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In vitro antagonism of rhizospheric fluorescent pseudomonads of coconut against *Ganoderma applanatum* and *Thielaviopsis paradoxa*, fungal pathogens of coconut

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Abstract

A total of 156 fluorescent pseudomonads were tested for antagonistic activity towards *G. applanatum* and *T. paradoxa* in a dual-plate assay on King's B agar plates. Eight percent of the total fluorescent pseudomonads showed antagonism towards *G. applanatum* (inhibition ranging from 39% to 73%) and 16% of the isolates inhibited *T. paradoxa* in the range of 20% - 76%. Moreover, eight percent of the fluorescent pseudomonads significantly inhibited both pathogens tested. They were found to possess some important biocontrol traits such as HCN, siderophores, ammonia and antibiotics. Among them fluorescent *Pseudomonas* spp. KiSF 17 and KiSF 16 exhibited strong antagonistic activity. They were identified as *P. aeruginosa* by Biolog® GEN III microplate identification system and confirmed by 16S rDNA sequencing. The present studies revealed the survival of useful bacterial antagonists in the rhizosphere of coconut palm, which have the potential to be used as a biocontrol agent.

Keywords: Antagonistic activity, coconut, fluorescent pseudomonads, *Ganoderma applanatum*, *Thielaviopsis paradoxa*

Introduction

Ganoderma applanatum and *Thielaviopsis paradoxa* are two fungal phytopathogens commonly present in soil. These find entry into coconut stem through growth cracks present on the stem or roots and result in disease condition. *Ganoderma* spp. causes basal stem rot in coconut leading to low productivity and death of palms. It also infects crops such as betel vine, tea and trees including *Acacia*, *Albizia*, *Mesua ferrea*, *Dalbergia* and *Grewia tiliifolia* (Bhaskaran, 2000). Stem bleeding disease in coconut is caused by *Thielaviopsis paradoxa,* which is also a serious disease in other palm species like *Areca catechu*, *Elaeis guineensis*, *Hyophorbe lagenicaulis*, *Phoenix africanus*, *Rhapis* sp., *Roystonea elata*, *Sabal palmetto*, *Syagus romanzoffiana* and *Washingtonia filifera* (Soytong *et al*., 2005). There has been success in the control of soil-borne pathogens by application of fungicides (Haas and Defago, 2005). However, it is desirable to integrate biological control agents into disease management programmes to minimize the environmental impact of agrochemicals. Many studies have reported antagonistic activity of fungi and bacteria against phytopathogens, and this is considered as a very appealing alternative to the use of chemical fungicides (Welbaum *et al*., 2004). Studies have been conducted for the biological control of basal stem rot disease and stem bleeding diseases of coconut using *Trichoderma* spp. as antagonists (Srinivasulu and Rao, 2007). *In vitro* antagonistic activity of rhizospheric and endophytic *Bacillus* spp. of coconut against *Ganoderma applanatum* and *Thielaviopsis paradoxa* was earlier reported (Priya *et al*., 2011). Endophytic bacteria from the roots of coconut were reported as antagonist against *Ganoderma* sp. which effectively inhibited 40% of mycelial growth (Rajendran *et al*., 2007).

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Fluorescent *Pseudomonas* spp. are common among the plant growth promoting rhizobacteria in the rhizosphere of many plants. Their ubiquitous nature, root colonizing capability, production of various antibiotics, which confer a competitive advantage and microbial fitness to survive in most environments are well documented (Haas and Keel, 2003). Hence, the screening of fluorescent pseudomonads for their development as biocontrol agents has great significance. In the present study, 156 fluorescent *Pseudomonas* spp. were evaluated for their antagonistic potential against two coconut fungal pathogens, *Ganoderma applanatum* and *Thielaviopsis paradoxa* with an objective to develop a potent antagonist against these pathogens. An attempt was also made to elucidate the biocontrol mechanisms like production of antibiotics, siderophores, chitinase and volatile metabolites involved in the antagonism.

