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Defense Related Enzyme Induction In Coconut By Endophytic Bacteria (EPC 5)

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Endophytic bacteria *Bacillus subtilis* (EPC 5) was isolated and tested *in vitro* along with *Pseudomonas fluorescens* (Pf1) and the fungus *Trichoderma viride* (Tv1) against *Ganoderma lucidum* (Leys) Karst, the causal agent of basal stem rot on coconut palm. The endophytic bacterial strains namely EPC 5 and EPC 8 showed higher vigor index (germination percentage, root and shoot length) and more inhibition against *G. lucidum* over un-inoculated control. These strains were confirmed as *Bacillus subtilis* by biochemical tests, cloning and sequencing of internal transcribed spacer (ITS) region. The *Bacillus subtilis* (EPC 5) along with *Pseudomonas fluorescens* (Pf1) and *Trichoderma viride* (Tv1) has been tried as bioconsortia against basal stem rot disease under greenhouse conditions. The soil application of bioconsortia enriched with farm yard manure (FYM) enhanced the coconut saplings growth under greenhouse conditions and showed higher induction of defense related enzymes like peroxidase, polyphenol oxidase, phenylalanine ammonia lyase and phenols when challenged with pathogen.

Keywords: coconut, basal stem rot (BSR), *Ganoderma*, endophytic bacteria, bioconsortia, Induced Systemic Resistance, defense enzymes.

Basal stem rot (BSR) disease in coconut plantation caused by *Ganoderma lucidum* (Leys) Karst. is a major limiting factor in coconut production (Bhaskaran et al., 1996) and known as Thanjavur wilt, bole rot, *Ganoderma* disease and Anabe roga in different states of India. It is a serious soil-borne disease occurring in numerous perennial, coniferous and palmaceous hosts. In East Coast Tall palms, the age groups of 5 to 30 years are generally more susceptible to the disease (43%) than younger trees (17%). All the coconut cultivars and hybrids of Kerala were infected by the pathogen and surviving plants showed symptoms of the disease (Rajamannar et al., 2000). Currently, chemical application through root feeding is commonly practiced; the disease could be delayed by adopting strategic management which is a labour-intensive procedure. But today, the adverse effects like toxicity, residual effect, resistance development have created a need for rapid development and implementation of effective biological products for disease management. Application of bioagents is the current interest for the management (Bhaskaran, 1990; Srinivasan et al., 2010). Many of them have been isolated from rhizosphere soil of different crops and tested against several fungal diseases. Among bioagents, the endo-

Abbreviations: EPC – endophytes coconut; BSR – basal stem rot

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phytes (Hallman et al., 1997; Rosenblueth and Martínez-Romero, 2006) which colonize the plant internally without doing substantial harm to the plant are gaining importance and will be used in the disease management (Wulff, 2000) with the members of *Pseudomonas, Bacillus* and *Azospirillum*. Induction of plant's defense genes by prior application of inducing agents is called induced resistance (Hammerschmidt and Kuć, 1995; van Loon et al., 1998). The defense gene products include peroxidase (PO), polyphenol oxidase (PPO) that catalyze the formation of lignin and phenylalanine ammonia-lyase (PAL) that is involved in phytoalexins and phenolics synthesis. Other defense enzymes namely β-1,3-glucanases (PR-2 family) and chitinases (PR-3 family) degrade the fungal cell wall and cause lysis of fungal cell. The antagonists which differ in their ecology could be combined so that they could effectively utilize the root exudates and survive in association and each biocontrol agent may use a different mechanism (Raupach and Kloepper, 1998) to fight the pathogen, thus better results would be achieved than with a single one (de Boer et al., 1999). It might broaden the spectrum of biocontrol activity, enhance the efficacy and reliability of control (Duffy and Weller, 1995) and allow the combination of various mechanisms without the need for genetic engineering. The combination of *Bacillus subtilis* strain GB03 (a growth-promoting agent), *B. amyloliquefaciens* strain IN937a (an inducer of systemic resistance) and chitosan and *P. fluorescens* CECT 5398 increased biometric parameters in tomato and pepper compared to individual treatments (Domenech et al., 2006). Mixtures of PGPR strains significantly reduced the severity of diseases compared to the non-bacterized control in tomato, pepper and cucumber (Jetiyanon and Kloepper, 2002). de Boer et al. (2003) combined *Pseudomonas* strains effective in siderophore-mediated competition for iron and induction of systemic plant resistance to improve control of *Fusarium* sp. wilt of radish whereas Barka et al. (2002) reported that the *Pseudomonas* sp. strain, PsJN induced plant growth in parallel with an antagonistic effect against *Botrytis cinerea* in grapes grown under *in vitro* conditions.

