Enhancement of NSIC Rc 192 Seedling Growth by Soil-based and Carbonized Rice Hull (CRH)-based Actinomycete Inoculants

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Abstract

A study was conducted to observe the effects of soil-based (SB) and carbonized rice hull (CRH)-based actinomycete inoculants on the seedling growth of rice variety NSIC Rc 192. The experiment was conducted under laboratory conditions using wet paper towels in petri dishes. The statistical design of the experiment was completely randomized (CRD) with three replicates per treatment. The actinomycete isolate, Streptomyces sp., used in the study was previously reported to produce indole-3-acetic acid (IAA), 1-aminocyclopropane-1-carboxylate (ACC) deaminase, and phosphatase. In this experiment, rice seeds were treated with soil-based and CRH-based actinomycete inoculants. Growth parameters such as shoot and root length and oven dry weight were measured 7 days after sowing (DAS). Inoculation with CRH-based and soil-based actinomycete inoculants significantly increased shoot length by 102.61% and 94.77%, respectively, relative to the uninoculated treatment at 7 DAS. Inoculation with CRH-based and soil-based actinomycete inoculants significantly increased root length by 113.24% and 98.53%, respectively, relative to the uninoculated treatment at 7 DAS. The highest shoot (4.5 mg) and root (3.5 mg) oven dry weight was observed at CRH-based inoculation while the lowest shoot (2.0 mg) and root (0.5 mg) was obtained at the uninoculated control. Regardless of the carrier used, actinomycete isolate can enhance the growth of rice seedlings. Both CRH and soil-based actinomycete inoculants significantly increased the shoot and root length and oven dry weight of rice seedlings.

***Keywords**:* actinomycetes, inoculant carrier, plant growth-promoting bacteria (PGPB), rice, seed germination

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1. Introduction

The actinomycetes are the group of most filamentous bacteria forming long filaments that stretch through the soil. They are prokaryotic, gram-positive, and most are mesophilic. The growth of actinomycetes is inhibited at pH 5.0 or higher and when the moisture content reaches 85-100% or flooding occurs (Prigent, 2012). Actinobacteria can solubilize phosphate, promote plant growth, and produce siderophore and phytohormone (IAA) (Jog *et al.* 2014). The use of plant growth-promoting bacteria, specifically actinomycetes, has been gaining the attention of the researchers in the past few years (Ahemad and Kibret, 2014). *Streptomyces*, a genus under actinomycete, is one of the many widely studied genera receiving increasing popularity because of its plant growth-promoting properties (Sousa and Olivares, 2016). Species under *Streptomyces* are well-known to be isolated from the soil around the roots which raises many concerns on the significance of the bacteria to its host (Sousa and Olivares, 2016). *Streptomyces* species have been isolated from different environments and was reported to exhibit a wide range of plant growth-promoting activities which include its ability to produce IAA, ACC deaminase, siderophores, and its phosphate-solubilizing activity (Sadeghi *et al.*, 2012; Abd-Alla *et al.*, 2013).

Several studies have reported the effectiveness of *Streptomyces* in improving and enhancing the growth and yield of different crops. In the study of Gopalakrishnan *et al*. (2013), *Streptomyces* inoculation significantly enhanced all the agronomic traits of sorghum and rice under greenhouse and field conditions, respectively. A similar study of Gopalakrishnan *et al*. (2015) also demonstrated the effectiveness of the same *Streptomyces* strains in enhancing the growth parameters, grain yield, and total dry matter of chickpea plant. However, information about the effect of the actinomycete during the germination of rice was not provided in these studies. One of the few studies that provided information on the impact of the actinomycete during the germination of rice showed that a siderophore-producing endophytic streptomycete isolated from roots of a Thai jasmine rice plant enhanced the growth of rice and mungbean plants (Rungin *et al*., 2012). The rate of rice growth during the early stage, especially after germination, is very critical since it will determine the success of crop establishment (Ogiwara and Terashima, 2001). Improving the plant growth and vigor during this stage is also essential for robustness and survival of the seedlings under adverse conditions (Vibhuti *et al*., 2015). Therefore, inoculation of actinomycete with an ability to produce ACC deaminase, IAA and phosphatase during early establishment of rice seedlings is beneficial. Hence, the objectives of this.

