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Characterization and Utilization of Silicate-Solubilizing Bacteria in Improving Rice Plant Growth and Yield

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ABSTRACT

Rice production has recently declined, primarily due to reduced soil fertility and the high cost of synthetic fertilizers. This necessitated exploring greener alternatives, such as silicate-solubilizing bacteria (SSB), which are vital for enhancing soil fertility by making silicate available for plant use. The objectives of this study are to screen selected bacteria from culture collections for silicate-solubilizing properties, characterize them for silicate-solubilizing properties, and evaluate the effect of silicate-solubilizing bacteria on rice growth and yield. The study was laid out in an RCBD with 2 silicate sources (Calcium Silicate and Kaolinite) and 3 bacterial strains (UPMC1341, UPMC1446, and UPMC383), with B0 serving as a negative control. An 8-treatment combination and a total of 24 experimental units were supplied with silicates, inoculated with 3 SSBs on day one, and re-inoculated at 55 days after transplanting (DAP). In the results, bacterial strains, most importantly UPMC1446, significantly increased plant height and the number of tillers (29 ± 2.08). UPMC1341 also increased the number of panicles/plant and the number of spikelets/panicle, 36±2.34, 211±2.25, respectively, in combination with Kaolinite. This research highlighted the efficacy of SSBs, most importantly UPMC1446 and UPMC1341, in solubilizing insoluble silicate, thereby enhancing rice growth and yield, particularly when supplied with kaolinite.

Keywords: **Silicate-solubilizing bacteria, organic acids, rice yield**

INTRODUCTION

Rice (*Oryza sativa*) is a vital global cereal crop; approximately 154 million hectares are harvested annually. Half of the world's population derives its nutrition from rice (Zhang *et al*., 2011), as it serves as the primary staple food and main calorie source for over three billion people worldwide; Latin America, Asia, and some parts of Africa in particular (Zhang *et al*., 2011; Chauhan *et al*., 2017; Cosslett *et al*., 2017). Recently, rice production worldwide has decreased. This reduction in rice productivity is mostly associated with declines in soil fertility, reduced arable land, climate change, the high cost of synthetic fertilizers, and the spread of unproductive rice varieties (Deng *et al.,* 2019). Silicon (~27%), which is the second most abundant element in the Earth's crust after oxygen (~47%), has been proven beneficial for many plant species' development and healthy growth, especially tropical graminaceous species such as rice (Liang *et al*., 2007). Although it might not be considered an essential element for higher plants. Silicon, which exists as monosilicic acid as a plant-available form of silicon in soil solution, strengthens plant cells, reducing water loss, less frost damage, and enhances more root growth, and equally improves the uptake and distribution of essential nutrients like potassium, phosphorus, calcium and iron within rice plants (Deshmukh *et al*., 2015).

Furthermore, in several plant species, including barley (*Hordeum vulgare*), tomato (*Solanum lycopersicum* L.), rice (*Oryza sativa*), wheat (*Triticum aestivum*), sugarcane (*Saccharum officinarum*), and citrus (*Citrus limon*), silicon has been noted for its potential to improve the turgidity of leaves, optimizing surface area for photosynthesis and alleviating the influence of several abiotic stresses like salt, metal toxicity, and drought (Naureen *et al*., 2015). Additionally, silicon helps reduce the development of various plant diseases in some pathosystems (bacterial and fungal diseases) by hardening the cell walls of leaf epidermal cells and accelerating the activation of the plant's natural defence mechanisms, thereby obstructing the entry of pathogenic fungi (Fauteux *et al*., 2005).

Rice is a silicon-loving crop that absorbs and stores available silicon in the soil. Hence, there is a need for adequate fertilization to achieve optimum production. Notwithstanding, the excessive use of formulated fertilizers is not only expensive for farmers but also a major contributor to air, soil, and water pollution through the discharge of nitrogen oxides and nitrates into these bodies (Savci, 2012; Deng *et al*., 2019). Unfortunately, when these three crucial bodies are polluted, they contribute to over 40% of deaths worldwide (Glick, 2012). Therefore, sustainable alternatives such as beneficial microorganisms offer a promising replacement for inorganic fertilizers. Hence, environmentally friendly, greener technologies, such as the use of beneficial microorganisms, such as silicate-solubilizing bacteria (SSB), should be considered.

