Shiv Chhatrapati Shikshan Sanstha's
Rajarshi Shahu Mahavidyalaya, Latur (Autonomous)
(Affiliated to Swami Ramanand Teerth Marathwada University, Nanded)
NAAC Accredited Grade "B++" CGPA(2.99)
ISO :9001:2008

Department Of Microbiology

Academic Year: 2021-2022

Project

Studies on Biocontrol Potential of a Rhizospheric Pseudomonas isolate against Fusarium roseum

Submitted By:

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2021-2022

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CERTIFICATION

This is to certify that **Miss. Sayyad Rukaiyya Kalimsab** a student of **B.Sc. T.Y** has completed her project work titled Studies on Biocontrol Potential of a Rhizospheric Pseudomonas isolate against Fusarium roseum and has submitted a satisfactory report under the guidance of **Dr.D.V. Vedpathak** for fulfillment of **B.Sc. TY** course of **"~~Swami Ramanand Teerth Marathwada University, Nanded.~~"** in the Academicyear 2021-2022.

Head of Dept.

Dr. K.G. Maske

Project Guide

Dr. D. V. Vedpathak

Principal

Dr.M.H. Gavhane

Rajarshi Shahu Mahavidyalay

(Autonomous) , Latur

DECLARATION

I declare that the project entitled Studies on Biocontrol Potential of a Rhizospheric Pseudomonas isolate against Fusarium roseum submitted by me for the degree of **B.Sc. TY** is the record of work carried out by me during the period from 2021-2022 under the guidance of **Dr.D.V. Vedpathak** the basis for the award of any degree, diploma, associate-ship, fellowship, titles in this or any other University or other institution of Higher learning. I further declare that the material obtained from other sources has been duly acknowledged in the project

Date:17-05-2022

Place:Latur

Rukaiyya

Signature

(Sayyad Rukaiyya Kalimsab)

Rukaiyya

ACKNOWLEDGEMENT:

There is always a sense of gratitude which one express towards others for their help and supervision in achieving the goals. This formal piece of acknowledgement is an attempt to express the feeling of gratitude towards people who helpful me in successfully completing of my project.

I would like to express my deep sense gratitude towards Principal **DR. MAHADEW GAHAVANE Sir** and our Vice- Principal **Dr. Sadashiv Shinde Sir** and **Dr. K. G Maske Madam** head of Microbiology department. I am deeply thankful to **Dr. D. V. Vedpathak Sir** who guided me to work honestly and to give valuable suggestion for improving my work.

Last but not least, above all no words can express my feelings to my parents, friends all those persons who supported me during my project. I would also like to thank God for his blessings showered on me during the completion of the project report.

Rukaiyya

Signature

Sayyad Rukaiyya Kalimsab

Studies on Biocontrol Potential of a Rhizospheric *Pseudomonas* isolate against *Fusarium roseum*

Abstract:

Fusarium roseum, is a fungal plant pathogen which causes fusarium head blight (FHB), a devastating disease on wheat and barley. The pathogen is responsible for billions of dollars in economic losses worldwide each year. Bio-control of this fungal phytopathogen by Plant Growth Promoting Rhizobacteria (PGPR) may be an ecofriendly option.

The present study deals with analyzing bio-control potential of *Pseudomonas* RSML23, a wheat rhizosphere soil isolate. The efforts are made to extract the blue coloured water soluble pigment by chloroform extraction method. The bacterial isolate and a blue coloured secondary metabolite produced by it exhibited considerable antifungal potential against *Fusarium roseum.*

Keywords: Biocontrol, Rhizosphere, *Pseudomonas* isolate, *Fusarium roseum*

Introduction:

fusarium head blight (FHB):

Fusarium roseum, is a fungal plant pathogen which causes fusarium head blight (FHB), a devastating disease on wheat and barley.[1]. Infection causes shifts in the amino acid composition of wheat resulting in shriveled kernels and contaminating the remaining grain with mycotoxins, mainly deoxynivalenol (DON), which inhibits protein biosynthesis; and zearalenone, an estrogenic mycotoxin. These toxins cause vomiting, liver damage, and reproductive defects in livestock, and are harmful to humans through contaminated food. Despite great efforts to find resistance genes against *F. graminearum*, no completely resistant variety is currently available. Research on the biology of *F. graminearum* is directed towards gaining insight into more details about the infection process and reveals weak spots in the life cycle of this pathogen to develop fungicides that can protect wheat from scab infection. [2].

