International Journal of Life Sciences
International Peer Reviewed Open Access Journal

Special Issue A 13, December 2019
ISSN:2320-7817(p) 2320-964X(0)

Original Article Open Access

Biochemical & molecular characterization of *Pseudomonas fluorescence* for divulging its plant growth promoting & biocontrol traits

Paliwal Kiran and Prakash Anil

Department of Microbiology, Barkatullah University, Bhopal – 462026
Email: kiranpaliwal881@gmail.com

Manuscript details:

Available online on
http://www.ijlsci.in

ISSN: 2320-964X (Online)
ISSN: 2320-7817 (Print)

Editor: Dr. Arvind Chavhan

Cite this article as:
Paliwal Kiran and Prakash Anil (2019) Biochemical & molecular characterization of *Pseudomonas fluorescence* for divulging its plant growth promoting & biocontrol traits, *Int. J. of. Life Sciences*, Special Issue, A13: 196-200.

ABSTRACT

Use of plant growth promoting bacteria for increasing the crop productivity could be an effective viable alternative for organic bio fertilizer. Plant growth promoting rhizobacteria influence the growth of plant by various mechanism. In the present investigation, rhizospheric isolate *Pseudomonas* was investigated for plant growth promoting & biocontrol traits. The isolated *Pseudomonas* was found to produce various biologically active compounds. Some of which are previously reported as effective plant growth promoters such as indole acetic acid (48 ug/ml), gibberlic acid (67ug/ml), extracellular enzymes such as amylase (20mm), lipase (10mm), phosphatase (5.7mm). Some secondary metabolites were also produced by this bacterium. Such as HCN, siderophore(87um/ml), Diacetyl Phloro Glucinol (119mg/lit.) which are recorded as potentially competent to the pathogenic fungi *Fusarium oxysporum* causing wilt of soybean. This dual efficiency of the isolated *Pseudomonas* could be a better alternative for chemical fungicides & fertilizer. This multifactorial potential could give a better result, if the large biomass of bacteria could be produced by modifying the basic media component. in this study it was found that the large amount of biomass could be produced by modifying the media component such as peptone2.5g/100ml, glucose (120Mm), proline (10Mm) along with basic King's Bmedia.

Key words: Secondary metabolites, bacterial antagonists, IAA, HCN PGPR

INTRODUCTION

Madhya Pradesh has a unique identity as the soya producing state of India. It produces 54% of the total production of soya in the country. The western and north-western parts of Madhya Pradesh are major soya producing areas. Comparatively, eastern and southern parts of Madhya Pradesh produce very little of it the rainfed potential of soybean in India is about 2.1 t/ha against the national average productivity of just 1.2 t/ha. Hence, large yield gaps exist between the potential and the actual yields harvested by the farmers. Narrowing of this yield gap may lead to doubling of soybean production.

National Agricultural Research System has so far been successful in meeting the research demands of agrarian and industrial community.

Use of chemical fertilizer is playing a significant role in increasing the crop productivity which fulfills the increasing global food demand. But the imbalanced use of chemical fertilizer & biocidal agents is having negative impact on the human health & environment. The application of chemical fertilizer is reaching to theoretical maximum use, beyond which there will be no further increase in the yield even after increasing the chemical fertilizer (berdiva 2015). Looking to the deleterious effect of agrochemical input, the plant growth promoting bacteria could be a better ecofriendly approach in the present scenario of intensified cropping system. Close association between soil & rhizobacteria is mandatory for proper plant growth & grain yield. Plant diseases account for ~13% of the world's crop production lost, nearly equivalent to $220 billion lost every year (Kandel et al. 2017).Among the crop pests, phytopathogenic fungi are the most common and cause a wide range of diseases to economically important plants (Mehnaz et al. 2013). Fusarium oxysporum, for example, is an important fungal pathogen known to cause vascular wilt diseases in more than 100 different species (Lopez-Berges et al. 2012).Secondary metabolites are low molecular weight compounds, less than 2.5 KDa produced during the idiophase of bacterial growth. Bacteria belonging to Pseudomonas, Bacillus and Streptomyces are prolific producers of secondary metabolites that include a wide array of naturally produced compounds viz., peptides, polypeptides, cyclic lipopeptides, polyketides, pyrroles, phenazines, phloroglucinols, lantibiotics, bacteriocins, lactones, macrolactone, anthracyclines, alkaloids, quinones, polyenes, pyrone, quinolones, isoquinoline, aminoglycosides, macrolides, bithiazoles, isocoumarins, aminosugars, phospholipids, siderophores and volatiles. These metabolites exhibit remarkable antimicrobial, plant growth regulatory, plant enzyme inhibitory, herbicidal, insecticidal and anti-parasitic properties. All these biological properties paved way for the use of these secondary metabolites as biocontrol agents in agriculture. so, in the present research diversified plant growth promoting & antagonistic activities are investigated as well as the optimized media component also used to enhance the biomass of the multipotent *Pseudomonas.*

