Antagonistic Activity of Bacillus subtilis against Rhizoctonia solani

P. Monisha, S. Sanjaygandhi and
L. Vengadeshkumar

Research Journal of Agricultural Sciences
An International Journal

P- ISSN: 0976-1675
E- ISSN: 2249-4538

Volume: 12
Issue: 04

Res Jr of Agril Sci (2021) 12: 1208–1210

CARAS

Res. Jr. of Agril. Sci. (July-Aug 2021) 12(4): 1208–1210
www.rjas.org

Short Communication

Antagonistic Activity of *Bacillus subtilis* against *Rhizoctonia solani*

P. Monisha[1], S. Sanjaygandhi*[2] and L. Vengadeshkumar[3]

Received: 01 Apr 2021 | Revised accepted: 22 Jun 2021 | Published online: 19 July 2021

***Key words*:** Rice, *Rhizoctonia solani*, *Bacillus subtilis,* Antagonistic activity

Rice sheath blight caused by *Rhizoctonia solani* Kuhn. The sexual stage of *R. solani* was *Thanatephorus cucumeris* (A.B. Frank) Donk), it is most important soil borne necrotrophic fungus and facultative parasite [1]. Rice cultivation started at the 15th century in South East Asia and spread to India, China and Japan. The first leading rice producer is China followed by India, Indonesia, Bangladesh, Vietnam, Thailand, Myanmar, Philippines, Brazil and Japan [2]. In the year of 2017, the average production of rice in India is 104.32 million Tones [3]. Generally, chemical methods is widely used for combating the sheath blight incidence but continuous uses of chemical fungicides leads susceptibility of crop and develop the resistance on pathogen towards the chemical [4]. Also, fungicides are very harmful to humans, environmental pollution and increasing the cost of production [5]. The increasing concern about the environmental impact of the usage of fungicides has led to an intense search for alternative plant protection strategies. Therefore, it has become necessary to adopt ecofriendly management for better crop health and high yield. The natural products can improve the ability of crop which may be very useful and reduce the diseases severity. Hence, the aim of present study is to evaluate the potential of biocontrol agents on the management of rice sheath blight disease.

Isolation and identification of pathogen

The diseased rice plants showing the typical symptom of sheath blight disease were collected from survey. The pathogens were isolated separately on potato dextrose agar (PDA) medium [6]. The infected portion of the sheath was cut into small bit, surface sterilized with 0.1% sodium hypochlorite solution for 1min and washed thrice with sterile distilled water. Further, a piece of specimen was transferred to Petri dishes containing Potato Dextrose Agar (PDA) medium. The plates were incubated at room temperature (28±2°C) for 7 days and the isolates were purified by single hyphal tip method. The identification of isolates of *R. solani* was confirmed and purified isolates were maintained on PDA slants for further studies.

Isolation and identification of Bacillus subtilis

Rhizoplane-colonizing *B. subtilis* was isolated from fresh roots of paddy grown in different locality of Cuddalore district and were designated as BS_1 to BS_7. The soil particles loosely adhering to the roots were teased out and used for the isolation of *B. subtilis*. A soil suspension was prepared from each rhizosphere sample by shaking one g of soil sample in 10 ml of sterile dist. water and serial dilutions were made. One ml of soil suspension from aliquot dilutions (10^{-5} to 10^{-8}) was aseptically added to sterile Petri dishes containing twenty ml of sterile King's B medium and incubated at 28 ± 2°C for 48 h after incubation, well separated individual colonies with yellow green and blue white pigments were marked and detected by viewing under UV light. Also, the colony and type of colony, shape of cell were observed. The individual colonies were picked up with sterile loop and transferred to fresh King's B slants and the pure cultures so obtained were stored in refrigerator at 4°C for further use.

Dual culture technique

The antagonistic activity of *B. subtilis* against *R. solani* was tested by dual culture technique [7]. Isolates of *B. subtilis* was streaked at one side of Petri dishes (one cm away from the edge) containing PDA. A 9mm mycelial disc from seven days old PDA culture of *R. solani* was placed at the opposite side of Petri dishes perpendicular to the respective bacterial and fungal antagonist and incubated at 28±2°C for 15 days. Petri dishes inoculated with fungal discs alone served as control. Three replications were maintained for each isolate. Observation on width of inhibition zone and mycelial growth of test pathogen was recorded and per cent inhibition of pathogen growth was calculated by using the formula proposed by Vincent [8].

$$I = C - T / C \times 100$$

Where;

