Research Journal of Agricultural Sciences
An International Journal

Res. Jr. of Agril. Sci. **12**(2): 470–473

P- ISSN: 0976-1675 | **E**- ISSN: 2249-4538

www.rjas.org

Research Paper

Antagonistic Efficiency of Indigenous Rhizospheric Bacteria against *Macrophomina phaseolina* causing Charcoal Rot in Soybean

Kriti Dave*[1], Madhulika Singh[2] and Ragini Gothalwal[3]

Received: 19 Jan 2021 | Revised accepted: 04 Mar 2021 | Published online: 17 Mar 2021

A B S T R A C T

Charcoal rot caused by *Macrophomina phaseolina* (Tassi) Goid. is one of the economically important diseases of soybean. 30-50% yield losses in soybean have been reported due to charcoal rot worldwide. Being a devastating disease, its management is of utmost concern for sustainable crop production. Chemical fungicides are neither ecofriendly nor safe for other life forms. Biological control is safe, economically and environmentally friendly approach to combat plant diseases. The present study aimed at isolating indigenous bacteria from soybean rhizosphere and evaluate their antagonistic activity against *M. phaseolina* by dual- culture technique. 20 bacterial isolates were able to inhibit *M. phaseolina in -vitro*. Isolate IKK7 and IOK16 exhibited strong inhibition (40.15% and 39.43%) while isolate ISKK64 moderately inhibited the fungus (28.79%) respectively. Potential isolates were identified on the basis of morphological and biochemical characters. The isolates IKK7 and IOK16 were gram positive rods and biochemical characters revealed that IKK7 and IOK16 belong to *Bacillus* species. The isolate ISKK64 was identified as *Serratia* species. These isolates have the potential to be used as biocontrol agents against *Macrophomina phaseolina*. Bioformulations utilizing the biocontrol traits can be developed for management of charcoal rot in soybean and other crops; thereby increasing crop productivity.

***Key words*:** *Macrophomina phaseolina,* Charcoal rot, Biological control, Biofungicide, Bioformulations

Soybean *Glycine max* (L.) Merrill is an important oil yielding crop worldwide, known as the "Golden Bean" of the 21st century [1]. It is a leguminous crop with high quality protein and oil content. It is majorly produced in USA, Brazil, India, Argentina and China. Its production ranks first among the oil seed crops in India. Madhya Pradesh is the largest soybean producing state in India named as Soya State. As per the reports of Directorate of Economics and Statistics, DAC and FW the area under cultivation of soybean in Madhya Pradesh was 5.4 million hectare with a production of 6.65 million tons (2016-2017). In present times, the major source of income for farmers is soybean having 95% marketable surplus. The low productivity of soybean is attributed to its susceptibility to various pathogens causing serious damages.

One of the major pathogen affecting its yield is *Macrophomina phaseolina* which causes charcoal rot in soybean. *Macrophomina phaseolina* (Tassi) Goid. is devastating fungus infecting many agronomically important crops viz. soybean, maize, sorghum and cotton [2]. It is a soil borne pathogen and infects the crop mainly due to moisture stress [3]. The yield loss in soybean crop due to *Macrophomina phaseolina* was reported between 30 to 40% by [4]. *M. phaseolina* being soil inhabitant remains in the soil as sclerotia for prolonged period of time causing infection under adverse conditions [5-7]. It infects soybean plant during vegetative developmental stages. Microsclerotia germinate at a temperature of 20-40°C and infect the plant. Soybean roots get infected early in the season then fungus systematically colonizes the root system and basal stem [8]. Microsclerotia are scattered throughout the tap roots and lower stem giving them charcoal like gray discoloration. Infected plants may wilt and prematurely die with dead leaves remaining attached to the petioles.

Management of charcoal rot is difficult due to saprophytic nature of *Macrophomina phaseolina*. Since it is soil borne pathogen and its propagules are distributed randomly in soil, it is difficult to be controlled by chemical fungicides [9]. Fungicides are expensive, harmful to beneficial microflora of the soil, toxic to humans and animals and cause pollution; their use is not an effective strategy to control fungal pathogens. Biological control proves to be a promising control measure for management of disease. Biocontrol involves the use of beneficial microflora bacteria, fungi and actinomycetes to suppress plant diseases. Rhizosphere provides the initial barrier to the root against pathogen attack; microbes in the rhizosphere are ideal for use as biocontrol agents [10]. BCA present in the rhizosphere colonize the root system to suppress disease and also promote plant growth;

*Kriti Dave
kriti_dave07@yahoo.co.in

[1]Department of Biotechnology and Bioinformatics Center, Barkatullah University, Bhopal - 462 026, Madhya Pradesh, India

[2]Department of Biotechnology, Sadhu Vasvani College, Bairagarh, Bhopal - 462 030, Madhya Pradesh, India

known as PGPR (Plant Growth Promoting Rhizobacteria). Biocontrol is ecofriendly, reduce environmental pollution as they decompose fast and do not leave any residue. Use of antagonistic microorganisms for inhibiting the growth of plant pathogens is practical and safe approach in various crops [11].

