

Isolation of Bacterial Strain Producing Blue-Green Pigment and It's Antimicrobial Activity Over *Fusarium oxysporum* and *E coli* DH5 α

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Abstract— Bacterial strain capable of producing extracellular blue-green pigment was isolated from soil samples. The blue-green extracellular supernatant showed antimicrobial effect against *F. oxysporum* and *E. coli* DH5 α on solid culture. Minimum inhibitory concentration (MIC) for both the microorganisms were measured by the Kirby-Bauer method. The extracellular supernatant had shown more inhibitory effect on *E. coli* DH5 α than *F. oxysporum* confirming better antibacterial efficiency than antifungal one. The characteristic blue colour of the chloroform extract obtained from the extracellular matrix of the bacterial culture indicates the pigment and the bacterial isolate to be pyocyanin and *Pseudomonas aeruginosa* respectively.

Keywords— Pyocyanin, *Pseudomonas aeruginosa*, *Fusarium oxysporum*, *E.coli* DH5 α , Minimum inhibitory concentration (MIC), Kirby-Bauer method

I. INTRODUCTION

In the recent years the study of microorganisms producing pigments is at interest of the scientific community. The pigments are usually secondary metabolite which exhibit virulence property. *Pseudomonas aeruginosa* produces a variety of redox-active phenazine compounds like pyocyanin, fluorescein, pyorubrin, and pyomelanin (El-fauly et al. 2015, Osama et al. 2019, Stephen et al. 1981). Pyocyanin is one of the important secondary metabolite pigments amongst the four pigment which *P.aeruginosa* produces which have a blue green colour (Jayaseelan et al. 2013). Pyocyanin is a member of a tricyclic compound phenazines, chemically it is 5-methyl-1-hydrophenazine which has a redox property. It has been reported to transform molecular oxygen to super oxide and hydrogen peroxide (H₂O₂) which exhibits toxicity in surrounding cellular environment. Therefore, presence of pyocyanin inhibit cellular growth by interacting with the cell membrane respiratory chain which in turn interferes with active transport system (Marrez and Muhammad 2020). The producer cells of *P. aeruginosa* protects itself from cytotoxic effect by higher level of catalase and peroxidase.

The precursor of the blue-green pigment pyocyanin and other phenazine is phenazine-1-carboxylic acid (PCA). The PCA gets subsequent conversion by two significant enzymes PhzS and PhzM to form pyocyanin (Jayaseelan et al. 2013).

Due to the secretion of the phenazine compounds including pyocyanin, *P. aeruginosa* has been reported to have antagonistic activity against Gram negative bacteria of Enterobacteraceae family and also Gram positive Bacillaceae family. The species also has the antifungal activity against human pathogens like *Candida* and *Aspergillus* along with phytopathogens like *Fusarium* and *Pythium* (Anjaiah et al., 1998). It has been reported by Islam et al., 2018 that phenazine compounds produced from the rhizosphere of plants is an attribute to the biological activity of *P. aeruginosa* against *Fusarium* (wilt of chickpea) and *Pythium* (damping-off of bean).

In present work bacterial strain has been isolated and screened for production blue-green pigment. The isolated strain was observed to have antimicrobial activity against bacterial strain and fungal phytopathogen. The crude chloroform extract have indicated the pigment to be pyocyanin which along with other extracellular compounds have sown the antimicrobial activity.

II. MATERIALS METHODS

Reagent: Nutrient Agar, Dry aliquot Chloroform, Hydrochloric Acid (HCL), Sodium Hydroxide (NaOH), Petroleum Ether were obtained from Sigma-Aldrich

Microbial culture: The Bacterial strain producing extracellular blue-green pigment were isolated from soil samples collected from cultivation land in the state of North 24 Paraganas of West Bengal, India during early November

after harvest. The soil sample was serially diluted in sterile water and spread plates of nutrient agar were done from 10^5 dilution. Single colonies were isolated and maintained in slants kept in 4°C . Glycerol stock of the strains were prepared by adding 25% sterile glycerol with overnight culture of each strains and the stocks were kept at -20°C till further use. The fungal strain of *Fusarium oxysporum* and *E. coli* DH5 α were procured from microbial type culture collection (MTCC), Chandigarh, India. Both the procured strains were cultured in nutrient agar media.

For analyzing antifungal activity of extracellular supernatant, Ouchterlony double diffusion method was followed on solid cultures of *F. oxysporum* and *E. coli* DH5 α . The crude extracellular supernatant collected after centrifugation was diluted up to $\frac{1}{2}$, $\frac{1}{4}$ and $\frac{1}{8}$ times. The crude supernatant along with three subsequent dilutions were added to the wells created in the spread plate of *F. oxysporum* and *E. coli* DH5 α respectively and kept overnight in 37°C . The inhibition zones were observed after 16hrs of incubation and the Minimum inhibitory concentration (MIC) of supernatant was estimated after incubation period.

