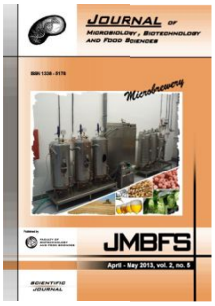


JMBFS

Journal of Microbiology, Biotechnology and Food Sciences

International peer-reviewed scientific online journal

Published by Faculty of Biotechnology and Food Sciences

Prasanna et al. 2013 : 2 (5) 2272-2276

OPTIMIZATION AND EVALUATION OF MICROBE FORTIFIED COMPOSTS AS BIOCONTROL AGENTS AGAINST PHYTOPATHOGENIC FUNGI

Ajinath S. Dukare, Radha Prasanna, Lata Nain, Anil Kumar Saxena*

Address(es): Dr. Radha Prasanna
[1]Division of Microbiology, Indian Agricultural Research Institute (IARI), New Delhi, India. Phone: 91 11 25847649; Fax: 91 11 25846420.

*Corresponding author: radhapr@gmail.com

ARTICLE INFO

Received 14.7.2012
Revised 5.2.2013
Accepted 5.2.2013
Published 1.5.2013

Regular article

OPEN ACCESS

ABSTRACT

A set of bacterial (B1-10) and cyanobacterial strains (C1-C14) were evaluated for their fungicidal activity against selected phytopathogenic fungi - *Fusarium solani*, *Fusarium oxysporum*, *Fusarium oxysporum lycopersici*, *Fusarium moniliforme*, *Pythium debaryanum* and *Rhizoctonia solani*. Further, they were used to amend rice straw compost and the filtrates were evaluated against the selected fungi after 10 and 20 days of incubation. Six promising strains, including three bacterial and three cyanobacterial strains were selected and characterised in terms of activity of hydrolytic enzymes. Interestingly, C12 strain (*Anabaena* spp.) showed highest activity of cellulase, chitosanase and β 1, 3 glucanase. These strains were then evaluated by optimization of inoculum levels (1-5%) in the rice straw compost. The strains B3, B5, C8 and C12 were observed to be most promising as they exhibited inhibition and significantly higher activity of microbiological parameters and hydrolytic enzymes at 1-2% level of inoculum in the compost. Further investigations are being undertaken to scale up the development of compost based biocontrol agents using these strains for evaluation at field level.

Keywords: Bacterial antagonists, biocontrol, compost, cyanobacteria, phytopathogenic fungi

INTRODUCTION

Fungal diseases of crop plants are one of the major concerns for agricultural production. Conventional practices to overcome this problem has been through the use of chemical fungicides, which have several adverse effects on the environment, besides being a major health hazard to humans and other non target biota. Therefore, there exists a pressing need to reduce the losses as a consequence of these fungal diseases by developing environment friendly practices, among which the use of microorganisms (including fungi) holds promise.

The potential use of naturally occurring bacteria, actinomycetes and fungi as a means of biological control has been addressed globally (**Yuen *et al.*, 1994**). Fluorescent Pseudomonads have been implicated in the control of several soil borne and wilt diseases in important crops such as wheat, rice and commercial fruits and vegetables. Among cyanobacteria, *Nostoc muscorum* is known to be effective against "damping off" disease caused by fungi (**Caire *et al.*, 1990**) and several *Anabaena* and *Calothrix* strains exhibit fungicidal activity against species of *Pythium, Fusarium* and *Rhizoctonia* (**Moon *et al.*, 1992**; **Prasanna *et al.*, 2008, Radhakrishnan *et al.*, 2009; Manjunath *et al.*, 2010**). Another eco-friendly alternative which is gaining much importance is the application of compost derived from agricultural/agro-industrial wastes, which are generally free of xenobiotics/excessive metal concentrations. Compost based suppression of a wide range of soil borne diseases has been demonstrated in several studies (**Hoitink, 1990; Temorshuizen *et al.*, 2006; Noble and Coventry, 2005**). However, the mechanism of biocontrol is complex. Disease suppression may involve competition for nutrients and ecological niches, or antagonism, antibioses/parasitism or induction of host resistance (**Hoitink and Boehm, 1999; Vallad *et al.*, 2003**). As a consequence, the degree of plant protection may vary diametrically among composts, besides being influenced by timing of application and maturity indices (**Temorshuizen *et al.*, 2006**).

Any biological control agent requires a carrier for its successful establishment and continued activity in soil. In this context, a wide number of carriers such as talc, vermiculite, plant based wastes have been utilised (**Nakkeeran *et al.*, 2005**). However there is limited information available on the use of compost as a carrier for biocontrol agents (**Scheuler *et al.*, 1989**).

The objective of our investigation was therefore to develop a compost based biocontrol agent against the selected phytopathogenic fungi, using paddy straw compost as a carrier for the bacterial / cyanobacterial culture and compare its potential against pure culture.

MATERIAL AND METHODS

Growth and maintenance of test and target fungal strains

The available germplasm of fourteen cyanobacterial strains **(Prasanna et al. 2008)** and ten bacterial strains (Supplementary Table 1) from the Division of Microbiology, IARI, New Delhi, were screened for the biocidal activity against selected phytopathogenic fungi - *Fusarium solani*, *Fusarium oxysporum*, *Fusarium oxysporum lycopersici*, *Fusarium moniliforme*, *Pythium debaryanum* and *Rhizoctonia solani* obtained from the Indian Type Culture Collection, Division of Plant Pathology, IARI, New Delhi.

