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Enhanced Rice Seedling Growth by *Clostridium* and *Pseudomonas*

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Abstract: Local isolates *Clostridium* sp. FWM1 and *Pseudomonas* sp. FA1 were studied with respect to their growth promoting effect on rice seedlings using a completely randomized design under greenhouse condition. Seedling growth components including rice seedling height, root length, wet weight, leaf number and biomass were used as parameters for this study. The results showed that *Clostridium* sp. FWM1 and *Pseudomonas* sp. FA1 significantly enhanced rice seedling wet weight and biomass. This suggests that *Clostridium* sp. and *Pseudomonas* sp. deserve to be considered as potential rhizobacterial inoculants for sustainable rice production.

Key words: *Clostridium* sp., *Pseudomonas* sp. rice, rhizobacteria

INTRODUCTION

Treatment of rice seedlings has been reported considered to be desirable due to its positive correlation to increase yield, resistant to disease and enhanced nutrients assimilation. Rice seedling growth can be promoted by Plant Growth Promoting Rhizobacteria (PGPR) which are found naturally as soil bacteria that actively colonize plant roots (Doni *et al*., 2013; Bhattacharyya and Jha, 2012; Kloepper *et al*., 1980). These bacteria include *Azotobacter*, *Bacillus*, *Pseudomonas* and *Clostridium* (Ahemad and Kibret, 2014; Brown, 1974; Kavidmandan and Guar, 1971).

Pseudomonas have many traits that make them suitable as a biocontrol and growth promoting agents. They can grow rapidly *in vitro* for mass production, utilize seed and root exudates, colonize and multiply in the rhizosphere and spermosphere environments (Weller *et al*., 2002). The application of *Pseudomonas* sp. that improves plant growth was recorded such as in tomatoes (Manikandan *et al*., 2010), *Piper nigrum* L. (Paul and Sarma, 2006) and potatoes (Leben *et al*., 1987). However, limited studies have been conducted on the effect of *Pseudomonas* sp. isolates on rice growth from the perspective of bacterial inoculation timing. Mirza *et al*. (2006) reported using *Pseudomonas* inoculants soaked with 5 week old rice seedling. Kandasamy *et al*. (2009), Naganandini *et al*. (2011) and Shruti *et al*. (2013) reported use of *Pseudomonas* as seed priming. Anhar *et al*. (2011) reported *Pseudomonas* as inoculants mixed with 10-day-old rice seedlings soil. *Pseudomonas* soaked with rice seedlings was mentioned as able to alleviate environmental stress (Doni *et al*., 2013; Moldenhauer and Slaton, 2004). Furthermore, the use of local microbial isolates to improve rice growth under specific local condition has been proven superior in enhancing rice growth and physiological characteristic (Doni *et al*., 2014).

Bacteria from the genus *Clostridium* are also included as PGPR (Gamalero and Glick, 2011). Sengupta and Chaudhuri (1991) stated that bacteria of the genus *Clostridium* classified as nitrogen-fixing bacteria. Further, Tsavkelova *et al*. (2006) reported that *Clostridium* sp. is able to produce gibberellin that can enhance plant growth. A study done by Polyanskaya *et al*. (2002) revealed that *Clostridium* sp. inoculation on cucumber and barley significantly enhanced the growth of barley and cucumber. In rice plants application, a laboratory experiment conducted by Yoshida *et al*. (1973) found that *Clostridium* sp. was not a nitrogen fixer under limited source of organic matter in the soil.

To date, there is little report on the ability of *Pseudomonas* sp. in enhancing rice seedling growth.

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Similar for *Clostridium* sp., no information found with respect to its ability on enhancing rice seedling growth. Thus, our objective is to examine the ability of local rice rhizosphere isolates *Clostridum* sp. FWM1 and *Pseudomonas* sp. FA1 in enhancing rice seedling growth.

MATERIALS AND METHODS

This experiment was conducted at the Fermentation Technology Laboratory and Greenhouse, School of Biosciences and Biotechnology, Faculty of Science and Technology, Universiti Kebangsaan Malaysia. A completely randomized design (with two treatments, one control and ten replications) was used for this experiment. The treatments were rice seedlings inoculated with *Clostridium* sp. FWM1 and *Pseudomonas* sp. FA1. Rice seedlings without any treatment were used as control.

***Clostridium* sp. FWM1 and *Pseudomonas* sp. FA1 suspension preparation:** Local isolates rhizobacteria were previously isolated from System of Rice Intensification (SRI) paddy field, Ledang, Johor, Malaysia namely *Clostridum* sp. FWM1 and *Pseudomonas* sp. FA1 were obtained from the Fermentation Technology Laboratory, School of Biosciences and Biotechnology, Faculty of Science and Technology, Universiti Kebangsaan Malaysia. Each isolate was grown separately in different media. *Clostridium* sp. FWM1 was grown on Reinforced Clostridial Agar (Oxoid Limited, UK) which was placed in a 3.5 L anaerobic jar (Merck KGaA, Germany) with Oxoid™ Anaerobic Gas Generating Kit (Oxoid Limited, UK) to create an anaerobic condition. *Pseudomonas* sp. FA1 was grown on King Agar B (Sigma-Aldrich Co. LLC., USA). Both *Clostridium* sp. FWM1 and *Pseudomonas* sp. FA1 were incubated for three days at 30°C temperatures. After three days of incubation, the bacterial cells were harvested using a loop and diluted with sterilized distilled water until the bacterial population density reached a value of 10^8 CFU mL^{-1}.

