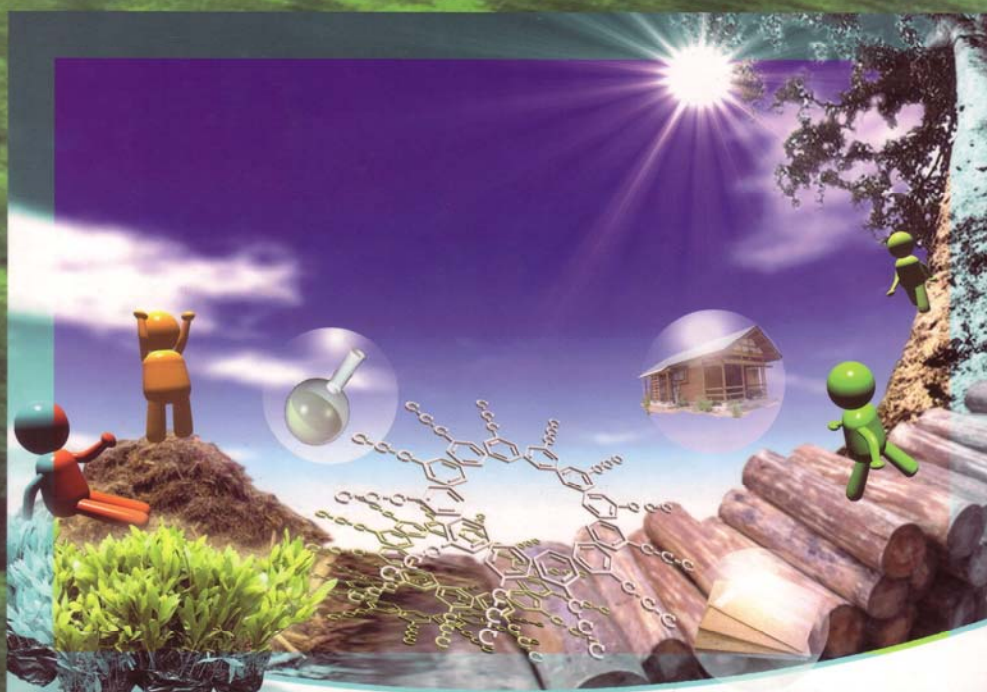


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ROLE OF OXALATE BIOSYNTHESIS IN GROWTH OF COPPER TOLERANT WOOD-ROTTING AND PATHOGENIC FUNGI

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Abstract Wood-rotting fungi produce oxalate while colonizing wood. Brown-rot fungi generally accumulate more oxalate than the white-rot type, which is the principal physiological difference between the two. For over two decades, investigators have examined the roles of oxalate in the chelation and reduction of lignin peroxidase and manganese peroxidase systems as well as the acid catalysis involved in the hydrolytic breakdown of cellulose, hemicellulose and calcium pectate during the process of decay caused by wood-rotting fungi. We recently proposed a novel metabolic mechanism for oxalic acid biosynthesis, which is an important metabolic means of acquiring the biochemical energy required for the growth of wood-rotting fungi. This paper discusses the role of oxalate biosynthesis in the growth of copper tolerant fungi under environmental stress. The roles of oxalate biosynthesis in other groups of microorganism are also described.

Key words: oxalate biosynthesis, copper-tolerant, wood-rotting fungi, pathogenic fungi, environmental stress

Introduction

We recently described the metabolic mechanism of oxalate biosynthesis in a copper-tolerant brown-rot fungus *Fomitopsis palustris* (previously known as *Tyromyces palustris*). We proposed this novel metabolic mechanism to explain how the fungus acquires the biochemical energy required for vegetative fungal growth through oxalate fermentation. Interestingly, the fungus efficiently converted glucose to oxalic acid, but to no other type of organic acid (Munir et al. 2001a). We proposed that this metabolic mechanism exists among brown-rot fungi, especially those that are copper tolerant. Furthermore, we purified and characterized the two key enzymes involved in the glyoxylate cycle, isocitrate lyase (ICL) and malate synthase (MS). These enzymes play crucial roles in the conversion of glucose to oxalate in *Fomitopsis palustris* (Munir 2002; Munir et al. 2002). We also recently reported that the fungus maintains normal enzymatic activities when grown in media supplemented with a different metal ion (Munir et al. 2004).