MATERIAL METHODS

Sample collection & isolation of pseudomonas strains

Microbial strains were isolated by the serial dilution method from the soybean rhizospheric soil. One gram of dried soil was weighed and added to 9 ml of double distilled water (dd H_2O) in a sterile test tube and shaken well using vortex mixer; this stock solution was then diluted serially up to the dilution of 10^{-5} and 0.1 mL of diluted sample was inoculated on surface of selective King's B agar and incubated at 30°C for 2 days. The purified colonies were preserved using standard preservation methods.

Collection of the fungal pathogens

Four potent fungal pathogen of the soybean were used for the study. The fungus *Fusarium oxysporum,* from MTCC Chandigarh. Fungal pathogens were stored in PDA agar & slants at 4°C for further use.

Biochemical characterization & molecular identification of the selected strain

selected strains were isolated on selective King B medium the strains were identified and characterized by morphological, cultural, and biochemical tests using further characterized by Gram staining and biochemical tests as per methodology described by (Krieg and Holf). The various tests performed were Oxidase, MR-VP, Indole, Citrate, Urease, Nitrate reduction and fermentation of various sugar. Identification was also confirmed by 16SrRNA genesequencin by using GAGTTTGATCCTGGCTCAG and AGAAAGGAGGTATCC-AGCC forward and reverse primer sequence, respectively. The amplified PCR product was analysed by neighbour joining method &identified culture shown maximum similarity to *Pseudomonas flouroscence*.

PGPR & biocontrol potential of isolated strain

For PGPR activity of pseudomonas were taken into consideration.

To determine the amounts of IAA produced by each isolate, a colorimetric technique was performed with Van Urk Salkowski reagent using the Salkowski's method (Ehmann, 1977). The isolates were grown in king, B broth (Himedia, India) and incubated at 28 °C for 4 days. The broth was centrifuged after incubation. Supernatant was reserved and 1ml was mixed with 2ml of Salkowski's reagent (2% 0.5 $FeCl_3$ in 35% $HCLO_4$ solution) and kept in the dark. The optical density (OD) was recorded at 530 nm after 30 min.

In this study *Pseudomonas* was examined for exploring antifungal activity of strains by dual culture assay using method described by (idris *et.al*) Ability of bacteria to produce siderophore was examined using method described by (Alexander and Zuberer 1991) Using (Bakker and Schippers 1987) method HCN production was determined. Qualitative cyanide determination were carried out by Lorck method modified by Alstrom. Isolates sub cultured on NA medium were supplemented with glycine (4/4 gl-1). The production of cyanide was detected 48h after inoculation, using picrate/Na2Co3 paper fixed to the underside of the Petri-dish lids which were scaled with parafilm before incubation at 28◦c. A change from yellow to orange, red, brown, or reddish brown was recorded as an indication of weak, moderate, or strongly cyanogenic potential.

For enzymatic analysis special media plate were used starch agar (amylase), tributyrin(lipase), pikovaskya agar for phosphatse.

Optimization of media component for maximum biomass production

Basic media king's B was supplemented with various carbon sources, nitrogen sources in different amount and optical density observed at 600nm. Various carbon sources, nitrogen sources & amino acid were taken into consideration .out of which glucose, peptone & proline showed maximum optical density.

RESULTS & DISCUSSION

Characterization of pseudomonas

The identified Pseudomonas were characterized for biocontrol &PGPR attributes. yellow coloured zone on CAS agar plate showed positive siderophore production. Development of pink colour in media, orange coloured Paper shows the positive results for IAA, HCN production. The bacteria effectively inhibited the growth of *Fusarium* on dual plate assay.

