

Characterization of the rhizosphere potassium-solubilizing *Cupriavidus taiwanensis* strain NKK10 by transcriptional profiling analysis

Youjun Zhang^{1,2}, Penghui Li³, Ruiling Zhang^{3,4*}, Xiaopeng Zhang², Hui Cong² and Lin Zhao^{1*}

¹ School of Environmental Science and Engineering, Tianjin University, Tianjin 300072, China

² Tianjin North China Geological Exploration Bureau, Tianjin 300170, China

³ School of Environmental Science and Safety Engineering, Tianjin University of Technology, Tianjin 300384, China

⁴ Tianjin TianBinTongSheng Environmental Technology Co., Ltd. Tianjin 300199, China

* Corresponding authors, E-mail: ruilingzhang@tjut.edu.cn; zhaolin@tju.edu.cn

Abstract

Potassium-solubilizing bacteria are microorganisms capable of dissolving potassium from potassium-containing minerals, with some species carrying the ability to promote plant growth. This study aims to explore the composition of potassium-solubilizing bacteria from the rhizosphere of wild *Lespedeza floribunda*, a representative Leguminosae plant, and the mechanisms underlying potassium release. Among the isolated bacteria, the *Cupriavidus taiwanensis* strain NKK10 exhibited highly efficient potassium solubilizing capacity from potassium feldspar, leading to potassium release of 0.35 and 0.57 mg/mL after 7 d and 14 d, respectively. Microscopic observation showed that the NKK10 cells were able to bind strongly to feldspar, resulting in the formation of a dense biofilm on the mineral surface. As revealed by mRNA sequencing and transcriptional profiling analysis, *C. taiwanensis* NKK10 drastically remodeled transcriptional profiling analysis in the feldspar medium, with upregulation of critical signaling pathways involved in interspecies interaction between organisms and biofilm formation in response to the stimulus of peptidoglycan synthesis. The results of this study extend our knowledge of the functional microorganisms in Leguminosae rhizospheric soil, and provide insights into the transcriptional regulation of potassium-solubilizing bacteria during the solubilization process.

Citation: Zhang Y, Li P, Zhang R, Zhang X, Cong H, et al. 2024. Characterization of the rhizosphere potassium-solubilizing *Cupriavidus taiwanensis* strain NKK10 by transcriptional profiling analysis. *Progress in Reaction Kinetics and Mechanism* 49: e004 <https://doi.org/10.48130/prkm-0024-0003>

Introduction

Potassium is often the critical nutrient for plant growth and agricultural productivity^[1,2]. In the plant body, potassium functions in abundant biochemical and physiological processes, e.g., enzyme activation, nutrient transport, signal transduction, regulation of the intracellular osmotic pressures, protein synthesis, and control of membrane polarity^[3–6]. Adequate potassium supply is essential for maintaining plant physiological processes including photosynthesis, enzyme activation, osmotic regulation, and assimilate translocation, which ultimately determine crop yield and quality^[7,8]. In conventional agricultural practices, water-soluble potassium fertilizers, primarily potassium chloride (KCl), potassium sulfate (K_2SO_4), and potassium dihydrogen phosphate (KH_2PO_4), are extensively applied to meet crop potassium requirements^[9,10]. However, the overuse of these water-soluble potassium fertilizers frequently result in the loss of potassium fertilizers, eutrophication, and soil degradation^[11–13]. To alleviate these problems, it is urgent to develop alternative potassium fertilizers for agricultural production.

Potassium feldspar is an aluminosilicate mineral with the common chemical structure of $K_2O \cdot Al_2O_3 \cdot 6SiO_2$, which contains high potassium contents with the percentage of K_2O being 16.9%^[14,15]. It is a non-water-soluble having the widest distribution and the largest reserves in the earth^[16,17]. With the increasing global demand of potassium fertilizers, many researchers have been devoted to transform potassium feldspar to potassium fertilizers^[18–20]. A series of strategies, e.g., hydrothermal treatment with CaO ^[21], thermal treatment with the assistance of sodium chloride, and calcium chloride^[22], $NaOH-H_2O$ digestion^[23,24], have been developed to release potassium from the feldspar mineral. However, these

strategies often entail high costs due to the use of alkaline sources and thermal energy and may frequently result in the generation of secondary pollutants. Therefore, adopting economic and environmental-friendly strategies is required for the sustainable use of potassium feldspar as a raw material in fertilizer production.

Potassium-solubilizing microbes (KSM) are microorganisms capable of converting insoluble potassium in minerals into plant accessible forms^[25,26]. These beneficial microorganisms not only solubilize mineral potassium but also promote plant growth through multiple mechanisms, particularly nutrient uptake enhancement and root system development^[27–30]. Numerous KSMs, including *Bacillus*, *Enterobacter*, *Priestia*, *Cladosporium*, *Aminobacter*, and *Sphingomonas*, have been previously identified^[25,31–33]. Recent studies have highlighted several key mechanisms by which potassium-solubilizing microbes mobilize mineral or insoluble potassium in soils. These mechanisms include the secretion of organic acids (such as citric acid, oxalic acid, and tartaric acid), which acidify the microenvironment to enhance potassium release, and the production of extracellular enzymes that facilitate mineral degradation^[25,31–33]. Additionally, biofilm formation has been recognized as an adaptive strategy that promotes close contact between microbes and mineral surfaces, enabling more efficient dissolution. Some strains also express mineral-binding proteins, such as feldspar-binding proteins, which directly interact with potassium-bearing minerals to improve nutrient availability. These mechanisms demonstrate the diversity and significance of potassium-solubilizing microbes in nutrient cycling and soil fertility enhancement^[34,35]. Notably, our recent study revealed that *Priestia megaterium* employs specific feldspar-binding proteins to enhance potassium solubilization^[36]. Transcriptional analyses have further demonstrated that potassium

limitation triggers comprehensive metabolic remodeling and upregulation of biofilm-related genes in these bacteria, suggesting sophisticated regulatory networks for mineral weathering

Rhizosphere microorganism plays a critical role in facilitating potassium release, which is essential for plant growth^[37,38]. To deeply investigate the composition of rhizosphere potassium-solubilizing bacteria and the mechanisms by which they respond to insoluble mineral potassium, highly efficient potassium-solubilizing bacteria were isolated and screened from the rhizosphere soil of wild *Lespedeza floribunda* plants. Subsequently, mRNA sequencing and transcriptional profiling analysis were conducted on the highly efficient potassium-solubilizing strain *Cupriavidus taiwanensis* NKK10, cultured in a medium with potassium feldspar as the sole potassium source. This study enhances our understanding of the composition of rhizosphere potassium-solubilizing bacteria and provides valuable insights into their potassium-solubilizing mechanisms at the transcriptional level.