The pathogen is responsible for billions of dollars in economic losses worldwide each year.[3]. The global intensification of wheat production will almost certainly be accompanied by a rise in Fusarium disease pressure [4]. Fusarium Head Blight was first described in 1809 by German mycologist Link (FHB). This infection has evolved into one of the most serious cereal diseases in the world, and research suggests that the likelihood of FHB epidemics has grown over the last century. Fusarium graminearum pandemic lines have accelerated disease progression, leading to large-scale epidemics and widespread mycotoxin contamination. [5–9]. Despite substantial efforts to regulate FHB and its persistent mycotoxins (e.g., deoxynivalenol-DON, zearalenone-ZEN) in major cereal crops, food, and feed, overall effective management of FHB remains a difficult task for various novel investigations. [10].

DON is a type of vomitoxin and, as its name states, is an antifeedant. Livestock that consume crops contaminated with vomitoxin become sick and refuse to eat anymore. Zearalenone is a phytoestrogen, mimicking mammals' estrogen. It can

be disastrous if it gets into the food chain, as zearalenone causes abortions in pregnant females and feminization of males. [11].

Photo 1: The fusarial wilt of Wheat

The Rhizosphere and its influence:

The soil surrounding the plant root where root exudate migrate and microbiological activity is exceptionally high is called rhizosphere. The surface of root is called rhizoplane. Plant root produce and release various exudates containing sugar, aminoacids, organic acids, fatty acids, vitamins, nucleotides and other organic matters that promotes growth of microorganisms. Therefore rhizospheric soil is characterized by greater number of microorganisms than soil

away from plant roots. The intensity of rhizospheric effects depends on the distance to which root exudates can diffuse. The number of microorganisms decreases continuously as the distance from the plant root increases. The term rhizosphere to soil ratio (R:S) indicates number of microbes in rhizospheric soil divided by number of microbes in soil free of plant root. R:S ratio is greater for bacteria (20:1) and less for fungi and actinomycetes. Effects of rhizosphere is almost negligible for algae and protozoa. It is because algae are photosynthetic and do not depends upon organic matter present in root exudates. On the other hand most bacteria cannot utilize relatively resistant to organic matter of soil and depends on easily available decomposable matter of root exudates. Therefore number of bacteria is exceptionally high in Rhizosphere

Role of rhizospheric microbes:

- Rhizospheric microorganisms are important for plant growth. They promote plant growth by various ways as given below;
- Some rhizospheric bacteria such as *Rhizobium, Azotobacter, Clostridium* etc. fix atmospheric nitrogen and make it available for plant gowth.
- Many phosphate solubilizing microbes such as Bacillus polymyxa found in rhizosphere release free phosphate from inorganic salt of phosphate. Free phosphate is important nutrient for plant growth.
- Several rhizospheric microbes (*Azotobacter, Arthrobacter, Pseudomonas, Agrobacterium)* produce growth hormone such as Gibberllin, Indole acetic acid (IAA) etc that promote plant growth.
- Many rhizospheric fungi are associated with plant root in the form of mycorrhiza. Mycorrhizal fungi promote plant growth by various ways.
- Rhizospheric microbes induce development of lateral root, root hairs development and mucilage secretion from plant root.
- Some rhizospheric microbes produces antibiotics and other antimicrobial chemicals that inhibit plant pathogens. Some time it may inhibit beneficial N2 fixing and phosphate solubilizing bacteria.

- Microorganisms also increase rate of exudate secretion. Exudate secretion from plant root helps in formation of soil aggregate that improve soil fertility.
- Some rhizospheric microbes eg *Pseudomonas* produces Siderophore. Siderophore is a chelating agent that tightly bind iron and make it unavailable for growth of pathogenic microorganisms.

Effect of plant root on rhizospheric microbes;

- Plant root usually promote growth of rhizospheric microbes. Sometimes plant root give minor unwanted effect to microorganism. Some of them are;
- Plant root produce exudate containing carbohydrate, aminoacids, nucleotide, vitamins etc that serves as food for growth of rhizospheric microbes.
- Some plant root produces antimicrobial chemicals such as glycosides, Zhydrocyanic acids and antifungal agents that inhibits growth of rhizospheric microorganisms.
- Plant root release CO2 during respiration that make habitat acidic and anaerobic.
- Some plant root produce chemicals that bring fungistasis. Fungistasis is referred to the inability of spore to germinate. For eg. Root of *Allium* produce alkylcystein sulfoxide that inhibit germination of sclerotia (spore) of *Sclerotium capivarum*. [12 & 13]

Plant growth promoting rhizobacteria (PGPR):

