



Isolation and Enumeration of Alkali resistant *Rhizobacterium* from Alkali soil of Ayodhya Region, Uttar Pradesh

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(Received: 25 October 2025; Revised: 27 December 2025; Accepted: 22 January 2026; Published online: 10 February 2026)

(Published by Research Trend)

DOI: <https://doi.org/10.65041/BiologicalForum.2026.18.2.10>

ABSTRACT: The present study aimed to isolate, enumerate, and identify rhizospheric root-associated bacteria from soil samples collected at three different sites. A total of approximately 95 bacterial isolates were obtained from rhizospheric soils using standard microbiological techniques, including serial dilution, streak plate, pour plate, spread plate methods, and total viable count analysis. Taxonomic characterization revealed the presence of 27 genera comprising 44 bacterial species. Successful identification was achieved for about 74.2% of the total isolates, indicating substantial bacterial diversity in the rhizosphere. Enumeration results showed that Gram-negative bacteria were exclusively dominant over Gram-positive bacteria in terms of total viable count per gram dry weight of soil. The colony forming unit (CFU/ml) values varied among the sites, with counts of 1.7×10^6 CFU/ml (Fz-1), 1.2×10^7 CFU/ml (Fz-2), and 3.0×10^7 CFU/ml (Fz-3), indicating site-specific variation in bacterial abundance. The dominance of Gram-negative bacteria highlights their adaptive advantage and ecological significance in the rhizospheric environment. Overall, the study provides valuable insights into the diversity, abundance, and distribution of rhizospheric bacteria and emphasizes their potential role in soil fertility and plant growth promotion.

Keywords: Rhizosphere, Rhizospheric bacteria, Root-associated bacteria, Gram-negative bacteria, Enumeration, CFU/ml.

INTRODUCTION

Alkaline soils are extensively distributed in semi-arid and arid climatic regions and occupy more than 25% of the global land surface (FAO, 2021; Singh *et al.*, 2025). These soils are typically porous, well-drained, and enriched with calcium carbonate (CaCO_3), leading to elevated pH conditions. High concentrations of Ca^{2+} in alkaline soils reduce phosphorus (P) bioavailability through precipitation of insoluble calcium-phosphate complexes, thereby limiting plant nutrient uptake and productivity (Raghavendra *et al.*, 2023; Kumar and Yadav 2025). In India, alkali soils are widely distributed, particularly in Uttar Pradesh, which accounts for the largest proportion of alkali-affected land (ICAR, 2022; Verma *et al.*, 2025). Alkaline soil occurs in the areas having little rainfall (more than areas of desert soils). They are known by various names, namely Reh, Kallar, Usar, Thur, Rakar, Karl, and Chopan. Excessive accumulation of sodium carbonates, bicarbonates, and chlorides in alkaline soils induces ionic toxicity and osmotic imbalance in plants. Elevated pH interferes with enzymatic activity, membrane stability, nutrient solubility, and metabolic pathways, resulting in reduced

plant growth and yield (Hasanuzzaman *et al.*, 2023; Zhao *et al.*, 2025). Alkalinity is often associated with salinity stress, and the combined effect significantly alters physiological and biochemical processes including photosynthesis, respiration, and nitrogen metabolism (Gupta *et al.*, 2022; Li *et al.*, 2025). "Plant bacterial infections" in the rhizosphere are the determinants of plant health and soil fertility. Interaction of plant growth promoting rhizobacteria (PGPR) with host plants is an intricate and interdependent relationship involving not only the two partners but other biotic and abiotic factors of the rhizosphere region (Dutta and Podile 2010). Plant growth promoting rhizobacteria (PGPR) are natural microflora of soil that are able to colonize plants roots and stimulates plant growth when applied to roots and other propagules (Egamberdieva *et al.*, 2010).

