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IN VITRO EVALUATION OF BACTERIAL ENDOPHYTES INFLUENCE ON *GANODERMA LUCIDUM* (LEYS) KARST. MYCELIAL GROWTH

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Abstract: Basal Stem Rot (BSR) disease caused by *Ganoderma lucidum* (Leys) Karst. is the most destructive disease and a major constraint in coconut production. Fifty five endophytic strains of bacteria were isolated from coconut roots of different regions. Among the isolates, EPC5 (Endophytes coconut), EPC8, EPC15, EPC29, EPC52 and Pf1 (Plant growth promoting rhizobacteria) promoted the rice seedling growth in roll towel and pot culture method. EPC5 (Plant growth promoting endophytic bacteria), Pf1 and *Trichoderma viride* (Plant growth promoting fungus) effectively inhibited the *G. lucidum* growth *in vitro*. When bioagents along with farm yard manure (FYM) were heaped for different days interval the population was increased in twenty days both in sterilized and unsterilized conditions.

Key words: coconut, endophytes, *Ganoderma,* farm yard manure

INTRODUCTION

Coconut, *Cocos nucifera* Linn., is known as "Kalpavriksha", is a major plantation as well as oilseed crop in the tropics of the world. India is the largest producer of coconut with an estimated production of 12,832.9 million nuts from 19.35 lakh hectares with productivity of 6,632 nuts/ha (All India Final Estimate of Coconut 2005). Coconut palm in spite of its hardiness is affected by a large number of diseases, among which basal stem rot (BSR) disease caused by *Ganoderma lucidum* (Leys) Karst. is the most destructive and a major limiting factor in coconut production especially in Tamil Nadu, Andhra Pradesh and other coconut growing states of India (Bhaskaran *et al*.

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1989). Currently, no cost-effective fungicide is available that gives guaranteed control. Development of biological control for basal stem rot disease is accepted as a durable and environment friendly alternative for agrochemicals. In biocontrol methods, the use of endophytic bacteria is emphasized by many authors. Endophytic bacteria have been shown to control *Fusarium oxysporum* f. sp. *vasinfectum* on cotton (Chen *et al.* 1995), *Clavibacter michiganensis* subsp. *sepedonicus*, the causal agent of bacterial ring rot of potato (Van Buren *et al.* 1993) and *Erwinia carotovora* subsp. *atroseptica* causing potato soft rot (Sturz and Matheson 1996). Endophytic *bacillus* amended with chitin promotes higher growth and suppresses bacterial blight incidence in cotton under greenhouse conditions (Rajendran *et al*. 2006). Garrett (1955) reported that *Trichoderma viride* and *Streptomyces* spp. were antagonistic to *G. lucidum*. Gunasekaran *et al*. (1986) and Bhaskaran *et al*. (1988) reported that *T. harzianum* and *T. hamatum* were found to be antagonistic to *G. lucidum* and application of neem cake encouraged the saprophytic soil microflora especially *Trichoderma* spp. in coconut basins and was effective in the control of *Ganoderma* disease. Soil application of *T. viride* and *Pseudomonas fluorescens* talc formulations at the rate of 200 g each/palm in combination with 50 kg FYM was found effective against the disease (Karthikeyan *et al*. 2005). Baker and Cook (1974) suggested that antagonists which differed in their ecology could be combined so that they could effectively utilise the root exudates and survive in association. With this background, the present study was undertaken to see the effect of bioagents individually and in combination against *G. lucidum in vitro*.

MATERIALS AND METHODS

Biocontrol agents from culture collection

Bioagents *viz*., *P. fluorescens* strain (Pf1), *T. viride* strain (TV1) was obtained from the Culture Collection Section, Department of Plant Pathology, Tamil Nadu Agricultural University, Coimbatore.

