Global Journal of Biotechnology & Biochemistry 6 (3): 95-101, 2011
ISSN 2078-466X

Screening of *Trichoderma* Spp. As Potential Fungal Partner in Co-Culturing with White Rot Fungi for Efficient Bio-Pulping

Susy Albert, Dhruvina Chauhan, Bhavika Pandya and Amee Padhiar

Department of Botany, Faculty of Science, The Maharaja Sayajirao
University of Baroda, Vadodara Gujarat, India

Abstract: Species of *Trichoderma* are ubiquitous in the environment and especially in the soil. *Trichoderma* species are well known to have antagonistic effect against plant pathogens and have been extensively studied as biological control agents against fungal pathogens. They are also known to produce xylanase which is an enzyme very important for brightening of paper in the pulp and paper industry. The objective of the present *in vitro* studies was to identify a potential compatible ligninolytic white rot fungi with *Trichoderma* species that can be utilized in practical application of co-culture especially for bio pulping in pulp and paper industry. Results showed that the two species of *Trichoderma* are compatible and grow well with the two ligninolytic white rot fungi *Daedeleopsis confragrosa* and *Phellinus pectinatus* proving to be potential fungal partners in co-culturing for efficient bio-pulping.

Key words: *Trichoderma viride* · *Trichoderma harzianum* · Paired interaction · Bio pulping

INTRODUCTION

Biobleaching is an important alternative used to reduce the use of chlorine and chlorine compounds in the bleaching process of pulp and paper industry. Biological bleaching is carried out using white rot fungi to degrade residual lignin in pulp by using ligninolytic enzymes such as manganese peroxidise and laccase or by using hemicellulolytic enzymes such as xylanases [1]. Isolation and screening of fungal strains suitable for biopulping have been performed in many laboratories worldwide since the 1970s. Lignocellulolytic enzymes producing fungi are widespread and include species from Ascomycetes (eg. *Trichoderma reesei*) and basidiomycetes such as white rot (eg. *Phanerocheate chrysosporium*) and brown rot fungi (eg. *Fomitopsis palustris*).

Paper obtained by fungal treatment appears to be yellowing. For brightening of cellulosic fibres xylanase enzyme is used. There are many fungi which produce xylanase. A combination of two such fungal co-culturing would help in obtaining cellulosic fibres of high quality. As a hypothesis co-culturing of fungi mean oxidative stress to both fungal partners and may accelerate fungal metabolic switch to secondary metabolism thus stimulating wood decay and production of lignin degrading enzymes.

Chi *et al*., (2007) nvestigated the effects of co-culturing two white rot fungi *Ceriporiopsis subvermispora* and *Physosporinus rivulosus* on the production of lignin degrading enzyme activities [2]. This was the first report on the effects of co-culturing of potential biopulping fungi on wood degradation and gives basic knowledge on fungal interactions during wood decay that can be utilized in practical applications.

Maijala (2005) evaluated the effects of co-culturing of three different white rot fungi promising in biopulping *Ceriporiopsis subvermispora, Phenerochaete chrysosporium* and *Pleurotus ostreatus* [3].

Evaluation of antagonistic effect of the selected fungal co-culture is of great importance in co-culture. Antagonistic effect of fungi is the consequence of one fungi counteracting the effects of another fungi. The opposing fungi cancel out each other's effects. Ascomycetes can suppress the growth of Basidiomycetes and some Basidiomycetes also can affect the growth of the Ascomycetes.

Corresponding Author: Susy Albert, Department of Botany, Faculty of Science,
The Maharaja Sayajirao University of Baroda, Vadodara Gujarat India.

