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Copper Tolerant Wood-Rotting Fungi under Environmental Stress

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ROLE OF OXALATE BIOSYNTHESIS FOR THE GROWTH OF COPPER TOLERANT WOOD-ROTTING FUNGI UNDER ENVIRONMENTAL STRESS

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Abstract

It has been widely accepted that wood-rotting fungi produce oxalic acid to the culture media and during colonization of wood. And the most significant physiological difference between the brown-rot and white-rot fungi is their ability to produce oxalic acid. Thus for more than two decades, various roles of oxalic acid in wood decay caused by wood-rotting fungi have been investigated from different aspects, such as in lignin peroxidase and manganese peroxidase systems, catalyst in hydrolytic breakdown of cellulose, and hemicellulose, and calcium pectate, and as reducing agent for generating Fenton type oxidation to breakdown cellulose. Recently we have proposed new metabolic mechanism for oxalic acid biosynthesis which is an important metabolic device for acquiring biochemical energy for the growth of wood-rotting fungi. This paper discusses the important role of oxalate biosynthesis for the growth of copper tolerant fungi under environmental stress condition. The important roles of oxalate biosynthesis in other group of microorganism are also described.

Keywords : Oxalate biosynthesis; copper-tolerant fungi; wood-rotting fungi; environmental stress

Introduction

Metabolic mechanism for oxalic acid biosynthesis in a copper tolerant brown-rot fungus *Fomitopsis palustris*, which was previously called as *Tyromyces palustris*, has been recently reported by our group. The new proposed metabolic mechanism plays an important role as a biochemical device for acquiring biochemical energy needed for the fungal growth. Interestingly, the fungus converted glucose to oxalic acid with high efficiency: only oxalate accumulated in the culture growth (Munir et al. 2001a). The metabolic mechanism has also been proposed to commonly occur in brown rot fungi especially for those known as copper tolerant fungi. Furthermore we successfully purified and characterized the two key enzymes, isocitrate lyase and malate synthase, playing crucial role and effectively keeping the metabolic conversion of glucose to oxalic acid in this wood-rotting fungus (Munir 2002; Munir et al. 2002). And recently, we have reported

that the fungus maintained the enzyme activities in normal level when it was grown in media supplemented with a different metal ion (Munir et al. 2004).

The important of oxalate biosynthesis in copper-tolerant wood rotting fungi

When fungi are grown under stress condition, e.g. in environment containing toxic metal ions, the fungi will experience various stresses, because toxic metal ion can cause different effect to the microbial cell, such as disrupting cell membrane, inhibiting transcription and translation processes, inhibiting enzyme activity and cell division, damaging DNA and denaturing the protein (Roanne & Pepper 2000). In order to minimize such effects during growth process, microbial cells have been known to develop active defense mechanisms. Gimmler et al. (2001) have proposed several mechanisms that are responsible for resistance of microorganisms to heavy metals, such as binding of heavy metals to cell walls, reducing transport of heavy metal across the plasma membrane, activating efflux of heavy metals (Tsai et al. 1997), compartmentation inside vacuoles, internal sequestration (complexation) by special protein (metallothioneins) and organic acids (Hayashi and Mutoh 1994; Macreadie et al. 1994).

From this point, we focus our discussion to the last mechanism above, i.e. the development of fungal resistance by organic acid produced by fungi. Oxalic acid as a major organic acid secreted by wood-rotting fungi is a strong chelating agent to precipitate many metal ions including calcium ions, copper ions etc. Precipitation of toxic metal ion out site of the cell by formation of metal - organic acid complex, such as metal-oxalate or crystal oxalate, has been commonly reported in wood preservation field. Sutter & Jones (1985); Murphy & Levy (1983) reported that oxalic acid produced by copper tolerant wood-rotting fungi has been known to detoxify copper-based wood preservatives into insoluble form of copper-oxalate, which is physiologically inert. Basically, copper compounds are highly effective fungicides and have been used traditionally in wood preservation. However many species of wood-rotting fungi that have been isolated are tolerant to these compounds (Tsunoda et al. 1997).