The bacterial cultures were grown at 20± 2^{0}C and maintained in the nutrient broth and on nutrient agar slants at 4^{0}C. The late log phase cultures of bacteria (48h) were utilised for compost amendment and evaluation of biocontrol potential. The cyanobacterial cultures were axenised by standard procedures (**Kaushik, 1987**) employing a set of antibiotics (Hi Media Laboratories, India). Such cultures were used for the further work after repeated sub culturing in nitrogen free BG-11 media (**Stanier *et al.*, 1971**) and grown under the light: dark cycle (16:8h) under white light (50-55 µmol photons / m / s) and 28 ± 2° C in nitrogen free BG-11 medium in Haffkine flasks. Six weeks old cultures were utilised for the fungicidal studies (**Prasanna *et al.*, 2008**). Potato dextrose agar medium (Hi Media Laboratories, India) was used for the growth and maintenance of the fungal cultures. Incubation was done at 28°C in an incubator (Lab Thermo LT-X Incubator Shaker, Kuhner AG, Basel).

In vitro fungicidal assay of bacterial and cyanobacterial cultures

Sterile 5mm discs (Hi Media Laboratories, India) were placed on the lawn of respective fungal strains. Twenty µL of filtrate of cyanobacteria/bacteria or 25 µL of cyanobacterial pellet was dispensed on the discs and incubated at 28°C in the incubator. Nystatin (Hi Media Laboratories, India) and sterile water were used as positive and negative controls.

Experimental set up

Pounded paddy straw compost (50 g) prepared by the method of **Gaind and Nain (2010)**, was taken in plastic containers and bacterial /cyanobacterial cultures were added such that 60% water holding capacity (WHC) was maintained. The physico-chemical characteristics of the paddy straw compost used is given as Supplementary Table 2. The bacterial cultures were added at the

rate of 1-2 x 10^6 CFU / mL. For the cyanobacterial cultures, the chlorophyll values were maintained in the range of 10-20 µg / mL, using the method of **Mackinney *et al.* (1967)**. Sterile water amended composts served as control. The containers were plugged with steristoppers to maintain aeration. Samples (10 g) were removed after 10 and 20 d of incubation and centrifuged after addition of sterile water. The samples were vortexed and the leachates used for fungicidal assay after centrifugation and passage through 0.45µ filters.

For the assay against phytopathogenic fungi, 20 µl of the leachate was inoculated on the sterile disc of 5mm and placed on the lawn of the selected phytopathogenic fungi. Observations on the development of inhibition zone were taken regularly up to 72 h incubation period. Positive and negative controls included nystatin and leachate from sterile water and medium amended composts respectively. The leachates of the amended composts were also tested for the activity of selected hydrolytic enzymes.

Enzyme assays

On the basis of preliminary screening a set of three efficient bacterial and three cyanobacterial strains were selected for amending the compost. The unamended/amended composts were analysed in terms of chitosanase, endoglucanase and cellobiase enzymes. along with controls-composts amended with sterile water / BG 11 medium / Nutrient Broth (Hi Media Laboratories, India).. The chitosanase activity of the culture filtrate was analysed by the spectrophotometric method, using glycol chitosan as the assay substrate. One unit (IU-International Unit) of chitosanase activity was defined as µmoles of amino sugars released per ml per min under the assay conditions (**Ohtakara, 1988**). The cellobiase (β-D-glucosidase*)* activity was determined spectrophotometrically at 430 nm by the method outlined by **Wood and Bhat (1988)** against the standard curve of p- nitrophenol. The β 1,3 glucanase activity culture of the filtrates of both bacteria and cyanobacteria was determined spectrophotometrically at 530 nm, using laminarin as the assay substrate by the method outlined by **Wood and Bhat (1988)** against the standard curve of N-acetyl glucosamine.

Microbial activity of composts

Alkaline phosphatase activity was assayed in composts (1 g) suspended in modified universal buffer (pH 11), along with 1 mL p- nitro phenyl phosphate (**Tabatbai and Bremner, 1969**). After incubation for 1 h at 37 ^{0}C, the enzyme reaction was stopped by addition of 0.5M $CaCl_2$ and 0.5M NaOH. The suspension was filtered through Whatman No 1 filter paper, the absorbance was measured at 440 nm and the enzymatic activity was expressed as µg p-nitro phenol released /g / compost / h. FDA (Fluorescein diacetate) hydrolysis assay was carried out using (100 mg) compost, suspended in Potassium Phosphate buffer (pH 7.6) and FDA (0.5 mg/ mL). After incubation for 2 h at 37° C, the reaction was stopped using acetone. The solution was filtered through Whatman No 1 filter paper and the absorbance of the supernatant taken at 490 nm using Fluorescein standard (**Green *et al*., 2006**). The values were represented as µg Fluorescein released / g compost /h.