Materials and methods

Materials

The rhizosphere loamy soil (depth 0–15 cm, 2 mm around the plant roots) of wild *floribunda* plants at the vegetative growth stage was sampled from the Limutai Mountain, Jizhou District, Tianjin, China (39°45' N, 117°05' E, altitude 997 m). The physical and chemical properties of rhizosphere soil were analyzed, revealing an organic matter content of 45 g/kg, total nitrogen of 1.8 g/kg, and total phosphorus of 1.6 g/kg, and a pH of 7.5^[39]. The control *Pseudomonas putida* strain KT2440 was sourced from the American Type Culture Collection (ATCC), USA. Feldspar, with a potassium content of 16.9%, was procured from Macklin, China. Safranin O was purchased from Dingguo, China, while other agents were purchased from Aladdin, China.

Isolation of potassium-solubilizing bacteria

The potassium-solubilizing bacteria were isolated from fresh rhizosphere soil collected from wild *Lespedeza floribunda* plants. Briefly, 1 g of soil was suspended in a liquid feldspar medium containing sucrose (10 g), Na₂HPO₄ (0.4 g), (NH₄)₂SO₄ (1 g), MgSO₄ (0.2 g), yeast extract (2 g), FeCl₃ (0.02 g), feldspar (5 g), distilled water (1000 mL) with a pH of 7.2^[36]. The soil suspension was then incubated at 30 °C with shaking for 48 h. After incubation, the cultured suspension was serially diluted with distilled water at 10-fold gradients. Aliquots of the diluted solutions were spread onto feldspar medium plates, and incubated at 30 °C for 48–72 h. Colonies were subsequently transferred to fresh plates for purification, resulting in the isolation of potassium-solubilizing strains designated as NKK1 to NKK15. To identify the phylogenetic relatedness of strain NKK10, its 16S rDNA sequence was obtained, and the sequence was submitted to the NCBI system (accession number OR53494). This sequence was then analyzed using the online BLASTN tool on the NCBI website and used to construct a phylogenetic tree via the MEGA11.0 software.

Potassium-releasing assays

To evaluate the ability of the strains to solubilize potassium from feldspar, they were cultivated in a liquid feldspar medium under shaking conditions at 30 °C for 7 s. After incubation, the cultures were centrifuged at 12000 rpm for 5 min to remove the feldspar and bacterial cells. The potassium concentration in the resulting supernatants was determined using an inductively coupled plasma–mass spectrometer (Elan drc-e, PerkinElmer, USA).

Bacterium staining and microscopy

Bacterial cells were cultured in liquid feldspar medium for 7 d. The feldspars with adsorbed bacterial cells were isolated by standing the cultures for 10 min, followed by sampling the pellets. These pellets were resuspended in distilled water, and safranin O was added to achieve a final concentration of 100 mg/L. After 10 min of staining, the feldspars were gently washed twice with distilled water and observed under a light microscope (BX43, Olympus, Japan). Additionally, the treated feldspars were sampled, fixed with 3% glutaric dialdehyde, dried using a fertilizing vacuum drier (SCIENTZ 10N, SCIENTZ, China), and estimated with a scanning electron microscope (TESCAN MIRA LMS, Czech).

To evaluate bacterial growth in the liquid feldspar medium, cultures were incubated for 0–12 d. The optical density of the cultures, measured after standing at room temperature for 10 min, was measured using a UV-Vis spectrometer (SmartSpec™ Plus, BioRad, USA). The number of viable bacterial cells on the feldspar stones at different time points was determined by the colony-forming unit (CFU) method^[40]. Cultures were serially diluted by distilled water, and the diluted solutions were plated onto the K⁺-rich medium plates. CFUs were counted after 3 d of incubation of the plates at 30 °C.

Construction of phylogenetic tree

The NKK10 cells were cultured for 3 d in a K⁺-rich medium (containing sucrose 10 g, K₂HPO₄ 0.3 g, NaCl 0.1 g, (NH₄)₂SO₄ 1 g, MgSO₄ 0.2 g, yeast extract 2 g, FeCl₃ 0.02 g, distilled water 1,000 mL, pH = 7.2). Genomic DNA was extracted from the cells using a soil DNA extraction kit ((Biomarker Technologies, Beijing, China). The 16S rDNA of the bacterial strains was amplified from the genomic DNA using primers 27F (5'-AGAGTTTGATCCTGGCTCAG-3') and 1492 R (5'-GGTACCTGTTACGACTT-3'). The PCR product was sequenced by Tsingke Biotech., China and the resulting sequence of NKK10 16S rDNA (accession number OR53494) was submitted to the NCBI BLAST system. Similar sequences were retrieved for construction of the phylogenetic tree of NKK10 via the MEGA11.0 software by using the Neighbor-Joining method^[36,41].

mRNA sequencing and transcriptional profiling assays

To investigate the differentially expressed genes (DEGs) of NKK10 during potassium solubilization, NKK10 cells were cultured for 7 d in liquid feldspar medium or a control K⁺-rich medium. Following incubation, bacterial cells were harvested by centrifugation at 12,000 rpm for 3 min. Total mRNA was extracted from the obtained cells by using an RNA extraction kit (Promega, Beijing, China), and corresponding cDNA was prepared with the assistance of poly-T oligo-attached magnetic beads to constructed the cDNA libraries. The libraries were then sequenced by using the Illumina NovaSeq platform to generate 150 bp paired-end reads (BioZeron, China) system. The obtained sequences were analyzed with the *C. taiwanensis* reference genomes in the NCBI database (www.ncbi.nlm.nih.gov/genome/1372?genome_assembly_id=171204). The differentially expressed genes were then assigned into the Gene Ontology (GO) terms and the Kyoto Encyclopedia of Genes and Genomes (KEGG) terms, by using the BioZeron analysis system and the online system of Biocloud (www.biocloud.net)^[42].

Statistical analysis

Each experiment in this study was performed with three replicates (n = 3). The values were shown in the figures with the means ± the standard errors. Differences between the groups were compared by the t-Student tests (*p* < 0.05). All statistical tests were performed with the SPSS software package (Version 20, IBM).

Results

The strain NKK10 exhibits the strongest capacity to release potassium from feldspar

After separation and purification, a total of 15 potassium solubilizing bacterial strains were obtained on the plate, coded NKK1~NKK15, for further study. After 7 d of culturing in liquid feldspar medium, the 15 strains led to the release of K^+ from potassium feldspar from 0.02 to 0.35 mg/mL (Fig. 1a). Among them, the strain NKK10 exhibited the highest potassium-releasing capacity, with the released potassium reaching 0.35 mg/mL (Fig. 1a). Time-dependent potassium release assays showed that the bacterium-free control group and the *P. putida* (the control *P. putida* strain KT2440) group only slightly released K^+ to 0.05 mg/mL even after 14 d. In contrast, the NKK10 group had remarkable potassium release from day 2 to day 10 and reached 0.57 mg/mL after 14 d (Fig. 1b). Therefore, the strain NKK10 was chosen as the high-efficiency potassium-solubilizing strain for further investigations.

