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Screening of Photosynthetic Bacteria, *Rhodospirllum centenum* for Stimulation of Rice Seed Germination

R. Vareeket [1]and K. Soytong[1]

[1]Department of Plant Production Technology, Faculty of Agricultural Technology, King Mongkut's Institute of Technology Ladkrabang, Bangkok 10520, Thailand

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Purple nonsulfure bacteria (PNSB) are being used in agriculture. Some of the PNSB isolates were reported as nitrogen-fixing microorganisms in flooded rice. A laboratory experiment was conducted to observe the effect of PNSB inoculation on seed germination. The experiment was conducted by using wet filter paper in petri dishes. The design of the experiment was completely randomized (CRD) with three replications. The tested isolates were the growth promoting microbes, SM41, SM61, SM72 and SM92 which were preliminary morphological identified as *Rhodospirillum* sp. The inoculated seeds were done with those isolates and the petri dish were incubated in the dark condition for seven days and subsequently subjected to light condition for another seven days. The seed germination and other related data were measured. The results showed that effect of tested photosynthetic bacteria of SM41, SM61, SM72 and SM92 on seedling growth of rice showed non-significant difference but significantly differed when compared to the non-treated control. The seed germination and growth of shoots were siginificantly different when compared to the non treated control. It was noted that the seedling root growth of rice var. RD41 gave a significant higher than rice var. SM92.

Keywords: photosynthetic bacteria, rice, PGP, seed germination

Introduction

Rice has always been the staple food of the Thai people, and it plays a crucial role as the essence of Thai life. Purple non-sulfur bacteria (PNSB) are a wide range of growth modes and able to grow under photoautotrophic, photoheterotrophic and chemoheterotrophic conditions (Imhoff and Truper, 2005). These groups of microbes are a cosmopolitan group which located in water bodies below the layer of oxygenic photosynthetic organisms such as: algae, aquatic plants and cyanobacteria (Oda *et al*., 2002). They can be found in habitats such as waste water ponds, sediments, moist soils, seawater pools and hypersaline environments (Okubo *et al*., 2006).

The major free-living, nitrogen-fixing microbial systems include the photosynthetic bacteria that inhabit floodwater and surface soil. These organisms absorb light through bacteriochlorophyll and carotenoid which are divided into two principal classes which include the green bacteria and

[1] **Coressponding Author:** Rungrat Vareeket **; E-mail:** rako2521@gmail.com

the purple bacteria (Poretsky, 2003). There are many reports on plant growth promotion and yield enhancement by plant growth promoting bacteria (PGPB), as well as growth promoting mechanisms such as solubilization of insoluble phosphates (Rodriguez and Fraga, 1999) and nitrogen fixation (Kennedy *et al*., 1997).

Purple nonsulfur bacteria (PNSB) are being searched and used in agriculture, although the precise mechanisms of growth promotion have not been elucidated (Elbadry *et al*., 1999). Some of the PNSB e.g. *Rhodopseudomonas*, *Rhodospirillum*, and *Rhodomicrobium* were reported as nitrogen-fixing microorganisms in flooded rice soils. Several studies were shown that the presence of PNSB in paddy soil may contribute to rice productivity. Harada *et al*. (2005) reported that inoculation of *Rhodopseudomonas palustris* can be increased the grain yield of rice while *Rhodobacter capsulatus* enhanced seedling growth, i.e. increasing shoot height of rice seedlings, regardless of rice variety (Elbadry and Elbanna, 1999).

In this research finding was to isolate the purple nonsulfur which isolated from wastewater and find out its efficacy of seed germination on rice var. RD41 and var. PL2.

Materials and Methods

Isolation of photosynthetic bacteria

The samples were randomly collected at Samutpralan Industrial Estate isolated from wastewater at Samutprakan province, Thailand. Sapmles were then isolated by using dilution plate method. The dilution was isolated by cross streak plate method in soft-agar tubes G-5 medium followed the method of Kohlmiler and Gest (1951). The tubes will be incubated under anaerobic condition with the illumination of 1,000-1,500 lux at room temperature (28-30 ^{o}C) for 7-12 days. A loopful of pinkish, reddish or brownish culture was transferred by single streak onto G-5 agar and incubated under the same conditions for 7 days to get pure culture. Single colony of each isolate was transferred to G-5 agar plate to obtain pure culture.

