Screening for P- and K- solubilizing, and siderophore producing capacity of rhizobacteria from Khao Dawk Mali 105 Aromatic Rice

K Chinachanta\textsuperscript{1,2} and A Shutsrirung\textsuperscript{2,3}

\textsuperscript{1} Doctor of Philosophy Program in Environmental Soil Science, Graduate School, Chiang Mai University, Thailand

\textsuperscript{2} Department of Plant and Soil Sciences, Faculty of Agriculture, Chiang Mai University, Thailand

\textsuperscript{3} E-mail: arawan.s@cmu.ac.th

Abstract. Low nutrient status and high salinity have been identified as significant constraints for KDML105 aromatic rice production in the TKR region, Northeastern, Thailand. The use of PGPR can provide green alternatives to synthetic fertilizer in this region. In this study, therefore, rhizobacteria were isolated from KDML105 rice and evaluated for their potential in P and K solubilization, and siderophore production. The results indicated that 52.0, 21.5, 17.9, and 21.1\% of 629 tested isolates, were able to solubilize P, K-mica, K-feldspar, and produce siderophore, respectively. At 0\% NaCl the highest amount of solubilized P (35.6 mg L\textsuperscript{−1}), K (49.5 mg L\textsuperscript{−1}), and hydroxamate-type siderophore (618.3 μg L\textsuperscript{−1}), were obtained from isolates ORF4-13, ORF15-23, and CRF16-3, respectively. Under salt stress, the amount of solubilized P of almost all the isolates increased with salt concentration up to 0.5\% NaCl and declined thereafter, as compared to the control. In contrast, the amount of solubilized K progressively decreased with NaCl concentration. On the average, ORF15-23 exhibited promising ability in P and K solubilization under salt stress. The promising isolates obtained in this study should be evaluated for their effects on rice nutrients uptake and growth before developing them as biofertilizer.

1. Introduction
Rice (\textit{Oryza sativa} L.) is the main staple diet in Thailand since it is eaten at almost every meal. Among different types of rice, aromatic rice particularly KDML105 variety is the most popular among Thai people as well as Asian, European, and American consumers [1]. Although KDML105 rice can be cultivated throughout the country, but the premium rice grain quality with strong and stable aroma is from the rainfed paddy field of Thung Kula Rong Hai (TKR) which occupied around 50\% of the KDML105 rice production of the Northeastern Thailand [2]. Unfortunately, the TKR region has been suffering from drought, excessive soil salinity (mainly NaCl), and infertile soils (mainly acidic sandy soils), which negatively affect the yield and grain aroma of the KDML105 rice. Furthermore, the soil problems are exacerbated by high agrochemicals application, climate change and limited rainfall which resulted in KDML105 rice yield and quality decline [3]. Native soil fertility evaluation in the TKR region indicated that low fertility occupies 37.95\% of the TKR soil in the year 2004 and increased up to 84.86\% in the year 2017. Chemical analysis of the representative native sandy soils collected from the TKR region revealed that the soil has a low available phosphorus (P) (<10 mg kg\textsuperscript{−1}) and low
exchangeable potassium (K) (<60 mg kg\(^{-1}\)) [4]. In general, soluble P added as chemical fertilizer, is rapidly converted into insoluble complex by precipitating with Ca in alkali soil and with Al and Fe in acidic soil [5]. Soil salinity reduces potassium (K\(^+\)) uptake due to strong competition with sodium cations (Na\(^+\)) at the root surface leading to K\(^+\) deficiency and plant growth impairment [6-7]. A field experiment with maze grown in acidic sandy soil of the Northeastern Thailand, indicated that N was the most limiting nutrient for its biomass, followed by P and K [8]. Besides these macronutrients (N, P and K) which limit rice productivity, micronutrients particularly iron (Fe) is also considerably deficient in rice [9].

Nutrient availability is an important soil attribute for plant growth, development, and productivity at the root-soil interfaces [10], where plant growth promoting rhizosphere microorganisms (PGPMs) are attracted and promoted to grow. The PGPMs help to make more nutrients available to the plants throughout the growing season as well as to resist both biotic and abiotic stresses. For these reasons, the utilization of plant growth promoting rhizobacteria (PGPR), including phosphate and potassium solubilizing bacteria (PSB and KSB) can be an effective alternative to chemical fertilizers for a sustainable improvement of KDML105 rice production in the TKR region. In addition, availability Fe to the crop plants is reduced in saline soils therefore the use of siderophore producing rhizobacteria for enhancing Fe supply to the host plant is also of important in salt stress area [11], including the TKR region.