The rhizosphere is the zone of the soil is influenced by the root. Complex gradient of substrate availability, water potential, and redox state distinguish this habitat from bulk soil, and constraints the distribution and the activity of the tremendously diverse rhizosphere microbiota (Kumar *et al.*, 2013). Root exudates include amino acids, organic acids, carbohydrates, sugars,

vitamins, mucilage, and protiens. The exudates launch signals and attract the microbes towards root by stimulating biological and physical interactions between roots and soil microorganisms (Brevik, 2012). They are formed by the weathering of rocks rich in minerals, like calcium, magnesium, and sodium, in acids like sulfurous acid. These minerals are transported by rivers to subsoil of plainer region. In Utter Pradesh they are found in drier areas of Chandauli district, Mirzapur district, Sant Ravidas Nagar district, and Varanasi district, Sultanpur. Uttar Pradesh having the largest alkali areas of 1.35 Mha account for 35.75% of total alkali affected area followed by Gujarat (14.36%), Maharashtra (11.21%), Tamil Nadu (9.4%), Haryana (4.86%), and Punjab (4.02%). Highly alkaline soil containing high amount of sodium chlorides and bicarbonates, this excessive concentration of alkaline and salt vicinity of plants cells leads to toxic effects showed in plants tissues in terms various metabolic reactions inside plants cells causes retardation of plant growth so that, major intermediates inhibited and metabolic reactions becomes altered to accomplished to another metabolic path. Thus, plants cells suffering stresses from alkaline and saline condition in soil also tend to be exposed to stresses by high PH-7. In excess, any form of salt or alkaline is deleterious for plant health and retardation to metabolic pathway. Alkaline condition in a soil is also sometime associated with salinity since, may saline environments have alkali or extremely alkaline PH values.

Soil has a huge versatility of microorganisms, belonging to different group of bacteria, fungi and Archaea. Among microbes, some are now well known for their inherent capability to tolerate different concentrations of alkali and salt and to promote plant growth as well. These Alkali-tolerant plant beneficial microbes have great importance in agriculture. They have shown their potential in improving crop productivity in arid and semiarid regions.

The genera *Pseudomonads*, *Bacillus*, *Enterobacter*, *Agrobacterium*, *Streptomyces*, *Klebsiella*, and *Ochrobacter* are best reported for improving the productivity of diverse crops under alkaline and saline conditions. The diazotrophic salt and Alkali-tolerant bacterial strains of *Klebsiella*, *Agrobacterium*, *Pseudomonads*, and *Ochrobacter* isolated from the root of halophytic plants.

MATERIALS AND METHOD

Hot plate Beakers, Flasks, Graduated cylinders, Inoculating loops, Test Tubes, Aluminum Foil, Pairs of scissors, Laminar air flow, Incubator, Autoclave, Micropipette, Fractional distillation setup, Microscope, Petri dishes, Slants, Bunsen burner, Electrical balance etc.

Study place: The laboratory work of this research was done in the department of microbiology, Dr. Ram Manohar Lohia Avadh University, Ayodhya.

Media Composition: Yeast Extract Manitol Agar (PH: 6.8-7.0)

Mannitol = 10.0 gm
 K_2HPO_4 = 0.5 gm
 $MgSO_4$ = 0.2 gm.

$CaCO_3$ = 0.1 gm.
 Yeast Extract = 1.0 gm.
 Congo- red = 2.5 ml.
 Agar = 20.0 gm.
 Distilled water = 1000 ml.

Different types of media were used for selective growth, enrichment culture, and indication of specific properties. Media Preparation and sterilization were done according to the protocol and standard recipe.

Sample collection: Soil sample were collected from rhizosphere and Non-rhizosphere from the agricultural land at the depth of 5.5 - 11.5 cm. Collected soil samples were stored in sterile polythene bags aseptically (Table 1).

Table 1: Sample collection from different areas.

| Sr. No. | Collection Area | Sample code |
|---------|-----------------|-------------|
| 1. | Milkipur Fz. | Fz-1 |
| 2. | RMLU | Fz-2 |
| 3. | Home | Fz-3 |

Fz = Faizabad

After collection the samples, pH was measured, 1g alkaline soil was serially diluted as (10^{-1} , 10^{-2} , 10^{-3} , 10^{-4} , 10^{-5} , 10^{-6} , 10^{-7}) from the sample and YEMA media (Fig. 2) was prepared for the isolation of *Rhizobacteria* to saw the growth of different *Rhizobacteria*.



Fig. 1. Yeast Extract Mannitol Agar Media (YEMA).

Then, samples were separately inoculated at YEMA by using streaking plate and pour plate technique and petri plates were incubated at 37°C for 24 hrs.

For Enumeration of bacteria through colony counter and Calculate Viable cells/g dry weight of collected soil sample.

To enumerate the bacterial cultures, standard plate count method was used. The number of viable cells per unit volume of a sample using yeast extract agar plates media was enumerated.