Dehydrogenase Activity was assayed using compost (1 g), incubated with Triphenyl tetrachloride (3 %) for 24 h in dark. Methanol was added to extract the triphenyl formazone (TPF) and the supernatant was filtered and absorbance taken at 485 nm (**Casida *et al*., 1964**). The values were expressed as µg of triphenyl formazan (TPF)/ g compost /day.

Statistical Analyses

The experimental data were tabulated and analysed using Statistical package for Social Sciences (SPSS Version 10.0). The data was recorded in triplicate for the selected parameters and subjected to ANOVA (analysis of variance) in accordance with the experimental design (completely randomized block design) using SPSS statistical software to quantify and evaluate the sources of variation. Correlation and treatment means were compared at 5% level of significance. Duncan's Multiple Range Test (DMRT) was employed to compare the mean performances of different strains for the specific parameters under study and the critical difference between treatments, calculated at 5% level of significance, denoted as CD (probability of 0.05) representing Critical Difference among the treatments and SEM (Standard error of Means) in the tables. Standard deviation values are depicted in the graphs as bars. Correlation analyses were undertaken using Microsoft Excel package.

RESULTS

All the bacterial strains inhibited the growth of *Fusarium moniliforme*, while B2-B8 strains also inhibited *Pythium debaryanum* (Tables 1 and 2). Strains B3, B4, B5 and B8 also inhibited the growth of *Rhizoctonia solani. Fusarium oxysporum lycopersici* was inhibited by the culture filtrate of bacterial strains-B3 B4, B5, B7, B9 and B10. *Fusarium solani* and *Fusarium oxysporum* were inhibited by only two (B8, B10) and three (B7, B9, B10) bacterial culture filtrates. Among the cyanobacterial cultures tested, the culture filtrate of the all the strains except C8, C9, C12 and C13 inhibited the growth of the *Fusarium solani*. The culture filtrates of C1-C8 strains inhibited the growth of *Fusarium moniliforme,* while *Fusarium oxysporum* was not inhibited by most of the cyanobacterial culture filtrates.

On the basis of the inhibition zone studies against phytopathogenic fungi and enzymatic profile generated, three bacterial and cyanobacterial strains were selected for amending the compost at different levels. The enzymatic profile (Table 3) of the selected six strains (B3, B4, B5, C4, C8 and C12) revealed that C12 strain (*Anabaena* spp.) exhibited highest activity of cellobiase, chitosanase and β 1, 3 glucanase followed by B3 and B5. High activity for cellobiase and β 1, 3 glucanase was shown by B5 and B3 strains and ranked after C12 in terms of activity of all the enzymes evaluated.

Table 1 Fungicidal activities of bacterial culture filtrate in terms of zone of inhibition against selected phytopathogenic fungi

Bacterial culture	Zone of inhibition					
	Fs	Fo	Fl	Fm	Pd	Rs
B1	-	-	-	+	-	-
B2	-	-	-	++	+	-
B3	-	-	+	++	+	++
B4	-	-	+	++	+	+
B5	-	-	+	++	+	++
B6	-	-	-	++	+	-
B7	-	+	+	++	+	-
B8	+	-	+	++	+	++
B9	-	+	-	++	-	-
B10	+	+	+	++	-	-
Nystatin	+	+	+	+	+	+

Legend: +, denotes 10-12mm ; ++, 12-15mm ; +++, >15mm; Fs, *Fusarium solani*; Fo, *Fusarium oxysporum*; Fl, *Fusarium oxysporum lycopersici*; Fm, *Fusarium moniliforme*; Pd, *Pythium debaryanum*, and Rs, *Rhizoctonia solani* .

Table 2 Fungicidal activity of cyanobacterial culture filtrate and pellet in terms of inhibition zone on the lawn of selected phytopathogenic fungi

Treatment/Strain	Culture filtrate (From six week old cultures)					
	Fs	**Fo**	**Fl**	**Fm**	**Pd**	**Rs**
C1(CF)	+	+	-	+	-	-
C1(P)	+	+	-	+	+	-
C2 (CF)	+	+	-	+	+	-
C2(P)	+	+	-	+	+	-
C3(CF)	+	+	-	+	+	-
C3(P)	+	-	+	-	+	-
C4(CF)	+	-	+	++	+	+
C4(P)	+	-	+	+	+	+
C5(CF)	+	-	+	+	+	+
C5(P)	+	-	-	+	+	-
C6(CF)	+	-	-	+	+	-
C6(P)	+	-	-	+	+	-
C7(CF)	+	-	+	+	+	-
C7(P)	+	-	+	+	+	-
C8(CF)	-	-	+	+	+	+
C8(P)	-	-	+	+	+	+
C9(CF)	+	+	-	+	-	-
C9(P)	+	+	-	+	-	+
C10(CF)	+	-	+	-	-	-
C10(P)	+	-	+	-	-	-
C11(CF)	+	-	-	-	+	-
C11(P)	+	-	-	-	-	+
C12(CF)	+	+	+	-	+	+
C12(P)	+	+	+	-	+	+
C13(CF)	-	+	-	+	+	+
C13(P)	-	-	-	+	+	+
C14(CF)	-	+	-	-	-	-
C14(P)	-	+	-	+	+	-
Nystatin	+	+	+	+	+	+

Legend: CF denotes culture filtrate, and P denotes pellet. Other details as given in Table 1

