Available online at www.ijpab.com

DOI: http://dx.doi.org/10.18782/2320-7051.7011

ISSN: 2320 – 7051

Int. J. Pure App. Biosci. **6 (5):** 738-741 (2018)

Research Article

International Journal of Pure & Applied Bioscience

OPEN ACCESS

Characterization of the Supernatants of the Growth of Potent Rhizobacteria

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Received: 14.08.2018 | Revised: 23.09.2018 | Accepted: 30.09.2018

ABSTRACT

A common screen for plant antimicrobial compounds consists of separating plant extracts by paper or thin-layer chromatography (PC or TLC). The effectiveness of this screening method, known as bioautography, depends on both the quality of the chromatographic separation. Screening for their plant growth-promoting properties, and metabolic characterization. In pigeon pea associated rhizobacteria displayed a large metabolic activity. And the antibiotics produced by PGPR include: butyrolactones, zwittermycin A kanosamine, oligomycin A, oomycin A, phenazine-1-carboxylic acid, pyoluteorin, pyrrolnitrin, viscosinamide, xanthobaccin, and 2,4-diacetyl phloroglucinol. 2,4-diacetyl phloroglucinol (2,4-DAPG) is one of the most efficient antibiotics in the control of plant pathogens and can be produced by various strains of Pseudomonas, one of the most common bacterial species of the rhizosphere. It has a wide spectrum of properties in that it is antifungal. The TLC methods apply to many types of plant extracts and other bacterial species (aerobic or anaerobic), as well as fungi, can be used as test organisms if culture conditions are modified to fit the growth requirements of the species.

Key words: *Antimicrobial compounds, Rhizobacteria, Thin layer chromatography, Metabolic characterization, Screening*

INTRODUCTION

Pigeon pea is one of the major pulse crops of India belonging to family, Fabaceae. It is widely used as a pulse, green vegetable, fodder and for a variety of other purposes[4]. The seed protein content of pigeon pea (21 %) compares well with that of other important grain legumes. Pigeon pea wilt is prevalent throughout the world but more important in India[3] and in eastern Africa[5].

Wilt is an important disease where in the plants get infected at early stage but the symptoms appear at different growth stages depending on the severity of infection. The yield loss depends on the stage at which the plants wilt. The disease is both seed and soil borne and reported to cause 30-100 per cent loss in grain yield[6] and may cause 100 per cent yield losses in susceptible genotypes.

Cite this article: Shreeja, A.N. and Chattannavar, S.N., Characterization of the Supernatants of the Growth of Potent Rhizobacteria, *Int. J. Pure App. Biosci.* **6(5):** 738-741 (2018). doi: http://dx.doi.org/10.18782/2320-7051.7011

The annual losses due to wilt have been estimated at $71 million in India and $5 million in eastern Africa. A common screen for plant antimicrobial compounds consists of separating plant extracts by paper or thin-layer chromatography (PC or TLC). The effectiveness of this screening method, known as bioautography, depends on both the quality of the chromatographic separation. Screening for their plant growth-promoting properties, and metabolic characterization. Use of beneficial microorganisms serves as viable alternative measure that do not pose risks to the environment along with better human health[2]. Utilization of plant growth promoting rhizobacteria (PGPR) is an eco-friendly method adopted for crop improvement and disease control in modern agricultural practices[1].

MATERIAL AND METHODS

Characterization of the supernatants of the growth of potent rhizobacteria

The rhizobacterial crude extract of supernatant was taken. The tested rhizobacterial strains were grown on 14 ml SCA / KB media for actinobacteria and fluroscent Psuedomonas in Petri plate respectively and incubated at 25°C for 24 hours. The test fungus (*F. udum*) was cultured on 14 ml PDA media in Petri plate at 22° C for 96 hours. The test is as per Wan *et al.*, using a double dish chamber containing a target fungi inoculated on upward dish of PDA (9 cm diameter) and 100 µL of rhizobacterial suspension on the lower dish of SCA / KB (9 cm diameter) . The chamber was sealed with parafilm and incubated at 22° C in darkness for 4 - 5 days. Fungitoxicity of volatile metabolites was monitored and then further, expressed by measuring the diameter of mycellial growth (mm).

