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Bioprospecting potassium solubilizing rhizobacteria from the unique Kuttanad paddy ecosystem as microbial soil health engineers

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Abstract
Rice, a vital starchy cereal the premier food crop of Kerala, India, with cultivation centered primarily in Palakkad and Alappuzha. Potassium is a major macronutrient for plant growth and has significant role in many functions like development, yield, and disease resistance in the plant system. However, the expanding cultivation in soils with low to medium potassium content, coupled with chemical fertilizer use, poses a significant threat to rice based cropping systems. One of the most prominent ways of improving plant utilization of potassium in the soil is to use potassium solubilizing microbes, which can make potassium ions available from minerals of both igneous and sedimentary origins. The present study involves isolation of rhizosphere bacteria, molecular identification and screening of their potassium solubilisation potential. Among 11 locally isolated bacterial strains, a single strain exhibited superior potassium solubilizing capability, as they converted insoluble potassium to soluble potassium. Further they were subjected to molecular identification and confirmed as *Enterobacter cloacae* showing 100% sequence similarity with reference strains in GenBank. The results strongly suggest that applying these rhizosphere bacteria to agricultural soils can significantly reduce chemical fertilizer dependence and enhance the sustainability of food production.

Keywords: Rhizosphere bacteria, Potassium solubilizers, *Enterobacter cloacae*, Biofertilizer

Introduction

Agricultural production faces extensive global losses due to abiotic and biotic stresses, including drought, salinity, and pathogen infection. Beyond these factors, nutrient deficiency represents a significant abiotic challenge. Potassium (K) is the most essential nutrient for plant growth and a critical macronutrient for enhancing plant stress tolerance [1]. Plants primarily uptake K in its soluble, ionic form K^+; however, plants can directly access only 1% of soil K; the remaining 90-98% is fixed in silicate minerals such as micas and feldspars, rendering it unavailable for plant growth [2]. Potassium deficiency leads to significant physiological issues, including reduced photosynthetic rates, shortened internodes, and decreased crop yields. Specific symptoms include the scorching of small grains and the blackening of tubers, such as potatoes [3].

To meet plant potassium requirements, chemical potash fertilizers are traditionally employed; however, their extensive use has led to soil precipitation and environmental degradation and detrimental to soil microflora. Over time, this practice degrades soil, human, and environmental health, ultimately diminishing crop yields [4]. In addition, nutrient leaching specifically Phosphorus, Potassium, and Nitrogen has been documented as a significant source of groundwater and waterway contamination especially in in high-rainfall regions and areas adjacent to drainage systems [5, 6]. Thus, implementing an alternative strategy that ensure competitive crop yields while maintaining environmental integrity is an urgent necessity.

Soil microorganisms play a fundamental role in mineral weathering by mobilizing nutrients from insoluble sources. A diverse array of microbes including fungi, bacteria, and actinomycetes solubilize potassium (K) minerals primarily through the excretion of organic acids [7]. Notably, potassium-solubilizing bacteria (KSB) facilitate the release of K from feldspar and alumina silicate minerals through mechanisms such as acidolysis, chelation, exchange reactions, and complexation, as well as via the decomposition of organic matter [8, 9]. Thus potassium-solubilizing microorganisms (KSMs) play a critical role in nutrient cycling by mobilizing mineral potassium from insoluble soil fractions into plant-available forms [10]. Furthermore, many of these microbes function as plant growth-promoting rhizobacteria (PGPR), establishing symbiotic relationships that enhance plant resilience and growth under both optimal and stressful conditions.

The application of potassium-solubilizing microbes (KSMs) as plant probiotics offers a sustainable alternative, enhancing crop quality, growth, and yield while reducing reliance on chemical inputs. In addition, the application of KSMs as bioinoculants offers a sustainable strategy to enhance soil fertility and reduce dependency on synthetic potassium-based chemical fertilizers. Given the importance of growth-limiting nutrient like potassium (K), the present investigation aimed to evaluate the potassium-solubilizing potential of rhizobacteria isolated from the paddy fields of Kuttanadu, highlighting their role as effective biofertilizers for improving soil health and boosting plant productivity through microbial inoculation.

