International Journal of Current Advanced Research
ISSN: O: 2319-6475, ISSN: P: 2319-6505, Impact Factor: SJIF: 5.995
Available Online at www.journalijcar.org
Volume 6; Issue 8; August 2017; Page No. 5194-5196
DOI: http://dx.doi.org/10.24327/ijcar.2017.5196.0670

Research Article

ANTIFUNGAL ACTIVITY OF BACILLUS SPECIES ISOLATED FROM AGRICULTURALLY CULTIVATED SOILS AGAINST FUNGAL PHYTOPATHOGENS FUSARIUM UDUM AND RHIZOCTONIA SOLANI AFFECTING PADDY AND PIGEON PEA CROPS

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ARTICLE INFO

Article History:

Received 20th May, 2017
Received in revised form 14th June, 2017 Accepted 26th July, 2017
Published online 28th August, 2017

Key words:

Biocontrol, Antagonism, phytopathogens, chitinase, siderophores.

ABSTRACT

The present research was conducted to test antifungal properties of soil bacteria. Soil plant microbe interactions are complex and there are many ways the outcome can influence plant health and productivity. These interactions may be detrimental, beneficial, or neutral to the plants. However, the focus of this work is to exploit the beneficial bacteria to enhance plant growth by biocontrol of fungal phytopathogens by different mechanisms. *Fusarium udum* and *Rhizoctonia solani* are soil borne fungal phytopathogens that affect paddy and pigeon pea crops in the early stages of cropping practices. Soil borne bacteria are well known for their antagonism, predation, and competition towards other microbes in consortia. These bacterial traits of antagonism and competition could suppress fungal phytopathogens enabling a sustainable method of crop protection and forfeits the classical chemical crop protection methods. Bacterial isolates such as Bacillus species present in enormous percentage in agriculture soils were isolated and tested for PGPR and fungal antagonistic traits. Eight bacillus species were tested for volatile HCN and Ammonia production, enzymes such as cellulase, proteases, chitinase productions, siderophore production. These bacillus species were further tested for fungal phytopathogen suppression in both qualitative and semi- quantitative method using dual plate assay methods. *Bacillus thuringenesis* and *Bacillus cereus* were found to suppress phytopathogens by inhibiting their growth both *in vitro* and *in vivo* methods. Hence these species of *Bacillus* enable the biocontrol methods of plant disease suppression.

INTRODUCTION

The term biological control was first coined by "Harry Smith" in relation to biological control of insects. In general terms biological control is defined as population levelling process in which the population of one species lowers the number of another species by mechanisms suchas predation, parasitism, pathogenisity or completion. Thus biological control refers to the use of one living organism to curtail the growth and proliferation of another undesirable organism. The widely coated definition of biological control of disease is: "The reduction in the amount of inoculums or disease producing activity of pathogen accomplished by or through one or more organisms." Some biocontrol PGPB have been found to produce enzymes including β-1,3 glucanase, chitinase, protease, cellulase, amylase that can lyse fungal cells and organic matter (Davis 1995). Chitin is a homopolymer of beta 1-4 linked N-acetyl D-glucosamine residues and can be degraded by microbial chitinases (Davis and Henrissat, 1995). These chitinases act mainly to degrade chitin for utilization by the bacteria as an energy source. In addition some chitinases of chitinolytic bacteria such as *Serratia marcescens*and *Enterobacter agglomerans* are potential agents for the biological control of plant diseases caused by various phytopathogenic fungi (Zhang, *et al.*, 2001). The enzymes inhibit fungal growth by hydrolyzing the chitin present in the fungal cell wall

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MATERIAL AND METHODS

Soil samples were collected from agricultural regions Kakinada areas of East Godavari District, Andhra Pradesh, India. Agriculturally cultivable field soils, paddy fields cultivated with pigeon pea, were dug into 60 cms depth for collection of soil samples. The sub surface soils from three different slots were collected and mixed in the sterile black polythene bags. Large and hard soil particles were removed and padded through a 2mm sieve to collect fine soil particles and stored at 4°C for 7-10 days in sterile ziploc polythene

bags. Their physico-chemical characteristics such as pH, EC (Electrical conductivity), Nitrogen content, available phosphorus and potassium were analyzed at SIFT (State Institute of Fisheries Technology), Kakinada, Andhra Pradesh.

Enumeration of bacteria and biochemical characterization

The bacterial colonies obtained in different dilutions were counted using a graduated Quebec colony counter (Dalal). The number of colonies were counted using the Misra and Miles drop count method (1938) on plates containing between 30-300 cfus. Serial dilutions were used to determine the number of bacteria in the original sample and were expressed as colony forming units (cfus) per gram of soil.

$$\text{Colony forming units} = \frac{Number\ of\ colonies}{Dilution} \times Volume\ factor$$

Bacterial enumeration was performed for all soil and compost samples that were diluted and tabulated as the average cfu/gm sample.

Biochemical characterization of bacteria isolated from soil is performed by using KB002 Hi Assorted Biochemical test Kit for gram negative rods and gram positive rods.

