Asian J. Exp. Sci., Vol. 22, No. 3, 2008; 373-384 **Review**

Biocontrol of Wood Decay by *Trichoderma* spp. – Retrospect and Prospect

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Abstract : A resurgence of interest in biological control of wood decay fungi due to recent revelations of its beneficial effects in protective wooden structures against decay fungi is in enormous increase. This has also been due, in large part, to increase public awareness and concern over the environmental impact of currently used chemical wood preservatives. Mechanisms of control, which have been attributed to *Trichoderma* spp., can be categorised into the following four types: competition for nutrients, production of inhibitory soluble metabolites, production of inhibitory volatiles and non-volatiles and mycoparasitism involving the production of lytic enzymes. *Trichoderma* spp. have been reported to produce siderophores, an iron chelating compounds. All the mechanisms of control of wood decay fungi have been discussed in the present review

Key words : Wood decay fungi, Biocontrol, *Trichoderma*, Competition, Mycoparasitism, Siderophores

Introduction

The microorganisms employed in biological control of fungi causing diseases of plants and wood rots are termed as antagonists and an antagonist is a microorganism that adversely affects another i.e. the target fungi causing rots and diseases growing in association with it (Baker and Cook, 1974). Fungi have got maximum attention as antagonists probably because of the fact that they are easy in handling and in identification compared to other microbes. It has been suggested that fungi serve as the most important antagonists of which the tendency of *Trichoderma* spp. and others to produce broad spectrum antibiotics is well known (Mukherjee *et al.* 1992).

Degradation of ground contact wood by wood decay microorganisms is a major problem for wood using industries. Wooden products have traditionally been protected against soft rot and the basidiomycetes through the use of chemical preservatives (Anon, 1994). Wood in ground contact is susceptible to a wide range of wood decaying microorganisms. As a result, timber intended for use in ground contact situation is generally treated using toxic chemicals such as copper chrome arsenic, which protect the wood against the effects of biodegradation. However, due to increasing awareness of the environmental impact of wood preservatives, and the introduction of more straight legislation over operations at treatment sites and the disposal of preservative treated wood, there has, over the last 25 years, been an upsurge of research into the potential of biological control as an alternative technology. During this time, a number of authors *(* Cook and Baker 1983; Nelson *et al.*

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1995; Bruce 1998) have reported on the use of biological control agents in agriculture, forestry and forest products.

More recently there has also been a very significant increase in the amount of research into biological control of wood decay fungi as an additional strategy to the use of chemical preservatives for wood protection (Freitag *et al.* 1991; Bruce 1992). This has mainly been due to the need for the wood preservation industry to develop more environmentally safe and acceptable technologies for wood protection at a time of heightened public concern on environmental issues (Philip *et al.* 1995).

The possibility of employing the antagonistic effects of some fungi against pathogenic fungi was first recognised more than 60 years ago (Weindling 1934). Successful application of biocontrol has since been reported in agriculture (Campbell, 1989), horticulture (Papavizas 1985) and in forestry (Risbeth 1975; Mercer and Kirk 1984).

Biocontrol in agriculture is usually designed to protect a crop against a narrow range of pathogens, possibly a single species, for a limited period of time, often one growing season, conversely, wood products more commonly need to be protected from a wide range of damaging microorganisms for the entire projected service life of the product. While this may be a relatively short period of some applications, e.g. paper pulp chip piles, in other instances the service life of the wooden product may be many years. Despite these obvious differences in requirements *Trichoderma* spp. have regularly been considered as potential biocontrol agents for use in both agricultural and wood preservation application.

The choice of a biological control agent is very much limited by the requirement for ecological compatibility between the control agent and its target. *Trichoderma* isolates are among the most widely researched biological control agents for the production of agricultural crops from a variety of plant diseases (Papavizas 1985).

Trichoderma is currently the most extensively researched biocontrol fungus in the field of forest products protection and has been shown on a number of occasions to provide effect against certain wood decay fungi through the production of various chemicals (Highley and Richard 1988; Bruce *et al.* 1984, 1996). Tucker *et al.* (1997) have shown that certain isolates of *Trichoderma* can protect wood against basidiomycete decay fungi.

