The Pharma Innovation Journal 2022; SP-11(6): 2451-2455

www.ThePharmaJournal.com

The Pharma Innovation

ISSN (E): 2277-7695
ISSN (P): 2349-8242
NAAS Rating: 5.23
TPI 2022; SP-11(6): 2451-2455

www.thepharmajournal.com
Received: 01-03-2022
Accepted: 04-04-2022

Paryotampal Kaur
Department of Agriculture, Guru Kashi University, Talwandi Sabo, Bathinda, Punjab, India

Amanpreet Singh Sran
Department of Agriculture, Guru Kashi University, Talwandi Sabo, Bathinda, Punjab, India

Manpreet Kaur Chahal
Department of Agriculture, Maharishi Markandeshwar University, Mullana, Ambala, Haryana, India

Gagandeep Singh Sohi
Department of Agriculture, Guru Kashi University, Talwandi Sabo, Bathinda, Punjab, India

Mamta beniwal
Department of Agriculture, Guru Kashi University, Talwandi Sabo, Bathinda, Punjab, India

Dalwinder Singh
Department of Agriculture, Guru Kashi University, Talwandi Sabo, Bathinda, Punjab, India

Anil Kumar
Department of Agriculture, Lovely Professional University, Phagwara, Jalandhar, Punjab, India

Bahaderjit Singh
Department of Agriculture, Guru Kashi University, Talwandi Sabo, Bathinda, Punjab, India

Corresponding Author
Paryotampal Kaur
Department of Agriculture, Guru Kashi University, Talwandi Sabo, Bathinda, Punjab, India

Studies on isolation of microbes from the rhizosphere zone of wheat and rice fields under Malwa and Majha region of Punjab

Paryotampal Kaur, Amanpreet Singh Sran, Manpreet Kaur Chahal, Gagandeep Singh Sohi, Mamta beniwal, Dalwinder Singh, Anil Kumar and Bahaderjit Singh

Abstract
Rhizosphere, the soil region nearest to plant root system, inhabits various microorganisms. Rhizosphere has the most density and diversity of microbes. The present work deals with the isolation of microorganisms from the rhizospheric soil of wheat and rice in Malwa and Majha region (Talwandi Sabo, Amritsar and Gurdaspur) of Punjab. The average number of bacteria ranges from 3 to 7 (per gm), fungi ranges from 3 to 8 (per gm), Actinomycetes 0 to 1 (per gm) and Yeast ranges from 0 to 4 (per gm) from parts of Punjab region. The fungal cultures isolated from all three parts were identified as *Alternaria* spp., *Aspergillus* spp., *Rhizopus* spp., *Fusarium* spp., *Penicillium* spp., *Cladosporium* spp., *Chrysosporium* spp. Thirty two bacterial cultures were isolated from all three parts of Punjab region. Ten fungal and fifteen bacterial cultures were selected for their antifungal activity against *Alternaria macrospora* during primary screening. Out of 15 bacterial isolates, three isolates B15, B14, B11 and out of ten fungal isolates five isolates *i.e. Rhizopus, Alternaria, Fusarium, Cladosporium and Aspergillus* shows good inhibitory effect against *Alternaria macrospora*. During secondary screening the bacterial isolate B15, B14 and B11 shows maximum zone of inhibition against *Pyricularia oryzae, Alternaria macrospora, Alternaria citri* and *Diplodia natalensis.* While fungal isolates control various pathogens but unable to control all the four pathogens. *Alternaria macrospora* was controlled by all the fungal isolates (selected during primary and secondary screening). Maximum zone of inhibition was shown by fungal isolate F10 (*Rhizopus* spp.) and minimum by F8 (*Aspergillus* spp.) against *Alternaria macrospora i.e.* 13mm and 8mm respectively. All the fungal isolates were unable to show inhibition zone against *Diplodia natalensis* and *Alternaria citri.* Considering the importance of rice and wheat crops and the cost of management of diseases, it is very important to study the microflora associated with these crops.

Keywords: Bacteria, fungi, isolation, rhizosphere

Introduction
The microorganisms are the main component of soil so they are more in soil than any other environment. The microorganisms include bacteria, fungi, algae, protozoa, nematodes and arthropods (Raaijmakers *et al.,* 2009) [9]. The diversity of microorganism depends on the availability of nutrients and their varied concentration (Willey *et al*., 2008) [12]. The microorganisms are higher in organically rich surface layers than in the underlying mineral soil. The estimation (quantitative and qualitative) of soil microorganism is necessary to know the role of different microorganisms (Arey 2010) [2].