Isolation of bacterial endophytes from coconut

Coconut root samples were taken and brought to the laboratory. Root sections (2–3 cm long) were made using a sterile scalpel. Root samples were surface sterilized with 1% sodium hypochlorite (NaOCl) in 0.05% triton X-100 for 10 min and rinsed four times in 0.02 M sterile potassium phosphate buffer pH 7.0 (PB). A 0.1 ml aliquot was taken from the final buffer wash and transferred to 9.9 ml tryptic soy broth (TSB) to serve as sterility check. Samples were discarded if growth was detected in the sterility check samples (agitating samples in TSB, Hi Media Code No. M 011, at 28±2°C) within 48 h. Each sample (0.5 g) was triturated with a sterile mortar and pestle in 9.5 ml of the final buffer wash. Serial dilutions up to (10^{10}) of the triturate were made in phosphate buffer. Each dilution of every sample was plated (0.1 ml) on three plates each of three different media: Tryptic soy agar (TSA-Hi Media, Code No. M290). Nutrient agar (NA g/l; peptone 5, beef extract 2 and agar 20, pH 5.0) and King's B Medium (g/l; proteose peptone 20, K_2HPO_4 1.5, Mg SO_4. $7H_2O$ 1.5, glycerol 20 ml and agar 15, pH 7.2) (King *et al*. 1954). The plates were incubated at 28±2°C for 48–72 h. At each sampling date and for each treatment, one representative of each bacterium, as evident from their colony type and morphology was transferred to fresh King's B Medium plates to establish pure cultures.

Preparation of bacterial suspension inoculum

The endophytic bacteria were grown on KB and NA broth with constant shaking at 150 rpm for 48 h at room temperature (28±2°C). The bacterial cells were harvested by centrifugation at 10000 rpm for 15 min, and bacterial cells were resuspended in phosphate buffer (0.01 M, pH 7.0). The concentration was adjusted using a spectrophotometer to approximately 10^8 cfu/ml (OD_{595} = 0.3) and used as bacterial inoculum (Thompson 1996).

Seed bacterization

Rice seeds (cv. ADT 46) were surface sterilized with two per cent sodium hypochlorite for 30 sec, rinsed in sterile distilled water and dried overnight under sterile air stream. Endophytic bacterial strains were inoculated in a conical flask into respective broth. Required quantity of seeds were soaked in bacterial suspension containing 3 x 10^8 for 2 h and dried under shade. The seeds without treatment with bacteria (instead of bacteria, water is used) are also maintained (control 1 and 2).

Plant growth – promotion

Plant growth – promoting activity of bacterial endophytic strains were assessed based on the seedling vigour index by the standard roll towel method (ISTA 1993). Rice seed bacterization was done as described earlier. Twenty seeds were kept over the presoaked germination paper. The seeds were held in position by placing another presoaked germination paper strip and gently pressed. The polythene sheet along with seeds was then rolled and incubated in growth chamber for 14 days. Three replications were maintained for each treatment. The root and shoot length of individual seedlings were measured and the germination percentage of seeds was also calculated.

Plant growth promotion was also tested in pot culture method. Bacterized seeds were sown in pots. Twenty seeds were maintained for each treatment. The root and shoot length of individual seedlings were measured and germination percentage of seeds was also calculated. The vigour index was calculated by using the formula as described by Baki and Anderson (1973).

Vigour index = per cent germination x seedling length (shoot length + root length)

In vitro* testing of endophytic bacterial strains for inhibition of mycelial growth of *G. lucidum

Bacterial endophytic strains were tested for their inhibition of mycelial growth of *Ganoderma* by following the dual culture technique (Dennis and Webster 1971). The bacterial culture was streaked at one side of Petri dish (1 cm from the edge of a plate) with PDA medium and mycelial disc (8 mm diameter) of seven days old culture of *G. lucidum* was placed on the opposite side in the Petri dish perpendicular to the bacterial streak. The plates were incubated at room temperature (28±2°C) for four days and the mycelial inhibition of pathogen was measured in millimeter.

Compatibility among biocontrol agents

PGPR strains were tested for their compatibility among each other by following the method described by Fukui *et al*. (1994). The compatibility was determined for *P. fluorescens*, *B. subtilis* strains and *T. viride* by using potato dextrose agar medium.

The bacterial strains of *B. subtilis* (EPC5) and Pf1 were streaked on the opposite side and *T. viride* disc placed at the center of the medium. Compatibility was tested by overgrowth or by inhibition of *P. fluorescens*, *B. subtilis* and *T. viride* strains by incubating at room temperature and by making observations over a period of 72 h.