2. Methodology

2.1 Time and Place of the Study

The experiment was conducted under laboratory conditions from June to July 2017 at the Philippine Rice Research Institute - Central Experiment Station (PhilRice-CES), Science City of Muñoz, Nueva Ecija, Philippines (15º 40’ N, 120º 53’ E, 57.6 masl). Experimental units were placed under laboratory temperature (28 ± 2 °C) with alternating light-dark cycles. Each of the three treatments in the study was done in triplicates.

2.2 Isolate Used

Actinomycete was isolated from Binangonan soil in Rizal, Philippines. This bacterium has been previously proven to produce plant growth-promoting compounds such as ACC deaminase, IAA, and phosphatase, which effectively promoted rice growth under laboratory room conditions (Cruz *et al*., 2014; Cruz *et al*., 2015c). The probable actinomycete identity is *Streptomyces mutabilis* with 98% of maximum identity based on 16S rDNA analysis (Cruz *et al*., 2015d). The bacterium was maintained on arginine-glycerol-salt (AGS) agar slants.

2.3 Seed Surface Sterilization

NSIC Rc 192 seeds were washed with tap water for five times to eliminate unwanted particles then soaked in 95% ethyl alcohol for 2 ½ minutes and washed with sterile distilled water five times. The seeds were then soaked in 30% sodium hypochlorite (NaClO) for 30 seconds and washed again with sterile distilled water five times to rinse off the 30% NaClO.

2.4 Preparation Soil-based and CRH-based Carriers

For the preparation of soil-based carrier, components (soil and charcoal) were pulverized, sieved, and mixed, following the ratio of 3 soil: 1 charcoal. One hundred grams (g) of the mixture was weighed and packed into autoclavable plastic bags and sterilized for 1 hour at 121 °C for three consecutive days.

For the preparation of CRH-based carrier, 100 g of CRH was weighed and packed into autoclavable plastic bags and sterilized for one hour at 121 °C for three consecutive days.

2.5 Preparation of Soil-based and CRH-based Inoculants

A loopful of actively growing *Streptomyces* sp. was inoculated into 50 mL and 100 mL of Arginine Glycerol Salt (AGS) broth and was incubated for five to seven days at room temperature (28-30 °C). After incubation, 50 mL and 100 mL of the culture broths were aseptically inoculated into the 100g sterilized soil-based and 100g CRH-based carriers, respectively, which brought the soil and CRH moisture to approximately field capacity.

2.6 Inoculant Suspension Preparation and Application

Five grams of each of the actinomycete inoculant were added to a separate 100 ml sterile distilled water in a beaker. The surface sterilized seeds were separately soaked in each inoculant suspension for 30 minutes.

2.7 Seedling of NSIC Rc192

CRH and SB inoculant suspensions were used to moist the previously sterilized paper towels in Petri plates. Twenty CRH-based and SB actinomycete inoculant-treated seeds were sown into the plates using sterile forceps. Seeds soaked in sterile distilled water served as the control. The experimental set-up was watered using sterile distilled water for seven days to maintain the moist environment.

2.8 Gathering of Data

Shoot and root length (cm) of five randomly sampled seedlings from each treatment was measured seven DAS (Agbodjato *et al.*, 2016; Gangwar, 2013; Gopalakrishnan *et al.*, 2013). The total oven dry weight was also recorded after 72 hours of oven-drying.

2.9 Statistical Analysis

Statistical analysis was performed using statistical analysis system (SAS) portable v.9.0. All data gathered were analyzed using one way analysis of variance (ANOVA) in a completely randomized design (CRD). Significant differences between treatments were determined using least significant difference (LSD) test at $p<0.05$.