Materials and methods

Collection of rhizospheric soil samples

Rhizospheric soil samples of yielding, healthy coconut palms were collected from different locations (five samples from each location) in Kerala, Karnataka, Tamil Nadu, Andhra Pradesh and Maharashtra states of India.

Isolation and identification of fluorescent *Pseudomonas* spp.

Ten gram of the collected rhizosphere soil samples were each added to 90 ml of sterile water blanks and shaken for 20 minutes at 180 rpm at 30°C in an incubator shaker (INNOVA 4335, USA). Ten-fold dilutions were made and 100 µL of the suspension from 10^{-1} and 10^{-2} dilution were spread plated on to King's B (KB) agar (King *et al*., 1954) and S2 agar (Gould *et al.*, 1985) and incubated at 30°C for 24 h. KB agar is a differential media and S2 agar is a selective media for fluorescent *Pseudomonas* spp. from other bacterial isolates. They were identified up to genus level by performing standard tests such as gram staining, oxidase test and arginine dihydrolase test. The isolated fluorescent *Pseudomonas* spp were maintained on KB agar slants stored at 4°C and one set was maintained at -22°C in KB broth with 15% glycerol.

Isolation and identification of fungal pathogens

Thielaviopsis paradoxa and *Ganoderma applanatum* were isolated from diseased portions of the stem (stem bleeding) and roots (basal stem rot) of coconut palm. Pathogens were identified by morphological, cultural characteristics and pathogenicity tests (Bhaskaran *et al*., 1994). These pathogens were grown and stored in potato dextrose agar (PDA) medium.

Selection of antagonists

Dual culture assay was followed for the primary screening of isolates against *Thielaviopsis paradoxa* and *Ganoderma applanatum.* Fungal cultures were grown on PDA plates and fluorescent pseudomonad isolates were raised in KB broth. Twenty four hour grown rhizobacterial isolates were applied towards the periphery of the KB agar plates as streaks and 2 days grown fungal discs were placed at the centre of the plate concurrently. Control plate was kept without bacterial inoculation. All the plates were incubated at 30°C. After 72 h incubation, radial growth of fungus was measured and percent inhibition over control was calculated by the formula Kr – ri / Kr x 100, where Kr is the radius of the control pathogen growth and 'ri' is the radius of the pathogen's growth towards the antagonist (Skidmore and Dickinson, 1976).

Characterization of potent antagonists

The potent antagonists were characterized for the production of siderophores, antibiotics, ammonia and HCN.

Production of siderophores: The CAS assay of Schwyn and Neilands (1987) was followed for the detection of siderophores. CAS agar was prepared from three solutions like Fe-CAS indicator solution, basal agar medium and 50% glucose solution. Isolates were spotted on CAS agar plates and observed for the development of orange halo around the colony.

Production of antibiotics: The ability of isolates to produce antibiotics was detected by agar well technique (Fuhrmann, 1994). Lawns were initiated by seeding the agar medium (TSA) with soil dilutions (1:10). Test culture supernatants were pipetted out to the wells on TSA plates and were incubated for 24 hrs. Sterile water served as control. Presence of inhibition zone around the well was observed and measured.

Production of ammonia: It was qualitatively detected by the method given by Cappuccino and Sherman (1992). About 30 µl of each sample was placed in cavity slides and one drop of Nessler's reagent (Merck) was added. The development of orange black precipitate was the indication of positive reaction.

Production of HCN: Bakker and Schipper's (1987) method was followed for testing HCN production by looking for colour change of filter paper, soaked in 2% sodium carbonate and 0.5% picric acid, from yellow to orange.

Production of chitinase: Chitinase activity was tested as given by Renwick *et al*. (1991) in a defined medium containing colloidal chitin as carbon source. Colloidal chitin was prepared freshly according to the procedure of Shimahara and Takiguchi (1988). Isolates were spotted and incubated at 30°C for 3 days. Presence of clearing zone was recorded and measured.

