Plant growth promoting rhizobacterial diversity in potato grown soil in the Gwalior region of India

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ARTICLE INFO

**Keywords:**
PGPR
Rhizosphere soil
Non-rhizosphere soil
Potato

ABSTRACT

There seems to be meager studies with regards to rhizo and non-rhizo microbial association with potato plant from the central India. Present study was undertaken to evaluate the microbial diversity of rhizospheric and non-rhizospheric isolates from three varieties of potato viz Kufri sindhuri, Kufri lauvkar and Kufri chipsona-3 procured from the Central Potato Research Station, Maharajpura, Gwalior. A total of 130 bacterial forms were isolated, and amongst these forty isolates were further characterized on their morphological basis, and those showing some of PGPR characteristics were identified to species level using VITEK-2 method. Various bacterial populations were found in potato rhizosphere and dominant presence was those of \textit{Bacillus subtilis}, \textit{Bacillus Megaterium} and \textit{Lysinibacillus sphaericus}. The non-rhizospheric soil was dominant in the forms like \textit{Aeromonas salmonicida}, \textit{Morxella group} and \textit{Bacillus coagulans}. Highest bacterial diversity was found in the rhizosphere soil of different potato cultivars than in the non-rhizospheric soil of potato.

1. Introduction

Microbial diversity of soils is being increasingly evaluated as indicators of soil fertility. There is an established relationship between microbial diversity and ecosystem sustainability [14]. A layer of soil just surrounding the plant roots is known as rhizosphere and it constitutes the important active area for root activity and metabolism. A large number of microorganisms such as bacteria, fungi, protozoa and algae abound the rhizosphere. Amongst these bacteria are one of the important component one. Certain bacterial species, which showed association with plant rhizosphere, are known to be involved in the enhanced plant growth, yield, and quality [4]. The presence of rhizobacteria in the rhizosphere therefore, can have a neutral, detrimental or beneficial effect on plant growth. Microbial activity may differ in mycorrhizosphere, hyphosphere, rhizosphere and bulk soils [2].

Microbial communities are now known to play key role in controlling biogeochemical cycling of nutrients in soil and as a consequence help plants to grow better [22, 24, 45, 5]. Soil microbial communities are often difficult to characterize, chiefly due to their complex phenotypes accompanied with genotypic diversity. Top soil bacterial populations can attain more than $10^{9}$ cells per g of soil [42], and most of these may generally be unculturabe. Soil microbial diversity is fundamental for sustaining above ground plant diversity and eco condition of a region [1, 27, 40]. Bacterial diversity involve species richness, numerical presence, evenness, and finally the distribution of their bacterial species [34]. This diversity and richness contribute to understanding in immense measure the soil health and sustainability. Thus the present study was to evaluate such microbial diversity around rhizosphere and non-rhizosphere of different potato cultivars.

2. Materials and methods

2.1. Experimental site

Three varieties viz Kufri sindhuri, Kufri chipsona-3 and Kufri lauvkar were assessed for their rhizospheric and non-rhizospheric microbial diversity based on the simple fact that these three varieties can complete their life cycle in three months. These work sites of varieties were

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https://doi.org/10.1016/j.btre.2022.e00713
Received 23 November 2021; Received in revised form 6 February 2022; Accepted 16 February 2022
Available online 17 February 2022
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generously provided by the Central Potato Research Station, Mahara-jpura, Gwalior, M.P. (26.22°N 78.18°E). The Institute is spread over an area of nearly 400 acres and is under the professional management of the Indian Council for Agricultural Research, New Delhi.

2.2. Isolation of PGPR from rhizosphere and non-rhizosphere (bulk) soil of potato varieties

Rhizospheric and non-rhizospheric bulk soil samples were collected from 6 to 25 cm depth from the plant surface using sterilised equipment and packed in sterile plastic bags. These were stored at 4 °C in the lab. Six different fields of three mentioned potato tuber variety with plants were selected for sampling and sampling of the soil was done and plants monitored at an interval of 15 days from sowing till harvesting.

2.3. Soil analysis

The soil pH was determined by the method of Jackson [21] with a slight modification. 10 gm of soil sample was suspended in 90 ml distilled water. The suspension was shaken vigorously and allowed to

| S/N | Potato varieties     | Soil pH | Soil EC (dS/m) |
|-----|----------------------|---------|---------------|
| 1.  | Kufri Sindhuri,      | 7.6     | 0.31          |
| 2.  | Kufri Chipsona-3     | 6.8     | 0.44          |
| 3.  | Kufri Lauvkar        | 6.3     | 0.36          |

Table 1
Soil pH and EC of different potato cultivar.