It appeared that very little information is available on the occurrence of PSB and KSB in salt-affected inland soils of the TKR region. Rhizobacteria isolated from KDML105 rice soil may be better adapted to the rice plants and provide better growth than rhizobacteria isolated from other regions since they have been already closely associated with the rice growing system and adapted to the stress environment as well. Therefore, the present investigation was aimed to isolate rhizobacteria from KDML105 rice rhizosphere grown in the TKR salt-affected soils of organic and conventional rice farming (ORF and CRF, respectively). The isolated KDML105 rice rhizobacteria were evaluated for their potential in P and K solubilization, and siderophore production. Only some selected isolates were quantitatively determined for their ability in solubilizing insoluble phosphate and potassium under various NaCl concentrations.

2. Materials and methods

2.1. Isolation of bacteria from rice rhizosphere soil

The 18 soil samples of KDML105 rice grown in Thung Kula Rong Hai (TKR) from our previous work [12] were used in the present study. Nine soil samples were from organic rice farming (ORF), and another nine soil samples were from conventional rice farming (CRF). On the average, the microbial populations of ORF system were higher than those of the CRF system (Table 1). Serial dilution of the rhizosphere-soil suspension was made (10\(^{-1}\)-10\(^{-5}\)) and plating technique was used to for bacterial isolation. In brief, an aliquot of 0.1 mL (10\(^{-3}\)-10\(^{-5}\)) of each sample was spread on egg albumin agar medium [13] plates. The plates were incubated at 30 °C for 3-5 days until rhizobacterial colony appearance. Rhizobacterial colonies appeared on egg albumin agar plate were selected and picked up based on the difference in the colony morphology. Single colony of each isolate was re-streaked onto nutrient agar (NA) plates to ensure pure culture isolation. The pure rhizobacterial cultures were preserved on NA slants for the short-term storage and 30% glycerol at -20°C for the long-term storage.
Table 1. Some soil physical and chemical properties of organic and conventional rice farming systems.

| site               | Sampling area* | Bacterial population (CFU g⁻¹ dry weight) | pH  | SOM (%) | Available P (mg kg⁻¹) | Exchangeable K (mg kg⁻¹) |
|--------------------|----------------|-------------------------------------------|-----|---------|-----------------------|--------------------------|
| **Organic rice farming** |                |                                           |     |         |                       |                          |
| 1 Prasat, Surin 1  |                | 2.10×10⁷                                  | 4.36| 1.03    | 10.86                 | 64.97                    |
| 2 Prasat, Surin 2  |                | 1.20×10⁷                                  | 4.78| 0.84    | 6.90                  | 131.91                   |
| 3 Prasat, Surin 3  |                | 1.60×10⁷                                  | 4.76| 1.12    | 7.08                  | 59.65                    |
| 4 Prasat, Surin 4  |                | 4.16×10⁷                                  | 4.52| 0.85    | 3.70                  | 60.92                    |
| 5 Thatum, Surin 1  |                | 4.99×10⁷                                  | 5.05| 1.88    | 37.53                 | 83.74                    |
| 6 Thammakul, Yasothon |               | 6.26×10⁷                                  | 5.14| 1.69    | 12.81                 | 49.10                    |
| 7 Kutchum, Yasothon |                | 8.51×10⁶                                  | 4.78| 1.06    | 37.53                 | 131.46                   |
| 8 Kutchum, Yasothon |                | 1.58×10⁷                                  | 5.10| 1.80    | 2.44                  | 34.08                    |
| 9 Kasetwisai, Roi Et |              | 2.57×10⁷                                  | 5.32| 2.41    | 4.31                  | 47.50                    |
| **Conventional farming** |              |                                           |     |         |                       |                          |
| 5 Prasat, Surin 5  |                | 1.37×10⁸                                  | 5.87| 0.74    | 139.16                | 19.7                     |
| 7 Thammakul, Surin 2 |               | 1.12×10⁸                                  | 5.23| 0.90    | 66.9                  | 56.68                    |
| 8 Rattanaburi, Surin |              | 1.43×10⁸                                  | 4.88| 0.77    | 8.34                  | 58.76                    |
| 12 Mahachnachai, Yasothon |           | 4.83×10⁸                                  | 5.96| 0.51    | 2.38                  | 9.01                     |
| 13 Silalat, Sisakat |                | 3.20×10⁸                                  | 5.63| 0.54    | 5.94                  | 106.86                   |
| 14 Sawannaphum, Roi Et |              | 5.72×10⁸                                  | 5.64| 0.30    | 24.39                 | 110.61                   |
| 15 Patumrat, Roi Et |                | 3.16×10⁸                                  | 5.37| 0.69    | 7.55                  | 159.49                   |
| 17 Phonsai, Roi Et |                | 1.48×10⁸                                  | 5.04| 1.03    | 6.46                  | 28.88                    |
| 18 Mahasarakham |                | 1.77×10⁸                                  | 5.06| 0.44    | 9.92                  | 55.56                    |