Identification of the potent antagonists by Biolog system

The potent antagonists (*Pseudomonas* sp. KiSF 16 and KiSF 17) were identified by Biolog® GEN III microplate identification system (Biolog, Hayward, CA, USA), which provided 94 phenotypic tests (71 carbon source utilization assays and 23 chemical sensitivity assays).

The test organism was inoculated on Biolog universal growth agar (BUGA) and incubated for 24 h at 33^0C. Cultures were transferred to inoculating fluid A (IFA) using Biolog inoculator and the inoculum density was adjusted to 98% T using Biolog turbidimeter. Cell suspension was then aseptically transferred in to a multi channel pipette reservoir. Using multi channel pipette, cell suspension was inoculated into Biolog Gen III microplates and incubated at 33^0C. The optical density at 590 nm produced from the reduction of tetrazolium violet in each well was read after 24 h using a Biolog microplate reader (version 5.1.1) in conjunction with the Microlog software. An identification was attained when it compared the pattern formed in the well with possible patterns in the database (Microstation/ Microlog Version 5.1.1). A species ID was called if the SIM and DIST values were >0.5 and <5.0, respectively.

16S rDNA sequence analysis of selected isolates

Genomic DNA of *Pseudomonas* sp. KiSF 16 and *Pseudomonas* sp. KiSF 17 were isolated by GenElute bacterial genomic DNA Kit (Sigma, USA) as per manufacturer's instructions. Bacterial 16S rRNA genes were amplified from all DNA samples by using the universal 16S rRNA gene primers F27 and R1492 (James, 2010). The amplifications were performed using Eppendorf Mastercycler gradient (Eppendorf, Germany). The reaction conditions included an initial denaturation of 3 min at 95°C followed by 30 cycles of 1 min at 94°C, 30 s at 55°C and 45 sec at 72°C with a final extension of 10 min at 72°C and then the samples were cooled to 4°C. Amplified products were resolved by electrophoresis on 0.8% agarose gels and the expected band of 1.5 kb was observed.

PCR amplicons were purified with a PCR purification kit (HiPurA PCR product purification spin kit, Himedia) to remove contaminants and sequenced using BDT v3.1 cycle sequencing kit (3730 x l96 capillary analyzer, Applied Biosystems). Forward and reverse sequences data were analysed using CodonCode Aligner software and consensus sequences of 1400 to 1450 bp of 16S rRNA gene were generated. These 16S rRNA gene sequences were used to carry out BLAST with the nr-database of NCBI genbank database.

Results and discussion

The populations of fluorescent *Pseudomonas* ranged from 3.18 to 4.56 log cfu g^{-1} soil (Table 1). From these soil samples, a total of 156 isolates were randomly selected for testing their antagonistic activity against the soil-borne pathogens *G. applanatum* and *T. paradoxa* on KB agar. The numbers of strains isolated from each site are given in Table 1. The isolates were labeled based on the place from where the soils were collected. All isolates were gram negative, oxidase positive, produced arginine dihydrolase and fluoresced under UV light indicating that they were all saprophytic fluorescent pseudomonads (Charigkapakorn and Sivasithamparam, 1987).

Antagonistic activity of these isolates was tested *in vitro* against the soil-borne pathogens *G. applanatum* and *T. paradoxa* on KB agar.

Table 1. Population, number of isolates selected and number of antagonistic fluorescent *Pseudomonas* obtained from the rhizosphere of coconut

