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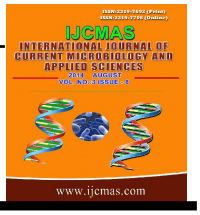

Original Research Article

Isolation and screening of Rice Rhizosphere Soil microorganisms for the production of IAA

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A B S T R A C T

The rhizosphere soil is source for high diversity of microorganisms which play a major role in the enhancement of the plant. The plant and microbe interaction influences the plant growth. In the present study 9 different rice rhizosphere soil samples were collected from different districts of Kolar, Hoskote, Ramnagar and Mysore. Isolations were done on nutrient agar media by serial dilutions and different colonies with morphological variation were selected from each dilution. A total of 53 isolates were differentiated and maintained as pure cultures on nutrient agar. Morphological and Biochemical characterization was carried out for all the individual isolates. Both gram positive bacilli and cocci along with gram negative bacilli and also gram positive branched colonies probably actinomycetes were isolated in the present investigation. A total of 57 Gram positive bacilli, 6 Gram negative bacilli, 47 Gram positive cocci and six actinomycetes were isolated of the total samples used in the current study. 53 isolates were screened for the IAA production and 6 isolates proved to produce IAA ranging from 0.535 - 14.05µg/ml. Further the organisms will be screened for production of growth promoting substances which can be used as natural biofertilisers in the enhancement of the yield of rice.

Keywords

Indole Acetic acid, Rhizosphere, *Oryza sativum*, Biofertiliser

Introduction

The rhizosphere soil possesses a large and diverse population of microorganisms mainly the bacteria. Bacteria in the rhizosphere influence plant growth because they affect soil chemical properties and interact with plant roots, where the influence can be beneficial, neutral, or deleterious (Russell1981, Morita 2000, Sakaietal. 2004). The microbes in the rhizosphere soil have the property to compose the complex Substances depending on the plant exudates (Kimura 1994, Rovira et al., 1974, Watt et al., 2006b). In few decades a large number of bacteria from the rhizosphere soil have been isolated and identified among which the common species of Pseudomonas, Azospirillum, Azotobacter, Klebsiella, Enterobacter, Alcaligens, Arthobacter, Burkholderia, Bacillus and Serratia have reported (Kloepper et al., 1989; Okon and Labandera-Gonzalez, 1994;Glick, 1995).

Rice (Oryza sativum) is one of the important source of calorie, it is a staple cereal to be consumed in large quantity throughout the world thus it is been called as queen among the cereals (Aadil Abbas *et al.,* 2011). Rice has been considered as one of the major crops after wheat and cotton throughout the world and it has been cultivated on area of 2590 thousand hectares with a production of 5720 thousand ton (Muhammad asim *et al.,* 2008). It provides half of total dietary carbohydrate, especially in Asian countries and it is suitable diet for more than three billion people, supplying 50-80% of their daily calorie intake (Khush, 2005). The genus Oryza is distributed in tropical and sub tropical regions of the world.

The bacteria isolated from rhizosphere soils, have proved to be beneficial to the plants by directly having an effect on nitrogen fixation (Han *et al*., 2005), solubilization of nutrients (Rodriguez and Fraga 1999), production of growth hormones, 1-amino-cyclopropane-1-carboxylate (ACC) deaminase (Correa *et al.,* 2004) and indirectly by antagonizing pathogenic fungi by the production of siderophores, β-1,3-glucanase, antibiotics, fluorescent pigments and cyanide (Pal *et al.,* 2001).

IAA producing microorganisms are known to promote root elongation and plant growth (Patten and Glick 2002). IAA-production by root associated bacteria is a major mechanism of plant growth promotion this has important implication for the development of the root system under the influence of rhizosphere microbes (Anton Hartmann *et al.,* 2009). IAA-biosynthesis of the plant growth promoting Rhizobacterium, *Azospirillum brasilense* was shown to be induced in rhizoplane colonizing bacteria (Rothballer *et al.,* 2005).