Materials and Methods

Sampling and Isolation of Rhizosphere Bacteria

Rhizosphere soil samples, including intact root systems of paddy (Oryza sativa L.), were collected from the different regions of Kuttanadu, Alappuzha. Samples (approximately 25 g) were secured in sterile, labeled plastic bags and transported to the laboratory. For bacterial isolation, the soil underwent serial dilution and was spread-plated onto Nutrient Agar (NA) medium [11]. Among the different colonies, 11 morphologically distinct, pigmented and prominent bacterial colonies were selected after a 24-hour incubation period at room temperature. The selected isolates, named SRB 1 to SRB 11 were purified, sub cultured and subsequently screened for potassium solubilization potential.

Screening for Potassium Solubilization

Eleven prominent bacterial isolates (SRB 1- SRB 11) were screened for potassium solubilization using a qualitative spot inoculation assay on Aleksandrov agar medium. The medium consisted of (per liter) : 5 g Glucose, 0.5 g $MgSO_4.7H_2O$, 0.005 g $FeCl_3$, 0.1g $CaCO_3$, 2g Potassium alumino silicate, 2 g Calcium phosphate and 20 g agar-agar with the pH adjusted to 7.2. Following 4 days of incubation at room temperature, colonies that exhibited rapid growth and a distinct solubilization zone on the agar plates were identified as potassium-solubilizing bacteria (KSB) during the primary screening [12].

Assessment of potassium solubilization efficiency (KE) of selected isolate

The primary screened rhizosphere bacterial isolate with prominent potassium solubilization potential (SRB 11) was purified three times, and again spot-inoculated onto the centre of Aleksandrov agar medium supplemented with an insoluble potassium source. Following an incubation period of 4 days at room temperature, the formation of a distinct halozone around the colony was measured to quantify the isolate's ability to mineralize insoluble potassium [13]. The Potassium solubilization efficiency (KE) was calculated as:

KE = Diameter of solubilisation halo/Diameter of the colony.

Further the rhizosphere bacterial isolate with prominent potassium solubilization potential was subjected to molecular identification.

Phylogenetic analyses of selected bacterial isolate SRB 11

The rhizosphere isolate exhibiting the highest potassium solubilization efficiency (SRB 11) was subjected to molecular identification via 16S rDNA sequencing. Total genomic DNA was extracted from the strain and utilized as a template for Polymerase Chain Reaction (PCR) amplification. The resulting PCR products were sequenced at the Rajiv Gandhi Centre for Biotechnology (RGCB), Thiruvananthapuram, Kerala, India. Sequence data were subsequently aligned using the BioEdit software and compared against the NCBI database using BLAST analysis [14].

Results

Isolation of Paddy Rhizosphere Bacteria

The primary phase of the study involved the systematic collection of rhizospheric soil the highly active zone surrounding the roots from paddy fields located in the Kuttanad region. This area is of particular interest due to its unique "below sea-level" farming ecosystem, which likely harbors specialized microbial communities. Following collection, the soil samples were processed and inoculated onto nutrient agar. The culture plates were then maintained at an ambient room temperature for duration of 24 hours, providing an optimal incubation window for the rapid development of bacterial colonies. After the incubation period, the plates were screened to identify the most representative microbial inhabitants of the paddy rhizosphere. From the diverse array of emerging growth, 11 prominent colonies were strategically selected for further investigation. This selection was not random but was based on a comprehensive assessment of their morphological diversity and distinct pigmentation (Fig. 1).

