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Original Article

"Rhizoproduct", a biofertilizer containing spores of *Bacillus cereus* strain RS87 for early rice seedling enhancement and with potential for partial fertilizer substitution

Kanchalee Jetiyanon[1]*, Sakchai Wittaya-Areekul[2], Pinyupa Plianbangchang[3], and Ornrat Lohitnavy[4]

[1] *Department of Agricultural Science, Faculty of Agriculture, Natural Resources, and Environment, Naresuan University, Mueang, Phitsanulok, 65000 Thailand.*

[2] *Department of Pharmaceutical Technology, Faculty of Pharmaceutical Science, Naresuan University, Mueang, Phitsanulok, 65000 Thailand.*

[3] *80, Sukhumvit 40, Sukhumvit Road, Phra Khanong, Khlong Toei, 10110 Thailand.*

[4] *Department of Pharmacy Practice, Faculty of Pharmaceutical Science, Naresuan University, Mueang, Phitsanulok, 65000 Thailand.*

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Abstract

The objectives of this study were to investigate the seed to seedling enhancement of Rhizo-product under laboratory conditions and to explore the efficacy of the product for partial fertilizer substitution in rice growth and yield production under submerged soil. Results showed that rice seedlings treated with Rhizo-product was significantly promoted compared to the non-bacterized control. Greenhouse experiments revealed that the growth parameters of all rice cultivars treated at 50% recommended fertilizer rate (RFR) and supplemented with Rhizo-product were better than, or equal to, the growth parameters of rice plants treated with full fertilizer rate (FFR) alone. Additionally, yield production of all rice cultivars receiving 50% RFR supplemented with the product was similar to rice treated with FFR alone. In conclusion, Rhizo-product stimulated early rice seed to seedling growth and exhibited growth and yield production at 50% RFR that was comparable to growth and yield production at FFR.

Keywords: Rhizo-product, early rice seedlings promotion, 50% chemical fertilizer replacement, rice yield enhancement

1. Introduction

Bacillus cereus strain RS87 was previously studied for plant growth enhancement in several plant families and examined in both greenhouse and field experiments (Jetiyanon, 2002; Jetiyanon & Plianbangchang, 2010; Jetiyanon & Plianbangchang, 2012). Multiple mechanisms of plant growth promotion by strain RS87 have been reported including indole-3-acetic acid (IAA) production, phosphate solubilization, siderophore production, and nitrogen fixation (Jetiyanon *et al.*, 2008; Jetiyanon & Plianbangchang, 2010; Jetiyanon, 2015).

Wetland or paddy rice cultivation is a major rice production system in Asia, including Thailand (Sahrawat, 2007). Nevertheless, this submerged rice growing is likely to result in losses of nitrogen due to several causes, such as denitrification, leaching, run-off, and volatilization (Patnaik, 1965; Pande & Adak, 1971; Ghosh & Bhat, 1998). Benefits of plant growth-promoting bacteria in the alleviation of the effects of such chemical problems have been evident (Rodriguez &

* Corresponding author.
Email address: kanchaleej@nu.ac.th; kanchaleej@gmail.com

Fraga, 1999; Sheng & He, 2006; Tripathi *et al.*, 2008; Berg, 2009; Yang *et al.*, 2009). Moreover, Jetiyanon and Plianbangchang (2012) reported that rice cultivars in small experimental paddy fields receiving 50% chemical fertilizer rate in combination with vegetative cells of strain RS87 gave similar growth and yield compared to those receiving the full chemical fertilizer rate alone. In these experiments, bacterial suspensions of fresh vegetative cells were routinely prepared for each experiment, causing tremendous difficulty for practical use. For this reason, an alternative formulation of strain RS87 needed to be developed. Being a Gram-positive bacterium, strain RS87 advantageously produces a resistant life stage, or "endospore". A film coating of seeds with endospores of strain RS87 has been successfully used to achieve early plant growth promotion in pepper and cucumber (Jetiyanon *et al.*, 2008). However, this formulation is only suitable for seeds sown in non-submerged soils.

Currently, we have developed a pellet formulation containing spores of strain RS87 on a laboratory scale for practical application on rice in submerged soils (Jetiyanon *et al.*, 2015). To establish the possible efficacy of this formulation of strain RS87, the product needs to be examined under the submerged system. The objectives of this study are (1) to investigate the potential of Rhizo-product containing spores of strain RS87 for early rice growth promotion from seeds under laboratory conditions and (2) to explore the efficacy of the product for partial chemical fertilizer substitution in growth enhancement and yield production in rice grown in submerged soil.