The importance of oxalate biosynthesis in copper-tolerant, wood rotting fungi

When grown under stress such as in an environment containing toxic metal ions, the fungi will be exposed to further stresses, because in microbial cells, toxic metal ions can disrupt the cell

membrane, inhibit transcription and translation processes, inhibit enzyme activity and cell division, damage DNA and denature proteins (Roanne & Pepper 2000). Microbial cells have therefore developed active defense mechanisms to minimize such effects during growth. Gimmler et al. (2001) have proposed several mechanisms to explain how microorganisms resist heavy metals, such as binding them to cell walls, reducing the amount of heavy metals transported across the plasma membrane, activating the efflux of heavy metals (Tsai et al. 1997), vacuolar engulfment and internal sequestration (complexation) by specific proteins (metallothioneins) and organic acids (Hayashi and Mutoh 1994; Macreadie et al. 1994).

Based on these studies, we focus our discussion on the development of fungal resistance through organic acid produced by the fungus itself. Oxalate, which is the major organic acid secreted by wood-rotting fungi, is a powerful chelating agent that precipitates many metal ions including calcium and copper. Toxic metal ions are extracellularly precipitated through the formation of metal-organic acid complexes such as metal- or crystal- oxalates. Sutter & Jones (1985) as well as Murphy & Levy (1983) reported that oxalic acid produced by copper tolerant wood-rotting fungi can detoxify copper-based wood preservatives into insoluble copper-oxalate, which is physiologically inert. Copper compounds are highly effective fungicides that have traditionally been used in wood preservation, but many species of wood-rotting fungi are tolerant to these compounds (Tsunoda et al. 1997).

We recently reported that the growth of both the brown-rot fungi *Fomitopsis palustris*, *Laetiporus sulphureus* and of *Coniophora puteana* is relatively improved compared with that of white-rot fungi when another metal ion at various concentrations is added to the culture media (Munir et al. 2004). The profiles of growth inhibition by metal ions for both white- and brown-rot fungi were essentially identical, in that a Co value of 1,000ppm was the most toxic. However the growth of the white-rot fungi, *Coriolus versicolor* and *Phanerochate chrysosporium* was considerably inhibited by 1,000ppm of copper, which was not true of any brown-rot fungi tested. Interestingly, a clear zone surrounded colonies of the brown-rot fungi grown in plates containing Cu but not those of the white-rot fungi (Fig. 1). The brown-rot fungi produce high levels of oxalate. These findings suggested that metal ions in that zone were solubilized by oxalate produced by the fungi and that a portion of them was precipitated on the fungal surface.

Oxalate is often produced by copper-tolerant fungi grown on media containing metal ions. Green and Clausen (2001) reported that most brown-rot copper-tolerant fungi including the genera *Coniophora*, *Laetiporus*, *Postia*, *Serpula*, *Tyromyces*, and *Wolfiporia*, increased the production of oxalate 2- to 17-fold when grown in wood preservative containing copper than in untreated wood. Importantly, metal ions such as Cu, Zn, and Al did not significantly inhibit enzyme activity when added to cultures (Munir et al. 2004). Brown-rot fungi produce oxalate (Munir et al. 2001b), ICL and MS are more active in brown- than in white-rot fungi and both enzymes play crucial roles in oxalate production. Thus, the glyoxylate cycle enzymes appear to play an important role in the growth of fungi in media containing metal ions.

The following illustration shows the relationship between the ability of wood-rotting fungi to produce oxalate and to colonize wood treated with copper.