The inoculum sample was spread across the plate and the colonies that were formed after incubation were counted. The colonies are referred to as colony forming units (CFU). Once the CFUs are counted on the plate, they were divided by the volume plated to determine the concentration of cells in the sample.

$$CFU/ml = \frac{\text{No. of colonies} \times \text{Dilution Factors}}{\text{Volume of Culture Plate}}$$

Select plates from the appropriate dilution, which contains colonies in the range of 30- 300 and make plate count using a colony counter and find out cell forming unit/ ml. Since, the dilution plates are replicates of each other determine the average of the triplicate microbial counts.

RESULTS

A transect survey was carried out in alkaline and sodic soil lands of Ayodhya district in Uttar Pradesh, India. The locations surveyed included Milkipur, RMLU, home. These locations were observed to have patches of barren sodic and alkaline soils with pH ranging from 7.5-8.9 showed in Table 2.

Table 2: Sample containing different pH- values.

| Sr. No. | Sample No. | pH value |
|---------|------------|----------|
| 1. | Fz-1 | 8.9 |
| 2. | Fz-2 | 7.6 |
| 3. | Fz-3 | 7.5 |

Fz = Faizabad

Isolated colonies appearing on agar plates (Fig. 2) were transferred to slants for further study.



Fig. 2. Bacterial growth at YEMA Media.

The taxonomic identities of 27 genera and 44 species from approximately 95 Rhizospheric root associated bacteria isolated from Rhizospheric soil samples, grown at three sites were determined. Determination of the bacterial isolates was several successful in the rhizosphere soil samples expressing an overall identification of about 74.2% of the total isolates. The data obtained from enumeration of bacterial culture, gram negative bacteria are exclusively dominant in contrast to gram positive bacteria in terms of total viable count per dry weight of soils. Above data comprises various CFU/ml values (Fz-1 = 1700000, Fz-2 = 12000000, Fz-3= 30000000 CFU/ml) showed in Table 3. Data obtained greater abundance of gram negative bacteria in rhizospheric soil in comparison to gram positive bacteria through various techniques included serial dilution, streak plate method, pour plate method, spread plate method and Total Viable Count (CFU/ml). Morphological characterization of bacterial colonies through different types of staining techniques like simple, negative and Gram staining were performed resulted in Table 4.

Table 3: Number of colonies of bacteria in each dilution plates.

| Sr. No. | Isolates code | Organisms | Dilution | No of colonies/ plates | Organisms/gm of soil (CFU) |
|---------|---------------|-----------|-----------|------------------------|-----------------------------------|
| 1. | Fz-1 | Bacteria | 10^{-4} | 17 | $17 \times 10^4 / 0.1 = 1700000$ |
| 2. | Fz-2 | Bacteria | 10^{-5} | 12 | $12 \times 10^5 / 0.1 = 12000000$ |
| 3. | Fz-3 | Bacteria | 10^{-6} | 3 | $3 \times 10^6 / 0.1 = 30000000$ |

Table 4: Colony morphology and Gram Staining.

| Sample code | Shape colonies | Colonies Size (mm) | Colony Colour | Texture | Elevation | Margin | Gram Stain | Motility test |
|-------------|----------------|--------------------|----------------|--------------|-----------|--------|---------------------|---------------|
| Fz-1 | Circular | 0.7×1.8 | White | Moist, Rough | Convex | Smooth | Gram Negative Rod | Positive |
| Fz-2 | Circular | 0.9×1.4 | White (Opaque) | Moist, Rough | Convex | Smooth | Gram Positive Cocci | Positive |
| Fz-3 | Circular | 0.8×1.5 | White | Moist, Rough | Convex | Smooth | Gram Negative Rod | Positive |

DISCUSSION

Alkaline soils are characterized by high pH, poor soil structure, low availability of essential nutrients such as phosphorus, iron, and zinc, and reduced microbial activity. These harsh conditions significantly limit plant growth and microbial diversity. Despite these constraints, the present study demonstrated the successful isolation and enumeration of alkali-resistant

rhizobacteria from alkali soil, indicating the presence of a well-adapted rhizospheric microbial community. Similar findings have been reported in alkaline and salt-affected soils, where specialized rhizobacteria persist through adaptive physiological and biochemical mechanisms (Egamberdieva *et al.*, 2019; Rizvi *et al.*, 2021).

