Available online at www.scholarsresearchlibrary.com

Scholars Research Library

Archives of Applied Science Research, 2013, 5 (1):1-4
(http://scholarsresearchlibrary.com/archive.html)

ISSN 0975-508X
CODEN (USA) AASRC9

In vitro screening of antagonistic effect of soil borne bacteria on some selected phytopathogenic fungi

Tharmila Sivanantham*[1], Vasanthakala Rasaiyah[1], Nalini Satkunanathan[2] and Arulanantham Christie Thavaranjit[1]

[1]*Department of Botany, Faculty of Science, University of Jaffna, Jaffna, Sri Lanka*
[2]*Department of Mathematics and Statistics, Faculty of Science, University of Jaffna, Jaffna, Sri Lanka*

ABSTRACT

Bacteria have the ability to produce a wide variety of metabolites with antifungal capabilities. The present study was assessed in order to find out the antagonistic ability of eight different soil borne bacterial strains, Escherichia coli, Klebsiella ozaenae, Pseudomonas maltophila, Bacillus circulans, Bacillus sphaericus, Bacillus coagulans, Serratia marcescens and Streptococcus spp.against some plant pathogenic fungi , Alternaria porri, Fusarium oxysporum, Sclerotium rolfsii and Botryodiplodia theobromae which were isolated from disease specimen. All isolates were subjected to primary screening against test organisms and further carried out to secondary screening using agar disc assay and slide culture techniques. Most of the selected bacteria exhibited promising antagonistic activity against tested organisms. Out of eight bacterial species P.maltophila and B.circulans revealed effective biocontrol potential against all tested fungi. Whereas Streptococcus spp induced the vegetative growth of S.rolfsii and F.oxysporum. In slide culture techniques, P.maltophila completely inhibited the spore's germination of B.theobromae and S.rolfsii while the S.marcescens and B.circulans produced 0% of spore germination on S.rolfsii. Hence, K.ozaenae and Streptococcus spp showed least effective on F.oxysporum and B.theobromae and S.rolfsii respectively. The experimental results demonstrated the fungicidal effect of bacterial species and revealed the possibility of these bacterial species to be used as bio control agents against these fungal species.

Key words: Antagonism, phytopathogenic fungi, soil borne bacteria.

INTRODUCTION

Fungal plant pathogens are the most important factors that cause serious losses to agricultural products every year. Therefore they have to be controlled to ensure the plant products quantitatively and qualitatively. Fungicides are commonly used to control the diseases in plants. Frequent uses of these chemicals are hazardous to humans and environment (1) and leads to environmental pollution. It is always better to adopt biological method as an alternative disease control method in order to reduce the hazards, which is also ecology conscious and eco-friendly.

Antifungal agents produced by microorganisms may be used as biocontrol agents.Some soil borne fungi, bacteria and actinomycetes have been identified and used as antagonistic microbes. A number of bacterial species have been tested as biocontrol agents. Antifungal metabolites produce by bacteria like *Pseudomonas* spp , *Bacillus* spp(2-5), *Serratia* spp (6)have been well documented for their antifungal activity. For instance, *Pseudomonas fluorescens* used against *Rhizoctonia* and *Pythium* damping off of cotton and *Bacillus subtilis* used for seed treatment(7) and *Serratia marcescens* used for growth inhibition of phytopathogens. The mechanisms underlying these bacterial antagonisms for plant pathogens involve antibiosis, competition for nutrients or space, enhancement of root and plant development, induction of plant resistance and/or inactivation of the pathogen's enzymes(8). Antibiosis, in particular, is the most important mechanism for control of plant disease. The present study was carried out to

evaluate the biological potential of eight different soil borne bacterial isolates against four different phytopathogenic fungi.

MATERIALS AND METHODS

Fungal and Bacterial isolates

Eight different species of soil borne bacteria, *Escherichia coli, Klebsiella ozaenae, Pseudomonas maltophila, Bacillus circulans, Bacillus sphaericus, Bacillus coagulans, Streptococcus* sp, *Serratia marcescens* were obtained from culture collection, Department of Botany, University of Jaffna, Sri Lanka. The plant pathogenic fungi, *Botryodiplodia theobromae, Alternaria porri, Fusarium oxysporum* and *Sclerotium rolfsii* were isolated from stem end rot of mango, purple blotch disease portion of onion and wilt disease of tomato, respectively.These were identified based on its vegetative and reproductive structures. Bacterial and fungal isolates were maintained on nutrient agar and Potato dextrose agar (PDA) slants respectively at 4^{o} C until used for further study.

