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ISOLATION, SCREENING, AND IDENTIFICATION OF POTASSIUM-SOLUBILIZING MICROORGANISMS FROM RUBBER PLANTATION SOILS IN PHITSANULOK PROVINCE

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Abstract

Potassium-solubilizing microorganisms have potential for improving potassium availability in agricultural soils, particularly in rubber plantations where a large proportion of soil potassium occurs in mineral forms that are not readily available to plants. This study aimed to isolate, screen, and identify native potassium-solubilizing microorganisms from rubber plantation soils in Wang Thong district, Phitsanulok province, Thailand. Rhizosphere soil samples were collected from rubber plantations managed under organic and chemical fertilizer systems. A total of 48 microbial isolates were obtained using Aleksandrov agar medium supplemented with mica as an insoluble potassium source. Among these isolates, 28 produced clear halo zones, indicating their ability to solubilize potassium from an insoluble mineral source. Based on radial clear zone formation, colony characteristics, and representation of sampling sources, 12 promising isolates were selected for quantitative evaluation in Aleksandrov liquid medium. After 14 days of incubation, soluble potassium concentrations ranged from 3.01 to 5.50 mg L⁻¹. The highest concentrations were observed in isolates No. 1, No. 10, and No. 11, with values of 4.93, 5.31, and 5.50 mg L⁻¹, respectively. These isolates were identified by 16S rRNA gene sequence analysis as *Bacillus siamensis*, *Pseudomonas orientalis*, and *Bacillus subtilis*. The results indicate that rubber plantation soils in Phitsanulok province harbor native potassium-solubilizing microorganisms with potential for further development as potassium-solubilizing biofertilizer candidates.

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Keywords: potassium-solubilizing microorganisms, rubber plantation soil, *Bacillus siamensis*, *Pseudomonas orientalis*, *Bacillus subtilis*, biofertilizer

1. Introduction

Rubber (*Hevea brasiliensis*) is one of the most important economic crops in Thailand and provides a major source of income for smallholder farmers in many regions, including Phitsanulok province [1,2]. Sustainable rubber production depends strongly on soil fertility management, particularly the availability of essential macronutrients such as potassium (K). Potassium is involved in plant physiological processes such as photosynthesis, osmotic regulation, stomatal control, carbohydrate transport, and stress tolerance; therefore, an adequate supply of K is important for maintaining rubber tree growth and latex productivity [3–5].

In many tropical agricultural soils, however, only a small proportion of total soil K is readily available for plant uptake. A large fraction of K is commonly fixed in mineral structures or occurs in insoluble K-bearing minerals such as mica and feldspar. As a result, rubber growers often rely on chemical K fertilizers to maintain soil fertility and crop production. Although chemical fertilizers are effective, continuous or excessive use may increase production costs and may contribute to nutrient imbalance or long-term changes in soil quality [6–9]. Alternative approaches that improve the availability of native soil K are therefore important for more sustainable nutrient management in rubber plantations.

Potassium-solubilizing microorganisms (KSMs) are beneficial microorganisms that can release K from insoluble mineral sources through several mechanisms, including organic acid production, proton release, chelation, complexolysis, and mineral weathering. These processes can increase soluble K in the rhizosphere and may improve nutrient availability for plants. Several bacterial genera, particularly *Bacillus* and *Pseudomonas*, have been reported as effective K-solubilizing and plant growth-promoting microorganisms in agricultural soils [6–14]. Because native microorganisms are already adapted to local soil and environmental conditions, their isolation and selection may provide useful candidates for developing locally suitable potassium-solubilizing biofertilizers.

Although KSMs have been studied in several crop production systems, information on native KSMs associated with rubber plantation soils remains limited, especially in smallholder rubber plantations in lower northern Thailand. Recent studies have shown that potassium-solubilizing

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bacteria can improve potassium release from mineral sources, enhance plant growth, and contribute to nutrient use efficiency in different agricultural systems [10–18]. In addition, rubber plantations are commonly managed under different fertilizer systems, such as organic and chemical fertilizer management, which may influence the occurrence and activity of K-solubilizing microorganisms in the rhizosphere. Screening native KSMs from these contrasting management systems can therefore provide useful information for selecting promising strains for future development as biofertilizer candidates.

Therefore, this study aimed to isolate and screen native potassium-solubilizing microorganisms from rubber plantation soils in Wang Thong district, Phitsanulok province. The specific objectives were to:

- i) isolate microorganisms capable of forming clear halo zones on Aleksandrov agar containing mica as an insoluble K source;
- ii) evaluate the potassium-solubilizing ability of selected isolates in Aleksandrov liquid medium; and
- iii) identify the most efficient isolates using DNA-based analysis.

