African Journal of Agricultural Research Vol. 5(11), pp. 1324-1330, 4 June, 2010
Available online at http://www.academicjournals.org/AJAR
DOI: 10.5897/AJAR09.541
ISSN 1991-637X © 2010 Academic Journals

Full Length Research Paper

Correlation between invasion by endophytic fungus *Phomopsis* sp. and enzyme production

Chuan-chao Dai*, Yan Chen, Lin-shuang Tian and Yang Shi

Jiangsu Engineering and Technology Research Center for Industrialization of Microbial Resources, Jiangsu Key Laboratory for Biodiversity and Biotechnology, College of Life Science, Nanjing Normal University, Nanjing 210046, China.

Accepted 12 May, 2010

The possible mechanisms of plant colonization by endophytic *Phomopsis* sp. strain B3 was examined in this study. The ability for the endophytic fungi to produce cavities in the surface of straw and the conditions influencing the laccase production were investigated. The results showed that endophytic fungus B3 could decompose plant materials by producing laccase to form a series of cavities on straw. Fermentation test indicated that using cellulose-Na as the optimal carbon source, NH_4Cl as optional nitrogen sources and keeping the culture temperature at 25°C could significantly increase the production of laccase. Both ways of using host leaf instead of potato extract as culture basis and adding NaCl to culture medium could inhibit the fungal production of laccase, but have little influence on the growth. Adjusting the product of laccase may be the key strategy for the strain B3's invasion.

Key words: *Phomopsis* sp., endophytic fungi, laccase, invasion, host.

INTRODUCTION

Endophytes are microorganisms that form symptomless infections within plants (Carroll, 1988; Guo, 2001). They have widespread existence in various plants. Although the mechanism is unclear, endophytic fungi actually played an important role in local ecology (Clay and Hoah, 1999; Omacini et al., 2001; Selosse et al., 2004). Presently, the route of endophytic fungal transmission could be mainly divided in two ways: horizontal and vertical transmission. Of the two groups of endophytes, systemic endophytes are transmitted vertically via the seeds, while non-systematic endophytes are transmitted horizontally with host colonization arising from the surrounding environment. As a common way of transmission, endophytic fungal vertical transmission could be described as seed reproduction, which is the same as the reproduction of most plant. However, the report of the mechanism of endophytic fungal horizontal transmission is rare. In this study, endophytic *Phomopsis* spp are non-systematic fungi lacking host specificity and have been used as model endophytes in many researches (Guo, 200, Horn et al., 1996; Murali et al., 2006; Dai et al., 2006). In an earlier study, the endophytic *Phomopsis* sp. strain B3 was isolated from *Bischofia polycarpa* (Dai et al., 2006). The fungus is thought to have a symbiotic relationship with rice, *Euphorbia pekinensis* and peanut, stimulating growth and acting as a pathogencide (Dai et al., 2005; 2010; Yuan et al., 2007). The fungus can colonize rice plants from infected mycelium in soil.

The question is how the fungus can become an endophyte when added into the environment. Several reports have suggested that some endophytic fungi invade plants via wounds made by insects, some enter the host through stomata and yet some just invade the host occasionally from wounds (Oliveira, 2004; Osono et al., 1999; Lemons et al., 2005). Whether endophytes can make wounds by themselves are not fully investigated. Based on the study's results (Shi et al., 2004; Yuan et al., 2007; Dai et al., 2008; 2010; Chen et al., 2010), the researchers hypothesized that *Phomopsis* strain B3 would possess the following characteristics: (1) capable of producing an enzyme(s) that facilitates entry through the surface of plant materials such as leaves or stems, (2) the plant materials could induce and fit the enzyme produced by the fungal strain, (3) the production of the enzyme can be inhibited when the fungus has entered the plant and (4) the cultural condition and temperature

*Corresponding author. E-mail: daichuanchao@njnu.edu.cn. Tel: 86-25-85891382 (O). Fax: 86-25-85891526.

for the production of the enzyme should be similar to those in the environment, which enable the endophyte invade and colonize its hosts.