State	Location (Districts/places)	Population of fluorescent-pseudomonads (log cfu/g soil)	Number of fluorescent pseudomonads isolated	Number of antagonists against	
				Ganoderma applanatum	*Thielaviopsis paradoxa*
Kerala	Kasaragod / HDMSCS, CPCRI	3.28	15	0	0
	Kasaragod / Kunnamkai	4.52	16	0	2
	Palakkad / Vadakkenchery	3.90	10	0	0
	Alappuzha / Chengannur	3.90	7	0	1
	Ernakulam / Thoppumpady	4.23	12	1	1
Tamil Nadu	Coimbatore / Vedapally, Veerakeralam, Nambianpalayam	3.20	2	0	0
	Pollachi / Aliyanagar	3.45	9	3	3
Karnataka	Tumkur / Siddapura	4.56	27	1	2
	Dakhshina Kannada / CPCRI, Kidu	3.70	33	5	10
Andhra Pradesh	East Godavari / Ambajipetta	4.26	9	1	3
Maharashtra	Ratnagiri / Bhatye	3.18	16	2	3
	Total number of isolates		**156**	**13**	**25**

Antagonists were evaluated based on the mycelial growth reduction and production of inhibition zones. Inhibition was clearly discerned by limited or suppressed growth of fungal mycelium in the area where the isolates and fungal pathogens contacted. Some produced inhibition zones between antagonists and pathogens (Fig. 1). Majority of the isolates tested did not show any antagonistic activity. A total of 25 from 156 isolates (16%) displayed antagonistic activity towards *Thielaviopsis paradoxa* and 13 from 156 isolates (8%) were active antagonistic bacteria towards *G. applanatum* on KB agar. It is reported that, in plant disease management programme, the use of a rapid method for screening efficient biocontrol agents is a prerequisite (Anith *et al.*, 2003). Among the 16% of the isolates that inhibited *Thielaviopsis paradoxa*, antagonistic activity was in the range of 20-76% mycelial growth inhibition. The maximum percent inhibition of 76% was showed by *Pseudomonas* spp. KiSF 17 and KiSF 16 folllowed by *Pseudomonas* sp. KiSF 21 (74%) isolated from rhizospheric soil collected from coconut palm growing at Kidu, Karnataka. Whilst the percent inhibition of fluorescent pseudomonads against *G. applanatum* ranged from 31% to 73%. The highest antagonistic activity against *Ganoderma applanatum* (73%) was found with the same isolate *Pseudomonas* sp. KiSF 17 followed by *Pseudomonas* sp. KiSF 16 (66%) from Kidu, Karnataka. The presence of antagonists supports earlier observations that agricultural soils possess some ability to suppress the activity of soil-borne plant pathogens due to the presence and activity of soil microorganisms (Adesina *et al.*, 2007). Antagonistic activity against *G. applanatum* and *T. paradoxa* was distributed among isolates obtained from all five states (Table 1). Higher percentage of antagonists was obtainted in rhizospheric soil of Tamil Nadu. About 27% (3 of the 11 isolates) of the Tamil Nadu isolates inhibited the growth of both pathogens, while it was lower among the isolates from Kerala. Out of 60 isolates, only one was found

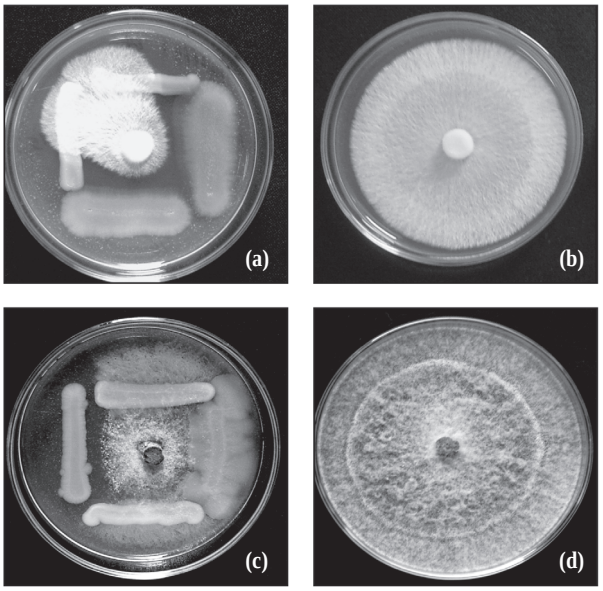

Fig. 1. *In vitro* antagonism of fluorescent pseudomonads against *G. applanatum* and *T. paradoxa* {(a) fluorescent pseudomonads against *G. applanatum* (b) *G. applanatum*, (c) Fluorescent pseudomonads against *T. paradoxa*, (d) *T. paradoxa*}

to be antagonist against *G. applanatum* and four against *Thielaviopsis paradoxa* (Table 1).