Trichoderma species are used in a wide range of commercial applications including the biological control of plant diseases [4, 5, 6]. *Trichoderma viride* and *Pleurotus ostreatus* showed increase in xyloglucanase activities when the fungi grow on microcrystalline cellulose as the sole carbon source [7]. *Trichoderma* spp. is known to produce enzymes with high xylanolytic activity. Different cyanoses and various components of their xylanolytic system have been identified and purified. Some of the xylanases have been characterized extensively with respect to their physicochemical, hydrolytic and molecular properties. Cellulose-free xylanase preparations have been tested successfully in industrial applications such as the pre bleaching of kraft pulps in the pulp and paper industry.

Future work on understanding the functional significance of xylanase multiplicity, the mechanisms of xylanase prebleaching and the structural conformation of xylanases could lead to improved or alternative applications of *Trichoderma* xylanases. The main aim of the present study was to identify two biopotential fungi which are not antagonist but compatible so as to suggest them for dual culture to increase the efficiency of biopulping. The fungal combination has to be such that one is ligninolytic and the other keeps up the quality of the cellulose fiber, especially the brightening property of the paper. Screening experiments for antagonism will also aid in identifying the fungi which can be used for co-culture in other different biotechnological applications.

MATERIALS AND METHODS

Source of the Fungal Isolates: The cultures of all the fungal isolates except *Phellinus pectinatus* and *Daedeleopsis confrogosa* used in the present study was procured from Forest Research Institute, Dehradun, India. Isolates of *Phellinus pectinatus* and *Daedeleopsis confragrosa* were isolated from fruiting bodies obtained from naturally growing habitat on the trunk of growing *Peltophorum* in the Arboretum of The Maharaja Sayajirao University of Baroda, Gujarat, India and dead wooden logs in Pavagadh Forest, Panchmahal district, Gujarat, India respectively. The basidiocarps were identified and authentically confirmed from Forest Research Institute, Dehradun.

Table 1: Characteristic features on 3rd-6th and 9th day of fungal isolates grown with its counterpart *Trichoderma harzianum*

Name of the fungi	3rd day	6th day	9th day
Irpex lectus (IL) (Fig. 1 A-C)	Growth of TH was faster than IL and both fungi came in to contact with each other	TH overgrown IL	Growth of IL was inhibited completely and killed by TH
Daedeleopsis confragrosa (LZ) (Fig. 1 D-F)	Growth of TH was very faster than LZ	Both fungi come in to contact with each other and overgrows each other	TH covered the whole petri plate LZ overgrows on TH indicatingcompatibility
Phellinus pectinatus (PHE) (Fig. 1 G-I)	Growth of TH was faster than PHE	Both fungi come in to contact with each other and TH overgrows PHE	TH covered the whole petri plate PHE overgrows on TH indicating compatibility
Pycnoporus sanguineus (PYS) (Fig. 1 J-L)	Growth of TH was faster than PYS	Both fungi came in to contact with each other and growth of PYS was inhibited	TH killed PYS and innoculum color of PYS changed from orange to black
Pleurotus eryngii (PE) (Fig. 2 A-C)	Growth of TH was faster than PE	TH overgrows PE and covers the whole petri plate and growth of PE was inhibited	Growth of PE was inhibited completely and killed by TH
Pleurotus florida (PF) (Fig. 2 D-F)	Growth of TH was faster than PF	TH overgrows PF and covers the whole plate but growth of PF was not inhibited	TH coveres the whole petri plate growth of PF occurred and killed byTH
Pleurotus ostreatus (PO) (Fig. 2 G-I)	Growth of TH was faster than PO	Both fungi came in to contact with each other and inhibited growth of PO	Growth of PE was inhibited completely and killed by TH
Pleurotus sajorkaju (PS) (Fig. 2 J-L)	Growth of TH was faster than PS	TH overgrows PF and covers the whole petri plate but growth of PS was inhibited	Growth of PS inhibited completely and killed by TH
Trichoderma viride (TV) (Fig. 2 M-O)	Growth of both fungi occurred equally	Both fungi came in to contact with each other and sporuation of TV started	Growth of both fungi stopped and could not grow further inhibition of both fungi occurred at the region of contact