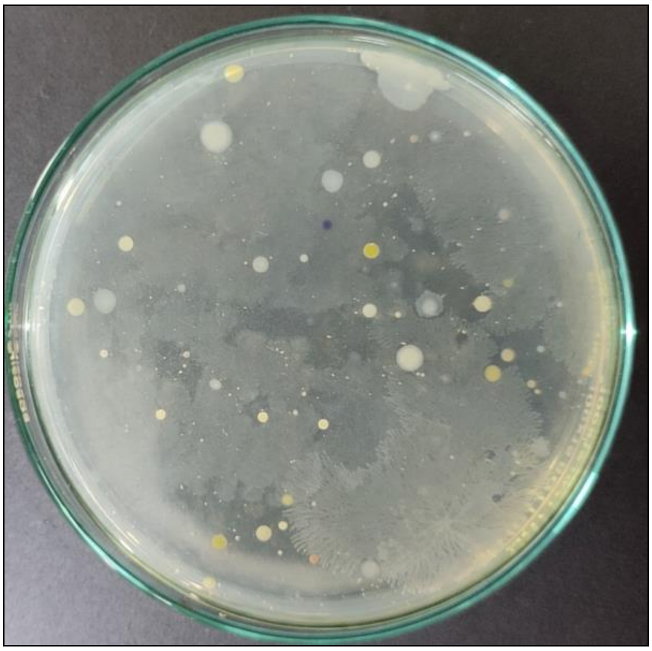

Fig 1: Morphological diversity of rhizospheric bacteria isolated from paddy soil

Screening and Selection of Potassium Solubilizing Bacteria

To evaluate the functional potential of these isolates, a qualitative screening was performed using the spot-inoculation method. The bacteria were inoculated onto Aleksandrov agar, a specialized diagnostic medium containing insoluble potassium aluminum silicate. This medium serves as a selective environment where only microorganisms capable of liberating inorganic potassium from mineral sources can thrive. The primary indicator of success in this assay is the formation of a clear halo zone (zone of solubilization) surrounding the bacterial colony. This transparency is caused by the microbial secretion of organic acids or other chelating agents, which chemically dissolve the opaque mineral particles in the agar.

After four days of incubation period, the plates were meticulously examined for visual evidence of mineral weathering. Of the 11 candidates tested, only isolate SRB-11 demonstrated a prominent and well-defined halo zone (Fig 2 a). The presence of this zone definitively identified SRB-11 as the most efficient potassium solubilizer within the experimental group. Further, this isolate was selected and purified (Fig 2 b) for further characterization.

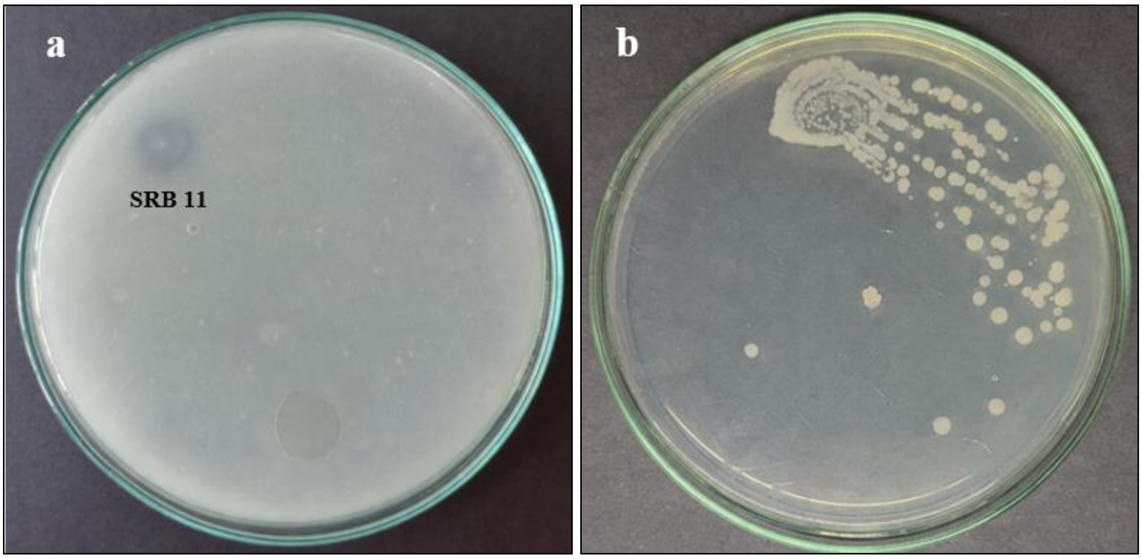

Fig 2: (a) Screening of potassium solubilizing potential of bacterial isolates on Aleksandrov agar medium (b) Pure culture of bacterial isolate SRB-11

Potassium solubilization efficiency of SRB 11

The evaluation of the potassium-solubilizing efficiency of isolate SRB-11 was conducted using a standardized qualitative spot-inoculation assay. The isolate was precisely inoculated at the center of Aleksandrov medium plate. During the incubation period, the metabolic activity of the bacterial colony specifically the secretion of organic acids results in the chemical dissolution of these insoluble minerals. The appearance of a transparent solubilization halo after incubation served as a qualitative indicator of potassium mineralization, which was further quantified to establish the isolate's solubilization index.

To quantify the mineral-mobilizing capacity of the isolate SRB-11, the Solubilization Index (SI) was determined using precise measurements of the growth on Aleksandrov agar. This index is a critical standardized metric in microbial physiology, as it normalizes the extent of mineral dissolution against the radial growth rate of the bacterial colony. By accounting for the colony size, the SI distinguishes between isolates that merely grow rapidly and those that possess a high specific activity for potassium release. Based on the empirical data derived from the experimental plate (Fig. 3), the following dimensions were recorded:

Colony Diameter (C): 1.2 cm, representing the central area of dense bacterial proliferation.