Recently we have reported that the growth of brown rot fungi *Fomitopsis palustris*, *Laetiporus sulphureus* and *Coniophora puteana* was relatively better than that of the white rot fungi when a different metal ion at different concentration was supplied to the culture media (Munir et al. 2004). The pattern of growth inhibition by metal ion both for the white-rot and the brown rot fungi was relatively the same, in which Co was more toxic

than others (at 1000 ppm). However the growth of white rot fungi, *Coriolus versicolor* and *Phanerochate chrysosporium* was very much inhibited by copper at 1000 ppm, which was not observed for all brown rot fungi tested. Interestingly, clear zone was observed surround the colony of the brown rot fungi when they were grown in Cu containing plate but it was not observed for the white rot fungi as we can see in Figure 1. The clear zone was only formed by brown rot fungi, which are widely known to produce higher amount of oxalic acid. Therefore, it is suggested that the metal ion in that zone was precipitated by oxalate the produced.



Figure 1. Growth of wood rotting fungi on copper containing plate. Upper row, brown rot fungi; bottom row, white rot fungi.

In many cases, it has been found that there was an increase production of oxalate when copper tolerant fungi were grown on metal ion containing media. Green & Clausen (2001) reported that most brown-rot fungi, known as copper-tolerant fungi including genus *Coniophora*, *Laetiporus*, *Postia*, *Serpula*, *Tyromyces*, and *Wolfiporia*, increased the production of oxalate 2 to 17 times higher when they were grown in copper-containing wood preservative than the production of oxalate when they were grown in untreated wood. Importantly, there was no significant inhibition of the enzyme activity by metal ions (Cu, Zn, and Al) when they were added to the culture growth (Munir et al. 2004). Then, because brown rot fungi have been known to produce oxalate (Munir et al. 2001b) and the activity of ICL and MS were found to be higher in the brown rot fungi than that in the white rot fungi and both enzymes play crucial role in the production of oxalate, it is

worthwhile to propose that the glyoxylate cycle enzymes play an important role for the growth of fungi in metal ion containing media.

The following illustration shows relationship between ability of wood rotting fungi to produce oxalic acid with their ability to colonize copper treated wood.

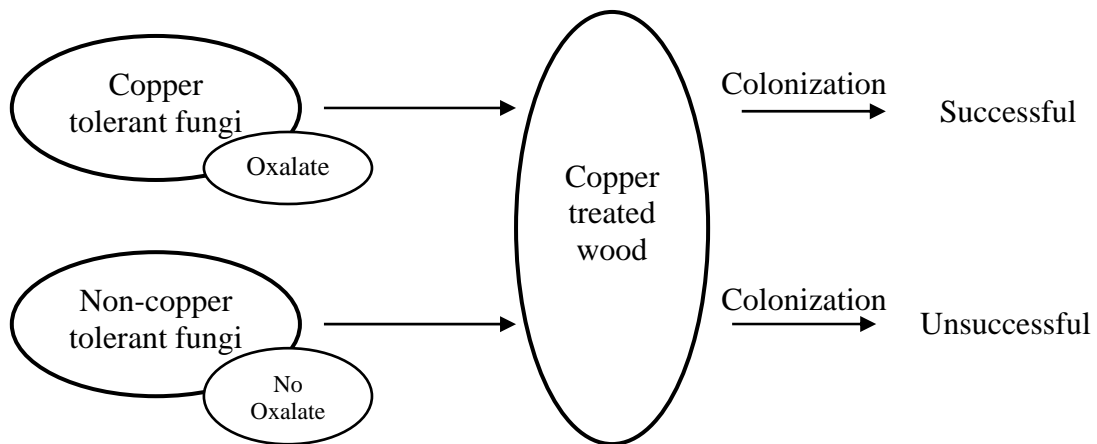


Figure 2. Proposed relationship between ability of wood rotting fungi to produce oxalic acid and their ability to colonize copper treated wood.

Before colonization is started, the fungi have to secrete oxalic acid to surrounding wood to minimize toxicity effect of copper through inactivation of copper compounds. The fungi, which are able to grow and colonize such copper-containing material, are commonly known as copper tolerant fungi, and brown rot fungi are more tolerant than white rot fungi. Further analyses have shown that the ability of fungi to colonize copper treated wood (successful) has a positive correlation with their ability to produce oxalate.