The strain NKK10 has high-growth biomass in the potassium feldspar medium

The growth of strain NKK10 was assessed in a feldspar medium. After 7 d of cultivation, the feldspars were isolated for microscope observation. While the control *P. putida* group showed sparse bacterial colonization on the feldspar, leaving its smooth surface of the feldspar, the NKK10 group exhibited extremely high levels of bacterial cells, with the cells forming dense biofilms on the feldspar (Fig. 2a, b). The NKK10 group showed much higher OD_{600} values and CFU than the control and the *P. putida*-treated group (Fig. 2c, d). For instance, on day 8, the NKK10 group exhibited an OD_{600} value of 0.88 and the viable bacterial cells at $10^{9.1}$ CFU/g stone. In contrast, both the control and the *P. putida*-treated group showed OD_{600} below 0.16, and viable cells counts below $10^{2.8}$ CFU/g stone (Fig. 2c, d). These results indicated that strain NKK10 maintained stable growth in a medium using potassium feldspar as the sole potassium source. This is consistent with the observation that NKK10 possesses a high capacity to release potassium from feldspar, efficiently supplying potassium to sustain bacterial cell growth.

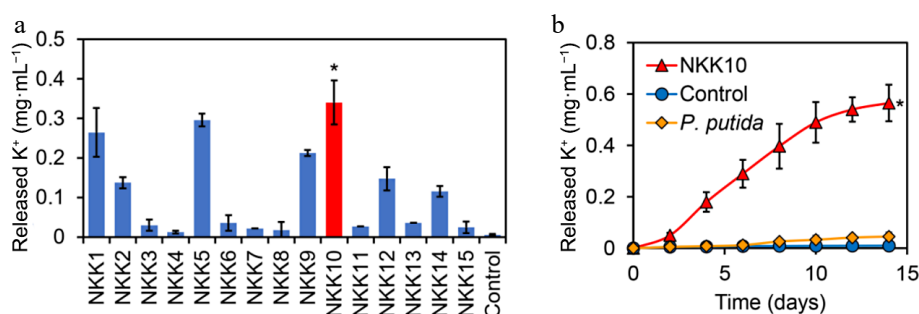


Fig. 1 Potassium release from potassium feldspar induced by the bacterial strains isolated from *Lespedeza floribunda* rhizosphere soil. (a) Potassium release induced by the strains from the isolated strains after 7 d of culturing. Control indicates the group with no addition of the bacterial strains in the potassium feldspar medium. (b) Time-dependent potassium release by the *P. putida* strain KT2440. The error bars indicate the standard errors of each group ($n = 3$). The asterisks (*) indicate significant difference between the NKK10 group and other groups ($p < 0.05$).

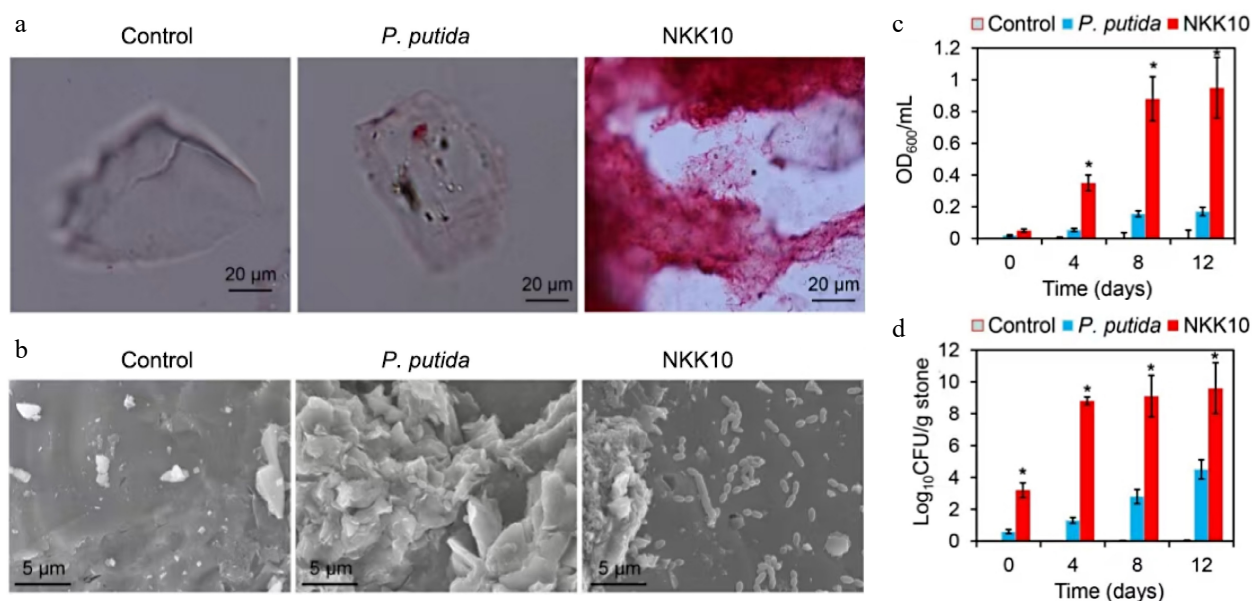


Fig. 2 Effect of potassium feldspar on growth of the *P. putida* KT2440 and NKK10. (a) Microscope images of feldspars after co-incubation of the strains for 7 d. The strains were incubated in the potassium feldspar medium, the feldspars were then sampled, and stained by safranin O for microscopy. (b) Scanning Electron Microscope (SEM) images of the feldspar after 7 d of treatment by the bacterial cells. The blue arrows indicate the distribution of bacterial cells on the feldspar surface. (c) Optical density of the cultures after 10 min of standing at the room temperature. (d) The number of viable bacterial cells on the feldspar stones at different time points. The error bars indicate the standard errors of each group ($n = 3$). (*) indicate significant difference between the NKK10 group and other groups ($p < 0.05$).

The potassium-solubilizing strain NKK10 belongs to *C. taiwanensis*

To determine the phylogenetic relatedness of strain NKK10, its 16S rDNA sequence was analyzed. As shown in Fig. 3, strain NKK10 is closely related to members of the *Cupriavidus* species, including *C. taiwanensis*, *C. nantongensis*, *C. malaysiensis*, and *C. pampae*. Especially, NKK10 shared the highest similarity (99%) with *C. taiwanensis* CD3, confirming its closest phylogenetic relatedness to *C. taiwanensis*.

