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IN VITRO INTERACTIONS BETWEEN *RIGIDOPORUS LIGNOSUS*, THE CAUSE OF WHITE ROOT DISEASE OF RUBBER AND SOME POTENTIALLY ANTAGONISTIC FUNGI

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ABSTRACT

The modes of action of fungi potentially antagonistic against *R. lignosus* were assessed *in vitro* by studying their ability a) to cause pre– or post–contact damage to *R. lignosus* hyphae in video taped interactions on thin purified water agar films and, b) to displace the established pathogen from woody inocula. The most antagonistic fungi were *Trichoderma* spp. which produced volatile or non–volatile inhibitors. Basidiomycetes such as *Trametes* sp. caused hyphal interference similar to that of *Phlebiopsis gigantea*, a commercial biocontrol agent of *Heterobasidion annosum* in pine stumps. Species of *Trametes* and *Aspergillus* partially or completely displaced *R. lignosus* from previously colonized woody inocula.

Key words: hyphal interference, antagonism, *Hevea brasiliensis*, *Rigidoporus lignosus*, biocontrol.

INTRODUCTION

White root disease of *Hevea brasiliensis* (Willd. ex Adr. & Juss) Mull. Arg., caused by *Rigidoporus lignosus* (Kl.) Imaz. is most destructive among the most of the rubber growing countries. The causal agent *R. lignosus* spreads through infected roots and mycelial aggregates (cords) through soil to nearby healthy trees causing new infections. Infection causes blockage of the vascular system of the tree which subsequently become unproductive for the industry. Infection of young plants depending on the severity of the infection, can cause a rapid death.

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Since 1930s many chemicals and control measures had been tested and recommended for the control of the disease (Napper, 1939; John, 1958; Riggenbach, 1961; Peries *et al.*, 1963; Peries, 1965; Fox, 1966; Gohet *et al.*, 1991). However, considering to the economy of the industry, the control of the disease by these methods is not profitable. Therefore, biological control of the pathogen can be an alternative for costly treatments.

The best example for successful biocontrol of a root pathogen was the control of *Heterobasidion annosum* root disease of pine trees by *Peniophora gigantea* (*Phlebiopsis gigantea*). Oidiospores of *P. gigantea* were successfully used by Rishbeth (1963) for inoculation of pine stumps directly after felling to protect against *H. annosum*. While checking the pathogen entering and advancing to healthy tissues it also replaced the pathogen to some extent from previously infected roots. The mode of action of *Phlebiopsis* on *H. annosum* was subsequently found to be contact-mediated inhibition, termed 'hyphal interference' (Ikediugwu *et al.*, 1970; Ikediugwu, 1976). They found no evidence that *Phlebiopsis* gained directly from this antagonism, i.e. no evidence that it acts as a mycoparasite. Instead hyphal interference was suggested to be a mechanism in which *Phlebiopsis* can antagonise other fungi that are potential competitors for the same underlying substrate.

Hyphal interference is a common feature among basidiomycetes. *R. lignosus* is also a basidiomycete, and therefore it is of interest to examine the effect of other basidiomycetes that antagonise by hyphal interference. In this paper we describe hyphal interactions of *R. lignosus* and other fungi potentially antagonistic to *R. lignosus* and efficacy of selected antagonistic fungi to displace *R. lignosus* from its established substrate. This research was conducted partially in Scotland, UK and in Sri Lanka.

MATERIALS AND METHODS

Culture media

Purified water agar (PWA): Bacto agar (Difco), 20g; distilled water, 1 liter.
Water agar (WA): agar(Oxoid No. 3) 20g; distilled water, 1 liter.

Fungi

The isolate of *Lantinus squarrosulus* (strain 55A) was obtained from France. *Trichocladium asperum* (ED1) was isolated from soils collected from Edinburgh, UK. Species of *Trichoderma* (isolates G1, DFTS, DZE6, KGRAO) and *T. longibrachiatum* (isolate DZE10) were isolated from soils collected in Sri Lanka on *Trichoderma* selective medium. *T. longibrachiatum* (isolate DZE11P), *T. koningii* (isolate G10P1), *Fusarium* sp. (isolate DFS4), *Aspergillus* sp. (isolate DFS12) and black toruloid fungus (isolate DFS18), *Penicillium* spp. (isolates DZS1, DFS3, DFS7) were isolated from soils collected in Sri Lanka by the precolonised agar plate method (refer to the text). *Phlebiopsis gigantea*, *T. harzianum* (strain TV12b), *T. viride* (strain TV62), *T. koningii* (strain R5,24) were obtained from the culture collection of ICMB, University of Edinburgh, UK. *Trametes* spp. (isolates ISO16, KIR8), *Schizophylum commune* (isolate ISO12) were isolated from fruit bodies collected from Sri Lanka.

