

## Full Length Research Article

# Qualitative and Quantitative Production of Secondary Metabolites from *Pseudomonas fluorescens* from Pseudolin-LF.

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In this work *Pseudomonas fluorescens* was isolated from commercially available Bio- control agent namely Pseudolin-LF and identified with suitable biochemical tests. This isolated *P. fluorescens* was cultivated in a laboratory scale bioreactor for obtaining secondary metabolite in King's B and Nutrient Broth with Glucose media. The centrifuged cell free substrates were tested for its antibacterial activity. The product quality of the crude extract obtained from two different media was analysed with UV-Spectrophotometer. UV- Vis spectrum and Antibacterial activity revealed that Nutrient Broth with Glucose medium might be better for qualitative and quantitative production of secondary metabolites.

**Key words:** *Pseudomonas fluorescens*, Secondary metabolites, Pseudolin-LF

## INTRODUCTION

Secondary metabolites are organic compounds that are not directly involved in the normal growth, development, or reproduction of an organism and it often plays an important role in defence and other interspecies differences. Secondary metabolites are used by human as medicines, flavourings, and recreational drugs (Barrios- González *et al.*, 2003). Research on microbial Secondary metabolite production and their applicability in controlling diseases is gaining some momentum in medical industries. The scope of developing these microbial secondary metabolites for commercial bio control agents such as medicine or chemical fungicide as an alternative to chemical fungicides; is gaining importance due to increased concerns on environmental pollution, pathogen resistance and high protection costs (Gurusiddaiah *et al.*, 1994). Earlier, several of these products have been developed and used as bactericides, fungicides, insecticides or acaricides in medical field and also in agriculture (Battu *et al.*, 2009). Microorganisms are gaining importance of study in for many years because of production of novel metabolites, which exhibits antimicrobial, antiviral, antitumor as well as anticoagulant properties. These secondary metabolites serve as model systems in discovery of new drugs (Rodrigues *et al.*, 2000). *Pseudomonas fluorescens* is a well-known bacteria for their bio control potential and it is belonging to the genus *Pseudomonas* is one of the most diverse Gram negative bacterial genera. *P. fluorescens* are Gram-negative rod shaped bacteria that inhabit the soil, plants, and water surfaces (Maleki *et al.*, 2010). The optimum growth temperature is between 25-30 degrees Celsius. Most of the *P. fluorescens*

*strains* present in the plant rhizosphere soil and produces a variety of secondary metabolites including antibiotics (Meera and Balabaskar, 2012). Pseudolin- LF is nothing but a commercial bio- control agent having *P. fluorescens* and it acts upon plant pathogens especially fungal pathogens which causes And this is also used to control weeds like downy brome of wheat. The present study focus on finding a suitable medium for qualitative and quantitative production of secondary metabolites against some bacterial species.

## MATERIALS AND METHODS

Isolation and identification of *Pseudomonas fluorescens*: Pseudolin- LF *P. fluorescens* were bought from Union agricultural society, Kadayam, Thirunelveli District. A loopful of Pseudolin-LF was inoculated on Pseudomonas Agar medium (King's B medium), incubated for 24 hours at 35<sup>o</sup>C (Gildo *et al.*, 2006) and then observed under UV- Tran's illuminator. The colonies that fluoresce at 365 nm were selected and further identification was carried out with various biochemical tests.

### Development of cultivation technology in bioreactor for production of metabolites from *P. fluorescens*

The isolated *P. fluorescens* were first inoculated in 100ml King's B medium in Erlenmeyer flasks. The selective media, King's B medium and Nutrient Broth with Glucose were prepared and transferred into bioreactor individually. Then the reactor was sterilized at 121<sup>o</sup>C, 15 lbs pressure, for 20 min (Ramyasmruthi *et al.*, 2012). After cooling the media was inoculated with pre-grown inoculum of *P. fluorescens*. The reactor was run under fixed parameters like temperature- 28<sup>o</sup>C, rpm-120, DO2-40%, pH-7.0 for 89 hours. This 89 hour