Table 2: Characteristic features on 3rd, 6th and 9th day of fungal isolates grown with its counterpart *Trichoderma viride*

Name of fungi	3rd day	6thday	9th day
Irpex lectus (IL) (Fig. 3 A-C)	Growth of TV was more than IL	TV over grows IL and completely covers the petri plate and sporulation of TV starts	Growth of IL was completely inhibited
Daedeleopsisconfragrosa (LZ) (Fig. 3 D-F)	Growth of TV was more than LZ	TV over grows LZ and completely covers the petri plate and sporulation of TV starts	Growth of LZ continues but at a very slow ratecompared to TV growth of both fungi remains unhindered
Pleurotus eryngii (PE) (Fig. 3 G-I)	Growth of PE was very slow as compared to TV	TV over grows PE and completely covers the petri plate and sporulation of TV starts and growth of PE was inhibited	Growth of PE was completely inhibited and killed by TV
*Pleurotus florida (*PF) (Fig.3 J-L)	Growth of TV was more than PF	TV over grows PE and completely covers the petri plate and sporulation of TV started and growth of PF was inhibited	Growth of PF was completely inhibited and killed by TV
Phellinus pectinatus (PHE) (Fig. 4 A-C)	Growth of TV was more than PHE	Both the fungi TV and PHE come in to contact and PHE over grows on TV both fungi are compatible	PHE still grows further and growth of TV also continued which indicates compatibility of both fungi
Pleurotus ostreatus (PO) (Fig. 4 D-F)	Growth of PO was not started even while TV covers the whole plate and sporulation also begins	Sporulation of TV occurred and growth of PO was inhibited	Sporulation of TV still occurred and growth of PO was inhibited completely and killed by TV
Pleurotus sajor kaju(PS) (Fig. 4 G-I)	Growth of PS was very slow as compared to TV	TV over grows PS and growth of PS was inhibited	Growth of PS was completely inhibited and PS killed by TV
Pycnoporus sanguineus (PYS) (Fig. 4 J-L)	Growth of TV was more than PYS	TV over grows PYS and completely coveres thewhole petri plate	Growth of PYS was completely inhibited and killed by TV and color of PYS innoculum changed from orange to black
Trichoderma harzianum (TH) (Fig. 4 M-O)	Both fungus grows equally on the plate	Both fungi come in contact with each other and sporulation of TV starts	Growth of both fungi stopped and could not grow further inhibition of both fungi occurred at the region of contact

Subculture of the Fungal Isolates: All the cultures were maintained on Potato dextrose agar (PDA) at 4 ±1°C. For further studies petri dishes containing potato dextrose agar medium were inoculated with 0.5 cm diameter agar plug, cut from the growing edge of colonies of the isolates and incubated in incubator at 25 ±1°C in dark with 70% relative humidity. Fruting bodies of *Phellinus pectinatus* and *Daedeleopsis confragrosa* were surface sterilized with 0.1% mercuric chloride, inoculated in petri-dish containing potato dextrose agar medium, under asceptic condition and incubated for 7 days at 25 ± 2°C. After development of colony these were subcultured on potato dextrose agar slants.

Paired Interaction Test to Detect Antagonistic Effect of the Different Fungi: *In vitro* antagonistic potential of the fungi was evaluated through dual culture technique. For the antagonistic effect the agar disc method was carried out on 3% malt extract agar. The isolates were screened for their antagonistic potential against the other fungal isolates on potato dextrose agar medium by measuring the relative growth rates as a function of the incubation period. Five mm mycelial discs by the help of cork borer was taken from the margin of young vigorously growing 7 days old culture of fungi was inoculated at the margin of the petri-dish (90mm) containing 20ml sterilized Malt Extract Agar medium at opposite sides of the each other and then incubated in dark at 25±2°C with 70% relative humidity for 4 weeks. Petri dishes inoculated with individual fungi were used as controls. Three replicates were used for each experiment. Photographs were taken on digital Sony Cyber shot model no. DSC-H2O. Observations were made daily to assess the growth of the cultures. Stages of growth at the 3rd, 6th and 9th day have been represented in Table 1 and 2.