Mostly bacteria and fungi are found in the rhizosphere. The rhizosphere is the narrow region of soil that is directly influenced by root secretion, and associated soil microorganisms known as microbiome. Rhizosphere has the most density and diversity of microbes (Clark, 1940). The rhizosphere differs from the bulk soil because of the activities of plant roots and their effect on soil organisms. Microorganisms play important role in various biological transformations taking place in soil and they also decompose the organic matter (Heritage *et al*., 1999) [4]. Soil bacteria and fungi are important in formation of soil aggregates. They protect plant from disease and intimately associated with plant growth and productivity.

Some commonly used beneficial microorganisms in agriculture include *Rhizobia, Mycorrhizae, Azospirillum, Bacillus, Pseudomonas, Trichoderma, Streptomyces* species. Rhizosphere bacterial communities have become a major focal point of research in recent years, especially regarding how they affect plants and vice versa (Philippot *et al*., 2013)

To solve new emerging disease problems and to advance in biotechnology, the study of microbial diversity is important. The use of biological fertilizers in recent times, is receiving attention mainly on account of increased global preference for natural organic products. To use environmental friendly microbes various steps are important i.e. isolation of microorganisms, screening for desirable characters, selection of efficient strains and production of inoculums. When these cultures are introduced into the natural environment, their individual beneficial effects are greatly magnified in a synergitic fashion. Microbial inoculants containing many kind of natural occurring beneficial microbes called "Effective microorganisms" has been used widely in nature and organic farming (Karthick *et al.*, 2011) [5].

Aim and Objectives

- Evaluation of microflora from rhizosphere soil of rice and wheat collected from Malwa and Majha region of Punjab.
- Identification and characterization of isolated microflora.
- Screening of antagonistic bacterial and fungal isolates against various fungal pathogens.

Material and Method

Table 1: Collection of rhizosphere soil samples from different area of Punjab (Malwa and Majha region)

Region	Soil sample	Areas	Isolation 1 (Feb 2019)	Isolation 2 (Sep. 2019)
Malwa	S1	Talwandi sabo, Bathinda (Punjab)	Rhizosphere of wheat	Rhizosphere of rice
Majha	S2	Amritsar (Punjab)	Rhizosphere of wheat	Rhizosphere of rice
	S3	Gurdaspur (Punjab)	Rhizosphere of wheat	Rhizosphere of rice

Soil samples were collected into small sterilized polythene bags and brought to laboratory for further studies.

Culture media used for isolation of rhizosphere soil microflora

Nutrient Agar (NA): For Bacteria and Yeast
Potato Dextrose Agar (PDA): For fungi and yeast

Isolation of rhizosphere microflora and screening of bacteria for antifungal activity

Identification of rhizosphere soil fungi

Fungal morphology were studied macroscopically by observing colony features (Color and Texture) in centre plate technique and microscopically by staining with lacto phenol cotton blue and observe under compound microscope for conidia, conidiophores, and arrangement of spores Aneja (2001) [1]. The fungi were identified with the help of literature Mukadam (1997) [7], Gilman (1957) [3] and Nagamani (2006) [8]

Identification of rhizosphere soil bacteria

The rhizospheric bacteria isolates were identified by studying their colony and Gram staining (microscopic morphology) described by Bergey's Mannual of systematic bacteriology.

Screening of bacterial and fungal isolates for antifungal activity

Primary screening

It determines the capability of the bacteria and fungus to produce antifungal without giving significant idea about the production or yield potential of the organism. Primary screening for antifungal activity done by well diffusion method.

Secondary screening

The active isolates selected from primary screening, were further subjected to secondary screening, against various other pathogenic fungi. Secondary screening for antifungal activity also done by well diffusion assay.