Strain NKK10 drastically alters transcriptional profiling analysis in feldspar medium

To elucidate the mechanism of potassium release by NKK10, transcriptional profiling analysis was performed on NKK10 cells cultured in feldspar medium, compared to those grown in a K⁺-rich medium control. The heat map revealed remarkable changes in gene expression in the feldspar medium (FD1-FD3) compared to the control group (CK1-CK3, Fig. 4a). Especially, in samples FD1 and FD3, the majority of genes were upregulated (Fig. 4a). A volcano plot further highlighted that 523 genes were upregulated, whereas only 81 genes were downregulated in the feldspar medium (Fig. 4b). These findings demonstrated that culturing NKK10 cells in feldspar medium strongly altered the transcriptional profiling analysis of this potassium-solubilizing bacterium.

The strain NKK10 exhibits changed GO map in feldspar medium

GO analysis was performed to illustrate the change in the GO map of NKK10 cells cultured in the feldspar medium. Regarding biological processes, culturing bacterial cells in the feldspar medium led to the upregulation of genes involved in biological regulation, inter-species interaction between organisms, localization, multi-organism process, reproduction, reproductive process, response to stimulus, and signaling (Fig. 5a). For molecular functions, the

feldspar-treated group exhibited increased expression of genes related to transcriptional regulator activity (Fig. 5a). These results indicated that NKK10 cells robustly activated a series of signaling pathways to strengthen intercellular interaction, thereby maintaining high metabolic activity and growth rate. In addition, the feldspar-treated group showed a comparable number of genes related to cellular components (CC, Fig. 5a), suggesting that the alteration of the potassium sources had minimal impact on cellular components. However, the dot map analysis revealed slight changes in the expression of the genes associated with transferase activity (transferring hexosyl groups), cytokinesis, acid-amino acid ligase activity, localization, and protein binding (Fig. 5b). In contrast, the expression of the genes related to cell movement, including bacterial-type flagellum basal body, bacterial-type flagellum-dependent swarming motility, chemotaxis, bacterial-type flagellum, and locomotion, was significantly changed, with an R-factor > 0.50, and P value < 0.01 (Fig. 5b). These results indicated that bacterial motility was significantly affected in the feldspar medium.

The strain NKK10 remodels metabolic pathways in feldspar medium

KEGG pathway analysis of transcriptional profiling was conducted in NKK10 cells. While pathways related to lysine/aminobenzoate/sugar metabolism were only slightly changed ($p > 0.12$, R factor < 0.25), several pathways were strongly impacted in feldspar medium ($p < 0.04$, Fig. 6a). These included ABC transporters, *Pseudomonas aeruginosa*-type biofilm formation, flagellar assembly, peptidoglycan biosynthesis, starch sucrose metabolism, and bacterial chemotaxis. For example, the pathways of peptidoglycan biosynthesis and *Pseudomonas aeruginosa*-type biofilm formation had R factors exceeding 0.38 and 0.25, respectively (Fig. 6a).

In the pathway of DAP-type peptidoglycan synthesis, the proteins MurC and MurD, which catalyze the conversion of UDP-GlcNAc to UDP-MurNAc-L-Ala- γ -D-Glu, as well as MurF, MraY, MurG, and ClassA

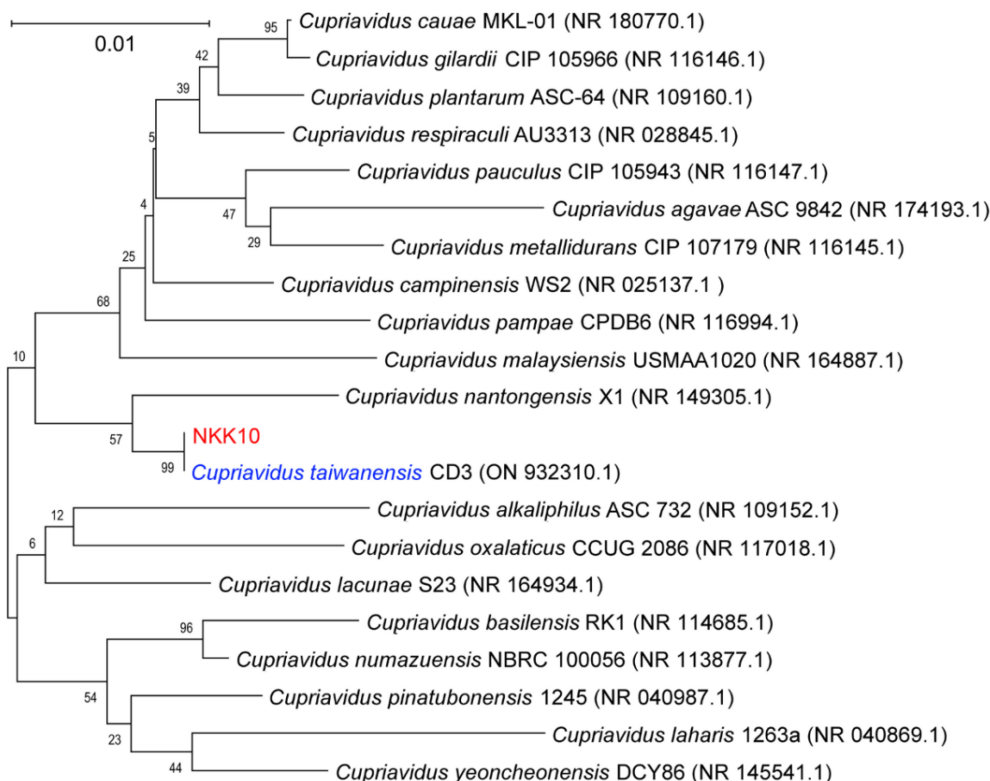
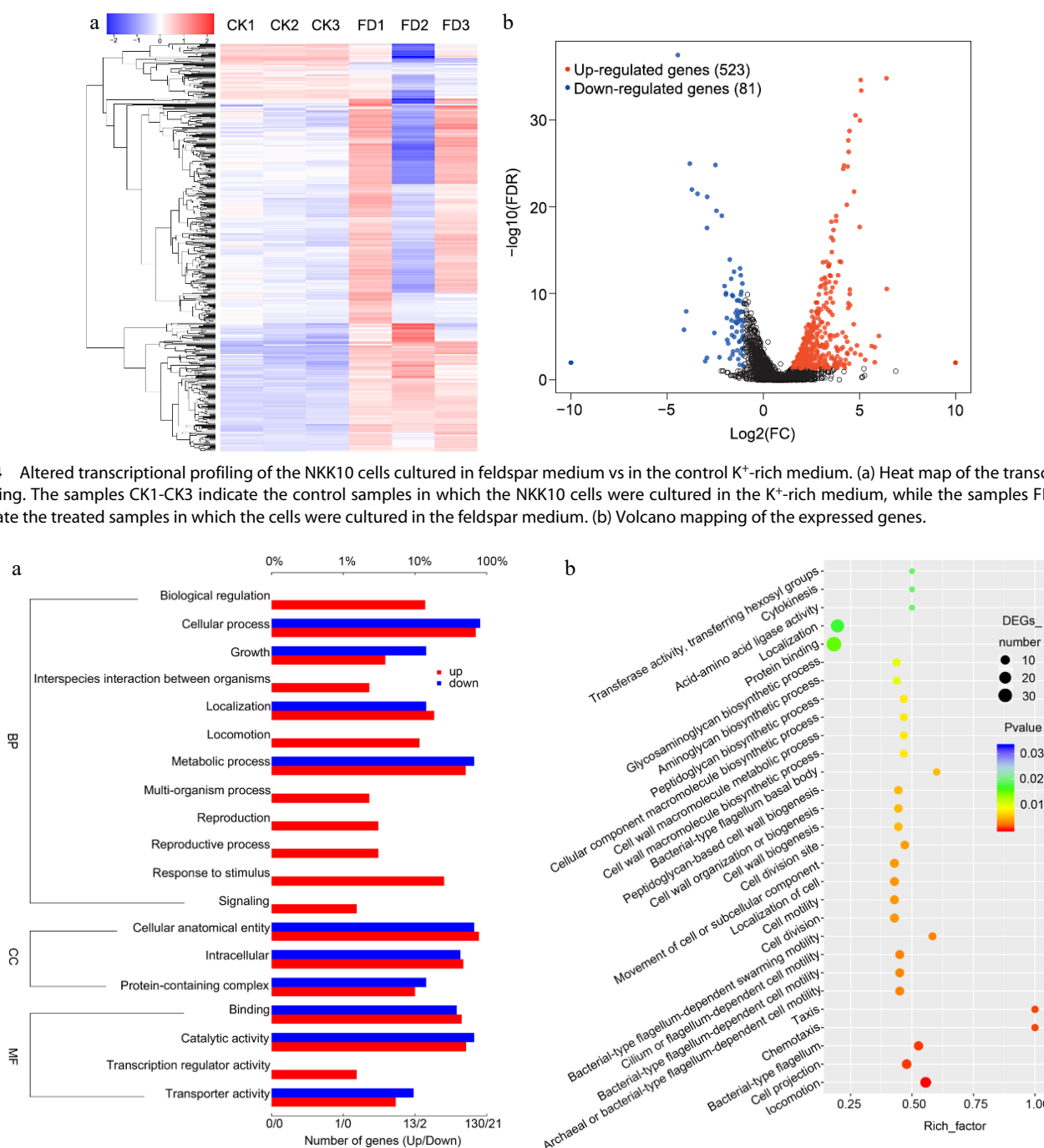