Trichoderma spp. have been a popular choice because they are well known to antagonise other fungi by a variety of active and passive mechanism. Included in the latter category would be the organism's ability to dominate substrates through its fast growth rate, prolific spore production, metabolic versatility and tolerance of environmental stresses particularly chemicals. *Trichoderma* are fast growing primary colonisers of wood capable of utilising the sugar present and thereby inhibiting the growth of decay fungi (Hulme and Shields 1972).

Trichoderma spp. have also been reported to produce soluble antifungal metabolites (Dennis and Webster 1971; Taylor 1976; Horvath *et al.* 1995), volatile organic compounds (Bruce *et al.* 1984, 1996; Wheatley *et al,* 1997), Chitinase and laminarinase (Bruce *et al. 1995*) and siderophores (Srinivasan 1993).

Mechanisms of Control

Mechanisms of control which have been attributed to *Trichoderma* spp. can be categorised into the following types – competition for nutrients (Hulme and Shields 1970), production of soluble metabolites (Dennis and Webster 1971a, Taylor 1976), production of inhibitory volatiles (Bruce *et al.*

1984), mycoparasitism involving the production of lytic enzymes (Elad *et al*. 1982; Chet 1990;Ozbay and Newmann, 2004).

Trichoderma spp. have been reported (Anke *et al*. 1991, Dutta *et al*. 2006) to produce siderophores (iron chelating compounds) and this may contribute to the biological control of wood decay fungi. Competition for iron via siderophore production has long been recognised as an important antagonistic trait of many biological control agents of plant pathogens (Neilands 1984*;* Leong 1986; Bossier 1988, Rane *et al*. 2005, Chincholkar *et al.* 2006, Machuca *et al;* 2007).

The research has shown that *Trichoderma* isolates are well able to control decay by a variety of basidiomycete in soil block and agar test systems and has highlighted the various control mechanisms, which the organisms may employ.

Mycoparasitism is a behavioural process involving a number of sequential stages including target location, lysis and nutrient acquisition. Production of the lytic enzymes and the factors, which influence the stages are, therefore, only one aspect, which will determine the potential of any likely *Trichoderma* isolate for the biological control of decay fungi (Bruce *et al*.1995). Mycoparasitism of plant pathogenic fungi by *Trichoderma* isolates has been well researched (Harman *et al*. 1981; Chet *et al*.1981; Chet and Elad 1982; Chakraborty *et al*. 2004) and is widely considered to be a major contributing factor to the biocontrol of *Trichoderma* spp. of a range of commercially important plant disease.

Mycoparasitism may be a significant mode of antagonism of *Trichoderma* isolates against wood decay fungi has been reported (Murmanis *et al.* 1988; Srinivasan 1993; Bruce *et al.* 1995; Kundu and Chatterjee, 2003). Little work however, has been reported on the importance of mycoparasitism in the biological control of wood decay fungi by *Trichoderma* isolates. Murmanis *et al.* (1988) regularly observed directed growth and hyphal interference by *Trichoderma* spp. towards basidiomycete fungi when the two organisms were allowed to interact in wooden blocks. After a period of time, the *Trichoderma* isolates had totally consumed the hosts cytoplasmic contents, indicating active mycoparasitism by the control agents.

Lytic enzymes including chitinase and laminarinase have long been recognised as being important in mycoparasitism of plant pathogen i.e fungi by *Trichoderma* spp. *(*Elad *et al.* 1982, Karasuda *et al.* 2003). While some researchers, including Herman and Hayes (1993) have attempted to use protoplast fusion to develop effective biocontrol strains, other researchers have concentrated on improving single antagonistic trait during strain development. Haran *et al.* (1993) considered that constitutive elevation of extracellular lytic activity could improve the natural capability of *T. harzianum* to attack pathogens and its consequent use as a biocontrol agent. One of the essential characters of fungal biological control agent to act as mycoparasites of fungal plant pathogen, is their ability to excrete hydrolytic enzymes. Fluorescent indicators and enzyme studies provided evidences for such enzyme activity leading to penetration of hypha by mycoparasites (Baker and Dickman 1993). Hydrolytic enzymes such as glucanase, chitinase, cellulase, xylanase, acid and alkaline phosphatase, esterase, lipase, leucinearylaminidase, á- and â- glucosidase, N-acetylglucoaminidase and protease are known to produce by *Trichoderma* upon induction (Elad *et al.,* 1982, Aziz *et al*.1993).