Preparation of substrate for the multiplication of bioagents

The organic substrate of dried cow dung was tested for the multiplication of *Pseudomonas*, *Bacillus* and *T. viride*. One hundred gram of substrate was weighed in polypropylene bag, moisture content was adjusted to 60% (w/v) by gravimetric method (Dutta and Das 1999). One set of treatments of the above substrate were sterilized and another set was kept without sterilization. For sterilization the substrates were autoclaved at 15lb psi for 1h on two consecutive days. The substrate was inoculated with 5 g of talc based bioformulation of *Pseudomonas*, *Bacillus*, *T. viride*. The substrate was incubated at room temperature.

Assessment of bioagents population in farm yard manure

One gram of sample from FYM was derived at regular intervals, and the population (CFU) of *Pseudomonas*, *Bacillus* and *T. viride* in the substrate and talc were counted at 10, 20, 30, 40, 50, 60 days intervals after inoculation by serial dilution techniques in selective medium for each bioagents.

Statistical analysis

The data were statistically analyzed (Rangasamy 1995) using the IRRISTAT version 92 developed by the International Rice Research Institute Biometrics unit, the Philippines (Gomez and Gomez 1984). The percentage values of the disease index were arcsine transformed. Data were subjected to the analysis of variance (ANOVA) at two significant levels (< 0.05 and < 0.01) and means were compared by Duncan's multiple range test (DMRT).

RESULTS

Isolation of endophytic bacteria

Totally fifty five isolates of endophytic bacteria were isolated from healthy coconut roots from different parts of Tamil Nadu.

Efficacy of bacterial endophytic strains in plant growth promotion

Coconut being a perennial crop, thus growth promotion by endophytic and rhizosphere bacteria was tested on rice crop. The growth promotion by bacterial endophyte isolates was assessed by treating the rice seeds with each bacterial isolate separately using their suspension. Endophytic bacterial isolates EPC5, EPC8, EPC12, EPC13, EPC15, EPC21, EPC29, EPC32, EPC52 and *P. fluorescens* strain Pf1 were found to increase the vigour index of rice seedlings significantly when compared to untreated control (Table 1).

***In vitro* screening of the endophytic bacterial strains against the pathogen**

Out of all endophytic bacterial strains, ten isolates were selected in preliminary screening for growth promotion. These isolates were tested for their efficacy by dual

Table 1. Effect of bacterial endophytes on rice seedling growth

Isolate	Vigour index	
	pot culture study	roll towel method
1	2	3
EPC 1	1483.49 y	1221.08 V
EPC 2	1097.47 M	979.98 U
EPC 3	864.48 X	1016.48 Y
EPC 4	1043.49 Q	1451.48 P
EPC 5	1943.37 d	2053.08 s
EPC 6	1615.35 o	2021.78 w
EPC 7	1056.48 P	1146.08 X
EPC 8	1934.39 e	2182.88 m
EPC 9	1139.43 K	1416.98 R
EPC 10	1671.48 m	1431.68 Q
EPC 11	1487.38 x	1625.08 L
EPC 12	1862.48 f	3524.48 b
EPC 13	1826.33 g	3549.48 a
EPC 14	1455.30 z	2007.48 x
EPC 15	1711.47 j	2035.78 uv
EPC 16	1294.47 F	1602.48 M
EPC 17	1575.46 q	2093.78 p
EPC 18	1431.45 B	1803.48 F
EPC 19	1663.48 n	2056.88 r
EPC 20	1518.35 v	2035.08 v
EPC 21	1807.47 h	3028.88 d
EPC 22	1532.46 t	2004.98 y
EPC 23	1592.28 p	1924.28 z
EPC 24	1448.48 A	2006.38 xy
EPC 25	1061.48 O	1711.68 I
EPC 26	904.50 V	1002.68 Z
EPC 27	1224.34 H	1629.08 K
EPC 28	1336.45 D	2121.88 n
EPC 29	2006.28 b	3379.48 c
EPC 30	1322.45 E	1851.68 D
EPC 31	1357.48 C	1673.88 J

