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Research Article

### Effect of salt-tolerant *Bacillus inoculum* on rice KDML 105 cultivated in saline soil

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#### Abstract

The use of plant growth promoting bacteria (PGPB) could increase the productivity of rice cultivated in saline soil areas. The effects of 3 groups of salt-tolerant *Bacillus inoculum* namely: single inoculum of *Bacillus megaterium* A12ag, a phosphate solubilizing bacterium (PSB); single inoculum of *Bacillus licheniformis* B2r, an ACC deaminase producing bacterium; and co-inoculum of *B. megaterium* A12ag and *B. licheniformis* B2r at mixture ratio 1:1, on germination of rice KDML 105 under laboratory saline conditions (0, 5, 10, 15, and 20 dS/m) were studied. The single inoculum of salt-tolerant PSB, *B. megaterium* A12ag, was the most efficient inoculum to enhance germination. The effects of salt-tolerant PSB, *B. megaterium* A12ag on growth of rice KDML 105 cultivated in saline soil were investigated under greenhouse experiments. Randomized complete block design was used for the experimental design with 7 treatments: using only bacterial inoculum; using bacterial inoculum and chemical fertilizer 16-16-8; using bacterial inoculum, chemical fertilizer 16-0-8 and rock phosphate 0-3-0; using only chemical fertilizer 16-16-8; using chemical fertilizer 16-0-8 and rock phosphate 0-3-0; using only chemical fertilizer 0-16-0; and no use of bacterial inoculums, chemical fertilizer and rock phosphate (control). The fertilizer (N-P-K) equivalent to 16-16-8 at the rate of 25 kg/rai was used. The results revealed that the use of salt-tolerant PSB, *B. megaterium* A12ag could increase the growth of rice and yield components. This study suggests there are potential benefits in using salt-tolerant *Bacillus* as a bioinoculant for cultivation of rice KDML 105 in saline soil.

**Keywords:** phosphate solubilizing bacteria, ACC deaminase producing bacteria, plant growth promoting bacteria, rice KDML 105, saline soil, Thailand

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## Introduction

Rice (*Oryza sativa* L.) is the most important cereal crop of Thailand. Thai jasmine rice or Khao Dawk Mali 105 (KDML 105) rice is the most famous Thai rice variety because of its aroma and tender cooking properties. It is popularly grown in the Northeast of Thailand. Unfortunately, this area has to contend with soil salinity and low soil fertility problems which affect the yield of rice production.

Ethylene is a plant hormone that regulates many physiological processes [1]. The stress conditions such as mechanical wounding, drought, flooding, heat, chilling, salinity and oxidative stresses could induce the biosynthesis of ethylene level by using 1-aminocyclopropane-1-carboxylate (ACC) as a precursor [2]. An increase in the ethylene level results in inhibition of plant root elongation [3]. The use of ACC deaminase producing bacteria is a promising approach to reduce the production of ethylene in plants grown under salt stress conditions.

Low soil fertility problem can be minimized by using fertilizer. Phosphorus (P) is one of the major essential macronutrients for plants and is applied to soil in the form of phosphatic fertilizers. However, a large portion of soluble inorganic phosphate applied to the soil as chemical fertilizer is immobilized rapidly, especially in saline soil, and then becomes unavailable to plants [4]. In addition, the use of chemical fertilizer is currently limited due to rising costs and environmental concerns. In this context, the use of biofertilizer containing phosphate solubilizing bacteria has received much attention.

Plant growth promoting bacteria (PGPB) represent a wide variety of soil bacteria which can stimulate growth of the host plant. The PGPB facilitate plant growth by providing plants with available forms of nutrients, reducing the production of stress induced ethylene and preventing phytopathogens (biocontrol) [5].

The present investigation aims to assess the effects of two PGPB namely: *B. megaterium* A12ag, a phosphate solubilizing bacterium; and *B. licheniformis* B2r, an ACC deaminase producing bacterium, on growth and yield of rice cultivated in saline soil.