Total Zone Diameter (Z): 2.8 cm, encompassing both the central colony and the surrounding transparent halo of mineral depletion.

The calculated Solubilization Index of 2.33 provides significant insight into the metabolic efficiency of SRB-11. An SI value significantly greater than 1.0 indicates that the diffusion of solubilizing agents—typically organic acids such as gluconic, citric, or oxalic acid—extends well beyond the physical boundary of the biomass. Such high efficiency *in vitro* serves as a strong indicator of the isolate's potential performance in the rhizosphere, where it could effectively convert non-exchangeable soil potassium into a bioavailable form (K^+) for plant uptake. Based on these quantitative findings, SRB-11 is a prime candidate for development into a specialized potassium biofertilizer to enhance soil fertility in paddy cultivation.

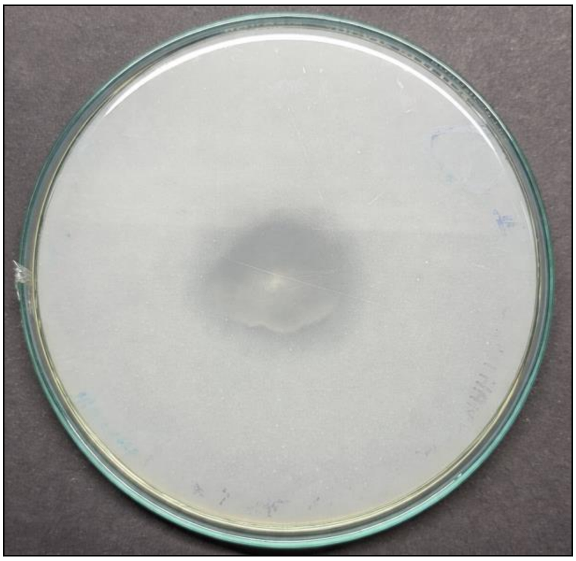

Fig 3: *In vitro* potassium solubilization efficiency of bacterial isolate SRB 11

Genetic Identification and Sequence Alignment

To achieve precise taxonomic classification, molecular identification was executed through the amplification and sequencing of the 16S ribosomal DNA (rDNA) gene, a gold standard molecular marker for bacterial phylogeny. Once the raw sequencing data was obtained, the resulting nucleotide sequences were processed and aligned using BioEdit Sequence Alignment Editor to ensure data integrity. To determine the identity of the isolate, the processed sequence was subjected to a homology search against the comprehensive global database maintained by the National Center for Biotechnology Information (NCBI) using the Basic Local Alignment Search Tool (BLAST).

The BLAST analysis of the specific isolate, designated as SRB-11, yielded a definitive result, demonstrating a 100% sequence identity with existing records of *Enterobacter cloacae* in the NCBI database. This high level of similarity confirms the isolate’s identity at the species level. To contribute to the global scientific community and provide a permanent reference for future studies, the verified 16S rDNA sequences were formally deposited into GenBank. Upon successful submission and curation, the sequence was assigned the unique official accession number PP748290.1

Discussion

The shift toward high-yielding varieties in intensive agriculture has addressed global food demands but at a significant environmental cost, including soil exhaustion and pollution. These challenges underscore the need for biocompatible, eco-friendly interventions that keep the One Health initiative intact [15, 16]. Efficient Rhizospheric Microorganisms specifically potassium-solubilizing microbes (KSMs) provide a sustainable alternative to chemical inputs, addressing both soil health and environmental safety [17]. Potassium is a primary macronutrient, surpassed only by nitrogen in its role in plant development. This critical physiological importance, coupled with the necessity for higher crop yields, has led to a significant increase in the demand for potassium fertilizers [18]. Insufficient application of inorganic K-fertilizers in potassium-deficient tropical soils compromises plant physiology, leading to lower yields and increased disease [19].