Methods

The *R. lignosus* (isolate RT1) used throughout this study was obtained from infected rubber roots collected from Ratnapura, a rubber growing area in the wet zone of Sri Lanka. The fungus was stored in 1 cm^3 elm wood blocks that had been previously autoclaved. Fungi antagonistic against *R. lignosus* were isolated from soil by the precolonised agar plate method described by Foley & Deacon (1985) or from soil plating on *Trichoderma* selective media (TSM) prepared as described by Papavizas (1982) with a slight modification, or from fruit bodies of wood decaying basidiomycetes collected from different sites of Sri Lanka (Jayasuriya, 1994). The soils used in this study also were collected from different agro-climatic zones of Sri Lanka. Most of them were red-yellow podzolic soils although some of them were reddish-brown earths. Scottish soils were clay loam soils. Each soil sample was bulked from 3-5 sub samples from the top 10 cm of the soil profiles as described above, then air-dried, sieved (2 mm mesh), mixed thoroughly and stored in polyethylene bags.

Preparation of woody inocula

Dried elm wood collected from the middle portion of logs was cut to sizes 1 or 2 cm^3, soaked in distilled water for 3 hours and autoclaved for 45 min. at 121 °C. The moisture content of the wood after autoclaving was 27-30% (w/w). All basidiomycetes were centrally inoculated on malt extract agar (MEA) plates (9 cm in diameter) and incubated until the colony margin reached the edge. Then the autoclaved wood blocks were aseptically placed on the mycelia of basidiomycetes. The lids were sealed with parafilm and were incubated for 8 weeks at 27±1 °C.

Assessment of different properties of fungi potentially antagonistic to *R. lignosus*.

All the isolated fungi were opposed against *R. lignosus* on dual membered plates as described by Dennis & Webster (1971) to recognize the species most antagonistic to *R. lignosus*. Those fungi which overgrew *R. lignosus* on culture media and/or caused inhibition of *R. lignosus* at a distance were considered as potential antagonists.

Inter-hyphal interactions of *R. lignosus* and antagonistic fungi were studied using video microscopy described by Laing & Deacon (1991). Sterile glass coverslips (38x38mm) were immersed in sterile molten purified water agar (PWA), and excess agar was drained off quickly such that only a thin layer of agar coated the coverslips and then the coverslips were laid horizontally on the surface of water agar (WA) plates. Agar discs (5 mm in diameter) of *R. lignosus* and antagonists were inoculated onto the agar films at different distances apart (e.g. 10–15 mm). The plates were sealed with parafilm and incubated overnight. Then the coverslips were removed from WA plates and inverted onto observation chambers coated in vaseline to seal the coverslips. The chambers were made from large microscope slides to which were glued lengths of glass capillary tubing (2 mm in diameter). The slides were placed under a microscope and interactions between *R. lignosus* and individual antagonists hyphae at the colony margins were observed by bright field microscopy using a x70 oil-immersion objective. The interactions were recorded using a video camera attached to a Leitz Orthoplan microscope. The equipment consisted of a Panasonic S-VHS F15 colour video camera, Panasonic S-VHS AG6720 video recorder with time laps facility and a Panasonic S-VHS BT M1420PY colour video monitor. Recordings were made on Master Broadcast S-VHS video tapes and video copy prints were obtained with a Mitsubishi video copy processor, model P61B.

Displacement of *R. lignosus* from wood blocks by antagonistic fungi in juxtaposed wood blocks.

Elm wood inocula prepared as previously described were paired with *R. lignosus* inocula using sterile rubber bands. The pairs of blocks were transferred to 9 mm diameter empty petri plates which were then filled with saturated sterile perlite. The dishes were then sealed with parafilm to maintain moisture, and incubated at 27

°C. Wood blocks of *R. lignosus* were paired with sterile wood blocks as control. Six replicates were employed for each antagonists isolate. After 10 weeks, when the control wood blocks (without antagonists) had been invaded by *R. lignosus*, the degree of colonisation of antagonists into the blocks precolonised by *R. lignosus* was recorded. The viability of *R. lignosus* on the surface of the original inoculum was studied by transferring mycelial fragments to MEA plates. Wood chips (slices 1–2 mm thickness) were aseptically cut from each block and transferred to MEA supplemented with benomyl, streptomycin sulphate and chlortetracycline (50, 30 and 30 μgml^{-1} respectively). The extent of outgrowth of *R. lignosus* from these wood chips was recorded. A scoring system similar to that used by Dowson *et al.* (1988) was used to assess the outgrowth of *R. lignosus* from the periphery of each wood chip on the agar (0= none; 1= trace; 2= outgrowth from up to one quarter of the periphery of the chip; 3= outgrowth from up to one half of the periphery of the chip; 4= outgrowth from up to three quarters of the periphery of the chip; 5= outgrowth from entire periphery of the chip).

RESULTS

Video microscopical analysis of mycelial interactions

The details of incidence and outcome of principal events in videotaped hyphal interactions between *R. lignosus* and fungi antagonistic against *Rigidoporus* is summerised in Table 1. *Phlebiopsis gigantea*, the commercial biocontrol agent for controlling *Heterobasidion* on pines was initially opposed on thin PWA against *R. lignosus*. Twenty four interactions of individual hyphae of *P. gigantea* with *R. lignosus* hyphae were studied, in which 19 caused post-contact damaged to *R. lignosus* hyphae (Fig. 2), but there was no effect before contact. In some cases *Phlebiopsis* hyphae branched at the point of contact with *R. lignosus* hypha. Post-contact coagulation of *R. lignosus* cytoplasm took place at 19± 2.7 (mean ± SEM) minutes, when *R. lignosus* tips touched the side of *P. gigantea* hypha, and at 28±3.4 minutes when *P. gigantea* tips touched the side of *R.lignosus* hyphae. The first sign of coagulation was observed as enlargement of individual vacuoles, which then coalesced, forming larger ones. In later stages, cytoplasm of *R. lignosus* streamed towards the hyphal tips and then coagulated. This coagulation was confined to particular hyphal compartments of *R. lignosus* which were in contact with *Phlebiopsis*.