With this background, the present study was undertaken at the Department of Plant Pathology, Centre for Plant Protection Studies, Tamil Nadu Agricultural University, Coimbatore, India to investigate the effect of endophytic bacteria *Bacillus subtilis* as bioconsortia with *Pseudomonas fluorescens* and *Trichoderma viride* (EPC5 + Pf1 + Tv1) for the management of basal stem rot disease under greenhouse conditions.

Materials and Methods

Coconut saplings

Coconut cultivar "East Coast Tall" saplings were obtained from the Coconut Research Station (CRS), Aliyar Nagar, TNAU, Tamil Nadu, India and the pathogen *Ganoderma* that was isolated from coconut (CRS-1) was used throughout the study. Bioagents namely *P. fluorescens* (Pf1) and *Trichoderma viride* (Tv1) were obtained from the Department of Plant Pathology, Tamil Nadu Agricultural University, Coimbatore, Tamil Nadu, India.

Bacterial endophytes

Coconut root samples (20.0 m Mean Sea Level, 10° 29'N, 79° 23'E, Veppankulam, Tamil Nadu, India with the soil type of sandy soil) were collected 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 a 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 ºC) 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). The plates were incubated at 28 ± 2 ºC 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.

Rice growth – promotion and in vitro *study*

Rice seed (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 KB broth. Required quantity of seeds were soaked in bacterial suspension containing 3×10^8 cells ml^{-1} for 2 h and dried under shade. The seeds without treatment with bacteria (instead of bacteria, water was used) were also maintained (control) (Thompson, 1996). 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. A polythene sheet along with seeds was then rolled and incubated in growth chamber for 14 days and the germination percentage, root and shoot length of seedlings was recorded (ISTA 1993). Bacterial endophytic strains were tested for their inhibition on mycelial growth of *Ganoderma* by following the dual culture technique. Biochemical tests were carried out to identify the isolates performing well in growth promotion and inhibition (Schaad 1992; Aneja 1993).

Greenhouse study

The bacterial strains either singly or as mixture were assessed for their efficacy in controlling *Ganoderma* infection under greenhouse conditions. A pot culture experiment was undertaken with the treatments (Table 1) in completely randomized design (CRD) with three replications.

Table 1

Effect of biocontrol agents on coconut seedling growth parameters challenged with *Ganoderma lucidum* under greenhouse conditions