The chromatography analysis was done in the laboratory. The silica gel plate was prepared by mixing Silica gel G in water and the fine slurry was made. Then, it was poured on glass plate uniformly. Later, it was air dried. Then, the plate was kept in hot air oven at 100° C for about one hour. Then, the sample was spotted leaving one cm from both the sides. Then, it was placed in a TLC chamber in which moving solvent ethyl actetate: methanol (4:1) was poured. Then, the movement of spot was noticed and the colour development was observed after ninhydrin spray. Further RF (Retention Factor) in centimeters was calculated.

RESULTS AND DISCUSSION

Metabolites production and colour development can be seen in (Plate 1) Highest sample movement was found in case of Pf218 (0.62 cm) and AUDT626 (0.77 cm) at eight minutes even the case is same with the Retention factor. Least sample movement was observed in control (0.00 cm) and there was no colour development and change in colour in case of control. So, these to found to have higher RF when compared to other samples (Table 1).

Rhizobacterial produce antifungal metabolites phenazine and pyocyanin. Presence of phenazine produce yellow green colour. The extracted antifungal metabolites from different isolates of *Pseudomonas* sp. gave RF value between the range of 0.10– 0.77 and also showed maximum absorption at wavelength i.e. at 254nm (Plate 2, 3 and Fig.1).

Table 1: Movement of bio-active metabolites produced by rhizobacterial supernatants at different time intervals in thin layer chromatography (TLC)

	Two minutes		Four minutes		Eight minutes	
Treatment	**Solute (cm)**	**R.F. (cm)**	**Solute (cm)**	**R.F. (cm)**	**Solute (cm)**	**R.F. (cm)**
AUDT626	1.00	0.50	1.75	0.47	5.00	0.77
AUDT502	0.70	0.35	1.50	0.37	3.75	0.47
Pf218	0.90	0.45	2.00	0.50	6.20	0.62
PCF(SS)1	0.20	0.10	0.90	0.22	2.00	0.25
Control	0.00	0.00	0.20	0.00	0.20	0.00

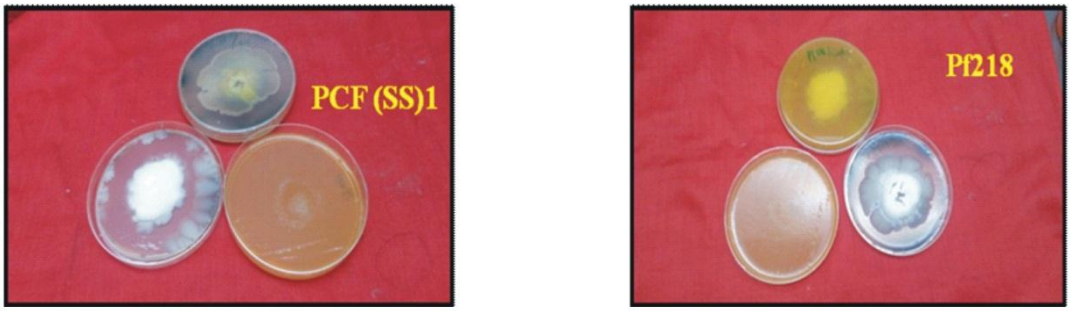

Plate 1. Production of metabolites by rhizobacteria in double-dish chamber method