2. Materials and Methods

2.1 Rhizo-product of *Bacillus cereus* strain RS87 and preparation of live bacterial suspension:

In this study, the tested product was formulated as granular pellets roughly 2-3 cm in diameter. The main active ingredient in the tested product for plant growth promotion was spores of *B. cereus* strain RS87 prepared with the procedure described by Jetiyanon *et al.* (2008). The pellets also contained clay powder, corn starch, and microcrystalline cellulose. There were approximately 10^8 spores of strain RS87 per one gram of tested product. *B. cereus* strain RS87 was maintained and prepared for experimental use as described by Jetiyanon *et al.* (2008). The bacterial concentration was then adjusted to 10^8 cells/ml.

2.2 Source of rice seed cultivars and clay soils

Three photoperiod insensitive Thai rice cultivars (*Oryza sativa* L. Phitsanulok 2 (PHS2), RD47, and RD49) were tested in this study. These rice cultivars were kindly provided by the Phitsanulok Rice Research Center, Rice Department, Ministry of Agriculture and Cooperatives, Thailand. Clay soils used in the greenhouse experiment were collected from growers' rice paddy fields next to Naresuan University, Phitsanulok Province. All soil samples were autoclaved at 121°C for 15 min before use.

2.3 Early seedling growth promotion

Rice seeds of each cultivar were surface sterilized in 3% NaOCl for 10 min and were then rinsed thrice with sterilized double distilled water (ddH_2O). Two grams of rice seeds of each cultivar were soaked in a suspension of the tested product of strain RS87 for 24 hrs. The suspension was prepared by combining three grams of tested product with 30 mL of sterilized ddH_2O in a 100 ml sterilized Erlenmeyer flask. The tested product was dispersed evenly using a magnetic bar on a stirrer. Seeds of each rice cultivar soaked in a sterilized ddH_2O, only, served as a non-bacterized control. Additionally, seeds of each rice cultivar soaked in live cells of the strain RS87 (10^8 cells/ml) served as a positive control treatment. Tested product suspensions and ddH_2O were discarded and seeds were further incubated in the incubator at 30°C covered with foil to prevent dehydration. Root and shoot development were observed daily. Ten rice seedlings of each cultivar from each treatment were randomly sampled six days after incubation for shoot and root measurement. The shoot height was measured from the seed germination site to the highest point of the shoot, while the root length was measured from the seed germination site to the end of the main root. The experiment was repeated.

2.4 Rice growth enhancement

All three rice cultivars (Phitsanulok 2 (PHS2), RD47, and RD49) were studied separately under greenhouse conditions. The experimental design for each tested cultivar was a randomized complete block consisting of four treatments replicated four times each. A 6-inch-diameter plastic pot containing five seedlings represented an experimental unit. All rice plants were grown to maturity in their pots. An experiment for each rice cultivar was conducted twice with each experiment consisting of four treatments: 100% (full rate or recommended fertilizer rate; RFR) with and without tested product, 50% RFR with tested product, and tested product alone. The average temperature in the greenhouse was 33°C during the day and 27°C at night. The relative humidity was approximately 80-85%. The daily photoperiod for all experiments was at least 11.5 hrs.

Rice seeds of each cultivar were surface sterilized and the tested product treatment was prepared and administered as previously described. A sterilized ddH_2O was served as the negative control treatment. Afterwards, tested product suspensions and ddH_2O were discarded,-and the seeds were further incubated at 30°C in the incubator for another six days. Rice seedlings of each cultivar in each treatment were then transplanted into plastic pots containing sterilized clay soil. Pots were flooded from the day of transplant and maintained with water above the soil line in each pot throughout the experiment. A commercial fertilizer, nitrogen (46-0-0)

was applied to rice plants 30 days after transplanting into the plastic pot. The RFR of nitrogen was applied 115 kg N ha^{-1}. The other treatments used 100% and 50% of these application rates supplemented concurrently with one gram of tested product per pot. The tested product alone was applied at one gram of tested product per pot. Growth parameters (plant height, stem size, numbers of leaves, and rice leaf blade size) in each treatment of each rice cultivar were observed daily and were recorded for 45 days after transplanting. All data were analyzed by analysis of variance (ANOVA) and the treatment means were separated using Fisher's protected least significant difference (LSD) test at $P \leq 0.05$ using SAS software (SAS Institute, Cary, NC, USA).

