Original Research Article

Phenotypic characterization of plant growth promoting microbial isolates from rice rhizosphere and phyllosphere

Abstract

The present investigation was carried during 2021-22 at Acharya Narendra Deva University of Agriculture & Technology, Kumarganj, Ayodhya (Uttar Pradesh). Results revealed that phenotypic characterization of plant growth promoting microbial isolates from rice rhizosphere and phyllosphere have significant effects on BD, pH and EC of the soil samples. Similarly microbial isolates have significant effect on organic carbon, microbial biomass carbon (MBC) and total protein content of the soil. After applying of microbial isolates on soil have magnificent impact on microbial population in terms of total rhizospheric bacterial population which is ranges between 0.77 - 8.4×10^6 cfu g^{-1} soil. Whereas, the soil fungal population found between 1.0 – 51.0 $\times10^3$ sfu g^{-1} of soil, soil actinomycetes population also influenced significantly (1.7- 97 $\times10^3$ cfu g^{-1} of soil). On the other hand total number of phyllospheric bacterial population of rice plant leaves and clumps were 0.53- 6.4×10^6 cfu g^{-1}, leaf fungal population 1.0- 73 $\times10^2$ sfu g^{-1} of sample, leaf Actinomycetes population 0.67- 1.2 $\times10^3$ cfu g^{-1} of sample. A total of 6 bacterial stains, 2 actinomycetes and 3 fungal strains were also isolated from the experimental rhizospheric soil. Similarly, 4 bacterial stains, 2 actinomycetes and 2 fungal strains were also isolated from the phyllosphere samples of the rice crop field by serial dilution effect on plating techniques. Plant growth promoting (PGP) traits was evaluation. The highest IAA production (23.75μg IAA ml^{-1}) was recorded under the RRS5 and followed by RRS3 which was produced at 18.76 μg IAA ml^{-1}. On the basis of these test results, 6 microbial strains (RRS3, RRS5, RRS9, RRS10, RPS4 and RPS8) showing the best results were selected for biochemical characterization. These potent microbial isolates showed effective positive responses to different Biochemical traits based on their varied PGP capabilities. It is concluded that isolated microorganisms can be used as an effective bio inoculants either individually or in different combinations for the formulation of different multi potent biofertilizers for plant growth promotion substance as well as control of plant diseases in rice crop.

Keywords: Actinomycete, Bacteria, Fungi, Phyllosphere, Plant growth promoting traits, Rice, Rhizosphere

Introduction

Rice (*Oryza sativa* L.) is an important stable cereal crop in India.it is cultivated on an area of 45 million hectare with a total production of 122 million tonnes and average productivity of 4.08 million tonnes ha^{-1}. In Uttar Pradesh, rice is growing on 5.74 million ha area with a total production of 15.52 million tonnes/year and average productivity

(27.04 q ha^{-1}). But comparatively it is lower that other states like Punjab (40.35 q ha^{-1}), Haryana (33.34 q/ ha) and West Bengal (28.51 q ha^{-1}). Therefore, there is urgent need to enhance productivity of this crop in the state by using of beneficial microorganisms in Rhizosphere and Phyllosphere (USDA 2022). Rice plays vital role in national food grain supply and contributed about 43% of total food grain production and 46% of total cereal production of the country. It is mainly grown in the states like Assam, West Bengal, Uttar Pradesh, Haryana, Punjab, Andhra Pradesh, Tamil Nadu and in other states of the country. It is cultivated globally across the divergent agro-ecosystems and is required divergent climatic and adhophic condition for its better growth. Therefore, there is urgent need to increase the yield of this crop through development of plant growth promoting isolates which can be promoted enzymatic activities in soil horizons. Diverse groups of microorganisms colonize the rhizospheric soils as well as the phyllosphere region of rice plant, including those that colonize the zone around the root (rhizosphere), some others that dwell on the root surfaces (rhizoplane) as well as those microbes found on the aerial parts (phyllosphere) (Knief *et al.*, 2011) have significant role on rice plant growth and development. Plant growth promoting rhizobacteria (PGPR) play a crucial role in stimulating plants growth substances in several ways including solubilization, mineralization and fixation of nutrients, phytohormone production, and pathogen suppression (Gupta *et al.* 2015). The groups of bacteria that are associated with plant roots have vital role on the rice plant development and yield.