1	2	3
EPC 32	1943.43 d	2258.48 i
EPC 33	1695.46 l	2825.48 e
EPC 34	1490.48 w	2097.88 o
EPC 35	1103.46 L	1854.48 C
EPC 36	1574.46 q	2049.08 t
EPC 37	1175.40 J	2454.48 g
EPC 38	1013.47 R	1232.98 U
EPC 39	1061.32 O	1518.08 N
EPC 40	1217.48 I	1875.48 A
EPC 41	1280.40 G	1831.48 E
EPC 42	764.44 Y	979.48 U
EPC 43	1615.42 o	2252.18 j
EPC 44	1224.37 H	1756.48 H
EPC 45	989.46 S	1859.38 B
EPC 46	1140.41 K	1452.08 P
EPC 47	983.48 T	1797.08 G
EPC 48	1084.50 N	1473.48 O
EPC 49	889.50 W	1157.48 W
EPC 50	1525.39 u	2200.98 l
EPC 51	1703.34 k	2037.18 u
EPC 52	1979.48 c	2653.58 f
EPC 53	1539.42 s	2216.28 k
EPC 54	1759.50 i	2201.08 l
EPC 55	1560.49 r	2085.48 q
Pf1	2024.43 a	2368.28 h
Control 1	939.46 U	1403.48 S
Control 2	1013.37 R	1390.88 T

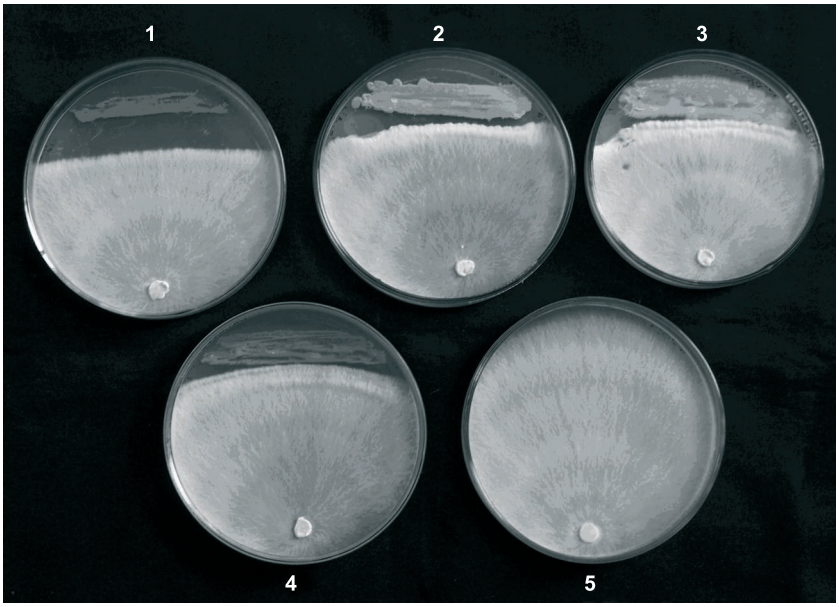
Values are mean of two replications
Control: Without endophytic bacteria or seeds treated with water (instead of bacteria water is used)
Data followed by the same letter in a column are not significantly different according to Duncan's multiple range test at p = 0.05

plate technique against *G. lucidum* along with Pf1. Among the ten isolates, five strains were found to inhibit the growth of *G. lucidum in vitro.* The strain, Pf1 caused high inhibition of *G. lucidum* followed by EPC5 (coconut root isolate) and EPC8 (coconut root isolate). The per cent inhibition was significantly higher in plates streaked with Pf1 (40.67%), EPC5 (33.80%) and EPC8 (29.16%) against control plates (Table 2, Fig. 1). The bioagents EPC5, Pf1 and *T. viride* were tested in combination against *G. lucidum in vitro.* These three bioagents effectively inhibited the growth of pathogen even up to one month whereas in control, *G. lucidum* overgrew the plate within 6 days after placing the disc (Fig. 2).

Table 2. *In vitro* antagonistic activity of bacterial endophytic isolates of coconut against *Ganoderma lucidum*