MATERIALS AND METHODS

Endophytic fungi

Endophytic fungi *phomopsis* sp. used in this research was isolated from stem's inner back of *Bischofia polycarpa* identified in this laboratory and named as B3 (Dai et al., 2006). *Phomopsis* sp. strains B3 was maintained at 4°C on potato dextrose agar (potato extract 200 g/l, glucose 20 g/l and agar 20 g/l), sub-cultured every month and preserved by this laboratory. Inoculum was prepared by three days culture at 25°C with 150 rpm shaking speed in 100 ml PDA medium containing potato extract (from 200 g potato of every liter) as basis and 20 g/l of glucose.

Culture medium

Seed medium: PDA medium containing potato extract (from 200 g potato of every liter) as basis and 20 g/l of glucose.

Basal medium used in plant material decomposition: enzyme assay and temperature test contained potato extract (from 200 g potato), 3 g/l KH_2PO_4 , 1.5 g/l $MgSO_4 \cdot 7H_2O$, 0.01 g/l vitaminB_1, 5 g/l corn flour, 20 g/l starch and 7 ml mixed solution of trace metals, which was composed of 7.8×10^{-3} mol/l Amino acetic acid, 2.9×10^{-3} mol/l $MnSO_4 \cdot H_2O$, 1.7×10^{-2} mol/l NaCl, 3.59×10^{-4} mol/l $FeSO_4 \cdot 7H_2O$, 7.75×10^{-4} mol/l $CoCl_2$, 9.0×10^{-4} mol/l $CaCl_2 \cdot 2H_2O$, 3.48×10^{-4} mol/l $ZnSO_4 \cdot 7H_2O$, 4×10^{-5} mol/l $CuSO_4 \cdot 5H_2O$, 2.1×10^{-5} mol/l $KAl(SO_4)_2 \cdot 12H_2O$, 1.6×10^{-4} mol/l H_3BO_3 and 4.1×10^{-5} mol/l $NaMnO_4$ (Kirk et al., 1986). The media for carbon source test contained one of its 20 g/l, 5 g/l bean cake powder as nitrogen source, 3 g/l KH_2PO_4, 1.5 g/l $MgSO_4 \cdot 7H_2O$, potato extract (from 200 g potato) as basis, 0.01 g/l vitaminB_1 and 7 ml mixed solution of trace metals. The carbon sources include: glucose, starch and wood, wheat germ and straw powders, corn flour, CMC-Na (cellulose-natrium) and CMC-Na+ glucose.

The media for nitrogen source test contained 5 g/l of nitrogen source, 20 g/l starch as carbon source, 3 g/l KH_2PO_4, 1.5 g/l $MgSO_4 \cdot 7H_2O$, potato extract (from 200 g potato) as basis, 0.01 g/l vitaminB_1 and 7 ml mixed solution of trace metals. The nitrogen sources include glycine, ammonia chloride, ammonia sulfate, yeast extract, urea and bean cake powder. The host extract medium contained host extract (from 200g *Bischofia polycorpa's* leaves every liter), 20 g/l glucose, 3 g/l KH_2PO_4, 1.5 g/l $MgSO_4 \cdot 7H_2O$, 0.01 g/l vitaminB_1 and 7 ml mixed solution of trace metals. The composition of NaCl pressure medium was same as basal medium and added NaCl at different levels (0.1 per level, from 0 - 0.5 mol/l).

Inoculum preparation and flask cultures

After transferring the strain B3 onto PDA plates, the plates were incubated at 28°C for 4 d. Inoculum was prepared as follows: 3 pieces (10 × 10 mm) of fungal clump (fungal culture on agar) were inoculated into a 250 ml flask containing 50 ml seed medium and cultured for 3 d with shaking (150 rpm) at 25°C (n = 4). The four different culture temperatures were compared at temperature test (20, 25, 30 and 40°C). Then, 5 ml seed were added to 50 ml basal medium in 250 ml flask at 25°C with shaking (150 rpm) for 4 days. The fungal biomass was harvested by filtration through filter paper and the biomass yield was determined using oven-dried (80°C) mycelium weight. The filtrate was then treated as a raw enzyme liquid. The biomasses of mycelium cultured on different carbon resources were not compared with the ones cultured on some carbon resources such as wood powder, which could not be determined precisely.