Trichocladium asperum (isolate ED1), an antagonist against *R. lignosus* isolated from British soils, also caused similar damage to *R. lignosus* hyphae on thin

PWA films (Fig. 1). Most of the antagonistic fungi isolated from Sri Lankan soils did not damage *R. lignosus* hyphae on PWA films. However, *Aspergillus* sp. (isolate DFS12) (Fig. 2), *Fusarium* sp. (isolate DFS 4) damaged *R. lignosus* hyphae on PWA films. Of the 22 interactions with *Aspergillus* sp. (isolate DFS 12), 14 led to post–contact coagulation of *R. lignosus* cytoplasm. It also caused lysis of *R. lignosus* cytoplasm at a distance of 860 μm. *Fusarium* sp. (isolate DFS 4) coagulated and lysed *R. lignosus* hyphae in three interactions studied (results on interactions at a distance are not cited in Table 1).

Trichoderma spp. isolated from soils of Sri Lanka, on TSM were also investigated for hyphal interactions against *R. lignosus*. Due to the large number of isolates available, only 6 isolates *viz*: G14, DFTS, KGRAO, DZE 6, DZE 10, G1, were selected for the investigation. They were selected on the basis of the results of other experiments which showed different levels of inhibitory capacities of different isolates, so that the selected group comprised with isolates with varied levels of inhibitive effects (Jayasuriya, 1994). Interpretation of the results was slightly difficult because no clear pattern of hyphal interactions was observed. For example, *T. longibrachiatum* (isolate DZE 10) which had the most inhibitory effect on *R. lignosus* by producing volatile metabolites (Jayasuriya, 1994) damaged *R. lignosus* hyphae only in 4 of the 15 interactions on thin PWA films. But *Trichoderma* sp. (isolate DFTS– had less effect by volatile inhibitory metabolites production) damaged more than did *T. longibrachiatum* (isolate DZE 10). Of the 9 post–contact interactions of *Trichoderma* sp. (isolate DZE 6) against *R. lignosus*, 5 cause damage to *R. lignosus* hyphae by coagulating its cytoplasm (Fig. 2). *Trichoderma* isolates G1 and DFTS caused lysis of *R. lignosus* hyphae at a distance of approximately 35 μm and 130 μm respectively. Three of 5 post–contact interactions of isolate G1 with *R. lignosus* led to damage of *R. lignosus* cytoplasm and 4 of 4 pre–contact interactions lysed *R. lignosus* hyphae. Five of 9 post–contact interactions involving isolate DFTS caused damage to *R. lignosus* hyphae (Fig. 1). Five of 5 pre–contact incidence lysed *R. lignosus* hyphae. Isolates KGRAO and G 14 coagulated *R. lignosus* cytoplasm in 3 of 8 and 5 of 10 post–contact interactions respectively.

Antagonistic basidiomycetes were also opposed against *R. lignosus* on thin PWA films. Among the 4 isolates, only *Trametes* sp. (isolate ISO 16) caused damage to *R. lignosus* cytoplasm (Fig. 2). Out of 25 post–contact interactions 3 caused coagulation and in 7 incidents *R. lignosus* cytoplasm was lysed (Fig. 2). Out of 4 pre–contact interactions 4 caused lysis of *R. lignosus* hyphae. The approximate distance between the two hyphae in these cases was 60 μm. Coiling of antagonists hyphae round *R. lignosus* hyphae was also observed (Fig. 3). *Trametes* sp. (isolate KIR 8) did not cause damage to *R. lignosus* but in some cases coiled round *R. lignosus* hyphae. Out of 15 contacts between *S. commune* (isolate ISO 12) and *R. lignosus*, none caused damage to *R. lignosus* hyphae. In interactions with *L. squarrosulus* (strain 55A), *R. lignosus* was damaged in 1 out of 12 interactions.

Displacement of *Rigidoporus* from precolonised wood blocks by potential antagonistic fungi.

A scoring system similar to that used by Dowson *et al.* (1988) was used to assess the combatant ability of decomposer basidiomycetes. As shown in Table 2, three control wood blocks *(R. lignosus* alone) gave rather low level of retrieval of *R. lignosus*, which complicated the interpretation of the experiment. However, it was evident that the antagonist isolated from soils by the precolonised agar plate method had different effects on the persistence of *R. lignosus* in the wood blocks. Some potential antagonists (e.g. isolates DFS 18: black toruloid mycelial fungus) evidently did not displace *R. lignosus* at all, whereas other antagonists [e.g. *Aspergillus* sp. (isolate DFS 12)] seemed markedly prevented outgrowth of *R. lignosus* from the wood blocks.