In our study, 13 (8%) fluorescent pseudomonads clearly inhibited the mycelial growth of both the pathogens (Table 2). *Pseudomonas* spp. KiSF 16 and KiSF 17 were found to be the most effective antagonists against both the pathogens tested. All of the isolates that exhibited antagonism against *G. applanatum* were also found to be antagonists of *T. paradoxa*. 12 more isolates were found to be antagonists against *T. paradoxa* but not inhibited *G. applanatum*.

the orange halo around the colony (Table 2). Pseudomonads are known to suppress soil-borne fungal pathogens by producing antifungal metabolites such as pyoluteorin, pyrrolnitrin, phenazines and 2, 4-diacetyl phloroglucinol. *Pseudomonas* spp. can indirectly suppress fungal pathogens by scavenging iron in the rhizosphere environment through the release of siderophores (Dwivedi and Johri, 2003). HCN production was detected in 3 of the isolates tested. These isolates changed the yellow colour of the filter paper to orange colour after 2 days of incubation. All the

Table 2. Antagonistic activity in terms of percent inhibition and mechanisms showed by 13 fluorescent *Pseudomonas* spp.

Isolates	% inhibition on KB		Siderophore (mm)	Antibiotics (mm)*	Ammoni-fication	HCN	Chitinase (mm)
	GA	TP					
Pseudomonas sp. RSF259	45	62	12	3	M	-	-
Pseudomonas sp. RSF 261	39	64	10	-	M	-	-
Pseudomonas sp. ASF 285	45	49	12	1	L	-	-
Pseudomonas sp. ESF 168	39	44	18	1	L	-	-
Pseudomonas sp. TSF 7	43	56	16	3	M	-	-
Pseudomonas sp. PoSF 313	43	51	18	3	M	-	-
Pseudomonas sp. PoSF 314	40	47	20	3	M	-	-
Pseudomonas sp. PoSF 315	40	51	-	2	M	-	-
Pseudomonas sp. KiSF12	34	49	16	-	L	+	-
Pseudomonas sp. KiSF 13	46	49	16	-	H	-	12
Pseudomonas sp. KiSF 16	66	76	18	4	H	+	-
Pseudomonas sp. KiSF 17	73	76	30	4	H	-	-
Pseudomonas sp. KiSF 28	31	56	18	1	H	+	-

GA - *Ganoderma applanatum*, TP - *Thielaviopsis paradoxa*, L - low, M - medium, H - high, + positive, - negative, mm- diameter in millimeter, *inhibition zone size to the periphery of the well in mm

In most of the antagonists, no physical contact with the fungi was observed; moreover, an inhibitory halo was observed suggesting the presence of fungistatic metabolites secreted by the bacteria. Studies on antifungal metabolites by these isolates revealed that 10 out of 13 efficient antagonists were antibiotic producers (Table 2) and twelve out of the 13 isolates produced siderophores. The potent antagonists *Pseudomonas* spp. KiSF 16 and KiSF 17 exhibited a maximum inhibition zone (4mm around the well) during antibiotic production test. This was one of the major mechanisms that might be involved in the antagonism of these isolates against the phytopathogens. It has previously been indicated that antibiosis is the general mode of antagonism observed for *Pseudomonas* spp. (Dwivedi and Johri, 2003). *Pseudomonas* sp. KiSF 17 showed the maximum siderophore production as evidenced by

tested isolates were found to be ammonifiers. The important role of volatile compounds like ammonia and HCN in biocontrol was reported by Brimecombe *et al*. (2001). Chitinase production was detected only in one (*Pseudomonas* sp. KiSF 13) of the isolates tested. Disease suppression mostly results from the simultaneous implementation of multiple mechanisms exhibited by one or several biocontrol agents (Francis *et al*., 2010).

