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A novel Physiological Aspect of Wood-destroying Basidiomycetes

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A NOVEL PHYSIOLOGICAL ASPECT OF WOOD-DESTROYING BASIDIOMYCETES
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Introduction

Metabolism of oxalic acid, which widely occurs in a wide variety of higher plants, cultures of molds, and even in human urine as an end product, has long been investigated from a medicinal viewpoint (Hodgkinson 1977). Oxalic acid has three chemical natures such as proton and electron sources, and a strong metal chelater, despite its simple chemical formula of $(\text{COOH})_2$. Due to its unique multiple chemical natures, it has been receiving much attention from various ecological aspects as follows: a) bioremediation of a wide variety of organic pollutants (Barr & Aust 1994) with lignin biodegradation systems (Akamatsu et al. 1990, Popp et al. 1990, Shimada et al. 1997), b) inactivation of copper-containing wood preservatives by wood-rotting fungi (Tsunoda 1997, Murphy & Levy 1983), c) detoxication of aluminum toxicity in Al-resistant buckwheat (Ma et al. 1997), d) crop damages caused by oxalic acid-producing phytopathogens (Maxwell & Bateman 1982), e) a biofertilizer effect of ectomycorrhizal fungi (Malczuk & Cromack 1982, Lapeyrie et al. 1987), and f) an electron source for nitrogen-fixation in symbiotic rhizobia in a legume plant (Trinchant et al. 1994). In addition, it is known as a general physiological trait that most of brown-rot basidiomycetes, including *Fomitopsis palustris*, accumulate oxalic acid at greater concentrations in culture fluid, whereas white-rot ones do not because they metabolize and/or decompose it by various mechanisms (Dutton et al. 1993, Green & Clausen 1999). Nevertheless, the white-rots were observed to accumulate Ca-oxalate during wood decay processes (Dutton Evans 1996).

Previously, we have reported the occurrence of the two oxalate-producing enzymes, glyoxylate oxidase and oxaloacetase, in wood-destroying fungus *F. palustris*. We have purified and characterized a novel flavoprotein glyoxylate dehydrogenase which catalyzes dehydrogenation of glyoxylate to oxalate in the presence of cytochrome c (Tokimatsu et al. 1998).

Quite recently, we have reported the unprecedented metabolic system which explains an important physiological role of the oxalate biosynthesis in a wood-rotting basidiomycete *F. palustris* (Munir et al. 2001). Reviewing the previous investigations, we would like to report here ubiquitous occurrence of the glyoxylate key enzymes, isocitrate lyase (ICL) and malate synthase (MS) among both white- and brown-rot basidiomycetes grown on glucose and the preliminary results of cDNA cloning for ICL of the brown-rot fungus *F. palustris*.

Results and Discussion

Figure 1 indicates that ICL is a central and pivotal enzyme in the metabolic cycles (A, B, C, and D) producing oxalate from glucose in the copper-tolerant fungus *Fomitopsis palustris*. In fact, inhibition of the key enzyme by the ICL inhibitor of itaconate caused a decrease in oxalate biosynthesis and the fungal growth (Munir et al. 2001). Thus, ICL will be a target enzyme of most of the copper-tolerant brown-rot fungi. However, it has not yet been examined whether the ICL enzyme generally occurs as a major constitutive enzyme in preference to isocitrate dehydrogenase (IDH) in TCA cycle (A) among many white- and brown-rot fungi. The preference of either of the two key enzymes determines the route to the TCA or to GLOX cycle, because these two enzymes are located at the branch-point leading to either of the cycles.

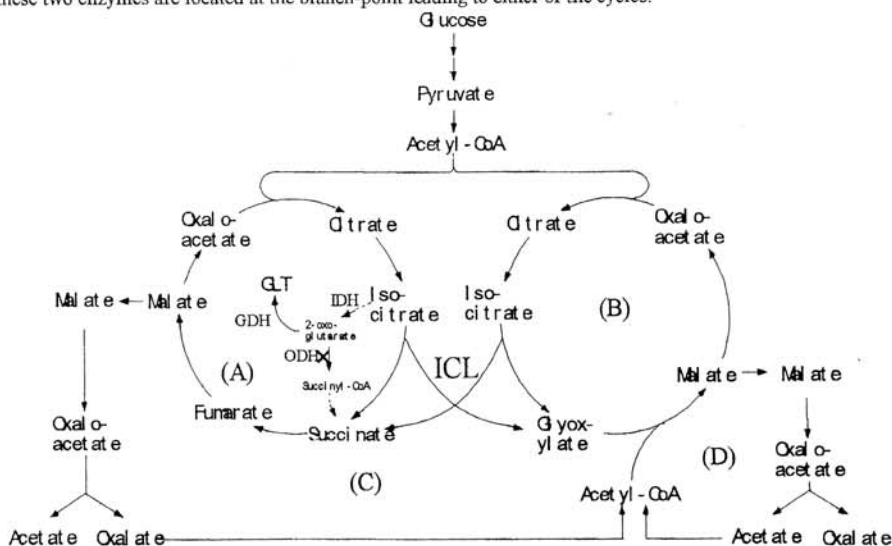


Fig. 1. Novel metabolic cycles linked with oxalic acid biosynthesis in the copper-tolerant fungus *F. palustris*.