When the antagonistic basidiomycetes were opposed against *R. lignosus* precolonised wood inocula, *R. lignosus* was partially or completely displaced by the invasion of the antagonists (Table 3). *Trametes* spp. (isolates ISO 16 and KIR 8) completely displaced *R. lignosus* from the inocula. The combating order (started with the maximum) of the test fungi against *R. lignosus* was as follows: *Trametes* sp (isolate ISO 16–rank 5) = *Trametes* sp. (isolate KIR 8–rank 5) > *L. squarrosulus* (strain 55A– rank 3.2) > *S. commune* (ISO 12–rank 0.2). As Dowson *et al.* (1988) noted the outcome of such interactions ranging from replacement of fungus 1 by fungus 2, through deadlock to replacement to species 2 by species 1 could often be altered by increasing the size of the inoculum base of one species relatively to other. Some fungi such as *Hypoloma fasciculare* replaced *Stecherinum fimbriatum* when the ratio between the respective inoculum sizes was 8:1 but *S. fimbriatum* replaced *H. fasciculare* when the ratio was 1:1. Therefore, perhaps the less combative fungi such as *L. squarrosulus* (strain 55A), *S. commune* (isolate ISO 12) in the present work may be more combative if they were opposed in larger sizes. The ratio used in present work was 1:1 for all combinations. As shown in Table 3 the degree of outgrowth of the antagonist from the wood blocks initially containing *R. lignosus* was assessed by the scoring system described earlier. *R. lignosus* grew abundantly from the control wood blocks in the absence of antagonists, and to a substantial degree from wood blocks juxtapposed to *S. commune* (isolate ISO 12) which did not invade *R. lignosus*

Table 1. *Summary of incidence and outcome of principal events in videotaped hyphal interaction between R. lignosus and antagonistic fungi.*

	***Rigidoporus* tip to antagonists side**					**Antagonists tip to *Rigidoporus* side**				
Antagonistic fungi (isolate)	Total interactions	coagulation *	lysis*	vacuoles enlarged *	hyphal stop*	Total interactions	coagulation*	lysis*	vacuoles enlarged *	hyphal stop*
S. commune (ISO12)	10			–	–	5	–	–	–	–
Trametes sp. (ISO16)	18	3(16%)	5(27%)	–	–	7	–	2(28%)	–	–
L. squarrosulus (strain 55A)	5	–	–	–	–	6	1(16%)	–	1(16%)	–
Trametes sp. (KIR8)	2	–	1(50%)	–	–	6	–	–	–	–
Aspergillus sp. (DFS12)	8	8(100)	–	–	–	12	4(33%)	–	–	–
Trichoderma sp. (DZE6)	4	–	1(25%)	–	–	5	1(20%)	3(60%)	–	–
T. longibrachiatum (DZE10)	4	1(20%)	–	–	–	9	3(33%)	–	2(22%)	–
Trichoderma sp. (isolate G1)	3		2(60%)	1(33%)	–	2	1(50%)	–	–	1(50%)
Trichoderma sp. (isolate G14)	3	3(100%)	–	–	–	5	–	–	–	–
Trichoderma sp. (DFTS)	4	–	–	–	4(100%)	5	4(80%)	1(20%)	–	–
Trichoderma sp. (KGRAO)	2	–	–	–	–	5	3(60%)	–	–	–
Fusarium sp. (isolate DFS4)	–	–	–	–	–	1	1(100%)	–	–	–
P. gigantea	13	12(92%)	–	–	–	11	7(63%)	–	–	–
T. asperum (isolate ED1)	2	1(50%)	–	–	–	–	–	–	–	–
G. virens (isolate G10P)	5	–	–	–	–	5	–	–	–	–
*T. longibrach*iatum (DZE11P)	8	8(100%)	–	–	–	–	–	–	–	–
Antagonist side to *Rigidoporus*	**side**									
Aspergillus sp. (DFS 12)	1	1(100%)	–	1(50%)	–	–	–	–	–	–
T. longibrachiatum (DZE 10)	2	–	–	–	–	–	–	–	–	–
Trichoderma sp. (isolate G14)	2	2(100%)	–	–	–	–	–	–	–	–
T. koningii (isolate G10P1)	–	–	–	–	–	12	9(75%)	3(25%)	–	–
Trichoderma sp. (KGRAO)	1	–	–	–	–	–	–	–	–	–

*All outcome of incidence are pertaining to *R. lignosus* mycelia.
Values in the paranthesies are percentage against the No. of interactions.

Table 2. Retrieval of *R. lignosus* from 4 replicate previously colonised wood blocks after incubation in the presence of an adjacent wood block colonised by a potentially antagonistic fungus isolated from soil by the precolonised plate method.