Treatments (Ganoderma was inoculated in the soil of all treatments except healthy)	Parameters											
	3 months old			6 months old			9 months old			One year old		
	Height (cm)	Girth (cm)	No. of leaves	Height (cm)	Girth (cm)	No. of leaves	Height (cm)	Girth (cm)	No. of leaves	Height (cm)	Girth (cm)	No. of leaves
P 15 g + FYM	119	9.55	2	130	9.9	3	132	12	3	138^{e}	14.75^{l}	4.5^{f}
B 15 g + FYM	108.25	10.35	3	115	10.75	3	116.5	12.55	3	118^{p}	15^{j}	5.5^{d}
T 15 g + FYM	98.25	10.3	2.5	105.5	11.2	2.5	109.5	12.5	3.5	128.5^{k}	14.9^{k}	5.5^{d}
P + B 15 g + FYM	117	11.6	3.5	123.5	12.0	3	129	12.5	3	132^{i}	17.75^{b}	6.5^{b}
P + T 15 g + FYM	106.5	9.15	3.5	118	9.75	3.5	123.5	11.8	4	139^{d}	15.25^{i}	5.5^{d}
B + T 15 g + FYM	118.5	9.85	3	125	11.05	3	130	11.2	3.5	131.5^{j}	16.25^{e}	5.5^{d}
P + B + T 15 g + FYM	129.5	11.05	3.5	139	11.85	3.5	142.5	13.8	4	154^{b}	17.75^{b}	6^{c}
P 30 g + FYM	129	10.25	3	133.5	11.0	2.5	137	12.5	3	135.5^{f}	15.5^{h}	5.5^{d}
B 30 g + FYM	108	8.9	3	110.5	9.75	2.5	114	11.15	3	127.5^{m}	16^{f}	6^{c}
T 30 g + FYM	104	9.25	2.5	111.5	9.4	3	119.5	11.55	3	127^{n}	15.75^{g}	5.5^{d}
P + B 30 g + FYM	106	10.5	3.5	113.5	11.15	2.5	121.5	16.55	3.5	134.5^{g}	15.25^{i}	5^{e}
P + T 30 g + FYM	118.5	8.65	3.5	125.5	10.35	2.5	129.5	12.6	3.5	133^{h}	16.75^{d}	6^{c}
B + T 30 g + FYM	103.5	10.25	3	108.5	11.45	3	113	13.45	3.5	133^{h}	17.5^{c}	6^{c}
P + B + T 30 g + FYM	130.25	10.65	3.5	140	11.9	3	144.5	14.1	4	156.5^{a}	20^{a}	7^{a}
FYM alone	126	11.65	3.5	129.5	12.05	3	134.5	12.2	4	107^{q}	14^{n}	5.5^{d}
Tridemorph (2 ml/100 ml)	97.5	10.10	3	101	11.2	3	104.5	12.6	3.5	143.5^{c}	14.5^{m}	5.5^{d}
Control	109	9.6	3	111.5	10.1	2.5	113.5	11.05	2.5	123.5^{o}	11.8^{p}	5^{e}
Healthy control	116.5	10.6	3	121	11.35	2.5	123.5	12.4	3	128^{l}	13.75^{o}	5^{e}

P – *Pseudomonas fluorescens* (Pf1), B – *Bacillus subtilis*, T – *Trichoderma viride* and FYM – 500 g.

Values are means of three replications. Parameters were analyzed for one year seedlings and the data followed by the same letter in a column are not significantly different from each other according to Duncan's multiple range test at $P = 0.05$

Ganoderma infection

Sorghum grains (250 g) were put into a polypropylene bag (12 × 8 inch) and sterilized at 121 °C for 15 min. The pure culture of *Ganoderma* isolate CRS-1 (Host: *Cocos nucifera*) was inoculated at the rate of three 8 mm mycelial disc separately on sorghum grains and allowed to mass multiply at 30 °C under dark room conditions for 12 days. At the time of planting the coconut saplings, *Ganoderma* multiplied in the sorghum grains was added to individual pots.

Talc-based powder formulation of strain and mixtures

The bacterial strains *Pseudomonas fluorescens* and *Bacillus subtilis* were grown separately in King's B (KB) and Nutrient agar (NA) broth. The bacteria were incubated in a rotary shaker at 150 rpm for 48 h at room temperature (28 ± 2 ºC). After 48 h of incubation, the broth containing 9×10^8 cfu/ml was used for the preparation of talc-based formulation. To the 400 ml of bacterial suspension, 1 kg of the purified talc powder (sterilized at 105 ºC for 12 h), calcium carbonate 15 g (to adjust the pH to neutral) and carboxymethyl cellulose (CMC) 10 g (adhesive) were mixed under sterile conditions, following the method described by Vidhyasekaran and Muthamilan (1995). After shade drying for over night, it was packed in polypropylene bags and sealed. At the time of application, the population of bacteria in talc formulation was 2.5 to 3 10^8 cfu/g. The fungal antagonist, *T. viride* was multiplied in molasses-yeast broth (30 ml molasses; 5 g yeast; plus water to a total volume of 1000 ml). The sterile broth was inoculated with an actively growing mycelial disc (9 mm) and incubated for 15 days. The biomass (3 ± 10^8 cfu ml^{-1}) along with the medium was incorporated into the sterile talc powder carrier material at the rate of 50 ml of suspension per 100 g of talc powder and thoroughly mixed with 500 mg CMC as described by Ramakrishnan et al. (1994). The mixture was shade-dried for 12 h and stored in polythene bags.