Table 3 Enzyme activity of selected cultures (IU/ml)

Name of organism	Cellobiase	Chitosanase	β 1,3 Glucanase
Pseudomonas sp.(B3)	0.229	0.626	0.458
Pseudomonas sp.(B4)	0.145	0.474	0.281
Bacillus sp.(B5)	0.208	0.543	0.432
Anabaena sp.(C4)	0.134	0.193	0.255
Anabaena sp.(C8)	0.165	0.285	0.339
Anabaena sp.(C12)	0.246	0.669	0.490
SEM	0.005	0.012	0.011
CD (5 %)	0.0138	0.033	0.0304

The culture filtrate and pellet amended compost of C4, C8, and C12 exhibited inhibition of most of the fungi tested (figure 1). Composts amended with B3 and B4 inhibited all the fungi tested after 10 and 20 d of incubation. The composts amended with B5 and B8 inhibited four of the fungi tested after 10 d incubation (Supplementary Table 3). Different levels of inoculum of the bacterial / cyanobacterial cultures were used to amend the composts. The strains B3, B5, C8 and C12 exhibited inhibition at 1-2% level of inoculums (Table 4). The microbial activity of the amended composts evaluated after 20 d revealed a significant enhancement over unamended compost at 1-3% level of inoculum in terms of activity of dehydrogenase, alkaline phosphatase enzymes and FDA (figure 2). Highest values were recorded in composts amended with the cyanobacterial strains C8 and C12. The activity of cellobiase, chitosanase and β 1, 3 glucanase in the amended composts also showed a significant increase over unamended compost (figure 3). In general, amendment with B5 and C12 strains exhibited highest values.

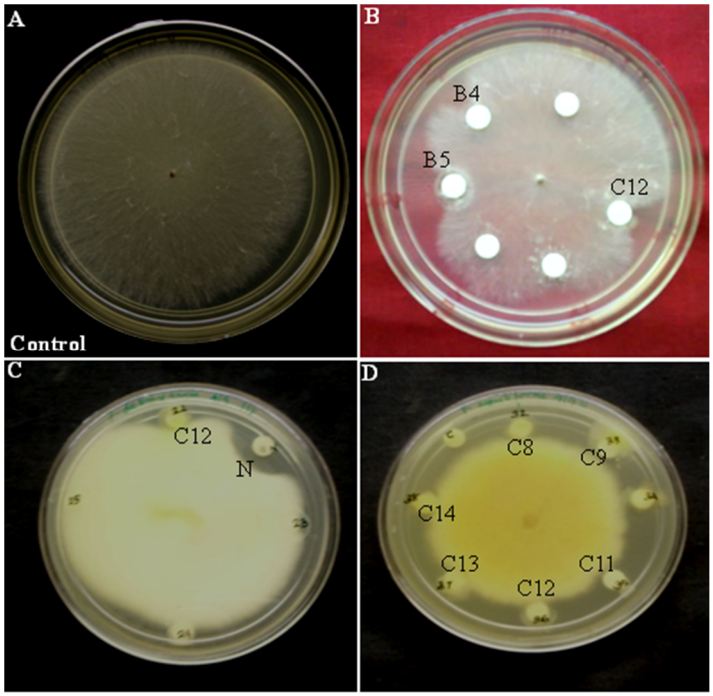
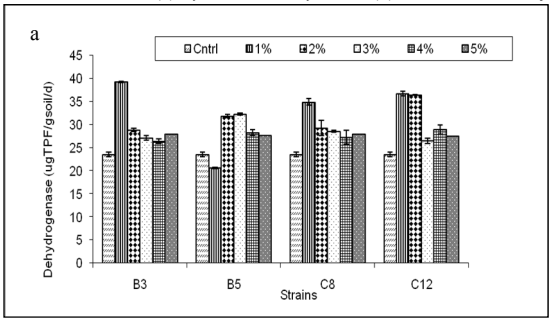


Figure 1 Zone of inhibition by leachates of bacterial / cyanobacterial cultures on the lawn of selected phytopathogenic fungi (a) *Rhizoctonia solani*-control plate (b) *Rhizoctonia solani* (c) *Pythium debaryanum* (d) *Fusarium moniliforme*



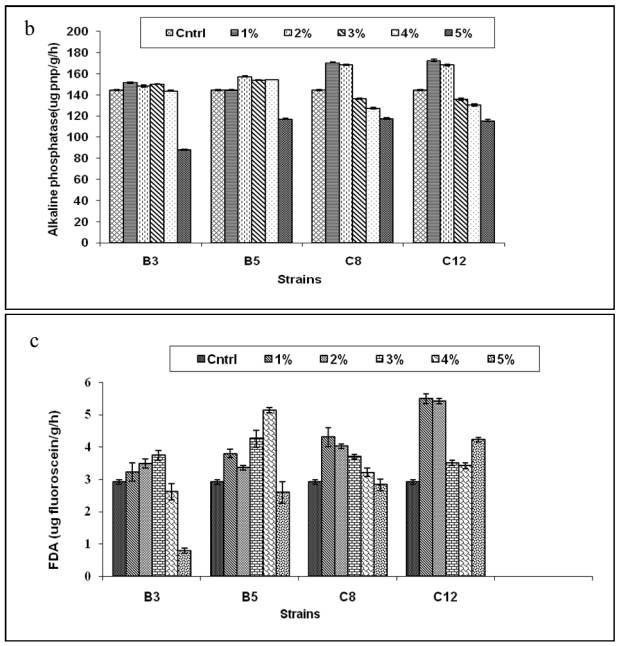


Figure 2 Microbial activity of compost inoculated with bacterial and cyanobacterial strains at different inoculum levels (1-5%)