## Materials and Methods

### *Microorganisms and inoculum preparation*

Bacterial strains used in this work were obtained from the previous work [6]. *Bacillus megaterium* A12ag, a phosphate solubilizing bacterium (PSB), was grown in MPVK agar [7], containing NaCl 500 mM. *Bacillus licheniformis* B2r, an ACC deaminase producing bacterium, was grown in DF salt minimal medium [8], containing ACC as a sole source of nitrogen and NaCl 500 mM. The growths were then incubated at 30°C for 24 h. Bacterial cells were scraped from the plates and diluted with phosphate buffer (0.1 M, pH 7.0) to obtain  $10^9$  CFU·mL<sup>-1</sup> for each single inoculum. For co-culture inoculum preparation, inoculum of *B. megaterium* A12ag and *B. licheniformis* B2r were mixed at ratio 1:1.

### **Determination of rice seed germination**

Rice (*Oryza sativa* L. ssp. *indica* cv. KDML105) seeds were surface disinfected by immersion in 70% ethanol and 3.0% (w/w) sodium hypochlorite for 5 min and 15 min, respectively. They were then washed thoroughly three times with sterile distilled water. These seeds were used for germination testing with 50 rice seeds per petri dish (15 cm). Each petri dish contained one sheet of paper which was moistened with 10 mL of 0.5% (w v-1) sterilized tricalcium phosphate solution and supplemented with NaCl to obtain an electrical conductivity (EC) at 0, 5, 10, 15 and 20 dS/m. Each treatment was performed 4 times. The seeds were grown under artificial light which provided light intensity at 2,000 lux for 16 h daily and at a temperature of 28±2°C. The two mL of single inoculum of *Bacillus megaterium* A12ag, single inoculum of *Bacillus licheniformis* B2r and co-culture of *B. megaterium* A12ag and *B. licheniformis* B2r were added to each petri dish on day ten. Germination was observed daily. The germination percentage and germination index were calculated at week three after planting.

### **Determination of rice growth and yield**

The experiment was conducted during November 2008 to April 2009. Saline soil (EC 3.3 dS·m<sup>-1</sup>) that was used for planting was collected from Mahasakham Province and autoclaved at 121°C psi for 90 min. Plastic pots were sterilized with 20% sodium hypochlorite solution and filled with autoclaved saline soil. The following treatments with 3 replicates were investigated: (1) using only bacterial inoculum; (2) using bacterial inoculum and chemical fertilizer 16-16-8; (3) using bacterial inoculum, chemical fertilizer 16-0-8 and rock phosphate (RP) 0-3-0; (4) using only chemical fertilizer 16-16-8; (5) using chemical fertilizer 16-0-8 and rock phosphate 0-3-0; (6) using only chemical fertilizer 0-16-0; and (7) no use of bacterial inoculums, chemical fertilizer and rock phosphate (control). The fertilizer (N-P-K) equivalent to 16-16-8 at the rate of 25 kg/rai was used. Ten-day-old rice seedlings were transplanted at the same depth (approx. 2.5 cm below the soil surface) in all pots. The pots were arranged in a randomized complete block design in the greenhouse. Water depth was controlled at 1 to 2 cm during the first week and at 8 to 10 cm thereafter. The seedlings were grown in the greenhouse under natural light, which provided a 15 h photoperiod at temperatures of 25-30°C. Rice plants were harvested on day 120 after planting. They were evaluated for shoot wet weight, shoot dry weight, nitrogen and phosphorus contents in shoot, tiller number per hill, panicle number per hill, number of grains per panicle and 100 grain weight.

### **Statistical analysis**

The data were analyzed with one way analysis of variance (ANOVA) and Scheffe tests to determine any significant differences between groups at  $p < 0.05$ . All statistical analyses were performed by using the SPSS 11.0 for Windows software (SPSS Inc., Chicago, IL, USA).