Spectrophotometric assay: The absorption spectra and the individual absorption were analyzed through spectrophotometer (Make: BioRad xMark microplate spectrophotometer) with keeping the extracellular supernatant as blank.

Centrifugation: Cells were separated through centrifugation (Make: RM-12C BL R-12M) in 13,000rpm for 15mins.

Extraction of pyocyanin: The extraction of the extracellular pyocyanin was extracted through solvent extraction method using chloroform following (Abou et al. 2018)

III. RESULT AND DISCUSSION:

The antibacterial effect was studied by applying Ouchterlony double diffusion method on *E. coli* plates. The Zone diameters were measured for all subsequent dilutions. The minimum inhibitory concentration (MIC) was observed at the $\frac{1}{8}$ dilution of the culture supernatant (Fig 1).

The antifungal effect was also measured by the same way as above for *Fusarium oxysporum*. The MIC has been observed at $\frac{1}{4}$ dilution in this case (Fig 2). The results show that the isolated strain is having antibacterial and also antifungal activity against Gram negative bacteria and the phytopathogen. The antibacterial activity of the extracellular matrix was found to be stronger than the antifungal activity. The bioactive compounds responsible for exhibiting the effect is yet to be analysed.

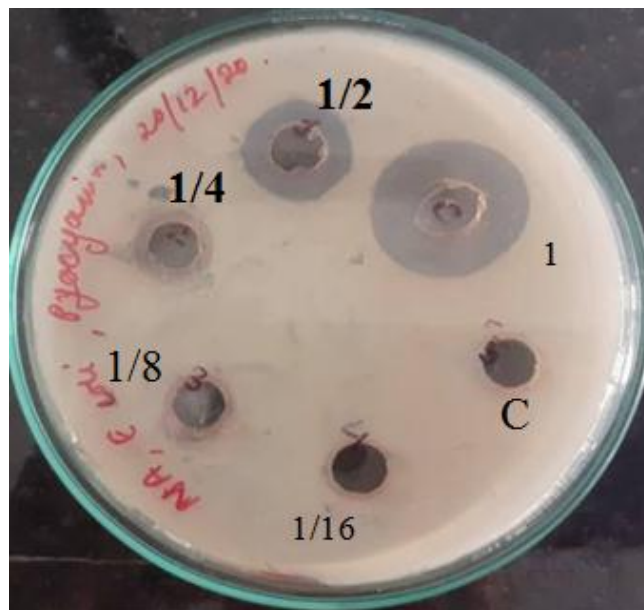


Fig 1: Determination of Minimum inhibitory concentration (MIC) of Pyocyanin over *E. coli* DH5 α by Ouchterlony double diffusion method. Different dilutions have been marked accordingly and the control was marked as 'C'.

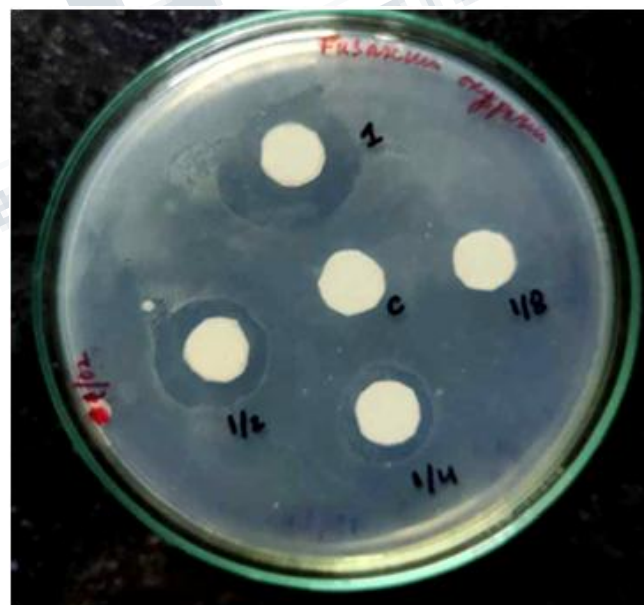


Fig 2 :- Determination of the Minimum inhibitory concentration (MIC) of extracellular supernatant of bacterial isolate on *Fusarium oxysporum* by disc diffusion method. Different dilutions have been marked accordingly and the control was marked as 'C'.

The crude chloroform extract of the extracellular supernatant was observed to be bright blue in colour. The intensity of the colour depends on the initial concentration of the pigment present in the extracellular matrix. The characteristic blue colour indicates the pigment to be pyocyanin and therefore the producer bacterial strain to be *Pseudomonas aeruginosa*. The UV-Vis spectrophotometric analysis of the crude cell supernatant have shown the

absorbance peak at 220 and 380nm where as the chloroform extract have shown to have the maximum absorption at 700nm. The absorbance peaks of the crude extracellular supernatant have indicated the presence of more than one bioactive compound whereas the chloroform extract has confirm the presence of pyocyanin among all the compounds present in them.