Silicate-solubilizing bacteria help solubilize abundant but insoluble silicates in soil, making silicon available for plant absorption and thereby boosting the growth and yields of rice under stress and non-stress conditions (Chaganti *et al*., 2023). SSB has been studied previously, but the underlying mechanism by which insoluble silicates are solubilized and essential nutrients, such as potassium and phosphorus, are released to enhance rice growth and yield remains relatively unexplored. This study explored the effects of specific strains of silicate-solubilizing bacteria in improving the growth and yield of rice plants. The two specific objectives of this study include: to screen selected bacteria from culture collections and characterize for silicate-solubilizing properties, and to evaluate the effect of silicate-solubilizing bacteria on rice growth and yield.

MATERIALS AND METHODS

Collection of bacterial strains

Sixteen (16) bacterial strains were used for this research, obtained from the Soil Microbiology Laboratory, Department of Land Management, Faculty of Agriculture, UPM (10), and the Microbial Culture Collection Unit (UNICC), Institute of Bioscience, UPM (6) (**Table 1**). These bacterial strains were characterized and screened for their abilities to solubilize silicate.

Characterization of SSB

To characterize the silicate-solubilizing bacteria, a silicate solubilizing test was conducted on the different bacterial strains. In brief, each strain was incubated on a silicate medium containing 10 g/L glucose, 2.5 g/L magnesium trisilicate [$Mg_2O_8Si_3$], and 20 g/L agar for 7 days at 28°C. Three (3) bacterial strains that displayed clear halo zones, signifying Si-solubilizing ability, were selected for this research (Vasanthi *et al*., 2013).

Plant inoculation test (glasshouse conditions)

This research used two silicate sources (calcium silicate and kaolinite) together with three selected silicate-solubilizing bacteria strains (SSBs), and B0, which served as a negative control. In total, there were eight (8) treatment combinations and three replications, yielding twenty-four (24) experimental units (**Table 2**).

Table 1. Sixteen Beneficial bacterial strains collected from the Soil Microbiology and Microbial Culture Collection Unit (UNICC)

Collection unit	Accession No.	Species
Soil Microbiology Lab	UPMC 382	*Bacillus spp.*
	UPMC 1446	*Bacillus spp.*
	UPMC 385	*Bacillus spp.*
	UPMC 1340	*Enterobacter spp.*
	UPMC 1341	*Enterobacter spp.*
	UPMC 1342	*Enterobacter spp.*
	UPMC 382	*Bacillus sp.*
	UPMC 383	*Bacillus sp.*
	UPMC 385	*Bacillus sp.*
	UPMC 386	*Bacillus sp.*
UNICC	UPMC 1405	*Bacillus tequilensis*
	UPMC 1406	*Bacillus aryabhattai*
	UPMC 87	*Bacillus sphaericus*
	UPMC 1109	*Bacillus subtilis*
	UPMC 1110	*Lysinibacillus xylanilyticus*
	UPMC 1111	*Alcaligenes faecalis*
	UPMC 1405	*Bacillus tequilensis*

Table 2: Treatment combination

Silicate Source	Bacterial Strains
Calcium Silicate (Ca_2SiO_4)	B0 = Negative control
	B1 = UPMC1341(*Enterobacter sp*)
	B2 = UPMC1446(*Bacillus sp*)
	B3 = UPMC383 (*Bacillus sp*)
Kaolinite ($Al_2Si_2O_5(OH)_4$)	B0 = Negative control
	B1 = UPMC1341(*Enterobacter sp*)
	B2 = UPMC1446(*Bacillus sp*)
	B3 = UPMC383 (*Bacillus sp*)

Experimental design

The experiment, which was conducted in Shelterhouse A2 at Ladang 15, Faculty of Agriculture, UPM, Serdang, Selangor, Malaysia, consisted of two factorial treatments, was laid out in a Randomized Complete Block Design (RCBD).

Pot experiment

400 kg of paddy soil was collected from Sawah Sempadan, Tanjung Karang, Selangor. The initial nutrient levels (N, P, K, Si) and pH were determined. For this experiment, 24 pots containing 13 kg of soil were used. In each pot, 2 rice seedlings (variety MR219) were planted. A standard fertilization scheme was followed: 1.9: 1.4: 1.3 g per 13 kg, equivalent to 170: 80: 150 kg per ha (Department of Agriculture, Malaysia). The plants were supplied with silicate compounds and inoculated with three silicate-solubilizing bacteria strains (SSBs). 4 ml of the bacterial inoculum, approximately 10^5 – 10^6 CFU/ml live bacterial cells, was inoculated according to the treatment at transplanting and re-inoculated at the panicle initiation stage [55 days after planting (DAP)].