RESULTS AND DISCUSSION

Observations of paired interaction tests are distinguished into three categories of compatibility:

- Where both fungi come in contact growing on the medium and growth of both fungal isolates are inhibited *i.e*. No further growth occurs once the two fungal isolates come in contact.
- Where the two fungal isolates in the paired interaction test come in contact and growth of one is inhibited by the other but it is not killed. The fungal isolate grows on the counterpart.
- The two fungal isolates in paired interaction test come in contact, one overgrows over the other and kills it.

A paired fungi was considered compatible once they come in contact and still each one grows over the other at its own pace with the formation of an overlapping zone which increases / advancing towards both the sides. The identification of characteristic features of the fungal isolates with its counterpart has been represented individually in Figures 1 to 4.

Abbreviations used to designate the different fungal isolates are represented below:

TV-*Trichoderma viride*	TH-*Trichoderma harzanium*
PS -*Pleurotus sajorcaju*	PO-*Pleurotus ostreatus*
PF-*Pleurotus florida*	PE -*Pleurotus eryngii*
IL-*Irpex lacteus*	PYS-*Pycnoporus sanguineus*
LZ -*Daedeleopsis confragrosa*	PHE -*Phellinus pectinatus*

Figures 1, 2 and 3, 4 represent the paired interaction growth characteristics of fungal isolates with TH and TV respectively. Based on the characteristic observations of the 3rd, 6th and 9th day (Tables 1 and 2) of the fungal isolates grown with its counterpart it could be depicted that both the species of *Trichoderma viz T.viridae and T. harzianum* is compatible with PHE and LZ. Data in Table 3 depicts the compatibility among the tested fungi and based on the observations an assessment of mycoparasitism conducted are represented.

Mycoparasitism of the fungal isolates has been assessed in the dual culture according to Highley *et al*., (1997) [8]. The assessment was done after 7 days of incubation at room temperature. Competition values