In vitro screening was very helpful as a first filter to identify potential biocontrol agents from a collection of rhizospheric fluorescent pseudomonads. Some fluorescent pseudomonads from the rhizospheric soil of coconut exhibited antagonistic activity against fungal pathogens. The production of antibiotics, siderophores and ammonia by most of the tested isolates could be considered as the mechanisms involved in the inhibition of

Table 3. Identification of the most efficient antagonists

Isolate	Conventional identification*	Biolog Identification			Molecular identification**	
		Identity	Similarity index	Distance	Best classified BLAST hit	% identity
KiSF17	*Pseudomonas* sp.	*Pseudomonas aeruginosa*	0.801	3.461	*Pseudomonas aeruginosa*	99%
KiSF16	*Pseudomonas* sp.	*Pseudomonas aeruginosa*	0.761	4.250	*Pseudomonas aeruginosa*	99%

*Based on biochemical tests, **Based on 16S rDNA sequence analysis

fungal growth. The *Pseudomonas* spp. KiSF 16 and KiSF 17 showed more inhibitory effect against *G. applanatum* and *T. paradoxa*. They were identified as *P. aeruginosa* by Biolog® GEN III microplate identification system with similarity index >0.75 (0.761 SIM for KiSF 16 and 0.801 SIM for KiSF 17). Sequence analysis of the 16S rRNA gene and BLAST sequence comparison confirmed the identity, validating the Biolog® GEN III microplate identification (Table 3).

The *in vitro* findings indicate the potential of these indigenous rhizobacteria as biocontrol agents due to the efficient inhibition of both of the coconut fungal pathogens tested. The results are promising but detailed studies are required to develop these antagonists as an efficient biocontrol agent. Their ability to prevent the disease development has to be investigated on coconut seedlings in field conditions. Also, it is imperative to test the pathogenicity of *P. aeruginosa* as many strains are known to be opportunistic human pathogens, before such trials are laid out.

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References

Adesina, M.F., Lembke, A., Costab, R., Speksnijder, A. and Smalla, K. 2007. Screening of bacterial isolates from various European soils for *in vitro* antagonistic activity towards *Rhizoctonia solani* and *Fusarium oxysporum*: Site-dependent composition and diversity revealed. *Soil Biol. Biochem.* **39**: 2818-2828.

Anith, K.N., Radhakrishnan, N.V. and Manomohandas, T.P. 2003. Screening of antagonistic bacteria for biological control of nursery wilt of black pepper *(Piper nigrum)*. *Microbiol. Res.* **158**: 91-97.

Bakker, A.W. and Schipper, B. 1987. Microbial cyanide production in the rhizosphere in relation to potato yield reduction and *Pseudomonas* spp.-mediated plant growth stimulation. *Soil Biol. Biochem.* **19**: 451-457.

Bhaskaran, R. 2000. Management of basal stem rot disease of coconut caused by *Ganoderma lucidum*. In: *Ganoderma Diseases of Perennial Crops.* (Eds.) Flood, J., Bridge, P.D. and Holderness, M. CABI Publishing, United Kingdom. pp. 121–126.

Brimecombe, M.J., De Liej, F.A. and Lynch, J.M. 2001. The effect of root exudates on rhizosphere microbial populations. In: *The Rhizosphere*. (Eds.) Pinton, R., Varanini, Z. and Nannipieri, P. Marcel Dekker, New York. pp. 95-140.

Cappuccino, J.C. and Sherman, N. 1992. *Microbiology: A Laboratory Manual*. Third edition. Benjamin/ Cummings publishing company. New York. 462 p.

Charigkapakorn, N. and Sivasithamparam, K. 1987. Changes in the composition and population of fluorescent pseudomonads on wheat roots inoculated with successive generations of root-piece inoculum of the take-all fungus. *Phytopathol.* **77**: 1002-1007.