2.5 Rice yield enhancement

All three rice cultivars (PHS2, RD47, and RD49) were also examined separately in the greenhouse for yield enhancement. The experimental design for each rice cultivar, as well as treatments, replications and greenhouse conditions were the same as those described in the rice growth promotion experiments. Rice seeds were sterilized, treated, and incubated with the same procedures as previously described. The rice seedlings of each cultivar in each treatment were transplanted into plastic pots (six inches in diameter) containing a sterilized clay soil, flooded, and maintained throughout the experiment as previously described. Two types of commercial fertilizer; nitrogen (46-0-0) and complex (15-15-15), were applied to rice plants depending on their growth stage. The RFR of nitrogen was applied 115 kg N ha^{-1} and the RFR of complex was applied 38 kg N, 16 kg P, 32 kg K ha^{-1}. The fertilizer application was split three ways: (1) 115 kg N ha^{-1} at 30 days after transplanting, (2) 38 kg N, 16 kg P, 32 kg K ha^{-1} at maximum tillering stage, and (3) 38 kg N, 16 kg P, 32 kg K ha^{-1} at panicle initiation. The other treatments used 100% and 50% of these application rates with one gram of tested product per pot applied concurrently. The tested product alone was applied at one gram of product per pot.

Tiller numbers in each treatment of each rice cultivar were also observed. During the harvesting period of each rice cultivar, water was drained from each plastic pot. The soil dried gradually while the whole rice panicles were approaching maturity. Rice panicles from each cultivar were harvested, and then were completely air dried in an oven (80°C) for 48 hrs. Number of panicles per pot, number of filled grains per main panicle, and grain yield of each rice cultivar were recorded. All data were analyzed by analysis of variance (ANOVA) and the treatment means were separated using Fisher's protected least significant difference (LSD) test at $P \leq 0.05$ using SAS software (Statistical Analysis System Institute [SAS]).

2.6 Shelf life of *B. cereus* strain RS87 in the tested product

Before shelf life testing of strain RS87 in the product, the product was randomly sampled and the original spore concentration of strain RS87 per gram of product was checked by serial dilution methodology. Then the product was divided into two portions. One portion was kept at 30°C and the other was kept at 50°C in the incubator for 12 months. Monthly, each portion of the product was randomly sampled and the viability of strain RS87 was checked onto tryptic soy agar by serial dilution methodology. The plates were incubated at 30°C for 24-48 hrs before checking the appearance of colony forming unit (CFU) of the bacterium for each dilution. The viability check of strain RS87 for each dilution was replicated three times.

3. Results

3.1 Seedling growth enhancement

Six days after incubation, all seed rice cultivars treated with either live cells of strain RS87 or the tested product generally had better growth promotion in terms of root length, plant height, and development of secondary leaf, compared to seeds in the non-bacterized control. For rice cultivar PHS2, seedlings treated with the tested product developed mean root length and mean plant height greater than seedlings treated with live cells of strain RS87. Both treatments had mean plant height and mean plant root length approximately 25% and 45%, respectively, significantly greater than seedlings treated with non-bacterized control (Table 1). At least 50% of seedlings treated with live cells of strain RS87 and the tested product developed secondary leaves, while mostly coleoptiles and some primary leaves appeared on the non-bacterized control (Figure 1a).

For rice cultivar RD47, neither tested product treatment nor treatment with live cells of strain RS87 had statistically significant differences in mean shoot height and root length, but both treatments did have significantly greater shoot height, at least 40% greater, and greater root length, about four times greater, compared to the non-bacterized control (Table 1). More than 80% of seedlings grown from seeds treated with the tested product and live cells of strain RS87 developed secondary leaves. While all seedlings in the non-bacterized control developed coleoptiles, 40% of these seedlings developed only primary leaves. Additionally, seedlings in both the tested product and live cells of strain RS87 treatments had developed several rootlets along the seminal roots. In contrast, only the seminal roots developed in seedlings of non-bacterized control. Moreover, many adventitious roots were noticeable in seedlings treated with the tested product (Figure 1b).