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time interval was calculated by growth curve method in which the stationary phase lasts upto 89 hours. The down streamed substrate from the reactor was centrifuged at 10000 rpm for 15 minutes to get cell free substrate. Identification of the number of compounds with Chromatographical method: Both Paper chromatography and Thin layer chromatography were performed for the identification of compounds. The paper chromatography was performed with Benzene: Acetic acid: Water (1:4:5) solvent system. The result was observed and the Rf value was calculated. And for Thin Layer Chromatography the slurry was prepared with the concentration of 1:2 (i.e. 20gm/40ml) with water and poured in a neat TLC plate. The Benzene: Acetic acid (95:5) solvent system was used (Fabio *et al.*, 2007). 35 $\mu$ l of crude sample was spotted on the TLC plate and allowed to migrate. The result was observed under UV - Trans illuminator at 360nm and Rf value was calculated.

### Antibacterial activity of crude extract containing secondary metabolites

The antimicrobial effect of the crude extract containing secondary metabolites was analysed by using Kirby- Bauer well diffusion method. The Muller- Hinton agar plates were prepared with a pH of  $7.2 \pm 0.2$ , and after solidification, four different bacterial pathogens viz., *Proteus vulgaris*, *Streptococcus* sp, *Vibrio* sp and *Salmonella* sp. were inoculated. Wells of 10mm diameter was made with the help of sterile well- puncher and the crude extract was poured into the well with different volumes (25  $\mu$ l, 50 $\mu$ l and 75 $\mu$ l). The plates were incubated at 37 $^{\circ}$ C for 24 hours and zone of inhibition was measured.

### UV-Visible Spectroscopic analysis of the crude extract

The UV/Vis Spectra for the crude extract was obtained from two different media using Double Beam UV/Visible Spectrophotometer (Systronics 2203). The baseline was corrected with respect to Distilled water in all the cases. The spectral range was scanned from 200-1000nm. The scan rate was kept constant (500nm / min) for complete experiment.

## RESULTS AND DISCUSSION

In the current investigation the organisms *Pseudomonas fluorescens*, well known for its various role in agriculture is employed. This *P. fluorescens* produce some bio-active compound known as secondary metabolites that are effective against fungal plant pathogens. The activity of this secondary metabolites against bacterial pathogens are scarce. Pseudolin - LF is a bio control agent having merely *Pseudomonas fluorescens* and is used to control plants fungal diseases. It is commercially available all over the world and it is used for crops like Paddy, Cotton, Wheat etc. This bio control agent is able to control plant fungal diseases like Tikka disease of groundnut caused by *Cercospora personata*, Blight of potato caused by *Phytophthora infestans*, Late blight of Paddy caused by *Puccinia graminis*, Apple Scap caused by *Venturia inaequalis*. The isolated *Pseudomonas* species were identified using Gram's staining, Motility, its Fluorescent ability and biochemical reactions like Gram staining, motility, IMViC technique. Its negative for Indole and Methyl red, Urea reduction ability was not present. It's positive for Voges-Proskaur, citrate, oxidase, and catalase and capable to

hydrolyse starch and Gelatin. All the above biochemical tests confirmed the isolated organism as *Pseudomonas fluorescens*. The Chromatographical analysis was done with standard operating procedures and Rf value was determined. There is more than 5 visible bands were observed while separation was done through paper chromatography. When TLC separation was carried out the solvent front was upto 13cm and the crude moved upto the distance of 7cm (NBwG) and 6.3cm (Kings B). There was a single prominent band for both the media spotted in benzene: acetic acid solvent system. Apart from that there was four bands which was obtained in both Chromatographical analysis.