Antagonistic assay

a) Preliminary test

Four different bacterial species were streaked as thick bands on four opposite edges on the PDA plates. Then 4 mm diameter disc of tested fungus was cut from of an actively growing culture by a sterile cork borer and placed onto the center of above PDA plates. The Petri dishes were sealed by parafilm and incubated at room temperature in dark for 2-3 days. Where mycelia disc on PDA medium without bacteria was maintained as control. The above procedure was carried out to eight soil borne bacteria and four selected fungi separately, and antagonistic effect showed by bacteria was noted as strong, moderate, weak and no effect (9).The experiment was conducted in three replicates.

b) Agar disc method.

0.1 ml of the test bacterial suspension (10^8 CFU/ml) was transferred to the center of the PDA plate using sterile pipette and spread by sterile glass spreader separately. Then 4 mm diameter of each mycelia disc was cut using a sterile cork borer and placed in the center of the above PDA plate separately under aseptic condition. Mycelia disc on PDA medium without bacteria was used as control. The cultures were incubated at room temperature in dark for 3-5 days and diameter of the fungal mycelia growth was measured(10). The experiments were carried out thrice.

c) Slide culture method

Spores suspension was prepared from 7-10 days of old culture of fungus separately and the numbers of fungal spores were counted by using haemocytometer. Likewise bacterial suspensions were prepared and their concentrations were adjusted to 10^8 CFU/ml by dilution technique. After that, by using micropipette, 25 µl of fungal spores suspension was mixed with the 25µl of the bacterial spores suspension on cavity slides. Then, cavity slide was placed in moist chamber made by placing sterile filter paper in the Petri dishes and incubated at room temperature for overnight. Spores mixed in sterile distilled water was used as control, percentage of germination was obtained using the formula(11).

$$\text{Percentage of germination} = \frac{\text{Number of germinated spores}}{\text{Total number of spores}} \times 100$$

Percentage of inhibition= 100 **–** **(**Percentage of germination)

Statistical analysis

The results for the antifungal activity were subjected to examine by using analysis of variance and Tukey test at P=0.05 using software SPSS Windows version 13.0.

RESULTS AND DISCUSSION

The results of preliminary test demonstrated that both *P.maltophila* and *B.circulans* had strong antagonism while *B.sphaericus* and *B.coagulans* showed the average. In addition to those, *K.ozaenae* clearly showed the weak inhibition among all tested fungi. On the other hand *Streptococcus* spp had no effect on all of the tested organisms. Furthermore, *E.coli* resulted average inhibition against *A.porri, B.theobromae* and *F.oxysporum* and it showed weak inhibition against *S.rolfsii.* According to the results, *A.porri* was the one which was highly inhibited by most of the soil borne bacteria (Table 1).

Table 1: Effect of soil borne bacteria on selected fungi in preliminary test.

Bacteria	Degree of antagonism on fungal vegetative growth			
	A.porri	***B.theobromae***	***S.rolfsii***	***F.oxysporum***
Pseudomonas maltophila	+++	+++	+++	+++
Bacillus circulans	+++	+++	+++	+++
Bacillus sphaericus	++	++	++	++
Escherichia coli	++	++	+	++
Serratia marcescens	+++	++	++	+
Streptococcus spp.	-	-	-	-
Klebsiella ozaena	+	+	+	+
Bacillus coagulans	++	++	++	++
Control	-	-	-	-

No inhibition: - (Fungal growth was similar to that of control)
Weak inhibition: + (Fungal growth was slightly inhibited by bacteria)
Average inhibition: ++ (Loosely arranged mycelial growth over the bacterial zone)
Strong inhibition: +++ (Fungal growth was completely inhibited before the bacterial zone)

Table 2: Effect of soil borne bacteria on selected fungal vegetative growth by agar disc method.

Bacteria	Mean diameter of fungal mycelia growth (cm)			
	A.porri	***S.rolfsii***	***B.theobromae***	***F.oxysporum***
P.maltophila	0.4 ± 0.05^{i}	2.17 ± 0.06^{f}	0.39 ± 0.03^{h}	0.42 ± 0.03^{f}
S.marcescens	3.76 ± 0.02^{f}	7.95 ± 0.05^{a}	4.85 ± 0.05^{e}	2.02 ± 0.03^{c}
E.coli	3.57 ± 0.06^{g}	3.45 ± 0.05^{e}	5.62 ± 0.03^{c}	1.86 ± 0.02^{cd}
K.ozaenae	4.65 ± 0.01^{e}	7.56 ± 0.02^{b}	5.66 ± 0.02^{c}	2.57 ± 0.02^{b}
B.sphaericus	6.47 ± 0.02^{c}	7.82 ± 0.22^{a}	5.33 ± 0.03^{d}	1.75 ± 0.05^{d}
B.coagulans	5.68 ± 0.03^{d}	6.07 ± 0.06^{c}	4.63 ± 0.03^{f}	1.90 ± 0.05^{cd}
B.circulans	2.68 ± 0.01^{h}	5.22 ± 0.03^{d}	2.13 ± 0.01^{g}	1.31 ± 0.01^{e}
Streptococcus sp	6.72 ± 0.08^{b}	7.94 ± 0.06^{a}	6.52 ± 0.02^{b}	3.42 ± 0.01^{a}
Control	8^{a}	8.02 ± 0.08^{a}	7.85 ± 0.05^{a}	2.72 ± 0.19^{b}