2. Materials and Methods

2.1. Study area and soil sampling

The study was conducted using rhizosphere soil samples collected from mature rubber plantations in Wang Thong district, Phitsanulok province, Thailand. The selected plantations represented two common fertilizer management systems used by local rubber growers: organic fertilizer management and chemical fertilizer management. Soil samples were collected from the rhizosphere of rubber trees at two depths, 0–15 cm and 15–30 cm, to represent the surface and subsurface root zones.

For each sampling point, soil adhering to or closely associated with rubber tree roots was collected as rhizosphere soil. Several subsamples were taken around the tree canopy area and then combined to form composite soil samples. The samples were placed in clean plastic bags, kept under cool conditions during transportation, and immediately transferred to the laboratory for microbiological analysis. A total of eight composite soil samples were used for microbial isolation,

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representing rubber plantation soils under different fertilizer management conditions and soil depths.

2.2. Isolation and qualitative screening of potassium-solubilizing microorganisms

Potassium-solubilizing microorganisms were isolated from the composite rhizosphere soil samples using serial dilution and spread-plate techniques. For each sample, 10 g of fresh rhizosphere soil was added to 90 mL of sterile distilled water and shaken thoroughly to obtain the initial soil suspension. Tenfold serial dilutions were then prepared from 10^{-2} to 10^{-7} .

An aliquot of 0.1 mL from appropriate dilutions was spread onto Aleksandrov agar medium supplemented with mica as an insoluble potassium source. The plates were incubated at 28 ± 2 °C for 7 days. Colonies that produced clear halo zones around their growth were recorded as putative potassium-solubilizing microorganisms. The appearance of a clear zone was used as a preliminary indicator of the ability of the isolate to solubilize potassium from the insoluble mineral source.

The colony diameter and the total diameter of the colony plus halo zone were measured after incubation. The radial clear zone was calculated using the following equation:

$$\text{Radial clear zone (cm)} = (\text{total diameter} - \text{colony diameter}) / 2 \quad (1)$$

where the total diameter represents the diameter of the colony together with the surrounding clear zone, and the colony diameter represents the diameter of the microbial colony itself. Isolates with larger radial clear zones were considered to have higher qualitative potassium-solubilizing ability. In total, 48 isolates were obtained, and 28 isolates showing distinct clear halo zones were selected for further evaluation.

2.3. Selection of promising isolates and quantitative potassium solubilization in liquid medium

After qualitative screening on Aleksandrov agar, isolates showing potassium-solubilizing potential were further considered for quantitative evaluation. The selection of 12 isolates for liquid culture evaluation was based not only on radial clear zone formation, but also on colony morphology, growth characteristics, and representation of isolates from different sampling sources and fertilizer management systems. Therefore, the liquid culture assay was used as a quantitative confirmation step to evaluate soluble potassium release under liquid medium conditions.

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Each selected isolate was inoculated into Aleksandrov liquid medium containing mica as an insoluble potassium source. The inoculated cultures were incubated at 28 ± 2 °C on a rotary shaker at 150 rpm. Uninoculated medium was used as a blank control to distinguish potassium released from the medium itself from potassium released through microbial activity. Samples were collected after 7 and 14 days of incubation.

At each sampling time, the cultures were centrifuged at 6,000 rpm for 10 min to separate microbial cells and insoluble mica particles from the liquid phase. The clear supernatant was collected for determination of culture pH and soluble potassium concentration. Culture pH was measured using a pH meter, while soluble potassium concentration was determined using a flame photometer. Soluble potassium was expressed as mg L⁻¹. The uninoculated shaken medium was used as a blank control to account for potassium released from the medium and mica particles in the absence of microbial activity. Isolates that produced the highest soluble potassium concentrations after 14 days of incubation, particularly in comparison with the blank control, were considered the most promising potassium-solubilizing isolates and were selected for DNA-based identification.

This liquid culture assay was conducted as a preliminary quantitative screening step, and the results were interpreted descriptively by comparing soluble K concentrations with the uninoculated blank control.

2.4. DNA analysis and identification of isolates

After quantitative screening in Aleksandrov liquid medium, the three isolates showing the highest soluble potassium concentrations were selected for molecular identification. These isolates were No. 1, No. 10, and No. 11. Prior to molecular identification, each isolate was purified by repeated streaking to ensure that a single bacterial culture was obtained.