Seed germination test.

Rice seeds var. RD41 and Pitsanulok 2 (PL2) were separately surface sterilized with 10 % ethanol for 5 minutes and followed by 2% sodium hypochlorite for 2 minutes, and rinsed thoroughly in sterilized distilled water. All bacterial isolates were grown in nutrient agar (NA) medium. Bacterial cells were collected twice into suspension with sterile distilled water, and the inoculation density of the bacterial suspension was

adjusted to $1x10^6$ cells/ml by using Optical density (OD) method (Koch, 1970). Seeds were separately inoculated by overnight soaking with suspensions of bacteria (10^6cfu/ml). After soaking, the air-dried seeds were used for germination test. Seeds were soaked only in sterilized distilled water served as the control. Four bacterial isolates were tested as treatments in the experiment. Ten seeds of both inoculated treatments and the control were placed onto sterilized Petri dishes containing filter paper (Whatman paper No. 102). The test was incubated at room temparature, with a cycle of 10 h dark and followed by 14 h of light fluorescent. Seed germination of rice was recorded for 3 days after inoculation, and the root and shoot lengths of the germinated rice seedlings were recorded after 7 days. The experiment was conducted using two factors factorial experiment in Completely Randomized Design (CRD) with four replications.

Seedling germination test

Seed germination was calculated with the following formula:

$$\text{Germination } \% = \frac{\text{Number of emerged seedlings}}{\text{Number of seeds sown}} \times 100$$

Vigour index = (mean root length + mean shoot length) × percentage germination
(Abdul-Baki and Anderson , 1973).

Results and discussion

Isolation and characteristics of the isolates

The samples were randomly collected at Samutpralan Industrial Estate isolated from wastewater at Samutprakan province, Thailand. Isolation of photosynthesizing bacteria was done by dilution plate method on the soft-agar tubes G-5 medium to obtain pure culture. Four bacterial isolates, SM41, SM61, SM72 and SM92 were found and morphologically identified as *Rhodospirillum centenum*. Colonies showed reddish color, shiny and convex colony. Gram-negative, motile and cells are spiral, 0.7-1.5 μm in wide.

As a result, Elbadry and Elbanna (1999) reported that NSB was identified as *Rhodospirillum* sp. which stated as as nitrogen-fixing bacteria can be isolated from paddy rice.

Effect of Rhodospirillum centenum on seed germination of rice

Result showed that seed germination of rice var RD41 in SM1was 45 %, and followed by SM 61, SM 72 which were 32 and 30 %, respectively and the non-tretaed control was 35 %. Seed germination of rice var. PL2 showed that SM41, SM61 and SM 72 were 12.5 % and followed by SM 92 which was 10 % when compared to the non-treated control was only 10 %. It was shown clearly that *Rhodospirillum centenum* isolate SM92 affected to rice var. RD41 which revealed the best for seed germination, shoot length (5.34 cm) and root length (8.35 cm) and vigor index was 479.33. Moreover, *Rhodospirillum centenum* isolate SM61 was the best for seed germination of 12.5 %, shoot length (3.61 cm0, root length (4.62 cm) and vigor index was 69.38 as seen in Figure 1.

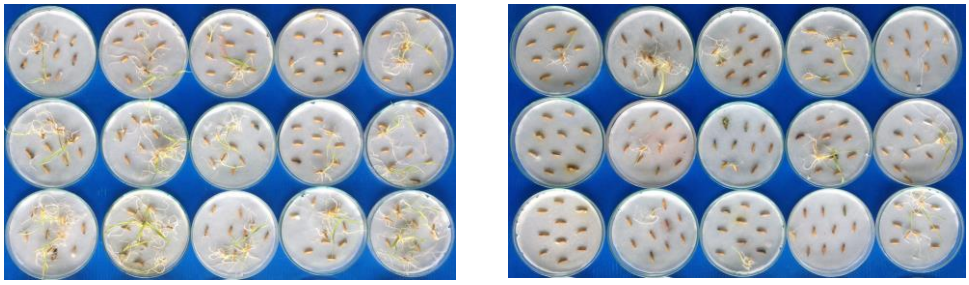

Figure 1. Seed germination of rice var. (A) RD41 and (B) PL2

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