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RESEARCH ARTICLE

ISOLATION AND PRODUCTION OF PLANT GROWTH PROMOTING SUBSTANCES BY *PSEUDOMONAS FLUORESCENS* FROM CHILLI RHIZOSPHERE SOIL

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ABSTRACT

Plants growth promoting rhizobacteria (PGPR) is one of the important group of microorganism involved in the production of growth promoting substances and enhance the growth and yield of crop either directly or indirectly. In the present study fifteen rhizosphere soil samples of chilli were collected in cuddalore district of Tamilnadu. The population of microbial groups such as bacteria, fungi and actinomycetes were estimated by serial dilution and pour plating method.The total bacterial population ranged from 1.33 to16.33x10^6 cfu g^{-1} of soil ,fungal population ranged from 7.33 to 13.00x10^4 cfu g^{-1} of soil and the antinomycetes population were ranged from 2.00 to 9.6633x10^5 cfu g^{-1} of soil. The occurence of *pseudomonas fluorescens* were also analysed and the population was ranged from 2.33 to 4.66x10^5 cfu g^{-1} of soil. The pseudomonas isolates were further studied for their efficiency of IAA and siderophore production. The maximum IAA was produced by the isolate DSP6 and siderophore was produced by the isolate DSP9.

Key words: Chilli, IAA, PGPR, *Pseudomonas,* siderophore..

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INTRODUCTION

Plant growth promoting rhizobacteria (PGPR) are free - living, soil - borne bacteria, which enhance the growth of the plant either directly or indirectly (Kloepper, J.W., F.M. Scher, 1985). The direct mechanisms involve nitrogen fixation, phosphorus solubilization, HCN production, production of phytohormones such as auxins, cytokinins and gibberellins and lowering of ethylene concentration (Glick, B and R. Ibid, 1995). It is also suggested that PGPR can also prevent the deleterious effects of stresses from the environment (Paul, D. and S. Nair, 2008 Globally), India contributes one fourth to world production of chilli with an area of 8.53 lakh ha and production of 8.74 lakh tonnes with a productivity of 1016 kg ha 1 Anonymous, 2007. In India, chilli is extensively grown in the states of Andhra Pradesh, Orissa, Maharashtra, West Bengal, Karnataka, Rajasthan and Tamil Nadu. Chilli, the fruit of *Capsicum annuum* L, is one of the most important commercial crops in India. With an annual production of 1.1 million tones, India is the largest producer of chilli in the world (Khan and Raj, 2006). Owing to its high cash value and consumption rate the annual trade of chilli is approximately 17% of total spice trade in the world (Ahmed *et al.,* 2000) and is about 33% in India. However, the yield of chilli in India is substantially low when the large area (930,000 hectares) of cultivation is considered (Bharathi *et al.,* 2004). A large amount of herbicides, pesticides and fertilizer were applied every year to achieve maximum productivity of chilli and to meet the growing demand, the use of chemical fertilizers in India has increased 170 times in last 50 years (FAO, 2010). This is a major environmental and health concern considering the deleterious impact of these chemical compounds on terrestrial and aquatic ecosystems. Plant growth promoting rhizobacteria (PGPR) constitute approximately 2-5% of the total rhizomicrobial population (Antoun and Kleopper, 2001). PGPR have been demonstrated to increase growth and productivity of many commercial crops including rice (Ashrafuzzaman *et al.,* 2009), wheat (Khalid *et al.,* 2004), cucumber (Maleki *et al.,* 2010), maize (Sandhya *et al.,* 2010), cotton (Anjum *et al.,* 2007), black pepper (Dastager *et al.,* 2010), and banana (Mia *et al.,* 2010). However, a few studies have isolated and characterized the PGPR and phosphate solubilizing bacteria from chilli rhizosphere (Ponmurugan and Gopi, 2006).This present study investigate the isolation and production of IAA and siderophore by *pseudomonas fluorescens* from rhizosphere soil.

MATERIALS AND METHODS

Survey for the collection of rhizosphere soilsamples of chilli

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Survey was conducted at different locations of cuddalore districts in Tamilnadu. Nearly fifteen rhizosphere soil samples were carefully collected, transported to the laboratory and soil samples were stored in refrigerator at 4°C for further studies.

