THE ROLE OF SIDEROPHORE PRODUCTION IN THE BIOLOGICAL CONTROL OF WOOD DECAY FUNGI BY *TRICHODERMA* SPP.

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ABSTRACT

In recent years biological control has received increasing attention as a means of protecting wooden structures against decay fungi. This has been due, in large part, to increased public awareness and concern over the environment impact of currently used chemical wood preservatives. Laboratory studies have indicated that *Trichoderma* isolates are able to antagonise wood decay fungi by a number of different mechanisms. These include passive antagonism through competition for available nutrients; active antagonism through the production of soluble metabolites and volatiles and mycoparasitism involving lytic enzymes. To date however, little has been reported regarding iron competition in wood as a possible antaponistic mechanism against wood decay fungi. Iron is in limited supply in wood, and recent papers have suggested that it may also play an important role in the biodeterioration process of timber. This paper examines the potential role of siderophores (iron chelators) in the biocontrol of wood decay fungi.

KEYWORDS: Wood, Fungi, Rot, Decay, Siderophore, Control, Iron.

INTRODUCTION

Iron is an indispensible nutrient for almost all living cells. It plays an essential role in respiration, nitrogen fixation, DNA and chlorophyll biosynthesis and other important enzymatic systems (Neilands, 1981). Although iron is one of the most common elements on Earth, its availability to living organisms is extremely low due to its profound insolubility. Under aerobic conditions iron is readily converted into its ferric form, which binds to hydroxide ions. The solubility product constant of ferric hydroxide is 10^{-38} M, and at biological pH becomes 10^{-17} M, which limits the concentration of free ferric ion to a value too low to sustain growth. Therefore, during the course of evolution, micro-organisms, plants and animals have developed have developed efficient sequestering mechanisms for ferric ion to ensure its utilisation (Neilands, 1981).

To cope with this extreme iron deficiency, micro-organisms 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 characterised siderophores, the majority can be divided into two main classes, the 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 siderphores (Neilands, 1984) production of phenolate type siderophores has recently been reported in wood decay basidiomycetes by Jellison *et al.* (1990). Siderophore production by *Trichoderma* spp. has also recently been reported by Anke *et al.* (1991) who recorded the production of hydroxamate type siderophores by these fungi.

Though siderophores are produced by virtually all micro-organisms the ability to sequester iron more efficiently than competitors is important for their survival. This may be dependent on a number of factors: 1) 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; Crowley *et al.*, 1991). All the above factors will play a role in competition for iron with other organisms. Due to such differences in affinity and production of siderophores by different fungi, iron competition can play an important role in a number of combative situations. Competition for iron is a way of life for soil micro-organisms and exerts a profound effect on the interactions among saprophytic microbes, pathogens and plants. the involvement's of siderophores produced by *Pseudomonas* species in suppressing soil-borne diseases is documented in numerous reports and reviews (Leong and Experts, 1989).

It is evident that siderphores play an important role in the biocontrol of many plant pathogenic fungi and bacteria (Neilands, 1984). To date however little has been published regarding iron competition between *Trichoderma* and wood decay basidiomycetes in wood as a possible antagonistic mechanism. If the *Trichoderma* can produce siderphores with an affinity constant that is greater than or equal to that of the basidiomycete siderophores they may deprive these decay fungi of iron and may affect their ability to survive in or degrade wood.

METHODS AND MATERIALS

One isolate of *T. auteoviride* (Rifai) IMI91968; four isolates of *T. viride* (Persoon ex S.F. Gray) including isomates IMI24039, and isolates 14, 40 and 60; one isolate of *T. harzianum* (Rifai) Isolate 25; three isolates of *T. psedokoningii* (Rifai) Isolate 51, 55 and 64; and one unidentified *Trichoderma* isolate 140, were used in study.

Interaction studies between these ten *Trichoderma* isolates, selected on the basis of preliminary studies (Srinivasen *et al.*, 1992) was carried out against two basidiomycetes. A brown rot fungus *Neolentinus legidus* FRL 7F and a white rot fungus *Trametes versicolor* MAD 697, were used and tested on both low nutrient

media (LNM) (see Srinivasen *et al.*, 1992) with higher iron (65 μM) (LNM+Fe) and low iron (0·65 μM) (LNM+Fe) to induce siderophore production. Death or survival of the basidiomycetes was determined by subsequently plating cores or mycelium from the interaction area onto selective media (3% malt extract agar containing benomyl (4 ppm)).