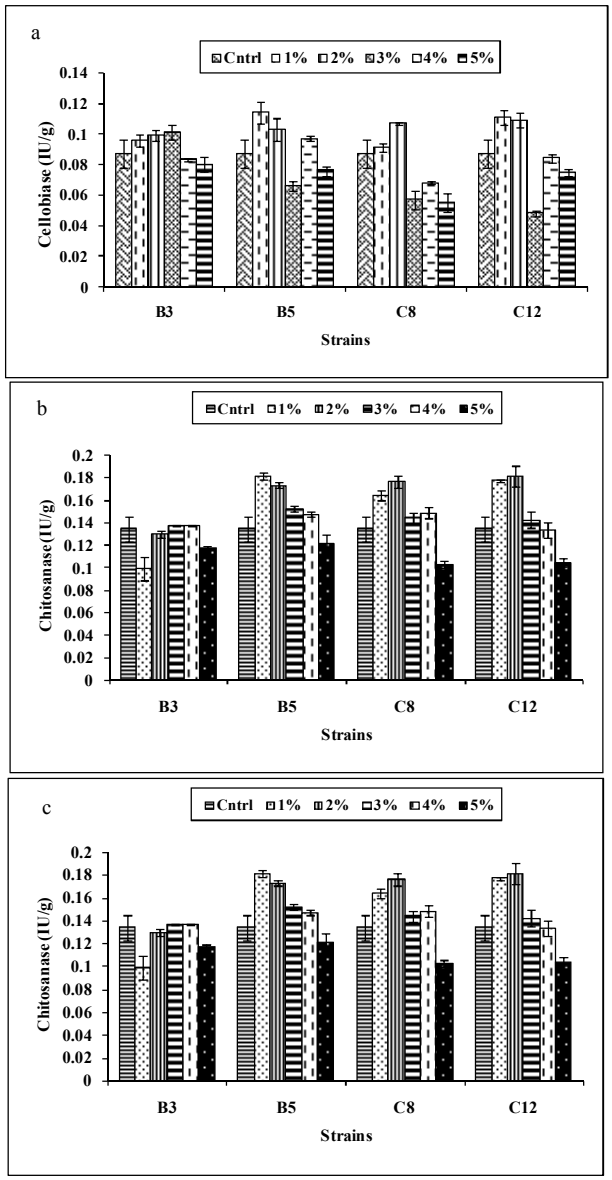


Figure 3 Activity of hydrolytic enzymes of the composts amended with the selected bacterial and cyanobacterial strains at different inoculum levels

Table 4 Effect of inoculum load on fungicidal activity of compost leachates

Strain	Inoculum rate	Zone of inhibition by compost inoculated with bacterial/cyanobacterial culture after								
		5d incubation			10d incubation			15d incubation		
		Rs	Pd	Fl	Rs	Pd	Fl	Rs	Pd	Fl
B3	1%	+	-	-	+	-	+	-	-	+
	2%	+	-	+	+	-	+	+	+	+
	3%	+	+	+	+	+	+	+	+	+
	4%	+	+	+	+	+	+	+	+	+
	5%	+	+	+	+	+	+	+	+	+
B4	1%	-	-	-	+	-	+	-	+	+
	2%	-	-	+	+	+	+	-	+	+
	3%	-	+	+	+	+	+	-	+	+
	4%	+	+	+	+	+	+	+	+	+
	5%	+	+	+	+	+	+	+	+	+
B5	1%	+	-	-	+	+	-	-	+	+
	2%	+	-	+	+	-	+	+	+	+
	3%	+	+	+	+	+	+	+	+	+
	4%	+	+	+	+	+	+	+	+	+
	5%	+	+	+	+	+	+	+	+	+
C4	1%	-	-	-	-	-	+	-	-	-
	2%	-	-	+	-	-	+	+	-	+
	3%	+	+	+	-	-	+	+	+	+
	4%	+	+	+	+	-	+	+	+	+
	5%	+	+	+	+	-	+	+	+	+
C8	1%	-	-	-	+	-	+	-	-	-
	2%	-	-	+	+	-	+	+	-	+
	3%	+	+	+	+	-	+	+	+	+
	4%	+	+	+	+	-	+	+	+	+
	5%	+	+	+	+	-	+	+	+	+
C12	1%	-	-	+	+	+	+	+	-	-
	2%	+	-	+	+	+	+	+	+	+
	3%	+	+	+	+	+	+	+	+	+
	4%	+	+	+	+	+	+	+	+	+
	5%	+	+	+	+	+	+	+	+	+
Control	Nystatin	+	+	+	+	+	+	+	+	+
	Unamended compost	-	-	-	-	-	-	-	-	-

Legend: Rs, *Rhizoctonia solani*; Pd, *Pythium debaryanum*; Fl, *Fusarium oxysporum lycopersici*

DISCUSSION

Among plant diseases, fungal infections represent more than 35%, which is a matter of serious concern to agricultural researchers and policy makers alike. Notwithstanding the importance of economically viable option of chemical fungicides, in recent years, environmental friendly options are being increasingly explored. It has been estimated that total losses as a consequence of plant diseases reach about 25% in developed countries, while in developing countries this can reach almost 50%. Management practices are known to regulate biological processes in soil and influence agricultural productivity and maintenance of soil fertility.