Plant Growth Promoting Rhizobacteria (PGPR) are the soil bacteria inhabiting the root surface as a population that competitively colonizes plant root and increases their growth and also reduces plant diseases. Few properties strictly associated through PGPR, are their properties of insistent migration and plant growth stimulation and their biocontrol ability. The use of PGPRs has been demonstrated to be an environmentally sound method of growing crop yields by facilitating

plant growth all the way through either a direct or indirect mechanism (14 & 15) The plant growth promoting rhizobacteria (PGPR), are characterized by the following inherent distinctiveness's: (i) they must be proficient to colonize the root surface (ii) they must survive, multiply and compete with other microbiota, at least for the time needed to express their plant growth promotion/protection activities, and (iii) they must promote plant growth (16). About 2–5% of rhizobacteria, when reintroduced by plant inoculation in a soil containing competitive microflora, exert a beneficial effect on plant growth(17)

Mechanism of Plant Growth Promotion by Rhizobacteria (PGPR):

Generally, PGPR promote plant growth directly by either facilitating resource acquisition (nitrogen, phosphorus and essential minerals) or modulating plant hormone levels, or indirectly by decreasing the inhibitory effects of various pathogens on plant growth and development in the forms of biocontrol agents. **Indirect mechanisms:** The application of microorganisms to control diseases, which is a form of biological control, is an environment-friendly approach. . The major indirect mechanism of plant growth promotion in rhizobacteria is through acting as biocontrol agents (18). In general, competition for nutrients, niche exclusion, induced systemic resistance and antifungal metabolites production is the chief modes of biocontrol activity in PGPR) (19)

Many PGPR have been reported to produce antifungal metabolites like, HCN, phenazines, pyocyanin, pyrrolnitrin, 2,4-diacetylphloroglucinol, pyoluteorin, viscosinamide and tensin (20).

Pseudomonas species:

Pseudomonas spp is a gram-negative, aerobic rod shaped bacterium, ubiquitous organism in nature and widespread in soil, water and many other environment. Pseudomonas spp. producing a variety of extra- cellular phenazine pigments. *P. aeruginosa* was a common environmental gram-negative Bacillus. It was an opportunistic human pathogen as well, was known for its ability to produce pigments [21]. *P. aeruginosa* was widely distributed in the environment; it was founded in soil, water, skin flora, and most man mode environments throughout

the world, and had thus colonized many natural and artificial environments [22]. Pyocyanin is a water soluble blue green phenazine nitrogen-containing heterocyclic compound. Pyocyanin is redox active secondary metabolite. It is an extracellular pigment which is produced by *Pseudomonas aeruginosa*.[23]

Objectives:

- To study the antifungal potential of *Pseudomonas* RSML23 isolate
- To produce and extract the antifungal metabolite produced by *Pseudomonas* RSML23
- To study the antifungal potential of the extracted metabolite produced by *Pseudomonas* RSML23

Materials and Methods:

Bacterial Isolate: The culture of *Pseudomonas* RSML23 species was obtained from bacterial culture collection of Rajarshi Shahu Microbiology Laboratory, Latur.

The culture was grown on Pseudomonas Isolation agar of Hi-Media (G/L of Peptic digest of animal tissue 20.000, Magnesium chloride 1.400, Potassium sulphate 10.000, Triclosan (Irgasan) 0.025, Agar 13.600, Final pH 7.0±0.2) for 48 hrs.

Gram stained to confirm the morphological characters and absence of contamination.

The culture was maintained on nutrient agar slants for further studies.

Fungal Isolate: The culture of *Fusarium roseum* is obtained from Department of plant pathology, college of Agriculture, Latur.

The cultures were grown on potato dextrose agar of Hi-Media (G/L of Potatoes, infusion from 200.000, Dextrose 20.000, Agar 15.000, Final pH 5.6±0.2) for 72 hrs. to confirm purity, growth pattern and observed under 45X on microscope, in lactophenol blue, to observe morphological characters.

The culture was maintained on PDA slants for further studies.

Antifungal activity of *Pseudomonas* RSML23

A loop full of active Pseudomonas RSML23 culture was spot inoculated at the centeron the surface of Glucose Nitrate salt Agar surface (G/L of Glucose 5.4, Sodium nitrate 1.5, Potassium dihydrogen phosphate 1.0, Magnesium sulphate 0.5, Agar 20 and pH 6.0). The fungal culture was streaked at a distance from spot. The plates were incubated at 30°C temperature for five days and observed for zone of inhibition around the bacterial colony.