Pure bacterial cultures were submitted to an external laboratory for 16S rRNA gene sequencing analysis. Genomic DNA extraction, polymerase chain reaction amplification of the 16S rRNA gene, and DNA sequencing were performed by the service laboratory according to its standard protocol. The obtained 16S rRNA gene sequences were compared with reference sequences deposited in the GenBank database using the BLASTn program. Bacterial identification was assigned based on the closest sequence similarity to reference bacterial species.

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3. Results

3.1. Isolation and qualitative screening of potassium-solubilizing microorganisms

A total of 48 microbial isolates were obtained from rhizosphere soil samples collected from rubber plantations under organic and chemical fertilizer management systems. Among these isolates, 28 isolates produced distinct clear halo zones on Aleksandrov agar medium containing mica as an insoluble potassium source. The formation of clear zones around the colonies indicated the ability of these isolates to solubilize potassium from the insoluble mineral source.

The 28 halo-forming isolates were distributed equally between the two fertilizer management systems, with 14 isolates obtained from organic fertilizer management and 14 isolates from chemical fertilizer management. The radial clear zone values after 7 days of incubation ranged from 0.20 to 1.20 cm. The mean radial clear zone was approximately 0.81 cm for isolates from the organic fertilizer system and 0.84 cm for isolates from the chemical fertilizer system. The radial clear zone values of all halo-forming isolates are presented in Table 1.

Table 1: Radial Clear Zone of Potassium-Solubilizing Isolates Obtained from Rubber Plantation Soils Under Organic and Chemical Fertilizer Management

Organic fertilizer management				Chemical fertilizer management			
No.	Isolate code	Radial clear zone (cm)		No.	Isolate code	Radial clear zone (cm)	
		3 days	7 days			3 days	7 days
1	T1/1	0.30	0.90	15	T5/1	0.40	0.90
2	T1/2	0.20	0.20	16	T5/2	0.40	1.00
3	T1/3	-	0.70	17	T5/3	0.30	0.50
4	T1/4	0.30	0.90	18	T5/4	0.20	0.50
5	T2/1	0.30	0.50	19	T6/1	0.40	0.80
6	T2/2	0.40	1.00	20	T6/2	0.40	0.90
7	T2/3	0.50	1.10	21	T6/3	0.40	0.60
8	T3/1	0.50	0.70	22	T7/1	0.50	1.10
9	T3/2	0.40	0.80	23	T7/2	0.30	0.60
10	T4/1	0.50	1.10	24	T7/3	0.40	0.90
11	T4/2	0.60	1.00	25	T8/1	0.60	1.20
12	T4/3	0.40	0.60	26	T8/2	0.30	0.80
13	T4/4	0.70	1.20	27	T8/3	0.50	0.90
14	T4/5	0.30	0.70	28	T8/4	0.60	1.00

Several isolates produced relatively large radial clear zones of 1.00 cm or greater. These included isolates T2/2, T2/3, T4/1, T4/2, and T4/4 from the organic fertilizer system, and isolates T5/2, T7/1, T8/1, and T8/4 from the chemical fertilizer system. Among all isolates, T4/4 and T8/1 showed the largest radial clear zone values at 1.20 cm after 7 days of incubation. These isolates showed strong qualitative potassium-solubilizing activity, supporting the need for further quantitative evaluation of representative isolates in liquid medium.

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Overall, the qualitative screening results showed that rubber plantation soils in Wang Thong district harbor native microorganisms capable of solubilizing potassium from mica. However, halo formation on agar medium should be interpreted as a preliminary screening indicator only. Further quantitative testing in liquid medium was therefore required to confirm the potassium-solubilizing ability of the selected isolates.

3.2. Quantitative potassium solubilization in liquid culture

The 12 selected representative isolates were further evaluated for their ability to solubilize potassium in Aleksandrov liquid medium containing mica as an insoluble K source. The uninoculated shaken blank was included to account for potassium released from the medium and mica particles during incubation. After 7 days of incubation, the pH values of the inoculated cultures ranged from 3.60 to 6.21, whereas soluble K concentrations ranged from 2.19 to 4.71 mg L⁻¹. After 14 days, soluble K concentrations ranged from 3.01 to 5.50 mg L⁻¹. The pH and soluble K concentrations of the selected isolates at 7 and 14 days of incubation are presented in Table 2.