Plant material decomposition test

Soil pots were employed for the test of plant material decomposition. 3 g peanut straws were put into each terylene cloth bag (10×10 cm). 5 ml B3 basal culture medium which contains 3 g B3 wet mycelium was added to some randomly chosen bags. Three treatments were used; (I) for sterilized soil added with terylene cloth bags which had added treated endophytic fungi, (2) treatment (II) for unsterilized soil added with terylene cloth bags which had added endophytic fungi and (3) treatment (III) for unsterilized soil added with terylene cloth bags which is without endophytic fungi and served as control (CK). Each treatment had five pots and when they were set outside, the temperature changed between 15 (night) – 258 0°C (day). Among the pots, one was selected from the plant material surface examination by a scanning electron microscope (SEM: JSM25610LV). The other four were used for chemical analysis (cellulose and lignin). The terylene cloth bags were implanted in the 600 g soil of the pots at 5 cm depth. There were two bags for each pot, one was harvested for 15 d and the other was for 30 d.

Enzyme assays

The extracellular medium of fungal cultures was used as enzyme source mentioned in the section "inoculum preparation and flask cultures". Laccase activity was measured by monitoring the oxidation of Benzidine at 600 nm, cellulase activity was determined by the filter paper hydrolysis-DNS (3,5-dinitrosalicylic acid) coloration at 520 nm and the polyphend oxidase assay was measured by the Brenzcatechin oxidation method (Shi et al., 2002). One unit of laccase activity and polyphend oxidase was defined as the amount of enzyme required to change OD value by 0.001 units per min at 600 and 200 nm, respectively. Lignin peroxidase was measured by the method of Tien et al. (1983). Manganese peroxidase activity was assayed by the oxidation of 1 Mm $MnSO_4$ in 50 mM sodium malonate and pH 4.5, in the presence of 0.1 mM H_2O_2. Manganic ions, Mn^{3+}, form a complex with malonate, which absorbs at 270 nm (Wariishi et al., 1992).

The determination of lignin and cellulose

The content of straw cellulose was determined by concentrated acid hydrolysis-DNS coloration of carbohydrate and straw lignin was determined by the method of concentrated sulfuric acid hydrolysis (Wang et al., 1987).

Data analysis

Variance analysis of results was performed by one-way ANOVA method using Origin 6.0 professional software. There are 4 triplicates for carbon resources, nitrogen resources, adding extract, NaCl and temperature treatment. Data of different C/N ratios on laccase production was the average of two triplicates.

RESULTS

Endophytic ability in producing wounds on the surfaces of peanut's straw

The decomposition experiment is to investigate the ability

Table 1. Decomposition rates (%) of plant materials by endophytic fungus B3.

Time (d)	15			30		
Treatment	**Total**	**Cellulose**	**Lignin**	**Total**	**Cellulose**	**Lignin**
(I)	36.76±3.45	33.62±4.31*	58.14±2.28**	48.25±2.82*	50.36±3.2**	61.76±3.45**
(II)	37.63±2.26	34.89±4.11**	53.55±2.81**	48.85±1.97*	53.09±4.82**	56.7±3.77**
(III) CK	30.76±6.64	26.23±1.85	43.64±2.17	37.39±6.2	31.98±7.2	49.14±0.98

*: significance at 0.05 level; **: significance at 0.01 level
I: sterilized soil and terylene cloth bags containing endophytic fungi; II: non-sterilized soil and terylene cloth bags containing endophytic fungi; (III) CK: soil and terylene cloth bags without endophytic fungi