Fig. 3 Phylogenetic tree of NKK10 constructed by the MEGA11.0 software with the Neighbor-Joining method.



PBP, which drives the subsequent transformation of UDP-MurNAc-L-Ala- γ -D-Glu to DAP-type peptidoglycan, were upregulated (Fig. 6b). Similarly, in the *Pseudomonas aeruginosa*-type biofilm formation pathway, upregulation was observed in proteins related to the Type 3 secretion system (PilJ, PilH, and PilG), the Type 6 secretion system (HSI-I), and polysaccharide synthesis (WspA, WspB/D & WspE, WspR, PelF) in the cells cultured in the feldspar medium were upregulated (Fig. 6c). These results indicate that NKK10 cells remarkably remodeled their metabolic pathways, particularly those related to cell wall synthesis and biofilm formation, to optimize metabolism and growth with feldspar as the sole potassium source.

Discussion

Composition of potassium-solubilizing bacteria in the rhizosphere soil

Potassium-solubilizing bacteria hold great promise in crop production due to their ability to reduce reliance on synthetic fertilizers and promote plant growth^[43,44]. Rhizosphere soil serves as a rich reservoir for the potassium-solubilizing bacteria^[45,46]. Numerous species, such as *Priestia megaterium*, *Klebsiella variicola*, *Agrobacterium tumefaciens*, *Enterobacter cloacae*, *Mesorhizobium* sp., *Paenibacillus* sp., *Arthrobacter* sp., *Burkholderia* sp., *Pseudomonas*