Selective media for bacterial growth and production of bioactive metabolites

The selected media (Nutrient Broth) and Potato Dextrose Broth (PDB) that allows the growth as well as high production of bioactive metabolites for bacterial and fungal strain. A loopfull purified bacteria were transferred in 250 ml flask containing 50 ml of nutrient broth (NB) and a loopfull purified fungi were transferred in flask containing PDB. The cultures were inoculated at 32 °C for bacteria and at 28 °C for fungi with 180 rpm shaking at rotator shaker for 2 days. After shaking 10-20 ml of each sample was harvested and centrifuged for 15 min. at 10,000 rpm for separation of supernatant. Then the supernatant was filtered through bacterial filter paper (Milipore filter 0.45µm) to get cell free samples. Antifungal activity of each cell free extract was determined using well diffusion assay (Schillinger and Lucke, 1989) [10] 50 to 200µm of sample extract having OD 1.1 was loaded and tested. The filterate was transferred aspetically into conical flasks and stored at 4 °C for further assay.

Well diffusion assay

Well diffusion assay was a modification of procedure described by Schillinger and Lucke (1989) [10]. PDA poured in petri dishes, after solidification wells of 5mm in a diameter were made using sterile cork borer and then the cells free filterates (100µl) were added separately in wells and tested fungal pathogens were spread on the surface of the cultivated PDA. The petri dishes were incubated at 25- 28 °C for 48-72 hours. After incubation the diameter of zone of inhibition around the wells was measured in millimeters scale to evaluate the antifungal activity of bacterial isolates and fungal isolates.

Result and Discussion

Quantitative data of rhizosphere soil microflora isolated from three different soils

To examine the number of bacteria, fungi, actinomycetes and yeast in per gram of rhizosphere soil of wheat and rice collected from the field of Guru Kashi University (Talwandi Sabo, Bathinda), the serial dilution and spread plate method was used under aspectic conditions. 10^{-4} and 10^{-3} dilutions were chosen for bacteria, fungi, actinomycetes and yeast. The number of fungi in the soil ranges from 6 to 8 (per gram), bacterial numbers 3to 7 (per gram), actinomycetes and yeast number 1(per gram)

In Amritsar region the number of bacteria, fungi, actinomycetes and yeast were range from 6,7,0,5 per gram of soil respectively.

In Gurdaspur region the number of bacterial culture were 5 (per gram) while the number of fungal culters were ranges

from 3 to 5 per gram of soil. The actinomycetes were not present there. The populations of yeast culters were range from 1 to 4 (per gram).

Morphological Identification of Isolated fungi

The fungal isolates from the rhizosphere soil of wheat and rice in three different areas of Punjab as shown in table 4, 5 and 6 were identified into 7 genera i.e. *Alternaria* spp., *Fusarium spp.*, *Rhizopus* spp., *Aspergillus* spp. *Penicillium* spp., *Cladosporium* spp. and *Chrysosporium* spp.. In 2016 Seth and co-workers conducted same study and isolate various strains of *Aspergillus* spp., *Fusarium* spp and *Alternaria* spp. from soil of wheat cultivated area of Uttar Pradesh.

Table 2: Isolated Fungi from three different area of Punjab (Malwa & Majha)

S. No.	Talwandi sabo	Amritsar	Gurdaspur
1	-	*Alternaria* spp.	*Alternaria* spp.
2	*Aspergillus* spp.	*Aspergillus* spp.	-
3	-	*Fusarium* spp.	*Fusarium* spp.
4	*Rhizopus* spp.	-	*Rhizopus* spp.
5	-	*Cladosporium* spp.	-
6	*Penicillium* spp.	-	-
7	-	*Chrysosporium* spp.	-

Identification of Fungal Isolates

The five fungal isolates i.e., F10, F16, F7, F18, and F8 which gave good result against *Alternaria* spp. were identified as *Rhizopus* spp., *Penicillium* spp., *Fusarium* spp., *Cladosporium and Aspergillus* spp. respectively.

Characterization and identification of active bacterial isolates

The three bacterial isolates B11, B15 and B14 found to have broad spectrum of antifungal activities were selected for further characterization on the basis of morphological colonies and staining.

Morphological characterization of Bacteria

The bacterial isolate B15 was white in color, B11 was creamish white and B14 was creamish in color. Their identification was made on the basis of further studies.

