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**A possible role of the key enzymes of the glyoxylate and TCA cycles,  
and gluconeogenesis pathway during fruit body formation  
in the wood-rotting basidiomycete, *Flammulina velutipes***

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### INTRODUCTION

Although a number of studies on carbon metabolism of basidiomycetes have been reported in relation to fruit body formation, no systematic enzymatic analysis of the tricarboxylic acid (TCA) and glyoxylate (GLOX) cycles, and gluconeogenesis has been conducted. Recently, we have found that the isocitrate lyase (ICL) and malate synthase (MS), the glyoxylate cycle key enzymes, occur in the mycelia of the brown-rot basidiomycete *Fomitopsis palustris*, that was grown on the glucose medium.<sup>1</sup> It has also been proposed that the wood-rotting basidiomycete acquires biochemical energy by oxidizing glucose to oxalate.<sup>2</sup> In this context, it is interesting to investigate changes in enzyme activities during the fruit body formation of the wood-rotting basidiomycete *Flammulina velutipes*. Here we report changes in activities of the key enzymes of the metabolic pathways to elucidate the carbon metabolism in *F. velutipes* mycelia at different stages of development.

### MATERIALS and METHODS

**Organism and Culture Conditions.** *Flammulina velutipes* Singer IFO 7777 strain was used in this experiment. The fungus was cultured in a 250 ml high-layer petri dish (polycarbonate, Iwaki Glass Co., Ltd) containing 50 ml potato glucose liquid (PGL) medium (pH 5.5). The medium was composed of the extract of 200 g potato and 20 g glucose in 1000 ml of distilled water. To form the mature fruit body, the fungus was first grown at 25°C for 14 days in the dark condition, and then transferred into the incubator at 16°C in the light (200 lux) until the fruit-bodies appeared.

**Preparation of Cell-Free Extracts and Enzyme Assays.** Cell free-extracts were prepared by homogenization of the 2.0-3.0 g of frozen mycelia in a cold mortar with a pestle in 0.1 M potassium phosphate buffer (pH 7.0) containing 1 mM DTT, 1 mM EDTA, and 1 mM PMSF with a small amount of sea sand. The homogenate was centrifuged at 14000 g at 4°C for 30 min. This manipulation was performed twice, and the combined supernatant obtained was used as the crude

enzyme solution. The all enzyme activities were determined spectrophotometrically by using double-beam spectrophotometer equipped with a temperature controller. Activities of ICL and MS were assayed by the methods of Dixon and Konberg.<sup>3</sup> Isocitrate dehydrogenase (IDH) and 2-oxoglutarate dehydrogenase (ODH) activities were determined by measuring the increase in absorbance at 340 nm due to the reduction of NAD or NADP, on the basis of the reported methods.<sup>4</sup> Fructose-1, 6-biphosphatase (FBPase) activity was assayed by the modified coupling assay method, as described by S. Pontremoli and E. Melloni.<sup>5</sup> Glutamate dehydrogenase (GDH) and L-3-hydroxyacyl CoA dehydrogenase (HCoADH) activities were determined by measuring the decrease in absorbance at 340 nm due to the oxidation of NADH/NADH and NADH, respectively, on the basis of the reported methods.<sup>4,6</sup> One unit of enzyme activity is defined as the amount of enzyme that catalyzes the formation of 1  $\mu$ mol product per minute or the consumption of 1  $\mu$ mol substrate per minute under the conditions described.

Protein concentrations were determined by the Bradford method using a protein assay kit with bovine serum albumin as a standard.<sup>7</sup>

## RESULTS and DISCUSSION

Figure 1 shows changes in the specific activities of the key enzymes of the glyoxylate cycle (ICL and MS) and gluconeogenesis pathway (FBPase) in mycelia. The level of the activity of FBPase remarkably increased during growth from the young fruit body to mature fruit body. The highest activity was observed at stage of the mature fruit body formation. The activity of MS began to increase at the stage of the vegetative growth, and then remarkably elevated during the stage of fruit body maturation. On the other hand, ICL activity showed at low levels in all stages of development. We have already reported that the glyoxylate cycle may play an anaplerotic function to support the TCA cycle in coordination with gluconeogenesis.<sup>1,8</sup> Table 1 shows that comparison of the activities of the enzymes related to gluconeogenesis in mycelia of the culture which formed fruit body or which did not form fruit body. The specific activities of ICL, MS, FBPase, IDH, and HCoADH in the former were greater than those in the latter, respectively (the enzyme activities in the former 1.7, 3.0, 3.0, 1.7, and 1.3 fold, respectively, as compared with the latter). In *F. velutipes* a few percents of mannitol and arabitol were detected in the fruit body.<sup>9</sup> These results indicate that

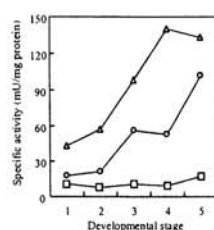


Fig 1. Changes in the specific activities of ICL, MS, and FBPase during the stages of fruit body formation. Circles, MS; squares, ICL; triangles, FBPase. Stage 1, vegetative mycelia; stage 2, primordia; stage 3, young fruit body; stage 4, immature fruit body; stage 5, mature fruit body.

Table 1. Comparison of specific activities of the enzymes related to gluconeogenesis in mycelia.

Enzyme	Enzyme activity *	
	A	B
ICL	17.2	10.0
MS	75.3	25.6
FBPase	171.4	56.4
IDH	203.0	120.5
HCoADH	43.1	34.0

\* Enzyme activities ( $\text{nmol min}^{-1} \text{mg}^{-1}$  protein) were determined for fungus grown for 28 days.

A) Mycelia of the culture with fruit body formed.

B) Mycelia of the culture without forming fruit body.

glyoxylate cycle links with gluconeogenesis to supply a source of carbon for the fruit body formation.

Figure 2 shows changes in the specific activities of the key enzymes of the TCA cycle (IDH and ODH) in mycelia. The activity of IDH (NADP<sup>+</sup>) began to increase at the stage of the primordium formation, and then remarkably elevated during the stages of the fruit body development. However, the activity of ODH, the

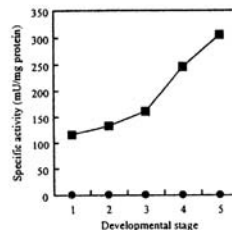


Fig 2. Changes in the specific activities of IDH and ODH during the stages of fruit body formation. Circles, ODH, squares, IDH. Stage 1-5, see the previous figure.

another key enzyme of TCA cycle, was not detected throughout any stages of development, although a few different extraction methods were attempted. In other basidiomycetes, such as *Pleurotus ostreatus*,<sup>10</sup> *F. palustris*,<sup>2</sup> and *Coprinus cinereus*,<sup>4</sup> the activity of ODH also was not detected. These results indicate that the classical TCA cycle may be deficient in the basidiomycete, *F. velutipes*.

## CONCLUSION

In *F. velutipes*, MS, as the key enzyme of the glyoxylate cycle was found to be good in coordination with FBPase, which supports gluconeogenesis to provide the carbon source required for fruit body formation (Table 1). Thus, in contrast to the previous finding, the glyoxylate cycle and gluconeogenesis may play an important role in carbon metabolism of fruit body formation. Furthermore, the results of this investigation have shown that TCA cycle may be deficient in basidiomycetes such as *F. velutipes* and *F. palustris*. However, further research remains to elucidate biochemical mechanisms controlled by the regulatory key enzymes involved in the carbon metabolism during the fruit body formation of basidiomycetes.

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