This is a standardized colorimetric identification system utilizing seven conventional biochemical tests and five carbohydrate utilization tests. The tests are based on the principle of pH change and substrate utilization. On incubation the organisms undergo metabolic changes which are indicated by a color change in the media that can be either interpreted visually or after addition of the reagent.

The organisms are cultured and purified. The purified inoculums in incubated in the test kit for 18-24 hours at the temperature of 37^{o}. Results are interpreted.

RESULTS AND DISCUSSION

Physicochemical characteristics of soils and composts

Analysis of the physicochemical characteristics of the soil and compost samples used for bacterial isolation is presented in Table 1.1. All soils and composts tested showed nearly neutral pH, with the exception of RSC which is lowest. Slight variations are recorded for electrical conductivity of composts and soils. Nitrogen content was found more in Rice straw compost (RSC) among the composts and in rhizoplane soil (RPS) among soils lowest was CFS. Available phosphorus levels were found to be slightly more in compost samples than in soils, whereas potassium and organic carbon levels were found to be slightly more in soils compared to composts (Results compared with standard values obtained from Agriculture Research Station, Kakinada). GVC was rich in all the three minerals (N, P, and K) that were tested. Overall, the tested parameters of the samples are not very different from standard values.

Bacterial enumeration of soils and biochemical characterization

Bacteria were enumerated and evaluated as colony forming units per gram of soil (cfus/gm) and were found to be highest in the Rhizoplane soil compared to Rhizosphere and Cultivable field soils which had moderate bacterial counts (Table 2). The distribution of bacterial genera most numerous in all the composts and soils as recorded in Table 2 *Bacillus* and P*seudomonas* species were the most numerous in composts and soils were seen. In soils, *Bacillus* sps., were predominant followed by *Pseudomonas* and *Enterobacter*.

Fungal antagonistic traits

HCN production by the bacterial isolates was recorded as a change in color of the filter paper from yellow to orange brown. The isolates of genera *Pseudomonas* (RB11, RB15, and RB22) were the best HCN producers compared to the other genera tested. The HCN producers were categorized based on the filter paper color change i.e. rapidly in 24 hours to brown (Pseudomonas isolates) or slow change of color from yellow to orange in 48hours (*Bacillus subtilis* RB1and *Bacillus cereus* RB13).

Chitinase production was observed via zones of chitin hydrolysis amended medium. Clearing of plates containing colloidal chitin as a sole source of carbon by the bacterium around the colony was used to measure chitin hydrolysis. Only three isolates showed chitinolytic activity; *Serratia marcesens (RB24), Bacillus cereus (RB13) and Enterobacter cancerogenus (RB17).* The isolates of genera Bacillus and Pseudomonas show siderophore production.

Tables 1a

Bacteria	Soil samples (cfu/gm)		
	RS	RPS	CFS
*Bacteria	$3.5X10^{6}$	$4X10^{6}$	$2.4X10^{6}$
Gm +ve rods	41±0.33	32±0.58	38±2.31
Gm –ve rods	26±1.75	28±0.23	22±1.76
Bacillus	19.1±0.58	36.1±0.67	31.2±0.9
Enterobacter	17.0±1.88	16.5±1.33	12.1±3.3
Serratia	10.7±3.23	10.0±0.16	8.6±0.11
Klebsiella	18.04±1.12	11.2±2.31	14.0±1.6
Pseudomonas	12.1±0.53	13.3±0.11	16.6±2.3
Azatobacter	4.4±2.53	4.6±0.03	5.9±0.03
Azospirillum	13.0±1.75	6.0±1.33	5.3±0.51
Rhizobium	7.8±2.21	4.6±0.01	8.2±0.03

Table 1b

BACTERIAL ISOLATES	*Siderophore production	*HCN production	*Chitinase production
RB1	-	+	-
RB6	-	-	-
RB8	-	-	-
RB13	+	+	+
RB9	-	-	-
RB17	-	-	+
RB19	-	-	-
RB30	+	-	-
RB3	-	-	-
RB24	-	-	+
RB11	+	++	-
RB15	+	+++	-
RB32	+	-	-
RB27	-	-	-

+ positive for presence of trait; - absence of trait
*+ weak HCN producer; ++moderate HCN producer;
+++strong HCN producer

CONCLUSIONS

Gram positive rods were abundant bacteria in all the soils and composts in our study. *Bacillus* and *Pseudomonas* were found to be the major genera found. The thermophilic and halophilic natures of these bacteria may explain their abundance in the composts and soils used in this study.

Thirty two isolates belonging to eight genera were identified based on colony and microscopic characteristics. The bacteria

were further identified to the species level using biochemical tests.

Pseudomonas fluorescens and Bacillus subtilus Bacillus cereus showed predominant antifungal traits of chitinase and HCN activity.

Acknowledgements

I thank DST WOS B scheme for funding this project.

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How to cite this article:

Usha Rani Ch *et al* (2017) 'Antifungal Activity of Bacillus Species Isolated from Agriculturally Cultivated Soils Against Fungal Phytopathogens Fusarium Udum and Rhizoctonia Solani Affecting Paddy and Pigeon Pea Crops', *International Journal of Current Advanced Research*, 06(08), pp. 5194-5196. DOI: http://dx.doi.org/10.24327/ijcar.2017.5196.0670

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