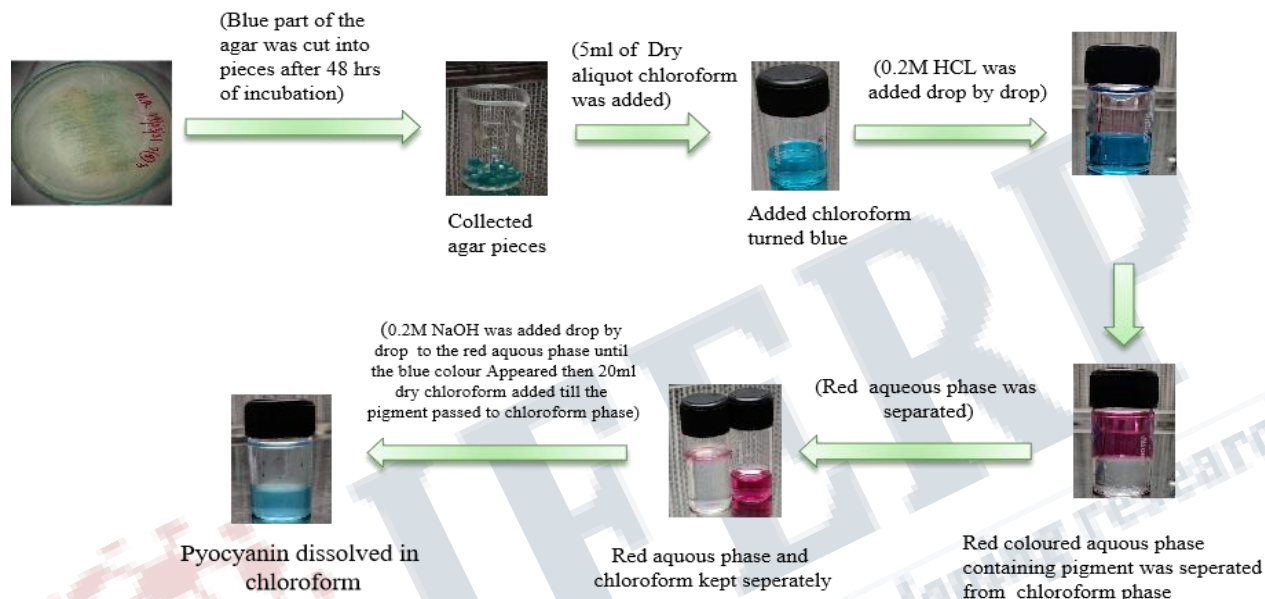


Fig 3: Extraction process of blue-green pigment from isolated bacterial strain.

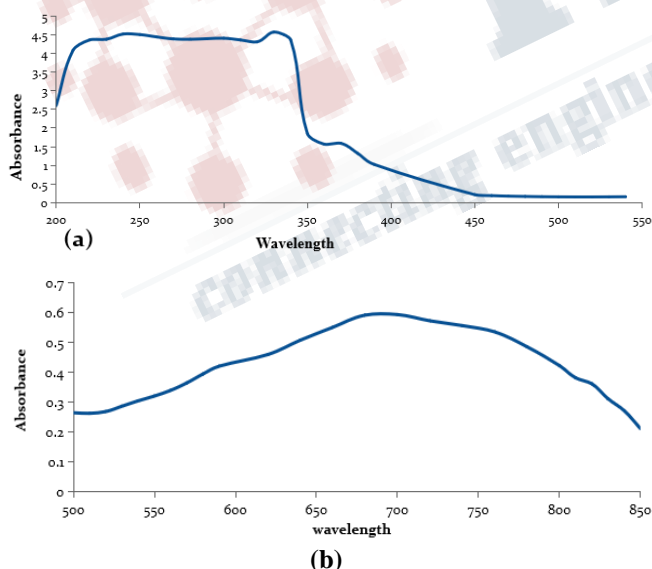


Fig 4: Absorption spectra of extracellular supernatant of bacterial isolate (a), Absorption spectra of chloroform extract of blue-green pigment (b)

IV. CONCLUSION

The isolated bacterial strain has antagonistic effect on Gram negative *E. coli* and also on wilt causing phytopathogen, *Fusarium oxysporum*. The blue green pigment secreted at the late log phase along with the other secondary metabolites present in the extracellular supernatant were responsible for the antimicrobial effect. The characteristic absorbance of the crude chloroform extract of the Blue-green pigment indicates that the bacterial isolate to be *Pseudomonas aeruginosa* and the pigment to be pyocyanin.

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