*Adapted from Chinachanta et al., 2020 [12]

2.2. Screening of rhizobacterial isolates for their plant growth-promoting activity

All the distinct rhizobacterial isolates obtained from KDML105 rice under experiment 2.1, were evaluated for their plant growth promoting (PGP) traits i.e., phosphate (P) and potassium (K) solubilization, and siderophore production. Each isolate was inoculated into nutrient broth (NB) (Hi Media, Mumbai, India) medium and incubated with shaking (120 rpm) at room temperature for 3 days. After that, the pure culture broth of each isolate was dropped on to each specific medium to evaluate their PGP traits. The specific media without rhizobacterial isolate was used as a control for each experiment. The evaluation of qualitative P and K solubilization, and siderophore production was ranked as follows; no clear zone (-), narrow clear zone (+), and wide clear zone (++). Triplicate samples were used for all the experiments, and data were presented as mean value of these triplicates.

2.2.1 Phosphate solubilization.

Firstly, the qualitative analysis of phosphate solubilizing ability of all the distinct rhizobacterial isolates were tested on Pikovskaya (PKV) agar plates containing 0.5% (w/v) tricalcium phosphate (Ca-P) [14]. Pikovskaya media contained the following ingredients (g L⁻¹): 10 g glucose, 0.2 g NaCl, 5 g Ca₃(PO₄)₂, 0.5 g (NH₄)₂SO₄, 0.2 g KCl, 0.1 g MgSO₄·7H₂O, 0.002 g MnSO₄·4H₂O, 0.002 g FeSO₄·7H₂O, 0.5 g yeast extract and 25 g agar, adjusted to pH 7.0 [14]. The 3-day-old culture broth (0.1 µL) of each isolate including the control (PKV broth without rhizobacterial strain) was dropped onto the PKV medium plate and incubated at room temperature (28-35 °C) for 7 days. The development of colonies on the surface of PKV plates surrounded by the clear zone indicated phosphate solubilization.

After the first screening, the isolates both from ORF and CRF that showed differences in Ca-P solubilization were selected (approximately not more than 10 isolates) to evaluate their ability by quantitative determination. Analysis of Ca-P solubilization was carried out by inoculating 1 mL of each selected isolate into 25 mL of PKV broth in 50 mL flasks and incubated at room temperature (28-35 °C) with shaking (120 rpm) for 7 days. Uninoculated PKV broth medium was used as a control. The supernatant of all the isolates was prepared by centrifugation at 10,000 rpm for 15 min to separate the culture broth and the cells. Released soluble phosphate in the supernatant was determined by the ascorbic acid color reagent. One mL of supernatant was mixed with 4 mL of color reagent (1:1:1:2 ratio of 3M H₂SO₄, 2.5% (NH₄)₂MoO₄, 10% ascorbic acid and distilled water), incubated in the dark for 30 minutes.
The optical density was measured at 820 nm using spectrophotometer (Thermo Scientific, mod. GENESYS 20, USA). The soluble phosphate concentration (mg L\(^{-1}\)) was calculated using standard curve equation.

2.2.2 Potassium solubilization. For the first screening, the qualitative analysis of potassium (K) solubilizing ability of all the distinct rhizobacterial isolates were tested on modified Alexandrov medium containing (g L\(^{-1}\)): 5.0 g glucose; 0.5 g MgSO\(_4\)\(\cdot\)7H\(_2\)O; 0.1 g CaCO\(_3\); 0.006 g FeCl\(_3\); 2.0 g Ca\(_3\)PO\(_4\); 3.0 g insoluble potassium source (K-feldspar or K-mica), and 20.0 g agar, adjusted to pH 7.5 [15]. The culture broth (0.1 µL) of each isolate including the control (NB without rhizobacterial strain) was dropped onto the Alexandrov medium plate and incubated at room temperature (28-35 °C) for 7 days. The appearance of colonies on the medium plates with clear zone around their colonies indicating the ability to solubilize potassium from insoluble potassium source [15].