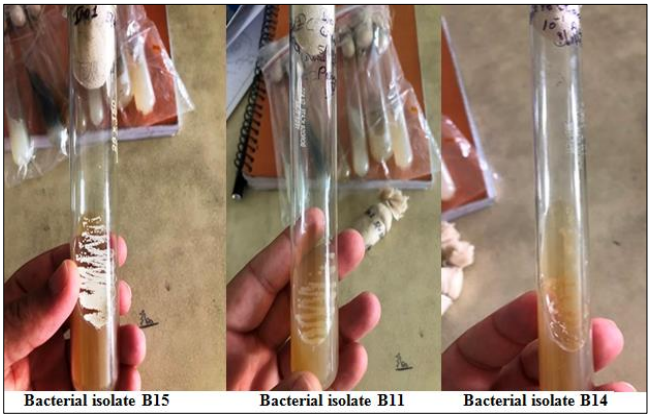


Fig 1: Morphological characterization of bacteria

Gram Staining

In gram staining studies both B14 and B15 bacterial isolates were identified as gram positive. The B11 isolate was identified as gram negative.

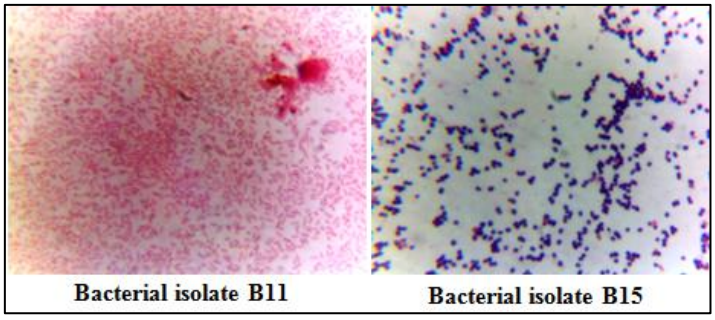


Fig 2: Gram staining Screening of bacterial and fungal isolates for Antifungal activity

Primary Screening

In the primary screening to check the antifungal activity, 15 bacterial isolates and 10 fungal isolates were tested against *Alternaria macrospora* pathogen due to culture availability in department Laboratory. Out of 15 bacterial isolates 3 isolates B11, B15 and B14 shows good inhibitory effect against *Alternaria macrospora* while in fungal isolates 5 isolates (F10, F16, F7, F18, F8) out of 10 shows good inhibitory effect against *Alternaria macrospora*. Both bacterial and fungal isolates which gave good inhibitory effect against *Alternaria macrospora* and selected for further secondary screening against various pathogen by well diffusion assay.

Secondary screening of bacterial and fungal isolates

In secondary screening the selected bacterial and fungal isolates were further tested against 4 pathogens (*Pyricularia oryzae*, *Alternaria citri*, *Alternaria macrospora* and *Diplodia natalensis*). All three bacterial isolates showed antifungal activity against *Pyricularia oryzae*, *Alternaria citri*, *Alternaria macrospora* and *Diplodia natalensis.* Bacterial isolate B11 shows antifungal activity against *Pyreculeria oryzae*, *Alternaria citri*, *Alternaria macrospora* and *Diplodia natalensis* with zone of inhibition 21mm, 13mm, 15 and 16 mm respectively. Isolate B14 shows antifungal activity against *Pyricularia oryzae*, *Alternaria citri*, *Alternaria macrospora* and *Diplodia natalensis* with inhibition zone 15, 20, 15 and 20 mm respectively. Zone of inhibition by bacterial isolate B15 against *Pyricularia oryzae*, *Alternaria citri*, *Alternaria macrospora and Diplodia natalensis* was 23, 16, 17 and 20 respectively (Table-3 and Fig.-3).

