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Bacterial Diversity in Wheat Rhizosphere and their Characterization

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ABSTRACT

The agriculture and agri-food sector is expected to move towards environmentally sustainable development, while increasing its productivity and simultaneously protecting the natural resource base for future generations. In view of its global significance in agriculture production and human health, wheat agroecosystem has been studied extensively from the viewpoint of bacterial diversity across various regions of the world. The race for producing more wheat by adopting intensive agronomic practices and applying more fertilizers is thought to have more adverse effects on the diversity of bacteria in the wheat fields. The global interest has been shifted towards beneficial microrganisms in bulk soil and rhizosphere in natural agroecosystems contributing soil health and plant productivity can be exploted as bioinoculants to increase more crop productivity. In the present study a gradual increase in population count (log_{10} cfu) of bacteria from 0d (6.83± 0.25) to 90d (8.50± 0.82) w as observed and thereafter population started declining. Bacillus was documented to be dominant population of zero day (71.42%). It constituted the dominant microflora of rhizosphere (37.5%) and in rhizoplane (49%) of 30d crop. Pseudomonas was the most dominant population (29.09%) of rhizosphere (62.5%) and in rhizoplane (40%) of 90d crop. All isolates recovered from 30d, 60d and 120d rhizoplane exhibited siderophore production. Maximum P-solubilizers were recovered from 60d rhizospheric samples. These isolates showed potency to be exploited as bioinoculants.

Key Words: Rhizosphere, Rhizoplane, Soil Health, Plant Productivity, Siderophore Production, P-solubilizers**.**

INTRODUCTION

A number of bacterial species associated with the plant rhizosphere belonging to genera *Azospirillum*, *Alcaligenes*, *Arthrobacter*, *Acinetobacter*, *Bacillus*, *Burkholderia*, *Enterobacter*, *Erwinia*, *Flavobacterium*, *Pseudomonas*, *Rhizobium* and *Serratia* are able to exert a beneficial effect on plant growth [1]. They promote growth of plant *via* acidolytic solubilization of minerals, chelation of iron, production of enzymes to mineralize N, S or P from organic

compounds to provide plants corresponding inorganic ions, nitrogen fixation, phytohormone production and exoploymer synthesis [2, 3, 4]. It is generally assumed that PGPR trigger an increase in root surface area which results in an increased mineral uptake and, in turn, enhances shoot biomass accumulation [5, 6]. PGPR improves plant growth either directly or indirectly: suppression of plant disease (Bioprotectants); improvement of nutrient acquisition (Biofertilizers), or phytohormone production (Biostimulants). But science of PGPR is still at its infancy.

MATERIALS AND METHODS

2.1 Collection of samples

Soil and root samples of wheat were collected aseptically in sterile plastic bags from a field in Manduwala, Dehradun. Samples were collected at different time periods viz., 0d, 30d, 60d, 90d and 120d.

2.2 Physical characteristics of soil

The various physical characteristics of soil viz., temperature, pH and moisture content were studied.

2.3 Recovery of microflora

2.3.1 Rhizospheric microflora

Rhizospheic soil was separated from roots of wheat with the help of brush in a petridish. 10g soil was placed in 100ml sterile Phosphate buffered saline (PBS) and was placed in shaker for 1h. 0.1 ml of appropriately diluted sample was spreaded on nutrient agar. All fractions were plated in triplicates. Plates were incubated in a BOD incubator at 28 ± 1^0C for 24.

2.3.2 Rhizoplane microflora

Rots were placed in sterile beaker containing autoclaved distilled water. It was shaken well and then 10-20 serial washings were given until clear root surface was exposed. Roots were placed with sterile forcep on nutrient agar and were incubated at 28 ± 1^0C for 24h.

2.4 Morphological characterization

Morphological characteristics viz., colony morphology (colour, chromogenesis, shape, margin, elevation and surface) and cell morphology (shape, gram reaction and arrangement) of recovered isolates were studied.

2.5 Biochemical Characterization

The various biochemical characteristics viz., Oxidase test, IMViC test, TSI test, Uresae test, Catalase test and nitrate reduction test were carried out according to Cappucino and Sherman (1992).

