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NOVEL STUDY ON THE CHARACTERIZATION OF RHIZOBACTERIA ISOLATED FROM THE SOIL OF RICE GROWING AREAS OF SINDH

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ABSTRACT

Rhizospheric bacteria have been reported to enhance and promote plant growth as well as to antagonize other pathogens. Present studies were conducted to search out such type of antagonistic bacteria. For this purpose, soil samples were collected from different rice growing areas of Sindh with the aim to find out bacteria that can enhance the growth of rice crop and antagonize the rice brown spot fungus *(Helminthosporium oryzae)*. Bacteria were isolated, purified, multiplied and morphologically characterized using light microscopy. Bacterial colonies were found to be small to large in sizes, they were raised round and spherical bearing green, white, offwhite, pale and raddish colours. The bacterial cells under microscope were seen as cocci, short to long rods. Out of all fourteen bacterial cells four were found motle, ten non-motilate, three gram negative and eleven gram positive. Thus, these studies may become helpful for further characterization and identification of bacteria to use them as biocontrol agents against diverse pathogens of different crops.

Keywords: Nutrient recycling, rhizobacteria, rice, Sindh.

INTRODUCTION

Rhizobacteria are root-colonizing bacteria that form symbiotic relationships with their host plants. They are important group of microorganisms used in biofertilizer. Biofertilization accounts for approximately 65% of the nitrogen supply to crops worldwide. These are also referred as plant growth-promoting rhizobacteria, or PGPRs, which have abilities to form different relationships with different species of host plants. The two major classes of relationships are rhizospheric and endophytic. Rhizospheric relationships consist of the PGPRs that colonize the surface of the root, or superficial intercellular spaces of the host plant, often forming root nodules.

Rhizobacteria, through, nitrogen fixation are able to convert gaseous nitrogen (N_2) to ammonia (NH_3) making it an available nutrient to the host plant which can

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support and enhance plant growth. The host plant provides the bacteria with amino acids so they do not need to assimilate ammonia (Willey, 2011). The amino acids are then shuttled back to the plant with newly fixed nitrogen. The rhizobacteria establish symbiotic relationship with its host plant. For the plant to be able to benefit from the added available nutrients provided by the rhizobacteria, it needs to provide a place and the proper conditions for the rhizobacteria to live. Creating and maintaining root nodules for rhizobacteria can cost between 12-25% of the plants total photosynthetic output. Legumes are often able to colonize early successional environments due to the unavailability of nutrients. Once colonized, though, the rhizobacteria make the soil surrounding the plant more nutrient rich, which in turn can lead to competition with other plants. The symbiotic relationship in short can lead to increase competition (Cain *et al.,* 2011). PGPRs enhance the availability of nutrients/minerals by solubilizing unavailable forms of nutrients and by the production of siderophores. Phosphorus can be plentiful in soil but is most commonly found in insoluble forms. Organic acids and phosphotases released by rhizobacteria found in plant rhizospheres facilitate the conversion of insoluble forms of phosphorus to plant available forms such as $H_2PO_4^-$.

Studies on some crops i.e. on sugar beet, it was found that some root-colonizing bacteria were deleterious rhizobacteria (DRB). It was also found that sugar beet inoculated seed with DRB had reduced germination rates, root elongation, plant growth, increased root lesions, root distortions, increased fungi infection. In one trial the sugar beet yield was reduced by 48% (Cain *et al*., 2011). Six strains of rhizobacteria have been identified as being DRB till now. These strains are in the genera *Enterobacter*, *Klebsiella*, *Citrobacter*, *Flavobacterium*, *Achromobacter*, and *Arthrobacter*. The presence of PGPRs has proven to reduce and inhibit the colonization of DRB on sugar beet roots. Plots inoculated with PGPRs and DRBs had an increase in production of 39%, while plots only treated with DRBs had a reduction in production of 30% (Suslow, 1982). Rhizobacteria have ability to control plant diseases caused by other pathogens. Diseases are suppressed through induced systematic resistance and through the production of anti-fungal metabolites. *Pseudomonas* strains have been genetically modified to improve plant growth and improve the disease resistance of agricultural crops. In agriculture, inoculant bacteria are often applied to the seed coat of 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.