The present study was carried out to isolate indigenous rhizospheric bacteria antagonistic to *Macrophomina phaseolina* causing charcoal rot in soybean. The bioefficacy of the isolates to inhibit *M. phaseolina* was evaluated under in-*vitro* conditions. The rhizospheric bacteria isolated can prove to be a promising solution for reducing disease incidence and preventing crop loss. Evaluation of their antagonistic traits and plant growth promoting activites can be exploited to formulate an efficient biofungicide with dual benefits of disease control and improving crop growth.

MATERIALS AND METHODS

Isolation of Rhizobacteria

Soil samples were collected from rhizosphere of healthy soybean fields of Indore, Madhya Pradesh. 1 gram of soil sample was suspended in 10ml of sterile distilled water and diluted serially upto 10^{-5} dilutions. 0.1ml of suspensions from 10^{-4} and 10^{-5} dilutions were spread on nutrient agar plates and incubated at 28°C for 24 hours. The isolates obtained were purified by quadrant streaking method repeatedly till single colonies were not observed. Pure and single colonies were picked and transferred to fresh nutrient agar slants and stored at 4°C for further studies.

In-vitro antagonistic activity of Rhizospheric Bacteria

The antagonistic activity of the isolates against *Macrophomina phaseolina* was evaluated by dual culture technique described by [12] with some modifications. A 5mm mycelial plug from five days old culture of *Macrophomina phaseolina* was placed at the center of the potato dextrose agar plates. Bacterial isolates were streaked 3 cm away from the mycelial plug. The plates were incubated at 28°C for 6-7 days. Plate inoculated with fungus only served as the control plate. The experiment was carried out in triplicates. Radial growth of fungus on control and treatment plates was measured and percent inhibition was calculated using formula:

$$I = \frac{C - T}{C} \times 100$$

Where;
I= Percent Inhibition
C= Radial growth of fungus in control plate (mm)
T= Radial growth of fungus in treatment plate (mm)

Morphological and Biochemical characterization

The potent bacterial antagonists screened were studied for their morphological and biochemical characterization. The colonies were examined for size, shape, pigmentation, opacity etc. Gram staining was performed to study gram reaction and morphology of the isolates. Biochemical tests were performed to identify the potential antagonists. Various tests, viz. catalase test, indole test, MR-VP test, citrate utilization, gelatin liquefaction, starch hydrolysis was studied as described by [13].

RESULTS AND DISCUSSION

Isolation of Rhizobacteria

Forty-eight (48) bacterial strains were isolated from rhizospheric soil samples of soybean. Indigenous bacteria from soybean were isolated and their antifungal activity was evaluated. Soil rhizosphere is rich in beneficial microflora involved in plant growth promotion. Many substances are released by plant roots providing nutrition to microorganisms. As a result, rhizosphere is the major site of microbial interactions. Indigenous bacteria are well adapted to the same environmental conditions as the plants; enabling their use as effective formulation for disease control and growth of regional plants [14].

In- vitro Antagonistic Activity

All of the 48 isolates were screened for their antagonistic activity against *Macrophomina phaseolina* by dual culture technique. 20 isolates were able to inhibit the growth of *M. phaseolina;* their percent inhibition in the range of 20% to 40.15%. The isolate IIK7 was found to be effective antagonist showing 40.15 % inhibition of *M. phaseolina* followed by IOK16 (39.43 % inhibition). Radial growth of pathogen and percent inhibition of five isolates is given in (Table 1).

The results of dual culture technique reveal that the rhizospheric soil of soybean is rich in antagonistic bacteria capable of retarding the growth of *Macrophomina phaseolina*. Rhizosphere of soil is abundant in beneficial microbes having immense potential to be used as biocontrol agents. [15] isolated antagonistic bacteria from rhizosphere of mungbean against *M. phaseolina* causing dry root rot in mungbean. Results show the potential of indigenous bacteria in controlling phytopathogens.