After the preliminary screening, the same selected isolates as used for quantitative soluble P determination in experiment 2.2.1, were selected to evaluate their ability by quantitative K determination. In this experiment, K-feldspar powder was added to liquid Aleksandrov medium as the sole source of K. Analysis of K-feldspar solubilization was carried out by inoculating 1 mL of each selected isolate into 25 mL of Pikovskaya broth in 50 mL flasks and incubated at room temperature (28-35 °C) with shaking (120 rpm) for 7 days. Uninoculated Aleksandrov broth medium was used as a control. The supernatant of each isolate was used to determine release soluble K by an atomic absorption spectrophotometer (AAS) (Spectra AA240 FS, Varian, New Jersey, USA). The soluble K concentration (mg L\(^{-1}\)) was calculated using standard curve equation.

2.2.3 Siderophore production. For the qualitative screening of siderophore producing ability of all the distinct rhizobacterial isolates, Chrome Azurol Sulphonate (CAS) agar was used [16]. The culture broth (0.1 µL) of each isolate including the control (NB without rhizobacterial strain) was dropped onto the CAS medium plate and incubated at room temperature (28-35 °C) for 7 days in a dark room [16]. The yellow-orange halo zone around the colony was a positive indication of siderophore production.

After the qualitative screening, the same selected isolates as used for quantitative soluble P in experiment 2.2.1 were selected to evaluate their ability in producing two types of siderophore. Siderophore type was determined by ferric perchlorate assay for hydroxamate type [17] and Arnow assay for catecholate type [18].

2.3. Influence of salt stress on P- and K- solubilizing ability
The experiment was conducted to evaluate the effect of different concentrations of NaCl on P- and K-solubilizing ability of selected rhizobacterial isolates (the same selected isolates as used for quantitative soluble P in 2.2.1. The NaCl concentration in this study was 0, 0.5, 1.0 and 3.0% NaCl (w/v). Quantitative determination of solubilized P and K under salinity stress was done according to the method described above [14, 15].

2.4. Statistical analysis
All data were analyzed using one-way analysis of variance (ANOVA) with LSD multiple range tests at the 0.05 probability level in Statistix 9.0. Arithmetic means were calculated for each of the three replicates separately.

3. Results
3.1. Isolation of bacteria from rice rhizosphere soil
The results indicated that the isolated rhizobacteria obtained from each site of ORF and CRF, showed differences in number of distinct colony morphology (shape, type, and color) on NA plates (Figure 1). Only isolates that appeared to have differences in their colony morphology were selected for further investigations. A total of 629 rhizobacteria were successfully selected from eighteen location of TKR. The number of morphologically different colonies appeared on egg albumin agar plates was 406 and
223 isolates (64.55% and 35.45% of the total tested isolates) for ORF and CRF system, respectively. The average number of selected rhizobacterial obtained from ORF and CRF system was 45.11 and 24.78 isolates, respectively (Figure 1, Table 2). All the selected colonies were then purified and used for screening of their plant growth promoting activities.

Figure 1. The number of morphologically different isolates obtained from rhizosphere soil of organic and conventional rice farming systems (ORF and CRF, respectively), different letters in the graph represents the average values are significantly different according to pairwise comparisons according to pairwise comparisons using the Tukey (HSD) test (p ≤ 0.01) (a) and the error bars represent the standard deviation of measurements for nine soil samples (nine samples of ORF and CRF, respectively) (b).

3.2. Evaluation for plant growth-promoting ability of rhizobacteria
All the selected colonies (629 isolates) were qualitatively screened for their P and K solubilization and siderophore production. Out of the total tested isolates, positive solubilizing activity seen as clear zone around the colony were recorded in 271, 88, and 77 isolates, for Ca-P, K-mica, and K-feldspar, respectively (43.08, 13.99, and 12.24% of the total tested isolates, respectively) (Table 2). Ninety selected rhizobacterial isolates were able to produce siderophore on CAS agar seen as orange zone around their colony accounting for only 14.31% of the total tested isolates (Table 1). On the average, the CRF isolates exhibited around 14% higher number of positive Ca-P solubilizing isolates than the ORF isolates. The CRF isolates also provided around twice higher percentage of positive isolates for K-mica, K-feldspar and siderophore activities than the ORF isolates (Table 2).