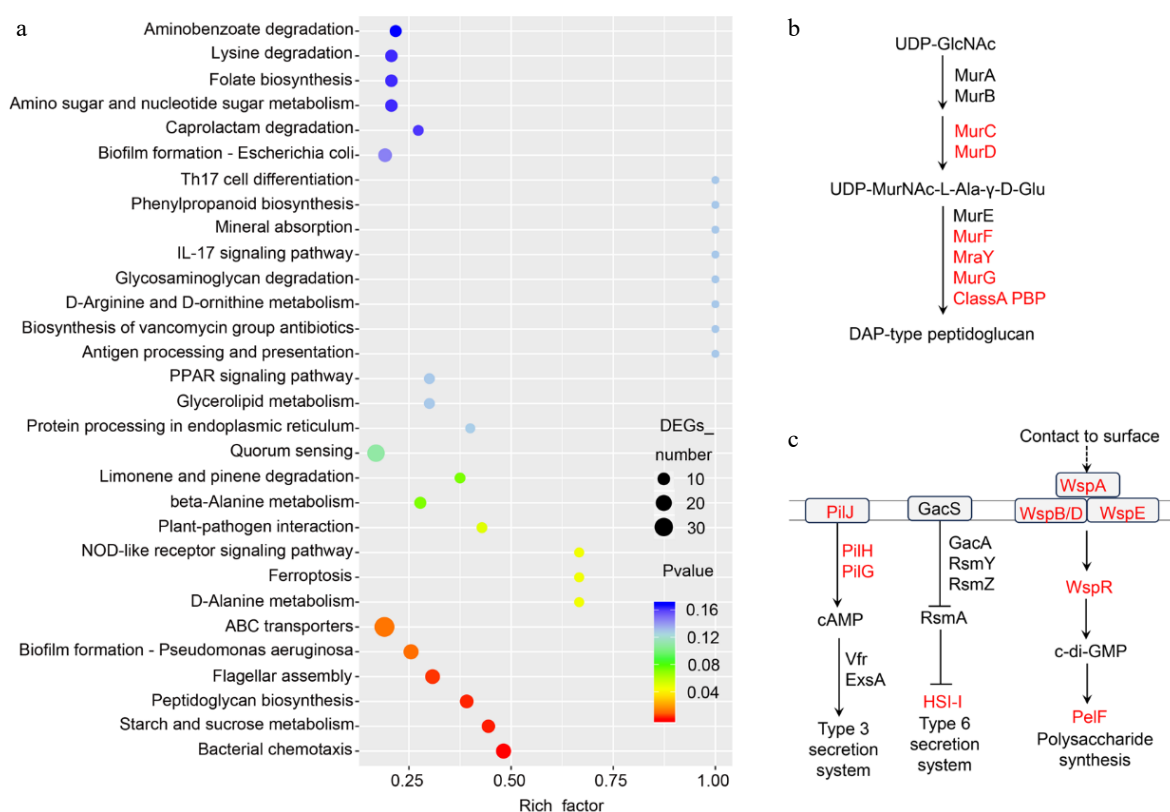


Fig. 6 KEGG analysis of the NKK10 transcriptional profiling in feldspar medium vs in the control K^+ -rich medium. (a) Dot map of KEGG analysis showing the gene groups with changed expression levels. (b) The proteins with up-regulated expression (indicated by red color) in the pathway of peptidoglycan biosynthesis. The numbers in the brackets indicate the \log_2 (fold change) values of the corresponding genes. (c) The proteins with up-regulated expression (indicated by red color) in the pathway of biofilm formation.

rhodesiae, and *Ralstonia mannitolilytica*, have been isolated from various plant rhizosphere soils^[36,47,48]. However, the diversity and functions of potassium-solubilizing bacteria in the rhizosphere of Leguminosae plants remain largely unexplored. In this study, rhizosphere soil from *Lespedeza floribunda*, a wild Leguminosae plant species, was sampled, leading to the isolation of potassium-solubilizing bacteria, including *C. taiwanensis*, a novel potassium-solubilizing bacterium. Traditionally, *C. taiwanensis* has been recognized for its high tolerance to heavy metal, having been isolated from heavy metal-contaminated soils^[48,49]. However, its additional role in potassium solubilization and nutrient cycling in the rhizosphere is demonstrated. Therefore, *C. taiwanensis* is not only a heavy metal-tolerant bacterium, but also a potential PGPM, which may be used for development of inocula to optimize the soil quality and promote the plant growth.

Role of biofilms during potassium solubilization

Bacterial biofilms are specialized aggregates of bacterial cells that integrate and communicate with one another through extracellular polymeric substrates (EPS)^[50,51]. These EPS, comprising polysaccharides, proteins, and nucleic acids envelop the cells, facilitating enhanced intercellular interaction and improving their tolerance to environmental stress^[52,53]. Recently, it has been discovered that biofilms formed by heavy metal-capturing bacteria play a crucial role in strengthening bacterium-plant host interaction and enhancing heavy metal removal efficiency^[54,55]. In this study, it was further found that the *C. taiwanensis* cells developed dense biofilms on the surface of feldspars for the solubilization of potassium, which is associated with the strong potassium-releasing capacity of this bacterium. Together, these findings implied that the regulation of bacterial biofilm may promote the activity of functional bacteria during agricultural production and environmental treatment.

Transcriptional regulation of potassium-solubilizing bacteria

Transcriptional regulation is a fundamental mechanism of living cells to intelligently respond to extracellular nutrition, stress, and other signals^[56,57]. When the cells encounter nutrients with low water solubility, they may remodel the transcriptional profiling, leading to production and secretion of extracellular enzymes, organic acids, and other extracellular components to dissolve the nutrients^[51,57,58]. Transcription of functional genes dominates bacterial transformation and utilization of extracellular substances^[59]. Herein, it was found that the *C. taiwanensis* cells, when they were cultured in feldspar medium, drastically remodeled transcriptional profiling for adapting to the potassium-depleted condition. As revealed by GO analysis, a series of gene groups, e.g., biological regulation, interspecies interaction between organisms, localization, multi-organism process, reproduction, reproductive process, response to stimulus, and signaling were upregulated in the feldspar medium as compared to the K^+ -rich medium. This suggested that the signal pathways involved in stress response and intracellular interaction were activated, resulting in a sensitive response to the feldspar sources. Further insights from the KEGG pathway analysis highlighted the activation of critical systems such as the Type III and Type VI secretion systems and polysaccharide synthesis pathways. These molecular mechanisms likely play a pivotal role in facilitating mineral attachment, biofilm stabilization, and the secretion of enzymes or organic acids that contribute to potassium release. For example, the upregulation of peptidoglycan biosynthesis-related proteins (e.g., MurC, MurD, MurF) and biofilm-associated proteins (e.g., WsbA, WsbB/D, PelF) underscores the bacterium's ability to remodel its cellular machinery for effective mineral weathering. Such adaptive responses demonstrate that *C.*

taiwanensis employs sophisticated transcriptional strategies not only to survive under potassium-limited conditions but also to actively modify its local environment to enhance nutrient availability. These findings provide a deeper understanding of the transcriptional regulation underlying potassium solubilization and highlight the strain's potential application in sustainable agriculture.

Conclusions

This study investigated the presence of potassium-solubilizing bacteria in the rhizosphere soil of wild *Lespedeza floribunda* and identified a novel high-efficiency potassium-solubilizing bacterium, *C. taiwanensis*, expanding the known diversity of functional soil microorganisms. It revealed the critical role of biofilm formation in bacterial potassium solubilization, demonstrating that *C. taiwanensis* forms specialized surface structures to enhance mineral weathering efficiency. Through transcriptional profiling analysis, this study uncovered the complex molecular networks regulating bacterial adaptation to mineral potassium, particularly the coordinated regulation of pathways related to cell wall synthesis, secretion systems, and biofilm formation. These molecular insights provide new strategies for improving microbial potassium-solubilization efficiency. Overall, the present research deepens the fundamental understanding of microbe-mineral interactions and offers valuable guidance for developing effective microbial agents to enhance soil quality and support sustainable agriculture.

Author contributions

The authors confirm contribution to the paper as follows: conceptualization, writing – original draft preparation: Zhang Y, Li P, Zhao L; data curation: Zhang Y, Li P, Zhang R; investigation: Zhang Y; validation: Zhang Y, Zhang R, Zhang X, Cong H, Zhao L; writing – review & editing: Zhang R, Zhang X, Cong H, Zhao L; formal analysis: Li P, Zhang R; resources: Li P, Zhao L; software: Li P, Cong H; methodology: Zhang R; visualization: Zhang R, Zhang X; project administration: Zhao L. All authors reviewed the results and approved the final version of the manuscript.

Data availability

The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

Acknowledgments

This work was supported by Tianjin Science and Technology Research Project (22YFYSHZ00310, 24YFXTHZ00170).

Conflict of interest

The authors declare that they have no conflict of interest.