Production of antifungal metabolite by *Pseudominas* RSML23

The growth from nutrient agar, was inoculated into king's B Broth (KB) (G/L of Peptone 20 g; Glycerol 10g; MgSo4 1.5 g; K2PO4 1.5 g) and incubated at 30°C on 120 rpm rotary shaker for 48 hours and were observed for color change. The Pigment was extracted using chloroform system.

Extraction of Pigment:

The broth culture was centrifuged at 5000 rpm for 10 minutes. The culture supernatants were transferred into new test tubes and extracted with chloroform (1:2) and the aqueous phase was removed.

Antifungal activity of the chloroform extract:

The *Fusarium roseum* culture was spread on the surface of Glucose nitrate salt agar. A well was dug with cork borer at the center. The well was filled with obtained chloroform extract. In a control plate the well was filled with chloroform. The plates were placed in refrigerator for half an hour for diffusion of the content in medium. The plates are then incubated at 30oC for 72hrs and observed for zone of inhibition.

Results and discussion:

***Pseudomonas* Isolate:** The *pseudomonas* isolate produced diffusible bluish green pigment on *Pseudomonas* isolation agar. The microscopic observation revealed Gram negative, non-spore forming small rods. These observations indicated that the culture procured from departmental laboratory is a pure culture.

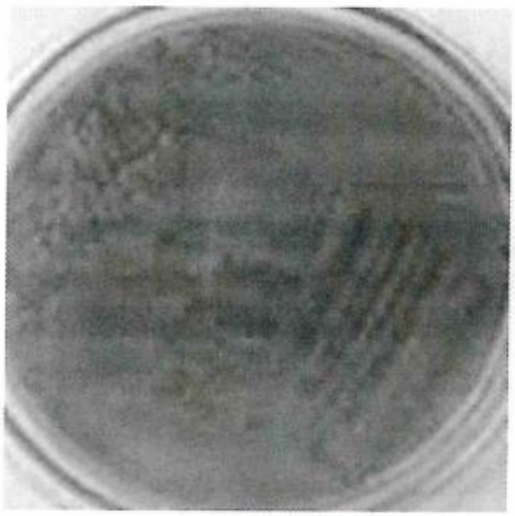

Photo 2: Growth of *Pseudomonas* isolate on Pseudomonas isolation agar

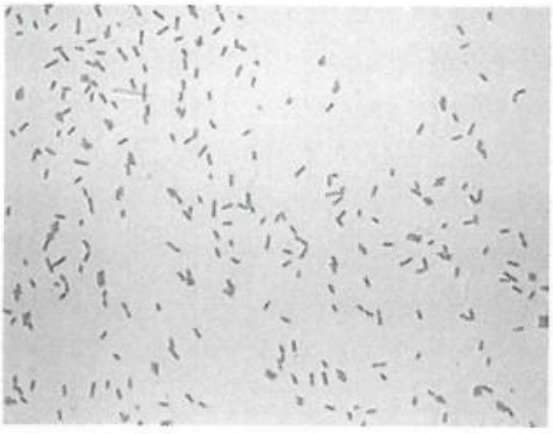

Photo 3: Gram staining of Pseudomonas isolate

Fungal isolate:

The cottony white growth with reddish coloration was observed on potato dextrose agar. Under high power objective in lacto phenol blue preparation, the mycelium and spores confirmed the isolate as *Fusarium roseum*

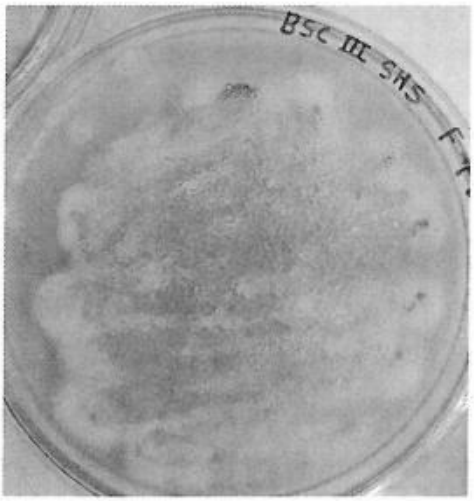

Photo4: Growth Fusarium roseum on PDA plate

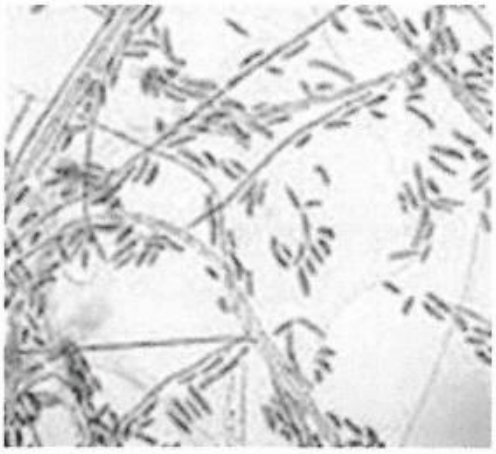

Photo 5: *Fusarium roseum* under 45 X in lacto phenol blue

Antifungal activity of *Pseudomonas* RSML23

The diffusible pigment produced by *Pseudomonas* RSML23 exhibited the inhibition of the growth of *Fusarium roseum* (Photo 6)