3. Results and Discussion

Table 1 shows the growth of rice as affected by actinomycete inoculation at seven DAS. Both treatments which include inoculation of *Streptomyces* sp. significantly increased the shoot and root length of the germinated rice relative to the uninoculated control (Figure 1).

Table 1. The growth of NSIC Rc 192 as affected by CRH-based and soil-based actinomycete inoculants at seven DAS

Treatment		Shoot length (cm)	Root length (cm)	Shoot oven dry weight (mg/3 seedlings)	Root oven dry weight (mg/3 seedlings)
1.	Uninoculated	3.06^{b}	2.72^{b}	2.00^{c}	0.50^{c}
2.	CRH-based actinomycete inoculant	6.20^{a}	5.80^{a}	4.00^{a}	3.50^{a}
3.	SB actinomycete inoculant	5.96^{a}	5.40^{ab}	3.30^{b}	2.80^{b}

[*]Values represent mean of five replications for length and three replications for dry weight. Values with the same letter within a column are not significantly different at P<0.05 according to LSD.

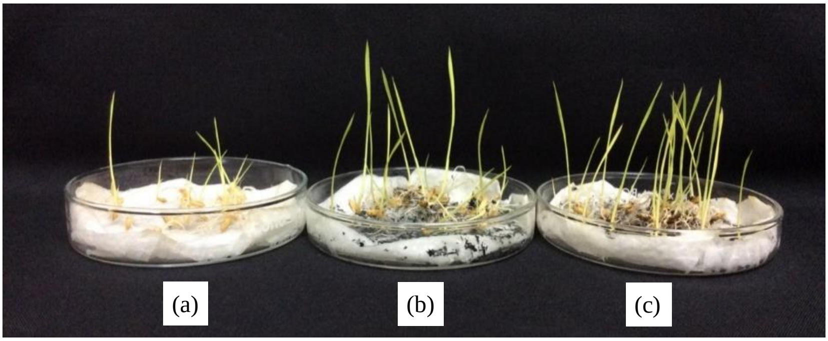

Figure 1. NSIC Rc 192 rice seedlings as affected by actinomycete inoculation: (a) uninoculated control, (b) CRH-based actinomycete inoculant and (c) soil-based actinomycete inoculant

Highest shoot length (6.20 cm) was obtained at rice seedlings treated with carbonized rice hull-based actinomycete inoculant while lowest (3.06 cm) was obtained at the uninoculated treatment. Biocharcoals, such as CRH, are rich source of carbon and are often used as carrier materials (Gaskin *et al*., 2008). Biocharcoal particles are stable, tiny in size (Chidumayo 1994), and increase the carbon content and fertility of the soil (Steinbeiss *et al*., 2009). Related studies by Cruz *et al*. (2015a) showed that initial population of actinomycete in CRH carrier increased by 2.2 x 104 cfu/g to 2.9 x 107 cfu/g (135, 169%) five days after inoculation (DAI). Survival of inoculum in a carrier is one of the major considerations in inoculant production. In this study, inoculation with CRH-based and soil-based actinomycete inoculants significantly increased shoot length by 102.61% and 94.77%, respectively, relative to the uninoculated control at seven DAS.

Highest (5.80 cm) root length was obtained due to CRH-based actinomycete inoculation. On the other hand, the lowest (2.72 cm) root length was obtained at the uninoculated treatment. Inoculation with CRH-based and soil-based actinomycete inoculants significantly increased root length by 113.24% and 98.53%, respectively, relative to the uninoculated control at seven DAS. In a study conducted by Saranya *et al*. (2011), biochar as inoculant carrier significantly increased the root growth, shoot growth, and yield of maize as compared to uninoculated control. One of the reasons for the success of microbial inoculation concerning plant growth promotion can be attributed to the number of viable cells available in the carrier material (Duquenne *et al*., 1999). On the other hand, a study conducted by Gaind and Gaur (1990) showed that the soil-charcoal mixture is a suitable carrier material in terms of the multiplication of phosphate-solubilizing bacteria that may be due to its higher porosity as compared with the other tested carriers.