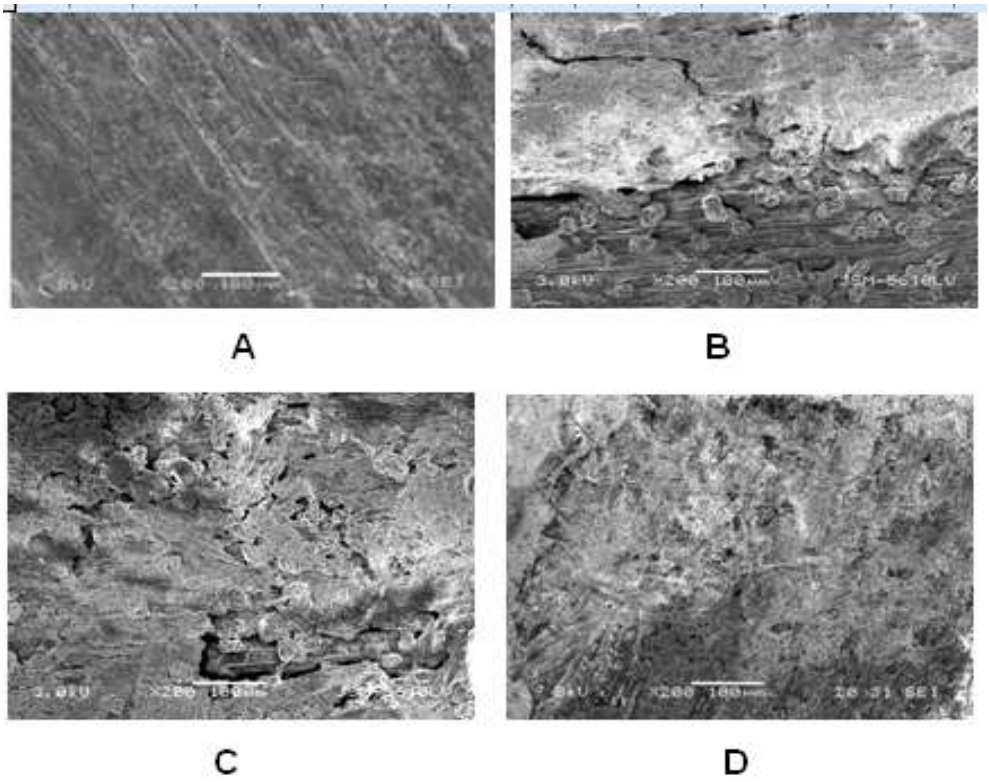

Figure 1. Surfaces of plant materials observed by SEM in 15 d. A: the original plant material; B: the plant material treated by B_3 in sterilized; C: the plant material treated by B_3 in non-sterilised soil; D: CK.

of endophyte in producing wounds in the surface of plant materials (Table 1). The results showed that the decomposition of treated plant materials with endophyte-treated samples was faster than the control. The difference was particularly prominent for lignin decomposition after 15 d, which was faster than that of cellulose. It is possible that strain B3 takes lignin component of the plant materials as an entry point for further decomposition. Figure 1 showed the plant material treated by strain B3 and their CK in 15 d. According to the photos, cavities (as entry points) could be found in the surface of the plant material and holes treated by endophytic fungi B3 were apparently bigger than that of CK.

The activities of decomposition enzymes after submerged culture

The activities of laccase, polyphend oxidase and cellulase of the strain under submerged culture were determined, while those of lignin and manganese peroxidase were too low to be detected. The activity of laccase which corresponds to the high lignin decomposition rate was the highest (Table 2).

Effects of carbon and nitrogen sources on laccase production

The effect of eight types of carbon sources including;

Table 2. Activities of decomposition enzymes under submerged culture (n = 3).

Enzyme	Laccase	Polyphend oxidase	Manganese peroxidase	Lignin peroxidase	Cellulase
Activity (U/ ml)	66.90 ± 3.21	17.03 ± 0.23	ND	ND	0.07± 0.01