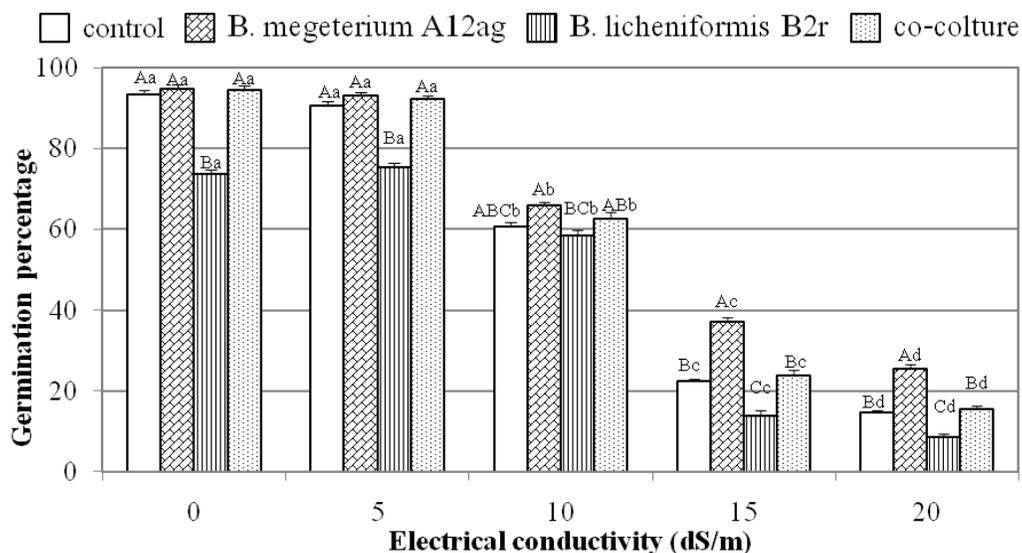
## **Results and Discussion**

### **Effect of salt-tolerant *Bacillus* on rice seed germination**

The effect of salt-tolerant *Bacillus* inoculum on rice seed germination under saline conditions is shown in Figure 1. The study on salt influencing rice growth found that increasing salinity caused a decrease in germination percentage in every treatment (Figure 1.) The reduction of rice seed germination was mainly due to the delayed-actions of salt on seed germination by interfering with the uptake of essential nutrients, the direct toxicity effects of salt ions [9].

The effect of the salt-tolerant PSB, *B. megaterium* A12ag on the germination of rice seeds grown under saline conditions showed trends in enhancing germination percentage over other inoculated and the non-inoculated treatments. When rice seeds were inoculated by *B.*

*megaterium* A12ag, a significant increase in germination was found at EC 10 dS/m. The increase in seed germination might be a result of bacterial activity which caused the increase in available phosphorus. It was then taken up by the plant for the emergence and development of the seedlings since only insoluble phosphate was applied as a source of nutrients for rice seeds. On the other hand, the inoculation of single inoculum of *B. licheniformis* B2r and inoculum of co-culture lowered the rice seed germination. These results could be due to the degradation of starch in the seed embryo by *B. licheniformis* B2r since it is known as a  $\mu$ -amylase producer [10]. Thus only the single inoculum of *B. megaterium* A12ag was selected and used for the evaluation of its effect on the growth and yield of rice KDML 105 grown under greenhouse conditions.



**Figure 1. Effect of salt-tolerant *Bacillus* on rice seed germination under artificial light of 2,000 lux for 16 hrs daily and at a temperature of  $28\pm 2^{\circ}\text{C}$ .**