Antagonists	Total score of *R.lignosus* retrieved from 4 replicates blocks*	Mean score ± SEM	Presence of *R. lignosus* on the surface of the wood blocks**
Control ***	32	2.8±0.4	About 60% blocks were covered
A.niger (isolate KGS3)	37	3.0±0.54	Not found
T. Koningii (strain R5,24)	45	3.7±0.37	Not reisolated from surface
T. viride (strain TV 62)	39	3.2±0.3	- do -
Aspergillus sp. (isolate DFS12)	12	1.0±0.56	- do -
Fusarium sp. (isolate DFS4)	24	2.0±0.47	- do -
Penicillum sp. (isolate DFS7)	40	3.3±0.48	reisolated from surface of all blocks
Penicillum sp. (isolate DFS3)	46	3.8±0.4	Not reisolated
Isolate DFS 18 (black toruloid mycelial fungus)	60	5.0±0	Not reisolated
Penicillium sp. (isolate DZS1)	52	4.3±0.25	Not reisolated

* Three slices (chips) of wood were removed from each wood block (replicate) and plated on agar semi-selective for *R. lignosus*. Out growth of *R. lignosus* from the periphery of each chip was scored from 1-5 (low to high) on described in the text.

** Presence of *R. lignosus* on the surface of wood blocks was assessed by transfering mycelial fragments from each block onto agar.

*** Rigidoporus precolonised wood block was kept adjacent with sterilised wood block.

DFS isolates were isolated from soils collected from the premises of the Rubber Research institute of Sri Lanka by the precolonised agar plate method.

DZS isolates were isolated from soils collected from the dry zone of Sri Lanka.

Table 3. Out growth of *R. lignosus* (left hand value) and of potential antagonists (right hand value) from wood blocks initially colonised by *R. lignosus* then placed adjacent to wood blocks containing basidiomycetes isolated from fruitbodies and from the collection.

Antagonistic basidiomycete (isolate)	Total score of *R. lignosus* retrieved from 4 replicates	Mean ± SEM of scores of 4 replicate wood blocks – 24 chips were removed altogether from all replicates*	Presence of *R. lignosus* mycelia on wood block surface
Control	120:0	5±0:0	All blocks were covered by thick mycelial mat of *R. lignosus*
S. commune (isolate ISO12)	96:4	4.8±0.09:0.2±0.09	Wood blocks were partially covered by both fungi.
Trametes sp. (isolate ISO16)	0:120	0:5±0	All wood blocks were completely covered by the antagonist, no trace of *R. lignosus* was reisolated from any block.
Trametes sp. (isolate KIR8)	0:120	0:5±0	– do –
L. squarrosulus (strain 55A)	45:78	1.8±0.4:3.2±0.4	– do –

* 4 wood chips were removed from each replicate wood block and out growth of fungi from the chips was scored 0–5 as explained in the text.

Fig. 1. Hyphal interactions of *R. lignosus* (Rl) and some antagonistic fungi on thin water agar films, post-contact coagulation of *R. lignosus* cytoplasm on contact with hyphae of (a) *Phlebiopsis gigantea*; (b) *Aspergillus* sp. (isolate DFS 12); (c) *Trichoderma* sp. (isolate DZE 6)- isolated from Sri Lankan soil on *Trichoderma* selective agar; (d) *Trichocladium asperum* (isolate ED1) isolated from Edinburgh soil by the precolonised agar plate method. Bar=10μm.