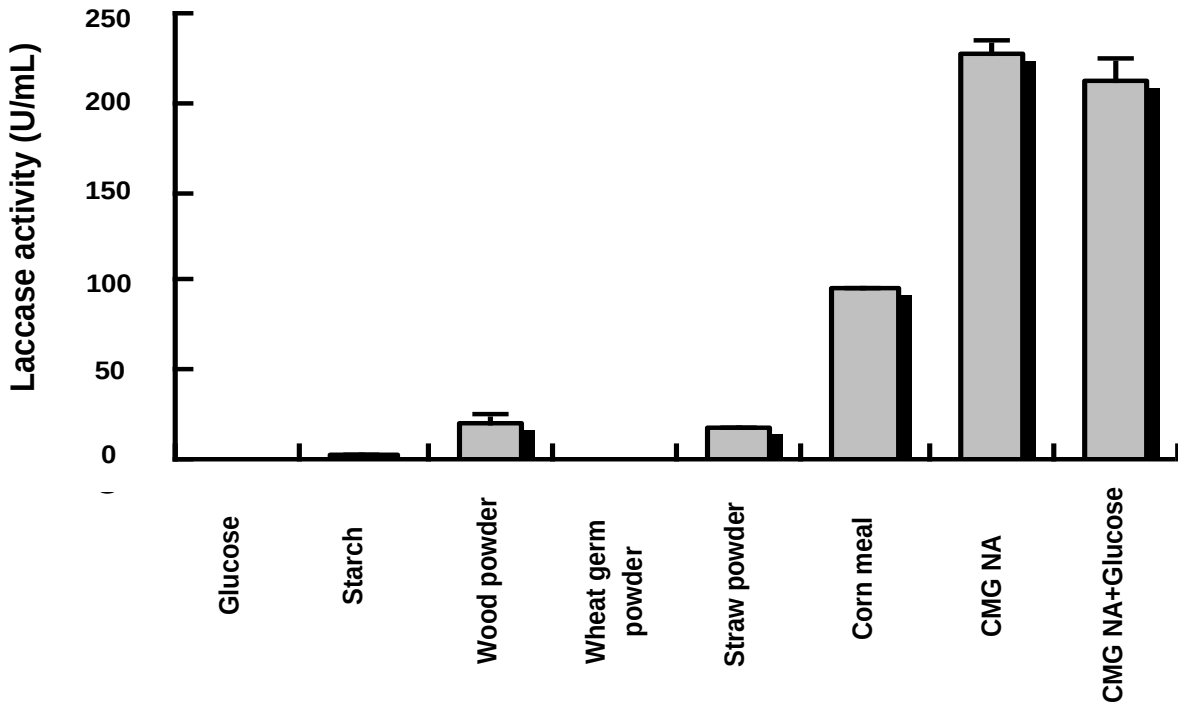

Figure 2. Effects of carbon resources to laccase activity

Table 3. Effects of different nitrogen resources to laccase activity and biomass.

Nitrogen sources	Glycine	Ammonia Chloride	Ammonia sulfate	Yeast extract	Urea	Bean cake powder
Biomass (g/l)	3.88±0.12	4.06±0.03	2.51±0.11	3.55±0.09	3.97±0.25	6.68±0.14
Laccase (U/ml)	92.52±4.41	126.50±1.09	119.45±2.98	114.15±8.09	4.40±0.18	122.28±2.40

glucose, starch, (wood, wheat germ and straw powders), corn flour, CMC-Na (cellulose-natrium) and CMC-Na + glucose, were compared in Figure 2. The best carbon source was CMC-Na, followed by CMC-Na + Glucose. Glucose and wheat germ powders were not ideal carbon sources. These results indicated that laccase production in strain B3 might be induced by CMC-Na. Due to cellulose which is a main component of peanut straw, the induction of laccase may reflect the speciality of this strain of endophytic fungi. Straw and wood powders did not effectively induce laccase as high as CMC-Na. Its reason was unknown, but may be related to the components of the powdered materials.

In the comparison of six types of nitrogen sources (glycine, urea, yeast extract, bean cake powder, ammonia sulfate and ammonia chloride) to starch as carbon source (Table 3), although the concentration of laccase induced by NH_4Cl was a little higher than other treats, all nitrogen sources demonstrated little changes to laccase production except for urea that inhibited it. The researchers also investigated the changes of laccase production with the concentration of carbon source using CMC-Na from 5 to 20 g/l and the concentration of nitrogen source using NH_4Cl from 0.5 to 2.0 g/l. The results showed that high concentration of carbon/nitrogen ratio, benefited laccase production (Table 4). When the medium was contained with 20 g/l CMC-Na and 1 g/l NH_4Cl, the laccase activity was as high as 225.3 U/ml in the fermentation broth. These results were consistent with those reported by Buswell et al. (1995) using

Table 4. Effects of different C/N ratios on laccase production and biomass of B3.