Fig. 1. Number of countable colonies in the rhizospheric soil of three potato varieties.

Fig. 2. Number of countable colonies in the non-rhizospheric (bulk) soil of three potato varieties.
settle overnight. Systronic pH meter analytic was used to determine pH.

The electrical conductivity of the soil was determined in the same suspension as used in the pH measurement, with the help of a conductivity meter following modified Jackson [21] method. The EC is expressed in deci Siemens per meter (dSm⁻¹) at 25 °C. Systronic analytic, conducting meter was used for the purpose.

### 2.4. Preparation of dilutions, inoculations and observations

In 250 ml capacity of conical flasks, 10 g of soil both from rhizo and non-rhizosphere separately were added in the already contained 90 ml of distilled water. The flask was shaken for 10 min on a rotary shaker. One ml of the shaken suspension was added to 9 ml distilled water in a test tube and again shaken for 2 min. This represented 10⁻¹ dilutions. Similarly, a series up to 10⁻¹ dilutions was prepared under aseptic conditions. An aliquot of this suspension was spread on the plates of solid Nutrient agar (NA) medium. Plates were incubated for 48 hr at 28 °C and the bacterial colonies observed. Isolated single colony was re-streaked on fresh NA medium plate and re-incubated. These bacterial pure cultures were maintained on the NA medium slants in glass culture tubes. All these isolates were maintained for longer durations at 4 °C in 70% nutrient broth and 30% glycerol in sterilized vials or eppendorf tube.

2.5. Standard plate count method (CFU)

Standard plate count method was used to enumerate the bacterial cultures. 100 μl inoculum from each sample and dilution was spread across the plate and the colonies that were formed after incubation were counted. The number of bacterial colonies in each were referred to as colony forming units (CFU). Colonies exhibiting good variable growth were selected for further streaking on fresh plates. Further purification and multiplication of isolates was done by streaking on fresh plates. The CFU was determined by the relation [43].

### Table 2

| S/N | Varieties | CFU/g soil in rhizo and non-rhizospheric (bulk) soil in three different varieties of Potato. |
|-----|-----------|--------------------------------------------------------------------------------------------------|
| 1   | KS        | 3.9 × 10⁷                                                                                       |
| 2   | KC-3      | 5.2 × 10⁷                                                                                       |
| 3   | KL        | 6.4 × 10⁷                                                                                       |

### Table 3

| S/N | Isolates | Gram stain | Color   | Shape         | Elevation | Surface | Opaqueness |
|-----|----------|------------|---------|---------------|-----------|---------|------------|
| 1   | PR1      | +ve        | Peach   | Small, Round  | Raised    | Smooth  | Opaque     |
| 2   | PR2      | +ve        | Off white | Oval         | Raised    | Smooth, Shiny | Opaque |
| 3   | PR3      | +ve        | Pink     | Small, round  | Raised    | Smooth | Opaque     |
| 4   | PR4      | +ve        | Off white | Irregular   | Middle flat edge swollen | Rough | Opaque |
| 5   | PR5      | +ve        | Off white | Oval         | Raised    | Smooth, Mucoid | Opaque |
| 6   | PR6      | +ve        | Off white | Irregular,   | Flat      | Smooth, shiny | Opaque |
| 7   | PR7      | -ve        | Light brown with pigmentation | Oval      | Raised    | Rough | Opaque     |
| 8   | PR8      | +ve        | Off white | Irregular   | Flat      | Rough | Opaque     |
| 9   | PR9      | +ve        | Off white | Irregular   | Flat      | Smooth | Transparent |
| 10  | PR10     | +ve        | Yellow   | Round       | Raised    | Smooth, Mucoid | Opaque |
| 11  | PR11     | +ve        | Peach    | Small, oval  | Raised    | Smooth | Opaque     |
| 12  | PR12     | +ve        | Off white | Irregular   | Flat      | Smooth Mucoid | Opaque |
| 13  | PR13     | +ve        | Off white with brown pigmentation | Round | Flat | Rough | Opaque     |
| 14  | PR14     | +ve        | Off white | Round       | Flat      | Rough | Opaque     |
| 15  | PR15     | +ve        | Off white | Irregular   | Flat      | Smooth, shiny | Opaque |
| 16  | PR16     | +ve        | Off white | Circular    | Middle flat edge swollen | Rough | Opaque |
| 17  | PR17     | +ve        | Off white | Circular    | Flat      | Smooth | Opaque     |
| 18  | PR18     | -ve        | Peach    | Round       | Raised    | Smooth, shiny | Opaque |
| 19  | PR19     | +ve        | Yellow   | Oval         | Flat      | Smooth | Opaque     |
| 20  | PR20     | +ve        | Off white | Irregular with brown pigmentation | Flat | Rough | Opaque     |
| 21  | PR21     | +ve        | Off white | Irregular   | Flat      | Smooth | Opaque     |
| 22  | PR22     | +ve        | Off white | Circular    | Flat      | Smooth, shiny | Opaque |
| 23  | PR23     | +ve        | Off white with brown pigmentation | Circular | Flat | Smooth | Opaque     |
| 24  | PR24     | +ve        | Purple   | Round with brown pigmentation | Raised | Rough | Opaque     |
| 25  | PR25     | +ve        | Brown with pigmentation | Oval | Raised | Smooth | Opaque     |