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Table 2: pH and Soluble Potassium Concentration of Selected Potassium-Solubilizing Isolates Grown in Aleksandrov Liquid Medium at 7 and 14 Days of Incubation

No.	Isolate	7 days		14 days	
No.	Isolate	pH	Soluble K (mg L ⁻¹)	pH	Soluble K (mg L ⁻¹)
	Blank	6.40	4.75	5.53	4.46
	Blank (NS)	6.36	4.38	-	-
1	T1/2	6.21	4.55	6.56	4.93
2	T1/5	6.15	4.61	6.32	4.64
3	T2/4	6.17	4.48	6.05	4.75
4	T4/2	3.60	3.94	6.29	3.27
5	T5/1	5.59	4.37	6.62	4.81
6	T5/2	4.46	2.19	4.44	3.01
7	T5/3	5.74	3.58	4.48	4.29
8	T6/1	5.64	4.32	6.39	4.60
9	T6/2	5.77	4.71	6.27	4.72
10	T6/3	4.97	4.13	6.24	5.31
11	T8/1	4.12	4.62	5.06	5.50
12	T8/4	5.39	4.61	6.53	4.57

Blank = uninoculated shaken control; Blank (NS) = uninoculated not-shaken control.

Although several isolates caused a clear decrease in culture pH at 7 days, their soluble K concentrations were not always higher than that of the blank control. The relatively high soluble K concentration in the blank control indicated that some K could be released from mica particles or the medium itself during shaking and incubation. Therefore, soluble K values were interpreted in comparison with the blank control. This result suggests that acidification alone did not directly correspond to measurable K release in the liquid medium during the early incubation period. The lower soluble K values observed in some inoculated treatments may have resulted from temporary microbial uptake of released K, adsorption of K onto microbial cells or insoluble particles, or differences among isolates in the type and amount of organic acids produced.

After 14 days of incubation, soluble K concentrations ranged from 3.01 to 5.50 mg L⁻¹, while the shaken blank control showed 4.46 mg L⁻¹. At this stage, several isolates produced soluble K concentrations higher than the shaken blank control. Among them, isolates No. 1, No. 10, and No. 11 showed the highest soluble K concentrations, with values of 4.93, 5.31, and 5.50 mg L⁻¹, respectively. These results indicate that potassium solubilization by the selected isolates was time-dependent and that only some isolates were able to increase soluble K above the uninoculated control under the conditions tested.

Among the selected isolates, isolate No. 11 showed the highest soluble K concentration after 14 days, followed by isolates No. 10 and No. 1. These isolates were therefore selected as the most promising potassium-solubilizing microorganisms for DNA-based identification. Overall, the

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liquid culture results showed that halo formation on agar medium was useful for preliminary screening, but quantitative evaluation in liquid medium was necessary to identify isolates with stronger potassium-solubilizing ability.

3.3. DNA-based identification of selected isolates

The three isolates that showed the highest soluble K concentrations in Aleksandrov liquid medium were selected for DNA-based identification. These isolates were No. 1, No. 10, and No. 11, which corresponded to isolate codes T1/2, T6/3, and T8/1, respectively. Based on 16S rRNA gene sequence comparison with the GenBank database, isolate No. 1 was closely related to *Bacillus siamensis*, isolate No. 10 to *Pseudomonas orientalis*, and isolate No. 11 to *Bacillus subtilis*. All three isolates showed high sequence similarity with their closest reference species ($\geq 99\%$), as shown in Table 3.

Table 3: DNA-Based Identification of the Most Efficient Potassium-Solubilizing Isolates Based on 16S rRNA Gene Sequence Similarity

Isolate No.	Closest species (16S rRNA)	Identity (%)
1	<i>Bacillus siamensis</i>	≥ 99
10	<i>Pseudomonas orientalis</i>	≥ 99
11	<i>Bacillus subtilis</i>	≥ 99

Isolate No. 1 = T1/2, isolate No. 10 = T6/3, and isolate No. 11 = T8/1.

The identified isolates belonged to the genera *Bacillus* and *Pseudomonas*, which are commonly reported as plant growth-promoting and mineral-solubilizing bacteria. These results indicate that the selected potassium-solubilizing isolates from rubber plantation soils were closely related to bacterial groups with known potential for nutrient solubilization. However, further physiological and plant-based evaluations are needed to verify their performance under practical rubber plantation conditions.

4. Discussion

The present study demonstrated that rubber plantation soils in Wang Thong district, Phitsanulok province, contained native microorganisms capable of forming clear halo zones on Aleksandrov agar supplemented with mica as an insoluble potassium source. From 48 microbial isolates, 28 isolates produced clear zones, suggesting that potassium-solubilizing microorganisms were present in rubber rhizosphere soils under both organic and chemical fertilizer management systems. The similar number of halo-forming isolates obtained from the two management systems indicates that

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both types of rubber plantation soil may serve as potential sources of native potassium-solubilizing microorganisms.