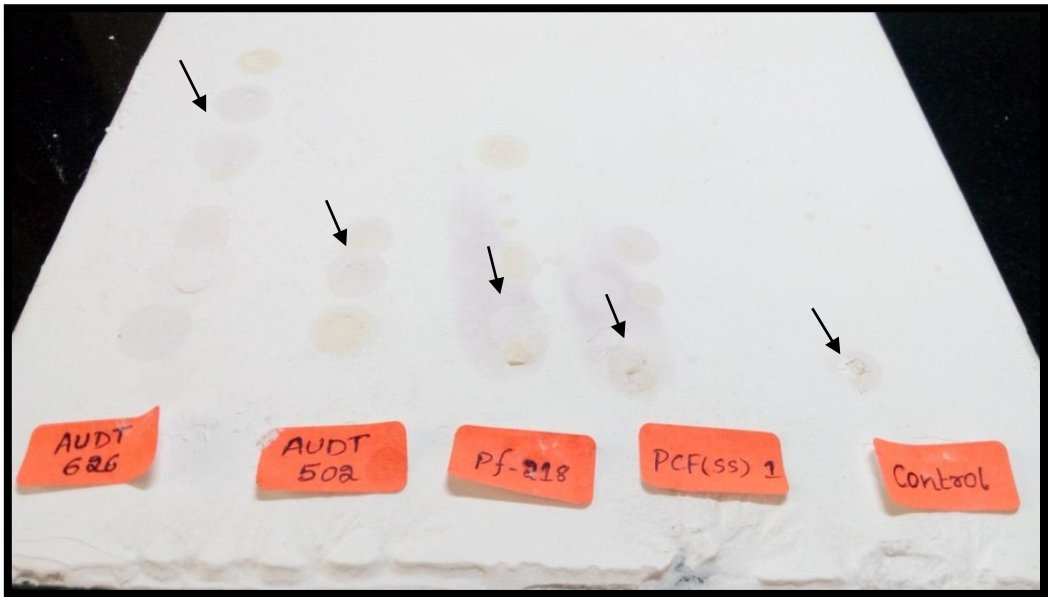

Plate 2. Movement of sample and purple colour development after ninhydrin spray in TLC plate (Arrows indicating purple spots)

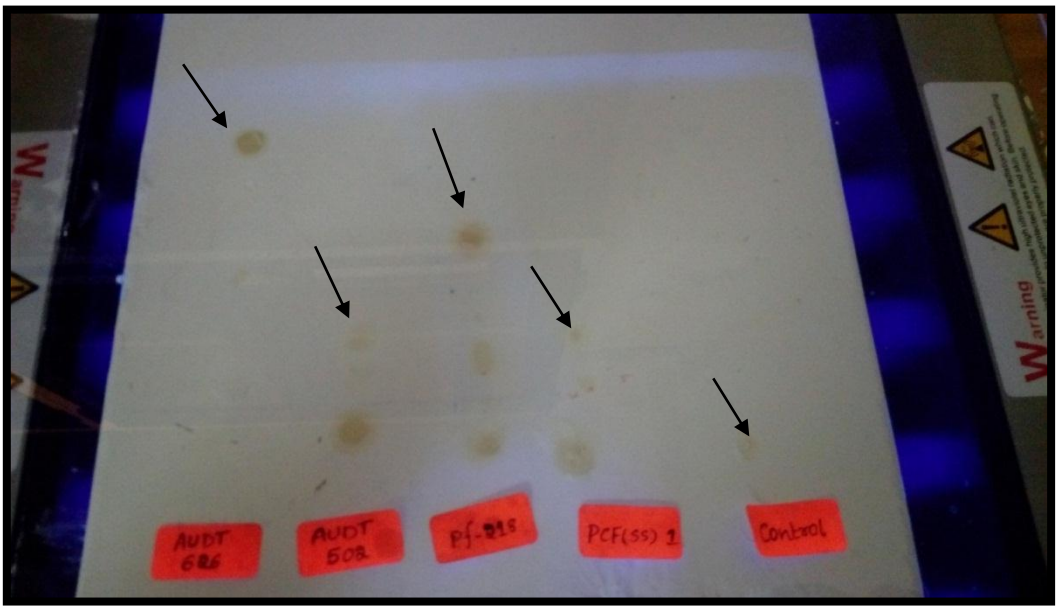

Plate 3. TLC Silica gel plate under UV transilluminator((Arrows indicating yellow spots)