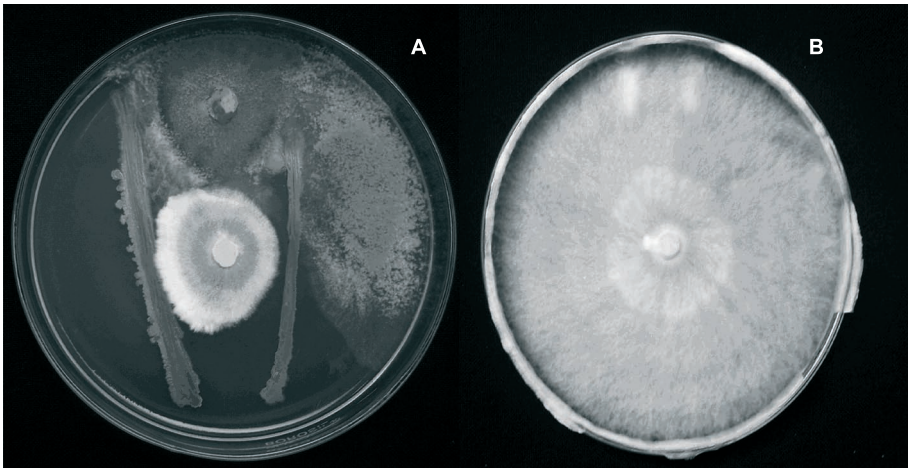
Isolates	Per cent inhibition over control
Pseudomonas fluorescens (Pf1)	40.67 (39.62) f
Bacillus (EPC5)	33.80 (35.54) e
Bacillus (EPC8)	29.16 (32.68) d
Pseudomonas (EPC15)	27.78 (31.80) d
Pseudomonas (EPC32)	4.75 (12.58) c
Pseudomonas (EPC52)	2.65 (9.37) b
Control	0.0 (0.0) a

Values are means of three replications
Data followed by the same letter in a column are not significantly different according to Duncan's multiple range test at p = 0.05
Values in parentheses are arcsine transformed



1. *Pseudomonas fluorescens* (Pf1)
2. *Bacillus* spp. (EPC5)
3. *Bacillus* spp. (EPC8)
4. *Pseudomonas* spp. (EPC15)
5. Control

Fig 1. *In vitro* antagonistic activity of bacterial endophytes against *Ganoderma lucidum* isolate CRS-1



A – 1. EPC5 (*Bacillus* spp.)
2. Pf1 (*Pseudomonas fluorescens*)
3. TV
4. *Ganoderma lucidum* (CRS-1)
B – Control (CRS-1)

Fig 2. *In vitro* antagonistic activity of bioconsortia against CRS-1

Compatibility among bioagents

Bioagents *viz*., Pf1, EPC5 and Tv were tested to establish their compatibility under *in vitro* conditions. After 48 h of streaking over the Petri plates, the interaction was observed. Among them, *Pseudomonas* showed more compatibility with *T. viride* and *Bacillus*. But *Bacillus* and *T. viride* were less compatible in plates whereas compatibility was observed in broth inoculation. Assessment of population of *Bacillus* and Pf1 in their combined inoculation in broth revealed that population was slightly decreased in combined inoculation compared to individual inoculation (Table 3). Similarly, assessment of mycelial dry weight of *T. viride* with the broth containing Pf1, *Bacillus* recorded a slight reduction of mycelial dry weight (Table 4). The result indicated the possibility of combined application of bioagents for the management of pathogens.

Table 3. Population of bioagents in consortia

Sl. No	Bioagents	Population (CFU X 10^6)
1	*Pseudomonas fluorescens* (Pf1)	157 a
2	*Bacillus* spp. (EPC5)	121 c
3	Pf1 in combination	131 b
4	*Bacillus* spp. in combination	112 d

Values are means of five replications
Data followed by the same letter in a column are not significantly different according to Duncan's multiple range test at p = 0.05

Table 4. Mycelial dry weight of *Trichoderma viride* in consortia

Sl. No.	Antagonist	Mycelial dry weight [g]
1	*T. viride* alone	0.637
2	*T. viride* in Pf1 and *Bacillus* inoculated broth	0.567

Population of bioagents in FYM

To test the population buildup of bioagents *in vitro,* the effective bioagent formulations were mixed with sterilized and unsterilized FYM separately and in combination and heaped for different time. The population of bioagents was assessed at different day intervals *viz.,* 10, 20, 30, 40, 50 and 60 days after mixing with FYM. The results showed that twenty days incubation was optimum for the maximum population buildup of *Bacillus*, *T. viride* and *Pseudomonas* in both sterilized and unsterilized FYM (Fig. 3).

