Mysore J. Agric. Sci., 50 (2) : 259-262 2015

In vitro Efficacy of Endophytic Bacteria Isolated from Aerobic Rice Varieties against *Rhizoctonia solani*

AMANDA SHYLLA AND M. K. SHIVAPRAKASH
Department of Agricultural Microbiology, College of Agriculture, UAS, GKVK, Bengaluru-560 065

ABSTRACT

Endophytic bacteria live inside the plants without causing any harm, promote plant growth and help in controlling plant diseases. An investigation was carried out *in vitro* to access the endophytic bacteria isolated from aerobic rice for biocontrol of *Rhizoctonia solani,* a causal organism of rice sheath blight. Out of the total isolates obtained from surface sterilized samples of the four genotypes of aerobic rice, only four isolates inhibited mycelial growth of *Rhizoctonia solani*. An isolate designated as AM65S2 showed highest antifungal activity (67.04% inhibition) and showed only 24 per cent disease incidence *in vitro*. Apart from showing antagonistic activity these isolates also fix nitrogen or solubilize phosphate. This work revealed that some endophytic bacteria from aerobic rice apart from acting as biocontrol agents also promote plant growth through biological nitrogen fixation and phosphate solubilization and will be added advantage to be used in microbial inoculation.

AEROBIC RICE cultivation is a water-saving technology, where adapted rice varieties are developed and grown under aerobic soil conditions. Aerobic rice holds promise for farmers who do not have access to enough water to grow flooded rice. The water use is 30 to 70 per cent less than in flooded rice (Bouman *et al*., 2006). Aerobic rice is indeed a future technology in context with climate change; however this system is still dependent on the excessive use of agrochemicals which could affect the soil biota, contaminate soil environment and ultimately human health.

Like flooded rice, aerobic rice too suffers from sheath blight caused by soilborne pathogen *Rhizoctonia solani*. It mainly attacks rice at the juvenile stages and cause serious plant losses. Currently, management of this disease is through chemical fungicides and cultural practices. Chemical fungicides not only create problems of fungicide resistance and increase contamination of the soil but also have adverse high toxicity on microbial communities. To avoid these negative effects, biological control of sheath blight using antagonistic microorganisms is a promising alternative.

Endophytic bacteria are those bacteria, which colonizes the interior of the plant parts without causing any harmful effect on host plant (Backman, 2008). They interact more closely with the host, with less competition for carbon sources and are in a protected environment. The most commonly reported mechanism for biocontrol by rhizospheric bacteria and endophytic bacteria is antagonism through predation, competition, and antibiosis. Unlike rhizospheric microbes, the endophytes have an alternative mechanism of biocontrol known as induced systemic resistance (ISR) where bacterial metabolites affect the plant in such a way as to increase the plants resistance to pathogens (Kloepper and Ryu, 2006).

Since aerobic rice is a new technology, more research is required with respect to disease management. In order to make this technology sustainable, less dependent on chemical pesticides, this work is planned to search for endophytic bacteria that resides inside the aerobic rice having antagonistic potential against *Rhizoctoia solani*.

The aerobic rice samples were collected during Kharif season 2014-15 from the aerobic rice research plot, Department of Plant Biotechnology, University of Agricultural Sciences, GKVK, Bengaluru. Four rice genotypes selected for the study were ARB6, IR64, AM65 and Jerigesana. Ten plants of each variety were collected in the heading stage. From each plant, 10 leaf segments, 10 shoot segments, and 10 root segments were analyzed.

The samples were surface sterilized, inoculated in nutrient agar and incubated at 30°C for two days. After incubation, the isolates were transferred to fresh

nutrient agar media and incubated at 30°C for 2 days. The transfer procedure was carried out 3–4 times to isolate single colony.

Dual culture technique was followed for *in vitro screening* of bio-control agents against *Rhizoctonia solani*, the zone of inhibition was measured and the percent inhibition of the growth of pathogen was calculated using the formula.

$$I = \frac{(C-T)}{C} \times 100$$

Where, I = Per cent inhibition

C = Growth of fungal pathogen in control (mm)

T = Growth of fungal pathogen in dual culture plate (mm)

The best isolates showing high inhibition of the pathogens were selected for plant infection study following the method given by Gravel (2005). Seeds of aerobic rice (ARB6) were surface sterilized and bacterisation of seeds was done according to Thompson (1996). The inoculated seeds were sown in the sick soil. Uninoculated seeds soaked in sterile distilled water served as control. The seedling trays were maintained under *in vitro* conditions. The number of healthy seedlings was recorded after 15 days. The experiment had five replications with 10 seeds / cup. The per cent disease incidence was calculated as (IP /TP) × 100, where, IP is the number of infected plants and TP is the total number of plants assessed.

The cell shape, Gram staining and test for the presence of endospores were carried out using the procedures given by Aneja (2006). Screening of the nitrogen fixing ability and phosphate solubilizing ability were tested on N-free Waksman's medium and Sperber's medium respectively.

A one-way analysis of variance (ANOVA) was performed on the data and significant differences between means were compared using Fisher's protected LSD test at P= 0.01.