Srinivasan (1993) compared weight losses in *Trichoderma* pre-treated wood blocks after exposure of wood-decay fungi against individual antagonistic responses (Volatiles, soluble metabolites, siderophores, chitinase and laminarinase) of the same *Trichoderma* isolates is an attempt to establish the likely importance of each mechanism during biocontrol *in situ* in wood. Large interstrain

and interspecies differences exist in the production of both laminarinase and chitinase by *Trichoderma* isolates (Bruce *et al.*1995). Sivan and Chet (1989) found that production of laminarinase and Chitinase enzyme by *T. harzianum* isolate could be induced by including the cell walls of suitable target fungi in the growth medium. These authors noted that cell walls from fungi known to parasitized by the bio-control agent gave greater induction of enzymes than cell wall material from other plant pathogens which were not susceptible to attack by the *Trichoderma* isolates. The cell walls of selected wood decay fungi can also act to induce production of lytic enzymes by *Trichoderma* isolate (Bruce *et al*. 1995*)*. In addition to direct competition for a soluble nutrient resource, *Trichoderma* isolates also produce a wide array of both fungi-static and fungicidal chemical compounds against wood decay fungi. Bruce *et al.* (1984) reported that some *Trichoderma* isolates produce volatile, which are fungistatic and fungicidal to a wide array of wood decay fungi.

Wheatley *et al.* (1997) reported that the composition of the growth media was a major determinant of the types and relative amounts of volatile organic compounds produced by *Trichoderma* isolates. Difference in volatile organic compound profiles when *Trichoderma* isolates were grown on different media were used to indicate which volatile organic compounds were most likely to be responsible for the inhibition of the wood decay fungi. Five volatile organic compounds were identified: 2-propanone; 2-methyl-1-butanal; heptanal; octanal and decanal *(*Bruce *et al*. 2000). Humphris *et al.* (1999) subsequently confirmed that a range of basidiomycetes was inhibited by octanal and heptanal at concentrations as low as 2.5 ppm.

Bruce *et al*. (2000) reported that aldehydes also (nonanal and decanal) inhibit the decay fungi when *Trichoderma* was grown on low nutrient medium. Other volatile organic compounds, however, may also be responsible as neither of these compounds was produced in another low nutrient medium yet volatiles from *T. aureoviride* still produced significant inhibition of the wood decay fungi on this medium.

Bruce *et al.* (1995) found that volatile organic compounds profile from *Trichoderma aureoviride* depended on the culture age, with 7-14 days old cultures of *Trichoderma* inhibiting decay fungi most. This culture age corresponded with an increase in the production of aldehydes and ketones. They also reported that production of aldehydes and other volatile organic compounds by *Trichoderma* was also affected by the specific amino acid content of the growth medium.

The inhibitory effect of *Trichoderma* volatiles is dependent on the type of amino acid added to growth and this was mirrored by corresponding changes in the volatile organic compound profiles (Bruce *et al.* 2000).

The range of amino acids in different timber species and cultivars or indeed in different sites within a tree may have a significant effect on the biological control of wood decay fungi by *Trichoderma* volatiles reported by Bruce *et al.* (2000). A proportion of the available nitrogen in sawn timber remains in soluble form and can migrate to evaporative surfaces as redistributed soluble nutrients (RSN). When the wood is dried (King *et al.*, 1974) RSN can have a significant influence on soft rot decay when such wood is in placed in soil contact (King *et al,. 1976*). Amino acid composition of this RSN material will also influence the array of volatile organic compounds produced by *Trichoderma* isolates when used for the biological protection of such material (Bruce *et al*. 2000). The volatile organic compound profiles from the *Trichoderma* isolates show that the volatile organic compounds produced are depended on both the media type and the *Trichoderma* isolate (Wheatley *et al*. 1997). Five compounds, i.e. 2-propanone, 2-methyl-butanal,

heptanal, octanal and decanal, were produced by *T. viride* and *T. pseudokoningii* in significantly higher proportion in the malt media and therefore may be important determinants of the inhibition (Wheatley *et al.* 1997).