Data collection

To evaluate the effects of SSBs on rice growth and yield, plant heights were measured at 30, 60, and 90 DAP, and rice tillers were counted at 55 DAP. Yield parameters, including the number of panicles per plant, number of spikelets per panicle, number of filled spikelets per plant, and 1000-grain weight, were determined at harvest.

Statistical analysis

Data collected were analyzed using the Two-way Analysis of Variance of RStudio software, version 4.4.3 (Posit team, 2025). Significant means were separated using Duncan's multiple-range test at the 5% significance level.

RESULTS AND DISCUSSION

Characterization of silicate-solubilizing bacteria

Based on silicate solubilization ability, three bacterial strains (UPMC1341 (*Enterobacter sp*), UPMC1446 (*Bacillus sp*), and UPMC383 (*Bacillus sp*) were selected out of the sixteen bacterial strains (**Figure 1**).

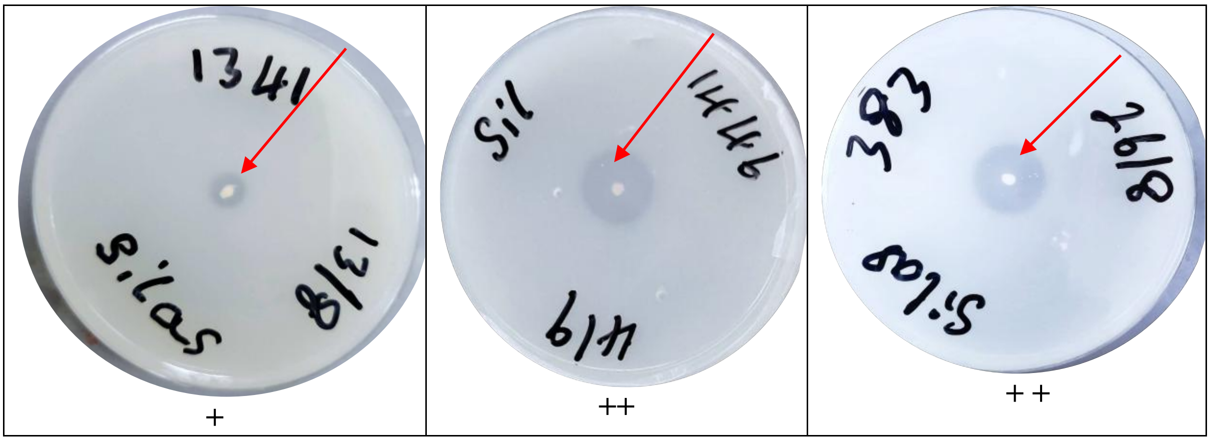


Figure 1. Three Strains of selected bacteria showing clear halo zones.
Note: +, ++ indicate the size of the halo zone, determined qualitatively

These three bacterial strains were selected for their ability to form a clear halo zone on silicate media. Research has shown that the halo zone formation indicates the bacteria's strength to solubilize insoluble silicate (Kang *et al*., 2017).

Plant tillers response

The number of tillers recorded with UPMC1446 (*Bacillus sp*) in combination with kaolinite among other bacterial strains was significantly different ($F = 14.96$; $df = 3,14$; $p<0.01$) Bacterial strain, UPMC1446 promoted the number of tillers in rice plants significantly, when applied in combination with Kaolinite as the source of insoluble silicate, as it recorded 29 ± 2.08 tillers, followed by the other two bacterial strains, UPMC383 (23 ± 3.06) and UPMC1341 (23 ± 1.53) compared with the negative control of 17 ± 0.58. This indicated that silicate-solubilizing bacteria (SSB) increased the number of tillers in rice plants (**Table 3**) by releasing silicon into the soil for rice uptake, a process activated by organic acids secreted by the bacteria. These results corroborate the findings of Peera *et al*. (2016), which showed similar beneficial trends.

