Sci.Int.(Lahore),28(4),4119-4122,2016 ISSN 1013-5316;CODEN: SINTE 8

RHYZOBACTERIA ASSOCIATED FROM RHYZOSPHERIC SOIL AND THERE ANTIFUNGAL ACTIVITY AGAINST BROWN SPOT FUNGUS (*HELMINTHOSPORIUM ORYZAE*)

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ABSTRACT: *The current research was studied to search out soil bacteria antagonistic to brown spot fungus Helminthosporium oryzae. Soil samples were collected from two different locations viz. district larkana (Dokri and Bakrni). Bacteria were isolated, purified multiplied and morphologically characterized. Antagonistic test between bacteria and test fungus were carried out potential bio-antagonistics were tentatively identified as pseudomonas spp. A total of 7 bacterial isolates were recovered from the cuticular surface and tested for antagonistic effect against test fungus. Among 14 strains one was found to be gram positive and remaining was found gram negative. A basic principle for the success of a biological control programme is a good adaptation of a given BCA to the local environmental conditions. The aim of this work was to evaluate the possibilities of exploitation of bacteria in the biocontrol of Brown spot of rice, being a devastating fungus to rice industry. Hence these studies will be helpful to control the brown spot of rice caused by Helminthosporium oryzae in sindh.*

Keywords: Rice, Rhyzobacteria, brown spot fungus, *Helminthosporium oryzae*

INTRODUCTION

Rice (*Oryza sativa*. L) is one of the major crops of the developing countries in the world and staple paddy rice for the people. It was recorded during fiscal year 2014 the annual rice production was 741.3 million tonnes [1]. Approximately, 90% of the total rice is produced in Asia [2]. Rice cultivation is found to be assaulted by different biotic factors including diseases and pests which detrimentally affect yield per unit area. Among the number of disquieting diseases, brown spot caused by *Helminthosporium oryzae* is most disastrous and reported in all paddies rice overall the countries [3]. Although this disease has been abandoned and overlooked as a major rice disease, but history reveals that the famous Bengal starvation in 1942 occurred anticipated to this ruinous problem which caused significant deficits to humanity [4]. Brown spot fungus disorders the plant at any growing stage, triggering seedling and adult plant infection. It appears on the foliage as scattered brown spot that coalesces and bring about withering and yellowing of the leaves. Moreover, it inhibits germination, triggering rotting of seeds, roots and coleoptiles. Poor germination and poor seedling vigor further cause extensive economic loss [5: 6: 7] Production losses due to this disease have been recorded in the range of 16% to 43% [8] This disease is referred as "poor rice farmer's disease" [9] as it has been observed on the rice crop facing drought and macro-nutrient deficiency [10]. [11] reported the occurrence of brown spot of rice in silicon deficient soils. Similarly [12] reported this disease on rice in soils deficient in potassium, manganese, magnesium, silicon, iron, or calcium. Different approaches such as the use of fungicides, biocontrol agents, resistant cultivars, agronomic practices and biotechnological methods can be employed to manage brown spot of rice [13]. Between all the control procedures biological control has been found most effective and environmentally sound. Rhizobacteria have been found most effective to control plant diseases that are caused by other bacteria and fungus. Disease is suppressed through induced systematic resistance and through the production of anti-fungal metabolites. *Pseudomonas* biocontrol strains have been genetically modified to improve plant growth and increase the disease resistance of agricultural crops. In agriculture, inoculants bacteria tend to be applied to the seed coat of plant seeds prior to being sown. Inoculated seeds are more likely to establish large enough rhizobacteria populations within the rhizosphere to produce notable beneficial effects on the crop. Hence, present studies were conducted to search out such bacteria from rice rhizosphere and to check their role against brown spot fungus *(Helminthosporium oryzae).*

MATERIALS AND METHODS

Survey and sampling of paddy seeds

Infected samples consisting of seeds of IR-6, IR-8, IR-9, Super Basmati, Shahkar, DR-82, DR-83, Dokri Basmati, Basmati 270, Basmati 385, Super Karnal, Danglo hybrid, Rachana hybrid and Maharani Hybrid rice varieties, were gathered from the Larkana areas of Sindh province. These types of rice varieties are common cultivated in Larkana district. These samples were located in paper bags which were accurately labeled and brought to the laboratory for isolation of disease causing fungi