Despite being a vital macronutrient and the eighth most abundant element in the Earth's crust (2.1%), potassium remains a limiting factor for plant growth because it exists primarily in insoluble mineral forms [20]. To overcome these constraints and maintain adequate potassium (K) levels for sustainable crop production, alternative indigenous sources are required to optimize plant uptake [21]. In this context, efficient soil microorganisms have been identified as key drivers of the natural K-cycle, facilitating the rapid mobilization of insoluble K-bearing minerals [22]. Many studies show that using natural soil microbes can help plants grow better. These microbes work by breaking down minerals to release potassium that plant can actually use [2, 23]. It was also reported that potassium-solubilizing microorganisms contribute to sustainable agriculture by suppressing pathogens, improving soil structure, and weathering silicate minerals to release K, Al, and Si. Furthermore, their production of bioactive substances and growth hormones provides direct physiological benefits to the plant [9].

Potassium-solubilizing microorganisms are vital bioresources that convert insoluble potassium into plant-available forms through several biochemical pathways. The process is primarily driven by acidolysis and chelation, mediated by the secretion of low-molecular-weight organic acids such as citric, gluconic, oxalic, and tartaric acids. These acids release protons (H^+) that displace K ions from mineral surfaces, while simultaneously forming metal-organic chelates with mineral cations via organic acids and extracellular polysaccharides (EPS). This dual action destabilizes the mineral lattice and accelerates weathering, effectively liberating soluble K. Supplemental mechanisms, including enzymolysis and capsule absorption; further enhance dissolution efficiency to ensure a steady nutrient supply [24, 25, 26].

The current study successfully identified K-solubilizing bacterial strain *Enterobacter cloacae* from paddy rhizosphere soil. The quantitative assessment of isolate *Enterobacter cloacae* on Aleksandrov medium confirms its status as a potent potassium-solubilizing microorganism (KSM). The formation of a distinct, transparent solubilization halo around the colony is a hallmark of mineral weathering, primarily attributed to the microbial secretion of organic acids. These organic acids facilitate acidolysis, where protons (H^+) displace potassium ions from the mineral lattice, and chelation, which stabilizes metal cations and prevents the re-precipitation of K [27, 28]. In addition, the result demonstrates that SRB-11 does not merely grow rapidly (C = 1.2 cm) but possesses a high specific metabolic activity for potassium mobilization. This distinction is critical; it suggests that even at low population densities in the rhizosphere, the isolate may exert a disproportionately large influence on nutrient availability. The SI of 2.33 compares favourably with various KSMs reported in recent literature. For instance, studies on Bacillus and Pseudomonas species in similar tropical soil profiles often report SI values ranging between 1.5 and 2.5 [9, 24]. For farmers dealing with K-deficient soils, the metabolic efficiency of SRB-11 offers a sustainable alternative to inorganic fertilizers. The ability of the isolate to create a significant zone (SI = 2.3 cm) implies that in a soil environment, the reach of the bacteria extends well beyond the point of inoculation.

The efficacy of *E. cloacae* in enhancing crop productivity has been extensively documented, encompassing its functional phenotypic, genotypic, and metabolomic characteristics [29, 30]. In addition high-performance liquid chromatography (HPLC) analysis of the organic acids produced by *Enterobacter spp*. revealed a predominance of gluconic acid, followed by malic, citric, succinic, and acetic acids, as well as gibberellic acid [6]. Therefore the integration of these microbial inoculants into agronomic practices provides a viable pathway for restoring soil microflora and improving the physical and chemical properties of the rhizosphere, ultimately driving higher crop performance.

Conclusion

Current farming practices are steadily draining agricultural soils of essential nutrients, with potassium (K) being among the most severely depleted. While chemical fertilizers can replenish these levels, their high cost and a general lack of awareness regarding potassium deficiency often prevent farmers from using them effectively. To achieve truly sustainable agriculture, we must learn to tap into the large reserves of potassium already present in the soil that are currently "locked" in mineral forms. This study highlights that specialized soil bacteria, known as Potassium Solubilizing Bacteria (KSB), act as natural recyclers. They use their own biological processes to break down minerals and release potassium in a form that plants can easily absorb. By adding these beneficial microbes back into the soil, we can boost crop yields and protect the environment from the harmful runoff associated with over-using chemicals. Although these "invisible helpers" are not yet widely recognized by the public or used in mainstream farming, they represent a major opportunity for the future of food security. The *Enterobacter cloacae* isolate identified in this study, indigenous to the unique paddy rhizosphere of the Kuttanadu region, demonstrates significant biofertilizer, providing a reliable and eco-friendly way to nourish crops for the long term.

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Statements and Declarations

The authors declare that we have no known competing financial interests or personal relationships that could have

appeared to influence the work reported in this paper. On behalf of all authors, the corresponding author states that there is no conflict of interest.

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