Effect of bioconsortia on coconut saplings

Bioagents in talc formulation were individually and in combination, as indicated in Table 1, mixed with fully decomposed farm yard manure and incubated in a shade room for 20 days with periodical racking, watering and then applied to pots at the interval of one month. In chemical check, coconut saplings were injected with 2 ml Tridemorph diluted with 100 ml water, whereas in control, the saplings were inoculated with pathogen only. In addition, saplings were maintained without any treatment (healthy control). All the treatments were replicated thrice in Completely Randomized Design. Periodical observations on sapling growth, girth and number of leaves were taken.

Assay of defense-related proteins and enzymes

One gram of root sample was homogenized with 2 ml of 0.1 M sodium citrate buffer (pH 5.0) at 4 °C. The homogenate was centrifuged for 20 min at 10 000 rpm. The superna-

tant was used as crude enzyme extract for assaying chitinase (Boller and Mauch, 1988) and ß-1,3-glucanase (Pan et al., 1991) activity. Enzyme extracted in 0.1 M sodium phosphate buffer (pH 7.0) was used for the estimation of peroxidase (PO) (Hammerschmidt et al., 1982), polyphenol oxidase (PPO) (Mayer et al., 1965) and phenylalanine ammonia-lyase (PAL) (Ross and Sederoff, 1992). Enzyme extract was stored in at –70 °C until used for biochemical analysis. The phenol content was estimated as per the procedure given by Zieslin and Ben-Zaken (1993) and expressed as catechol equivalents mg^{-1} of protein.

Activity gel electrophoresis

The isoform profile of PO and PPO were examined by discontinuous native poly-acrylamide gel electrophoresis using tris buffer (native-PAGE) (Laemmli, 1970). Root samples were collected at the 4th day after the pathogen challenge for PO and at the 5th day after the pathogen challenge for PPO analysis during that time the activities of PO and PPO were at a maximum, respectively. The protein extract was prepared by homogenizing 1 g of root samples in 2 mL of 0.1 M sodium phosphate buffer pH 7.0 and centrifuged at 16 000 g for 20 min at 4 ºC. The protein content of the sample was determined by the Bradford method (Bradford, 1976). Samples (50µ g protein) were loaded onto 8% poly-acrylamide gels (Sigma, USA). After electrophoresis, PO isoforms were visualized by soaking the gels in a staining solution containing 0.05% benzidine (Sigma, USA) and 0.03% H_2O_2 in acetate buffer (20 mM, pH 4.2) (Sindhu et al., 1984). For assessing PPO isoforms profile, the gels were equilibrated for 30 min in 0.1% *p*-phenylenediamine followed by addition of 10 mM catechol in the same buffer (Jayaraman et al., 1987).

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. The percentage values of the disease index were arcsine transformed. Data were subjected to analysis of variance (ANOVA) and means were compared by Duncan's Multiple Range Test (DMRT).

Results

Bacterial endophytes

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

Rice growth promotion and in vitro *screening*

Among the isolates that promoted high growth of rice, five strains were found to inhibit the growth of *G. lucidum in vitro*. The strain, Pf1 showed high inhibition to *G. lu-*

cidum followed by EPC5 (coconut root isolate) and EPC8 (coconut root isolate). The bioagents EPC5, Pf1 and *T. viride* were also 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* covered the plate within 6 days after placing the disc.