Microorganisms, including fungi and cyanobacteria represent a "green" option and are being included in organic farming practices, not only as biofertilizers, but also as biocontrol agents (**Kulik, 1995; Nakkeeran *et al*., 2005; Prasanna *et al*., 2010**). Although it is difficult to quantify the existence of competition among coexisting microbial populations in limiting disease development, significant levels of disease suppression has been observed in several studies (**Kulik, 1995; Hoitink and Boehm, 1999; Vallad *et al*., 2003**). Our investigation aimed at selecting a set of promising bacterial and cyanobacterial strains exhibiting fungicidal activity and evaluating the biocontrol potential of paddy straw composts fortified with effective cyanobacterial/ bacterial strains under laboratory conditions.

A set of ten bacterial cultures (B1-B10) and fourteen cyanobacterial cultures (C1-C14) were evaluated for their fungicidal activity against selected phytopathogenic fungi *viz Pythium debaryanum, Rhizoctonia solani, Fusarium oxysporum* and *Pythium aphanidermatum*. Earlier investigations have shown that most of the fungicidal compounds are secondary metabolites, hence late log or stationary phase cultures exhibit greater activity (**Kulik, 1995; Hoitink and Boehm, 1999; Vallad *et al*., 2003**). Therefore, in our study 48h old bacterial cultures were analysed for their fungicidal activity against *Pythium debaryanum, Rhizoctonia solani, Fusarium oxysporum* and *Pythium aphanidermatum*. On the basis of previous investigations, maximum fungicidal activity was observed in cyanobacterial cultures which were more than 4 weeks old (**Singh *et al*., 1999; Prasanna *et al*., 2008; Radhakrishnan *et al*., 2009**), hence 6 weeks old cyanobacterial cultures were used in this studies.

Among the cultures tested, all the bacterial strains and the culture filtrates of C1-C8 strains inhibited the growth of *Fusarium moniliforme*, while B2-B8 strains and the culture filtrates of C3, C4, C11, and C12 also inhibited *Pythium debaryanum*. Most of the biocidal compounds of the cyanobacteria are known to be extracellular in nature and the culture filtrate was therefore utilised for analyses of metabolites (**Prasanna *et al*., 2008**). However, the culture pellet being the reservoir of membrane bound toxins/ endotoxins can also to show fungicidal activity, as illustrated in the present study. Further studies are needed to evaluate pellet based biocidal compounds.

The selected set of effective cyanobacterial and bacterial cultures were used for amending the rice straw compost. The culture filtrate and pellet amended compost of C4, C8, and C12 exhibited inhibition of most of the fungi tested. As regards the performance of bacterial cultures, when amended to composts, B3 and B4 inhibited all the fungi tested after 10 and 20 d of incubation. The degree of inhibition was in general, greater after 10 d as compared to 20 d incubation. These observations prompted us to optimise the dosage level, as it may be possible that by 20 d, the amount of culture remained insufficient to inhibit the growth of fungi.

The enzymatic profiles of the selected six strains (B3, B4, B5, C4, C8 and C12) also provided interesting pointers regarding the potential of hydrolytic enzymes of these strains being responsible for fungicidal activity. Our earlier studies had shown that several *Anabaena* strains show fungicidal activity which could be correlated with the production of hydrolytic enzymes and the homologue for chitosanase was detected in two *Anabaena* strains (**Prasanna *et al*., 2008; Prasanna *et al*., 2010**). The production of hydrolytic enzymes and their role in biocontrol has been reported earlier for bacterial strains (**Singh *et al*., 1999; Grover *et al*., 2009**). On the basis of the inhibition zone studies against phytopathogenic fungi and enzymatic profile generated, three bacterial and cyanobacterial strains were selected for amending the compost at different levels.

A range of inocula levels (1-5%) were used and the observations revealed the diversity among the cultures being evaluated. The culture of B5 showed inhibition of *Rhizoctonia solani* from 1% level onwards; *Fusarium oxysporum lycopersici* and *Pythium debaryanum* were inhibited at 2 and 3% level respectively after 5 d incubation (Table 5). In general, with increase in the period of incubation, a lower level of inoculum was effective i.e. a positive correlation

between percent inoculum and fungicidal activity was observed. The strains B3, B5, C8 and C12 were found to be most promising as they exhibited inhibition at 1-2 % level of inoculum.

Analyses of microbial activity as a function of enzymes such as dehydrogenase, alkaline phosphatase are considered as functional criteria for evaluating quality of composts and significant as indicators of soil health (**Frankenberger and Dick, 1983**). The evaluation of the amended composts in our study revealed their potential in terms of enhanced microbiological activity (dehydrogenase, FDA, alkaline phosphatase) as well as the activity of hydrolytic enzymes (chitosanase, cellobiase and endoglucanase). Such indices reveal that the amendment of compost with selected microorganisms with biocontrol properties can be a useful endeavour which can lead to the development of integrated pest management strategies for environmentally sustainable agriculture.