### Table 4

| S/N | Isolates | Gram stain | Color   | Shape         | Elevation | Surface | Opaqueness |
|-----|----------|------------|---------|---------------|-----------|---------|------------|
| 1   | PB1      | +ve        | Off white | Irregular    | Flat      | Rough | Opaque     |
| 2   | PB2      | +ve        | Yellow   | Round, Raised | Smooth, shiny | Opaque |
| 3   | PB3      | +ve        | Off white | Circular    | Flat      | Smooth | Opaque     |
| 4   | PB4      | +ve        | Off white | Circular    | Flat      | Rough | Opaque     |
| 5   | PB5      | -ve        | Light peach | Round      | Raised    | Smooth | Opaque     |
| 6   | PB6      | -ve        | Light yellow | Round   | Raised    | Smooth | Opaque     |
| 7   | PB7      | -ve        | Peach     | Circular    | Flat      | Smooth | Opaque     |
| 8   | PB8      | +ve        | Light yellow | Round, Slightly raised | Smooth | Opaque     |
| 9   | PB9      | +ve        | Off white | Circular    | Flat      | Smooth | Opaque     |
| 10  | PB10     | +ve        | Off white | Irregular   | Flat      | Smooth | Opaque     |
| 11  | PB11     | -ve        | Off white | Circular    | Slightly raised | Smooth | Opaque     |
| 12  | PB12     | -ve        | Off white | Irregular   | Flat      | Smooth, Mucoid | Opaque |
| 13  | PB13     | -ve        | Off white | Circular    | Flat      | Smooth | Opaque     |
| 14  | PB14     | -ve        | Off white with brown pigmentation | Round | Raised | Rough | Opaque     |
| 15  | PB15     | -ve        | Light yellow | Circular | Slightly raised | Smooth | Opaque     |
CFU/g = Average no of colonies/inoculation volume plated (ml) × Dilution Factor

2.6. Bacterial identification

Bacterial identifications were done using VITEK-2 method at Supratech Micropath Laboratory, Ahmadabad. VITEK 2 is a fully automated system that performs bacterial identification and antibiotic susceptibility testing. The reagent cards have 64 wells that can each contain an individual test substrate. Each card has a pre-inserted transfer tube used for inoculation. Cards have bar codes that contain information on product type, lot number, expiration date, and a unique identifier that can be linked to the sample either before or after loading the card onto the system.

Four reagent cards are available for the identification of different organism classes as follows:
1. GN - Gram-negative fermenting and non-fermenting bacilli
2. GP - Gram-positive cocci and non-spore-forming bacilli
3. YST - yeasts and yeast-like organisms
4. BCL - Gram-positive spore-forming bacilli

3. Results

The physiochemical soil (pH and EC) analysis of different varieties of potato soil in the CPRI, Gwalior showed that the soil pH for different cultivars of potato KS, KC-3 and KL ranged 7.6, 6.8 and 6.3 respectively whereas EC (Electrical conductivity) ranged respectively as 0.35, 0.44 and 0.36 dS/m (Table 1).

3.1. Serial dilution and microbial count

In serial dilution from $10^1$ to $10^6$ three sets of the plates that were incubated at 37°C showed well developed colonies and therefore used for the purpose of colony count. Colonies counts were descended from $10^3$ to $10^5$. These microbial colonies on agar planting at $10^3$ to $10^5$.
dilutions were found appropriate for enumeration of colonies. Amongst them 10^3 dilution showed maximum number of countable colonies. 10^5 dilutions always showed lower colony count. This was true for soil from all potato cultivars. Comparatively, rhizospheric soil had more bacterial colony count than the non-rhizospheric one (Figs 1 & 2Table 2) this was a trend presented by all the three potato cultivars. Subsequently bacterial density as CFU/g too was higher in the rhizospheric than non-rhizospheric soil of the three potato cultivars.