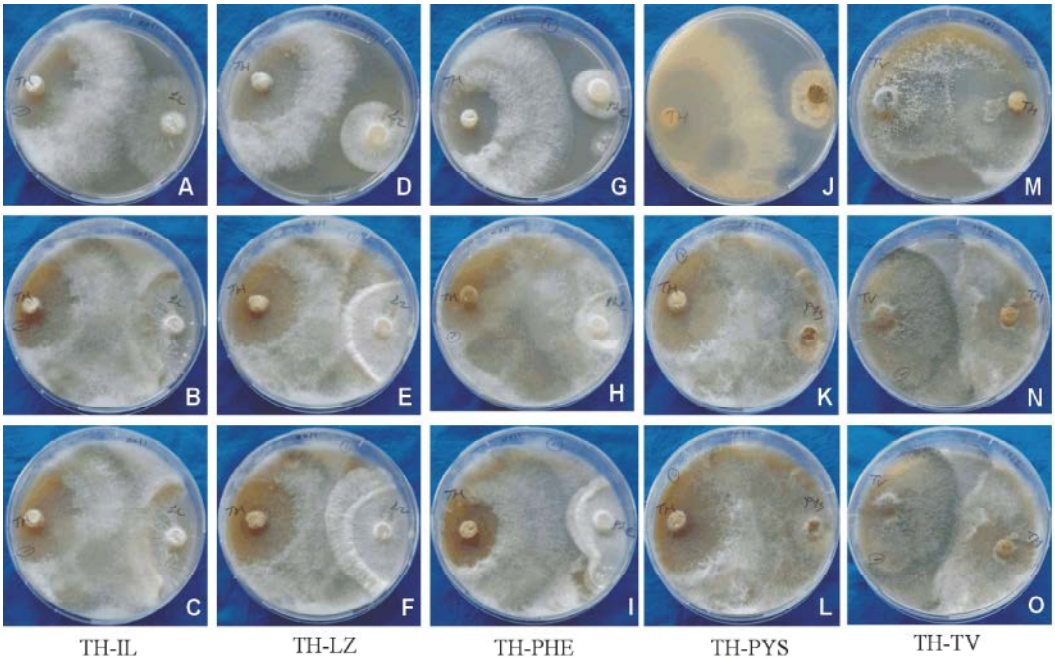

Fig. 1: Paired interaction test of *Trichoderma harzianum* with the other fungal isolates
a-c: (TH-IL) *Trichoderma harzianum* - *Irpex lacteus*
d-f: (TH-LZ) *Trichoderma harzianum* - *Daedaleopsis confragosa*
g-i: (TH-PHE) *Trichoderma harzianum* - *Phellinus pectinatus*
j-l: (TH-PYS) *Trichoderma harzianum* - *Pycnoporus sanguineus*
m-o: (TH-TV) *Trichoderma harzianum* - *Trichoderma viride*

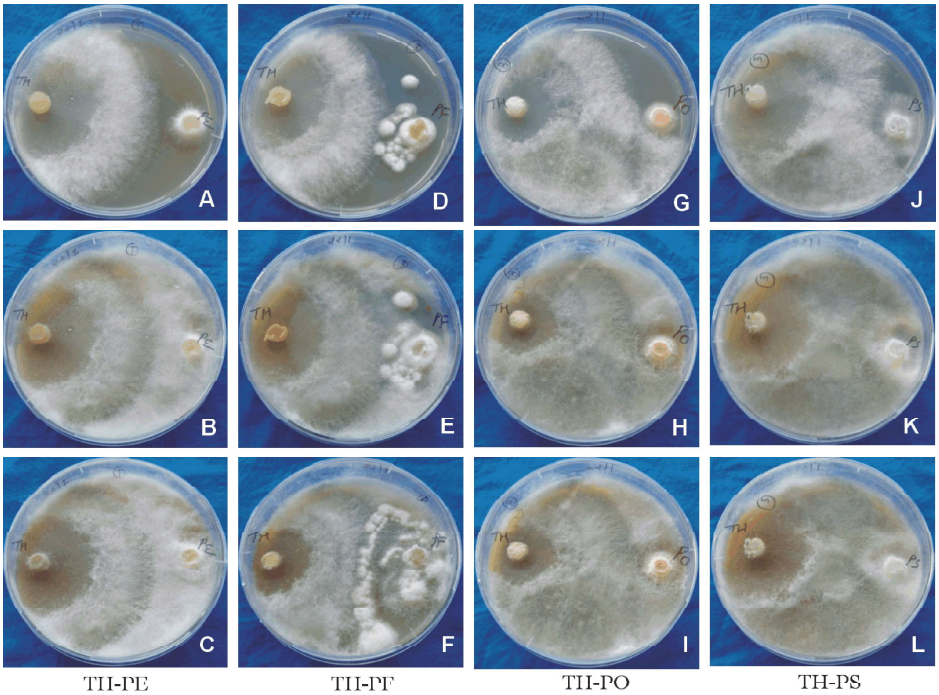

Fig. 2: Paired interaction test of *Trichoderma harzianum* with the other fungal isolates
a-c: (TH-PE) *Trichoderma harzianum* - *Pleurotus eryngii*
d-f: (TH-PF) *Trichoderma harzianum* - *Pleurotus florida*
g-i: (TH-PO) *Trichoderma harzianum* - *Pleurotus ostreatus*
j-l: (TH-PS) *Trichoderma harzianum* - *Pleurotus sajor*

