

**Antagonistic activity of *Rhizobium* species against phytopathogenic microorganisms****\*Manoja Das, Sumitra Mandal, Rajsekher Padhy and Pragyna P Tripathy****ABSTRACT**

Green gram (*Vignaradiata* L.) is a popular edible legume of India. The plant is cultivated by the local farmer as cash crops. *Rhizobium* sp. was isolated from the root nodules of green gram. The pure culture of the isolated bacterium was carried out at the laboratory with Yeast- Extract Mannitol (YEM) Agar medium. The characterization of the bacterium was made by morphological and biochemical tests. Production of siderophores by the *Rhizobium* bacterium was qualitatively measured by Chrome Azurol S (CAS) agar plate method. Antagonistic behaviour of the *Rhizobium* isolates against two phytopathogenic microorganisms such as *Rhizoctonia solani* and *Rhizoctonia oryzae* were studied by the agar co-cultivation method. The zone of inhibition was recorded for both the pathogens are 46% and 51% respectively. The results indicated that *Rhizobium* species are capable to resist the growth of both the studied phytopathogens at *in vitro* condition. Therefore, *Rhizobium* sp. is a good candidate to control the growth of both the studied phytopathogenic microorganisms and can be used as a biocontrol agent for the same.

**Keywords:** Biocontrol; *Rhizobium* species; phytopathogens; legume plant**MS History:** 13.03.2021 (Received)-20.05.2021 (Revised) - 21.05.2021 (Accepted).**Citation:** Manoja Das, Sumitra Mandal, Rajsekher Padhy and Pragyna P Tripathy. 2021 Antagonistic activity of *Rhizobium* species against phytopathogenic microorganisms. *Journal of Biopesticides*, 14(1):27-31.**INTRODUCTION**

Green gram (*Vignaradiata* L.) is a legume plant belongs to family leguminosae and is cultivated by the local farmer as rabi crops. The crop plants are facing a lot of stresses during their growth in the environment. These stresses are directly impact on the production of the crop (Pandey *et al.*, 2017). Out of the range of stresses, the biotic stress is very frequent to the plants. The stress causes profound loss to the yielding of plant (Achuo *et al.*, 2006). It is estimated that about 33% of a reduction of yield has been incurred due to attack of pest (Duveiller *et al.*, 2007). Therefore, a reliable and effective method of control of pest is very essential to get hold of more production. Generally the pests are controlled by using of chemical pesticides. Most of the chemical pesticides are degraded naturally after application on the crops or when they come to soil. One of the major causes of soil and water pollutants is the use of pesticide to the crop (Aktar *et al.*, 2009). Due to improper use of pesticides by farmers, they cause serious

problem to the ecosystem. These pesticides are harmful to the plants and animals as well, including human beings. Sometimes, several pesticides persist for a longer period at the ecosystem and accumulate in the body or organ of plant and animal particularly those organisms are positioned at the higher level of the food chain (Chau *et al.*, 2015). Therefore, it is quite desirable to replace the chemical pesticides with suitable organic pesticides which have no adverse effect to the ecosystem.

Biopesticides are preferable because they do not cause any harm to the environment and also control the pests as par with the chemical pesticides (Meena and Mishra, 2020). These are organic compounds are obtained from microorganisms or the plants or their products which are used against pest to protect the crops. It is therefore desirable to use biocontrol sort of pest management (Bagheri *et al.*, 2018). It is reported that certain bacteria can be used as a biocontrol agent because these bacteria can manufacture various type of metabolites such as enzymes, antibiotics, siderophore setc like

antimicrobial metabolites (Singh *et al.*, 2017). Lacuna should be highlighted. In the present study *Rhizobium* sp. was isolated from root nodule of legume plant (green gram) and screened as a biocontrol agent against two destructive phytopathogens such as *Rhizoctonia solani* and *Rhizoctonia oryzae*.

## MATERIALS AND METHODS

### Isolation of *Rhizobium* strains

The roots of Green gram (*Vigna radiata* L.) were collected by uprooting the plant and then immediately brought to the laboratory for isolation of *Rhizobium* bacteria. The larger sized root nodules separated from the root of the old plant, the surface sterilized with 5% (w/v) sodium hypochlorite (NaOCl<sub>2</sub>) for 5 minutes followed by 0.3% of mercury chloride (HgCl<sub>2</sub>) for 3 minutes. The root nodules were put in a test tube and crushed with a sterilized glass rod to extract the bacterium. The extract was serially diluted with double distilled water and spread on the Yeast- Extract Mannitol (YEM) agar medium (Mallika *et al.*, 2018). The agar plates were kept at 27 ± 2° C for 48 h. The bacterial colonies appeared in the agar plate were vigilantly observed.