The objective of this present study was to isolate and identify the diversity of bacteria from the rhizosphere of nine different rhizosphere soil from Oryza sativum plants grown in south Karnataka and to check its ability to produce IAA.

Materials and Methods

Sample collection: Rhizosphere soil of Oryza sativum were collected from different locations in south Karnataka by uprooting the plant and collecting the root along with the soil in clean sterile ziplock cover bags and transported to laboratory for further analysis.

Isolation of Bacteria from the rhizosphere soil: The bacteria were isolated by serially diluting the rhizosphere soil and inoculating 100µl of the diluted sample onto the nutrient agar medium. The plates were then incubated at 30ºC for 24-48 hours for the growth the bacteria. All the procedures were carried out in sterile conditions.

Culture Maintenance: The bacterial colonies were isolated individually on nutrient agar plates and slants for further studies.

Indole Acetic Acid (IAA) production: Bacterial strains isolated were inoculated into nutrient broth and incubated at 37ºC for 24 hours. 100µl of the bacterial inoculum was then inoculated in the nutrient broth supplemented with 5mg/ml tryptophan and incubated at 37ºC for 72 hours. After 72 hours the amount of IAA production was determined by taking 1.5ml bacterial broth culture which was centrifuged at 12,000 rpm for 5 minutes. 1ml of the supernatant was added to 2ml Solawaski's reagent (50 ml, 35% perchloric acid; 1 ml 0.5M FeCl. And incubated for 25 minutes till the color density reaches its maximum. The optical

density of the color was read in UV-spectrophotometer at 530 nm. The amount of IAA produced per milliliter culture was estimated using a standard curve.

Results and Discussion

Isolation of Micro-organisms from soil sample

Isolation was carried out according to the standard isolation procedure from all the nine soil samples. All the isolates were characterized morphologically on the basis of its form, elevation, margin, pigment, biochemical tests and Gram staining characteristics. The Gram's staining data are given in the Table 1. A total of 57 Gram positive bacilli, 6 Gram negative bacilli, 47 Gram positive cocci and six actinomycetes were isolated of the total samples used in the current study. Similarly Subramaniam Gopalakrishnan *et al.,* 2010 isolated a total of 360 bacteria from the rhizospheres of a system of rice intensification (SRI) fields.

Screening for IAA: The screening of 53 bacterial isolates for the production of IAA was carried out where in 7 isolates proved to have the ability to produce IAA. IAA production was maximally produced from the sample R-3, R-5, R-13, R-14, R-20, R-47 & R-53 with the concentration of 11.83,5.98,14.05,12.17,12.66,0.535 & 5.83 µg/ml respectively (Figure 2).

Similarly Farah Ahmad et al., 2008 isolated a total of 72 bacteria belonging to *Azotobacter, fluorescent Pseudomonas* , *Mesorhizobium* and *Bacillus* were from different rhizospheric soil and plant root nodules in the vicinity of Aligarh and determined the production of IAA and observed that more than 80% of the isolates of *Azotobacter , fluorescent Pseudomonas* and *Mesorhizobium cicero* produced IAA, whereas only 20% of *Bacillus* isolates was IAA producer.

These findings of IAA production in isolates are in agreement with other workers (Gonzalez-Lopez et al., 1986; Jagnow, 1987; Nieto and Frankenberger, 1989).

Table.1 Isolation Data of microorganisms from different rhizosphere soil

Location /Area	Gram positive Bacilli	Gram positive cocci	Gram negative bacilli	Actinomycetes
Mulbagal	6	8	1	2
Kolar	7	8	0	2
Hoskote	6	4	3	0
Ramanagara	4	3	2	0
channapattna	3	7	0	0
Maddur	7	6	0	0
Mandya	9	4	0	2
Srirangapatna	11	2	0	0
Mysore	4	5	0	0
Total	**57**	**47**	**6**	**6**

Table.2 IAA production of the isolates

Sl.No	Sample	IAA production (µg/ml)
1.	R-3	11.83
2.	R-5	5.98
3.	R-13	14.05
4.	R-14	12.17
5.	R-20	12.66
6.	R-47	0.535
7.	R-53	5.83