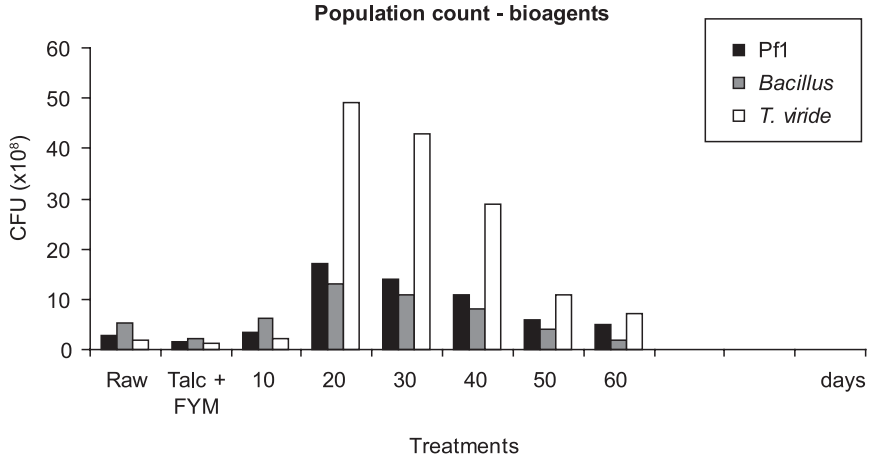


Fig. 3. Population load of bioagents in farm yard manure (FYM) under *in vitro* conditions

DISCUSSION

The coconut, primarily a small holder's crop is now cultivated throughout the humid tropics. In India, most of the acreage under coconut palm lies in the four southern states *viz*., Kerala, Karnataka, Tamil Nadu and Andhra Pradesh. Coconut palms were affected by many pests and diseases. Among them, basal stem rot (BSR) disease caused by *G. lucidum* is the most destructive one which is widespread in nature (Bhaskaran *et al*. 1989; Lattiffah *et al*. 2002). Basal stem rot can be controlled effectively by management practices in the early stages of disease development. Though the efficacy of the biocontrol agents Pf1 and *T. viride* has been studied individually (Karunanithi *et al*. 2004), their combined effect and their molecular mechanisms on the management of BSR of coconut have not been studied. Hence, the present study was undertaken to investigate the effect of three bioagents *viz*., *Bacillus*, Pf1 and *T. viride* individually

and in combination for the management of basal stem rot disease. Fifty five endophytes were isolated from coconut roots. Similarly, endophytic bacterial isolates of *Bacillus* (39%), *Pseudomonas* (27.6%), *Corynebacterium* (16.7%), *Actinomyces* (11.1%) and *Staphylococcus* (5.6%) with enzymatic activity in solid media isolated from *Jacaranda decurrens* (a medicinal plant) were reported (Carrim *et al*. 2006). In the present study, endophytic bacteria from the roots of coconut palms EPC5, EPC8, EPC15, EPC29 and EPC52 were found to increase the vigour index of rice seedlings significantly when compared to untreated control. Hallman *et al.* (1997) reported that most of the endophytic bacterial strains are capable of promoting plant growth. Endophytic bacteria colonize a broad spectrum of plant species and plant parts (Sturz *et al.* 1997). *Bacillus* species are among the most common bacteria found to colonize plants endophytically (Mahaffee and Kloepper 1997). In the present study, EPC5 and Pf1 effectively inhibited the growth of *G. lucidum in vitro.* Bhowmik *et al*. (2002) reported that seed bacterization with endophyte, Endo PR8 was found to be the most effective to reduce the cotyledonary infection by *Xam*. The endophytic *Bacillus* spp. CY22 isolated from balloon flower produced iturin A with antifungal activity against *Rhizoctonia solani, Pythium ultimum* and *Fusarium oxysporum* (Cho *et al.* 2003). Endophytic bacteria *viz*., *B. amyloliquefaciens* produces surfactin, iturin, bacillomucine, azalomycin F, *B. subtilis* produces surfactin and arthrobactin and *B. pumilus* produces surfactin, amphomycin, arthrobactin and valinomycin which are effective against black rot of crucifers caused by *X. campestris* pv. *campestris* (Monteiro *et al*. 2005). *Trichoderma* spp. are known to produce large quantities of fungistatic metabolites *viz*., trichodermin, dermin, trichciridin, trichobrachin, etc. (Bruckner *et al.* 1990) which are active against many soilborne plant pathogens. In our study, *T. viride* strongly inhibited the growth of *G. lucidum in vitro. B. subtilis* (EPC5) showed compatibility with *P. fluorescens* strain Pf1, was less compatible with *T. viride*, Pf1 compatible with *T. viride* in Petri plate assay. In broth inoculation, EPC5 and Tv showed compatibility. In our study, the population of bioagents *Bacillus*, *P. fluorescens* and *T. viride* mixed with farm yard manure, heaped for different interval days was maximum after twenty days. The microbial biomass in soil or compost mixtures increased in 22 days after the addition of compost to the soil (Saison *et al*. 2006). In this work similar to soil application along with FYM suppressed the soilborne pathogen *G. lucidum.* In the present study, *T. viride* population was more followed by Pf1 and *Bacillus.* Similarly species of *Trichoderma* have a high growth rate and are often among the first colonizers of fresh substrates. Their maximum temperature for growth is in the range 30–40°C (Rifai 1969).