The importance of oxalate biosynthesis in plant pathogen

Role of oxalic acid produced by plant pathogens has been obtained big attention in field of plant pathology. Oxalic acid produced by fungal plant pathogen during infection causes a reduction of pH and induces the activity of polygalacturonase in hydrolysis of pectate component in plant cell wall (Margo et al. 1984). In many cases, such as in *Rhizoktonia soliani* and in *Sclerotium rolfii*, it was found that polygalacturonase only active in the presence of oxalate. Furthermore this group has reported that mutant of *Sclerotinia schlerotiorum* (not producing oxalate) was unable to cause disease in plant. Therefore it is

suggested that there is a positive correlation between virulence and the ability to produce oxalate in pathogenesis of plants as describe in the following illustration.

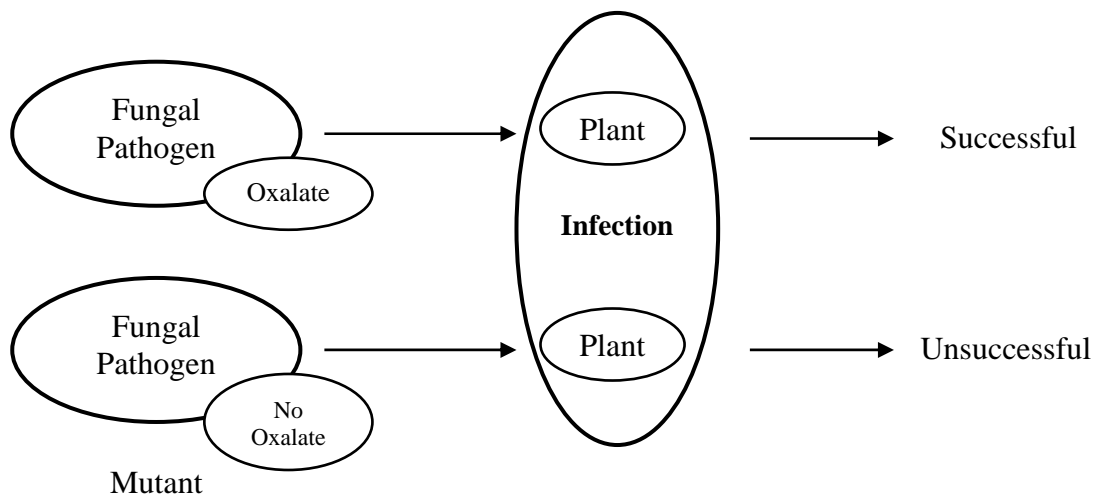


Figure 3. Proposed relationship between ability of fungal pathogen fungi to produce oxalic acid and their ability to cause infection in host plant.

Recently, it has been reported that bacterial plant pathogens defective in glyoxylate cycle enzymes were unable to cause disease. Bacterial pathogen *Rhodococcus fascians* defective of malate synthase was unable to cause gall in various plants (Vereecke et al. 2002) and fungal pathogen *Leptosphaeria maculans* isocitrate lyase mutant was unable to cause disease on *Brassica napus* (Idnurm & Howlett 2002). Both group have proposed that there is a positive correlation between activity of glyoxylate cycle enzymes and the ability of pathogen to cause disease. Unfortunately they did not discuss on the production of oxalate as well as role of the enzymes in the synthesis of oxalate in these bacteria.

In pathogenic microbes such as *Mycobacterim tuberculosis* and *Candida albicans*, isocitrate lyase is required for virulence. The enzyme has been found to play a crucial role for survival and proliferation of pathogens in host tissue, which normally serves various environmental stresses. Mutant strains of isocitrate lyase attenuate pathogen persistence and virulence and unable to cause the diseases (Lorenz & Fink 2001). McKinney et al. (2000) have reported that persistence and virulence of *Mycobacterium* in macrophage (in mice) requires glyoxylate cycle and ICL.

Conclusion

On the basis of this discussion, it is proposed that when grown under environmental stress condition, wood rotting fungi particularly brown-rot increase the synthesis of oxalic acid by increasing the activity of enzymes involved in metabolic mechanism such as isocitrate lyase as a pivotal enzyme. The success in finding specific inhibitors for the important enzyme on metabolic mechanism might be able to overcome development copper-tolerant fungi. Thus, it is suggested that the enzyme is the important target in searching environmentally benign wood preservatives.

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