#### ***Effect of salt-tolerant Bacillus inoculums on rice growth and yield components***

The effect of salt-tolerant *Bacillus* inoculum on rice growth and yield components, cultivated in saline soil is shown in Table 1. Treatments with an inoculation of *B. megaterium* A12ag significantly increased the shoot wet-dry weight and N-P content in shoots over the treatments with no bacteria ( $p < 0.05$ ). In addition, the treatment that used only bacterial inoculums resulted in the highest growth and yield of rice (Tables 1 and 2). The highest plant nutrient content was also observed in this treatment. These results may be due to the fact the bacterium could increase the availability of phosphorus and stimulate the assimilation of N and P in the plant tissue [11]. These findings were similar to Meunchang *et al.* [12], who reported that rice yield was increased significantly because of inoculation with PSB. On the other hand, treatments that used only chemical fertilizer (T4) and control (T7) had no significant effect on tiller number per hill, panicle number per hill and 100 grain weight. Results of this study suggest the use of salt-tolerant PSB as being a suitable bacterial inoculant to increase the growth and yield of rice grown in saline soil. In addition, the utilization of biofertilizer can reduce the use of chemical fertilizer and the cost of agricultural production. For long term application, it can provide a healthy soil with less chemical accumulation.

**Table 1. Effectiveness of *B. megaterium* A12ag on growth of rice and N-P contents in rice shoots.**

Treatment	Wet weight (g/pot)	Dry weight (g/pot)	Nitrogen (% per pot)	Phosphorus (% per pot)
Bacterial inoculum (T1) only	220.55±17.80 <sup>A</sup>	71.67±2.87 <sup>A</sup>	1.394±0.143 <sup>A</sup>	0.156±0.004 <sup>A</sup>
Bacterial inoculum + N-P-K 16-16-8 (T2)	163.62±20.36 <sup>B</sup>	54.66±2.66 <sup>C</sup>	1.259±0.068 <sup>AB</sup>	0.140±0.025 <sup>B</sup>
Bacterial inoculum + N-P-K 16-0-8 + RP 0-3-0 (T3)	199.42±3.07 <sup>AB</sup>	62.89±1.35 <sup>B</sup>	1.147±0.065 <sup>AB</sup>	0.156±0.006 <sup>A</sup>
N-P-K 16-16-8 (T4) only	38.94±7.19 <sup>D</sup>	12.31±1.18 <sup>E</sup>	0.983±0.203 <sup>B</sup>	0.128±0.028 <sup>C</sup>
N-P-K 16-0-8 and RP 0-3-0 (T5)	79.50±8.70 <sup>CD</sup>	23.77±3.33 <sup>D</sup>	1.204±0.052 <sup>AB</sup>	0.112±0.004 <sup>D</sup>
N-P-K 0-16-0 (T6) only	84.95±14.43 <sup>C</sup>	26.13±0.31 <sup>D</sup>	1.033±0.068 <sup>B</sup>	0.103±0.010 <sup>E</sup>
Control (T7)	46.85±2.76 <sup>CD</sup>	21.08±1.05 <sup>D</sup>	1.056±0.161 <sup>B</sup>	0.090±0.011 <sup>F</sup>

ABC values with the same letter within column indicate no significant difference with  $p \geq 0.05$

**Table 2. Effectiveness of *B. megaterium* A12ag on rice yield components.**

Treatment	Tiller number per hill	Panicle number per hill	Number of grains per panicle	100 grain weight (g)
Bacterial inoculum (T1) only	9.00±1.00 <sup>A</sup>	29.00±1.73 <sup>A</sup>	76.67±2.08 <sup>A</sup>	2.757±0.047 <sup>A</sup>
Bacterial inoculum + N-P-K 16-16-8 (T2)	7.67±0.58 <sup>A</sup>	24.33±1.53 <sup>A</sup>	62.67±2.08 <sup>BC</sup>	2.585±0.013 <sup>A</sup>
Bacterial inoculum + N-P-K 16-0-8 + RP 0-3-0 (T3)	8.67±1.53 <sup>A</sup>	25.33±1.53 <sup>A</sup>	68.33±0.58 <sup>AB</sup>	2.648±0.111 <sup>A</sup>
N-P-K 16-16-8 (T4) only	3.00±1.00 <sup>B</sup>	5.67±2.08 <sup>B</sup>	34.00±2.65 <sup>F</sup>	2.496±0.170 <sup>AB</sup>
N-P-K 16-0-8 and RP 0-3-0 (T5)	2.67±0.58 <sup>B</sup>	7.33±3.21 <sup>B</sup>	53.67±1.53 <sup>D</sup>	2.511±0.084 <sup>AB</sup>
N-P-K 0-16-0 (T6) only	3.00±1.00 <sup>B</sup>	9.00±0.00 <sup>B</sup>	56.67±0.58 <sup>CD</sup>	2.201±0.139 <sup>B</sup>
Control (T7)	3.00±0.00 <sup>B</sup>	7.67±0.58 <sup>B</sup>	43.67±4.73 <sup>E</sup>	2.485±0.089 <sup>AB</sup>