Table 3. Interaction effects of bacterial strains and silicate types on rice plant tiller production (Mean ± SD).

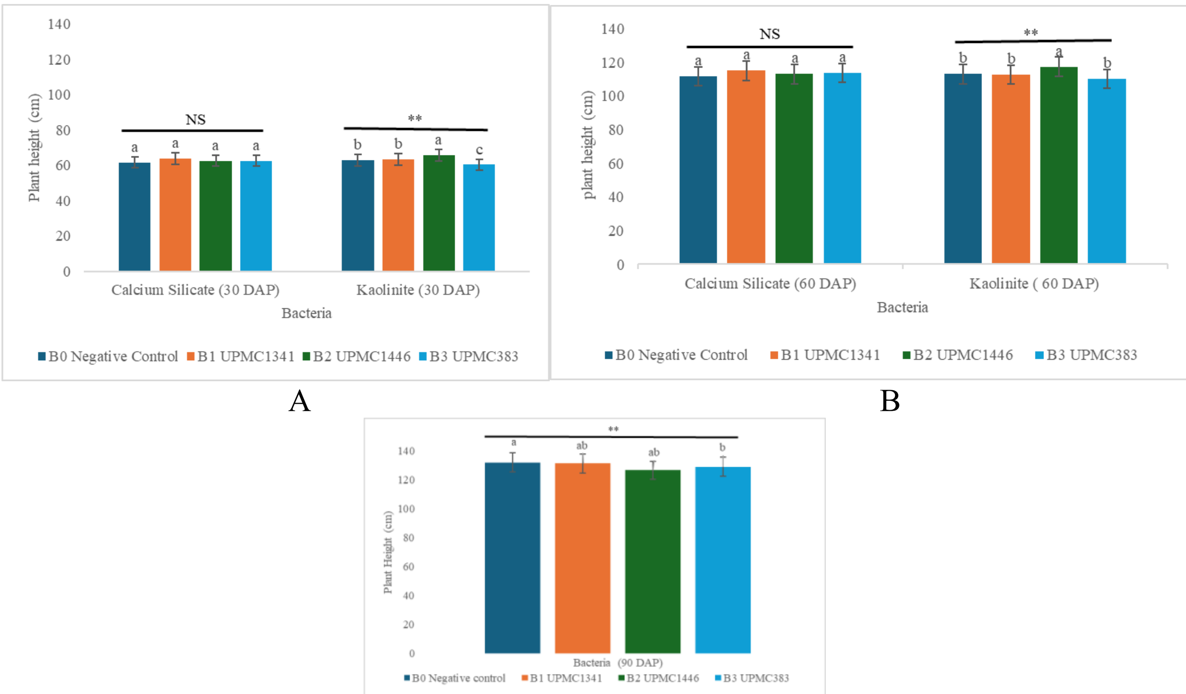
Silicate Type	Tiller number			
	No Bacterial	UPMC1341	UPMC1446	UPMC383
Calcium Silicate	23 ± 1.00^{a}	24 ± 0.58^{a}	22 ± 1.00^{a}	22 ± 2.00^{a}
Kaolinite	17 ± 0.58^{c}	23 ± 1.53^{b}	29 ± 2.08^{a}	23 ± 3.06^{b}

Note: Means carrying different letters indicate significant differences at $p<0.05$.

The three bacterial strains significantly increase the number of tillers in rice plants, especially when combined with Kaolinite, with the highest tiller production coming from rice plants inoculated with UPMC1446. This is consistent with previous research, which found that beneficial bacterial inoculations enhanced rice plant tiller production with and without fertilizer; however, some of the increases were not statistically significant (Ali-Tan *et al.*, 2017).In contrast, the interaction of bacterial strains with calcium silicate shows no significant difference amongst the treatments, as they all resulted in uniform tiller numbers across the treatments: B0 (23 ± 1.00), UPMC1341 (24 ± 0.58), UPMC1446 (22 ± 1.00), UPMC383 (22 ± 2.00) (**Table 3**). This shows that the bacterial strains are likely to produce more tillers in combination with kaolinite than with calcium silicate because of the ability of the organic acids, such as citric acid, released by these bacterial strains into the soil, which easily solubilize kaolinite and made silicon readily available for plant absorption, thereby increasing the growth of rice.