Table 2. Number of KDML105 rice rhizobacterial isolates performing positive plant growth promoting abilities.

| Site No. | Sampling area | Total tested isolates | Number of isolates performing positive activity |
|----------|---------------|-----------------------|-----------------------------------------------|
|          |               |                       | Calcium phosphate solubilization | Potassium solubilization | Siderophore production |
|          |               |                       | K-mica | K-feldspar | K-mica | K-feldspar |
| Organic farming | | | | | | |
| 1 | Prasat, Surin 1 | 49 | 24 | 7 | 7 | 9 |
| 2 | Prasat, Surin 2 | 34 | 14 | 3 | 4 | 10 |
| 3 | Prasat, Surin 3 | 50 | 17 | 3 | 4 | 5 |
| 4 | Prasat, Surin 4 | 61 | 16 | 2 | 1 | 11 |
| 6 | Thatum, Surin 1 | 49 | 26 | 4 | 2 | 4 |
| 9 | Kutchum, Yasothon 1 | 35 | 18 | 10 | 5 | 0 |
| 10 | Kutchum, Yasothon 2 | 36 | 16 | 1 | 2 | 4 |
| 11 | Kutchum, Yasothon 3 | 32 | 6 | 1 | 1 | 0 |
| 15 | Kasetwisai, Roi Et | 60 | 18 | 9 | 11 | 0 |
| Total | | 406 | 155 | 40 | 37 | 43 |
| Percent positive of ORF isolate (%) | | 38.18 | 9.85 | 9.11 | 10.59 |
From qualitative screening for Ca-P solubilization of 629 isolates, nine isolates that showed differences in solubilizing level seen as no clear zone (−), (isolate ORF15-19), narrow clear zone (+) (isolates ORF10-12, ORF15-20, ORF15-23, and CRF16-3), and wide clear zone (++) (isolates ORF4-13, CRF5-8, CRF14-15, and CRF17-18), were selected to evaluate their ability by quantitative determination. The examples of clear zone (−, +, and ++) appearing on plates were shown in Figure 2.

![Figure 2](image-url)
One isolate (ORF15-19) exhibiting no clear zone, four isolates exhibiting narrow clear zone (ORF10-12, ORF15-20, ORF15-23, and CRF16-3), and four isolates exhibiting wide clear zone (ORF4-13, CRF5-8, CRF14-15, and CRF17-18), were selected for quantitative evaluation of Ca-P solubilization. The ability of the nine selected isolates to solubilize Ca-P was tested in PKV broth. The same selected isolates were used for the quantitative measurement of the K solubilization and siderophore production (Table 3).

The lowest solubilized P was obtained with isolate ORF15-19 which showed no clear zone on the PVK agar plate. Among the tested rhizobacteria, isolates that show narrow clear zone gave moderate value of solubilized P (17.47 to 25.03 mg L\(^{-1}\)) (ORF15-20, ORF15-23, and CRF16-3), except for isolate ORF10-12 (31.63 mg L\(^{-1}\)). On the average, the high amount of solubilized P was obtained from isolates performing wide clear zone (ORF4-13, CRF5-8, CRF14-15, and CRF17-18), with values of 35.58, 31.63, 36.64 and 34.97 mg P L\(^{-1}\), respectively. The pH of the culture PVK broth was decreased from 7 to around 5 in high P solubilizing isolates, suggesting that organic acids may be produced by the rhizobacteria and responsible for the release of P from Ca-P (Table 3).

All the nine selected rhizobacterial isolates were able to solubilize K-feldspar. It appeared that isolates performing wide clear zone on Alexandrov agar plate (ORF10-12, ORF15-23, CRF5-8, CRF14-15, and CRF16-3) exhibited higher K solubilization (38.63 to 49.45 mg L\(^{-1}\)), than isolates performing narrow clear zone (28.65 to 37.33 mg L\(^{-1}\)). Isolate ORF15-23 exhibited the highest K solubilization (49.45 mg L\(^{-1}\)). The pH of the culture Alexandrov broth was decreased from 7.5 to around 5.21-6.48 (Table 2).

The five selected rhizobacterial isolates producing orange zone around colony ORF4-13, ORF10-12, ORF15-19, ORF15-20, and CRF16-3, exhibited markedly higher siderophore (hydroxamate and catecholate types) production than isolates with no clear zone (Table 3). The amount of hydroxamate type produced by positive and negative orange zone isolates were 64.17 to 618.33 μg L\(^{-1}\), and 0.50 to 22.50 μg L\(^{-1}\), respectively. The amount of catecholate type produced by positive and negative orange zone isolates were 16.49 to 34.39 μg L\(^{-1}\), and 0.04 to 14.74 μg L\(^{-1}\), respectively. Rhizobacterial isolate CRF16-3 gave the highest value of siderophore production both for hydroxamate and catecholate types (Table 3).