3.2. Bacteria morphological characteristic

An approximately 400 colonies were observed. On the basis of their colony structure and morphology 130 bacterial isolates testing on the Pikovskaya agar medium [38] modified with 1% methyl red containing tricalcium phosphate [36] showing phosphorus solubilisation character were used for further studies which they were qualify as PGPR. A total of 40 morphologically distinct isolates were observed. Among these 25 isolates were from rhizosphere and 15 from the non-rhizosphere (bulk soil). Rhizospheric bacteria, presented 90% gram +ve and 10% gram -ve whereas the non-rhizospheric were presently 40% gram +ve and 60% gram -ve. Most of the colonies were off white colonies having both smooth and rough texture. The rest of the other colonies were presented with yellow, pink, brown and peach colorisation, and some either mucoid or non-mucoid showed yellow, brown and green pigmentation (Tables 3 and 4). Gram positive forms were dominantly rod shaped bacillus organized as single, diplobacilli, short cum long chains, and/or as coci. Gram negative form too were either rod shaped bacillus, coci, diploccoci, and/or as clusters in arrangement (Fig 3a,b,c,d,e).

All the 40 isolates were further subjected to characterisation and identification at Supratech Micropath Laboratory, Ahmadabad. Based on the numerical probability and calculation of confidence of similarities the form identification probability are present in the Tables 5 and 6. Therefore, the probability range from 99% to 86% and confidence are referred in their descending order, as excellent, very good, good, acceptable, and low. Based on this therefore, the generic distribution tentatively was shown to be as these of Bacillus with 20 form, Neisseria, Pseudomonas, Sphingobacterium and actinomycetes with one form each in the rhizosphere and Bacillus with 6, Aeromonas with 3, Morrella with 2 and Pseudomonas, Sphingomonas, Sphingobacterium with single form each in non rhizospheric soil.

3.3. Bacterial diversity distribution

The highest bacterial population was found in rhizosphere soil of Kufri luvakar and lowest in Kufri sindhuri whereas in non rhizosphere soil highest bacterial population was found in Kufri luvakar and lowest in Kufri chipsona-3. The count wise highest count numbers shown were those of Bacillus subtilis and Bacillus Megaterium followed by Lysinibacillus sphaericus in the rhizospheric soil and in non-rhizospheric soil the highest bacterium count was presented by Aeromonas salmonicida followed by Bacillus coagulans and Morrella sp. (Table 7).

4. Discussion

[32] have said that the relation between biodiversity, which can simply be defined as the numerical presence of species in a certain system, relates to the functional dynamics of the soil and therefore, is part of prime concern to be conserved so as to maintain its role in a functional biosphere. In the present study therefore, higher no of countable colonies of bacterial forms in neutral pH and lower in number in low pH needs, considered attention in potato cultivar soils. pH, as is known, determines the availability of nutrients, thus can have a strong effect on physiological processes, such as root exudations containing signal molecules which consequently, affect the microbial communities in the plant rhizosphere.

Soil pH, aeration, and physicochemical characteristics are jointly responsible for creating specific soil environment, thereby, the rhizosphere microbial communities [11, 17, 29] [13]. have reported reduced bacterial diversity with increase in soil pH. Role of soil salinity induced bacterial diversity, is suggested as a cause of environmental stress [8]. Bacterial identification by VITEK-2 method as employed in this study has been used by other workers too for bacterial identification [12, 25, 26, 31, 35]. The diverse microbial community was found in three different potato cultivars KS, KC-3 and KL at different time intervals [20], and [18] have reported that the, rhizospheric bacterial community of varieties ‘Monalisa’ and ‘Asterix’ were phylogenetically more similar at the early first and second samplings. Thus suggesting that their root signals may be selecting similar bacterial groups. Multiplicity of groups shown by the other cultivars, influence rhizosphere associated microbial communities during early development and then diversifying subsequently as shown by the present three cultivars.

[6] and [9] have produced detailed reviews regarding biotic and abiotic factors such as soil type, seasons, plant developmental stage, proximity to root, root architecture, plant species, and cultivars that can affect the structure of microbial communities in the rhizosphere. Various other studies have established that the influence on rhizospheric microbial communities is a synergic effect of both the plant species and the plant genotypes [5, 6, 41, 44, 46, 47]. This observation therefore, can also aptly explain the PGPR community structure variation in the present cultivars too.