The ability of rhizobacteria to survive under alkaline stress is largely attributed to their capacity to maintain intracellular pH homeostasis. Alkali-tolerant bacteria regulate ion transport systems, particularly Na^+/H^+ antiporters, and produce buffering compounds that protect cellular enzymes from pH-induced denaturation (Rizvi *et al.*, 2021). Additionally, the secretion of extracellular polymeric substances (EPS) enhances cell aggregation and surface attachment, creating a protective microenvironment against alkaline stress. These adaptive traits explain the considerable bacterial population recovered during enumeration in the present study.

The rhizosphere plays a critical role in supporting microbial life in alkaline soils. Root exudates, consisting of sugars, amino acids, organic acids, and secondary metabolites, provide carbon and energy sources that stimulate microbial growth and activity. This rhizosphere effect leads to higher microbial density near plant roots compared to bulk soil, even under extreme pH conditions (Zhang *et al.*, 2022). The results of the present study are consistent with earlier reports showing increased abundance and diversity of alkali-tolerant rhizobacteria in the rhizosphere of plants growing in alkaline soils.

Alkali-resistant rhizobacteria are often classified as plant growth-promoting rhizobacteria (PGPR) due to their beneficial effects on plant health. These bacteria contribute to plant growth by solubilizing insoluble phosphates, fixing atmospheric nitrogen, producing phytohormones such as indole-3-acetic acid (IAA), and synthesizing siderophores that improve iron uptake under alkaline conditions (Kumar *et al.*, 2020; Singh & Jha 2023). Such functional traits enable plants to overcome nutrient deficiencies commonly observed in alkali soils. Therefore, the presence of alkali-resistant rhizobacteria, as observed in this study, suggests a strong potential for improving crop productivity in alkali-affected regions.

Industrial effluents, particularly distillery effluent, contain high organic load, melanoidins, and recalcitrant compounds that pose environmental challenges if untreated. However, when properly treated, these effluents can serve as a source of organic carbon and nutrients for soil improvement. The challenges associated with distillery effluent treatment and its microbial bioremediation have been extensively reviewed, highlighting the role of microorganisms in reducing toxicity and improving environmental compatibility (Patel *et al.*, 2023a).

Recent studies emphasize the use of native, site-specific rhizobacterial isolates rather than introduced strains for soil reclamation and sustainable agriculture. Indigenous alkali-tolerant rhizobacteria are better adapted to local soil and climatic conditions, enhancing their survival and functional efficiency when applied as bioinoculants (Singh & Jha 2023). The enumeration and isolation of such native bacteria in the present study provide a valuable resource for future screening of functional traits and biofertilizer development.

In addition, the findings align with global efforts to promote eco-friendly soil management practices. According to FAO and ITPS (2024), enhancing soil

microbial diversity is essential for restoring degraded and alkali-affected soils. The present study contributes baseline data on alkali-resistant rhizobacteria and supports their role in sustainable land management. However, further molecular characterization and functional evaluations of the isolates are required to fully understand their ecological roles and agricultural potential.

Alkaline soils are characterized by high pH (usually > 8.0), excessive exchangeable sodium, and poor physical structure, which collectively reduce nutrient availability and microbial activity. Such soils commonly exhibit deficiencies of micronutrients such as iron, zinc, and manganese, leading to poor crop productivity. Sustainable remediation of alkaline soils therefore requires biological approaches that enhance nutrient cycling and organic matter stabilization.

Microbial decolorization and degradation of melanoidin pigments play an important role in reducing the phytotoxic effects of effluent before soil application. For instance, yeast strains such as *Candida tropicalis* have demonstrated significant melanoidin decolorization efficiency, thereby reducing environmental load (Patel *et al.*, 2023b). Similarly, bacterial strains such as *Bacillus nitratireducens* have shown promising results in effluent decolorization and organic pollutant degradation (Patel *et al.*, 2023c). The use of *Pseudomonas putida* in spent wash treatment further confirms the potential of microbial systems in reducing pollutant concentration and enhancing biodegradability (Patel *et al.*, 2023d). In the context of alkaline soils, treated effluent enriched with beneficial microbial consortia may contribute to improved soil aggregation, enhanced enzymatic activity, and better nutrient solubilization. Microorganisms can help in organic acid production, which may slightly lower soil pH in microenvironments and improve micronutrient availability. Furthermore, microbial biomass contributes to soil organic carbon, which is often deficient in alkaline soils.