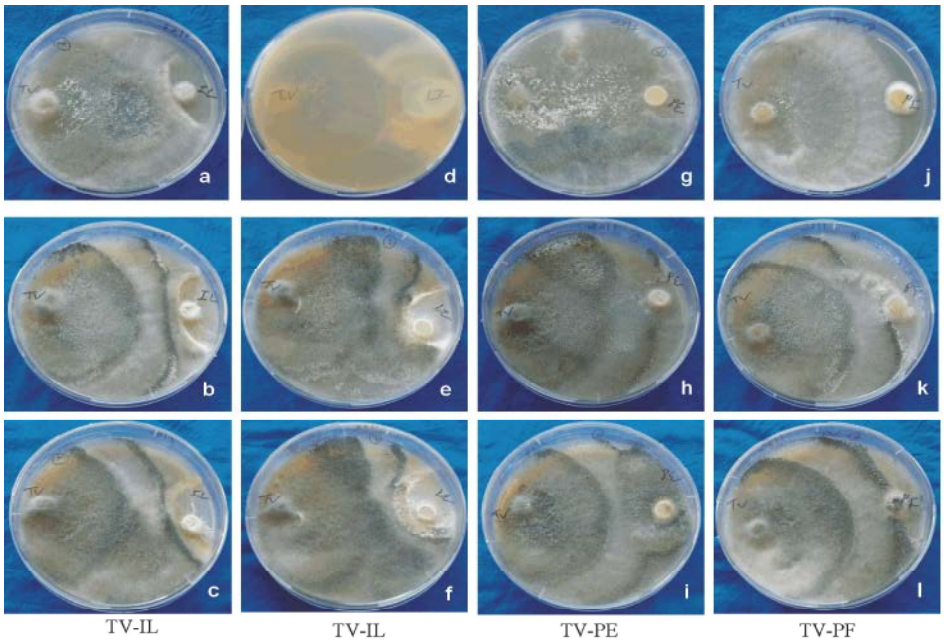

Fig. 3: Paired interaction test of *Trichoderma viride* with the other fungal isolates
a-c: (TV-IL) *Trichoderma viride* - *Irpex lacteus*
d-f: (TV-LZ) *Trichoderma viride* - *Daedaleopsis confragosa*
g-i: (TV-PE) *Trichoderma viride* - *Pleurotus eryngii*
j-l: (TV-PF) *Trichoderma viride* - *Pleurotus florida*