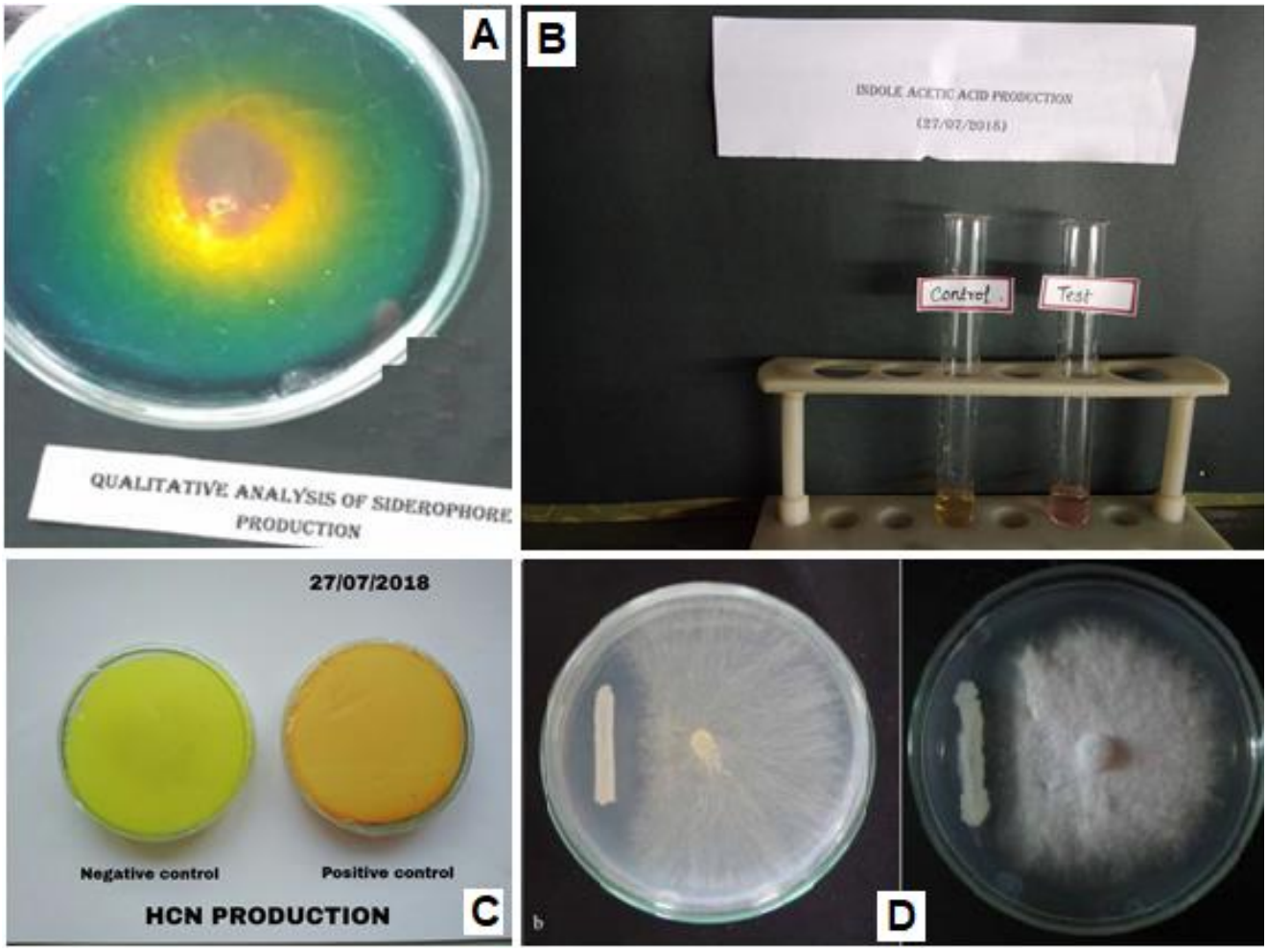
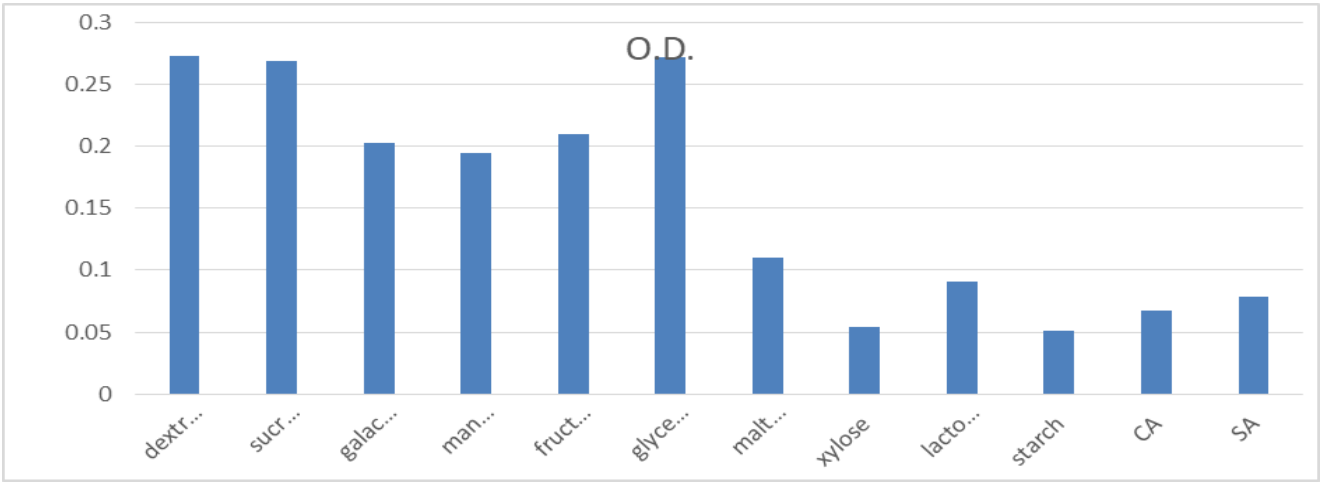
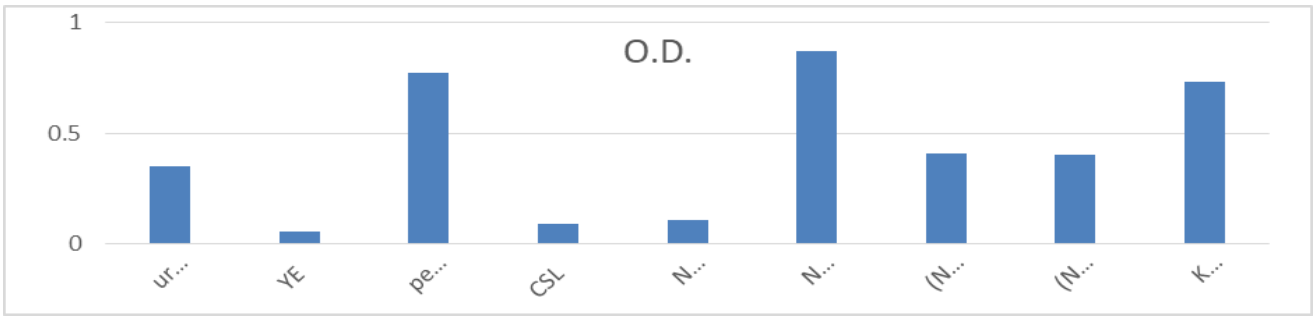