Dates

Received 29 October 2024; Revised 24 November 2024; Accepted 26 November 2024; Published online 31 December 2024

References

- Johnson R, Vishwakarma K, Hossen MS, Kumar V, Shackira AM, et al. 2022. Potassium in plants: growth regulation, signaling, and environmental stress tolerance. *Plant Physiology and Biochemistry* 172:56–69
- Srivastava AK, Shankar A, Nalini Chandran AK, Sharma M, Jung KH, et al. 2020. Emerging concepts of potassium homeostasis in plants. *Journal of Experimental Botany* 71:608–19
- Santos EF, Mateus NS, Rosário MO, Garcez TB, Mazzafera P, et al. 2021. Enhancing potassium content in leaves and stems improves drought tolerance of eucalyptus clones. *Physiologia Plantarum* 172:552–63
- Sardans J, Peñuelas J. 2015. Potassium: a neglected nutrient in global change. *Global Ecology and Biogeography* 24:261–75
- Gessler A, Schaub M, McDowell NG. 2017. The role of nutrients in drought-induced tree mortality and recovery. *New Phytologist* 214:513–20
- Sardans J, Peñuelas J. 2021. Potassium control of plant functions: ecological and agricultural implications. *Plants* 10:419
- Jaiswal DK, Verma JP, Prakash S, Meena VS, Meena RS. 2016. Potassium as an important plant nutrient in sustainable agriculture: a state of the art. In *Potassium Solubilizing Microorganisms for Sustainable Agriculture*, ed. Meena VS, Maurya BR, Verma JP, Meena RS. New Delhi, India: Springer. pp. 21–29. doi: 10.1007/978-81-322-2776-2_2
- Liu K, Ma BL, Luan L, Li C. 2011. Nitrogen, phosphorus, and potassium nutrient effects on grain filling and yield of high-yielding summer corn. *Journal of Plant Nutrition* 34:1516–31
- Zörb C, Senbayram M, Peiter E. 2014. Potassium in agriculture – Status and perspectives. *Journal of Plant Physiology* 171:656–69
- elov SV, Danyleiko YK, Glinushkin AP, Kalinitchenko VP, Egorov AV, et al. 2021. An activated potassium phosphate fertilizer solution for stimulating the growth of agricultural plants. *Frontiers in Physics* 8:618320
- Saljnikov E, Lavrishchev A, Römbke J, Rinklebe J, Scherber C, et al. 2022. Understanding and monitoring chemical and biological soil degradation. In *Advances in Understanding Soil Degradation*, ed. Saljnikov E, Mueller L, Lavrishchev A, Eulenstein F. Cham: Springer International Publishing. pp. 75–124. doi: 10.1007/978-3-030-85682-3_3
- Lu M, Powlson DS, Liang Y, Chadwick DR, Long S, et al. 2021. Significant soil degradation is associated with intensive vegetable cropping in a subtropical area: a case study in southwestern China. *SOIL* 7:333–46
- Hunke P, Mueller EN, Schröder B, Zeilhofer P. 2015. The Brazilian Cerrado: assessment of water and soil degradation in catchments under intensive agricultural use. *Ecohydrology* 8:1154–80
- Manning DAC. 2010. Mineral sources of potassium for plant nutrition. A review. *Agronomy for Sustainable Development* 30:281–94
- Kronenberg AK, Yund RA, Rossman GR. 1998. Reply to the comment by Robert H. Doremus on "Stationary and Mobile Hydrogen Defects in Potassium Feldspar". *Geochimica et Cosmochimica Acta* 62:379–82
- Harris MJ, Salje EKH, Guttler BK, Carpenter MA. 1989. Structural states of natural potassium feldspar: An infrared spectroscopic study. *Physics and Chemistry of Minerals* 16:649–58
- Hellmann R, Zhai Y, Robin E, Findling N, Mayanna S, et al. 2021. The hydrothermal alkaline alteration of potassium feldspar: A nanometer-scale investigation of the orthoclase interface. *Chemical Geology* 569:120133
- Ma H, Yang J, Su S, Liu M, Zheng H, et al. 2015. 20 Years advances in preparation of potassium salts from potassic rocks: a review. *Acta Geologica Sinica (English Edition)* 89:2058–71
- Sanz Scovino JI, Rowell DL. 1988. The use of feldspars as potassium fertilizers in the savannah of Colombia. *Fertilizer research* 17:71–83
- Ciceri D, Close TC, Barker AV, Allanore A. 2019. Fertilizing Properties of Potassium Feldspar Altered Hydrothermally. *Communications in Soil Science and Plant Analysis* 50:482–91
- Ciceri D, de Oliveira M, Allanore A. 2017. Potassium fertilizer via hydrothermal alteration of K-feldspar ore. *Green Chemistry* 19:5187–202
- Jena SK, Mohapatra S, Mohanty B, Nayak AP, Palatasingh S. 2024. Thermal treatment of K-feldspar for potassium recovery using NaCl–CaCl₂ mixture and its mechanism study. *Chemical Engineering Communications* 211:251–62
- Ma X, Yang J, Ma H, Liu C, Zhang P. 2015. Synthesis and characterization of analcime using quartz syenite powder by alkali-hydrothermal treatment. *Microporous and Mesoporous Materials* 201:134–40
- Yang J, Ma H. 2016. Zeolitization of potassic syenites by alkali-hydrothermal treatment and its mineralization significance. *Acta Mineralogica Sinica* 36:38–42
- Jini D, Ganga VS, Greeshma MB, Sivashankar R, Thirunavukkarasu A. 2024. Sustainable agricultural practices using potassium-solubilizing