However, long-term field studies are necessary to evaluate ecological safety, heavy metal accumulation, and crop response before large-scale application. The soil of Milkipur, RMLU and Home sites sampled in the present study had PH-values that ranged from 7.5 to 9.0. Cultivated nitrogen-fixing bacterial community from native rhizosphere samples and represented members of the genera *Pseudomonads*, *Bacillus* were commonly found in alkaline soils. These bacteria are often identified as dominant taxa of cultivable microbial populations from rhizosphere of various crop plants. The widely studied *Pseudomonads*, *Bacillus* genus represents one of the most diverse genera in the plant rhizosphere soil populations and these species can be characterized with the ability to tolerate unfavorable conditions including Rhizobacterial populations.

In addition, the majority of the isolates were salt tolerant up to 3% NaCl, alkali-tolerant, PH-tolerant. Salt-tolerant, Alkali-tolerant, and PH-tolerant traits of these strains might be of some significance for its survival in high salt accumulated and alkaline soils. In this study indicated that habitat had a strong influence on the diversity of N_2 -fixing species that were the most abundant among the isolates in plants rhizosphere.

However, from above data (CFU) the composition of the rhizobacterial community associated with plants roots is influenced by a variety of sites, soil PH and types and environmental factors. Although it well known that many species of rhizobacteria can contribute to plant growth and health in many ways, there are few studies concerning the alkali-tolerant strain of Phosphate solubilizing and Nitrogen fixing bacteria. These strains could be useful in the formulation of new inoculants, improving the cropping systems into which it can be most profitably applied. The identification and the isolation of Rhizobacteria from alkali soil which combine the ability to solubilize phosphate and to produce plant growth could also significantly increase the productivity of crops in crops in alkali soils.

CONCLUSION

The present investigation demonstrated a high diversity and abundance of rhizospheric root-associated bacteria across three sampling sites. The isolation of 95 bacterial strains representing 27 genera and 44 species reflects the richness of the rhizospheric microbial community. Successful identification of over 74% of the isolates indicates the effectiveness of the applied microbiological techniques. Enumeration data clearly showed the dominance of Gram-negative bacteria over Gram-positive bacteria in rhizospheric soils, suggesting their superior adaptability to root-associated environments. The variation in CFU/ml values among sites further indicates that local soil conditions influence microbial population dynamics. These findings highlight the ecological importance of Gram-negative rhizobacteria in maintaining soil health and supporting plant growth. The study provides a strong foundation for future functional and molecular characterization of rhizospheric bacteria with potential applications in sustainable agriculture and biofertilizer development.

FUTURE SCOPE

Future studies should focus on molecular identification of the isolated rhizospheric bacteria using 16S rRNA sequencing and genomic tools to validate taxonomy and functional potential. Detailed evaluation of dominant Gram-negative isolates for plant growth-promoting traits such as nitrogen fixation, phosphate solubilization, siderophore production, and phytohormone synthesis is required. Since CFU variation among Fz-1, Fz-2, and Fz-3 indicates site-specific effects, correlation of microbial diversity with soil physicochemical properties should be undertaken. Greenhouse and field trials are necessary to assess their efficiency under natural conditions. Development of effective microbial consortia and stable biofertilizer formulations will enhance practical application. Integration of metagenomic approaches can further clarify rhizosphere dynamics and support sustainable soil fertility management strategies.

Acknowledgments. The authors would like to thank the Head, Department of Microbiology, Dr. Ram Manohar Lohia Avadh University, Ayodhya for providing the necessary laboratory facilities to carry out the research work. Thanks, is also extended to the Vice Chancellor of Dr. R.M.L. Avadh University Ayodhya as well as Vice Chancellor of Maharaja

Ganga Singh University, Bikaner, Rajasthan for the necessary support rendered for the work.

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How to cite this article: Ajad Patel, Ranjan Singh, Abhishek Vashishtha, Gautam Kumar Meghwanshi, Soni Tiwari, Prashant Singh, Adarsh Kumar and Akanksha Yadav (2026). Isolation and Enumeration of Alkali resistant *Rhizobacterium* from Alkali soil of Ayodhya Region, Uttar Pradesh. *Biological Forum*, 18(2): 65-70.