**Table 3.** Amount of phosphate and potassium solubilization, and siderophore production by the nine selected KDML105 rice rhizobacterial isolates.

| Rhizobacterial isolate | Phosphate (Ca-P) solubilization | Potassium (K-feldspar) solubilization | Siderophore production |
|------------------------|---------------------------------|--------------------------------------|------------------------|
|                        | Clear zone                      | Amount of soluble P\(^{+}\) (mg L\(^{-1}\)) | pH | Clear zone | Amount of soluble K\(^{+}\) (mg L\(^{-1}\)) | pH | Orange zone | Hydroxamate-type (μmol L\(^{-1}\)) | Catecholate-type (μmol L\(^{-1}\)) |
| Control\(^a\)          | -                               | 3.41 f                                | 6.90 | -          | 2.80 d                                | 7.02 | -          | 3.16 ef                                | 1.45 d |
| ORF 4-13               | ++                              | 35.58 a                               | 5.15 | +          | 37.33 bc                              | 5.74 | ++         | 110.83 c                               | 26.14 b |
| ORF 10-12              | +                               | 31.63 ab                              | 5.29 | ++         | 39.38 abc                             | 5.67 | ++         | 162.50 b                               | 16.49 c |
| ORF 15-19              | -                               | 11.62 c                               | 6.84 | +          | 34.62 bc                              | 6.24 | +          | 64.17 d                                | 19.12 c |
| ORF 15-20              | +                               | 25.03 c                               | 6.23 | +          | 30.69 c                               | 6.35 | +          | 83.33 d                                | 16.84 c |
| ORF 15-23              | +                               | 20.96 cd                              | 6.94 | +          | 49.45 a                               | 5.21 | -          | 22.50 e                                | 14.74 d |
| CRF 5-8                | ++                              | 31.63 ab                              | 5.16 | ++         | 44.81 ab                              | 5.22 | -          | 2.53 ef                                | 4.43 d |
| CRF 14-15              | ++                              | 36.64 a                               | 5.29 | ++         | 38.63 abc                             | 6.12 | -          | 1.67 ef                                | 3.13 c |
| CRF 16-3               | +                               | 17.47 de                              | 6.83 | ++         | 43.02 ab                              | 5.12 | ++         | 618.33 a                               | 34.39 a |
| CRF 17-18              | +                               | 34.97 a                               | 5.14 | +          | 28.65 c                               | 6.48 | -          | 0.50 f                                 | 0.04 d |

\(^a\) Clear zone was qualitatively evaluated on specific agar media.

\(^b\) P and K solubilizing ability was quantitatively analysed in the specific culture broth media.

\(^c\) The mean values in the Table were determined after 7 days of incubation. The initial pH values at 0 day of all the treatments was 7.0, and 7.5 for PVK and Alexandrov broth, respectively.

\(^d\) All the treatment means (3 replications) were subtracted from the inoculated control (without rhizobacterial isolate). Different letters in the columns represents the average values are significantly different according to pairwise comparisons using the Tukey (HSD) test (\(p \leq 0.01\)).
3.3 Effects of NaCl concentrations on phosphate and potassium solubilizing ability of selected rhizobacterial isolates

The amount of P and K of solubilized by nine selected rhizobacterial isolates under salt stress, was evaluated using PVK and Aleksandrov broth plus 0, 0.50, 1, and 3% NaCl (w/v). The responses of the rhizobacterial strains under salinity stress conditions were evaluated by monitoring changes in phosphate and potassium solubilization. Analysis results show significance interaction (P≤0.01) between rhizobacterial strains and NaCl concentrations for all study variables (Table 4).

| Source of Variance | P-solubilization | K-solubilization |
|--------------------|------------------|------------------|
| Rhizobacterial isolates (A) | ** | ** |
| NaCl concentration (B) | ** | ** |
| A x B | ** | ** |
| LSD_{0.05} for (AxB) | 4.1161 | 3.2350 |
| CV% | 30.10 | 20.51 |

** Significant at the 0.01 probability level

The phosphate solubilization potential of KDML105 rice rhizobacteria may play a key role in increasing the bioavailability of soil phosphorus under salinity stress. We therefore evaluated the phosphate solubilization by rhizobacterial isolates under different NaCl concentrations. Among the tested isolates, the results indicated that, at 0% NaCl, ORF4-13, ORF10-12, CRF 5-8, CRF14-15, and CRF17-18 solubilized high amounts of P (31.63 to 36.64 mgP L\textsuperscript{-1}), followed by CRF16-3, ORF15-20, and ORF15-23, which solubilized medium amounts of P (17.47 to 25.03 mgP L\textsuperscript{-1}), and ORF15-19 which solubilized the lowest amount of P (11.62 mgP L\textsuperscript{-1}) (Figure 3). Most of the tested isolates, when exposed to high salt level, solubilize more P than at 0% NaCl however the solubilized P started to decrease at >0.5% NaCl. Among all the tested isolates, the highest P-solubilization was observed in ORF15-23 (54.15 mgP L\textsuperscript{-1}) at 0.5% NaCl, followed by CRF14-15 (50.13 mgP L\textsuperscript{-1}) at 0.5% NaCl.