Regarding dry weight, both treatments with actinomycete inoculation significantly increased the oven-dried shoot and root weight relative to the uninoculated control. The highest shoot (4.5 mg) and root (3.5 mg) oven-dry weight were obtained due to CRH-based inoculant. The lowest shoot (2.0 mg) and root (0.5 mg) were obtained at the uninoculated control. Inoculation with CRH-based and soil-based actinomycete inoculants significantly increased the shoot oven dry weight by 100% and 65% respectively, relative to the uninoculated treatment at seven DAS. Similarly, inoculation of actinomycete with CRH-based and soil-based actinomycete inoculants significantly increased the root oven dry weight by 600% and 460% respectively, relative to the uninoculated treatment at seven DAS. Related studies by Suralta *et al*. (2017) shows that seminal root length of rice seedlings was 145% greater in actinomycete-inoculated rice seeds than in uninoculated ones at seven days after germination. It suggests that inoculation with actinomycete promotes shoot and root growth of rice seedlings during germination stage.

Related studies reported that *Streptomyces* sp. can enhance the growth of different plants including rice, sorghum, wheat, corn, cucumber, tomato, etc. Gopalakrishnan *et al*. (2013) observed that *Streptomyces* sp. strains significantly enhanced all PGP parameters including root length, volume and dry weight, and yield parameters over the uninoculated control in both sorghum and rice under greenhouse and field conditions, respectively. The probable reasons for the enhancement of morphological parameters on both *Streptomyces* sp.-treated sorghum and rice could be the ability of the bacteria used to produce IAA and siderophore and or chitinase, lipase, and β-1,3-glucanase.

A study conducted by Hanapi *et al*. (2014) provided positive results on the use of bacteria to enhance the growth of two varieties of rice. *Nitrosomonas europaea* showed better performance on root and shoot length when combined *Rhodopseudomonas palustris* and *Acinetobacter* sp., respectively. Similarly, laboratory experiments conducted by Gangwar (2013) used *P. fluorescens,* a rhizobacterium, as a bioagent on the growth of rice plant and the results showed that the root and shoot length of the rice plant inoculated with the bacteria was significantly higher than the chemical treatment under laboratory conditions. The same results were obtained from the glasshouse conditions; aside from the increased root and shoot length, *P. fluorescens* exhibited superiority compared to the chemically treated rice plants in terms of the increment of fresh and dry root and shoot weight.

In terms of inoculant formulation, scientists are now using different carriers. The soil is usually used, but other materials like CRH can also be a potential inoculant carrier. CRH is a waste product in rice farming, hence is more practical and environment-friendly to use.

The CRH-based and soil-based carriers used in this study had a pH of 7.85 and 6.6, respectively. In a study by Tang *et al*. (2003), actinomycetes were able to survive within the pH range of 6.0-10.0. In terms of the physiological mechanism, cell organelles in the cytoplasm of most organisms are neutral. Enzymes work best at pH close to that of the environment.

In this study, significant differences in terms of effectiveness to rice seedling growth were observed between the two microbial inoculant carriers (soil-based and CRH-based carriers) relative to the uninoculated control. Both CRH and soil-based actinomycete inoculants significantly increased the shoot and root length and oven dry weight of rice seedlings. A previous study on survival test of actinomycete proved that both soil and carbonized rice hull are potential microbial inoculant carriers (Cruz *et al*., 2015a and Cruz *et al*., 2015b).

4. Conclusion and Recommendation

Inoculation with CRH-based and soil-based actinomycete inoculants enhanced the growth of rice seedlings. This finding supports that the actinomycete, *Streptomyces* sp., used in the study can improve the rice seedling growth in addition to the other studies which showed similar results. However, it is recommended that further study should be made on the promising actinomycete to firmly establish its effectiveness under field conditions where environmental factors cannot be controlled.

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