Table 1 Table showing radial growth and percent inhibition of the isolates

Name of the isolate	Radial growth of pathogen (mm)	Percent inhibition (%)
IIK6	59.33	32.57%
IIK7	52.66	40.15%
IOK16	53.33	39.43%
IKAK32	56	36.36%
ISKK64	62.66	28.79%
Control	88	NA

The radial growth of pathogen in mm is the average of triplicates

Morphological and Biochemical Characterization

The antagonistic isolates were characterized on the basis of morphological and biochemical characters. Colonial characters like size, shape, pigmentation, texture and opacity of these isolates were observed. Gram staining results revealed IIK7 as gram-positive rod-shaped bacteria. Colonial characters and gram reactions of five isolates is shown in (Table 2). All the five isolates were able to liquefy gelatin and were positive for catalase activity. Catalase protects the cell from oxidative damage by reactive oxygen species. Bacteria showing catalase activity must be resistant to harsh environmental, chemical and mechanical conditions; thereby surviving stressful conditions [16]. This characteristic seems effective for their use as BCA. All the isolates were positive for VP test and

starch hydrolysis except for ISK64 which was unable to hydrolyze starch. All the five isolates were negative for indole production. The biochemical characters of these five isolates are summarized in (Table 3). On the basis of morphological and biochemical characters bacterial isolates IKK7 and IOK16 showing strong inhibition are found to be *Bacillus* species while the isolate ISKK64 exhibiting moderate inhibition belongs to *Serratia*. Mostly bacteria isolated from rhizospheric soil belong to genera *Bacillus*, *Serratia*, *Pseudomonas* and *Arthrobacter*. These genera of rhizospheric bacteria have proved to be excellent biocontrol agents against soil- borne plant pathogens [17]. *Bacillus* species have been reported as the first successful biocontrol agent used to suppress plant pathogens and insects [18]. *Bacillus* species form endospores which remain dormant for a long period under unfavorable environmental conditions make them efficient colonizers [19].

Table 2 Morphological Characters of the bacterial isolates

Isolate	Size	Pigmentation	Morphology	Gram reaction
IIK6	Large	No	Rods	Gram positive
IIK7	Small to medium	No	Rods	Gram positive
IOK16	Large	No	Rods	Gram positive
IKAK32	Small	Yellowish	Rods	Gram positive
ISKK64	Small	Orange	Rods	Gram negative

Table 3 Biochemical Characters of the bacterial isolates

Isolate	Catalase test	Citrate utilization	Indole production	Methyl Red	Vogues Prosekaur	Gelatin lique-faction	Starch hydrolysis
IIK6	+	-	-	-	+	+	+
IIK7	+	+	-	-	+	+	+
IOK16	+	+	-	-	+	+	+
IKAK32	+	-	-	-	+	+	+
ISKK64	+	+	-	-	+	+	-

Pic: https://crops.extension.iastate.edu

Fig 1 Charcoal rot (*Macrophomina phaseolina*) in soybean

The present study reveals *Bacillus* species IKK7 and IOK16 as potent biocontrol agents exhibiting significant antagonistic activity against *M. phaseolina* (40.15% and 39.43% respectively). Similar results were obtained from the rhizospheric strain *Bacillus* B20b isolated from chickpea soils [20]. B20b inhibited *Rhizoctonia bataticola* (sclerotial stage of *M. phaseolina*) infecting chickpea (percent inhibition reported to be 36.75%). Antagonistic activity of *Bacillus subtilis* CTS-G24 against *Fusarium oxysporium f. sp. ciceri* and *Macrophomina phaseolina* causing wilt and dry root rot in chickpea respectively [21]. *Bacillus subtilis* CTS- G24 was checked for siderophore production. 64% of siderophore units

was produced in succinate medium. It suggests production of siderophore as the underlying mechanism used by CTS- G24 to control *F. oxysporum* and *M. phaseolina*. Our present study suggests the application of both the bacterial isolates IIK7 and IOK16 as effective biocontrol agents in controlling charcoal rot infection in soybean.

Biochemical studies of isolate ISKK64 confirms it as *Serratia* species. ISKK64 is antagonistic to *M. phaseolina*; percent inhibition observed to be 28.79%. It is able to control *M. phaseolina* infection moderately under in- *vitro* conditions. *Serratia* being ubiquitous in nature; is found in water, soil and humans. Rhizosphere of soil has been well documented for existence of *Serratia*. It has been found to be an efficient BCA. Antifungal activity of *Serratia marcescens* indigenous to tea rhizosphere for controlling *Rhizoctonia solani* infection in tea has been studied by [22]. *S. marcescens* strain ETR17 was found effective against phytopathogen under *in-vitro* as well as *in-vivo* conditions. *Serratia* can be an effective BCA for management of plant diseases.

CONCLUSIONS

Charcoal rot caused by *Macrophomina phaseolina* is a major disease affecting soybean production. The present study unveils the potential of *Bacillus* and *Serratia* species in inhibiting *Macrophomina phaseolina* infecting soybean. These isolates can be further studied for the antifungal metabolites involved in antagonism. Plant growth promoting traits of the isolates will be studied to formulate an effective biofertilizer/biopesticide. Co-inoculation of these isolates in the field might prove to be an efficient approach for management of charcoal rot disease in soybean. An effective bioformulation involving these microbes can be prepared for sustainable agriculture.