Figure 3. Effect of salt concentration on P solubilizing ability of selected rhizobacterial isolates in nutrient broth after 7 days of incubation. The error bars represent the standard deviation of measurements for three replications.

Notes: All the treatment means were subtracted from the inoculated controls.
The dissolution of insoluble K in soil can negatively affected by high salinity. Potassium-solubilizing bacteria might improve K solubility in soil as well as salt tolerance in rice. Therefore, in the present study, the K solubilizing activity of nine rhizobacterial isolates under different NaCl% was investigated. The results indicated that as salinity increased, the amount of solubilized K in the solution progressively decreased for all the rhizobacterial isolates. The results indicated that at 0% NaCl, ORF15-23, CRF5-8 and CRF16-3 provided high amounts of solubilized K (43.02 to 49.45 mgK L⁻¹), followed by ORF4-13, ORF10-12, ORF15-19, ORF15-20, and CRF 14-15 which produced medium amounts of solubilized K (30.69 to 39.38 mgK L⁻¹); CRF17-18 produced the lowest amount of solubilized K (28.65 mgK L⁻¹) (Figure 4). On the average isolate ORF15-23 exhibited highest K solubilization at all NaCl level with values ranged from 29.9 to 49.4 mgK L⁻¹. Isolate ORF4-13 provided similar values of solubilized K as ORF15-23 under 0.50 to 3.0% NaCl (Figure 4).

**Figure 4.** Effect of salt concentration on K solubilizing ability of rhizobacterial isolates in nutrient broth after 7 days of incubation. The error bars represent the standard deviation of measurements for three replications.

Notes: All the treatment means were subtracted from the inoculated controls.

### 4. Discussion

Infertile acidic sandy soil, drought and salinity stresses are major limiting factors for the yield and quality of KDML105 aromatic rice cultivated in the TKR region. Synthetic fertilizers application for KDML105 rice production has led to soil fertility degradation and rice quality decline. The utilization of PGPRs is considered an eco-friendly alternative to hazardous synthetic fertilizers as well as a better alternative to solve the soil problems. Phosphorus plays a vital role in every aspect of plant growth and development, but it is often in forms that unavailable for plant uptake [19], particularly in saline soil that P deficiency is often occurred [20]. Insoluble soil P can be available to the plants via phosphate solubilizing bacteria (PSB). The indigenous strains RK24 and RK33 isolated from cabbage rhizosphere, were able to produce oxalic acid and phytase enzyme in the culture broth with the solubilized P of 12.84 and 89.1 mg L⁻¹, respectively [19]. From the present experiment it was observed that around 50% of the tested isolates (629 isolates) could solubilize Ca-P. The amount of P solubilized by the nine selected isolates was 11.62 to 36.64 mg L⁻¹, and the pH reduction in the culture broth was observed suggesting organic acid(s) was released by rhizobacterial isolates. Production of organic acids e.g., citric, lactic, and oxalic acid, by PSB resulted in dissolution of inorganic/insoluble soil phosphates [21-22]. In general, high soil salinity suppresses plants P uptake and reduces the available P by sorption processes.
and the solubility of the Ca-P minerals [23]. Interestingly, in the present study, Ca-P solubilization of most of the tested isolates was enhanced at 0.5% NaCl with the highest percentage increase of 258.3% by ORF15-23, as compared to the control (0% NaCl). In addition, ORF15-23 and CRF14-15 solubilized more P at 1.0% NaCl than 0% NaCl. This phenomenon implied that P is necessary to cope with excessive salinity. Phosphorus was found to enhance salt tolerance in tomato with higher-than-normal P levels required when tomatoes are grown in saline environments [24].