2.6 Functional Characterization

The functional diversity amongst recovered isolates was studied by qualitative screening of their ability to solubilize phosphorus and siderophore production.

2.6.1 Phosphorus Solubilization

Isolates exhibiting clearing zone on Pikovaskya's agar after 96-120h of incubation were considered as positive.

2.6.2 Siderophore production

It was assayed according to Schwyne and Neilands [7]. Isolates exhibiting an orange halo zone on Chromeazurol S agar after 48-72h of incubation were considered as positive.

RESULTS

The physical parameters viz., temperature, pH and moisture content of soil were studied at different time intervals. The temperature varied from, 12±0.40^{0}C (0d) to 35±0.10^{0}C (120d). pH of the field did not vary significantly. It varied from slightly acidic i.e., 6.00±0.05 (0d) to 7.10±0.04 (120d). Moisture content (%) of the field varied from 60±0.25 (0d) to 31±0.25 (120d).

Characteristics	Time Period of sampling (days)				
	0	30	60	90	120
Temperature (^{0}C)	12.00±0.40^{0}C	18.00±0.30^{0}C	24.00±0.50^{0}C	32.00±0.20^{0}C	35±0.10^{0}C
pH	6.00±0.05	6.25±0.06	6.50±0.03	6.79±0.02	7.10±0.04
Moisture content (%)	60.00±0.25	54.00±0.20	45.00±0.26	40.00±0.12	31±0.25

Culturable Diversity

Structural Diversity

A significant difference in the population count of rhizobacteria was observed from 0d to 120d (Fig. 1). A gradual increase in population count (log_{10}cfu) of recovered bacteria from 0d (6.83±0.25) to 90d (8.50±0.82) was observed. A decline in population count (7.60±0.54) was observed on 120d. Distinct bacterial morphotypes were isolated.

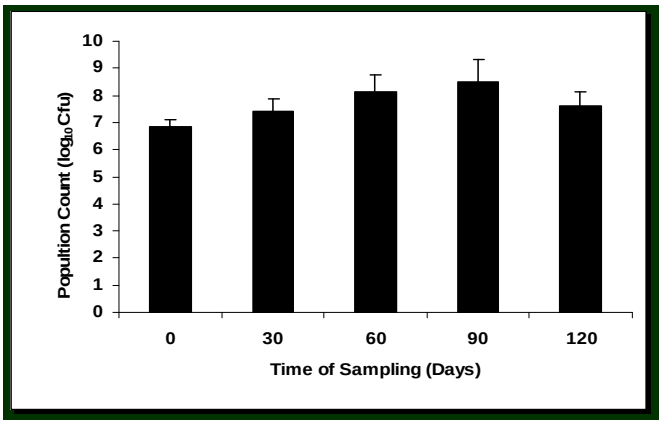

Fig.1: Population structure of wheat rhizobacteria

Bacterial morphotypes were selected on the basis of their colour, chromogenesis, morphological characteristics viz., colony morphology (shape, size, margin, elevation and surface) and cell morphology (gram's reaction, cell shape and arrangement). The recovered bacterial morphotypes were identified on the basis of their morphological characteristics and biochemical characteristics according to Bergey's manual of systematic bacteriology [8].

Bacillus was documented to be dominant population of zero day (75%) (Fig.2). It constituted the dominant microflora of rhizosphere (37%) and in rhizoplane (49%) of 30d crop. *Pseudomonas* was the most dominant population of rhizosphere in 60d RS (30%) and in rhizosphere of 90d (62.5%) and in rhizoplane (40%) of 90d crop. In 120d crop *Bacillus* and *Pseudomonas* were dominant (33.3%) in rhizosphere while the abundance of *Pseudomonas* (25%) was similar to that of *Micrococcus*, *Corynebacterium* and *Bacillus* in rhizoplane (Fig.2).