- microorganisms (KSMs) in coastal regions: a critical review on the challenges and opportunities. *Environment Development and Sustainability* 26:13641–64
26. Bakhshandeh E, Pirdashti H, Lendeh KS. 2017. Phosphate and potassium-solubilizing bacteria effect on the growth of rice. *Ecological Engineering* 103:164–69
 27. Ali AM, Awad MYM, Hegab SA, Gawad AMAE, Eissa MA. 2021. Effect of potassium solubilizing bacteria (*Bacillus cereus*) on growth and yield of potato. *Journal of Plant Nutrition* 44:411–20
 28. Wang Y, Yan X, Su M, Li J, Man T, et al. 2022. Isolation of potassium solubilizing bacteria in soil and preparation of liquid bacteria fertilizer from food wastewater. *Biochemical Engineering Journal* 181:108378
 29. Liu S, Tang W, Yang F, Meng J, Chen W, et al. 2017. Influence of biochar application on potassium-solubilizing *Bacillus mucilaginosus* as potential biofertilizer. *Preparative Biochemistry & Biotechnology* 47:32–37
 30. Khatoun Z, Huang S, Rafique M, Fakhar A, Kamran MA, et al. 2020. Unlocking the potential of plant growth-promoting rhizobacteria on soil health and the sustainability of agricultural systems. *Journal of Environmental Management* 273:111118
 31. Chen Y, Yang H, Shen Z, Ye J. 2022. Whole-genome sequencing and potassium-solubilizing mechanism of *Bacillus aryabhattai* SK1-7. *Frontiers in Microbiology* 12:722379
 32. Meena VS, Maurya BR, Verma JP, Aeron A, Kumar A, et al. 2015. Potassium solubilizing rhizobacteria (KSR): Isolation, identification, and K-release dynamics from waste mica. *Ecological Engineering* 81:340–47
 33. Santosh S, Velmourugane K, Idapuganti RG, Manikandan A, Blaise D. 2022. Potassium Solubilizing Potential of Native Bacterial Isolates from Cotton Rhizosphere of Rainfed Vertisols. *National Academy Science Letters* 45:209–12
 34. Mali SD, Attar YC. 2021. Formulation of cost-effective agro residues containing potassium solubilizing bacterial bio-inoculants using response surface methodology. *Biocatalysis and Agricultural Biotechnology* 35:102113
 35. Lang J, Ye M, Luo Y, Wang Y, Shi Z, et al. 2024. Effects of potassium-solubilizing bacteria on growth, antioxidant activity and expression of related genes in *Fritillaria taipaiensis* P. Y. Li. *Phyton - International Journal of Experimental Botany* 93:789–806
 36. Wu X, Zhao Z, Zhao Z, Zhang Y, Li M, et al. 2023. Analysis of the potassium-solubilizing *Priestia megaterium* strain NK851 and its potassium feldspar-binding proteins. *International Journal of Molecular Sciences* 24:14226
 37. Enagbonma BJ, Fadiji AE, Ayangbenro AS, Babalola OO. 2023. Communication between plants and rhizosphere microbiome: exploring the root microbiome for sustainable agriculture. *Microorganisms* 11:2003
 38. Mahmud K, Missaoui A, Lee K, Ghimire B, Presley HW, et al. 2021. Rhizosphere microbiome manipulation for sustainable crop production. *Current Plant Biology* 27:100210
 39. Zhao Z, Liu L, Sun Y, Xie L, Liu S, et al. 2024. Combined microbe-plant remediation of cadmium in saline-alkali soil assisted by fungal mycelium-derived biochar. *Environmental Research* 240:117424
 40. Bhuyan S, Yadav M, Giri SJ, Begum S, Das S, et al. 2023. Microliter spotting and micro-colony observation: A rapid and simple approach for counting bacterial colony forming units. *Journal of Microbiological Methods* 207:106707
 41. Hall BG. 2013. Building phylogenetic trees from molecular data with MEGA. *Molecular Biology and Evolution* 30:1229–35
 42. Zhao Z, Sun Y, Wang H, Yu Q. 2023. Regulation of cadmium-induced biofilm formation by artificial polysaccharide-binding proteins for enhanced phytoremediation. *Chemosphere* 342:140156
 43. Dong X, Lv L, Wang W, Liu Y, Yin C, et al. 2019. Differences in distribution of potassium-solubilizing bacteria in forest and plantation soils in myanmar. *International Journal of Environmental Research and Public Health* 16:700
 44. Sattar A, Naveed M, Ali M, Zahir ZA, Nadeem SM, et al. 2019. Perspectives of potassium solubilizing microbes in sustainable food production system: A review. *Applied Soil Ecology* 133:146–59
 45. Parmar P, Sindhu SS. 2013. Potassium Solubilization by Rhizosphere Bacteria: Influence of Nutritional and Environmental Conditions. *Journal of Microbiology Research* 3:25–31
 46. Yaghoubi Khanghahi M, Pirdashti H, Rahimian H, Nematzadeh G, Ghajar Sepanlou M. 2018. Potassium solubilizing bacteria (KSB) isolated from rice paddy soil: from isolation, identification to K use efficiency. *Symbiosis* 76:13–23
 47. Zhang C, Kong F. 2014. Isolation and identification of potassium-solubilizing bacteria from tobacco rhizospheric soil and their effect on tobacco plants. *Applied Soil Ecology* 82:18–25
 48. Xiao Y, Wang X, Chen W, Huang Q. 2017. Isolation and Identification of Three Potassium-Solubilizing Bacteria from Rape Rhizospheric Soil and Their Effects on Ryegrass. *Geomicrobiology Journal* 34:873–80
 49. Chien CC, Wang LJ, Lin WR. 2014. Polyhydroxybutyrate accumulation by a cadmium-resistant strain of *Cupriavidus taiwanensis*. *Journal of the Taiwan Institute of Chemical Engineers* 45:1164–69
 50. Mahto KU, Das S. 2022. Bacterial biofilm and extracellular polymeric substances in the moving bed biofilm reactor for wastewater treatment: A review. *Bioresource Technology* 345:126476
 51. Cheah YT, Chan DJC. 2022. A methodological review on the characterization of microalgal biofilm and its extracellular polymeric substances. *Journal of Applied Microbiology* 132:3490–514
 52. Lee KWK, Periasamy S, Mukherjee M, Xie C, Kjelleberg S, et al. 2014. Biofilm development and enhanced stress resistance of a model, mixed-species community biofilm. *The ISME Journal* 8:894–907
 53. Silva NBS, Marques LA, Röder DDB. 2021. Diagnosis of biofilm infections: current methods used, challenges and perspectives for the future. *Journal of Applied Microbiology* 131:2148–60
 54. Feng LA, Liang B, Zeng X, Shi C, Yin H, et al. 2022. Engineered bacterium-binding protein promotes root recruitment of functional bacteria for enhanced cadmium removal from wastewater by phytoremediation. *Water Research* 221:118746
 55. Yin H, Chen Y, Feng Y, Feng L, Yu Q. 2022. Synthetic physical contact-remodeled rhizosphere microbiome for enhanced phytoremediation. *Journal of Hazardous Materials* 433:128828
 56. Yi L, Dong X, Grenier D, Wang K, Wang Y. 2021. Research progress of bacterial quorum sensing receptors: Classification, structure, function and characteristics. *Science of The Total Environment* 763:143031
 57. Dar D, Dar N, Cai L, Newman DK. 2021. Spatial transcriptomics of planktonic and sessile bacterial populations at single-cell resolution. *Science* 373:eabi4882
 58. Xiao B, Lian B, Sun L, Shao W. 2012. Gene transcription response to weathering of K-bearing minerals by *Aspergillus fumigatus*. *Chemical Geology* 306-307:1–9
 59. Xuan W, Beeckman T, Xu G. 2017. Plant nitrogen nutrition: sensing and signaling. *Current Opinion in Plant Biology* 39:57–65



Copyright: © 2024 by the author(s). Published by Maximum Academic Press, Fayetteville, GA. This article is an open access article distributed under Creative Commons Attribution License (CC BY 4.0), visit <https://creativecommons.org/licenses/by/4.0/>.