For rice cultivar RD49, seedlings treated with the tested product had the greatest mean shoot height (~37 mm) followed by seedlings treated with live cells of strain RS87 (~27 mm), and the non-bacterized control treatment (~22 mm). Seedlings treated with live cells of strain RS87 developed the longest mean root length (~34 mm) compared with seedlings in the non-bacterized control (~27 mm) and seedlings treated with the tested product (~22 mm). There was statistically sig-

Table 1. Early growth promotion of shoot height and root length of three Thai rice cultivars treated with the tested product 6 days after incubation.

Treatment[w]	PHS2		RD47		RD49	
	Mean[x] shoot height(mm)	Mean[y] root length (mm)	Mean shoot height(mm)	Mean root length (mm)	Mean shoot height(mm)	Mean root length (mm)
Nonbacterized control	28.10b[z]	30.05b	21.50b	15.65b	22.45c	27.00b
Lived cells RS87	37.95a	54.40a	36.10a	47.05a	26.85b	34.25a
Tested product	39.50a	55.60a	39.05a	43.45a	36.75a	22.40c
$LSD_{0.05}$	3.35	4.52	3.25	4.04	2.65	3.77

[w]Nonbacterized control, seeds treated with sterilized ddH_2O; Lived cells RS87, seeds treated with bacterial cell suspensions (10^8 cells/mL) of *Bacillus cereus* strain RS87; Tested product, seeds treated with rhizo-product suspensions (10^8 spores/gram product). [x]Mean shoot height of each rice cultivar are from two separate experiments. [y]Mean of primary root length of each rice cultivar are from two separate experiments. [z]Values followed by a different letter(s) within a column are significantly different at $P \leq 0.05$, according to Fishers's protected least significant difference test.

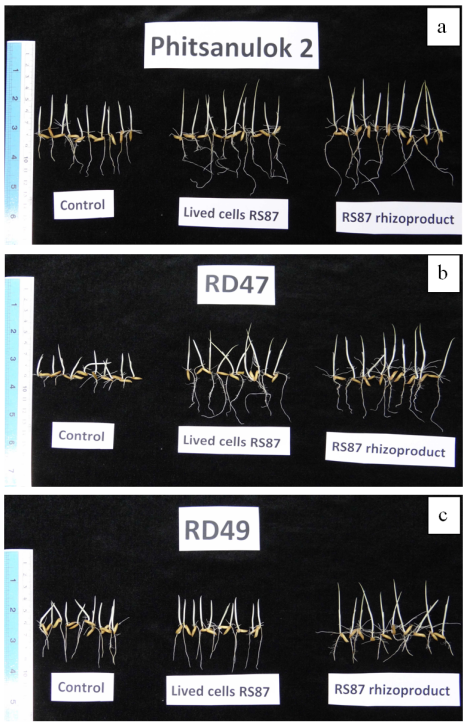

Figure 1. Enhancement of shoot height and root length of three rice cultivars (a) Phitsanulok 2, (b) RD47, and (c) RD49 six days after incubation with the tested product.

nificant difference ($P \leq 0.05$) in mean shoot height and mean root length among all three treatments (Table 1). There was sixty percent emergence of secondary leaves from seedlings treated with the tested product, while no such leaves emerged in the other two treatments. Furthermore, the adventitious roots and rootlets developed only in seedlings treated with the tested product but were not observed in the other two treatments (Figure 1c).

3.2 Rice growth promotion in vegetative stage

After transplanting into sterile soil in plastic pots, seedlings of three rice cultivars generated from seeds soaked with the tested product generally developed better and grew faster than those seedlings in the non-bacterized control. Fifteen days after fertilizer and/or tested product application, for PHS2 cultivar, growth parameters such as plant height, stem size, leaf size and numbers of leaves in rice treated with 100% RFR in combination with the tested product and rice treated with 50% RFR in combination with the tested product were significantly greater than rice treated with 100% RFR alone. It was noticed that in this plant growth stage, other growth parameters of rice treated only with the tested product were similar to rice receiving 100% RFR alone (Table 2).

For RD47 cultivar, rice treated with 100% RFR supplemented with tested product generally exhibited the greatest plant height, stem size, leaf size, and leaf numbers. Stem size and leaf numbers of this treatment were significantly bigger and greater than treatment receiving 100% RFR alone. Most growth parameters of rice treated with 50% RFR in combination with tested product and rice treated only with tested product were similar to rice treated with 100% RFR alone (Table 2).