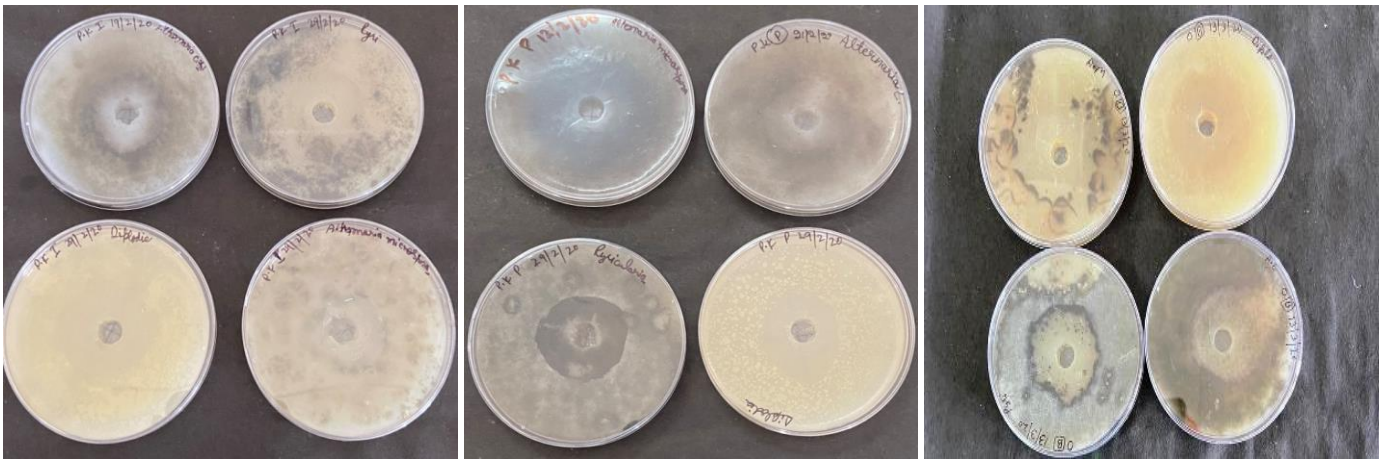
Alternaria macrospora was controlled by all selected fungal isolates during secondary screening. Maximun zone of inhibition was shown by fungal isolate F10 and minimum by F8 against *Alternaria macrospora* i.e. 13mm and 8mm respectively. All the fungal isolates were unable to show inhibition zone against *Diplodia natalensis* and *Alternaria citri* (Table-4 and Fig.-4).

Table 3: Inhibition Zone by bacterial isolates against various pathogens

S. No.	Tested pathogen	Inhibition zone by isolate B11 (in mm)	Inhibition zone by isolate B14 (in mm)	Inhibition zone by isolate B15 (in mm)
1	*Pyricularia oryzae*	21	15	23
2	*Alternaria citri*	13	20	16
3	*Alternaria macrospora*	15	15	17
4	*Diplodia natalensis*	16	20	20

Table 4: Inhibition Zone by different Fungal isolates against various pathogens

S. No.	Test phytopathogen	Inhibition zone by F10	Inhibition Zone by F16	Inhibition Zone by F7	Inhibition Zone by F18	Inhibition Zone by F8
1	*Pyricularia oryzae*	-	-	10mm	11mm	-
2	*Alternaria citri*	-	-	-	-	-
3	*Alternaria macrospora*	13mm	9mm	9mm	10mm	8mm
4	*Diplodia natalensis*	-	-	-	-	-



A- Bacterial isolate B15 B- Bacterial isolate B14 C- Bacterial isolate B11

Fig 3: Inhibition Zone by bacterial isolates against various pathogens.

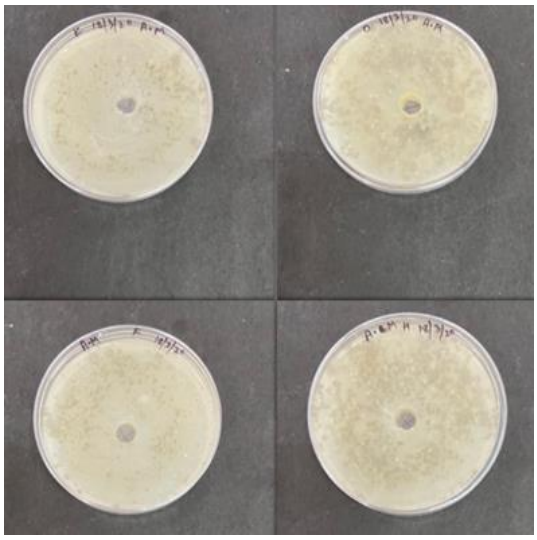


Fig 4: Inhibition zone by different fungal isolates against

Alternaria macrospora

Total 36 cultures of fungi were isolated from all three parts of Punjab region. Maximum number of fungus was present in the rhizosphere rice soil of Talwandi Sabo while the rhizosphere soil of rice of Gurdaspur region had least fungal cultures. The fungal cultures isolated from rhizosphere of wheat and rice were *Alternaria* spp., *Fusarium* spp., *Rhizopus spp*., *Aspergillus* spp., *Penicillium* spp., *Cladosporium* spp. and *Chrysosporium* spp. The isolated bacterial culture from the rhizosphere of wheat and rice were 32 in number. Actinomycetes were only present in rhizospheric wheat soil of Talwandi Sabo (Bathinda) which was only one in number. Total 11 cultures of yeast were isolated from all three regions. The Gurdaspur regions have maximum number of yeast i.e. 4 while Talwandi Sabo region have least number i.e 1.