Nitrogen source (g/l)	Carbon source (g/l)							
	20		15		10		5	
	Laccase activity (U /ml)	Biomass (g/l)	Laccase activity (U /ml)	Biomass (g/l)	Laccase activity (U /ml)	Biomass (g /l)	Laccase activity (U /ml)	Biomass (g/l)
5	26.77	3.88	45.52	3.85	13.83	2.65	3.02	2.62
2	36.52	2.96	55.62	3.44	15.02	3.07	16.6	2.73
1	225.32	2.87	85.87	2.63	19.63	4.13	56.79	2.54
0.5	221.27	2.68	135.57	2.45	23.59	3.30	60.07	1.35

Table 5. Effects of *Bischofia polycarpam* extract on laccase activity and biomass of B3.

***Bischofia polycarpam* extract (ml/l)**	**0**	**200**	**400**	**600**	**800**	**1000**
Biomass (g/l)	2.35±0.03	3.49±1.06	4.29±0.04	5.63±0.36	7.51±0.57	8.06±0.13
Laccase (U /ml)	46.89±4.37	0	0	0	0	0

Table 6. Effects of NaCl on laccase activity and biomass of strain B3.

NaCl (mol/l)	0	0.1	0.2	0.3	0.4	0.5
Biomass (g/l)	5.12±0.74	5.00±0.16	5.07±0.69	5.91±0.99	7.94±0.04	6.09±0.48
Laccase (U /ml)	46.89±4.37	21.01±4.92	0.02±0.02	0	0	0

Table 7. The effect of temperature to laccse activity and biomass (n = 4).

Temperature (°C)	Biomass (g/l)	Laccase (U /ml)
20	6.69±0.12	176.09±58.81
25	6.89±0.05	136.63±1.97
30	4.85±0.09	55.65±9.57
40	0	0

Lentinula (Lentinus) edodes (1995). When the medium consisted of carbon 20 g/l and NH_4Cl 1 g/l, the biomass reached the peak of 4.13 g/l.

Effects of host extract or NaCl on laccase production

To observe the effects of the host chemical composition on laccase production, the extracts of host *Bischofia polycarpa* leaves were used as substitute for potato extract (Table 5). Although the biomass increased with increasing extract, the laccase activity could not be detected. These might be interpreted as an adaptation between endophytic fungi and its host. The nutrition from the host might benefit the growth of endophytic strains, but it seemed to inhibit the production of laccase. This presumption was consistent with the situation that endophytic fungi live in between plant cells without destroying the cells (Oses et al., 2006). The effects of osmotic pressure on the production of laccase were determined by adding different concentrations of NaCl from 0.1 to 0.5 mol/l (Table 6). Production of laccase dropped dramatically with the presence of NaCl and could not be detected when NaCl concentration was higher than 0.2 mol/l. However, the biomass of fungi increased with the addition of NaCl, which indicated that strain had the ability to resist saline conditions.

Effects of culture temperature on laccase production

The effects of four culture temperatures (20, 25, 30 and 40°C) were examined and the results showed that 20°C was the optimal culture temperature for the production of laccase (Table 7). The biomass was the greatest at 25°C and the fungus did not grow at 40, therefore, the temperature from 20 to 25°C was probably the most

favorable for inducing endophytic fungi to invade the host.

DISCUSSION

There must be a period for endophytes outside the plant to transmit horizontally. The question of how endophytes then get back into a plant is very interesting. In this study, *Phomopsis* sp. B3 which has an extensive host range was examined. For this reason, the researchers hypothesize that the endophyte can produce enzymes to make the necessary wound, allowing mycelium to invade the plant. From the study, it is suggested that endophyte and perhaps others can produce decomposition enzymes and create entry points on the surface of plants. For strain B3, laccase is the main enzyme capable of doing this. It is not yet clear why the endophyte does secrete the decomposition enzyme after it invades the plant. The research study showed that NaCl and host-leaf extracts inhibit the production of laccase. The researchers suggest that the endophyte does not produce the decomposition enzyme until there is an appropriate carbon source available. This could explain why endophytic *Stagonospora* sp. can penetrate the cell walls of root epidermis, but did not proliferate in the cortex of *Phragmites australis* (Gao et al., 2006). Studies show that some endophytic fungi can produce laccase or utilize plant material such as lignin or cellulose (Carroll and Petrini, 1983; Koide et al., 2005a, 2005b; Lemons et al., 2005; Osono and Takeda, 1999; Oses et al., 2006). These support the research findings.