* S. Sanjaygandhi
✉ premasivajothi@gmail.com
[1-3] Department of Plant Pathology, Faculty of Agriculture, Annamalai University, Annamalai Nagar - 608 002, Chidambaram, Tamil Nadu, India

I - Per cent inhibition
C- Mycelial growth of pathogen in control
T- Mycelial growth of pathogen in dual plate

Poisoned food technique (Radial growth)

The culture filtrates of the fungal and bacterial antagonists were separately incorporated into sterilized PDA and King's B medium at 5, 10, 15 and 20 per cent by adding the calculated quantity of the culture filtrates to the medium by means of a sterile pipette. The PDA medium without the culture filtrate served as control. The amended media were transferred to sterile Petri dishes separately @ 15ml and allowed to solidify. Each plate was inoculated at the centre with a fifteen-days old (9 mm) PDA culture disc of *R. solani* and incubated at room temperature (28±2°C) for fifteen days. The radial growth of the mycelium was measured after fifteen days of incubation. The results were expressed as per cent growth inhibition over control.

Table 1 Evaluation of native isolates of *B. subtilis* on the growth of *R. solani* under *in vitro* (Dual culture and poisoned food technique)

Isolates	Linear growth of *R. solani* (mm)		Per cent growth inhibition	Mycelial dry weight (mg/50 ml broth)				
	Inhibition zone	Growth		10%	20%	30%	40%	Mean
BS_1	54.82 (47.76)	35.18 (36.37)	60.91^{e}	321	284	167	68	210.00^{f}
BS_2	58.45 (49.86)	31.55 (34.17)	64.94^{d}	294	249	136	50	182.25^{d}
BS_3	62.72 (52.36)	27.28 (31.48)	69.68^{a}	206	174	100	35	128.75^{a}
BS_4	51.63 (45.93)	38.37 (38.27)	57.36^{f}	342	298	176	79	223.75^{g}
BS_5	59.32 (50.37)	30.68 (33.68)	65.91^{c}	282	226	128	44	170.00^{c}
BS_6	55.10 (47.92)	34.90 (36.21)	61.22^{e}	302	263	142	52	189.75^{e}
BS_7	61.24 (51.49)	28.76 (32.43)	68.04^{b}	256	215	115	40	156.50^{b}
Control	-	90.00 (71.56)	-	390	390	390	390	390^{h}

Poisoned food technique (Mycelial dry weight)

The culture filtrates of the fungal and bacterial antagonists were separately incorporated into sterilized PDA and King's B broth respectively at 10, 20, 30 and 40 per cent by adding the calculated quantity of the culture filtrates to the broth. The PDA broth without the culture filtrate served as control. The amended media were dispensed in 250 ml Erlenmeyer flasks, autoclaved at 1.4 kg / cm^2 for 20 min and cooled. Each flask was inoculated separately with a 15-day old nine mm PDA culture disc of *R. solani* and incubated at room temperature (28±2°C) for fifteen days. Three replications were maintained for each medium. After incubation the mycelial mat was filtered through a pre weighed Whatman No. 1 filter paper and then dried in hot air oven at 60°C till a constant weight was obtained. The mycelial dry weight was calculated by subtracting from the weight of the filter paper and recorded. The results were expressed as per cent growth inhibition over control.

Table 2 Effect of culture filtrate of native *B. subtilis* on the mycelial growth of *R. solani* (Poisoned food technique)

Isolates	Mycelial growth (mm)							
	5%	Percent inhibition over control	10%	Percent inhibition over control	15%	Percent inhibition over control	20%	Percent inhibition over control
BS_1	60.17	33.14^{f}	57.20	36.44^{f}	42.27	53.03^{f}	35.25	60.83^{f}
BS_2	49.11	45.43^{d}	47.10	47.66^{d}	30.92	65.64^{d}	23.15	74.27^{d}
BS_3	35.24	60.84^{a}	30.72	65.86^{a}	16.24	81.95^{a}	10.18	88.68^{a}
BS_4	65.92	26.75^{g}	60.15	33.16^{g}	44.62	50.42^{g}	41.82	53.53^{g}
BS_5	46.61	48.21^{c}	38.73	56.96^{c}	29.72	66.97^{c}	19.72	78.08^{c}
BS_6	57.28	36.35^{e}	52.35	41.83^{e}	38.72	56.97^{e}	30.24	66.40^{e}
BS_7	38.54	57.17^{b}	36.21	59.76^{b}	19.56	78.26^{b}	15.44	82.84^{b}
Control	90.00	-	90.00	-	90.00	-	90.00	-

Evaluation of native isolates of Bacillus subtilis against Rhizoctonia solani

The present investigation was taken under to study the effect of bacterial antagonist *B. subtilis* against sheath blight disease incidence. Among the seven isolates of *B. subtilis*, BS_3 was recorded the maximum inhibition of *R. solani* (69.68). Findings well endorsed by earlier workers [9-10]. This may be due to antifungal compounds produced by mycobacillins, iturins, bacilliomycins, surfactins. The bacterial antagonistic against reduced growth of the pathogen [11].