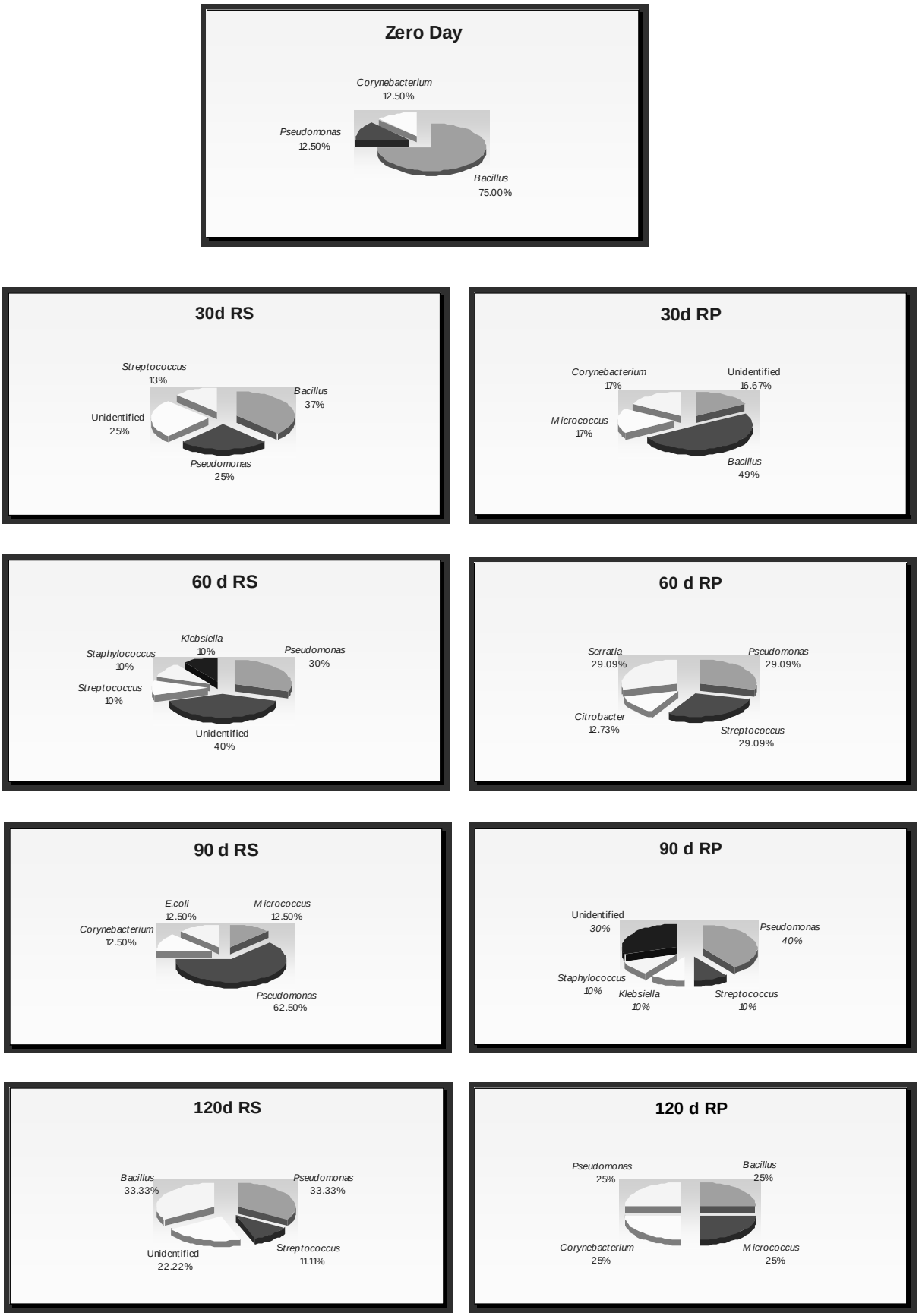

Fig.2: Distribution of wheat rhizobacteria in different days of wheat crop

Functional Diversity

All isolates recovered from 30d, 60d and 120d rhizoplane exhibited siderophore production. Maximum P-solubilizers were recovered from 60d rhizospheric samples. These isolates showed potency to be exploited as bioinoculants.

