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INVITRO SCREENING OF PLANT GROWTH PROMOTING SUBSTANCES FROM THE RHIZOSPHERIC BACTERIA OF PADDY FIELDS

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Abstract

Plant growth promoting bacteria are found on the surfaces of roots, and associated with the roots that can promote the growth of the plant both qualitatively and quantitatively. Many bacteria such as *Pseudomonas* and *bacillus* were found in the different rhizosphere soil of paddy fields. *Psedudomas fluoresecence* were isolated in the paddy fields of rhizosphere soil. This isolate was biochemically tested and screened for the growth promoting substances like such as indole acetic acid, hydrogen cyanide and siderophores. This result showed that the organism can produce both the growth promoting and inhibiting substances.

Keywords; Indole 3 acetic acid, siderophores, Hydrogen cyanide and plant growth promoting substances.

1. Introduction

Soil is the abode for different micro-organisms, which live in complex network. The rhizosphere is the thin layer of soil surrounding plant roots and the soil occupied by the roots supports large active groups of bacteria known as Plant growth promoting rhizobacteria (PGPR) (Glick 1995). Plant growth promoting rhizobacteria includes the sps of *Pseudomomas, Bacillus, Klebsills, Enterobacter Azospirillum,* Azotobacter that are free-living, soil- borne bacteria and can also be beneficial to the plant by stimulating growth. Among these organisms, *Pseudomonas species* especially fluorescent *Pseudomonads* are suitable to be used as agricultural bio control agents because

they can produce large amounts of secondary metabolites to protect plants from phytopathogens by production of the siderophores, cyanide, antibiotics (Arshad *et al.,* 1992). Siderophores are low molecular weight (<10 KD) iron binding compounds synthesized by many prokaryotic cells, certain fungi and plants in large quantity under iron limited conditions. These siderophores scavenge the iron from the enviornmnet and makes available to the cells. siderophore which plays a vital role in stimulating plant growth and in controlling several plant disease, they function as a bio control agent by depriving the pathogen from iron nutrition, thus resulting in increased yield of crop (magadalena et.al., 2019). The plant growth promoting substances by producing the indole 3- acetic acid, gibberlins, cytokinins and ethylene.

Our present work is focused on the isolation of Pseudomonas florescence from the rhizosphere of paddy fields and to investigate the invitro detection of plant growth promoting and inhibiting substances from the bacteria.

2. Materials and methods

2.1 Collection of soil sample

The soil sample collected from the rhizosphere of paddy fields, at Chemudugunta village besides Nellore Town, Andhra Pradesh, India. The soil was air dried under shade for isolation of *Pseudomonas* bacteria.

2.2 Isolation of bacteria from the rhizosphere soil of paddy fields

1gm of dried soil is diluted with 10ml of sterile double distilled water and performed serial dilution. Using King's B Medium (selective medium), *Pseudomonas fluorescens* can be isolated by pouring 100µl of soil sample onto sterile King's B Medium Agar plates and Incubated for 72 hours at 28ºC.

2.3 Morphology and biochemical characterization of isolate

The isolate based on micromorphological observation and biochemical characterization were identified. The morphological tests involved, were simple staining and Gram staining. Isolate was biochemically characterized by Indole Test, Methyl Red Test, Voges-Proskauer Test, Gelatin test, Catalase Test, Citrate test as per standard procedures (Aneja, 2001).

2.4 Invitro screening for the qualitative analysis of plant growth promoting substances

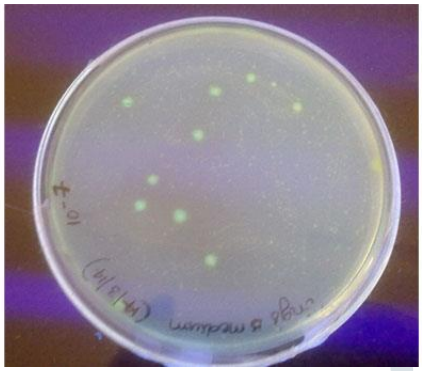
2.4.1 Indole Acetic Acid (IAA)

Qualitative analysis for IAA was performed by using the Loper and Scroth (1986) method. Test bacteria was inoculated in the King's B medium with tryptophan (0.1 mg/ml) and incubated at 28ºC for 48 h.

Fully grown Culture was centrifuged at 3000 rpm for 30 min. the supernatant (2 ml) was mixed with 2 drops of Orthophosphoric acid and 4 ml of Solawaski's reagent (50 ml of 35% Perchloric acid; 1 ml of 0.5M $FeCl_3$). Development of pink color indicates the production of IAA (Ehmann, 1977).

2.4.2 Hydrogen cyanide (HCN)

Pseudomonas fluorescens was screened for HCN production by using the method Lorck (1983). The King's B Medium with (glycine) 4.4 g/l and the bacteria were streaked on the modified agar medium. Place the Whatman filter paper soaked in Alkaline picrate solution (2% Sodium carbonate and 0.5% Picric acid) for 10 min was placed on the top of the plates. Plates were sealed with paraffin and then Incubate for 48-72 hours at 28ºC. Development of orange to red colour indicates the production of HCN (Lorck 1948).



2.4.3 siderophores

Bacteria were assayed for the detection of siderophores by using chrome azuorol sulphonate (CAS) assay. *Pseudomonas fluorescens* was grown in Standard Succinate Medium [(SM) (Meyer and Abdullah, 1978) consisting of K_2HPO_4-6 gm/lit, KH_2PO_4-3 g/L, $(NH_4)_2SO_4$-1 g/L, $MgSO_4.7H_2O$-0.2 g/L, Succinic acid-4 g/L]. The pH was adjusted to 7 at 28ºC with constant shaking at 120 rpm for 24-48 h. culture was centrifuged at 6000 rpm for 15 min. To the supernatant add 0.5 ml of CAS solution and mixed gently. Then add 10µl of 0.2 M 5-Sulphosalicylic acid and mixed, stand for few minutes (Guan et al, 2011).