LITERATURE CITED

1. Meena P, Maravi PS, Vyas MD. 2017. Performance of soybean genotypes under varying plant densities. *Soybean Research* 15(2): 61-64.
2. Kumar P, Gaur VK, Meena AK, Paliwal SK, Meena AK. 2015. Evaluation of antagonists isolated from rhizosphere of Mungbean against *Macrophomina phaseolina*. *International Journal of Therapeutic Applications* 33(4): 2531-2536.
3. Arya VK, Vishunavat K, Himanshu N. 2004. Detection, location and transmission of seed – borne inoculums of *Macrophoimna phaseolina* in charcoal rot in soybean. *Jr. Mycol. Pl. Pathology* 34(2): 233-237.
4. Yang XB, Navi SS. 2005. First report of charcoal rot epidemics caused by *Macrophomina phaseolina* in soybean in Iowa. *Plant Diseases* 89: 526.
5. Gangopadhyay S, Wyllie TD, Teague WR. 1982. Effect of bulk density and moisture content of soil on the survival of *Macrophomina phaseolina. Plant and Soil* 68(2): 241-247.
6. Short GE, Willie TD, Bristow PR. 1980. Survival of *M. phaseolina* in soil and residue of soybeans. *Phytopathology* 70: 13-17.
7. Sheikh AH, Ghaffar A. 1979. Relation of sclerotial inoculums density and soil moisture to infection of field crops by *Macrophomina phaseolina*. *Pakistan Journal of Botany* 11: 185-189.
8. Mengistu A, Smith JR, Ray JD, Bellaloui N. 2011. Seasonal progress of charcoal rot and its impact on soybean productivity. *Plant Disease* 95: 1159-1166.
9. Kumari P, Khanna V, Sharma P, Kumari S. 2015. Metabolites in control of *Rhizoctonia bataticola*. *Journal of Pure and Applied Microbiology* 9(1): 749-756.
10. Weller DM. 1988. Biological control of soil borne plant pathogens in the rhizosphere with bacteria. *Ann Rev of Phytopathology* 26: 379-407.
11. Patel ST, Anahosur KH. 2001. Potential antagonism of *Trichoderma harzianum* against *Fusarium* spp., *Macrophomina phaseolina* and *Sclerotium rolfsii*. *Jr. Mycol. Plant Pathology* 31: 365-366.
12. Morton DJ, Stroube WH. 1995. Antagonistic and stimulatory effects of soil microorganisms upon *Sclerotium rolfsii*. *Phytopahtology* 45: 417-420.
13. Aneja KR. 2011. *Experiment in Microbiology, Plant Pathology and Biotechnology*. New Age International Publisher. pp 253-272.
14. Patil S, Bheemaraddi MC, Channappa T, Shivannavar, Gaddad SM. 2014. Biocontrol activity of siderophore producing *Bacillus subtilis* CTS-G24 against wilt and dry root rot causing fungi in chickpea. *IOSR, Journal of Agriculture and Veterinary Science* 7(9): 63-68.
15. Kumar A, Saini P, Shrivastava JN. 2008. Production of peptide antifungal antibiotic and biocontrol activity of *Bacillus subtilis*. *Indian Jr. Exp. Biology* 47: 57-62.
16. Patil S, Shivannavar CT, Bheemaraddi MC, Gaddad SM. 2015. Antiphytopathogenic and plant growth promoting attributes of *Bacillus* strains isolated from rhizospheric soil of chickpea. *Jr. Agr. Sci. Tech* 17: 1365-1377.
17. Joseph B, Patra RR, Lawrence R. 2007. Characterization of plant growth promoting rhizobacteria associated with chickpea (*Cicer arietinum* L.). *Int. Jr. Pl. Production* 1: 141-152.
18. Powell KA, Justum AR. 1993. Technical and commercial aspects of biological control products. *Pesticide Science* 37: 315-321.
19. Fritze D. 2004. Taxonomy of the genus *Bacillus* and related genera: The serobic endospore forming bacteria. *Phytopathology* 94: 1245-1248.
20. Viso NP, Montecchia M, Zilli C, Balestrasse K, Carmona M. 2015. Evaluation of native bacteria and manganese phosphite for alternative control of charcoal root rot of soybean. *Microbiological Research* 180: 40-48.
21. Choudhary D. 2011. Plant growth-promotion (PGP) activities and molecular characterization of rhizobacterial strains isolated from soybean (*Glycine max* L. Merril) plants against charcoal rot pathogen *Macrophomina phaseolina. Biotechnological Letters* 33: 2287-2295.
22. Purkayastha GD, Mangar P, Saha A, Saha D. 2018. Evaluation of the biocontrol efficacy of a *Serratia mercescens* strain indigenous to tea rhizosphere for the management of root rot disease in tea. *Plos One* 13(2): 1-27.