Plant height response at 30, 60, and 90 DAP

Figure 2A shows the rice plant height response to bacteria interaction with silicates at 30 DAP. Plant height response to the bacterial strains' interaction with kaolinite is significantly different (F = 7.36; df = 3,14; p<0.01) The three bacterial strains significantly increased the rice plant height at 30 DAP; this increment was more prevalent when the bacterial strain, *Bacillus sp* (UPMC1446), was combined with Kaolinite as opposed to when it was combined with calcium silicate (**Figure 2A**). This indicated that UPMC1446 significantly promoted rice plant development when supplied alongside insoluble silica (Kaolinite), compared with other strains (UPMC1341 and UPMC383) and the negative control (B0) (Kang *et al.*, 2017).



C

Figure 2. Interaction effects of three bacterial strains at each silicate (Calcium silicate and kaolinite) at 30 and 60 DAP (A and B), and main effects of bacterial strains at 90 DAP (C). Means with different letters are significantly different at P<0.05.

The responses to bacterial treatments were more variable with kaolinite than with calcium silicate at 60 DAP (**Figure 2B**). At 60 DAP, the height of rice plants was significantly different with UPMC1446 interactions with kaolinite, among other bacterial strains (F = 7.93; df = 3,14;

p<0.01). UPMC1446 positively influences rice plant height when combined with kaolinite, indicating a potentially synergistic interaction with kaolinite compared with other strains and the negative control. In contrast, calcium silicate neutralizes, resulting in similar plant heights regardless of the bacterial strain. This supports previous findings by Lee *et al*. (2019), who reported that silicate-solubilizing bacteria increased rice plant height, although the difference was not significant. These results illustrated the ability of silicate-solubilizing bacteria to increase rice plant height at 30 and 60 days after transplanting when combined with Kaolinite, as they showed a greater affinity for solubilizing insoluble kaolinite and making it available for rice absorption, thereby promoting rice growth. These findings align with that of Chandrakala *et al*. (2019) and Kang *et al.* (2017), which demonstrated that SSB (e.g., *Rhizobium* sp. IIRR-1 and *Burkholderia eburnea* CS4-2), which were isolated from japonica rice, increased the availability of both Silicon and phosphorus in paddy soil that was supplied with insoluble sources of silicate, which resulted in higher growth of rice plants. **Figure 2C** shows the main effect of bacteria on the height of the rice plant at 90 DAP. Inoculation with bacterial strains had no significant effect on height at 90 DAP ($p \geq 0.05$). The bacterial treatments exhibited a more pronounced effect on plant height, as evidenced by statistically significant differences between the bacterial strains and the negative control (**Figure 2C**). While the negative control resulted in the highest rice plant height (132.11cm), UPMC1446 recorded the lowest plant height (126.67cm), followed by UPMC383 (129.17cm) and UPMC1341 (131.50 cm). This implies that while the bacterial-treated stands were primarily focused on panicle initiation and flowering during this period, the control stands were still prioritizing vertical growth over tillering and flowering.

Yield parameter responses

Table 4 shows the main effects of bacterial strains on the number of panicles per plant, the number of spikelets per plant, and the weight of 1000 grains of rice. Inoculation with bacterial strains significantly affects growth parameters ($p<0.05$). Bacterial inoculation significantly increased rice growth parameters, with greater prevalence observed with UPMC1341 for the number of panicles/plant and number of spikelets/panicle, and with UPMC1446 for 1000-grain weight (**Table 4**). The results indicated that bacteria-treated stands increased yield parameters compared with the negative control stands. In terms of panicles per plant, UPMC1341 had the highest (36±2.34), followed by the negative control (31±1.81). Among all bacterial strains, UPMC1341 exhibited the highest number of spikelets per panicle (211 ± 2.25), whereas the negative control showed the lowest value (178 ± 20.43). In terms of 1000-grain weight, UPMC1446 produced a significantly higher weight than the other treatments. This demonstration of increased yield parameters resulting from bacterial inoculation agrees with Chandramani *et al*. (2009) and Peera *et al*. (2016). The results showed that silicate-solubilizing bacteria significantly improve yield parameters (**Table 4**).