Identification

Biochemical tests showed that the isolates EPC5, EPC8 were belonging to *Bacillus* genera. Further, cloning and sequencing of 16s rDNA of EPC 5 and EPC 8 confirmed that they belong to *Bacillus subtilis* (Accession No. EF139862 for EPC 5; EF139863 for EPC 8). Pf1 strain also acted as endophyte already characterized from our laboratory.

Growth promotion in coconut seedlings

Coconut seedlings treated with endophytic bacteria in different combinations promoted seedling growth under greenhouse conditions. Endophytic *Bacillus* combined with *Pseudomonas* and *T. viride* at the rate of 30 g (10 g each) per seedling promoted the growth of seedling (height of seedling), girth and number of leaves compared to 15 g of individual, combined application and other treatments in cultivar ECT coconut seedlings. After one year of seedling growth, the bioagents mixture EPC5 + Pf1 + Tv at 30 g recorded seedling height of 156.5 cm, girth of 20 cm and seven leaves when compared to the height (123.5 cm), girth (11.8 cm) and five leaves of inoculated control seedling (Table 1).

Induction of defense enzymes in phenylpropanoid pathway

The assay of defense enzymes revealed that PGPR mixture bioformulations induced a greater amount of enzymes than individual and the control plants. EPC5 + Pf1 + Tv treated coconut seedlings challenged with *G. lucidum* recorded higher levels of PO activity up to five days of inoculation and declined thereafter throughout the experimental period of nine days. In healthy control, lesser activity was observed (Fig. 1a). Combined application of bioagents bioformulation led to the enhanced activity of PPO compared to individual applicaton. EPC5 + Pf1 + Tv recorded higher PPO activity up to the fifth day after challenge inoculation followed by EPC5 + Tv compared to other treatments. The control seedlings recorded less induction of PPO throughout the experimental period of nine days and showed steep decline on the fifth day after challenge inoculation (Fig. 1b).

PAL activity was significantly higher in bioagents treated coconut seedlings challenged with *G. lucidum* than the untreated inoculated seedlings. The enzyme activity reached the maximum on third day of challenge inoculation and thereafter it declined. In control seedlings, the activity slowly increased and started declining after the third day (Fig. 1c). The application of mixture of bioagents resulted in increased accumulation of phenolics than the individual bioagent application. The phenolic activity was increased significantly in the seedlings treated with mixture of bioagents than the control seedlings due to the colonization of growth promoting bacteria and fungus by re-isolation, plating (Fig. 1d). The effect of various bioagent isolates on chitinase activity in coconut seedlings