Several researchers have highlighted the use of compost for disease supressiveness, besides their role as organic manures (**Chaturvedi *et al*., 2009; Hegde *et al*., 2005**). The extract of composts have also been employed for biocontrol and shown promise especially in Late blight control of potato (**Ghorbani *et al*., 2005**). **Marcos *et al*. (1995)** evaluated commercial compost for their effect on rhizosphere flora and emphasized that composts have the potential to protect plant against soil borne root pathogens. However, composts are known to show inconsistent supressiveness to soil borne diseases (**Hadar and Mandelbaum, 1992**). In our study, amendment of compost with bacterial and cyanobacterial strains improved their consistency and effectiveness as biocontrol agents. Further studies have been undertaken in pot experiments (**Dukare *et al*., 2011**) which have illustrated their promise and field level evaluation of the promising microbe fortified composts is in progress.

CONCLUSION

With problems associated with the use of tremendous chemicals for plant disease control receiving increasing attention due to multiple problems including environmental pollution, ecological imbalance and development of resistance in pathogens, our investigation clearly illustrates the potential of microbe (cyanobacteria/bacteria) amended compost as a suitable alternative for sustainable and safe agriculture.

Acknowledgments: The authors are thankful to the Post Graduate School and Director, Indian Agricultural Research Institute, New Delhi, India for providing for fellowship towards M.Sc. program of the senior author. The study was also partly funded by the AMAAS Network Project on Microorganisms, (Themes: Microbial Genomics and Nutrient Management) granted by the Indian Council of Agricultural Research, New Delhi. We are also grateful to the Division of Microbiology, IARI, New Delhi for providing necessary facilities for undertaking this study.

REFERENCES

CAIRE, G.Z., DE, M.S., DE CANO, M.C.Z., DE MULE, DEHALPERIN, D.R. 1990. Antimycotic products from the cyanobacterium *Nostoc muscorum* against *Rhizoctonia solani*. *Phytopathology,* 5, 1-4.

CASIDA, L.E.JR., KLEIN, D.A., SANTORO, T. 1964. Soil Dehydrogenase Activity. *Soil Science,* 98, 371-376.

CHATURVEDI, S., KUMAR, V., SATYA, S. 2008. Composting effect of *Pongamia pinnata* on tomato fertilization. *Archives of Agronomy and Soil Science*, 55, 535-546.

DESIKACHARY, T.V. 1959. Cyanophyta - ICAR Monographs on algae. Indian Council of Agricultural Research. New Delhi.

DUKARE, A.S., PRASANNA, R., DUBEY, S.C., CHAUDHARY, V., NAIN, L., SINGH, R., SAXENA, A.K. 2011. Evaluating novel microbe amended composts as biocontrol agents in tomato. *Crop Protection*, 30, 436 - 442.

FRANKENBERGER, JR.W.T., DICK, W.A. 1983. Relationship between enzyme activities and microbial growth and activity indices in soil. *Soil Science Society of America Journal*, 47, 945 –951.

GAIND, S., NAIN, L. 2010. Exploration of composted cereal waste and poultry manure for soil restoration. *Bioresource Technology*, 101, 2996-3003.

GHORBANI, R., WILCOCKSON, S., LEIFERT, C. 2005. Alternative treatment for late blight control in organic potato: antagonistic microorganisms and compost extract for activity against *Phytophthora infestans*. *Potato Research*, 48, 181-189.

GREEN, V.S., STOTT D.E., DIACK M. 2006. Assay of fluorescein diacetate hydrolytic activity-optimization of soil samples. *Soil Biol. Biochem.,* 38, 693-701.

GROVER, M., NAIN, L., SAXENA, A.K. 2009. Comparison between *Bacillus subtilis* RP24 and its antibiotic defective mutants. *World Journal of Microbiology and Biotechnol*ogy, 25, 1329-1335.

HADAR, Y., MANDELBAUM, R. 1992. Suppressive compost for biocontrol of soilborne plant pathogens. *Phytoparasitica,* 20, 113-116.

HEGDE, G.V., HIREMATH, S.M., KEREMANE, B.G. 2005. Potential of tree borne oil seed spices, *Polygamics, Madhuca, Calophyllu*m and *Simarouba*. BAIF Institute for Rural Development, Karnataka, Tiptur.

HOITINK, H.A.J. 1990. Production of disease suppressive compost and container media and microorganism culture for use therein. U.S. Patent 4, 900, 348:13.

HOITINK, H.A.J., BOEHM, M.J. 1999. Biocontrol with the context of soil microbial communities: a substrate-dependent phenomenon. *Annual Reviews of Phytopathology*, 37, 427–446.

KAUSHIK, B.D. 1987. Laboratory Methods for blue green algae. Associated Publishing Company, New Delhi.

KULIK, M.M. 1995. The potential for using cyanobacteria (blue green algae) and algae in the biological control of plant pathogenic bacteria and fungi. *European Journal of Plant Pathology*, l01, 585-599.