Enumeration of microorganisms from the rhizosphere soil samples of Chilli

The rhizosphere soil samples collected were serially diluted up to 10^{-} 6 dilution to determine the rhizosphere bacterial, fungal and actinomycete populations. The 10^{-} 6 dilutions were plated on sterile petri plates containing Soil Extract Agar (SEA) medium for the growth of bacterial colonies at 37°C for two days, 10^{-} 4 dilutions were plated on sterile petri plates containing Martin's rose bengal agar medium (RBA) for the growth of fungal colonies at 28 ± 1°C for 3 days and 10^{-} 5 dilutions were plated on sterile petri plates containing Ken knight's agar medium (KKA) for the growth of actinomycete colonies at 30±2°C for 5 to 7 days. The number of bacterial, fungal and actinomycete colonies in the respective plates were counted.

Enumeration of *Pseudomonas* fluroscens

Pseudomonas population was enumerated from the rhizosphere soils of chilli plant by serial dilution and plating technique). The soil samples were serially diluted up to 10^{-4}dilution. One ml of aliquots of last dilution were plated in using Sperber' shydroxy apatite medium. The plates were incubated up to two weeks at 28±2°C . The Bacterial colonies showing clear zone were enumerated and expressed as cfu g^{-1} of oven dry soil.

Estimation of indole acetic acid (IAA)

A quantity of 100 ml of nitrogen free malate broth (without bromothymol blue indicator) for Azospirillum and Nutrient broth for Pseudomonas isolates were prepared and sterilized. Freshly prepared, filter sterilized solution of L-tryptophan was added to each flask to a final concentration of 100 mg l^{-} 1. One ml of culture broth containing 108 cells ml-1 of plant growth promoting rhizobacterial isolates were inoculated to each flask and incubated at 37°C in dark for seven days at stationary condition. After incubation, the cultures were centrifuged at 6,000 rpm for 5 min to remove the bacteria cells. The supernatant was brought to pH 2.8 with 1 N HC1.

Fifteen ml of the acidified supernatant was taken in 100 ml conical flask and to it equal volume of diethylether was added and incubated in dark for 4 hrs. IAA extraction was done at 4°C in a separating funnel using diethylether. The organic phase was discarded and the solvent phase was pooled and evaporated to dryness. To the dried material, 2 ml of methanol was added, pooled and the IAA present in the methanol extract was determined using the method of Gorden and Paleg (1957). To 0.5 ml of the methanol extract, 1.5 ml of distilled water and four ml of Salper's reagent (1.0 ml of 0.5 M FeCl3 in 50 ml of 35 per cent perchloric acid) were added and incubated in dark for one hour. The intensity of pink colour developed was read at 535 nm in a spectrophotometer. From a standard curve prepared with known concentrations of IAA, the quantity in the culture filtrate was determined and expressed as µg 25 ml^{-} ¹ of culture medium.

Estimation of siderophore production

Siderophore production by the plant growth promoting bacteria was estimated by standard method.Nitrogen free malate broth and Nutrient broth were prepared for Azospirillum and Pseudomonas isolates respectively and dispensed in 100 ml quantities in 250 ml Erlenmeyer flasks and sterilized. One ml of standard inoculum of bacterial isolates was added into each flask and incubated at 37°C for 7 days. After 7 days of incubation, the culture broth was centrifuged at 10,000 rpm for 20 min. The supernatant was used for estimation of siderophores. Twenty ml of culture supernatant was taken and the pH was adjusted to 2.0 with dilute HC1. To 20 ml of supernatant, 20 ml of ethyl acetate was added and extraction was done twice. Hathway reagent (1ml of 0.1 M ferric chloride and 1 ml of 0.1 N HC1 was added to 100 ml of distilled water and to this 1 ml of 0.1 M potassium ferricyanide was added) was prepared. Five ml of the assay solution was added with 5 ml of Hathway reagent and absorbance was determined at 560 nm with sodium salicylate as standard for the estimation of salicylate type of siderophore. To measure the catechol type siderophore, 5ml of the assay solution was added with 5 ml Hathway reagent and absorbance was determined at 700 nm with 2, 3 dihydroxy benzoic acid (DHBA) as a standard. One µ mole of 2, 3-DHBA gave an absorbance of 0.75. From the absorbance value of the sample, the quantum of siderophore produced was calculated and expressed as µg ml^{-} 1 of culture filtrate.

