MECHANISMS FOR OXALIC ACID DECOMPOSITION AND TRANSPORT IN WOOD-ROTTING FUNGI

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Wood-rotting fungi play an important role in global carbon recycling because they are the major organisms that degrade wood, the most abundant biomass on Earth. Wood cell walls are primarily composed of lignocelluloses, and wood-rotting fungi are grouped into white- and brown-rot fungi on the basis of their ability to degrade this material. White-rot fungi can completely mineralize all wood cell wall components, including lignin. On the other hand, brown-rot fungi metabolize cellulose and hemicellulose without causing depolymerization of lignin [1], although they alter lignin *via* hydroxylation and demethylation [2].

Another biochemical feature that distinguishes the two types of fungi is the amount of oxalate accumulated in culture media. Brown-rot fungi accumulate a large amount of this acid, whereas white-rot fungi do not, but rather decompose it to CO_2 [3, 4].

It is widely assumed that this difference in oxalate accumulation results from different metabolism of oxalate as follows: 1) White-rot fungi degrade oxalate during fungal growth, but most brown-rot fungi do not. 2) Brown-rot fungi possess greater abilities to biosynthesize and transport oxalate than white-rot fungi. However, with regard to 1), the occurrence of both oxalate decarboxylase (ODC, EC 4.1.1.2) catalyzing the conversion of oxalate to formate, and formate dehydrogenase (FDH, EC 1.2.1.2) catalyzing oxidation of formate to CO_2, have never been reported in the same strain of white-rot fungus.

Regarding 2), Munir *et al.* proposed a new concept of oxalate biosynthesis in the wood-rotting basidiomycete *Fomitopsis palustris.* They suggested that oxalate biosynthesis is a fermentation process to acquire energy by oxidizing glucose to oxalate during vegetative mycelial growth [5]. It is essential that oxalate is exported from the cells, because the activities of several indispensable enzymes in the tricarboxylic acid (TCA) and glyoxylate (GLOX) cycles, are inhibited by oxalate [6–8]. However, oxalate transport has not been studied in *F. palustris*.

In this context, it is important to elucidate in detail the mechanisms of oxalate metabolism and transport, to gain insight into the physiological differences between white- and brown-rot fungi.

The first objective of this study is to elucidate the possible role of oxalate decomposition in the white-rot fungus *Ceriporiopsis subvermispora* in relation to fungal growth and lignin degradation.

The author found that *C. subvermispora* possessed two pathways for oxalate decomposition. Each pathway had a different role in this fungus. During vegetative growth, oxalate was decomposed *via* formate to CO_2 by ODC and FDH. It was suggested that this reaction contributed to ATP supply for fungal growth, because FDH also catalyzes the formation of NADH, leading to ATP production. On the other hand, during stationary phase, oxalate was decomposed by oxalate oxidase (OXO, EC 1.2.3.4), which probably supplies H_2O_2 for lignin degradation [9].

FDH of *C. subvermispora*, which was named CsFDH, was purified to clarify its role in oxalate metabolism. This was the first FDH purification from filamentous fungi. Two cDNAs encoding this enzyme were cloned; *CsFDH1* and *CsFDH2.* On the basis of gene expression analysis, *CsFDH1* is strongly suggested to be the main contributor to CsFDH production [10].

The second objective of the present study is to identify an oxalate transporter in *F. palustris*.

A cDNA encoding a protein conferring oxalic acid resistance on *F. palustris* was isolated from the fungus by functional screening of yeast transformants. This cDNA, *FpTRP26* (*Fomitopsis palustris* thioredoxin-related protein 26 kDa), conferred resistance to oxalic acid specifically on the transformant, concomitantly with a decrease in oxalic acid in yeast cells [11]. Furthermore, the author isolated a cDNA from *F. palustris*, *FpOAR* (*Fomitopsis palustris* oxalic acid resistance protein), encoding a membrane protein with oxalate transport activity (Watanabe *et al.* manuscript in preparation).

Based on the results of these studies, different roles of oxalate decomposition and transport between white- and brown-rot fungi are proposed. As shown in Fig. 1, both fungi acquire energy by bicycle mechanisms, in which TCA and constitutive GLOX cycles are essential. In addition to the bicycle mechanism, the white-rot fungus *C. subvermispora* decomposes oxalate to acquire energy and supply H_2O_2 for lignin degradation (Fig. 1A). By contrast, the brown-rot fungus *F. palustris*

efficiently exports large amounts of oxalate, mediated by FpOAR and FpTRP26. The exported oxalate decomposes cellulose yielding the carbon source for fungal growth (Fig. 1B).

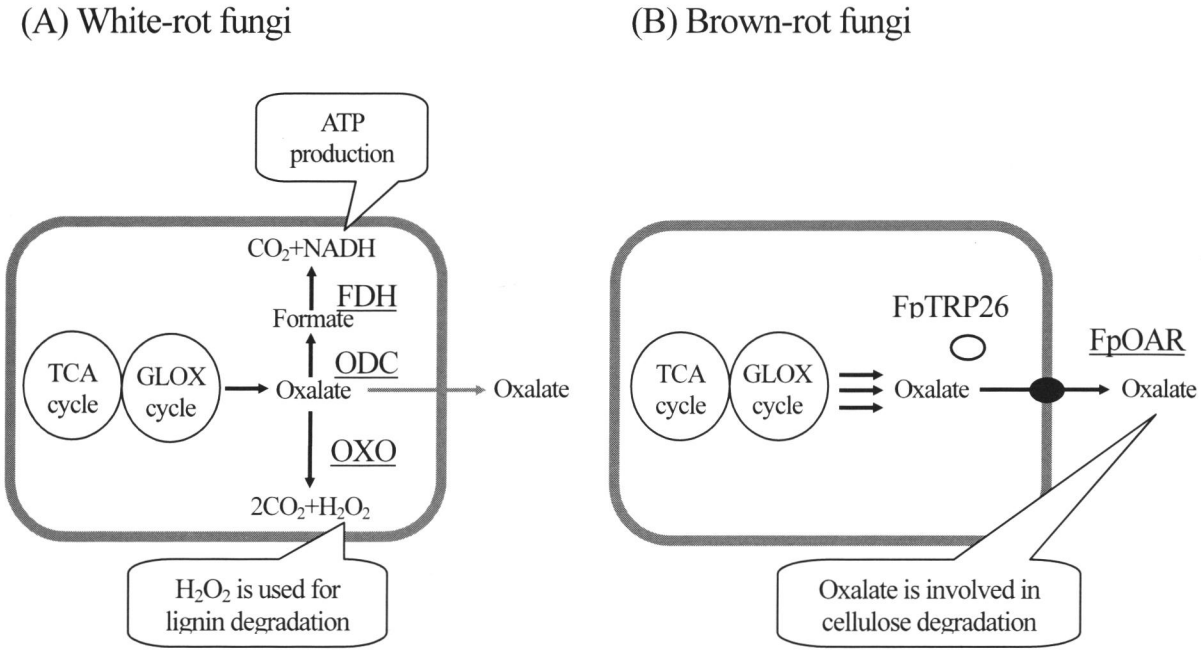

Fig. 1. Possible physiological roles of oxalate decomposition and transport in white-rot fungi (A) and brown-rot fungi (B).

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