CONCLUSIONS

Of all the tested rhizobacteria, bacterial endophytes and fungi, EPC5, Pf1, Tv1 showed the most effective inhibition against *G. lucidum* and their efficacy increased by the combination of these antagonists.

REFERENCE

Baker K.F., Cook R.J. 1974. Biological Control of Plant Pathogens. W.H. Freeman and Co., San Francisco, 433 pp.

Baki A.A.A., Anderson J.D. 1973. Vigour determination in soybean seed by multiple criteria. Crop Sci. 31: 630–633.

Bhaskaran R., Ramdoss N., Ramachandran T.K. 1988. Biological control of Thanjavur wilt disease of coconut. Indian Coconut J. 19 (6): 3–8.

Bhaskaran R., Rethinam P., Nambiar K.K.N. 1989. Thanjavur wilt of coconut. J. Plant. Crops. 17: 69–79.

Bhowmik B., Singh R.P., Jayaram J., Verma J.P. 2002. Population dynamics of cotton endophytic *Pseudomonas*, their antagonism and protective action against the major pathogens of cotton. Indian Phytopath. 55 (2): 124–132.

Bruckner H., Reinecke C., Kripp T., Kieb M. 1990. Screening isolation and sequence determination of a unique group of polypeptide antibiotics from filamentous fungi. Proc. Int. Mycol. Cong. Regensburg, Federation Republic Germany, p. 224.

Carrim A.J.L., Barbosa E.C., Vieira J.D.G. 2006. Enzymatic activity of endophytic bacterial isolates of *Jacaranda decurrens* Cham. (Carobinha-do-campo). Braz. Arch. Biol. Technol. 49 (3): 353–359.

Chen C., Bauske E.M., Musson G., Rodriguez-Kabana R., Kloepper J.W. 1995. Biological control of *Fusarium* wilt on cotton by use of endophytic bacteria. Biol. Control 5: 83–91.

Cho S.J., Lim W.J., Hong S.Y., Park S.R., Yun H.D. 2003. Endophytic colonization of balloon flower by antifungal strain *Bacillus* sp. CY22. Biosci. Biotechnol. Biochem. 67 (10): 2132–2138.

Dennis C., Webster J. 1971. Antagonistic properties of species groups of *Trichoderma* 1. Production of non-volatile antibiotics. Trans. Br. Mycol. Soc. 57: 25–39.

Dutta P., Das B.C. 1999. Effect of seed pelleting and soil application of *Trichoderma harzianum* in the management of stem rot of soybean. J. Mycol. Pl. Pathol. 29 (3): 317–322.

Fukui R., Schroth M.N., Hendson M., Hancock J.G. 1994. Interaction between strains of *Pseudomonads* in sugar beet sphermospheres and the relationship to pericarp colonization by *Pythium ultimum* in soil. Phytopathology 84: 1322–1330.

Garrett S.D. 1955. A century of root disease investigation. Ann. Appl. Biol. 42: 211–219.

Gomez K.A., Gomez A.A. 1984. Statistical Procedure for Agricultural Research. John Wiley and Sons, New York, p. 680.

Gunasekaran M., Ramdoss N., Ramiah M., Bhaskaran R., Ramanathan T. 1986. Role of neem cake in the control of Thanjavur wilt of coconut. Indian Coconut J. 17 (1): 7–12.

Hallmann J., Quadt-Hallmann A., Mahaffee W.F., Kloepper J.W. 1997. Bacterial endophytes in agricultural crops**.** Can. J. Microbiol. 43: 895–914.

ISTA. 1993. Proceedings of the international Seed Testing Association, International Rules for Seed Testing. Seed Sci. Technol. 21: 25–30.