Table 4. Main Effect of Bacteria Inoculation on Yield Parameters (Mean ± SD)

Treatment	No. of Panicle/Plant	No. of Spikelet/Panicle	1000 Grains Weight (g)
Negative control	$31±1.81^{c}$	$178±20.43^{b}$	$21±1.73^{b}$
UPMC1341	$36±2.34^{a}$	$211±2.25^{a}$	$22±1.67^{b}$
UPMC1446	$32±3.78^{bc}$	$193±37.89^{ab}$	$25±1.05^{a}$
UPMC383	$34±2.48^{ab}$	$197±17.97^{ab}$	$21±1.05^{b}$

Note: Means carrying different letters indicate significant differences at p<0.05.

The interaction between bacterial and silicate sources was significant in the percentage of filled spikelets when UPMC1341, UPMC383, and UPMC1446 were combined with

Calcium silicate compared to the control (**Figure 3**). The results showed that a significantly ($P \leq 0.05$) higher percentage of filled spikelets was observed in the UPMC1341 interaction with kaolinite, compared with other inoculated bacterial strains and the negative control (**Figure 3**). UPMC1341 showed a higher significance level than other bacterial strains when combined with Kaolinite, indicating that silicate-solubilizing bacteria, particularly UPMC1341, significantly increased the percentage of filled spikelets.

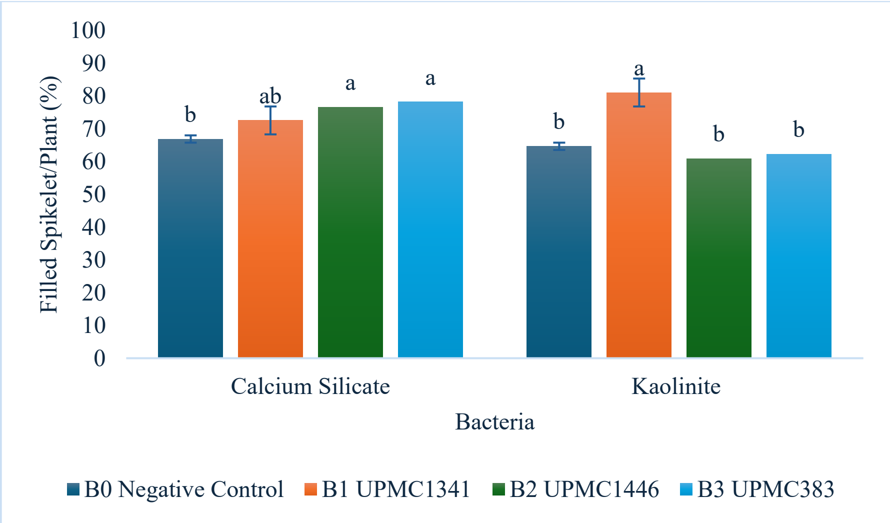


Figure 3. The interaction effect of bacterial strains and silicate on the filled spikelet. Means carrying different letters indicate significant differences at $p<0.05$.

This significant improvement in both rice plant growth and yield under bacterial strains and silicate treatment is associated with the release of silicon and other essential elements, such as potassium and phosphorus, by organic acids, including gluconic, citric, oxalic, and acetic acids. These acids help acidify the soil by lowering its pH, enabling the dissolution of silicate compounds and the release of silicon as monosilicic acid for uptake by the rice plant (Shivaraj *et al*., 2022). This indicated that the ability of SSBs to solubilize insoluble silicate is limited by bacterial strains and soil conditions, as Si solubilization is more efficient in acidic soils than in alkaline soils (Chandrakala *et al*., 2019).

CONCLUSION

This research highlighted the efficacy of beneficial bacterial strains, most notably Bacillus sp. UPMC1446 and Enterobacter sp. UPMC1341, which can readily solubilize insoluble silicate, promotes rice plant growth and yield, particularly when supplied with kaolinite as the silicate source. This research serves as a preparatory phase for in-depth analysis and trials to determine the impact of these SSBs on other rice plant growth and yield parameters. Given these findings, rice farmers can potentially improve crop yield and plant health by adopting these bacterial treatments, especially the combination of UPMC1446 with kaolinite. This approach can lead to more efficient use of soil silicate, enhancing plant growth during critical developmental stages and ultimately increasing agricultural productivity. More research is needed to determine the impact of bacterial inoculation on Si uptake, soil respiration, plant lignin content, and the processes involved in the release of potassium and phosphorus during the solubilization of insoluble silicates.

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