Humid and rainy conditions and temperature between 20 to 25°C are suitable for endophyte transmission. These are consistent with the research laccase production results. As lignin is located outside cellulose, the researchers suppose that enzyme production is sufficient for the intrusion of the endophyte. The entry holes could be made through decomposition of lignin and cellulose. The optimum conditions for laccase production in strain B3 were very different from those reported by Wang et al. (2006) for *Monotospora* sp. For *Monotospora* sp. the optimum conditions included a culture temperature of 30°C, maltose as carbon source and ammonium tartrate as nitrogen source. Also, the research results differed from the reports of Xiao et al. (2001) on a white rot fungus and these may reflect B3's host range and endophyte speciality. Further studies should be conducted on the transmission processes in strain B3 and the techniques used in the present study may be employed for other endophytes.

ACKNOWLEDGEMENTS

This work was financially supported by the fund from National Natural Science Foundation of China (No. 30970523, 30770073). The authors express their great thanks to anonymous reviewers and editorial staff for their time and attention.

REFERENCES

Buswell JA, Cai Y, Chang ST (1995). Effect of nutrient nitrogen and manganese on manganese peroxidase and laccase production by *Lentinula (Lentinus) edodes*. FEMS Microbiol. Lett.. 128: 81-88.

Clay K, Holah J (1999). Fungal endophyte symbiosis and plant diversity in successional fields. Sci, 285: 1742-1744.

Carroll G, Petrini O (1983). Patterns of substrate utilization by some fungal endophytes from coniferous foliage. Mycol, 75(1): 53-63.

Carroll G (1988). Fungal endophytes in stems and leaves: from latent pathogen to mutualistic symbiont. Ecol. 691: 2-9.

Chen Y, Dai CC, Wang XX, Zhang B, Ju Q (2010). Effects of endophytic *fungus Phomopsis* sp. on decomposition of plant *(Atractylodes lancea*) Litters and activity of degrading enzymes in soil. Acta pedologica sinica 47(3): 134-141.

Dai CC, Yu BY, Zhao YT, Dong C, Jiang JH (2005). The rapid propagation of medicinal plant *Euphorbia pekinensis*. Guihaia 25(2): 152-155.

Dai CC, Yu BY, Zhao YT, Jiang JH, Yang QY (2006). The screening and identification of endophytic fungi from four species of family *Euphorbiaceae* and the strain sp. antibacterial activity. J. Nanjing Forestry University (Natural Sciences Edition) 30(1): 79-83.

Dai CC, Xie H, Wang XX, Li PH, Li YL,Zhang TL (2010). The effects of intercropping with medicinal plants and addition of endophytic fungi on soil microflora and peanut yield. Acta ecologia sinica 30(8): 2105-2111.

Dai CC, Yuan ZL, Yang QY, Shi QS, Li X (2008). The effect of increasing production of endophytes *Phomopsis* B3 on rice. Agric. Sci. Technol. 9(1): 39-42

Faeth SH (2002). Are endophytic fungi defensive plant mutualists. OIKOS 98: 25-36.

Freeman S, Fodriguez RJ (1993). Genetic conversion of a fungal plant pathogen to a nonparhogenic, endophytic mutualist. Sci. 260: 75-78.

Gao KX, Mendgen K (2006). Seed-transmitted beneficial endophytic *Stagonospora* sp. can pentrate the walls of the root epidermis, but does not proliferate in the cortex, of *Phragmites australis*. Can. J. Bot. Canadienne de botanique 84(6): 981-988.

Guo LD (2001). Advances of researches on endophytic fungi. Mycosystema, 20(1): 148-152.

Horn WS, Simmonds MSJ, Schwartz RE, Blaney WM (1996). Variation in production of phomodiol and phomopsolide B by *phomopsis* spp. Mycologia 88(4): 588-595.

Kirk TK, Croan S, Tien M (1986). Production of multiple liginases by *Phanerochaete chrysosporium*: effect of selected growth conditions and use of a mutant strain. Enzyme Microb. Technol. 1986 8: 27-32.