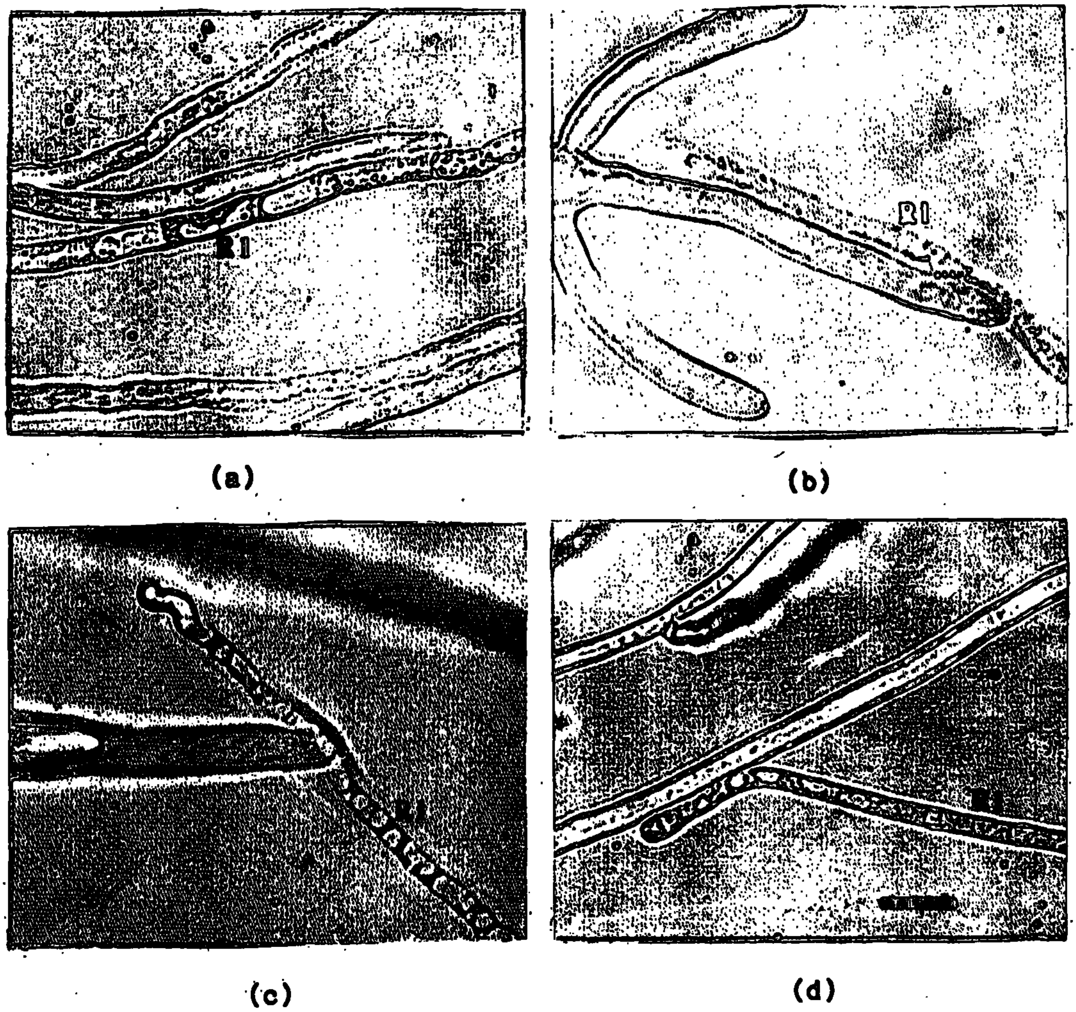
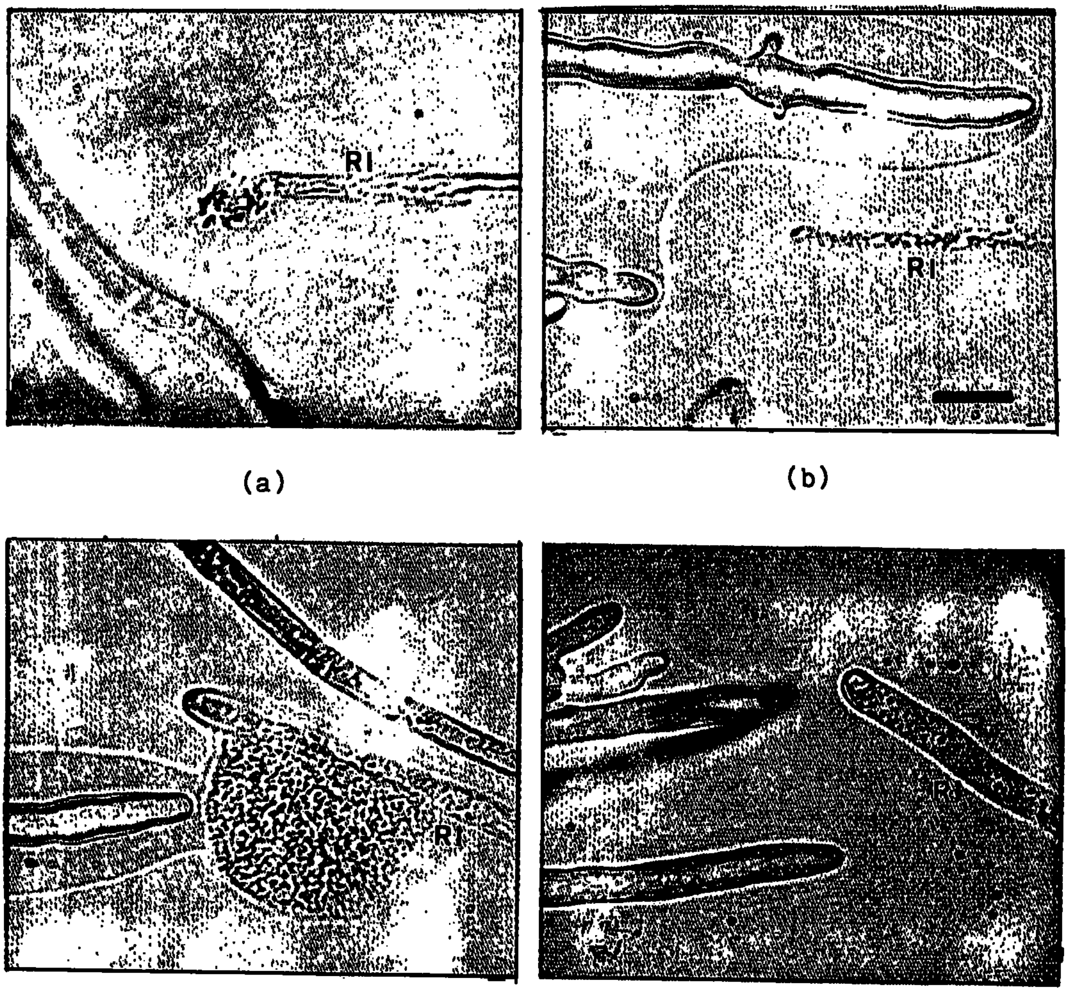


Fig. 2. Hyphal interactions of *R. lignosus* with some antagonistic fungi on thin water agar films: Pre-contact lysis of *R. lignosus* (Rl) hyphae by (a) *Trametes sp.* (Iisolate ISO 16) lysis of *Rigidoporus* hypha; (b) *Trichoderma koningii* (isolate G10P1)-coagulation of *Rigidoporus* cytoplasm; (c) *Trichoderma koningii* (isolate G10P1)-lysis of *Rigidoporus* cytoplasm; (d) *Trichoderma* sp. (isolate DFTS). Bar=10μm.