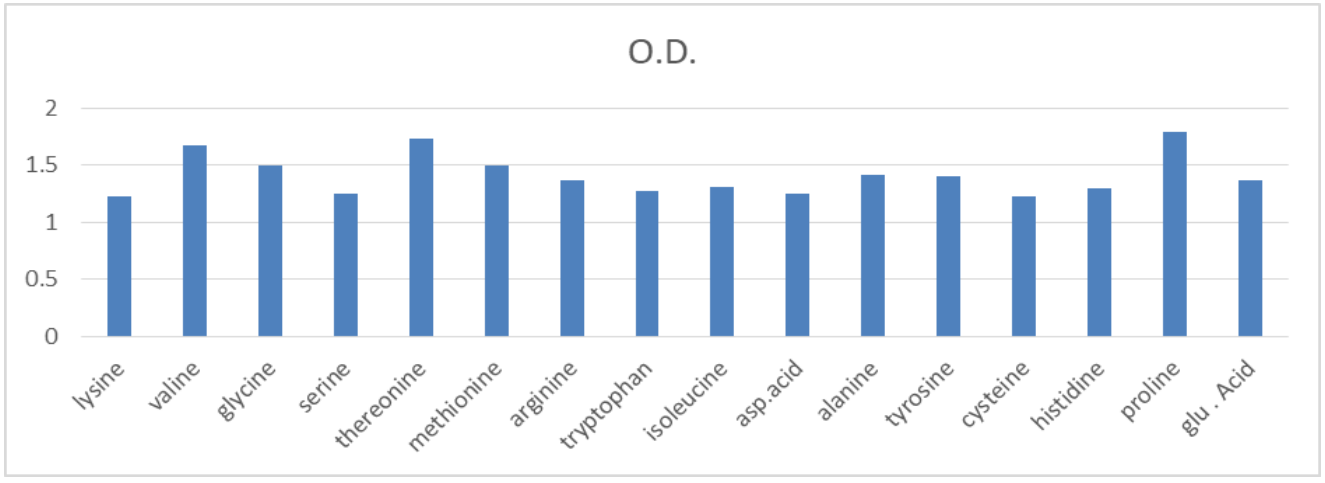
Fig. 1 A: Siderophore production, B. IAA Production, C. Siderophore production, D: dual culture assay