Plant growth promoting rhizobacteria enhance plant growth directly or indirectly, but the precise mechanisms involved have not all been characterized till now (Kloepper, 1993). Direct mean of plant growth promotion by PGPR can be established in the absence of plant pathogens or other soil microflora, while indirect mechanisms involve the capability of PGPR to decrease the injurious effects of plant pathogens on crop yield. PGPR have been reported to directly enhance plant growth by number of mechanisms: fixation of atmospheric nitrogen (Zakry *et al.,* 2012), production of siderophores, solubilization of minerals such as phosphorus, and synthesis of phytohormones. PGPR strains may use one or

more of these mechanisms in the rhizosphere. Molecular methodologies (microbial and plant mutants) used to synthesize specific phytohormones have increased phytohormone synthesis as a direct mechanism of plant growth enhancement by PGPR (Glick and Bernard, 1995). PGPR that synthesize auxins and cytokinins or that interfere with plant ethylene synthesis have been identified. Keeping in view the plant growth promotion activity and antagonistic activity of bacteria present studies were conducted to search out soil bacteria from different localities.

MATERIALS AND METHODS

Collection of soil samples

Soil samples were collected from two localities (Dokri and Bukho Dero) of Sindh province of Pakistan. Samples of both Dokri and Bukho Dero location were further processed for isolation, purification and characterization of bacteria.

Isolation of bacteria

Isolation of bacteria was done as described by Kasimpur *et al.* (2004). For this 200 gram soil from rice field was taken, from which bacteria were isolated. One gram of soil was mixed in 20 ml test tubes containing 9 ml autoclaved water. The soil suspension was mixed and dilutions were made up to 10^{-8}. 0.1ml of each dilution was spread on Nutrient Agar plates. Then these plates were incubated at 28 ± 3^{0}C for three days. Bacterial cultures were purified by streaking method which involves spreading of bacteria across on agar plate and allowing them to incubate at a certain temperature for a period of time.

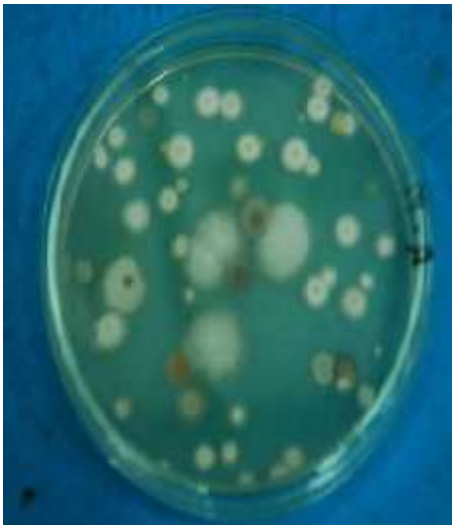

Plate 1. Bacterial colonies on Nutrient Agar.

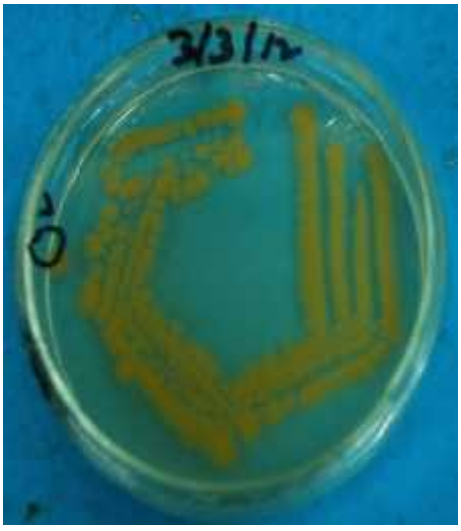

Plate 2. Purified bacterial isolate.

Purification of bacterial cultures

Characterization of rhizobacteria isolates

Purified single bacterial colonies and their respective bacterial cells were morphologically characterized on the basis of shape, color, size and orientation. The bacterial cells were also differentiated on the basis of their reactions. i. e. positive or negative by gram staining, according to the method described by Schaad (1988).