There are distinct differences in bacterial form between bulk (non rhizosphere) and rhizosphere soil [7, 10, 30] and the probable reason seems that in rhizosphere, roots exudate chemicals which are helping the bacteria to flourish abundantly. In the present study Bacillus sp. was shown to be abundant both in rhizospheric soil than in the bulk soil. This being followed by Aeromonas, Morrella sp. and Pseudomonas [28], have reported Bacillus as a dominant form genus in the tuber rhizosphere of

| Bacterial form rhizospheric soil | Potato cultivar | Kufri sindhuri | Kufri luvakar | Kufri chipsona-3 | Total |
|----------------------------------|----------------|---------------|---------------|-----------------|-------|
| Bacillus subtilis                | 2              | 2             | 6             |                 |       |
| Brevibacillus firmus             | 2              | 0             | 0             |                 |       |
| Aerobic actinomycetes spp.       | 1              | 0             | 0             |                 |       |
| Micrococcus latus                | 0              | 0             | 1             |                 |       |
| Lyssinibacillus sphaericus       | 0              | 3             | 1             |                 |       |
| Bacillus megaterium             | 0              | 3             | 3             |                 |       |
| Sphingobacterium                | 0              | 0             | 1             |                 |       |
| thalophilum                      |                |               |               |                 |       |
| Neisseria animaloris            | 0              | 1             | 0             |                 |       |
| Pseudomonas stutzeri            | 0              | 1             | 0             |                 |       |
| Alicyclobacillus                | 0              | 1             | 0             |                 |       |
| acidoterrestris                 |                |               |               |                 |       |

| Total                            | 5              | 11            | 9             |                 | 25    |

| Bacterial form non rhizospheric soil | Potato cultivar | Kufri sindhuri | Kufri luvakar | Kufri chipsona-3 | Total |
|-------------------------------------|----------------|---------------|---------------|-----------------|-------|
| Morrella group                      | 2              | 0             | 0             |                 |       |
| Bacillus coagulans                 | 1              | 0             | 1             |                 |       |
| Pseudomonas stutzeri               | 1              | 0             | 0             |                 |       |
| Bacillus lentus                     | 0              | 0             | 1             |                 |       |
| Bacillus pumilis                   | 0              | 0             | 1             |                 |       |
| Aeromonas salmonicida              | 0              | 2             | 1             |                 |       |
| Acinetobacter lwofli               | 0              | 0             | 1             |                 |       |
| Bacillus vulgaris                  | 0              | 1             | 0             |                 |       |
| Bacillus smithii                   | 0              | 1             | 0             |                 |       |
| Sphingomonas paucimobilis          | 0              | 1             | 0             |                 |       |
| Sphingobacterium                   | 0              | 0             | 1             |                 |       |
| thalophilum                        |                |               |               |                 |       |

| Total                              | 4              | 6             | 5             |                 | 15    |
sweet potato. Various species of Bacillus constitute major populations in the rhizospheres of chrysanthemum [15], of barley [33], and that of grass [16].

In this study too high number of colonies was found in rhizosphere compared to non-rhizosphere (bulk) soil and also different species of Bacillus, Pseudomonas and other forms.

70 bacteria species were reported isolated from the rhizosphere of potato cv [23]. too have reported 25 morphologically distinct bacterial isolates belonging both to Gram +ve and Gram –ve groups from the manna potato field. They further reported that in potato these beneficial microbes mainly belonged to the genera of Bacillus, Pseudomonas, and Penicillium, along with actinomycetes and yeast. This study too recorded that on morphological basis viz shape of colonies, color and elevation and then surface distinctions, the bacterial isolates belonging to Gram +ve and Gram –ve bacteria. Bacteria belonging to the Bacillus and Pseudomonas groups are known to be growth promoters [19, 57].

5. Conclusion

The study shows the presence of bacterial diversity in the rhizospheric and non-rhizospheric soil of different potato cv. KS, KC-3 and KL. The extent of the microbial diversity in soil is consistent with the soil health and quality. Soil microorganisms, such as bacteria, play important roles in soil fertility and promoting plant health, and can be employed and tested to be PGPR consortium potato plant.

Declaration of Competing Interest

All authors declare that they have no conflict of interest

Acknowledgement

The Authors are thankful to the Head, School of Studies in Botany, Jiwaji University, Gwalior, for providing support and necessary facilities. Special thanks are due to the Director and Staff, especially Dr. Sanjay Sharma, of CPRS, Gwalior, for providing the potato varieties and the facilities on their fields.

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