Rice plant inoculation to bacterial suspension: Rice seeds of the variety MRQ74 were surface sterilized with 70% ethanol, followed by 5% sodium hypochlorite and washed by sterilized distilled water. The rice seeds were grown in autoclaved lateritic soil under greenhouse condition with ambient temperatures of 26-34°C that placed in a seedling tray. Ten five-day old rice seedlings were selected for each treatment then soaked in the respective microbial suspension in a flask containing 10^8 CFU mL^{-1} for 15 min. Rice plants soaked in distilled water served as control. The treated rice plants were grown singly in 15×15 cm plastic containing autoclaved lateritic soil. Water was maintained at 2 cm level from the soil surface and actively aerated by physically breaking up the soil surface once every ten days.

Measurement of rice growth components: Rice seedling growth components were measured after 15 days after transplanting. Plant height (cm) was measured from ground level to the tip of the longest leaf and leaf number was counted for each treatment and control. Root length (cm) was measured from the base of the stem to the longest root using a ruler and rice seedling wet weight (g) was measured using a digital scale. Rice biomass (g) measurement was done after rice roots were dried in the oven at a temperature of 65°C for seven days.

Statistical analyses: All data was statistically analyzed using one-way analysis of variance (ANOVA). The significance of the effect of the treatment was determined using F-test and to determine the significance of the difference between the means of the treatments, Least Significant Difference (LSD) was calculated at 5% probability level.

RESULTS

Rice seedlings wet weight and biomass treated with *Pseudomonas* sp. FA1 and *Clostridium* sp. FW1 were significantly greater than the control (untreated). However, it was not significant with respect to the plant height, root length and leaf number (Table 1, Fig. 1). The results revealed that *Pseudomonas* sp. FA1 has the highest ability to enhance rice biomass among all the treatments. On the other hand, *Pseudomonas* sp. FA1 and *Clostridium* sp. FW1 have equal ability in enhancing rice seedling wet weight.

Table 1: Effect of *Clostridium* sp. FWM1 and *Pseudomonas* sp. FA1 on rice seedling growth

Treatments	Height (cm)	Root length (cm)	Wet weight (g)	Leaf number	Biomass (g)
Pseudomonas sp. FA1	30.8ns*	9.6ns	1.54^{a}**	5.4ns	0.59^{a}
Clostridium sp. FW1	30.4ns	12.3ns	1.30^{a}	4.6ns	0.35^{b}
Control	23.2ns	8.6ns	0.63^{b}	4.4ns	0.12^{c}

*ns: Not significant, **Means with the same letters within the column do not differ significantly according to LSD ($p<0.05$)

Fig. 1: Use of *Pseudomonas sp.* FA1 and *Clostridium* sp. FWM1 resulted in better growth compared to control

DISCUSSION

The enhancement of rice seedling wet weight was double in rice inoculated with *Pseudomonas* sp. FWM1 compared to control. This result agrees with the finding of Leben *et al*. (1987) which reported the enhancement of tomato wet weight inoculated with *Pseudomonas fluorescens*. Xu and Gross (1986) reported that the enhancement of tomato growth by *Pseudomonas* sp. by mechanisms that enhance phosphorus availability to tomato plants. Furthermore, Rodriguez and Fraga (1999) stated that the ability of *Pseudomonas* sp. to solubilize insoluble inorganic phosphate compounds is a key factor for this bacterium to enhance plant growth.

Our study also reveals the ability of *Clostridium* sp. FWM1 to increase wet weight and biomass of rice seedlings compared to control. This study is valuable because, to date, there is no report regarding the application of *Clostridium* sp. in enhancing the growth of rice or other crops. The mechanisms employed by PGPR like the ability to synthesize plant-growth promoting metabolites, stimulate of root growth, produce IAA, control of plant stress and facilitate resource acquisition (nitrogen, phosphorus and essential minerals) are possible mechanisms employed by *Clostridium* sp. to enhance plant growth (Ahemad and Kibret, 2014; Lugtenberg and Kamilova, 2009; Polyanskaya *et al*., 2002).

CONCLUSION

The present study concludes that *Clostridium* sp. FWM1 and *Pseudomonas* sp. FA1 significantly enhance rice seedling wet weight and biomass. This study suggests that *Pseudomonas* sp. and *Clostridium* sp. could be used as plant growth promoters in increasing rice productivity.

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