Values are mean±SD. Values with different superscript on the same column show significant (P<0.05) difference.

The present study showed that most of the tested bacterial strains exhibited varying degree of antagonistic effect against all pathogenic fungi. In this trial, *Pseudomonas maltophila* showed highest antifungal activity against all tested fungi cultivated in PDA medium, it may be due to the production and secretion of antifungal compounds that was able to reduce the growth of fungi. Former study have investigated the antifungal potential of *Pseudomonas flourescence* againstpathogenic fungi, *Alternaria cajani*, *Curvularia lunata*, *Fusarium sp.*, *Bipolaris sp.* and *Helminthosporium sp*(12). Hence, Rakh studied the highest antagonistic activity of *P.monteilii* on *S.rolfsii* by producing diffusible antibiotic, volatile metabolites, hydrogen cyanide and siderophore(13). Furthermore, our study also showed that the mycelial growth of *B.theobromae* was dramatically reduced by the *P.maltophila* which was followed by *B.circulans*, *B.coagulans*, *S.marcescens* and *B.sphaericus* respectively. And also *E.coli* and *K.ozaenae* exhibited equal antagonistic effect on it. Previously, similar growth inhibitory ability of *Bacilli* species, *Bacillus subtilis* and *B. polymyxa* in *in vitro* have been reported against wood decaying fungi (14). Yadav has also reported cytosolic proteins of *Escherichia coli* are responsible for antifungal potential against pathogenic strains of *Aspergillus fumigatus*, *A. flavus*, *A. niger* and *Candida albicans* (15). Another study also mentioned that the mycelia growth of many species of *Aspergillus,Penicillium and Fusarium* were inhibited by the antifungal potential of *Bacillus* sp, *Pseudomonas* sp and *Streptococcus* sp(16-17). In case of *Streptococcus* sp exhibited least antagonistic activity against *A.porri* and *B.theobromae* compared to other bacterial strains. At the same time , although there was no significant difference between *Streptococcus* spp and control on the mycelia growth of *S.rolfsii*, the result showed the significant (P<0.05) difference on the growth of *F.oxysporum* where the growth of *F.oxysporum* on *Streptococcus* treated PDA medium was noticed to be higher than the growth on control plate (Table2). Besides this, the results obtained from this study demonstrated that there was a significant difference (P<0.05) between most of the bacterial antagonistic activity against each and every fungus. However, some of them such as *S.marcescens*, *B.sphaericus* and *Streptococcus* sp. didn't show any significant difference among the antagonistic activity on *S.rolfsii* whereas the fungi revealed approximately equal growth on the above bacteria treated PDA plate as well as on control plate(Table2). In the case of *F.oxysporum*, the bacterial species, *Streptococcus* greatly induced the growth of the above fungi while the *B.circulans* significantly reduced the growth of the fungi next to *P.maltophila*.(Table2).

From the conducted studies, *P. maltophila* showed completely (0%) inhibitory effect on spores germination of *B.theobromae* and *S.rolfsii*. Whereas *B.circulans* and *S.marcescens* also had 0% of inhibitory effect only on spores germination of *S.rolfsii*. On the other hand least inhibitory effect on spores germination of both *B.theobromae* and *S.rolfsii* were revealed by *Streptococcus* sp. While the germination of *F.oxysporum* was induced by the *K.ozaenae* only, the *P.maltophila* hugely inhibited the germination of the fungi which was followed by the *B.sphaericus*, *B.coagulans* and *S.marcescens* respectively (Table3).

Table 3: Effect of soil borne bacteria on fungal spores germination in slide culture method.