Koide K, Osono T, Takeda H (2005). Colonization and lignin decomposition of *Camellia japonica* leaf litter by endophytic fungi. Mycoscience 46: 280-286.

Koide K, Osono T, Takeda H (2005). Fungal succession and decomposition of *Camellia japonica* leaf litter. Ecol. Res. 20: 599-609.

Lemons A, Clay K, Rudgers JA (2005). Connecting plant-microbial interactions above and belowground: a fungal endophyte affectes decomposition. Oecol, 145: 595-604.

Murali TS, Suryanarayanan TS, Geeta R (2006). Endophytic *phompsis* species: host range and implication for diversity estimates. Can. J. Microbiol. 52(7): 673-680.

Oliveira ALM, Canuto EL, Silva EE, Reis VMJ, Baldani I (2004). Survial of endophytic diazotrophic bacteria in soil under different moisture levels. Braz. J. Microbiol. 35: 295-299.

Omacini M, Chaneton EJ, Ghersa CM, Muller CB (2001). Symbiotic fungal endophytes control insect host parasite interaction web. Nature 409: 78-80.

Osono T, Takeda H (1999). Decomposing ability of interior and surface fungal colonizers of beech leaves with reference to lignin decomposition. Eur. J. Soil Biol. 35(2): 51-56.

Oses R, Valenzuela S, Freer J, Baeza J, Rodrı′guez J (2006). Evaluation of fungal endophytes for lignocellulolytic enzyme production and wood biodegradation. Int. Biodeterior. Biodegrad. 57: 129-135.

Rudgers JA, Koslow JM, Clay K (2004). Endophytic fungi alter relationship between diversity and ecosystem properties. Ecol. Lett. 7: 42-51.
Saikkonen K, Wäli P, Helander M, Faith SH (2004). Evolution of endophyte-plant symbioses. Trends Plant Sci. 9(6): 275-280.
Selosse MA, Baudoin E, Vandenkoornhuyse P (2004). Symbiotic microorganisms, a key for ecological success and protection of plant competes. Rendus Biol. 327: 639-648.
Shi Y, Dai CC, Lu L, Yu BY (2002). Comparison of the extracellular enzyme activities of the endophytic fungi in four species of medicinal plants from *Euphorbiacea*. J. Plant Resour. Environ, 11(2): 17-20.
Shi Y, Dai CC, Wu YC, Yuan ZL (2004). Study on the degradation of wheat straw by endophytic fungi. Acta Scientiae Circumstantiae. 24(1): 144-149.
Stierle A, Strobel G, Stierle D (1993). Taxol and taxane production by *Taxomyces andreanae,* an endophytic fungus of Pacific yew. Sci, 260: 214-216.
Tien M, Kirk TK (1983). Lignin degrading from *Phanerochaete chrysosporium*: purification, characterization and catalytic properties of unique H_2O_2-requiring oxygenase. Proc. National Aca. Sci. 81: 2280-2284.
Wang JW, Wu JH, Huang WY, Tan RX (2006). Laccase production by *Monotospora* sp., an endophytic fungus in *Cynodon dactylon*. Bioresour. Technol. 97(5): 786-789.
Wang WY, Xu WY (1987). Process for hemicellulose, cellulose and lignin in the solid substrate fermentation of lingocellulose. Microbiology 14(2): 81-84.
Wariishi H, Valli K, Gold MH (1992). Manganese(II) oxidation by manganese peroxidase from the basidiomycete *Phanerochaete chrysosporium*. J. Biol. Chem. 267: 23688-23695.
Xiao YZ, Zhang M, Wu J, Wang YP, Hang J, Zeng WY, Shi Y (2001). Factors of laccase producing and fermentation conditions by a new white-rot fungus AH2822. Chinese. J. Biotechnol. 17(5): 579-583.
Xie LH, Wang GH,Yang MH (2006). Endophytic fungi and their effects on the ecological adaptability of host plants. J. Fungal Res. 4(3): 98-106.
Yuan ZL, Dai CC, Li X, Tian LS, Wang XX (2007). Extensive host range of an endophytic fungus affects the growth and physiological functions in rice (*Oryza sativa* L.), Symbiosis 43: 21-28.