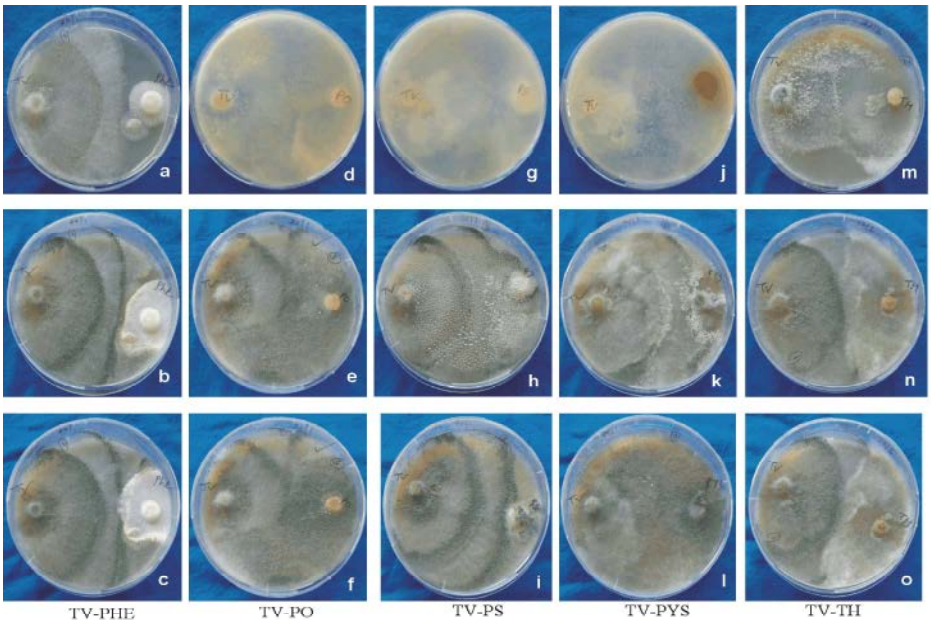

Fig. 4: Paired interaction test of *Trichoderma viride* with the other fungal isolates
a-c: (TV-PHE) *Trichoderma viride* - *Phellinus pectinatus*
d-f: (TV-PO) *Trichoderma viride* - *Pleurotus ostreatus*
g-i: (TV-PS) *Trichoderma viride* - *Pleurotus sajorcaju*
j-l: (TV-PYS) *Trichoderma viride* - *Pycnoporus sanguineus*
m-o: (TV-TH) *Trichoderma viride* - *Trichoderma harzianum*

Table 3: Result of paired interaction tests showing compatibility / nona compatibility of the co-cultures and mycoparasitism of the fungal isolates

Fungal isolates		TV	TH	PS	PO	PF	PE	IL	PYS	LZ	PHE
TV	Compatibility	-	-ve	-ve	-ve	-ve	-ve	-ve	-ve	+ve	+ve
	Mycoparasitism	-	3	3	3	3	3	3	3	3	3
TH	Compatibility	-ve	-	-ve	-ve	-ve	-ve	-ve	-ve	+ve	+ve
	Mycoparasitism	2	-	3	3	3	3	1	3	2	1

+ ve indicates compatible, -ve indicates non compatibility.

Mycoparasitism rate 0 = no overgrow; 1= slow overgrow; 2= fast overgrow; 3= very fast overgrow

(mycoparasitism rate) was assessed as 0=no overgrow; 1=slow overgrow; 2=fast overgrow; 3=very fast overgrow and deadlock of the white rot fungi.

Lethal effect as percent was measured by the ability of the fungal isolate to eliminate the other fungi during the incubation time of 3 weeks. *Trichoderma* belonging to class Ascomycetes is one of the most widely studied genera of fungal biocontrol agents [9]. Highley and Recard (1988) found that on a malt agar medium, isolates of *Trichoderma viren*, *T. harzianum, T. polysporum* completely inhibited the growth of several white and brown rot fungi and killed them [10]. TV is known to produce the antibiotic gliotoxin, an epidithiodi kelopiperazene. Brian and Hemming [11] and Howell *et al*. [12] have demonstrated an association between gliotoxin production and *in vivo* inhibition. Wood decay test were done also to evaluate the decay resistance. In dual cultures of *T. virens* and *Irpex lacteus, T.virens* rapidly was found to overgrow and kill the decay fungi [13]. Paranthaman *et al*. [14] studied the effect of fungal co-culture for the biosynthesis of Tannic acid and Gallic acid from grape waste under solid state fermentation. The co-culture *Penicillium chrysogenum* and *Trichoderma viride* produced highest activity of 84±U/g/min than other organisms.

Trichoderma atroviridae was tested for its antagonistic potential against white rot wood decay fungi *Ganoderma adspersum, Ganoderma lipsiense, Inonotus liespidus, Polyporus squamosus* and Asomycete *Kretzschmaria densta* [15]. *Trichoderma atroviridae* was

consistently and highly competitive against most wood decay fungi with the exception of *Polporus squamosus* which showed resistance against antagonism in laboratory tests.

In the present study TV very rapidly overgrow and killed all the white rot fungi except *Daedeleopsis confragrosa* (LZ) and *Phellinus pectinatus* (PHE). The rate of growth of their white rot fungi is slower but they are compatible to grow with TV. The overlapping zone is very prominently observed (Fig. 3, 4) even after 7 days of inoculation.