In paper chromatography the bands were not easily distinguishable, but in TLC the strong band in the Rf of 1.85 (Nutrient broth with glucose) and 2.06 (King's Medium) differentiated the bands. Further the antimicrobial activity of the crude from from both the media was tested against *Vibrio* sp, *Streptococcus* sp, *Salmonella* sp and *Proteus vulgaris*. The crude with secondary metabolites exhibited high inhibitory effect against all the selected bacterial pathogens and especially the crude from NBwG (75 $\mu$ l) had higher inhibitory activity against *Streptococcus* sp. (Fig 2). And the the 75  $\mu$ l of the crude obtained from the Kings B medium had high inhibition effect against *Salmonella* sp., (Fig 1). When UV-Vis spectrum was performed to find the quality of the crude gained from Nutrient Broth with Glucose and Kings medium B, the maximum adsorbance was around 230-260nm in both substrates. But the  $\lambda$ -max of NBwG was higher than Kings B medium. The  $\lambda$ - max of Kings B medium was 2.47 and 2.69 for NBwG medium (Fig 3).

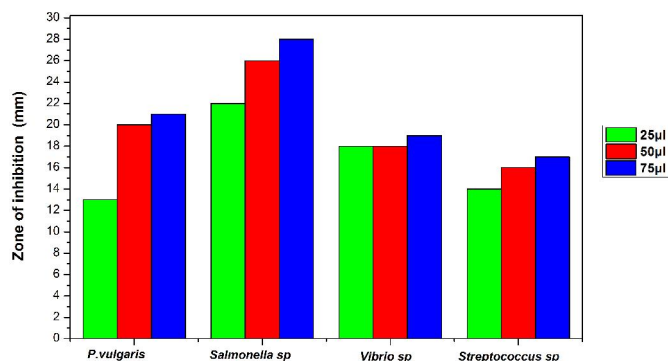


Fig. 1. Antibacterial activity of the extract obtained from King's B medium

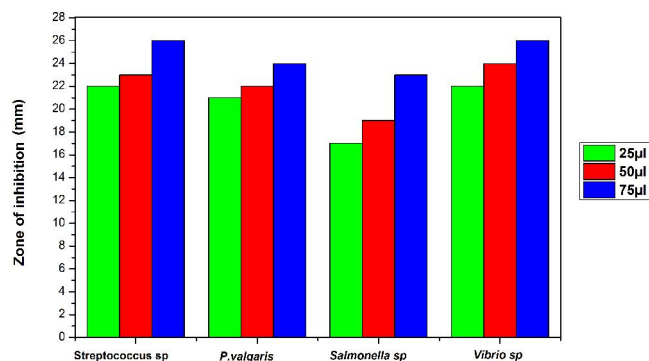


Fig. 2. Antibacterial activity of the extract obtained from NBwG medium

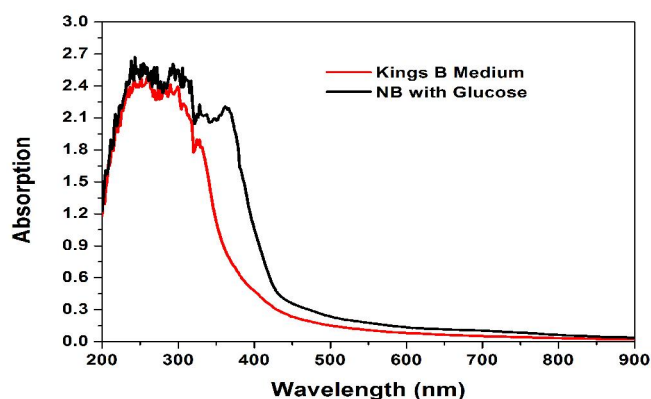


Fig. 3. Compare the quality of the crude extracts from different Medias using UV-Vis spectrophotometer

### Conclusion

From the results obtained we are able to assume that NBwG might be a better medium for higher amount of secondary metabolites production than the King's B medium. Antibiotic production by fluorescent *Pseudomonas* sp is recognized apart from its plant disease management feature. Further work when geared up may give benefits to researcher in a new area.

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