Survey and sampling of Rhizospheric soil

Soil samples were collected from several rice growing area of Sindh such as Larkana (Dokri) and Bakrani. These soil samples are generally collected in the region of sindh province these samples were put in paper begs which were properly labeled and brought to the laboratory for isolation of Rhizobacteria for antagonistic tested

ISOLATION AND PURIFICATION OF FUNGI

Isolation of pathogen was carried out from contaminated grains and panicles. Discolored grains were collected from the experimental field of NIA, Tandojam. The infected grains were separated from panicles and kept in petri plates. Seeds were surface sterilized with 5% sodium hypochlorite solution for two minutes and put on fresh potato dextrose agar (PDA). Fifteen sterilized seeds were kept in three PDA plates (10 seeds/plate) and these plates were incubated at 28 ^{0}C for 5-7 days. Pathogen was purified through taking little bit of fungus and transferring them in next PDA plate. Identification of pathogen was done by studying colony characteristics, spore shape and size following the Method

4120 ISSN 1013-5316;CODEN: SINTE 8 Sci.Int.(Lahore),28(4),4119-4122,2016

described in the Technical Bulletin of the Seed Borne Diseases and Seed Health Testing of Rice [14;15]. The pure cultures of the isolated pathogen were preserved at 5 ^{0}C on PDA slants for further studies Isolation of rhizobacteria was also done from the same soil according to [16]. Soil from rhizosphere of rice plants was taken, from which rhizobacteria was isolated. One gram of soil was mixed in the 20 ml test tube containing 9 ml autoclaved saline. The suspension was mixed and dilutions were made up to 10^{-8}. 0.1ml of each dilution was spread on Lauria Bertani plates and plates were incubated at 28 ± 3 ^{0}C until colony development was observed and purification was done by streaking method.

GRAM STAINING

Gram's stain is method which is used to separate bacteria into two groups, the Gram-negative and Gram-positive types. The stain and procedure are as follows:

1. Stain one minute in crystal violet.
2. Wash brief in water.
3. Cover or immerse for one minute in Gram's iodine solution:
4. Wash briefly in water and blot dry.
5. Decolourize in 95% ethyl alchohol for 30 second with slight agitation.
6. Wash in water and counterstain with safranin for 10 seconds.
7. Wash dry, mount and examine.

By this method, some bacteria will retain the violet stain and be dark, and other will be distained and appear red.

ANTAGONISTIC ACTIVITY OF BACTERIA AGAINST *HELMINTHOSPORIUM ORYZAE*

A total of 7 bacterial isolates were recovered from the cuticular surface and tested for antagonistic effect against test fungus. A first, screening to evaluate the effect of potential antagonists on the fungal growth was carried out by a central disk test assay [17]. Briefly, the fungal strain was cultured on Potato Dextrose Agar (PDA) for 7 days at 28º C and a 7 mm mycelium disk from the sporulation area was cut and transferred to the centre of a PDA plate. At the same time bacterial culture was streake opposite to test fungus. Then Petri plates were incubated at 28^{0}C. Data was recorded when growth of both the microorganism initiated.

RESULTS AND DISCUSSIONS

Several fungus growths appeared on PDA plates. Only *Helminthosporium* was considered for further studies. It was purified and identified using light microscope. Moreover samples collected from Dokri and Bakrani were processed for isolation of bacteria. Among 14 strains one was found to be gram positive and remaining were found gram negative. The bacteria retarding *Helminthosporium* growth in antagonistic test were tentatively identified as *Pseudomonas* spp. Biological control is an fascinating alternative using antagonistic bacteria has been reported as an attractive alternative as the ability to antagonize the pathogen which is effective for colonizition [18]. Gram-negative bacteria genera Pseudomonas has been utilized frequently [19; 20; 21]. Whereas. Gram-positive Bacillus species contain several benefits as for the use of biological control agents (BCA). The antagonistic effect process various types of antimicrobial compounds, such as antibiotics (e.g., bacilysin, iturin, mycosubtilin) and siderophores [22]. Secondly, they have the capability to induce growth and defense responses in the host plant [23]. Additionally, the Bacillus develop resistant spores resistant to UV light, allowing them to control the harmful conditions, and produce formulation for commercial purposes [18]. This biological control method is a basic principle and good adaptation of a given BCA to the local environmental conditions.. In this sense, the aim of this work was to evaluate the possibilities of exploitation of bacteria in the biocontrol of Brown spot of rice, being a devastating fungus to rice industry