Similarly TH also is a very rapidly growing fungus which killed all the white rot decay fungi except LZ and PHE. The overlapping zone in TH-LZ is more than TH-PHE (Fig. 1). TH (*T. virens* Miller) rapidly overgrows *Irpex lacteus* and kills it in the once it has come in contact within 7 days of inoculation. Compatibility of *Trichoderma* species (TH and TV) with PHE and LZ is a positive result for our present study as our aim is to select a co-culture which is potential for biopulping. *Trichoderma* is well known to produce xylanase enzyme and PHE and LZ are well known selectively delignifying white rot wood decay fungi, together it would increase the process of delignification as well as maintain brightness property of the fibers. However wood decay tests and chemical analysis have to be performed to confirm the potential of co-culturing these fungi.

ACKNOWLEDGEMENTS

The authors are thankful to the University Grants Commission, New Delhi for providing the financial assistance under which this work was conducted. We thank the Head, Department of Botany, for providing us with the necessary facilities for conducting the work.

REFERENCES

1. Casimir-Schenkel, J., S. Davis, A. Fiechter, B. Gygin, E. Murray, J. Perrolax and W. Zimmermann, 1995. Pulp bleaching with thermostable xylanase of *Thermomonospora fusca*. US Patent No. 5407827, 18.04.95.
2. Chi, Y., A. Hatakka and P. Maijala, 2007. Can co-culturing of two white-rot fungi increase lignin degradation and the production of lignin degrading enzymes. International Biodeterioration and Biodegradation, 59(1): 32-39.
3. Maijala, P., 2005. Co-culturing of white-rot fungi on wood - potential in biopulping. Seminar on forest pathology. Finnish Forest Research Institute, 4/04/2005.
4. Hjeljord, L. and A. Tronsmo,1998. *Trichoderma* and *Gliocladium* in biological control: an overview. In *Trichoderma* and *Gliocladium*, Eds., G.E. Harman and C.P. Kubicek, Taylor and Francis, London, UK, 2: 131-152.
5. Harman, G.E., 2006. Overview of mechanisms and uses of *Trichoderma* spp. Phytopathol., 96: 190-194.
6. Howell, C.R., 2003. Mechanisms employed by *Trichoderma* species in the biological control of plant diseases: the history and evolution of current concepts. Plant Diseases, 87: 4-10.
7. Radford, A., P.J. Stone and F. Taleb, 1996. Cellulase and amylase complexes. In The mycota III Biochemistry and MolecularBiology, Eds., R. Brambl and G.A. Marzluf, Springer - Verlag, pp: 269-294.
8. Highley, T.L., H.S.A. Padmanabha and C.R. Howell, 1997. Control of wood decay by *Trichoderma (Gliocladium) virens*. II. Antibiosis. Material und Organismen, 31: 158-166.
9. Cook, R.J. and K.F. Baker, 1983. The nature and practice of biological control of plant pathogens. American Phytopathological Society, pp: 539.
10. Highley, T.L. and J. Ricard, 1988. Antagonism of *Trichoderma* spp. and *Gliocladium virens* against wood decay fungi. Material und Organismen, 23: 157-169.
11. Brian, P.W. and H.G. Hemming, 1945. Gliotoxin, a fungistatic metabolic product of *Trichoderma viride*. Annals of Applied Biol., 32: 214-220.
12. Howell, C.R., R.D. Stipanovic and R.D. Lumsden, 1993. Antibiotic production by strains of Gliocladium virens and its relation to the biocontrol of cotton seedling diseases. Biocontrol Science and Technol., 3: 435-441.
13. Highley, T.L., 1997. Control of wood decay by *Trichoderma* (*Gliocladium*) *virens I*. Antagonistic properties. Material Und Organismen, 31: 79-89.
14. Paranthaman, R., R. Vidyalakshmi, S. Murugesh and K. Singaravadivel, 2009. Effects of Fungal Co-Culture for the Biosynthesis of Tannase and Gallic Acid from Grape Wastes under Solid State Fermentation. Global J. Biotechnology and Biochemistry, 4(1): 29-36.
15. Schubert, M., S. Fink and F.W.M.R. Schwarze, 2008. Evaluation of *Trichoderma* spp. as a biocontrol agent against wood decay fungi in urban trees. Biological Control, 45: 111-123.