MACKINNEY, G. 1941. Absorption of light by chlorophyll solutions. *Journal of Biological Chemistry*, 140, 315-322.

MANJUNATH, M., PRASANNA, R., NAIN, L., DUREJA, P., SINGH, R., KUMAR, A., JAGGI, S., KAUSHIK, B.D. 2009. Biocontrol potential of cyanobacterial metabolites against damping off disease caused by *Pythium aphanidermatum* in solanaceous vegetables. *Archives of Phytopathology and Plant Protection,* 43, 666-677.

MARCOS, A., DE BRITO-ALVAREZ, M.A., GAGNE, S., ANTOUN, H. 1995. Effect of compost on rhizosphere microflora of the tomato and on the incidence of plant growth promoting rhizobacteria. *Applied and Environmental Microbiology*, 61, 194-199.

MOON, S.J., CHEN, L., MOORE, R.E., PATTERSON, G.M.L. 1992. Calophycin, a fungicidal cyclic decapeptide form the terrestrial blue green algae *Calothrix fusca*. *Journal of Organic Chemistry,* 57, 1097-1103.

NAKKEERAN, S., FERNANDO, W.G.D., SIDDIQUI Z.A. 2005. Plant growth promoting rhizobacteria formulations and its scope in commercialization for the management of pests and disease In Z.A. Siddqui, Editor, *PGPR: Biocontrol and Biofertilization*, Springer, Dordrecht, The Netherlands. p. 257-296.

NOBLE, R., COVENTRY, E. 2005. Suppression of soil-borne plant diseases with compost: a review. *Biocontrol Sciience and Technology,* 15, 3-20.

OHTAKARA, A. 1988. Chitosanase and B-N-Acetyl Hexosamine from *Pycnosporus cinnabarinus*. *Methods in Enzymology,* 168, 464-468.

PRASANNA, R., LATA-TRIPATHI, R., GUPTA, V., MIDDHA, S., JOSHI, M., ANCHA, R., KAUSHIK, B.D. 2008. Evaluation of fungicidal activity of extracellular filtrates of cyanobacteria-possible role of hydrolytic enzymes. *Journal of Basic Microbiology*, 48, 186-194.

PRASANNA, R., GUPTA, V., NATARAJAN, C., CHAUDHARY, V. 2010. Allele mining for chitosanases and microcystin-like compounds in *Anabaena* strains. *World Journal of Microbiology and Biotechnology,* 26, 717-724.

PRASANNA, R., SOOD, A., JAISWAL, P., NAYAK, S., GUPTA, V., CHAUDHARY, V., JOSHI, M., NATARAJAN, C. 2010. Rediscovering Cyanobacteria as valuable sources of bioactive compounds. *Applied Biochemistry and Microbiol*ogy, 46, 133 -147.

RADHAKRISHNAN, B., PRASANNA, R., JAISWAL, P., NAYAK, S., DUREJA, P. 2009. Modulation of biocidal activity of *Calothrix* sp. a*nd Anabaena* sp. by environmental factors. *Biologia*, 64, 881-889.

SCHUELER, C., BIALA, J., VOGTMANN, H. 1989. Antiphytopathogenic properties of biogenic wastes compost. *Agric. Ecosyst. Environ*., 27, 477- 482.

SINGH, P.P., SHIN, Y.C., PARK, C.S., CHUNG, Y.R. 1999. Biological control of *fusarium* wilt of cucumber by chitinolytic bacteria. *Phytopathology,* 89, 92-99.

STANIER, R.Y., KUNISAWA, R., MANDEL, M., COHEN-BAZIRE, G. 1971. Purification and properties of unicellular blue green algae (Order: Chroococcales). *Bacteriological Reviews,* 35, 171-305.

TABATABAI, M.A., BREMNER, J.M. 1969. Use of p-nitrophenyl phosphate for assay of soil phosphatase activity. *Soil Biology and Biochemistry*, 1, 301-307.

TEMORSHUIZEN, A.J., VAN RIJN, E., VAN DER GAAG, D.J., ALABOUVETTE, C., CHEN, Y., LGERLOF, J., MALANDRAKIS, A.A., PAPLOMATAS, E.J., RAMERT, B., RYCKEBOER, J., STEINBERG, C., ZMORA-NAHUM, S. 2006. Suppressiveness of 18 composts against 17 pathosystems: variability in pathogen response. *Soil Biology and Biochemistry,* 38, 2461-2477.

VALLAD, G.E., STONE, A., GOODMAN, R.M., STEVENSON, W.R., COOPERBAND, L.R. 2003. Paper mill sludge and compost effects on disease incidences in a vegetable rotation. University of Wisconsin-Madison. *alfi.soils.usc.edu.*

WOOD, T.M., BHAT, K.M. 1988. Methods for measurements of cellulase activity. In *Methods in Enzymology,* 160, 87-112.

YUEN, G.Y., CRAIG, L.M., KERR, E.D., STEADMAN, J.R. 1994. Influences of antagonist population levels, blossom development stage and canopy temperature on the inhibition of the *Sclerotinia sclerotiorum* on dry edible bean by *Erwinia herbicola*. *Phytopathology,* 84, 495–501.