Effect of culture filtrate of B. subtilis on the mycelial growth of Rhizoctonia solani

The culture filtrate of all the *B. subtilis* isolates inhibited the growth of *R. solani*. Generally, an increase in the concentration of the culture filtrate were reduced the growth of the pathogen. Among the isolates tested, BS_3 was

found to be most inhibitory to the growth of *R. solani*. The crude antibiotics were produced by *B. subtilis* for controlling the mycelial growth of *Rhizoctonia solani* by poison food technique [12-13].

SUMMARY

In the present study the efficacy of biocontrol agent against *Rhizoctonia solani*. The biocontrol agent was *B. subtilis* is most effective against rice sheath blight pathogen. The screening of seven isolates of *B. subtilis* against *R. solani* on nutrient agar plates. All the isolates significantly reduced the mycelial growth of the pathogen. Among the isolates of *B. subtilis* (BS_3) appeared to be the most effective against the test pathogen showing 69.68 per cent inhibition of colony growth, followed by BS_7, BS_5 isolates. The results of the experiment showed the superiority of *B. subtilis* (BS_3) and hence the same was used for subsequent studies.

LITERATURE CITED

1. Wang AJ, Zheng AP. 2018. Characteristics and control measures of rice sheath blight. *Chinese Rice* 3: 124-126.
2. Li XP, Ma DW, Chang W, Liang CB, Zhao HX, Guo JX, Song CY, Pan GJ. 2018. Linkage disepuilibrium analysis of rice sheath blight resistance markers of rice grown in the cold region of northeast China. *Genetika* 3: 943-958.
3. IRRI. 2016-2017. International Rice Research Institute Annual Report for www.irri.org/resources/publications/annual-reports/annual-reports-2016-2017
4. Ghewande MP, Nandagopal V. 1997. Integrated pest management in groundnut (*Arachis hypogeal L*.) in India. *Integrated Pest Management Reviews* 2: 1-15.
5. Zhang F, Zeng D, Zhang CS. 2019. Genome-wide association analysis of the genetic basis for sheath blight resistance in rice. *Rice* 12: 9. https://doi.org/10.1186/s12284-019-0351-5
6. Ainsworth GC. 1961. *Dictionar of Fungi*. Commonwealth mycological institute Kew Surrey England. pp 547.
7. Dennis C, Webster. 1971. Antagonistic properties of species groups of Trichoderma production of non-volatile antibiotics. *Trans. Brit. Mycology* 57: 25-39.
8. Vincent JM. 1927. Distribution of fungal hyphae in the presence of certain inhibitors. *Nature* 159: 850.
9. Rajkumar K, Naik MK, Amaresh YS, Chennappa G. 2018. Bio efficacy of *Bacillus subtilis* against major pathogen of chilli *Colletrotrichum capsici* causing fruit rot of chilli. *Int. Jr. Curr. Microbiol. App. Sci*. 7(7): 2681-2686.
10. Abarna T, Abdul Raheem AH, Abhishek R, Livingstone, Abichandra K, Abikannan A, Suthin RT. 2019. Bio efficacy of bacillus subtilis and plant extracts against *Colletotrichum capsici* (Syd.) Butler and Bisby under *in vitro* condition. *Jr. of Applied Sciences and Computations* 6(4): 2110-2123.
11. Ben KS, Kilani FO, Dammak M, Khiareddine H, Remadi M, Tounsi S. 2015. Efficacy of *Bacillus subtilis* V26 as a biological control agent against *Rhizocotnia solani* on potato. *Jr. Plant Biol. Pathology*. pp 1631.
12. Gong A, Li H, Yuan Q, Song X, Wei W, He J, Zhang J, Liao Y. 2015. Antagonistic mechanism of Iturin A and Plipastatin A from *Bacillus amyloliquefaciens* S76-3 from wheat spikes against *Fusarium graminearum. PLoS ONE* 10(2): e0116871.
13. Torres M, Brandan C, Sabate D, Petroselli G, Balsells R, Audisio M. 2017. Biological activity of the lipopeptide-producing *Bacillus amyloliquefaciens* PGPBacCA 1 on common bean *Phaseolus vulgaris* L. pathogens. *Jr. Biol. Control* 105: 93.