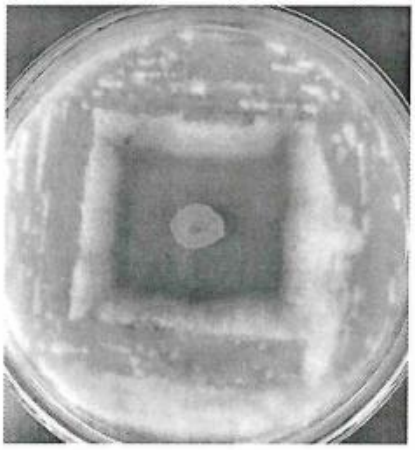

Photo 6: Antifusarial activity of *Pseudomonas* RSML23

Production of antifungal metabolite by *Pseudominas* RSML23

TThe appearance of blue green coloured pigment in the medium after 48 hours of incubation at 30°C indicated production of pyocyanin, a well-known secondary metabolite of *Pseudomonas aeruginosa* (photo 7)

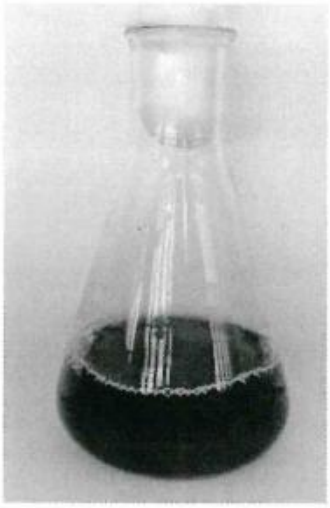

Photo 7: Production of blue coloured water soluble pigment

Extraction of Pigment:

The blue coloured pigment was extracted by the addition of chloroform from the cell free supernatant. The pigment was separated as a blue color compound at the organic phase. The chemical nature of pyocyanin was confirmed with the appearance of red color upon addition of 0.2N HCl

Photo 8: Chloroform extraction of blue coloured pigment

Antifungal activity of the chloroform extract:

A considerable zone of inhibition is observed on GNS agar after 48 hours around the well. It exhibited the antifungal potential of the water soluble blue pigment.

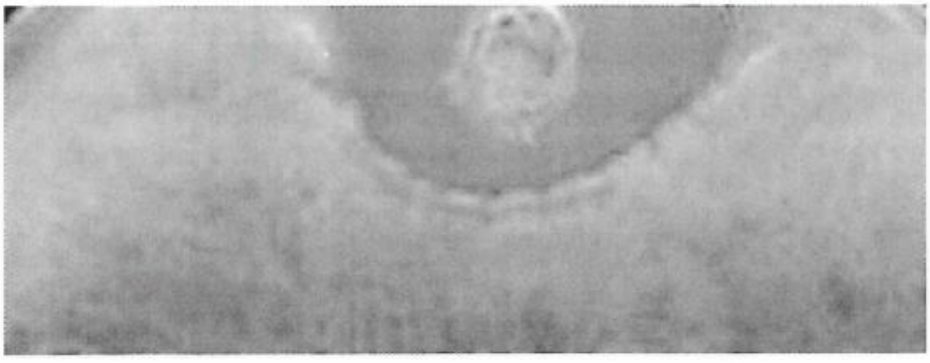

Photo 9: Zone of inhibition around the extracted pigment filled well

Similar antifungal activities of *Pseudomonas aeruginosa* and its blue pigment were reported against *Aspergillus fumugatus, aspergillus flavus* and *Fusarium oxysporum* [23, 24& 25]

Conclusion:

According to this study, *Pseudomonas* RSML23 and the extracted pigment successfully inhibited the growth of Fusarium roseum, In vitro. This investigation could be expanded to include purified product to assess its antifungal properties. Although *Pseudomonas* RSML23 as a fungal growth inhibitor has been shown to be beneficial in the lab, further research and implementation in agriculture is required.

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