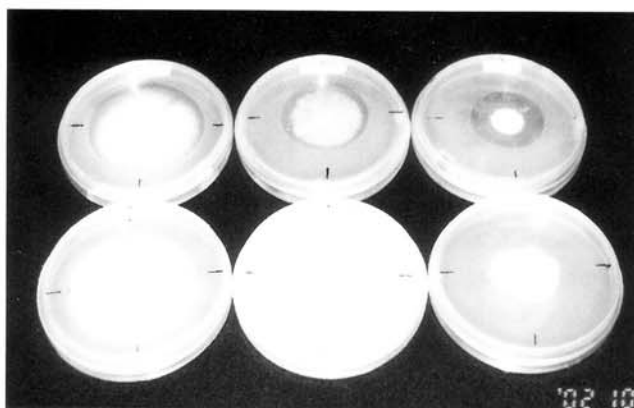


Fig. 1 Growth of wood-rotting fungi on plates containing copper. Upper row, brown-rot fungi; bottom row, white-rot fungi.

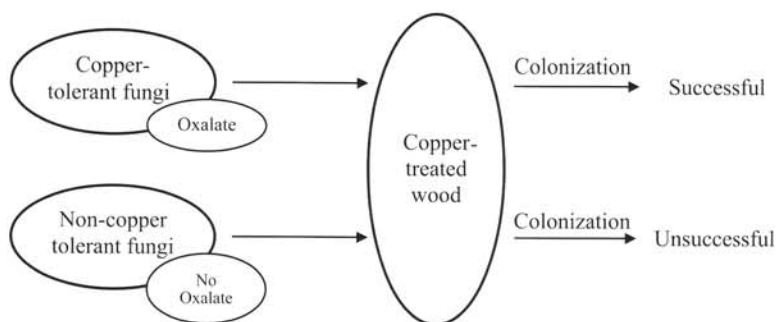


Fig. 2 Proposed relationship between ability of wood-rotting fungi to produce oxalic acid and to colonize wood treated with copper.

Before colonization begins, a fungus must secrete oxalate into the surrounding wood to minimize toxic effects via inactivating copper compounds. Fungi that can grow and colonize wood under such circumstances are commonly known as being copper tolerant, 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 is positively correlated with their ability to produce oxalate (Green and Clausen 2001)

Pathway of oxalic acid biosynthesis compared with TCA and GLOX cycles

Because the mechanisms of oxalate biosynthesis are directly related to the metabolism of acetyl-CoA (Munir et al. 2001a), we compared the mechanisms of oxalic acid biosynthesis with those of the TCA and glyoxylate cycles (Fig. 3). In the TCA or Krebs' cycle (A), 1 mol of acetyl-CoA is oxidized to 2 mol of CO₂, during with the generation of biochemical energy. In the glyoxylate or Kornberg's cycle (B), 2 mol of acetyl-CoA are converted to 1 mol succinate, which is usually transferred to the TCA cycle. Therefore, the glyoxylate cycle is also known as the anaplerotic cycle. In the pathway of oxalic acid metabolism shown at the bottom of Fig. 3, 2 mol

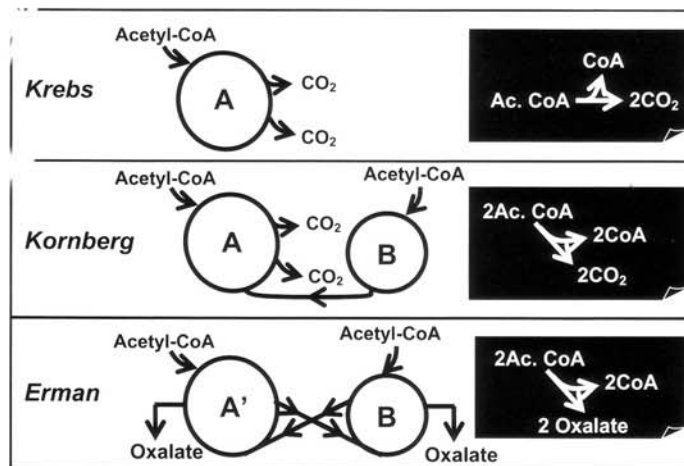


Fig. 3 TCA cycle and its analogues.

