oxidase and peroxidase as has been reported earlier [Mehnaz, S., B. Weselowski, F. Aftab, S. Zahid, G. Lazarovits and J. Iqbal. 2009.]. The maximum IAA production was observed in the stationary phase of growth [19]. Production of IAA is influenced by culture conditions, growth range and availability of substrates and varies greatly among different species [Vijila, K. 2000.].

. These PGPR isolates prefer 7 to 7.5 pH, 37° C temperature for maximum IAA production. Previous works suggest that *Pseudomonas*, *Burkholderia* and *E.coli* are mesophilic bacteria Auxin production by all isolates also increased when culture medium supplemented with an IAA precursor; tryptophan which confirm the results of other scholar (Mutluru and Konada, 2007). Several reporters suggested the role of tryptophan in the enhancement of IAA production [Khalid, A., M.Arshad and Z.A. Zahir. 2004] Maximum IAA production of 1300 ppm was obtained when 1% L- tryptophan was used in *Pseudomonas* sp. Which supports the work of [Balaji, N., S.S. Lavanya , S. Muthamizhselvi and K. Tamilarasan]. Our test isolates can produce IAA in absence of tryptophan also which support the work of Karnwal (2009) who tested Fluorescent Pseudomonas isolates for their ability to produce indole acetic acid in pure culture in the absence and presence of L-tryptophan and found that for both strains produce IAA. The isolates produced IAA in pH range 7-7.5. The probable reason could be the neutral or slight alkaline nature (pH 7.5) of the dhaincha rhizosphere from where these strains were isolated.

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IV. CONCLUSION

From this study, it is clear that several naturally occurring PSB isolates from the rhizosphere of dhaincha are capable of producing a significant amount of IAA, capable of solubilizing inorganic phosphates and IAA production increased when the cultural conditions are optimized. The significance of the study could be stated as the potential of these IAA producing and phosphorus solubilizing isolates and optimization of the parameters for IAA production. It was found that maximum IAA production was recorded at pH 7, 37° C temperature and 1gm/Lit L-tryptophan concentration and the best 3 IAA producing isolates are *Escherichia coli* DACG2 (GenBank Accession number JN858966) *Pseudomonas fluorescens* strain DACG3 (GenBank Accession No. KP641168) *Burkholderia sp.* DACG1 (GenBank Accession No. JN639877). Study for IAA production will flourish the growth and ultimately IAA production in the field and prevent environmental pollution by avoiding excessive applications of industrially produced fertilizers to cultivated fields. Further studies are required to use these PSB isolates as a bioinoculum for the better productivity of crops which are non-hazardous to the environment as well as to the rapidly growing World's human population who has threatened the World's food security.

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