

# Proceedings



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## **The Fourth International Wood Science Symposium**

2 - 5 September 2002 • Serpong, Indonesia

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LIPI - JSPS Core University Program  
In The Field of Wood Science

Research Center for Physics, LIPI, Indonesia  
Wood Research Institute, Kyoto University, Japan

## A New Glucose Metabolism in Wood-rotting Fungi

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Wood-rotting fungi known to have capability to degrade wooden materials utilize glucose for their growth and development. Unlike in other groups of organism that owe their energy acquisition by oxidizing glucose to carbon dioxide, the wood-rotting fungus *Fomitopsis palustris* was discovered to acquire biochemical energy by converting glucose to oxalic acid. Enzymatic analyses showed that the fungus did not have a classical tricarboxylic acid cycle because of low activity of isocitrate dehydrogenase and no activity of 2-oxoglutarate dehydrogenase. Malate dehydrogenase, which had the highest activity among the detected enzymes, is suggested to play an important role in generating NADH by oxidation malate to oxaloacetate as the direct precursor of oxalate. Furthermore, analysis of organic acids showed that no any other organic acid other than oxalic acid secreted to the culture fluid. Thus it has been proposed that oxalate is the major product of glucose metabolism in wood rotting fungus *F. palustris*.

### Introduction

Wood-rotting fungi including white- and brown-rot fungi have been known as the potent degraders of lignocelluloses materials. One of the most devastating forms of wood decay is caused by brown-rot fungi, which preferentially utilize cellulose and hemicelluloses rather than lignin. At the early stage of decay process, the growing hyphae invading wood secrete the agents that cause a rapid depolymerization in glucosyl residues of cellulose chain from 10,000 to 15,000 to about 200 (Cowling and Brown 1969), which further decrease in strength properties of brown-rotted wood. On this point, oxalic acid, which has been known to be produced by a wide variety of wood-rotting fungi, has been implicated to play a crucial role (Green *et al.* 1991). Acidic condition or reduction of pH causes a non-enzymatic degradation of cellulose (Koenigs 1974~ Kirk *et al.* 1991~ Shimada *et al.* 1994). On the other hand, oxalic acid produced by plant pathogen *Sclerotium roflsii* during pathogenesis works synergistically with endopolygalacturonase, which is activated at lower pH (Punja and Jenkins 1984).

Biosynthesis of oxalic acid has been investigated in many plant species and in numerous fungi. Although the specific metabolism may differ from one organism to the others, in most cases, the precursors of oxalate are derived from the intermediates of the tricarboxylic acid (TCA) cycle. In *Oxalis per-caprae*, glyoxylate is oxidized to oxalate by FMN-dependent oxidation~ glyoxylate is presumably produced from isocitrate by the action of isocitrate lyase (Millard *et al.* 1965). *Aspergillus niger* synthesizes oxalate from oxaloacetate by the action of oxaloacetate hydrolase known as oxaloacetase (Lenz *et al.* 1976). The wood-rotting fungus *Fomitopsis palustris*, which secretes large amount of oxalate, synthesizes oxalate from glyoxylate and oxaloacetate (Munir *et al.* 2001a).

Nevertheless, none has been reported on systematic biochemical analysis of glucose metabolism for oxalic acid biosynthesis in wood-rotting fungi, except in our previous paper (Munir *et al.* 2001a). We have discovered that wood-rotting fungus *F. palustris* oxidizes glucose to oxalate by a coupling metabolic system of TCA and glyoxylate cycles. This paper describes the important role of a new glucose metabolism pathway in conjunction with oxalic acid biosynthesis in wood-rotting fungi.

### Materials and Methods

**Chemicals.** All chemical and biochemical reagents were of reagent grades. NADP, acetyl coenzyme A, DL-isocitric acid, and 2-oxoglutaric acid were obtained from Nacalai Tesque (Kyoto). Oxalic acid, acetic acid, and glucose assay kits were purchased from Boehringer (Mannheim, Germany).

**Organism and culture conditions.** The prominent oxalate-producer *Fomitopsis palustris* (Berkeley et Curtis) Murill, which is a Japanese Industrial Standard fungus for wood- preservative efficacy tests, was used in this study. The fungus was grown on a modified Kirk's medium with 2% (w/v) glucose and 24 mM ammonium tartrate as the carbon and the nitrogen source, respectively (Kirk *et al.* 1978) and on a peptone-containing medium with 2% glucose (Munir *et al.* 2001 b)

**Preparation of cell-free extracts and determination of proteins.** Cell-free extracts were prepared from the fungal mycelia in the same way as previously reported (Munir *et al.* 2001b). Protein concentrations were determined by the Bio-Rad method (Bradford 1976) with bovine serum albumin as the standard.

**Determination of glucose and organic acids.** Glucose, oxalic acid, and acetic acid content in the culture fluid was determined enzymatically by using the glucose, oxalic acid, and acetic acid assay kits, respectively, following the methods provided by Boehringer. Other organic acids including oxalic acid were analyzed by GC-MS.

**Enzyme assays.** All enzyme activities in the fungal extracts were determined spectrophotometric ally using a double-beam spectrophotometer (Hitachi model U-3000, Hitachi, Tokyo). Each of the assayed enzymes was carried out following the reported methods or a modification of the standard methods. The assay conditions were described in the previous paper (Munir *et al.* 2001a). The enzyme activities were expressed in terms of specific activity (nmol min<sup>-1</sup> mg<sup>-1</sup> protein). One unit of enzyme activity is defined as the amount of enzyme that catalyzes the formation of 1 μmol product per minute or the consumption of 1 μmol substrate per minute under the conditions described.