RESULTS AND DISCUSSION

Isolation and characterization of rhizobacteria

Several colonies appeared on Nutrient Agar (N.A) medium. Among them, 14 colonies were selected from samples of both the locations i.e. Bukho Dero and Dokri. The selected bacterial colonies were purified. Colonies were found to be small, medium and large in size; their shapes were raised, round, spherical bearing green, white, off-white, pale and reddish colors. Out of seven bacterial cells isolated from Bukho Dero soil, one was found to be motile and remaining six were found to be non-motile. Moreover, two were found to be gram negative and five gram positive on staining. Out of seven bacterial cells isolated from Dokri soil, three were found to be motile and four non-motile. Moreover, six were found to be gram positive and one gram negative (Tables1-4). The preliminary work for the morphological characterization of soil bacteria isolated from both Bukhu Dero and Dokri soils was done with the objective to search out plant growth promoting bacteria possessing antagonistic properties. Investigation for further characterization and identification of these soil bacteria and their application *in-vivo* and *in-vitro* is needed, because if these bacteria are found well then these can be used as biocontrol agents.

Soil bacteria are also able to control plant diseases that are caused by other bacteria and fungi. Diseases are 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 improve the disease resistance of agricultural crops. In agriculture, inoculant *Pseudomonas* bacteria are often applied to the seed coat of 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. Biological control using antagonistic bacteria has also been reported as an attractive, alternative tactic due to their ability to antagonize the pathogen by different modes of action, and to effectively colonize distinct plant habitats (Raaijmakers *et al.,* 2002). Most attention has been focused on the use of Gram-negative bacteria belonging to genera *Pseudomonas* or *Erwinia* (Cartwright *et al*., 1995; Braun-Kiewnick *et al,*. 2000; Shoda, 2000; Slininger *et al*., 2000; Costa *et al*., 2001).

Table 1. Colony characteristics of rhizobacteria isolated from Bukho Dero soil.

Strain No.	Origin	Size	Shape	Color	Orientation/ Border
JAG-1	Rice Soil	Small	Raised	Off white	Irregular
JAG-2	Rice Soil	Small	Plane	White	Smooth
JAG-3	Rice Soil	Small	Plane	White	Smooth
JAG-4	Rice Soil	Small	Raised	Brighten white	Smooth
JAG-5	Rice Soil	Small	Raised	Off-white	Irregular
JAG-6	Rice Soil	Small	Greenish	Off-white	Irregular
JAG-7	Rice Soil	Small	convex	Off-white	Irregular

Table 2. Cell characteristics of rhizobacteria isolated from Bukho Dero soil.

Strain No.	Origin	Size	Shape	motility	Gram reaction
JAG-1	Rice Soil	Small	Rod	Non motile	-
JAG-2	Rice Soil	Small	Cocci	Non motile	+
JAG-3	Rice Soil	Small	Cocci	Non motile	-
JAG-4	Rice Soil	Small	Cocci	Motile	+
JAG-5	Rice Soil	Small	Rods	Non motile	+
JAG-6	Rice Soil	Small	Rods	Non motile	+

Table 3. Colony characteristics of rhizobacteria isolated from Dokri soil.

Strain No.	Origin	Size	Shape	Color	Orientation/ Border
MRT-1	Rice Soil	Medium to large	concentric	Egg white	Smooth
MRT-2	Rice Soil	Medium to large	Centrally Raised	Off-white	Irregular
MRT-3	Rice Soil	Large	Plane	Off-white	Smooth
MRT-4	Rice Soil	Medium	Raised	white	Smooth
MRT-5	Rice Soil	Small	Plane	Reddish brown	Irregular
MRT-6	Rice Soil	Medium	Wavy	Off-white	Smooth
MRT-7	Rice Soil	Small	Raised and Round	Pale	Wavy

Table 4. Cell characteristics of rhizobacteria isolated from Dokri soil.

Strain No.	Origin	Size	Shape	Motility	Gram reaction
MRT-1	Rice Soil	Small	Rods	Motile	+
MRT-2	Rice Soil	Small	Round	Non motile	+
MRT-3	Rice Soil	Small	Rods	Motile	+
MRT-4	Rice Soil	Small	Cocci	Motile	+
MRT-5	Rice Soil	Small	Round	Non motile	+
MRT-6	Rice Soil	Small	Curved and round	Non motile	-
MRT-7	Rice Soil	Long	Oval shaped	-	+

CONCLUSION

These preliminary studies will be helpful to identify rhizobacteria having abilities to enhance plant growth as well as with best antagonistic activities. Then these can be used in any biocontrol strategy for protection of any agricultural crop.

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