Volatile organic compounds from *T. viride* and *T. pseudokoningii* growth on malt agar media constantly inhibited the growth of four wood decay fungi : *Neolentinus lepideus* (Fr:Fr), Readhead and Ginns (FPRLTG), *Postia placenta* (Fr.) M. Lars at Lomb. (FPRL 280), *Gloeophyllum trabeum* (Pres:Fr) Murr. (FPRL 108N) and *Trametes versicolor* (=*Coriolus versicolor*) (L:Fr.) Pilat (FPRL 28G), effects were negligible when the isolates were shown on a minimal agar medium (Wheatley *et al.* 1997). A total of 72 different volatile organic compounds were recorded from *T. aurioviride*. Not all the volatile organic compounds were however produced by all of the different aged cultures of *Trichoderma* and the volatile profile of the fungus changed significantly with time (Bruce *et al*.1996). All the basidiomycetes (*Neolentinus lepideus, Postia placenta, Glocophylum trabeum and Coriolus versicolor)* showed a similar pattern of inhibition when exposed to the volatiles from the different aged *Trichoderma* cultures. Levels of inhibition increased to a maximum when the *Trichoderma* culture was 1-2 weeks old and then progressively decreased in 2-3 week and 4-5 week old cultures which may indicate that any active inhibitory compound is produced or reaches its maximum concentration in the gaseous fraction at around this time. The composition of the *Trichoderma* volatiles changes with time and many new compounds are only produced in cultures reaching 2-3 weeks old. Many of these compounds are likely to represent secondary metabolites produced by the *Trichoderma* after the active phase of growth. The inhibition of wood decay fungi may of course be associated with the production of single compound rather than a general shift in the category of volatile compound produced. Time of maximum inhibition of the basidiomycetes of between 7-14 days also corresponds wth the appearance if various hydrocarbons and esters and any one or combination of these may account for the levels of inhibition of the decay fungi (Bruce *et al.* 1996).

Trichoderma isolates can inhibit and kill wood decay fungi by the release of volatile organic compounds, their production dependent on the identity of the *Trichoderma* isolate (Srinivasan e*t al.* 1992), the age of the colony (Bruce *et al.* 1996) and based on the growth media (Srinivasan *et al.* 1992; Wheatley *et al.* 1997)

Volatile production by *Trichoderma* isolates *in situ* in wood will act as a long-term production strategy for biocontrol agents in wood. Identification of the active volatile organic compounds may, however, provide a more environmentally acceptable strategy for the remedial treatment of established decay in wooden structures. A fuller understanding of the nature of volatile organic compounds and the factors that influence their production will also provide a much greater understanding of the microbial interactions that occur in microbial ecosystems in substrates such as wood and soil (Wheatley *et al.* 1997). This has also important implications for the use of *Trichoderma* isolates as biological control agents of wood decay fungi where the age of *Trichoderma* may be crucial in determining the level of inhibition achieved by the volatile organic compounds. The significant shifts in volatile profiles seen with *T. aureoviride* between each of the time intervals indicates that although inhibitory volatiles may play an important role in mycological ecosystems their effects would appear to be transient (Bruce *et al.*, 1996).