All growth parameters of RD49 cultivar receiving 100% RFR supplemented with tested product also were the greatest compared to the other treatments. In addition, stem size and leaf size of this treatment was significantly greater than rice treated with only 100% RFR. Except for stem size which was greater, most growth parameters including plant height, leaf size and number of leaves of rice receiving 50% RFR in combination with tested product were similar to plants receiving 100% RFR alone. While stem size and number of leaves were similar to rice receiving 100% RFR alone, plant height and leaf size in treatments receiving only tested

Table 2. Growth promotion of various rice vegetative growth parameters of three Thai rice cultivars responding to a half-rate of fertilizer in combined with the tested product 45 days after transplanting

Treatment[r]	Mean plant growth parameters											
	PHS2				RD47				RD49			
	Plant height[s] (cm)	Stem size[t] (mm)	Leaf size[u] (mm)	No. of leaves	Plant height (cm)	Stem size (mm)[t]	Leaf size (mm)[u]	No.of leaves	Plant height (cm)	Stem size (mm)[t]	Leaf size (mm)[u]	No. of leaves
100% Chemical fertilizer only	45.40b[x]	7.35c	9.40b	7.65b	47.88ab	7.20b	8.35a	8.00b	47.76a	7.50b	9.60b	8.25ab
100% Chemical fertilizer+Tested product	51.41a	8.77ab	10.72a	8.25a	50.54a	8.10a	8.65a	8.40a	50.99a	8.65a	10.50a	8.40a
50% Chemical fertilizer+ Tested product	51.03a	9.02a	10.55a	8.25a	48.86ab	8.15a	8.45a	7.90b	49.45a	8.70a	9.35b	8.05b
Tested product only	48.01ab	8.05bc	8.70b	7.20b	45.35b	7.65ab	7.30b	7.65b	43.79b	7.80b	8.00c	7.65b
$LSD_{0.05}$	4.55	0.96	0.99	0.48	4.34	0.82	0.70	0.37	3.51	0.75	0.70	0.32

Note: Values followed by a different letter(s) within a column are significantly different at $P \pounds 0.05$, according to Fishers's protected least significant difference test. [r]Each rice cultivar was separately executed. There are four replications (5 plants/rep.) per treatment in each experiment. [s]Mean plant heights of each rice cultivar are from two separate experiments. The plant height is measured from the soil surface up to the highest leaf of each plant. [t]Means of stem size of each rice cultivar are from two separate experiments. The stem size is measured from main stem above the water surface about 3-4 centimeters. [u]Means of leaf size of each rice cultivar are from two separate experiments. The leaf size is measured at the mid-leaf of the 7th or 8th fully expanded leaf of each plant.

product were significantly less than rice receiving 100% RFR alone (Table 2).

3.3 Rice yield enhancement

In general, the leaf color intensity of rice is proportional to the amount of chemical fertilizer received. Leaf color intensity of rice treated with 100% RFR supplemented with the tested product observed by naked eyes was somewhat greener than rice receiving only 100% RFR. Nevertheless, it was noticed that leaf color intensity of rice receiving 50% RFR in combination with the tested product was almost the same as the leaf color intensity of rice treated with 100% RFR alone. Rice receiving only the tested product had small yellowish-green leaves and the least plant canopy compared to the other treatments (data not shown). For all rice cultivars, the flag leaf emerged from the main stem at approximately 75 days after transplanting and the flag leaf emerged from the tillering stems about 8-19 days later.

After reaching maturity, all rice cultivars receiving 100% RFR in combination with the tested product had developed the maximum mean number of panicles per pot. All rice cultivars receiving 50% RFR supplemented with the tested product produced significantly less panicles per pot ($P \leq 0.05$), compared to rice receiving 100% RFR with and without tested product, approximately 35-45% and 33-43% , respectively. There was no statistically significant difference in mean number of panicles per pot between rice receiving 100% RFR with or without the tested product (Table 3). It was observed that all rice cultivars receiving 50% RFR in combination with the tested product generated a significantly greater number of filled grains per main panicle compared to rice receiving 100% RFR in combination with the tested product and 100% fertilizer alone, approximately 10-15% and 13-18%, respectively (Table 3). Even though the rice receiving 100% RFR with and without the tested product produced a great number of panicles per pot, several spikelets (especially from panicles generated from tillering stems) were infertile. Almost all spikelets from the main panicle of rice receiving 50% RFR in combination with the tested product fully developed and were filled with grains (data not shown). All rice cultivars receiving 100% RFR supplemented with the tested product gave the greatest yield. Nevertheless, rice receiving 50% RFR with the tested product gave somewhat lesser yield than the treatment receiving 100% RFR alone. However, this difference was not statistically significant. No tillering was observed in any of the three rice cultivars treated with the tested product alone. In addition, the number of filled grains per main panicle in this treatment was about 33-52% (depending on rice cultivar) which was significantly less than the other treatments. Moreover, about 10-15% of the spikelets originating from the main panicle were infertile (data not shown). This resulted in the least mean grain yield of all rice cultivars for this treatment compared to the other treatments (Table 3).