In general, the amount of soluble K in soil is very small as major part of K in soil exists in form of insoluble K-minerals. Excessive soil salinity in salt-affected soils causes ion toxicity reduces potassium (K\(^+\)) uptake by roots due to strong competition between K\(^+\) and Na\(^+\) thus impair plant growth [6-7]. A study has shown that bacterial isolates could solubilize potassium mineral in liquid Aleksandrov broth medium with the amount of 13.71 to 23.88 mg L\(^{-1}\) [25]. The amount of released-K obtained by rice rhizobacterial isolates in this study showed slightly higher values (28.65 to 49.45 mg L\(^{-1}\)) than that found by Maurya et al. [25]. However, under salt stress conditions (>0 to 3.0% NaCl) in this study, the ability of the rhizobacterial isolates to solubilize K was markedly reduced with salt concentrations. This result was in accordance with Mohammad et al. [26] who concluded that the K solubilizing activity of the three tested isolates decreased significantly as salinity increased. Regardless of the decrease in K solubilizing ability of the rhizobacterial isolates under salt stress, it was obvious in this study that several isolates still could release an appreciable amount of K from the K mineral, particularly ORF15-23 (35.9, 33.9 and 29.9 mg K L\(^{-1}\) at 0.5, 1.0, and 3.0% NaCl, respectively). In addition, it was observed that NaCl application might release K from the soil minerals and increased the K concentration in soil solution [27]. The co-inoculation of PSB and KSB together with rock P and K provided the highest availability of P and K in soils with the percentage increase of 36% P and 31% K, as compared to the control. These applications also increased N, P and K uptake in pepper and cucumber [28]. The P (<10 mg kg\(^{-1}\) and K (<60 mg kg\(^{-1}\)) availability of the native TKR soils were very low, and the chemical analysis of the representative sandy soils of Northeastern Thailand indicated that the available fractions of N, P and K corresponded to only 2.2, 4.6 and 2.6% of the total amount, respectively [8]. These results indicated that there are a huge amount of insoluble P and K fraction remaining (>90% of the total amount) in the native TKR soils which could be biologically solubilized at the root-soil interfaces, by promising PSB and KSB obtained in this study, thus a high potential to use them to improve rice growth and quality grown in the TKR soils.

Micronutrients are as important as macronutrients since they involved in the key physiological processes of the plants. Deficiency of micronutrients particularly iron (Fe) is one of the major problems for rice production resulting in poor yields and reduced nutritional quality thus severe malnutrition problems in rice-consuming populations [9, 29]. Availability of iron in soils is limited due to the oxidizing conditions which divalent ferrous iron Fe\(^{2+}\) (soluble form) oxidized to trivalent ferric iron Fe\(^{3+}\) (insoluble form), and the very low solubility of the iron minerals. Furthermore, availability of Fe to plants is reduced in saline soils. Siderophores are biogenic chelators with extremely strong affinity for ferric iron and when Fe\(^{3+}\)-siderophore complex is formed, it becomes available for plants uptake. Siderophore producing rhizobacteria may represent a promising alternative to increase Fe supply to plants and on the mitigation of saline stress [11]. In the present study, all the rice rhizobacterial isolates could produce both hydroxamate- and catecholate-type siderophore with considerably lower values of the latter was observed for all the isolates. Very high amount of hydroxamate-type siderophore was obtained from CRF16-3 (618.33 μg L\(^{-1}\), followed by ORF10-12 (162.5 μg L\(^{-1}\)) suggesting a high potential of these isolates to apply as bioagent for Fe improvement in rice. It was found that hydroxamate-type siderophores are mainly produced by rhizobacteria while catechol-type siderophores are mainly produced by endophytic bacteria. The inoculation of these siderophore producing bacteria increased plant growth grown in heavy metals contaminated soils suggesting that siderophores producing bacteria may reduce the toxicity of heavy metals [30]. The use of several siderophore producing PGPR strains increase in iron uptake by rice and led to double iron content of the rice grain as compared to the control [29]. The siderophores producing PGPR improve Fe content in the crop plants, reduce toxic metals and act as a potential biocontrol agent against root pathogens [31].
Base on the results of the current study, it can be concluded that isolate ORF15-3 exhibited a promising ability in solubilizing insoluble P and K under salt stress, while isolate CRF16-3 gave a considerable high amount of hydroxamate-type siderophore production. These isolates, therefore, can be developed as bioinoculant to apply in KDML105 rice production in salt-affected inland of the TKR region. However, all the nine isolates selected in this experiment should be further evaluated for their ability in other PGP traits such as N₂ fixing and IAA producing abilities as to gain more information for the best selection of rhizobacterial isolate(s) for the improvement of rice production. In addition, experiment(s) with rice under realistic field conditions is needed to confirm the benefit of PGPR inoculation for rice production.

5. Conclusions
The nine selected rhizobacteria isolating from salt-affected inland of the TKR region exhibited multiple PGP traits. Under normal conditions (0% NaCl), the three isolates, CRF14-15, ORF15-23, and CRF16-3 provided the highest P, K solubilization, and two types of siderophore production, respectively. However, on the average, under salt stress (0.5 to 3.0% NaCl) isolate ORF15-23 appeared to perform the best P and K solubilizing activities than the rest of the tested isolates. These KDML105 rhizobacterial isolates may be potentially beneficial biofertilizers and should be tested more in field conditions to confirm their potential to use as biofertilizers inoculants.

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