Diffusible (non-volatile) antibiotic activity of the *Trichoderma* spp. was more potential than the volatile antibiotics (Bunker and Mathur, 2001). Suzukacillin (Ooka *et al.*,

1966), alamethicine (Meyer and Reusser 1967), U-21963 i.e. dermadin (Pyke and Dietz 1966; Meyer, 1966) and viridepyronone (Evidente *et al.,* 2003) are some of the antibiotics extracted from culture filtrates of *T. viride.*

The production of antifungal metabolites e.g harzianolide (Avent *et al.,* 1992) volatiles e.g. Sequisterpenes (Claydon *et al*., 1987) hydrolytic enzymes e.g chitinase and laminarinase (Srinivasan *et al.,* 1992) and Siderophores (Anke *et al.*1991, Srinivasan *et al.,* 1992; Rane *et al*., 2005) have all been implicated in *Trichoderma* action. To cope with the extreme iron deficiency, microorganisms have developed high-affinity-systems for ferric transport which consists of two components a) the secretion of siderophores, i.e., iron-regulated, low-molecular weight (500 to 1500 daltons) ferric specific chelators and b) the elaboration of membrane receptor molecules which bind the siderophores and transport them into the cell. Although considerable variation exists among the several dozen charecterised siderophores, the majority may be divided into two main classes, then hydroxamates and the catecholates (Phenolates) (Hider, 1984).

Bacteria are known to produce both types (Lankford, 1973) and though fungi were thought to produce only hydroxamate siderophores (Neilands, 1984), production of phenolate type siderophores has recently been reported in wood wood-decay basimycetes by Jellison *et al*. (1991). Siderophore production by *Trichoderma* spp. has also recently been reported by Anke *et al.* (1991), who recorded the production of hydroxamate type of siderophores by these fungi.

Though siderophores are produced by virtually all microorganism the ability to sequester iron more efficiently than competitors is important for their survival. This may be dependent on a number of factors : i) the different types of siderophores produced (hydroxamate or phenolate type); 2) concentration of the siderophores produced 3) metal binding properties of the individual siderophore structure (Hider 1984; Neilands 1981; Crowly *et al.,* 1991). It has become clear from examining the mechanisms involved in wood degradation that, iron plays an important role in biodegradation both as a component of the extra cellular heme enzymes involved in white rot degradation (Pszezynski *et al*. 1988) and possibly in brown rot organisms during non-enzymatic iron/hydrogen peroxide catalysis of cellulose degradation (Murmanis *et al*. 1988).

It has been known for sometime that basidiomycetes can produce hydroxamate type siderophores (Neilands, 1984) and recently *Trichoderma* spp. have also been shown (Anke *et al.,* 1991) to produce this type of siderophore. Jellison *et al*. (1981) also identified for the first time that wood decay basidiomycetes can also produce phenolate siderophores and while this characteristic was considered to be unique to these fungi the results of TLC and NMR analysis of siderophores during this study has shown that *Trichoderma* spp. are also capable of producing phenolate siderophore compounds. In the natural environment where there is shortage of iron e.g in wood or he rhizosphere microorganisms have to compete with others to acquire the iron with the aid of their siderophores (Crowley *et al.* 1991). The ability of the organisms to sequester the iron depends on the type of siderophores produced and it has been established that hydroxamate type siderophores have a lower formation constant or iron binding ability, than the phenolate type siderophores (*Neilands, 1981; Hider, 1984*). The results indicate that the rate of siderophore production influences outcome of interaction between wood decay basidiomycetes and *Trichoderma,* and it is evident from the CAS agar plates that *Trichoderma* isolates show varying levels of production as indicated by the halo size and the optical density values respectively (Payne, 1994; Schwyn and Neilands, 1997; Milagres *et al.,* 1999; Dave and Dube, 2000, Yeole *et al.,* 2001; Kundu, 2002). The fact that both basidiomycetes