Table-1. Colony Characteristics of Soil bacteria isolated from Dokr

Strain No.	Location	Host	Size	Shape	Color	Orientation/Border
1	Dokri	Rice Soil	Medium	Filaments	Off-white	Irregular
2	Dokri	Rice Soil	Small	Round	Off-white	Wavy
3	Dokri	Rice Soil	Small	Raised and round	Pale	Smooth
4	Dokri	Rice Soil	Medium	Flat and round	Off-white	Irregular
5	Dokri	Rice Soil	Small	Flat	Yellow	Smooth
6	Dokri	Rice Soil	Large	Raised and round	Off-White	Smooth
7	Dokri	Rice Soil	Medium	Flate and Round	Off-white	Smooth

Table-2. Colony Characteristics of Soil bacteria Isolated from Bakrani

Strain No.	Origin	Host	Size	Shape	Color	Orientation/Border
1	Bakrani	Rice Soil	Medium	Raised and Round	Pale	Smooth
2	Bakrani	Rice Soil	Small	Round	Off-white	Wavy
3	Bakrani	Rice Soil	Small	Raised and irregular	Pale	Smooth
4	Bakrani	Rice Soil	Small	Flat and round	Off-white	Smooth
5	Bakrani	Rice Soil	Large	Raised and round	Off-white	Smooth
6	Bakrani	Rice Soil	Medium	Flat and round	Off-White	Smooth
7	Bakrani	Rice Soil	Small	Round	Orange	Smooth

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Table-3. Morphological characteristics of bacterial cell Isolated from Dokri Soil

Strain No.	origion	Host	Size	Shape	Motility	Gram reaction
1	Dokri	Rice Soil	Long	Rods	motile	-
2	Dokri	Rice Soil	Small	Round	Non motile	-
3	Dokri	Rice Soil	Medium	Cylinderical	Non motile	-
4	Dokri	Rice Soil	Small	Beet like	Non motile	_
5	Dokri	Rice Soil	Small	Round in chain	Motile	-
6	Dokri	Rice Soil	Medium	Round	Non motile	-
7	Dokri	Rice Soil	Small	Curved rods	Non motile	-

Table-4. Morphological characteristics of bacterial cell Isolated from Bakrani **Soil**

Strain No.	Origion	Host	Size	Shape	Motility	Gram reaction
1	Bakrani	Rice Soil	Medium	Rods	Non motile	+
2	Bakrani	Rice Soil	Small	round	Non motile	-
3	Bakrani	Rice Soil	Medium	Curved Rods	Non motile	-
4	Bakrani	Rice Soil	Small	Beet like	Non motile	-
5	Bakrani	Rice Soil	Small long	Curved rods	Motile	-
6	Bakrani	Rice Soil	Medium	Round	Motile	-
7	Bakrani	Rice Soil	Small	Round	Motile	-

Table 5. Bioantagonistic activity of Rhizobacteria against *Helminthosporium Oryzae*

S.No	Day 3	Day-4	Day-5	Day-6
1	-	-	-	-
2	-	-	-	-
3	-	-	-	-
4	-	-	-	-
5	-	-	-	-
6	++	++	++	++
7	+	+	+	+
8	-	-	-	-
9	-	-	-	-
10	-	-	-	-
11	-	-	-	-
12	-	-	-	-
13	++	+++	+++	+++
14	+	+	+	+

Antagonism between *Helminthosporium oryzae* and bacterial bio-control agent. (+++ very good inhibition; ++ good inhibition; + poor inhibition; - no-inhibition)

CONCLUSION

Antagonistic activity of bacteria against *Helminthosporium oryzae* showed reliable effects at Dokri and Bakrani.The Bacillus was able to resist environmental effects and will be useful as a formulation not only for commercial purpose but also beneficial biological control against Brown spot of rice which is s disaster spreading over rice crop. Hence, rice industry could utilize this antagonistic activity of bacteria and produce some products and secure our rice crop.

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