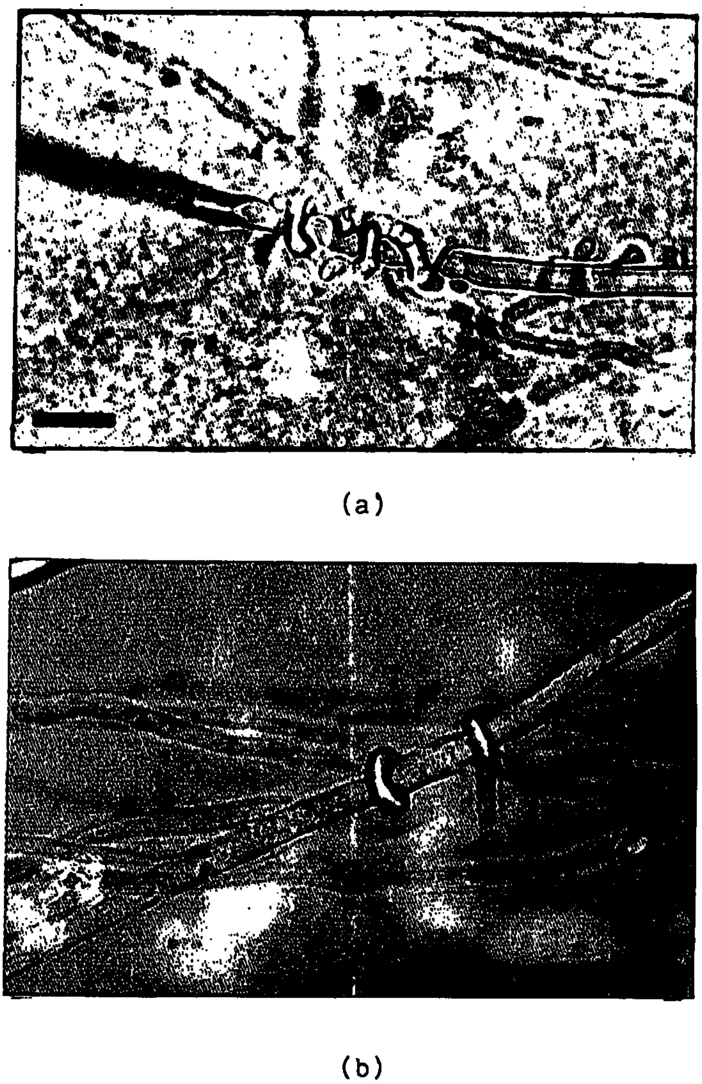
(a)

(b)

(c)

(d)

Fig. 3. Hyphal interactions of *R. lignosus* with some antagonistic fungi on thin water agar films: (a) coiling of *Trametes* sp. (isolate ISO 10), hypha round *R. lignosus*; (b) coiling of *Lantinus squarrosulus* (strain 55A) hypha round *R. lignosus* (Rl). Bar=10μm.



blocks. *Rigidoporus* was partially displaced from wood blocks by *L. squarrosulus* (strain 55A) and completely displaced by *Trametes* spp. (isolates ISO 16 and KIR 8).

DISCUSSION

Pre-contact antagonism at close range depends on the inhibitory activity of diffusible compounds, but quantification or analysis of these compounds and evaluation of their inhibitory capacity in nature are difficult. It is likely that antibiotic production only occurs in nutrient rich microsites, and towards the end of vegetative growth when one or more essential nutrients becomes limiting (Demain, 1972). In contrast to this 'direct' post-contact inhibition may not require an abundant energy source and it could be simulated *in vitro* by studies on water agar films using video-microscopy to analyse individual hypha-hypha contacts. Using this technique, behaviour of individual hyphae through films of water agar was studied by Laing & Deacon (1991) who found that *Pythium oligandrum* had no pre-contact effect on other fungi even in the 30 seconds before contact, but rapidly parasitised hyphae of a range of pathogens after hyphal contacts.

As *R. lignosus* is also a basidiomycete, video microscopy was used to study the antagonistic activity of *P. gigantea* against *R. lignosus*. Different types of contacts between the two fungi were studied- e.g. when *Phlebiopsis* hyphal tips contacted the sides of *R. lignosus* hyphae and *vice-versa*. Results showed that after mean time of 19 and 27 minutes from the first hyphal contact, when the *Rigidoporus* hyphal tip was touching the side of *Phlebiopsis* hypha or *vice-versa*, the hyphae of *R. lignosus* stopped the growth and the cytoplasm became granulated or vacuolated. Branching of *Phlebiopsis* from points of contacts was observed in a number of contacts. All the observed events were compatible with hyphal interference as reported by Ikediugwu *et al.* (1970), Ikediugwu (1976) and Ikediugwu & Webster (1970b). Notably, *P. gigantea* was never seen to penetrate and grow inside the antagonised hyphae of *R. lignosus*. In contrast to this, Laing & Deacon (1991) found that *P. oligandrum* stopped the hyphae of *Fusarium oxysporium*, *Botrytis cineria* and *Rhizoctonia soloni* within 4-7 min. after contact, causing explosive hyphal lysis or coagulation of cytoplasm, and it often penetrated the hyphae. This is compatible with parasitism of other fungi whereas, *P. gigantea* evidently is not a parasite of other fungi.