Enzymatic index of *Pseudomonas*: Enzymatic index of *Pseudomonas* shown the maximum enzymatic index for amylase.

Enzymes	Hydrolysis zone diameter (In c.m.)	Growth diameter (In c.m.)	Enzymatic Index
Amylase	3.1	2	**2.5**
Protease	-	0.5	0.5
Cellulase	1	2.2	1.59
Pectinase	-	.8	.8
Chitinase	.8	3.5	1.2
Lipase	-	1.0	1.0







Optimized carbon sources, nitrogen sources & amino acid for growth

Various carbon sources, nitrogen sources & amino acid were taken into consideration .out of which glucose, peptone & proline showed maximum optical density. The results are better depicted in the graphs given below.

CONCLUSION

The multifarious characterstics of *Pseudomonas* could be a better sustainable alternative for the agrochemicals. These characterstics potential of this microbe could give better impact in the field .If the large biomass of these bacteria is increased in the modified media component. The present investigation was based on basic characterization of isolated strain & steps taken for efficient biomass production.

Acknowledgement:

The authors would like to acknowledge NCIM, pune culture identification services. The facilities for experimental analysis by Barktullah university are highly acknowledged

Conflicts of interest: The authors stated that no conflicts of interest.

REFERENCES

Alstrom S and Burns RG (1989) Cyanide production by rhizobacteria as a possible mechanism of plant growth inhibition. *Biol. Fertil. Soils*. 7: 232-238.

Brian C Louden, Daniel Haarmann and Aaron M Lynne (2011) Siderophore use of Blue Agar CAS Assay for Siderophore DetectionJ Microbiol Biol Educ. 2011; 12(1): 51–53.Published online 2011 May 19. doi: 10.1128/jmbe.v12i1.249

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

Kandel SL, Firrincieli A, Joubert PM, Okubara PA, Leston ND, McGeorge KM, Mugnozza GS, Harfouche A, Kim SH, Doty SL (2017) An in vitro study of bio-control and plant growth promotion potential of salicaceae endophytes. Front Microbiol 8:386

Kreig NR and Holf JG (1984) Bergeys Manual of Systematic Bacteriology. William and Wilkins, Baltimore, USA

Lopez-Berges MS, Capilla J, Turra D, Schafferer L, Matthijs S, Jochl C, Cornelis P, Guarro J, Haas H, Di Pietro A (2012) HapX-mediated iron homeostasis is essential for rhizosphere competence and virulence of the soilborne pathogen Fusarium oxysporum. Plant Cell 24:3805–3822.

Lorck H (1948) Production of hydrocyanic acid by bacteria. Physiol. Plant. 1: 142-146.

Mehnaz S, Saleem RSZ, Yameen B, Pianet I, Schnakenburg G, Pietraszkiewicz H, Valeriote F, Josten M, Sahl HG, Franzblau SG, Harald G (2013) Lahorenoic acids A-C, ortho-dialkyl-substituted aromatic acids from the biocontrol strain Pseudomonas aurantiaca PB-St2. J Nat Prod 76:135–141.

Schwyn B, Neilands JB. Universal chemical assay for the detection and determination of siderophores. Analytical Biochem. 1987;160:47–56. doi: 10.1016/0003-2697(87)90612-9.