Bacteria	Mean percentage of fungal spores germination		
	F.oxysporum	***B.theobromae***	***S.rolfsii***
P.maltophila	15.51±0.02^{i}	0	0
S.marcescens	37.05±0.09^{f}	29.48±0.06^{f}	0
E.coli	67.72±0.02^{b}	69.50±0.02^{c}	62.17±0.29^{b}
K.ozaenae	73.60±0.02^{a}	63.89±0.03^{d}	50^{c}
B.sphaericus	31.98±0.03^{h}	24.17±0.29^{g}	12.30±0.44^{e}
B.coagulans	35.31±0.9^{g}	29.97±0.06^{e}	37.33±0.29^{d}
B.circulans	48.39±0.04^{d}	7.02±0.03^{h}	0
Streptococcus sp	38.57±0.24^{e}	72.09±0.10^{b}	62.33±0.29^{b}
Control	51.03±0.06^{c}	75.90±0.02^{a}	100^{a}

Values are mean±SD. Values with different superscript on the same column show significant (P<0.05) difference.

Previous experimental results indicated that *Pseudomonas* sp produces antibiotics such as HCN, phycocyanin, pyrolnitrin and pseudomonic acid which are responsible for the inhibition of fungal spores germination (11). Another study also mentioned that *Bacillus* sp also able to produce antibiotics, bacilysin, iturin and mycosubtilin and siderophores which are suppressing fungal spores germination (18). And also *Serratia marcescens* has an antagonistic activity through antibiosis (19).

Moreover, in the case of spore germination of *S.rolfsii*, the bacterial species, *E.coli* and *Streptococcus* sp had no any significant difference among them which are responsible for the induction of spore's germination of above fungi.

CONCLUSION

P.*maltophila* and *B.circulans* had more antagonistic effect on vegetative growth of most of the tested fungi. *P.maltophila* showed high inhibitory effect on fungal spore germination among the tested fungi and least effect was shown by *Streptococcus* spp. *P. maltophila*, *B.circulans* and *S.marcescens* showed 100% inhibition of spore germination on *S.rolfsii*. Therefore *P.maltophila* and *B.circulans* could be used as bio control agents against the phytopathogenic fungi. Furthermore, the feasibility of plant disease management can be confirmed in field studies by using these microbes.

REFERENCES

[1] RJ Cook ;K.Baker. *American phytopathological society*, **1983**,539.
[2] C Moita; SS Feio; L Nunes;MJM Curto ;JC Roseiro. *International Biodeterioration & Biodegradation*, **2005**, 55, 261-269.
[3] S Siddiqui; ZA Siddiqui; I Ahmad. *World J. Microbiol. & Biotechnol*, **2005**,21, 729-732.
[4] J Nourozian; HR Etebarian;G. Khodakaramian. *BiologicalSci. and Technol*, **2006**, 28, 29-38.
[5] E Christy Jeyaseelan; S Tharmila ; K Niranjan. *Archives of Applied Science Research*, **2012**,4(4),1623-1627.
[6] A Ordentlich; Y Elad;I Chet. *Phytopathology*, **1988**, *78*, 84–87.
[7] GN Agrios. Plant pathology, 5th ed., Elsevier Academic Press, Oxford, UK,**2005**.
[8] GE Harman; *Plant Disease*, **2000**, 84,377-393.
[9] WD Boer; PJAK Gunnewiek; P Lafeber; JD Janse; BE Spit; JW woldendrop. *Soil biology and Biochemistry* ,**1998**, 30, 193-203.
[10] SM Matar; EL Kazzar; EE Wagih; HE Moustafa. *Microbiology* , **2009**,8,53-61.
[11] R Srivastava. *The international journal of Microbiology*, **2009**,7, 2.
[12] R Srivastava; Shalni. *Electronic J. Environmental,Agriculture and Food Chemistry*, **2008**,7, 2789-2796.
[13] RR Rakh; SL Raut; MS Dalvi; VA Manwar. *Microbiology*,**2011**,3,26-34.
[14] P Melent'ev;L Helisto, Yu Kuz'mina; NFGE Aktuganov; T Korpela. *Applied Biochemistry and Microbiol*, **2006**, 42,1, 70-75.
[15] V Yadav;R Mandhan; Q Pasha; S Pasha; A Katyal; AK Chhillar; J Gupta; R Dabur GL Sharma. *J. Med. Microbiol*, **2007** ,56,637-644.
[16] J Nourozian; HR Etebarian ; G Khodakaramian. *Songklanakarin J. Sci. and Technol*, **2006**, 28, 29-38.
[17] C Munimbazi; LB Bullerman. *J. Appl. Microbiol*, **1998**,84, 959-968.
[18] M Shoda. *J. Bioscience and Bioengineering*, 2000,89, 515-521.
[19] J Braz . *Microbiology*. **2006**,37,1517