The radial clear zone values varied among isolates, ranging from low to relatively high values. This variation suggests that potassium-solubilizing potential differs among native microbial isolates, even when they originate from the same crop system. However, halo formation on solid medium should be interpreted only as a preliminary screening indicator. The size of a clear zone may be influenced by microbial growth rate, diffusion of organic acids or other metabolites in the agar, and interaction between the isolate and the insoluble K source. Therefore, quantitative evaluation in liquid medium was necessary to further distinguish isolates with stronger potassium-solubilizing ability.

In liquid culture, the selected isolates showed different patterns of pH change and soluble K release. Several isolates reduced the pH of the medium after 7 days, but their soluble K concentrations were not always higher than that of the blank control. This indicates that medium acidification alone was not sufficient to explain measurable K release during the early incubation period. The lower soluble K concentrations observed in some inoculated cultures may be related to temporary uptake of released K by microbial cells, adsorption of K onto cell surfaces or insoluble particles, or differences in the type and amount of organic acids produced by each isolate. Similar observations have been reported in studies of potassium-solubilizing bacteria, where the ability to form halos on agar did not always correspond directly with soluble K concentration in liquid medium [7,10,13,14].

After 14 days of incubation, isolates No. 1, No. 10, and No. 11 showed the highest soluble K concentrations, reaching 4.93, 5.31, and 5.50 mg L⁻¹, respectively. The soluble K concentrations observed in this study were relatively low compared with some previous reports [7,10,11,13,17,18], which may be attributed to differences in K-bearing mineral sources, incubation period, medium composition, microbial species, and analytical methods. This result suggests that potassium solubilization by these isolates was time-dependent and became more evident after a longer incubation period. The increase in soluble K after 14 days may be associated with continued microbial metabolism, production of organic acids, proton release, chelation, and mineral weathering processes that promote the dissolution of K-bearing minerals such as mica. These mechanisms have been widely reported as important pathways by which potassium-solubilizing microorganisms release K from insoluble mineral sources [6–9,13,14].

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The three selected isolates were identified as *Bacillus siamensis*, *Pseudomonas orientalis*, and *Bacillus subtilis* based on 16S rRNA gene sequence comparison. Members of the genera *Bacillus* and *Pseudomonas* are commonly reported as plant growth-promoting bacteria with the ability to solubilize mineral nutrients, including potassium and phosphorus. Their presence among the most promising isolates in this study is therefore consistent with previous reports on mineral-solubilizing microorganisms in agricultural soils [6,8,10–14,17,18]. Nevertheless, identification based on 16S rRNA gene sequences indicates taxonomic relatedness, while functional performance still needs to be confirmed through further physiological testing and plant-based evaluation.

Overall, the results of this study support the usefulness of a stepwise screening approach for selecting native potassium-solubilizing microorganisms from rubber plantation soils. Initial screening on Aleksandrov agar allowed the detection of halo-forming isolates, while quantitative testing in liquid medium provided clearer evidence of soluble K release. The selected isolates, particularly *B. siamensis*, *P. orientalis*, and *B. subtilis*, may be considered promising candidates for further development as potassium-solubilizing biofertilizer resources. Further physiological and plant-based evaluations are still required to verify their effects on soil K availability, plant nutrient uptake, and rubber production under practical plantation conditions.

5. Conclusion

This study demonstrated that rubber plantation soils in Wang Thong district, Phitsanulok province, contained native microorganisms with potassium-solubilizing potential. Among 48 microbial isolates obtained from rhizosphere soils under organic and chemical fertilizer management systems, 28 isolates produced clear halo zones on Aleksandrov agar containing mica as an insoluble potassium source. This indicates that potassium-solubilizing microorganisms were present in rubber plantation soils under both management systems.

Quantitative evaluation in Aleksandrov liquid medium showed that soluble potassium release varied among the selected isolates. After 14 days of incubation, isolates No. 1, No. 10, and No. 11 produced the highest soluble potassium concentrations, with values of 4.93, 5.31, and 5.50 mg L⁻¹, respectively. These isolates were identified by 16S rRNA gene sequence analysis as *Bacillus siamensis*, *Pseudomonas orientalis*, and *Bacillus subtilis*.

The results suggest that these three native isolates are promising candidates for further evaluation as potassium-solubilizing biofertilizer resources for rubber plantation soils. Further

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plant-based evaluation is needed to confirm their effects on soil potassium availability, plant nutrient uptake, and rubber production under practical plantation conditions.

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