Table 3. Number of panicles, number of filled grains per main panicle, and grain yield of three Thai rice cultivars at different fertilizer rate in combined with the tested product after harvest.

Treatment[r]	Mean number of panicles per pot			Mean number of filled grains per main panicle			Mean grain yield (gram)		
	PHS2	RD47	RD49	PHS2	RD47	RD49	PHS2	RD47	RD49
100% Chemical fertilizer only	9.25a[v]	7.62a	8.87a	78.55b	74.40b	70.55b	17.34ab	14.83b	16.00ab
100% Chemical fertilizer+Tested product	9.62a	8.37a	9.12a	83.82b	77.90b	73.75b	19.39a	17.14a	17.92a
50% Chemical fertilizer+Tested product	5.25b	5.25b	5.87b	93.25a	91.45a	81.22a	15.94b	13.74b	14.37b
Tested product only	5.00b	5.00b	5.00b	48.40c	44.55c	46.97c	6.64c	6.75c	6.94c
$LSD_{0.05}$	0.88	0.97	0.93	5.34	5.85	4.78	2.07	1.61	2.04

Note: Values followed by a different letter(s) within a column are significantly different at *P*£0.05, according to Fishers's protected least significant difference test. [r]Each rice cultivar was separately executed. There are four replications (5 plants/rep.) per treatment in each experiment. "Means of number of panicle per pot, means of number of filled grain per main panicle, and means of grain yield of each rice cultivar are from two separate greenhouse experiments at maturity.

3.4 Longevity of strain RS87 in the product

Results showed that strain RS87 spores in the product remained viable at both 30°C and 50°C and could survive throughout the 12 month test period with almost the same bacterial populations (~10^8 CFU/gram of product) as the freshly prepared product (Table 4).

4. Discussion

In this study, it has been shown that growth of Thai rice seedlings treated with the tested product was significantly promoted compared to the non-bacterized control. The root length, shoot height, and the appearance of the secondary leaf of two rice cultivars (PHS2 and RD47) treated with the tested product were enhanced almost equally to those treated with live cells of strain RS87. Although root length of the RD49 cultivar seedlings treated with the tested product was significantly inferior to that of seedlings treated with live cells. However, this was compensated for by enhancement in some growth parameters such as shoot height, rootlets, adventitious roots and the appearance of the secondary leaves. This finding suggests that the tested product could also be applied as a seed treatment to promote early rice seedling growth before transplanting.

Microorganisms incorporated into biofertilizers used for rice crops are mainly *Azospirillum*, *Azotobacter*, phosphobacteria, *Rhizobium*, *Trichoderma* sp. and mycorhizal fungi

Table 4. Survival of *Bacillus cereus* strain RS87 spores in the product kept at 30°C and 50°C for twelve months.

Incubation time (month)	Populations of strain RS87 in the product kept at 30°C (CFU/gram product)	Populations of strain RS87 in the product kept at 50°C (CFU/gram product)
0	3.18×10^8	3.25×10^8
1	3.01×10^8	3.17×10^8
2	2.91×10^8	3.19×10^8
3	2.89×10^8	3.11×10^8
4	2.81×10^8	3.14×10^8
5	2.92×10^8	2.97×10^8
6	2.83×10^8	2.85×10^8
7	2.87×10^8	2.88×10^8
8	2.73×10^8	2.76×10^8
9	2.75×10^8	2.79×10^8
10	2.71×10^8	2.72×10^8
11	2.82×10^8	2.74×10^8
12	2.77×10^8	2.65×10^8

Note: The value in the table is the means of three replications.