of acetyl-CoA are converted to 2 mol of oxalic acid by coordinating a modified TCA cycle (A') with a glyoxylate cycle (B). Since the result of this investigation is quite significant to the general metabolism of acetyl-CoA, Dr. Mikio Shimada has referred to this mechanism as the bicyclic "Erman's cycle".

The importance of oxalate biosynthesis in plant pathogenicity

The role of oxalic acid produced by plant pathogens has recently undergone considerable scrutiny by plant pathologists. Oxalate produced by fungal plant pathogens during infection reduces the pH and induces polygalacturonase activity in the hydrolysis of pectate components in plant cell walls (Margo et al. 1984). Polygalacturonase is only active in *Rhizoktonia soliani* and in *Sclerotium rolfsii* in the presence of oxalate. Furthermore, Margo et al. have reported that a mutant of *Sclerotinia sclerotiorum* (not an oxalate producer) does not cause plant diseases, indicating a positive correlation between virulence and the ability to produce oxalate in plant pathogenicity as described below.

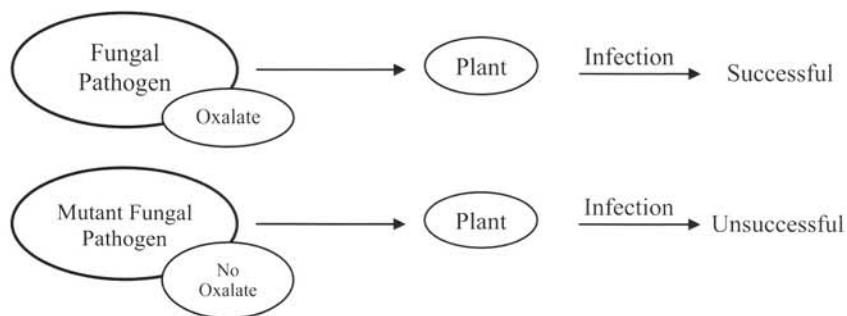


Fig. 4 Proposed relationship between ability of fungal pathogens to produce oxalic acid and to infect host plants.

Bacterial plant pathogens that are defective in glyoxylate cycle enzymes do not cause disease. The bacterial pathogen, *Rhodococcus fascians*, which is defective in malate synthase does not cause gall in various plants (Vereecke et al. 2002) and an isocitrate lyase mutant of the fungal pathogen, *Leptosphaeria maculans* does not cause disease on *Brassica napus* (Idnurm and Howlett 2002). Both groups have proposed a positive correlation between the activity of glyoxylate cycle enzymes and the ability of a pathogen to cause disease. However, they did not discuss the production of oxalate in addition to the role of the enzymes in the synthesis of oxalate by these bacteria.

Isocitrate lyase is required for the virulence of pathogenic microbes such as *Mycobacterium tuberculosis* and *Candida albicans*. This enzyme plays a crucial role in the survival and proliferation of pathogens in host tissue, which normally provides various environmental stressors. Mutant strains of isocitrate lyase attenuate pathogen persistence and virulence and cannot cause diseases (Lorenz & Fink 2001). McKinney et al. (2000) have reported that the persistence and virulence of *Mycobacterium* in mouse macrophages require the glyoxylate cycle and ICL.

Conclusions

On the basis of this discussion, we propose that when under environmental stress, wood-rotting fungi, particularly brown-rot species, increase the synthesis of oxalic acid. This is achieved by increasing the activity of enzymes pivotal to the metabolic mechanism such as isocitrate lyase. Identifying specific inhibitors of these important enzymes might be able to overcome the development of copper-tolerant fungi.

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