The per cent inhibition of Sheath blight of rice by endophytic bacteria is presented in Table I. Out of total endophytes isolated from different parts of aerobic rice varieties only four isolates shows antagonism against *Rhizoctonia solani in vitro*. AM65S2 isolate shows significantly highest per cent inhibition (67.04%) when compared to reference strain (53.12%) and other

TABLE I

In vitro evaluation of bacterial endophytes against mycelial growth of Rhizoctonia solani

Variety	Source	Isolates	Per cent inhibition
IR64	Root	IR64R1	46.4 ^{bc}
ARB6	Root	ARB6R1	29.86 ^{d}
ARB6	Shoot	ARB6S2	40.68 ^{cd}
AM65	Shoot	AM65S2	67.04 ^{a}
Reference strain		*Pseudomonas fluorescens*	53.12 ^{b}
SEm			1.74
CD (0.01)			15.67

Note: R-Root,S-Shoot and the number followed is the isolate number
Different letters in superscripts indicate significantly different values.

isolates. The isolate ARB6R1 shows poor antagonistic activity (29.8%) and was not selected for *in vitro* plant infection study. This result is in accordance with Shabanamol and Jisha (2014) where they isolated five endophytic diazotrophic bacteria from rice grown under flooded condition which showed an antagonistic effect against *Rhizoctonia solani* and inhibition of the mycelial growth ranged from 30-70 per cent.

Three isolates designated as AM65S2, IR64R1 and ARB6S2 were selected for the plant infection study. The *In vitro* per cent disease incidence was given in Fig. 1. The highest per cent disease incidence was observed in control (92%) which was on par with the isolate ARB6S2 (88%). Seeds treated with IR64R1 showed 64% disease incidence and the best isolate was AM65S2 (24% disease incidence) where the plants showed healthy growth even after 15 days of sowing in infested soil.

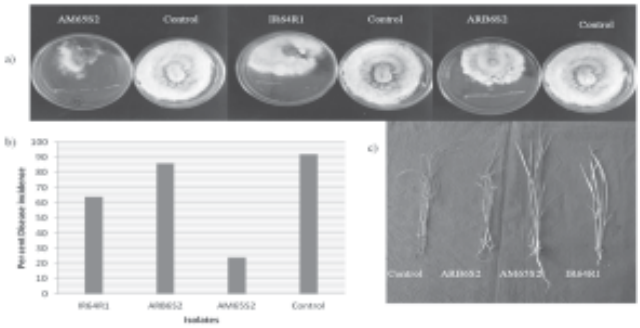

Fig. 1: Effect of seed bacterisation with endophytic bacteria against *Rhizoctonia solani*

a) Endophytic isolates selected for *in vitro* plant infection study, b) Per cent disease incidence of *Rhizoctonia solani* shown by the isolates after 15 days, c) Antifungal activity of rice plants treated with endophytic bacteria.

The characteristics of four antagonistic isolates are given in Table II. Three among the four isolates are Gram negative rod shaped bacteria and one isolate (IR64R1) is a Gram positive rod shaped bacterium. IR64R1 and ARB6R1 which are root endophytes have both nitrogen fixation and phosphate solubilization properties. ARB6S2 has only nitrogen fixation property and AM65S2 is a phosphate solubilizing endophytic bacterium.

The present study revealed that aerobic rice harbored endophytic bacteria which are antagonist against *Rhizoctonia solani* at the same time promoting plant growth through biological nitrogen fixation and

TABLE II

Characteristics of the biocontrol endophytic bacteria isolated from aerobic rice against Rhizoctonia solani

Variety	Sources	Isolates	Colony characteristic in nutrient agar	Microscopic charatristies			Nitrogen fixing ability	Phosphate solubilizing activity
				Cell shape	Gram reaction	Endospore formation		
IR64	Root	IR64R1	Circular, cream, glistening	Rod	+	+	+	+
ARB6	Root	ARB6R1	Circular, red pigmented, glistening	Rod	-	-	+	+
ARB6	Shoot	ARB6S2	Circular, Yellow pigmented, glistening	Rod	-	-	-	+
AM65	Shoot	AM65S1	Circular, creamish, glistening	Rod	-	-	+	-

phosphate solubilization. Hence, the isolates could be used for further studies regarding field inoculation, understanding the mechanisms of biocontrol and molecular characterization which will add more information to the study.

References

ANEJA, K. R., 2006, Experiments in Microbiology, Plant Pathology and Biotechnology. 4th Edition. New Delhi, p. 245-275.

BACKMAN, A. AND SIKORA, R., 2008, Endophytes: an emerging tool for biological control. *Biol. Control,* **26**:1–3.

BOUMAN, M., WANG, M., ZHAO, F. AND CHEN, B., 2006, Performance of aerobic rice cultivars under irrigated conditions in North China. *Field Crops Res.,* **97**: 53–65.

GRAVEL, V., MARTINEZ, C., ANTOUN, H. AND TWEDDELL, R. J., 2005, Antagonist microorganisms with the ability to control *Pythium* damping-off of tomato seeds in rockwool. *BioControl*., **50**: 771–786.

KLOEPPER, W. AND RYU, C. M., 2006, Bacterial endophytes as elicitors of induced systemic resistance. In: SCHULZ, B. J. E., BOYLE, C. J. C. AND SIEBER, T. N. (ed.), Microbial Root Endophytes. Springer Verlag, Berlin, p.33-52

SHABANAMOL, S. AND JISHA., 2014, Assessment of rice endophytic diazotrophic bacteria for biocontrol of rice sheath blight. *Indian Streams Research Journal*, **3:** 1- 6.

THOMPSON, C., 1996, Evaluation of bacterial antagonist for reduction of summer patch symptoms in Kentucky blue grass. *Plant Dis.,* **80**: 856–862

(*Received :* May, 2016 ***Accepted :*** June, 2016**)**