(Ali *et al.*, 1995; Rodrigues *et al.*, 2008; Baset Mia & Shamsuddin, 2010; Javaid, 2011; Banayo *et al.*, 2012). Commercial biofertilizer products available in the market mostly use endophytic bacteria for rice cultivation; free living microorganisms are rarely applied. In this study the tested product formulated as a spherical shaped pellet contained spores of a free-living *Bacillus cereus* strain RS87, as the active component for rice growth and yield promotion. The formulated product easily disintegrates in water, and since *Bacillus* spp. bacteria are facultative anaerobes, the tested product's active component is capable of living in the low oxygen conditions of submerged soil rice culture.

Banayo *et al.* (2012) evaluated three commercial biofertilzers, Bio-N® (BN), BioGroe® (BG), and BioSapark® (BS) in irrigated rice for their effect on grain yield at different fertilizer rates. They reported that rice variety PSB Rc18 treated with biofertilizer Bio-N® (containing *Azospirillum lipoferum* and *A. brasilense*) applied concurrently with 50% recommended fertilizer rate consistently provided grain yield similar to, or better than, rice receiving only 100% recommended fertilizer rate. Live cells of our strain RS87 had previously demonstrated the potential to replace 50% recommended fertilizer rate and still maintain yield production of various Thai rice cultivars in both the greenhouse and a small experimental paddy field (Jetiyanon & Plianbangchange, 2012). The tested product of strain RS87 studied here not only demonstrated early rice-seed growth enhancement but also substituted for at least 50% chemical fertilizer while maintaining similar rice growth promotion during the vegetative stage and grain yield during reproductive stage compared to plants receiving a full rate of fertilizer alone.

Since strain RS87 produces a resistant life stage, the bacterium in the tested product could survive at least 12 months and resist high temperature. Such features are clear of our tested product compared to Bio-N®, because the latter had a shelf life of three months (Banayo *et al.*, 2012) and could not be stored at high temperature.

The particular mechanism for plant growth promotion demonstrated by free living rhizobacteria in some commercial biofertilizer products such as Azogreen-m® (Okon & Labandera-Gonzalez, 1994) and Bio-N® (Banayo *et al.*, 2012) has been attributed to nitrogen fixation, while production of plant hormones and phosphate solubilization has been suggested as the growth enhancement mechanism for BioGroe (Paredes & Go, 2008). *Bacillus cereus* strain RS87 in the tested product studied here provides several mechanisms for plant growth enhancement: IAA production (Jetiyanon *et al.*, 2008), phosphate solubilization and siderophore production (Jetiyanon & Plianbangchang, 2010), and nitrogen fixation (Jetiyanon, 2015). These multiple growth promotion mechanisms of strain RS87 in the tested product could account for the 50% reduction of chemical fertilizer for comparable rice growth and yield.

Recently, different spore concentrations of *B. cereus* strain RS87 have been tested for oral toxicity on Wistar rats. No mortality and no signs of toxicity were observed after a single oral administration of the bacterial spores at all tested doses during 14-day study period (Lohitnavy *et al.*, 2014). Thus, it can be concluded that the product is "non-toxic" and can be considered safe in humans, as well.

In conclusion, the results of the experiments with the tested product strain RS87 suggest that it can promote early growth of rice seeds and maintains rice growth and yield production at half the standard chemical fertilizer rate. Future studies in rice paddy fields, including community microbial interactions of strain RS87, will be conducted to determine whether the tested product is environmentally friendly and can be used for sustainable rice production with partial chemical fertilizer replacement

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References

Ali, S., Hamid, N., Rasul, G., & Malik, K. A. (1995). Use of biofertilizers to enhance rice yield, nitrogen uptake and fertilizer-N use efficiency in saline soils. *Pakistan Journal of Botany, 27*, 275-281.

Banayo, N. P. M., Cruz, P. C. S., Aguilar, E. A., Badayos, R. B., & Haefele, S. M. (2012). Evaluation of biofertilizers in irrigated rice: Effect on grain yield at different fertilizer rates. *Agriculture, 2*, 73-86.

Baset Mia, M. A., & Shamsuddin, Z. H. (2010). Rhizobium as a crop enhance and biofertilizer for increased cereal production. *African Journal of Biotechnology, 9*, 6001-6009.

Berg, G. (2009). Plant-microbe interactions promoting plant growth and health: perspectives for controlled use of microorganisms in agriculture. *Applied Microbiology and Biotechnology, 84*, 11-18.

Ghosh, B. C., & Bhat, R. (1998). Environmental hazards of nitrogen loading in wetland rice fields. *Environmental Pollution, 102*, 123-126.