Karthikeyan G., Karpagavalli S., Rabindran R., Natarajan C. 2005. Biological control of basal stem rot disease in coconut. The Planter 81 (957): 777–784.

Karunanithi K., Sarala L., Rabindran R., Kalaimani T., Manickam G. 2004. Effect of biocontrol agents on the management of basal stem rot (BSR) of coconut. 26th Annual Conference of ISMPP and National symposium on advances in fungal diversity and host-pathogen interactions. Goa University, October 7–9, 2004, p. 53.

King E.O., Ward M.N., Raney D.E. 1954. Two simple media for the demonstration of pyocyanin and fluorescein. J. Lab. Clin. Med. 44: 301–307.

Lattiffah Z., Harikrishna K., Tan S.G., Tan S.H., Abdullah F., Ho Y.W. 2002. Restriction analysis and sequencing of the ITS regions and 5.8S gene of rDNA of *Ganoderma* isolates from infected oil palm and coconut stumps in Malaysia. Ann. Appl. Biol. 141: 133–142.

Mahafee W.F., Kloepper J.W. 1997. Temporal changes in the bacterial communities of soil, rhizosphere and endorhiza associated with field-grown cucumber (*Cucumis sativus* L.). Microbial. Ecol. 34: 210–223.

Monteiro L., Mariano R.L., Souto-Maior A.M. 2005. Antagonism of *Bacillus* spp. against *Xanthomonas campestris* pv. *campestris*. Braz. Arch. Biol. Technol. 48: 23–29.

Rajendran L., Saravanakumar D., Raguchander T., Samiyappan R. 2006. Endophytic bacterial induction of defence enzymes against bacterial blight of cotton. Phytopathol. Mediterr. 45: 203–214.

Rangasamy R. 1995. A Text Book of Agricultural Statistics. New Delhi, India: New Age International publisher Ltd, 269 pp.

Rifai M.A. 1969. A revision of the genus *Trichoderma*. Micol. Papers 116: 1–56.

Saison C., Degrange V., Oliver R., Millard P., Commeaux C., Montange D., Roux X.L. 2006. Alteration and resilience of the soil microbial community following compost amendment: effects of compost level and compost-borne microbial community. Environ. Microbiol. 8: 247–257.

Sturz A.V., Matheson B.G. 1996. Populations of endophytic bacteria which influence host-resistance to *Erwinia* induced bacterial soft rot in potato tuber. Plant Soil 184: 265–271.

Sturz A.V., Christie H.R., Matheson B.G., Arsenault W.J., Buchman N.A. 1997. Biodiversity of endophytic bacteria which colonize red clover nodules, roots, stems and foliage and their influence on host growth. Biol. Fert. Soils 25: 13–19.

Thompson D.C. 1996. Evaluation of bacterial antagonist for reduction of summer patch symptoms in Kentucky blue grass. Plant Dis. 80: 856–862.

Van Buren A.M., Andren C., Isbimaru C.A. 1993. Biological control of bacterial ring rot pathogen by endophytic bacteria isolated from potato. Phytopathology 83, p. 1406.

POLISH SUMMARY

OCENA *IN VITRO* WPŁYWU BAKTERII ENDOFITYCZNYCH NA WZROST GRZYBNI *GANODERMA LUCIDUM* (LEYS) KARST.

Zgnilizna podstawy pnia palm kokosowych (BSR) wywoływana przez *Ganoderma lucidum* (Leys) Karst. jest najgroźniejszą chorobą powodującą bardzo duże straty w produkcji orzechów kokosowych. Z korzeni palm kokosowych pochodzących z różnych lokalizacji wyosobniono 55 szczepów bakterii endofitycznych. Z nich endofity EPC5, EPC8, EPC15, EPC29, EPC52 oraz rizobakteria Pf1 stymulowały wzrost siewek ryżu w zwijanym teście bibułowym i w doświadczeniu wazonowym. Bakterie endofityczne EPC5 stymulujące wzrost roślin, rizobakterie Pf1 stymulujące wzrost roślin i grzyb *Trichoderma viride* również stymulujący wzrost roślin, inhibowały skutecznie wzrost patogena *G. lucidum in vitro*. Gdy zmieszano je z obornikiem i pozostawiono przez różną ilość dni, ich populacja zwiększała się w ciągu 20 dni zarówno w warunkach sterylności podłoża, jak i w warunkach niesterylnych.