Dwivedi, D. and Johri, B.N. 2003. Antifungals from fluorescent pseudomonads: Biosynthesis and regulation. *Curr. Sci.* **85**: 1693-1703.

Francis, I., Holsters, M. and Vereecke, D. 2010. The Gram-positive side of plant–microbe interactions. *Environ. Microbiol.* **12**(1): 1–12.

Fuhrmann, J.J. 1994. Isolation of soil microorganisms producing antibiotic compounds. In: *Methods of Soil Analysis, Part 2, Microbiological and Biochemical Properties.* (Ed.) Weaver, R.W. Soil Science Society of America, Madison, Wisconsin. pp. 379-405.

Gould, W.D., Hagedorn, C., Badinelli, T.R. and Zablotowicz, R.M. 1985. New selective media for enumeration and recovery of fluorescent pseudomonads from various habitats. *Appl. Environ. Microbiol*. **49**: 28-32.

Haas, D. and Défago, G. 2005. Biological control of soil-borne pathogens by fluorescent pseudomonads. *Nature Rev. Microbiol.* **3**: 307-319.

Haas, D. and Keel, C. 2003. Regulation of antibiotic production in root colonizing *Pseudomonas* spp. and relevance for biological control of plant disease. *Annu. Rev. Phytopathol.* **41**: 117–153.

James, G. 2010. Universal Bacterial Identification by PCR and DNA Sequencing of 16S rRNA Gene. In: *PCR for Clinical Microbiology - Part 3* (Eds.) Margret Schuller, Theo P. Sloots, Gregory S. James, Catriona L. Halliday and Ian W.J. Carter., Springer, New York. ISBN 978-90-481-9038-6. pp. 209-214.

King, E.O., Ward, M.K. and Raney, D.E. 1954. Two simple media for the demonstration of pyocianin and fluorescein. *J. Lab. Clin. Med*. **44**: 301-307.

Priya, G., Gupta, A., Gopal, M., ChandraMohanan, R., Litty, T. and Thomas, G. V. 2011. Antagonistic activity of coconut rhizospheric and endophytic *Bacillus* spp. against *Ganoderma applanatum* and *Thielaviopsis paradoxa. J. Plantn. Crops* **39**(2): 278- 284.

Rajendran, L., Karthikeyan, G., Raguchander, T. and Samiyappan, T., 2007. *In vitro* evaluation of bacterial endophytes influence on *Ganoderma lucidum* (leys) Karst. mycelial growth. *J. Plant Protection Res.* **47(**4): 425–436.

Renwick, A., Campbell, R. and Coe, S. 1991. Assessment of *in vivo* screening systems for potential biocontrol agents of *Gaeumannomyces graminis. Plant Pathol*. **40:** 524-532.

Schwyn, B. and Neilands, J.B. 1987. Universal chemical assay for the detection and detrmination of siderophores. *Anal.Biochem.* **160**: 47-56.

Shimahara, K. and Takiguchi, Y. 1988. Preparation of crustacean chitin. In: *Methods in Enzymology.* (Eds.) Wood, W. A. and Kellog, S. T. Academic press, London. pp. 417-423.

Skidmore, A.M. and Dickinson, C.H. 1976. Colony interactions and hyphal interference between *Septena nodurum* and phylloplane fungi. *Trans. Br. Mycol. Soc.* **66**: 57-64.

Soytong, K., Pongak, W. and Kasiolarn, H. 2005. Biological control of *Thielaviopsis* bud rot of *Hyophorbe lagenicaulis* in the field. *J. Agric.Technol.***1**(2)**:** 235-245.

Srinivasulu, B. and Rao, D.V.R. 2007. *Coconut Diseases*. International Book Distributing Company, Lucknow. 114 p.

Welbaum, G., Sturz, A.V., Dong, Z. and Nowak, J. 2004. Fertilizing soil microorganisms to improve productivity of agro-ecosystems. *Crit. Rev. Plant Sci.* **23**: 175-193.