Table 1. Microbial status in the rhizosphere soil samples from commercially grown area of chilli

S.No	Name of the locations	Bacteria 1 X 10□ cfu g -1	Fungi 1 X 10□ cfu g -1	Actinomycetes 1 X 10□ cfu g -1
1.	Annamalai nagar	13.66	8.33	7.66
2.	Mutlur	12.33	7.66	5.33
3.	Puthuchatiram	10.33	6.33	6.33
4.	Setthiyathopu	11.66	5.00	5.66
5.	Nanjalour	10.66	7.33	6.66
6.	Sivayam	14.33	5.66	5.00
7.	Kumaratchi	5.66	4.33	9.33
8.	Neivasal	16.33	11.00	8.66
9.	Yellaeri	15.66	13.00	7.66
10.	Lalpettai	1.33	6.66	2.00
11.	Kattumanarkoil	9.00	8.33	8.33
12.	Anaikarai	7.33	10.66	7.33
13.	Neduncheri	13.66	12.33	6.00
14.	Kurinchipadi	6.00	11.33	9.66
15.	Panruti	12.66	8.00	3.00

Table 2. Enumeration of pseudomonas fluroscens from the rhizosphere soils of chilli

S.No	Locations	Population (1 X 10□ cfu g^{-1} on oven Dry Weight)
		Pseudomonas fluroscens
1.	Annamalai nagar	6.00
2.	Mutlur	3.00
3.	Puthuchatiram	5.33
4.	Setthiyathopu	5.66
5.	Nanjalour	7.33
6.	Sivayam	6.00
7.	Kumaratchi	8.66
8.	Neivasal	4.33
9.	Yellaeri	3.66
10.	Lalpettai	3.33
11.	Kattumanarkoil	7.00
12.	Anaikarai	6.33
13.	Neduncheri	2.66
14.	Kurinchipadi	8.33
15.	Panruti	5.33

Table 3. Indole acetic acid (IAA) and Gibberellic acid (GA_3) producing potential of *Pseudomonas* isolated obtained from the rhizosphere soils of chilli

S.NO	Name of the Isolates	IAA (25 ml^{-1})
1.	DSP-1	64.87
2.	DSP-2	39.19
3.	DSP-3	35.09
4.	DSP-4	74.87
5.	DSP-5	71.99
6.	DSP-6	78.05
7.	DSP-7	23.04
8.	DSP-8	71.49
9.	DSP-9	75.33
10.	DSP-10	76.11
11.	DSP-11	76.28
12.	DSP-12	76.73
13.	DSP-13	57.90
14.	DSP-14	59.07
15.	DSP-15	71.45
	SEd	2.1226
	CD(p=0,05)	4.3122

Table 4. Siderophore production by *Pseudomonas* isolates obtained from the rhizosphere soils of chilli

S.NO	Name of the isolate	Siderophore content ($\mu g\ ml^{-1}$)	
		Catechol Type	Salicylate Type
1.	DSP-1	6.54	7.43
2.	DSP-2	7.79	7.15
3.	DSP-3	6.84	8.17
4.	DSP-4	5.11	8.20
5.	DSP-5	7.30	7.55
6.	DSP-6	6.73	8.14
7.	DSP-7	5.15	7.57
8.	DSP-8	8.00	8.23
9.	DSP-9	8.89	8.33
10.	DSP-10	7.31	7.01
11.	DSP-11	5.36	7.12
12.	DSP-12	4.88	5.13
13.	DSP-13	7.96	6.77
14.	DSP-14	4.11	5.02
15.	DSP-15	5.98	6.53
	SEd	0.3747	0.3024
	CD(p=0.05)	0.8650	0.6989

RESULTS AND DISCUSSION

Totally fifteen rhizosphere soil samples were collected from Cuddalore district in Tamil Nadu (Table 1) and the results reveled that bacteria population ranged from 1.33 to 16.33 x 10 ^{6} cfu g^{-1} of soil and antinomycetes from 2.00 to 9.66 x 10 ^{5} cfu g^{-1} of soil Table 2 reveled the population of pseudomonas fluorescence and the population raised from 2.33. to 5.66 x 10 ^{4} cfu g^{-1} of soil. Then totally fifteen isolates were taken for further studies and designated to DSP15. Then thee isolates were analyzed for their efficiency to produce Indale acetic acid (IAA) and sidewphore production. Among the isolates, the isolate DSP 6 produced maximum IAA of 78.05 µg of 25 ml^{-1} of broth.

All the isolates showed IAA production and it ranged from 23.04 to 78.05 µg of 25 ml^{-1} (Table 3).The similar results was observed by Berg G(2009) and reported that pseudomonas sp produce 68.73 of 25 ml^{-1} of broth. Likewise, siderophore production of catechol type and salicylate type was analysed for the isolates and it was observed that the isolateDSP9 produced maximum of 8.89 µg ml^{-1} of broth for catechol type and 8.33 µg ml^{-1} of broth for salicylate type From the study,it was concluded that the PGPR pseudomonas fluroscens isolates DSP6 and DSP9 can be potentially used for chilli crop to increase yield and growth.

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