### Results and Discussion

**Metabolism of glucose to oxalate in *Fomitopsis palustris*.** To start with, the amounts of glucose consumed and oxalic acid produced were determined during cultivation of the oxalate producing fungus *F. palustris*, showing a strong correlation between glucose consumption and oxalic acid production; the decrease of investigate the activity of decarboxylating enzymes, isocitrate dehydrogenase (ilIH) and 2- oxoglutarate dehydrogenase (ODH), which are both the common enzymes in TCA cycle, in comparison with the activity of isocitrate lyase (ICL) known as the key enzyme of

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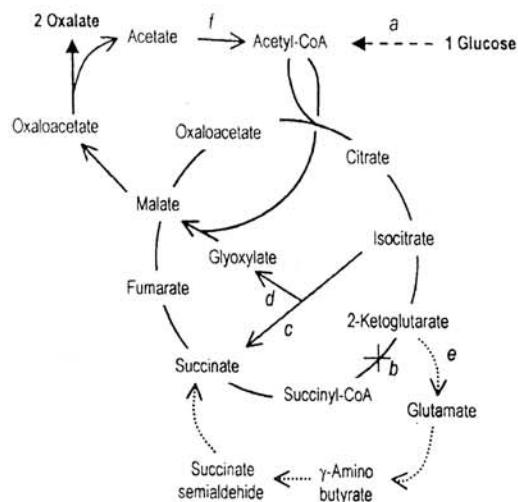


Figure 1. A proposed metabolic pathway for the conversion of glucose to oxalate in the wood-rotting fungus *F. palustris*. (a), glycolytic pathway; (b), TCA cycle; (c), a modified of TCA cycle; (d), glyoxylate cycle; (e), GABA route; (f) acetate-recycling route.

Furthermore, we detected the activities of all 12 enzymes involved in the metabolic system in this fungus. Table 2 shows that all the enzymes except for ODH were active to a varying degree and that the greatest activity was found for malate dehydrogenase (MDH); 2639 and 5840 nmol min<sup>-1</sup> mg<sup>-1</sup> protein in the peptone-containing (a) and ammonium tartrate-containing (b) medium, respectively. The reason for the high activity of MDH may be that the enzyme is intrinsically required for catalyzing dehydrogenation of malate to oxaloacetate in the modified TCA cycle (cycle c), glyoxylate cycle (cycle d), and acetate recycling route (route j) as shown in the metabolic pathway in Figure 1. Furthermore, acetyl-CoA synthase was detected in a significant amount. In fact, free accumulating acetate was not detected at all by rigorous enzymatic analysis. Moreover, no other free organic acid could be detected by GC-MS analysis. Thus, it is concluded that acetate and other intermediary organic acids were not leaked out of the metabolic system into the culture fluid, but metabolized to oxalate with high efficiency.

**Physiological importance of the metabolism of glucose to oxalate.** Based on physiological and enzymatic analyses of the metabolism of glucose, a new metabolic mechanism for oxalate biosynthesis in *F. palustris* was discovered as shown in Figure 1 (a modified form of the previously reported ones) (Munir *et al.* 2001a). The metabolic mechanism consisting of the TCA and glyoxylate cycles cooperatively couple with each other and also coordinate with the acetate-recycling routes to bring the liberated acetate back into the metabolic system via acetyl-CoA. Since the activity of OXA was much higher than that of GLOXDH, glyoxylate may not be oxidized effectively to oxalate but may be condensed with acetyl-CoA to form malate by MS.

Theoretically, the metabolic system in Figure 1 indicates that 2 mol acetyl-CoA, derived from 1 mol glucose through the glycolysis pathway (pathway a), enter cycle c

and *d* first and finally exit as oxalate of the end product from recycling route *f*. A physiological role of the new metabolic system is primarily to oxidize acetyl-CoA to yield oxalate, which accumulates in the culture fluid, because this fungus does not contain oxalate-decomposing enzyme systems such as oxalate oxidase (Aguilar *et al.* 1999) and oxalate decarboxylase (Dutton *et al.* 1994).

Importantly, MDH, which had extraordinarily high activity, plays a major role in generating energy (NADH, equivalent to 3 ATP) by oxidation of malate to oxaloacetate, which is the direct precursor of oxalic acid. Thus, evidently, oxalate production is coupled with energy production. Stoichiometrically, the overall oxidation of 2 mol acetyl-CoA, yielding 2 mol oxalate as proposed in Figure 1 results in the generation of a net amount of 4 NADHs and 2 ATPs or production of 14 ATPs, on the assumption that no other metabolic pathway oxidizes acetyl-CoA (Munir *et al.* 2001a). If it is assumed that conversion of glucose to acetyl-CoA follows the normal glycolytic pathway, conversion of 1 mol glucose to 2 mol oxalate will generate 28 ATPs or lower than that of the net ATP generated when organisms utilize normal TCA cycle. Therefore, it has been speculated that the more oxalate is produced the higher biochemical energy will be acquired by the fungus. Because the majority of wood-rotting fungi produce oxalate, the metabolic system proposed for this fungus may be a general feature for the brown- and white-rot fungi. Then it has been proposed that the wood-rotting fungus *F. palustris* oxidizes glucose to oxalate to acquire biochemical energy.

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