ABC values with the same letter within column indicate no significant difference with  $p \geq 0.05$

## Conclusions

Application of salt-tolerant PSB, *B. megaterium* A12ag had a significant promoting effect on the germination of rice KDML105 seed under saline conditions. The bacterium also increased the growth and yield of rice cultivated in saline soil over the treatments with no use of bacterial inoculum. This study suggests the great potential of using *B. megaterium* A12ag as bacterial inoculant for production of biofertilizer.

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## References

1. Bleecher, A.B. and Kende, H. (2000). Ethylene: a gaseous signal molecule in plants. **Annual Review of Cell Development Biology**, Vol. 16, pp. 1-18.
2. Morgan, P.W. and Drew, M.C. (1997). Ethylene and plant response to stress. **Physiologia Plantarum**, Vol. 100, pp. 620-630.

3. Mayak, S., Tirosh, T. and Glick, B.R. (2004). Plant growth-promoting bacteria that confer resistance to water stress in tomatoes and peppers. **Plant Science**, Vol. 166, pp. 525-530.
4. Goldstein, A.H. (1986). Bacterial solubilization of mineral phosphates: historical perspectives and future prospects. **American Journal of Alternative Agriculture**, Vol. 1, pp. 57-65.
5. Goldstein, A.H., Liu, S.T., (1987). Molecular cloning and regulation of a mineral phosphate solubilizing gene from *Erwinia herbicola*. **Biotechnology**, Vol. 5, pp. 72-74.
6. Chookietwattana, K. (2003). Diversity of halophilic bacteria in saline soil at Nong Bo Reservoir, Mahasarakham Province, Thailand. Ph.D. Thesis. Nakhon Ratchasima: Suranaree University of Technology.
7. Son, H.J., Park, G.T., Cha, M.S. and Heo, M.S. (2006). Solubilization of insoluble inorganic phosphates by a novel salt- and pH- tolerant *Pantoea agglomerans* R-42 isolated from soybean rhizosphere. **Bioresource Technology**, Vol. 97(2), pp. 204-210.
8. Penrose, M.D. and Glick, R.B. (2003). Methods for isolating and characterizing ACC deaminase-containing plant growth-promoting rhizobacteria. **Physiologia Plantarum**, Vol. 118, pp. 10-15.
9. Flowers, T.J. and Flowers S.A. (2005). Why does salinity pose such a difficult problem for plant breeders. **Agricultural Water Management**, Vol. 78, pp. 15-24.
10. Haq-Ikram-ul, Ashraf, H., Qadeer, M.A. and Iqbal, J. (2005). Pearl millet, a source of alpha amylase production by *Bacillus licheniformis*. **Bioresource Technology**, Vol. 96, pp. 1201-1206.
11. Vessey, J.K. 2003. Plant growth promoting rhizobacteria as biofertilizers. **Plant and Soil**, Vol. 255, pp. 571-586.
12. Meunchang, S., Thongra-ar, P., Sanoh, S., Kaewsuralikhit1, S. and Ando, S. (2006). Development of rhizobacteria as a biofertilizer for rice production. International workshop on sustained management of the soil-rhizosphere system for efficient crop production and fertilizer use 16<sup>th</sup>-20<sup>th</sup> October, 2006, Land Development Department, Bangkok Thailand. pp. 1-7.