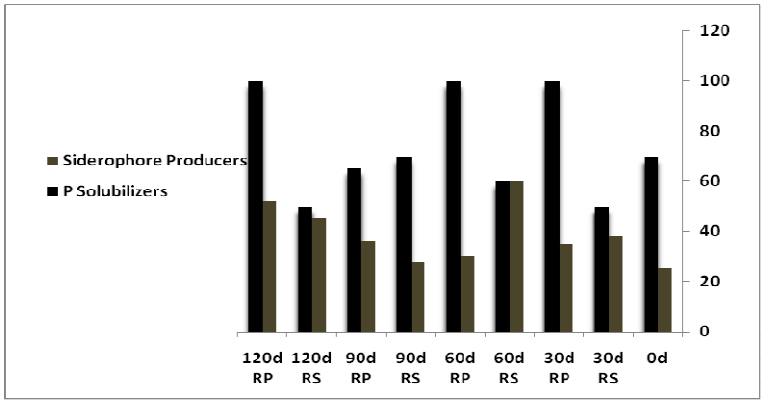

Fig.3: Functional distribution of recovered rhizobacteria

DISCUSSION

The rhizosphere, considered to be a hot spot of bacterial diversity , harbours bacterial flora whose diversity is mainly expressed in terms of functions adapted to the root presence, and in particular to favour plant growth. This is in turn beneficial to the whole rhizosphere microbiota through the highly nutritive and energetically rhizodepositions. This microbiota consists, besides bacteria, of mycorrhizal fungi and bacteria grazers working in stable synergy [1]. A continued exploration of the natural biodiversity of soil microrganisms and the optimization and manipulation of microbial interactions in the rhizosphere of crops represents a prerequisite step to develop more efficient bioinoculants.

The population profile indicated that population to be reaching at maximum level upto 90d and thereafter it declined. The dominance of Bacilli and Pseudomonas correlated well with Gaur *et al*. [9] and Mittal [10]. The appearance and disappearance of species observed with the age of crop was indicative of the fact that the effectiveness of any species to colonize rhizosphere depends upon its ability to acquire nutrients as observed by Aragno [1] that iP.fluorescens genes involved in nutrient acquisition, stress response, or secretion had elevated levels of expression during rhizosphere colonization To come to an improved understanding of the factors affecting the ability of bacteria to colonize the rhizosphere, the plant should be taken into account. It is very important to understand how rhizobacteria exert their beneficial effecr on plants.

In order to determine the potentiality of recovered rhizobacteria so that they can be used as bioinoculants, the functional characteristics viz., production of siderophore and solubilization of phosphorus were studied. Siderophore was found to be most prominent characteristic in 30d, 60d and 120d RS and RP fractions. Siderophore can be either directly or indirectly involved in influencing plant growth by chelation of iron which is present in very low amount in soil and that too in bound form. Siderophore can trap traces of insoluble iron (III) and form stable complexes. P solubilization was documented to be a major characteristic possessed by 60d RS and 90d RS isolates. Phosphorus is an essential element for plant development and growth making up about 0.2% of plant dry weight. Plants acquire P from soil solution as phosphate anions. However, phosphate anions are extremely reactive and may be immobilized through precipitation with

cations such as Ca^{2+}, Mg^{2+}, Fe^{3+} and Al^{3+}, depending on the particular properties of a soil. In these forms, P is highly insoluble and unavailable to plants [11]. As a result, the amount available to plants is usually a small proportion of this total. A considerably higher concentration of phosphate solubilizing bacteria is commonly found in the rhizospheric soil. The principal mechanism for mineral phosphate solubilization is the production of organic acids, and acid phosphatases play a major role in the mineralization of organic phosphorus in soil. The production of organic acids by phosphate solubilizing bacteria had been well documented [9, 1]. Strains from the genera Pseudomonas, Bacillus and Rhizobium are among the most phosphate solubilizers. In 60d and 90d RS Pseudomonas was observed to be the dominant population and thus contributing to high number of PSB in RS fractions. The abundance of a particular functional type in samples was indicative of the predominance of the role played by the microflora in their niche. However, no clear relationship could be estabilished between the structural and functional diversity as RS fractions were found to be structurally more diverse but RP fractions were found to be functionally more diverse and rich.

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