### Subculture and identification of the bacteria

The isolated *Rhizobium* bacterial strain was sub cultured at the laboratory using the same medium. The isolated bacterium was morphologically and biochemically characterized following the Bergey's Manual of Systematic Bacteriology. The pure culture of the bacterium was maintained at the laboratory for future study.

### Assay of antimicrobial compounds

*Rhizobium* species produce metabolites known as siderophore, an antimicrobial compound. The siderophores synthesised by the culture bacteria was qualitatively measured by the Chrome Azurol S (CAS) agar plate method. Chrome Azurol S agar medium was commercially obtained from Hi Media Private Limited, Mumbai. The agar plates were prepared following the standard procedure (Arora and Verma, 2017). *Rhizobium* isolates were transferred onto the CAS agar plates and then incubated at 28° C for 72 h. Change of colour of the medium was recorded.

### The in vitro study of the zone of inhibition:

*Rhizobium* bacterium was screened for its antimicrobial efficacy against two experimental phytopathogens such as *Rhizoctonia solani* and *Rhizoctonia oryzae*. The phytopathogens were collected from the local Agriculture Centre and maintained at the laboratory with the suitable medium. The phytopathogens in pure form was placed centrally on a Yeast- Extract Mannitol agar plate and then isolated *Rhizobium* isolate was placed in opposite sides of the phytopathogens following standard procedure (Sharma and Das, 2010; Das, 2011). The agar plates were incubated at room temperature for 5 days. The zone of inhibition developed by *Rhizobium* sp. due to resist of growth of phytopathogenic in agar culture was recorded. The average values are calculated in the replica of three sets of experiments.

### Scanning Electron Microscope study

Phytopathogenic microorganisms growing towards the *Rhizobium* sp. at the zone of interaction was analysed with Scanning Electron Microscope (SEM) to reveal the cause of death of the phytopathogenic microorganisms. A small piece of agar disc (1 mm thickness) was cut out from the interaction zone, treated with 2% osmium tetroxide for 24 h. Then photograph was taken by scanning electron microscopy at different angles.

## RESULTS AND DISCUSSION

The *Rhizobium* strains were isolated from root nodules of green gram using YEM media. Pink coloured bacterial colonies were appeared in the culture medium after 48 h (fig. 1).



**Figure 1.** *Rhizobium* bacterial colonies in the culture medium after 48 h of incubation

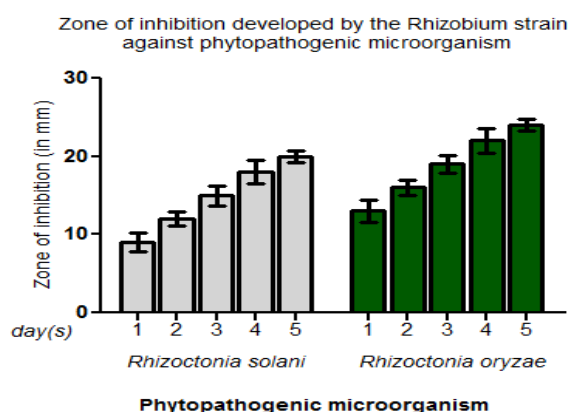
The development of pink colour bacterial colonies is the preliminary confirmation of isolation of *Rhizobium* bacteria. The characterization of the isolated bacterial strain was made with their morphological and biochemical analysis. The results of the analyses are presented in table 1.

**Table 1.** The characterization of the isolated *Rhizobium* bacterial strain with their Morphological and biochemical analysis.

Characters analysed	Result
Shape	Rod shaped
Motility	Positive
Gram staining	Negative
Indole Production	Positive
Methyl Production	Negative
Voges–Proskauer	Positive
Citrate utilization	Negative
H <sub>2</sub> S production	Negative
NO <sub>3</sub> Production	Positive
Catalase	Positive
Urase activity	Positive
Oxidase	Positive
Starch Hydrolysis	Positive
Gelatin Liquefaction	Negative

The shape of the bacterium was rod shaped and tested gram negative. The indol production and Voges–Proskauer test showed positive whereas methyl production and citrate utilization were negative. Similarly, other biochemical testes such as NO<sub>3</sub> production, catalase production, Urease activity, Oxidase, Starch Hydrolysis were found positive. H<sub>2</sub>S production and gelatin liquefaction were found to negative. From the study, it is confirmed that the isolated bacterium was *Rhizobium* strain. To analyse the production of siderophore by the isolated *Rhizobium* sp., the bacterial strain was transferred to Chrome Azurol S (CAS) agar plate.