IV Results and Discussion

4.1 Isolation of Pseudomonas florescent from Kings medium

Bacteria was isolated and Kings media a selective media was used for the isolation of pseudomonas fluorescent. Fig 4.1 shows that Round and yellowish green colour colonies were identified.

Fig 4.1 Yellowish green colour colonies on Kings media indicates the presence of *Pseudomonas fluorescens*

4.2 Biochemical tests for the identification of pseudomonas

The organism showed positive test for the biochemical tests like gelatin test, citrate, catalase and negative for the tests like indole, methyl red and voges -proskauer (Table 1).

S.NO.	Biochemical Tests	*Pseudomonas fluorescens*
1	Fluorescent Test	+
2	Indole's Test	_
3	Methyl Red Test	_
4	Voges-Proskauer Test	_
5	Gelatin Test	+
6	Citrate Test	+
7	Catalase Test	+
8	Gram's Staining	Gram negative

4.3 Invitro screening for the qualitative analysis of plant growth promoting substances.

4.3.1 Indole 3-acetic acid: The isolate was able to produce the IAA and was checked with Salkowski reagent, and the development of pink colour was observed with 0.1 mg/ml of tryptophan in the presence of kings B medium. Most of the IAA producing microbes belongs to the gram negative bacteria (Datta and Basu, 2000). Few certain gram positive bacteria such as bacillus was known to produce the IAA 9Wahyudi *et al.,* 2011)

4.3.2 Detection of Hydrogen cyanide: The colour change from orange to red colour due to release of hydrocyanic acid. HCN is the secondary metabolite. playa a very important role in the suppression of disease in the various crops. It was commonly produced by the pseudomonas in the rhizosphere soil of tobacco fields to suppress the agent that causes the black root rot of tobacco. ad playa a very important role in the suppression of disease in the various crops (Ramette et al., 2006).

4.3.3 Qualitative analysis of siderophore by CAS assay: The isolate showed the colour change from blue to orange colour in few minutes. Siderphores acts as antibacterial agent for the treatment of iron deficiency diseases. In recent years many siderophores producing microbes have been discovered like protochelin by

Azotobactervinelandii; Rhizobactin by *Rhizobium meliloti,* and pyoverdine by *Pseudomonas fluorescens* and so on (Yaxun sun et al 2021)

Conclusion:

In this study concludes the bacteria Pseudomonas flourescenc was isolated from the rhizosphere soil of paddy fields by using selective media called Kings B media. Then the bacteria was identified by using both morphological and biochemical tests and concluded that the bacteria has the ability to produce the IAA, siderophores and Hydrogen cyanide that has beneficial effects on the crop growth and yield.

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References

1. Arshad, M., Frankenberger, W.T. Jr. 1992. Microbial production of plant growth regulators. In: Metting FB Jr(eds). Soil Microbial Ecol, Marcel Dekker Inc., New York. pp: 307-347.
2. Aneja, K.R. 2001. Experiments in microbiology plant pathology and biotechnology 4th Edition.102, 106, 112, 245-275, 278.
3. Datta, C., Basu, P. 2000. lndole acetic acid production by a Rhizobium species from root nodules of a leguminous shrub *Cajanus cojan.* Microbiol. Res. 155, 123 - 127.
4. De Serrano LO, Camper AK, Richards AM (2016) An overview of siderophores for iron acquisition in microorganisms living in the extreme. Biometals 29:551–571.
5. Ehmann, A. 1977. The Van Urk-Salkowski reagent-a sensitive and specific chromogenic reagent for silica gel thin-layer chromatographic detection and identification of indole derivatives. Journal of Chromatography. 132, 267-276.
6. Guan, L.L., Kanoh, K., & Kamino, K., (2011). Effect of exogenous siderophores on iron uptake activity of marine bacteria under iron-limited conditions, App. & Env. Microbio., 67(4), 1710-1717.
7. Glick, B.R., 1995. The enhancement of plant growth by free living bacteria. Can. J. Microbiol. 41, 109–114.

8. Magdalena AB, Mathilde M, Edith J (2019) Chapter five-siderophores: from natural roles to potential applications. J Adv Appl Microbiol 106:193–225.
9. Lorck, H., 1948. Production of hydrocyanic acid by bacteria. Physiol. Plant. 1, 142–146.
10. Ramette, A., Loy, M. and Defago, G. 2006. Genetic diversity and biocontrol protection of fluorescens pseudomonas producing phloroglucinols and hydrogen cyanide from swiss soils naturally suppressive or conducive to Thieviopsis basicola mediated black rot of tobacco. FEMS Microbial Ecol., 55(3): 369-381.
11. Sun, Y., Wu, J., Shang, X. *et al.* Screening of Siderophore-Producing Bacteria and Their Effects on Promoting the Growth of Plants. *Curr Microbiol* **79**, 150 (2022).
12. Wahyudi, A.T., Astuti, R.P., Widyawati, A., Meryandini, A., Nawangsih, A.A. 2011. Characterization of *Bacillus* sp. strains isolated from rhizosphere of soybean plants for their use as potential plant growth for promoting Rhizobacteria. Journal of Microbiology and Antimicrobials. 3, 34-40.