The results indicate that bacterial isolates i.e. B11, B14 and B15 found to have broad spectrum of antifungal activities against selected pathogens while fungal isolates also effective against various pathogens but no one fungus is able to control all the four pathogens. Hence fungal isolates were unable to

control all the four pathogens. *Alternaria macrospora* was controlled by all the fungal isolates (selected during primary and secondary screening). Maximum zone of inhibition was shown by fungal isolate F10 (*Rhizopus* spp.) and minimum by F8 (*Aspergillus* spp.) against *Alternaria macrospora i.e.* 13mm and 8mm respectively during secondary screening. All the fungal isolates were unable to show inhibition zone against *Diplodia natalensis* and *Alternaria citri.*

Conclusion

In our investigation, *Alternaria macrospora* was controlled by all the fungal isolates (selected during primary and secondary screening). Three bacterial isolates from Amritsar such as B15, B14 and B11 gave good antagonistic activity against *Pyricularia oryzae*, *Alternaria macrospora*, *Alternaria citri* and *Diplodia natalensis* while fungal isolates also effective against various pathogens but no one fungus is able to control all the four pathogens. Hence fungal isolates were unable to control all the four pathogens. From the results it was observed that the three bacterial isolates suppress the major field disease such as rice blast, alternaria blight of cotton and Pre-harvest fruit drop of citrus.

Acknowledgements

The authors are grateful to Guru Kashi University, Talwandi sabo, Bathinda (Punjab) for providing necessary facilities and we are also thankful to Dr. Dilip Monga, Ex. - Head & Principal Scientist (Plant Pathology), Indian Council of Agricultural Research-CICR, Regional Station (Haryana), India for their valuable guidance and useful suggestions.

References

1. Aneja KR. Biochemical activities of microorganisms, Experiments in Microbiology, Plant pathology and biotechnology, New age International Publishers. 2001;4:157-162.
2. Arey NC. Manual of environmental analysis, Ane Books PVT. 2010, 424.
3. Gilman JC. A manual of soil fungi. 2nd Ed. The lowa state University press. Ames. lowa. 1957, 1-450.
4. Heritage J, Evans EGV, Killington RA. The microbiology of soil and nutrient cycling. In: Microbiology in action. Cambridge University Press, U.K. 1999, 289.
5. Karthick S, Namsivagam R, Narendrakumar G, Kumar AJ. Evaluation of effective microoraganisms (EM) for treatment of domestic sewage. Journal of Experimental Science. 2011;2(7):30-32.
6. Laurent P, Jos M, Philippe L, Wim H. 2013.
7. Mukadam DM. Te Illustrated kingdom of Fungi, Akshar Ganga Prakashan, Aurangabad (M. S.) India. 1997.
8. Nagamani A, Kunwar IK, Monoharachary C. Handbook of soil fungi, I. K. International Private limited, 2006.
9. Raaijmakers JM, Paulitz TC, Steinberg C, Alabouvette C, Moenne-Loccoz Y. The rhizosphere: A playground and battlefield forsoilborne pathogens and beneficial microorganisms. Plant Soil. 2009;321:341-361.
10. Schillinger U, Lucke FK. Antibacterial activity of *Lactobacillus* isolated from meat. App. Enviorn. Microbiol. 1989;55:1901-1906.
11. Seth RK, Alam S, Shukla DN. Isoaltion and Identification of soil fungi from wheat cultivated area of Uttar Pradesh. Journal of Plant Pathology and Microbiology. 2016;7:384.
12. Willey JM, Sherwood LM, Woolverton CJ. Prescott, Harley and Klein's microbiology, 7th edn. McGraw-Hill Companies, Inc., America, New York, 2008, 667-713.