and *Trichoderma* are capable of producing both phenolate and hydroxamate type siderophores implies that competition for iron may play an important role in the antagonistic mechanism between these organism and therefore may be very significant for the bicontrol of wood decay fungi by the *Trichoderma* spp. (Srinivasan, 1993). Due to very low concentration of iron in wood, siderophore competition for iron between *Trichoderma* and the decay fungi may be a very significant antagonistic mechanism. It is believed that cellulose and hemicellulose are two large to diffuse into wood (Highley *et al.* 1988*)* and that they can degrade wood only if preceded by the non-enzymatic iron/hydrogen peroxide system that catalyses the oxidation of cellulose as proposed by Koenigs (1974). The iron needed for these purposes is sequestered by siderophores (Jellison *et al.* 1991). They have also shown that siderophores of *Gloeophyllum trabeum* are capable of altering the structure and erystallinity of cellulose compounds and siderophore competition from an antagonist may prevent wood decay fungi from efficiently degrading wood. Recently the study of the ability of wood decaying fungi to produce high affinity iron binding compounds of the siderophore type has received much attention, because it has been suggested that these compounds play an important role in the initial stages of wood cell wall decay process (Enoki *et al.* 1997; Goodell *et al.* 1997). It is reported that lectins were shown to be involved in the recognition of *Trichoderma* spp. and their host fungus, while chitinase (EC-3, 21.4) is involved in the degradation of the host cell wall (Chet and Inbar 1994).

The cell wall degrading enzymes chitinase and â–1,3 glucanase were recovered from *T. harzianum* cultures. There was evidence that the mechanisms of antagonism employed by *T. harzianum* were competition, lysis ad hyperparasitism (mycoparasitism) (Godwin Egein and Arinzae 2001; Shoulkamy *et al.* 2006)

Recently, the antagonistic properties of purified chitinolytic and glucanolytic enzymes from the biocontrol fungi *Trichoderma harzianum* and *G. virens* have been described, and evidence provided that these cell walls degrading enzymes may act synergistically with antibiotics. Recombinant DNA technology, allowing the construction of genetically modified biocontrol agents will be useful for evaluating the role of specific compounds in biocontrol and creating improved biocontrol organisms. (Pietro, 1995). The chitinolytic enzymes from *T. harzianum* appeared to be biologically more active than enzymes from other sources and more effective against a wider range of fungi have been reported (Lorito *et al.,* 1993). The *Trichoderma* based formulation has been shown to strongly inhibit the growth of the common brown rot fungus *Lentinus lepideus* under controlled laboratory condition, either because of the production of inhibitory volatiles (Bruce *et al.* 1984) or the deposition of non-volatile compounds in the wood cell wall.

The recently developed biocontrol formulation Binab, which contains *Trichoderma polysporum* (Link) and *T. harzianum* Rifai; Morris *et al.,* 1986 has received extensive attention in Europe. If *Trichoderma* spp. will grow in heartwood and sapwood of timber spp. then the possibilities for biological control using *Trichoderma* isolates to inhibit the growth of basidiomycete organisms which colonize the wood, will be greatly enhanced and increase the service life of a number of timber products (Philip *et al.,* 1995). The antagonistic properties of different *Trichoderma* isolates and their ability to kill basidiomycete vary between isolates and have also been shown to be dependent on growth substrate composition (Srinivasan *et al.,* 1992).

T. viride isolate (T60) has been reported to reduce the level of Sapstain in the field (Brown and Bruce, 1999). He has also reported the inhibition of soft rot and the decay caused by basidiomycete by *T. viride*. *Trichoderma* is currently the most extensively researched biocontrol fungus in the field of

products protection and has been shown in a number of occasions to provide a protection effect against certain wood decay fungi (Highley et *al.* 1988) and protect wood against basidiomycete decay fungi (Brown and Bruce, 1999).

Conclusion

Inadequate understanding of microbial ecology, factors leading to sustained performance of biocontrol agent in natural environment and lastly the expectation that biocontrol agent will substitute for chemical in terms of instant results were the main causes by which biocontrol of wood decay has not been fulfilled its promise. But to develop sustainable systems of wood decay protection, the role of biological control method is unquestionably pivotal. To avoid chemical hazards that deplete and degrade the resources of environment, wood rot management approach through biocontrol agents has gained more attention recently. Emergence of biotechnology, genetic engineering and plant immunization technology will provide better solutions to control wood deterioration problems and to develop broad-spectrum durable resistance of wood against decay fungi. It is now well accepted that biocontrol of wood rot fungi is an eco-friendly means which has got distinct possibilities of successful exploitation in wood and timber industry.

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