MATERIALS AND METHODS: A Field experiment was conducted at Acharya Narendra Deva University of Agriculture & Technology, Kumarganj, Ayodhya (Uttar Pradesh) during kharif season of 2021-22, which is located at 81.837461 ^{0}N latitude and 26.545184 ^{0}E longitude and mean sea level height 322 feet. Samples from rice rhizosphere and phyllosphere were randomly collected from each site. Twelve samples were collected from entire paddy field at 70 days after transplanting (DAT). Samples were collected just before the fruiting stage in the crop. Whole paddy plant was carefully uprooted along with adhering soil, without breaking the secondary and tertiary roots and followed by chopping off the whole shoots. The samples of shoots and roots of the rice plant along with the adhering rhizospheric soil was placed in to separate polythene bags and then labelled and tied in order to minimize the evaporation loses. The roots were shaken to dislodge and separate loosely adhering soil aggregates around primary, secondary and tertiary roots and the adhering soil was collected and stored in a refrigerator at 4^{0}C temperature. The shoot samples were directly taken in laminar flow hoods and the leaves and stem samples cut into small pieces with the help of surface sterilized scissors and imprinted aseptically into nutrient agar and PDA (Potato Dextrose Agar) plates were kept separately for screening of rice phyllosphere bacteria, fungi and actinomycetes, respectively. Soil samples were analysed for determination of pH, EC, organic carbon content, microbial biomass carbon (MBC) and total protein content, a portion from each stored composite soil sample was taken for air drying, grinding followed by passing through 2 mm sieves before physico-chemical analyse of the soil. Collected soil samples of Rhizospheric were estimated for the counting of microbial population through using method described by Aneja (2018). By preparation of serial dilutions up to 10^{-8} and further plating into culture media plates specific for bacteria, fungi and actinomycetes. Viable microbes were developed into colonies or spores on respective media after incubation. The number of colonies was same as the number of organisms contained in the sample multiplied by the dilution factor.

$$\text{Organisms per g of soil samples} = \frac{\text{Number of colonies(Average of 3 replicated)}}{\text{Amount plated X Dilution factor}}$$

The following common culture media was used for plating of serially diluted rhizospheric and phyllospheric samples for counting of bacterial colonies, fungal spores and actinomycetes spores. The culture media was used for plating the dilutions and for isolating, purification and sub-culturing of microbial colonies were listed as their respective nutrient composition. The common culture media was used for plating of serially diluted rhizospheric and phyllospheric samples for counting of bacterial colonies, fungal spores and actinomycetes spores.

RESULT AND DISCUSSION

The highest total bacterial count (8.4×10^{6} cfu g^{-1} soil) was recorded in RRS6 treatment. Whereas, RRS10 treatment had the lowest number of bacterial population (0.77×10^{6}cfu g^{-1} soil). However, highest fungal population (51 x 10^{3} sfu g^{-1} soil) was found in the RRS2 treatment while the least number of fungi (1 x 10^{3} sfu g^{-1} soil) were observed in RRS1 treatment. The actinomycetes population in the tested soil samples ranges between 97 x 10^{3} cfu g^{-1} with RRS3 to 1.7 x 10^{3} cfu g^{-1} soil with RRS6. Similarly, in phyllosphere samples, the maximum bacterial population was recorded(6.4x10^{6} cfu g^{-1} sample) in RPS4 and the lowest bacterial count was in sample of RPS7 (0.53 x 10^{6} cfu g^{-1}).The maximum fungal population was recorded (32 x10^{2} sfu g^{-1})in RPS2 and the lowest fungal count was observed in RPS1 (1 x 10^{2} sfu g^{-1}) sample. But the actinomycetes population in casein starch agar plates was varied among the samples with no colonies detected in plates which were prepared with the samples like RPS1, RPS3, RPS6 and RPS7, respectively. Among the sample plates of actinomycetes could not be detected. However, highest number of actinomycetes were noticed in RPS4 (1.2 x 10^{3} cfu g^{-1} sample) and lowest was observed in RPS2 (0.67 x 10^{3} cfu g^{-1} sample). The variation inmicrobial load of rhizospheric soil samples have been found due to addition of inputs with varying levels of organic ingradients and other soil inputs. The low microbial load and their diversity in phyllosphere may be attributed due to biocontrol activities of the resident phyllosphere bacteria, fungi and actinomycetes. Etesami (*et al*. 2017) also isolated gram-positive bacteria from rhizosphere and inside the roots of rice; they characterized them for their plant growth promoting (PGP) traits and antifungal activity against some rice plant pathogenic fungi. The results showed the endophytic and rhizosphere isolates had different PGP traits and antifungal activity. Only one rhizosphere isolate and one endophytic isolate showed highly inhibitory effects against the mycelial growth of all fungal rice pathogens tested. The best bacterial isolates, based on multiple PGP traits and inhibitory effects against the mycelial growth of all fungal rice pathogens, were identified. Krishna (*et al.* 2012) have showed that population of bacteria, fungi and actinomycetes decreased significantly with increased soil depth. The depth wise profile of total microbial abundance was well matched with the vertical profiles of soil nutrients and organic carbon. Microbial numbers were highest in surface soil where organic matter and nutrients were highest. Further, the presence of more plant roots in the top soil had a strong effect on microbial load.