Fusarium sp. (isolate DFS 4) and *Aspergillus* sp. (isolate DFS 12) caused significant damage to *R. lignosus* hyphae by coagulating its cytoplasm respectively in 100% and in 63% pre- and post-contact interactions. *Aspergillus* sp. (isolate DFS 12) also caused lysis and tip burst of *R. lignosus* hyphae at a distance of approximately 86 μm. When *Trichoderma* and *Gliocladium* spp. isolated from Sri Lankan soils by the precolonised agar plate method were tested, *T. koningii* (isolate G10P1) and *T. longibrachiatum* (isolate DZE11P) damaged *R. lignosus* hyphae respectively in 12 of 12 pre-contact and in 8 of 8 post-contact interactions. Therefore, it was evident that, some of the fungi isolated by the precolonised agar plate method (*Trichoderma*, *Aspergillus*, and *Fusarium* spp.) were able to cause post-contact damage to *R. lignosus* on thin PWA films.

The activities of antagonistic basidiomycetes on thin PWA films were slightly different from *Trichoderma* spp. Of the 5 isolates employed to investigate mycelial interactions against *R. lignosus*, only *Trametes* sp. (isolate ISO16) caused damage to *R. lignosus* hyphae in pre- and post-contact interactions. *Trametes* spp. (isolates KIR 8 and ISO16) with an equal efficiency displaced *R. lignosus* from precolonised wood inocula, the incidence of which was considered as the most important aspect in successful biocontrol. However, it was evident that *Trametes* sp. (isolate KIR8) had no effect in mycelial interactions with *R. lignosus* on thin PWA films. *Trametes* sp. (isolate KIR8) was also more efficient on degrading elm wood than did by other antagonistic basidiomycetes (Jayasuriya, 1994).

Coiling of some antagonistic basidiomycetous fungi round *R. lignosus* hyphae was noted in some interactions studied on thin PWA films. There may be some effects on *R. lignosus* as it may interfere with extension of *R. lignosus* hyphae. Stoppage of *R. lignosus* hyphae may also occur as a result of coiling the antagonists hyphae round *R. lignosus* hyphae. Similar effects were reported by Berry *et al.* (1993) in *Pythium* spp.

Results also showed that some of the basidiomycetous antagonists had the potential to displace *R. lignosus* from the precolonised wood inocula. However, most of the antagonistic fungi isolated from Sri Lankan soil by the precolonised agar plate method did not displace *R. lignosus* completely from precolonised wood inocula, while some of them partially displaced *R. lignosus*. It was evident that *Trametes* sp. (isolate KIR8) had no post-contact effect against *R. lignosus* on thin PWA films. Traquair & Mckeen (1977) reported hyphal interference by *Trametes hispida* Bagl. in dual cultures with *Hirschioporus* species and other non-basidiomycetous fungi. They also mentioned its overgrowth on colonies of sensitive fungi. However, only *Trametes* sp. (isolate ISO 16) had a similar effect on *R. lignosus* when they were opposed on thin PWA films. The effect of *Trametes* sp. (isolate KIR8) was different. It had no pre-contact or post-contact effect on *R. lignosus* but successfully invaded the *R. lignosus*-colonized wood blocks and completely displaced the pathogen. *L. squarrosulus* was reported to be antagonistic to *R. lignosus* (Sudirman *et al.*, 1992). It displaced *R. lignosus* on artificial inocula. However, data herein showed that *L.*

squarrosulus had relatively less potential for displacing *R. lignosus* from woody inocula. Tzean & Estey (1978) reported *Schizophyllum commune* as a destructive mycoparasite on several phytopathogenic and nematode trapping fungi. It also coiled round host hyphae and fruiting structures. However, *S. commune* (isolate ISO12) had no effect on *R. lignosus* when opposed on thin PWA films, but it partially displaced *R. lignosus* from precolonised woody inocula.

CONCLUSIONS

It may not be possible to determine the potential antagonism of a fungus as antagonistic to a particular pathogenic fungus entirely based on results of hyphal interactions of the two fungi. It was evident that the method of isolation of fungi antagonistic against *R. lignosus* did not determine the potentiality of such fungi. Therefore, the method of isolation would not be a correct prediction for successful isolation of biocontrol agents.

The displacement of the pathogen from its established food base by the biocontrol agent is important in successful biological control. The pathogen in the food base may need to be weakened before introducing the biocontrol agent if the complete displacement of the pathogen by antagonist is to be achieved. This approach was followed by Munnecke *et al.* (1981) to control *Armillaria mellea* in soil by weakening the pathogen with methyl bromide, so that naturally occuring antagonistic fungi could easily invade the weakened pathogen inoculum.

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