Detection of siderphore production by the *Trichoderma* isolates was also carried out with the aid of special dye medium, CAS (chrome azurol S) agar (Schwyn and Neilands, 1987). When a strong chelator such as a siderophore removes the iron from the dye, its colour turns from blue to orange. When the CAS complex is incorporated into agar plates, orange halos around the colonies are indicative of siderophore excretion.

A few *Trichoderma* spp. selected on the basis of their ability to show killing effect against both the basidiomycetes and their ability to produce siderphores were grown on iron free LNM to induce production of siderophores. The hydroxamate and phenolate type siderophores produced were isolated and purified after minor modification of the procedures by Fekete *et al.* (1989) and Jellison *et al.* (1991) for the respective siderophore types. Thin layer chromatography (TLC) and nuclear magnetic resonance (NMR) were carried out on the purified samples for characterisation of the siderophore type and molecular configuration of chelating sites.

RESULTS AND DISCUSSION

Results of the interaction studies on LNM+Fe and LNM+low Fe between the ten *Trichoderma* isolates and the two basidiomycetes show that browning of the basidomycetes (indicating cell lysis) at the interaction zone was heavier in the low iron than the high iron media (Figure 1). In some instances the outcome of the interaction varied between the two media. This was found to be particularly true with the brown rot fungus. Interactions that exhibited a lethal effect against basidiomycetes in the low iron media area obviously influenced by the concentration of iron in the medium and therefore it can be assumed that this lethal effect is due to siderophore competition for iron between the two organisms.

Iron and other metals such as manganese, play a role in the biological degradation as essential elements for fungal metabolism and growth. And it has become clear from examining the mechanisms involved in wood degradation that, iron also plays an important role in biodegradation both as a component of the extracelular heme enzymes involved in white-rot degradation (Paszczynski, *et al.*, 1988) and possibly in brown-rot organisms during non-enzymatic iron-hydrogen peroxide catalysis of cellulose degradation (Murmanis *et al.*, 1988). To solubilise and sequester iron for biodegradation vasidiiomycetes need the aid of siderophores, and competition for this iron may therefore prove essential for the survival of these fungi.

It has been known for sometime that basisiomycetes 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.* (1991) also identified for the first time that wood decay basidiomycetes can also produce phenolate siderphores 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. area also capable of producing phenolate siderophore compounds. In the natural environment where there is shortage of iron e.g. in wood or the rhizosphere, micro-organisms have to complete with others to acquire the iron with the aid of siderophores (Crowley *et al.*, 1991). The ability of the organisms to sequester the iron depends on the type of siderphores 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 of our study 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. The fact that both basidiomycetes and *Trichoderma* are capable or producing both phenolate and hydroxamate type siderphores implies that competition for iron may play in important role in the antagonistic mechanisms between these organisms and therefore may be very significant for the biocontrol of wood decay fungi by the *Trichoderma* spp.

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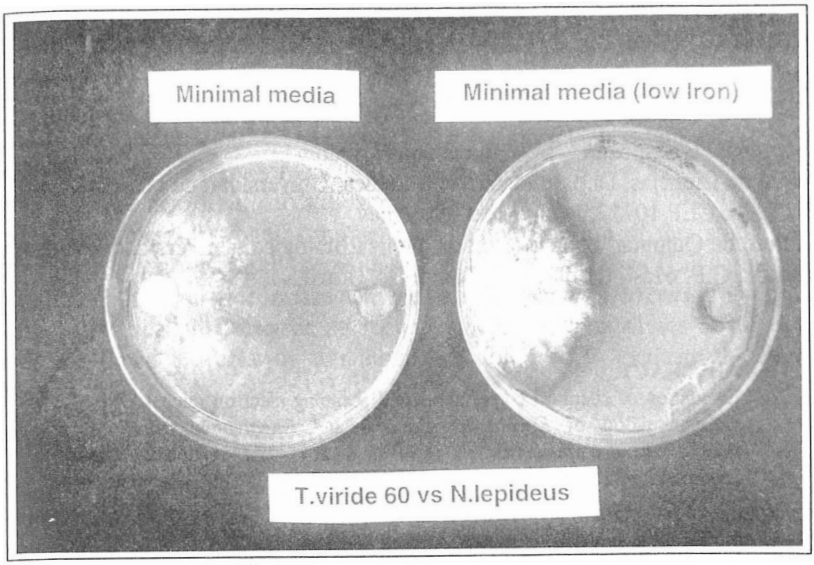

Figure 1. Interaction of *T. viride* species (isolate 60) with the brown rot fungus *N. lepideus* on the low nutrient media (left) and low nutrient media with reduced iron (right). Note the increased browning in the low iron media. Basidiomycete inoculated on the left and *Trichoderma* on the right side of the plate.

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