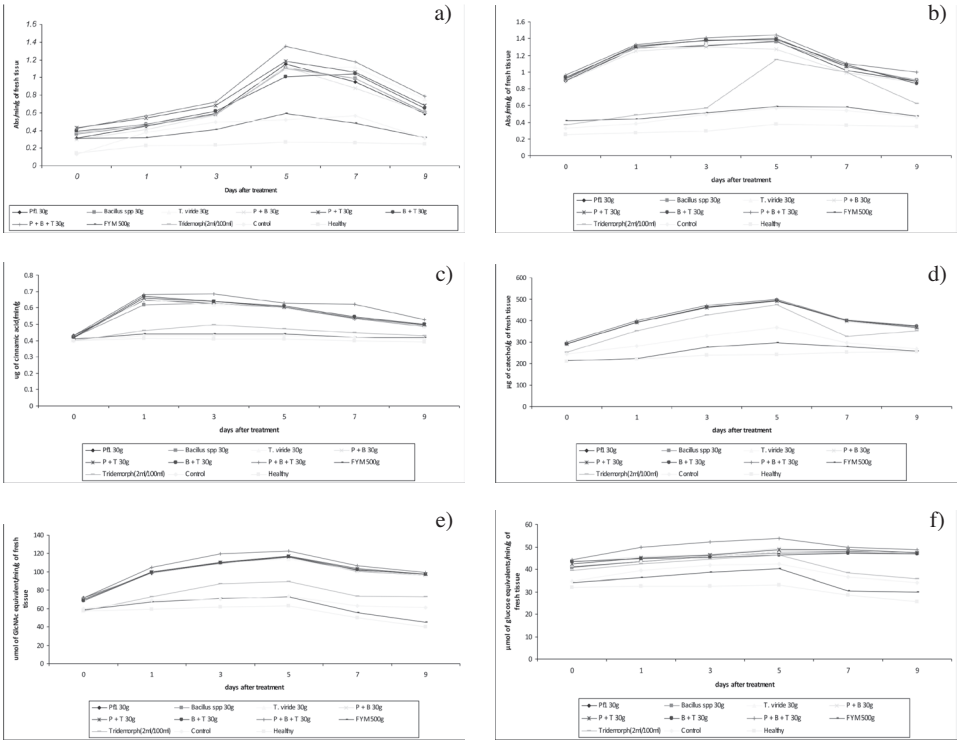

Fig. 1. Induction of (a) peroxidase, (b) polyphenol oxidase, (c) phenylalanine ammonia lyase, (d) total phenols, (e) chitinase and (f) β-1,3-glucanase in coconut seedlings treated with bioagents against *G. lucidum*

with or without *G. lucidum* was observed. Among the different treatments, EPC5 + Pf1 + Tv recorded higher activity of chitinase than the inoculated control seedling (Fig. 1e). The results showed that the activity of β-1,3-glucanase was maximum in bioagents treated coconut seedlings challenged with *G. lucidum* than the other treatments. The induction was more in EPC5 + Pf1 + Tv whereas in control the enzyme activity increased but not to the level of bioagents treated seedlings (Fig. 1f).

Native PAGE analysis

Native PAGE analysis of the peroxidase revealed that EPC5 + Pf1 + Tv treated coconut seedlings challenged with basal stem rot pathogen induced additionally four PO isoforms *viz.,* PO3, PO4, PO6 and PO8 with more intensity compared to inoculated control and other treatments. In case of chemical treatment, PO7 is additionally induced (Fig. 2).

Bioagents individually and in combination treated coconut seedlings showed different PPO isozyme patterns. The enzyme extract from the bioagents treated seedling challenged with *G. lucidum* showed nine PPO isoforms *viz.,* PPO1, PPO2, PPO3, PPO4, PPO5, PPO6, PPO7, PPO8 and PPO9 while in the control plants only three isoforms

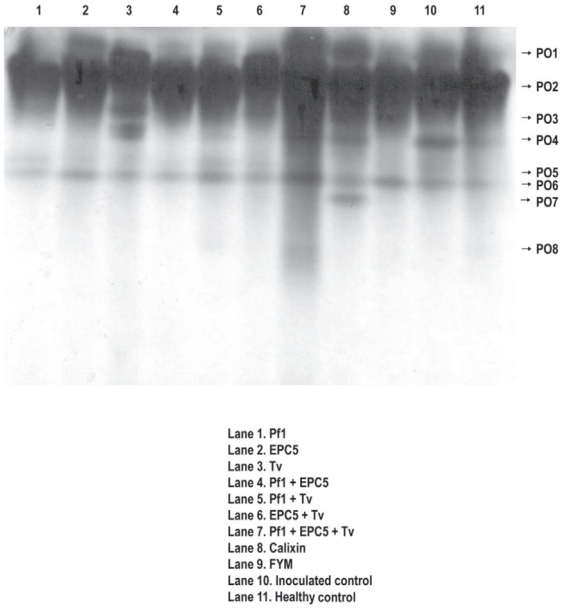

Fig. 2. Inducion of peroxidase isoforms in coconut seedlings treated with bioconsortia against *Ganoderma* infection