Javaid, A. (2011). Effects of biofertilizers combined with different soil amendments on potted rice plants. *Chilean Journal of Agricultural Research, 71*, 157-163.

Jetiyanon, K. (2002). Potential use of natural rhizobacteria for plant growth promotion and yield increase. In Thailand Toray Foundation (Ed.), *The 10th Anniversary Thailand Toray Science Foundation*. Bangkok, Thailand: Thailand Toray Foundation.

Jetiyanon, K. (2015). Multiple mechanisms of *Enterobacter asburiae* strain RS83 for plant growth enhancement. *Songklanakarin Journal of Science and Technology, 37*(1), 1-8.

Jetiyanon, K., & Plianbangchang, P. (2010). Dose-responses of *Bacillus cereus* RS87 for growth enhancement in various Thai rice cultivars. *Canadian Journal of Microbiology, 56*(12), 1011-1019. Retrieved from http://www.nrcresearchpress.com/journal/cjm

Jetiyanon, K., & Plianbangchang, P. (2012). Potential of *Bacillus cereus* strain RS87 for partial replacement of chemical fertilisers in the production of Thai rice cultivars. *Journal of the Science of Food and Agriculture, 92*, 1080-1085.

Jetiyanon, K., Wittaya-Areekul, S., & Plianbangchang, P. (2008). Film coating of seeds with *Bacillus cereus* strain RS87 spores for early plant growth enhancement. *Canadian Journal of Microbiology, 54*(10), 861-867.

Jetiyanon, K., Wittaya-Areekul, S., Plianbangchang, P., & Lohitnavy, O. (2015). Pellet formulation containing rhizobacteria. Thai Petty Patent No. 1503000365 (Transcript in Thai; submitted to Department of Intellectual property on March 20, 2014).

Lohitnavy, O., Jetiyanon, K., Plianbangchang, P. & Wittaya-areekul, S. (2014). *In Vivo* safety assessment of the plant growth-promoting rhizobacterium *Bacillus cereus* RS87 and rhizoproduct. *British Biotechnology Journal, 4*, 1141-1148.

Okon, Y. & Labandera-Gonzalez, C. A. (1994). Agronomic applications of *Azospirillum*: an evaluation of 20 years worldwide field inoculation. *Soil Biology and Biochemistry, 26*, 1591-1601.

Pande, H. K., and Adak, N. K. (1971). Leaching loss of nitrogen in submerged rice cultivation. *Experimental Agriculture, 7*, 329-336.

Paredes, J. C., & Go, I. (2008). Putting biofertilizers to good use. *BioLife, 5*, 4-10.

Patnaik, S. (1965). N^{15} tracer studies on the transformation of applied nitrogen in submerged rice soils. *Proceedings of the Indian Academy of Sciences Plant Science, 61*, 25–30.

Rodriguez, H., & Fraga, R. (1999). Phosphate solubilizing bacteria and their role in plant growth promotion. *Biotechnology Advances, 17*, 319-339.

Rodrigues, E. P., Rodrigues, L. S., Oliveira, A. L. M., Baldani, V. L. D., Teixeira, K. R. S., Urquiaga, S., & Reis, V. M. (2008). *Azospirillum amazonense* inoculation: effects on growth, yield and N_2 fixation of rice (*Oriza sativa* L.). *Biotechnology Advances, 17*, 319-339.

Sahrawat, K. L. (2007). Soil fertility advantages of submerged rice cropping systems: A review. *Agroecology and Sustainable Food Systems, 31*, 5-23.

Sheng, X. F., & He, L. Y. (2006). Solubilization of potassium-bearingmineralsby a wild-type strain of *Bacillus edaphicus* and its mutants and increased potassium uptake by wheat. *Canadian Journal of Microbiology, 52*(1), 66-72.

Tripathi, R. D., Dwivedi, S., Shukla, M. K., Mishra, S., Srivastava, S., Singh, R., Rai, U. N., & Gupta, D. K. (2008). Role of blue green algae biofertilizer in ameliorating the nitrogen demand and fly-ash stress to the growth and yield of rice (*Oryza sativa* L.) plants. *Chemosphere, 70*, 1919-1929.

Yang, J., Kloepper, J. W., & Ryu, C. M. (2009). Rhizosphere bacteria help plants tolerate abiotic stress. *Trends in Plant Science, 14*, 1-4.