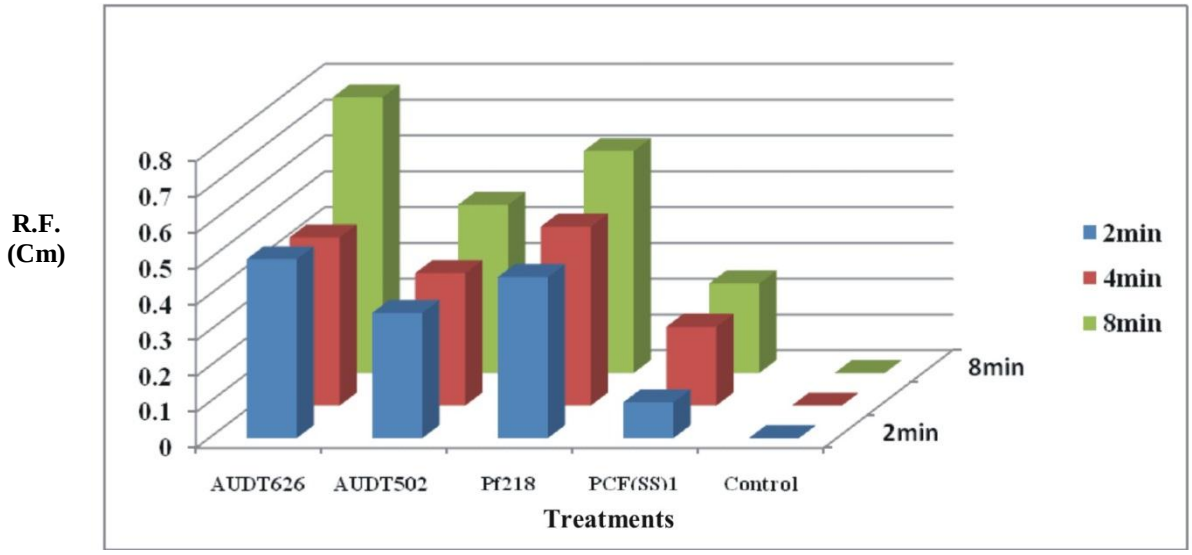

Fig. 1: Movement of bio-active metabolites produced by rhizobacterial supernatants at different time intervals in TLC

REFERENCES

1. Dutta, S., Morang. P. and Nishanth Kumar, S., Fusarial wilt control and growth promotion of pigeon pea through bioactive metabolites produced by two PGPR's. *World J. Microbiol. Biotechnol.*, **30:** 1111-1121 (2014).
2. George, E., Nishant Kumar, S. and Jacob, J., Characterization of the bioactive metabolites from PGPR and their exploitation as Antimicrobial and PGP agents. *Appl. Biochem. Biotechnol*., **15:** 1593- 1599 (2010).
3. Kannaiyan, J. and Nene, Y. L., Influence of wilt at different growth stages on yield loss in pigeon pea. *Trop. Pest Manag.*, **30:** 62-71 (1981).
4. Nene, Y.L. and Sheila, V.K., Pigeon pea: Geography and importance, The Pigeon pea., 1-10 (1990).
5. Okiror, M. A., Genetics of resistance to *Fusarium udum* in pigeonpea [*Cajanus cajan* (L.) Millsp.]. Indian J. Genet. Plant. Breed., **62:** 218–220 (2002).
6. Reddy, M.V., Sharma, S.B. and Nene, Y.L., Pigeon pea: Disease Management. In:Nene, Y.L., Hall, S.D. and Sheila, V.K., (eds.). The Pigeon pea. C. A. B. *International*., 303-348 (1990).