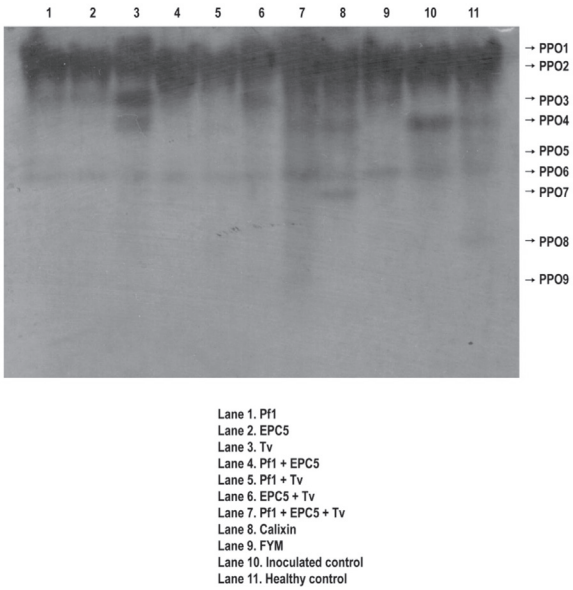

Fig. 3. Inducion of polyphenol oxidase isoforms in coconut seedlings treated with bioconsortia against *Ganoderma* infection

PPO2, PPO4 and PPO6 were induced with low intensity. Interestingly in EPC5 + Pf1 + Tv treated and challenged seedlings, four additional isoforms (PPO3, PPO4, PPO8 and PPO9) were induced. The induction of new isoforms was well pronounced in this treatment compared to other treatments (Fig. 3).

Discussion

Coconut palms were affected by many pests and diseases. Among them, the basal stem rot (BSR) disease caused by *Ganoderma lucidum* is widely present in nature (Ramadoss, 1991). The pathogen is soil borne and spread to nearby palms by root contact. The majority of farmers are practicing chemical application as root feeding for the management of this pathogen. This may lead to adverse effects like toxicity, residual effect and resistance development. To reduce this, bioconsortia approach using different bioagents has been tried in our study. The result showed that the endophyte is successfully isolated from root region of coconut plantation. Cho et al. (2003) stated that endophytic colonization of balloon flower by *Bacillus* sp. CY22 was without any harm to the root.

Bacillus species are among the most common bacteria found to colonize plants endophytically (Lilley et al., 1996; Mahafee and Kloepper, 1997) and it is likely that their endophytic ability could play an important role in the biocontrol of vascular plant pathogens (Nejad and Johnson, 2000). In our study, fifty-five bacterial endophytes in total were isolated from the roots of coconut palms and the isolates EPC5, EPC8, EPC15, EPC29 and EPC52 were found to increase the vigour index of rice seedlings. Certain growth promoting substances and secondary metabolites produced by both fungal and bacterial biocontrol agents might be responsible for the better plant growth (Saravanakumar et al., 2007; Shanmugaiah et al., 2009). EPC5, Pf1 effectively inhibited the growth of *Ganoderma lucidum in vitro* which supports the finding of Pandey et al. (2006) that reported that the antagonistic strain of *Pseudomonas putida* (B0) isolated from a sub-alpine exhibited antifungal activity against phytopathogenic fungi in Petri dish assays and produced chitinase, ß-l,3-glucanase, salicylic acid, siderophore and hydrogen cyanide. An array of antifungal compounds including iturin produced by the *Bacillus* is responsible for the inhibitory effect on plant pathogens (Gumede, 2008). Various phenotypic and molecular methods have been developed and used for characterizing bacterial isolates. The result showed that the EPC 5 and EPC 8 belonged to *Bacillus subtilis* based on biochemical tests, cloning and sequencing of 546 bp region (Rajendran et al., 2008). Similarly, Tilak and Srinivasa Reddy (2006) identified isolates from maize as *Bacillus cereus* and *B. circulans* by biochemical characteristics and profile of fatty acids. The soil borne filamentous fungus *Trichoderma virens* is a biocontrol agent with a well known ability to produce antibiotics, parasitize pathogenic fungi and induced systemic resistance in plants. These are among the most studied fungal biocontrol agents and are successfully used as biopesticides (Harman et al., 2004). In our study, *T. viride* strongly inhibited the growth of *G. lucidum in vitro*. Similarly, Chakrabarty et al. (2013) reported that *T. viride* was most effective (66.55%) among three different species of *Trichoderma* tested under dual culture study (*T. harzianum* 63.99% and *T. virens* 62.12%) over control after 96 hrs of incubation.