Table.3 Standard value of IAA

Concentration of IAA (µg/ml)	OD at 530 nm
10	**0.362**
20	**0.609**
30	**0.851**
40	**1.101**
50	**1.374**
60	**1.494**
70	**1.699**
80	**1.887**
90	**2.071**
100	**2.133**

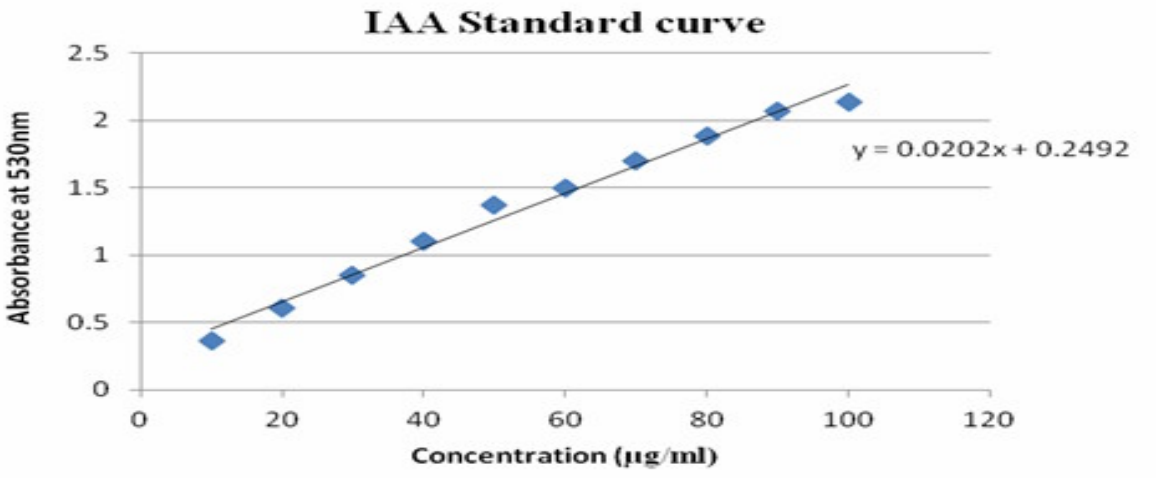

Figure.1 Standard Graph of IAA

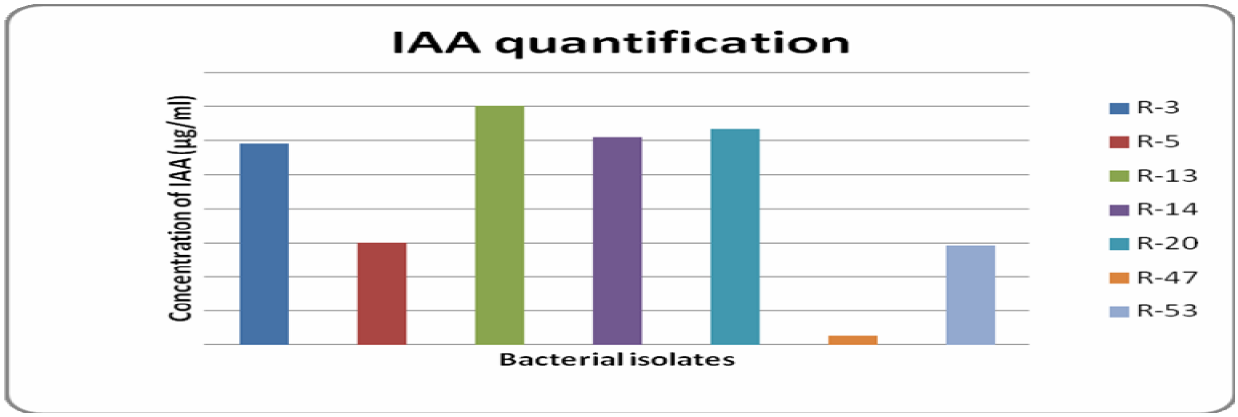

Figure.2 IAA production by the bacterial isolates

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