Table 1 Microbial population in soil and leaf samples

Treatment	**Soil**			**Treatment**	**leaf**		
	Bacteria (cfu g^{-1} soil)	**Fungi (sfu g^{-1} soil)**	**Actinomycetes (cfu g^{-1} soil)**		**Bacteria (cfu g^{-1} soil)**	**Fungi (sfu g^{-1} soil)**	**Actinomycetes (cfu g^{-1} soil)**
RRS1	1.3×10^{6}	1×10^{3}	6×10^{3}	RPS-1	1.0×10^{6}	1×10^{2}	ND
RRS2	6.0×10^{6}	51×10^{3}	50×10^{3}	RPS-2	4.2×10^{6}	32×10^{2}	0.67×10^{3}
RRS3	2.5×10^{6}	26×10^{3}	97×10^{3}	RPS-3	2.0×10^{6}	21×10^{2}	ND
RRS4	ND	26×10^{3}	6.5×10^{3}	RPS-4	6.4×10^{6}	6.0×10^{2}	1.2×10^{3}
RRS5	7.2×10^{6}	3×10^{3}	8.1×10^{3}	RPS-5	0.58×10^{6}	21×10^{2}	ND
RRS6	8.4×10^{6}	11×10^{3}	1.7×10^{3}	RPS-6	0.53×10^{6}	24×10^{2}	ND
RRS7	0.78×10^{6}	32×10^{3}	2.2×10^{3}	RPS-7	0.62×10^{6}	73×10^{2}	ND
RRS8	0.82×10^{6}	30×10^{3}	2.4×10^{3}	RPS-8	2.9×10^{6}	30×10^{2}	1.18×10^{3}
RRS9	0.81×10^{6}	44×10^{3}	2.1×10^{3}	RPS-9	3.3×10^{6}	26×10^{2}	1.05×10^{3}
RRS10	0.77×10^{6}	50×10^{3}	1.8×10^{3}	RPS-10	2.3×10^{6}	24×10^{2}	ND
RRS11	0.82×10^{6}	41×10^{3}	2.2×10^{3}	RPS-11	1.8×10^{6}	18×10^{2}	1.07×10^{3}
RRS12	0.98×10^{6}	36×10^{3}	2.0×10^{3}	RPS-12	2.6 x 10^{6}	12 x 10^{6}	0.74×10^{3}

Where, ND: No colonies detected on the plates

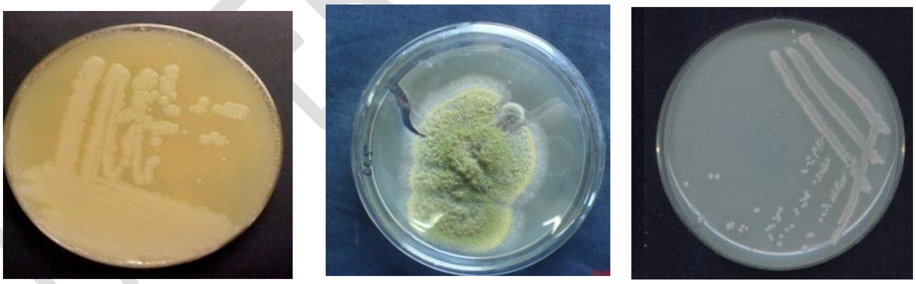

Fig 1 General view of Bacteria, Fungal and Actinomycetes isolates from the rice

CONCLUSIONS:

It is inferences that rhizospheric samples have the highest total bacterial count (8.4×10^{6} cfu g^{-1} soil) with RRS6. Apart from this highest fungal population (51 x 10^{3} sfu g^{-1} soil) was found in the sample of RRS2. The actinomycetes population in the tested soil samples ranges from 97 x 10^{3} cfu g^{-1} to 1.7 x 10^{3} cfu g^{-1} soil recorded in sample

RRS6.Phyllosphere samples were consisted highest total bacterial count (4.2×10^{6} cfu g^{-1}) in RPS2. The highest fungal population in plant sample (73×10^{2} sfu g^{-1}) was observed in RPS7 sample. From the experimental results a total of 6 bacterial, 2 actinomycetes and 3 fungal strains were isolated from rhizospheric soil samples whereas 4 bacterial, 2 actinomycetes and 2 fungal strains were isolated from Phyllosphere samples of rice crop by serial dilution plating technique.

REFERENCES:

Aneja K R. 2018. Laboratory manual of microbiology and biotechnology. 2nd edition. New age International Publishers, New Delhi. Pp:242-70.

Etesami H and Alikhani H A. 2017. Evaluation of gram-positive rhizosphere and endophytic bacteria for biological control of fungal rice (*Oryza sativa L.*) pathogens. *European Journal of Plant Pathology* **147**(1): 7-14.

Gupta G, Parihar S, Ahirwar N, Snehi S and Singh V 2015**.** Plant growth promoting Rhizobacteria (PGPR): current and future prospects for development of sustainable agriculture. *Journal of Microbial & Biochemical Technology* **32**(6):1-29.

Knief C, Delmotte N and Vorholt J A 2011. Bacterial adaptation to life in association with plants-A proteomic perspective from culture to in situ conditions. *Proteomics*. **11**(15): 3086-05.

Krishna M P, Rinoy Varghese and Hatha M A A. 2012. Depth wise variation of microbial load in the soils of midland region of Kerala: a function of important soil physicochemical characteristics and nutrients. *Indian Journal of* Education *and Information Manage*ment **3**: 126-129

USDA. 2022. World Agriculture Production United States Department of Agriculture.