Pretreatment of cotton cotyledons with *Trichoderma* spp. provided high levels of protection to the foliar pathogen, *Colletotrichum* sp. and involved in the induction of resistance through the activation of plant defense mechanisms (Djonovic et al., 2006). Application of single biocontrol agent can have limitations with regard to consistency and efficacy in different environments (Gumede, 2008). Several works suggest that combinations of biocontrol agents could be more effective in controlling soil-borne pathogens than a single agent (Pierson and Weller, 1994; Duffy et al., 1996; Singh et al., 1999; Domenech et al., 2006 and Thilagavathi et al., 2012).

With the above advantages, the endophytic bacteria EPC 5 along with Pf1 and Tv1 as bioconsortia has been tried for the management of basal stem rot disease in coconut plantations. The bioconsortia promoted higher sapling growth and produced more accumulation of defense related enzymes compared to individual application. Similarly, Raupach and Kloepper (1998) demonstrated that the use of more than one biocontrol agent could be more effective than the use of a single one. It was observed that soil application of bioconsortia enriched with FYM promoted the coconut sapling growth *viz*., seedling height, girth, leaf area and number of leaves significantly under greenhouse conditions. It was supported by De Silva et al. (2000), leaf area and stem diameter of high bush blueberry were increased by applying *Pseudomonas fluorescens* Pf5. Esitken et al. (2003) found that application of *Bacillus* strain OSU 142 as foliar spraying at full bloom stage significantly increased the shoot length, yield and the nutrient element composition in apricot trees compared to control. Colonization of plant roots with certain beneficial microbes causes the induction of a unique physiological and biochemical state in plants called "priming" (Conrath et al., 2006). In the present study, bioconsortia treated coconut seedlings challenged with the basal stem rot pathogen showed higher induction of peroxidase activity with the induction of eight peroxidase isoforms. Recently, several peroxidase genes were cloned and studied in cotton plants during compatible and incompatible interactions with *X. campestris* pv. *malvacearum* (Delannoy et al., 2003). The PPO activity was higher in bioconsortia compared to individual treatments. Specific isoforms were induced in seedlings treated with bioconsortia formulation after challenge inoculation with pathogen and their expression was prominent when compared to untreated control seedling. Similarly, Chen et al. (2000) reported that various rhizobacteria and *P. aphanidermatum* induced the PPO activity in cucumber root tissues. PPO transcript levels increased in young leaves of tomato when matured leaflets were injured (Thipyapong and Steffens, 1997). The maximum accumulation of PAL upto 5 days of challenge inoculation of pathogen coincides with the time which is normally favourable for the pathogen. The increased activity of PAL may also be constituted for enhancing the resistance in coconut seedlings against basal stem rot disease. Chen et al. (2000) reported that high levels of PAL were induced in cucumber roots inoculated with *Pythium aphanidermatum* but roots treated with *Pseudomonas corrugata* had initially higher levels of PAL and levels were lower after challenging the plant with *P. aphanidermatum.* In the present study, increased activity of chitinase, β-1,3-glucanase and total phenols were recorded in seedlings treated with bioconsortia. Maurhofer et al. (1994) reported that induction of systemic resistance by *P. fluorescens* was correlated with the accumulation of β-1,3-glucanase and chitinase. Benhamou et al. (2000) reported that an endophytic

bacterium, *Serratia plymuthica* induced the accumulation of phenolics in cucumber roots following infection by *P. ultimum.*

Conclusions

Biochemical and molecular tools in this study helped to identify *Bacillus* strains (EPC 5 and EPC 8) from different ecosystems of Tamil Nadu. The management of soil borne pathogen namely BSR is particularly complex, because the disease occur in dynamic environment at the interface of the root with the soil. The use of endophytic bacteria as bioconsortia (EPC5 + Pf1 + Tv1) enriched with FYM performed well against basal stem rot disease in coconut saplings by activating defense enzymes.

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