The bacterial colonies changed the colour of the medium from blue to yellow after 24 h of incubation. This indicates that the *Rhizobium* species has capable of producing the antimicrobial secondary metabolites siderophore (Boiteau *et al.*, 2016). To study the antibacterial activity the isolated *Rhizobium* strains are co-cultured with phytopathogenic fungi in Yeast-Extract Mannitol agar plate. To each well 0.1 mL of 10<sup>4</sup> cells per of *Rhizobium* isolates was

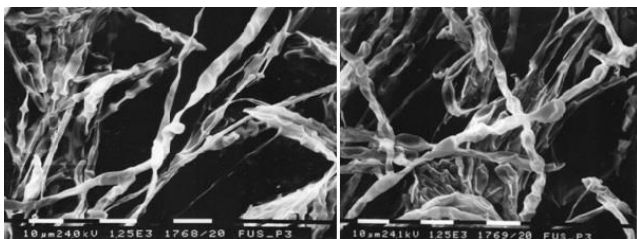


**Figure 2.** Percentage of the zone of inhibition developed by the *Rhizobium* strain against phytopathogenic microorganism

added. The zone of inhibition developed by the *Rhizobium* strain against the phytopathogenic microorganism represented in Figure 2.

Each value is the average of 5 replicates. The *Rhizobium* sp. had strong antagonistic behaviour against all the studied phytopathogens such as *Rhizoctonia solani* and *Rhizoctonia oryzae*. The zone of inhibition was initially observed after 24 hours of incubation in both the phytopathogenic fungi and then it was gradually increased up to 120 h (i.e. 5 days) of incubation. The maximum zone of inhibition was recorded for phytopathogenic fungi *Rhizoctonia solani* and *Rhizoctonia oryzae* was 20±0.8 mm and 24±0.7 mm respectively. The antagonistic behaviours of *Rhizobium* sp. against phytopathogenic microorganism have been reported by many investigators (Deshwal *et al.*, 2003; Khan *et al.*, 2008; Al-Ani *et al.*, 2012). *Rhizobium* species produce a secondary metabolites siderophores which is an iron chelating compound (Ghavami *et al.*, 2017). Therefore, *Rhizobium* causes iron deprive to the phytopathogenic microorganism which is required for their growth and pathogenesis. Apart from that *Rhizobium* species are also produce rhizobactin an antimicrobial compound during its growth (Damien *et al.*, 2001). Therefore, the antimicrobial compounds siderophores or rhizotoxins or both the compounds thereof subdued the growth of the studied phytopathogenic microorganism. Thus,

the growth of the phytopathogenic microorganism was inhibited. Phytopathogenic fungi were analysed with scanning electron micrograph (Fig. 3).



**Figure 3.** Scanning Electron Micrograph (SEM) of Phytopathogenic fungi interacted with *Rhizobium* strain.

The photograph indicates that the fungal pathogens have deformities and fragmentation in their hyphae. There is a clear sign of swelling and presence of pores on the hyphal wall. Therefore, the *Rhizobium* sp. has competent to control the growth of both the studied phytopathogenic microorganisms.

Biological or biocontrol is an eco-friendly method where insects, mites, weeds and plant diseases are controlled using other organism. The technique does not emit any hazardous chemical to the environment. It is based on the principle of predation, parasitism, herbivory or antagonistic effect. The technique is considered as the better method of pest control in compared to the chemical method of control of pest. In the piece of investigation, *Rhizobium* sp. is screened as a biocontrol agent against two common phytopathogens such as *Rhizoctonia solani* and *Rhizoctonia oryzae*. *Rhizobium* species was isolated from the root nodules of the green gram cultivated by the local farmers as a cash crop in their field. The isolation of the *Rhizobium* bacterium was made with Yeast- Extract Mannitol (YEM) agar medium. Production of the antimicrobial secondary metabolites siderophores was qualitatively measured by Chrome Azurol S (CAS) agar medium assay. The isolated *Rhizobium* strain is capable of producing siderophores an iron chelating compound. The *in vitro* study of antagonistic behaviour of the isolated *Rhizobium* sp. against *Rhizoctonia solani* and *Rhizoctonia oryzae* was studied by agar plate co-cultured method. It is found that the isolated *Rhizobium* species has capable to resist the growth of both the studied pathogenic

microorganisms. The scanning electron micrograph photograph of the pathogenic microorganisms indicated that there is a swelling, breakage and pores on the fungal hyphae. Therefore it can be concluded that *Rhizobium* species is a good candidate for biocontrol mediator against *Rhizoctonia solani* and *Rhizoctonia oryzae*, the two studied phytopathogenic microorganisms.

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