The examination of the key enzyme distribution among the wood-rotting fungi shows that only 3 fungi (*Ganoderma applanatum*, *Lentinus edodes*, and *Pycnoporus cinnabarinus*) out of 14 white-rot fungi tested and 3 fungi (*F. palustris*,

L. sulphureus, and *Poria cocos*) out of 7 brown-rot fungi exhibit greater ICL activities than IDH activities, which indicates that as a rule ICL occurs at higher ratio in a group of brown rotters, whereas IDH is major in a group of the white-rotters tested in this experiment.

Thus, it has been established that unique TCA cycle is operative with GLT pathway and the GLOX cycle in wood decay fungi, because ODH is commonly lacking in both white- and brown-rot fungi. It is likely that the oxalate producing brown rotters have the preference of the GLOX cycle to the TCA cycle during vegetative growth.

As to the cDNA cloning of ICL of the brown-rot fungus *F. palustris*, although the N-terminal residues of the amino acid sequence of ICL has not been clarified yet, the preliminary investigation has revealed that the nucleotide sequence shows 73% and 69% homology to those of the basidiomycete *Coprinus cinereus* and the ascomycete *Aspergillus nidulans*, respectively. The analysis of the amino acid sequence of ICL showed that it has 78% and 64% identity compared with those of *Coprinus cinereus* and *Aspergillus nidulans*, respectively (Nishide 2002).

Presently, our research on the cellular localization of the GLOX key enzymes and oxalate producing enzymes is under way and further investigations remain to elucidate the mechanism for intracellular transportation of oxalate which is secreted into the extracellular site.

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Abstract

We have reported that the copper tolerant brown-rot fungus, *Fomitopsis palustris*, acquires metabolic energy by use of the constitutively-occurring Kornberg's glyoxylate (GLOX) cycle coordinating with oxalate biosynthesis during glucose oxidation (Erman Munir et al. *Proc. Natl. Acad. Sci. USA*, (2001) 98, 11126–11130), because it has been demonstrated that the brown-rot fungus has the two key enzymes (isocitrate lyase and malate synthase) of GLOX cycle although it was grown on glucose media. Furthermore, this fungus does not have the normal TCA cycle, lacking 2-oxoglutarate dehydrogenase (ODH) which is a key enzyme of the TCA cycle of most living things. This paper reports that most wood decay fungi tested lack ODH and that much greater activities of glutamate dehydrogenase (GLT) compensating the absence of ODH were detected from both white- and brown-rot fungi. The preliminary experiment for the cDNA cloning of isocitrate lyase (ICL) indicated that the nucleotide sequence shows 73% and 69% homology to those of the basidiomycete *Coprinus cinereus* and the Ascomycete *Aspergillus nidulans*, respectively

БИОПОВРЕЖДЕНИЯ И РАДИАЛЬНЫЙ ПРИРОСТ ДУБА ЧЕРЕШЧАТОГО

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Основными факторами биоповреждения листьев дуба является комплекс листогрызущих насекомых и возбудитель мучнистой росы. После объедания листья весенних побегов вредителями дуб дает побеги возобновления (компенсаторная реакция), которые сильно поражаются мучнистой росой. Позже патоген активно развивается на листьях второго прироста («кивановых» побегах). Мучнистая роса дуба – одна из болезней XX века – известна в России с 1909 г. (Головин, 1960), когда была зафиксирована внезапная вспышка сильного поражения дуба черешчатого. Возбудитель болезни оказался вирулентным, агрессивным и очень жизнеспособным. Массовое образование конидий на листьях дуба в течение сезона вегетации обеспечивало широкое распространение патогена, который через два года поражал дуб на всей территории европейской части России и на Кавказе. Адаптация гриба к растению-хозяину проходила в течение 11 лет, от 1912 г., когда впервые были обнаружены единичные клейстотеции сумчатой (половой, зимующей) стадии, замыкающей полный цикл его развития, до 1920г., в котором клейстотеции обнаруживались в массе и повсеместно.