

An Enzymatic Study of an Oxalate Producing System in Relation to the Glyoxylate Cycle in White-rot Fungus *Phanerochaete chrysosporium**¹

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Introduction

It has been well established that oxalate produced from white-rot and brown-rot fungi plays several important roles in wood decay processes¹. Furthermore, it is a commonly known physiological trait that most brown-rot basidiomycetes, including *Fomitopsis* (formerly called *Tyromyces*) *palustris*, accumulate oxalate in culture media, whereas white-rot ones do not. However, the enzymatic study of an oxalate producing system in white-rot fungi has not been fully studied, although glyoxylate oxidase from *Coriolus versicolor* has been reported². Thus, the purpose of this study was to investigate the changes in the activities of glyoxylate dehydrogenase (GDH), formate dehydrogenase (FDH), and glyoxylate cycle enzymes such as isocitrate lyase (ICL) and malate synthase (MS) in a white-rot fungus *Phanerochaete chrysosporium*, as glyoxylate cycle has been reported to distribute among many wood rotting basidiomycetes^{3,4}.

Materials and Methods

Phanerochaete chrysosporium (ATCC 24725) was grown statically in the Kirk's basal medium⁵ containing Glucose as a sole carbon source and ammonium tartrate as a nitrogen source. The crude enzymes including glyoxylate dehydrogenase (GDH)⁶, formate dehydrogenase (FDH)⁷, isocitrate lyase (ICL)⁸, malate synthase (MS)⁹ were

assayed spectrophotometrically.

Results and Discussion

Total activity of GDH obtained at 33°C (108 pkat/flask) was 3 times that obtained at 38°C. Mycelia grew well at 33°C than 38°C, whereas the FDH activities at 33°C and 38°C were almost equal (500 pkat/flask). The specific activity of GDH in low nitrogen culture (LN, 2.4 mM of NH_4^+) was 17 pkat/mg protein, which was twice that found in high nitrogen culture (HN, 24 mM). However, almost equal total GDH activities (92 pkat/flask) were observed in the both cultures. Addition of Tween 80 (1%, w/v) resulted in a 2-fold increase in specific activity of GDH (33 pkat/mg protein). Thus, it was found that the highest GDH activity was obtained from the culture with 2.4 mM ammonium ion at 33°C in the presence of Tween 80.

During the cultivation of the fungus, the accumulation of oxalate reached the maximum level on day 4, but decreased thereafter (Fig. 1(A)). The highest GDH activity was obtained on day 2, whereas the glyoxylate cycle enzymes, ICL and MS, showed the maximum activity on day 4 and 2, respectively (Fig. 1(B)). Thus, taking into consideration of the results, the reason why highest amounts of oxalate accumulated on day 4 is not clear but probably because large amounts of glyoxylate could be supplied by ICL. The results also suggest that

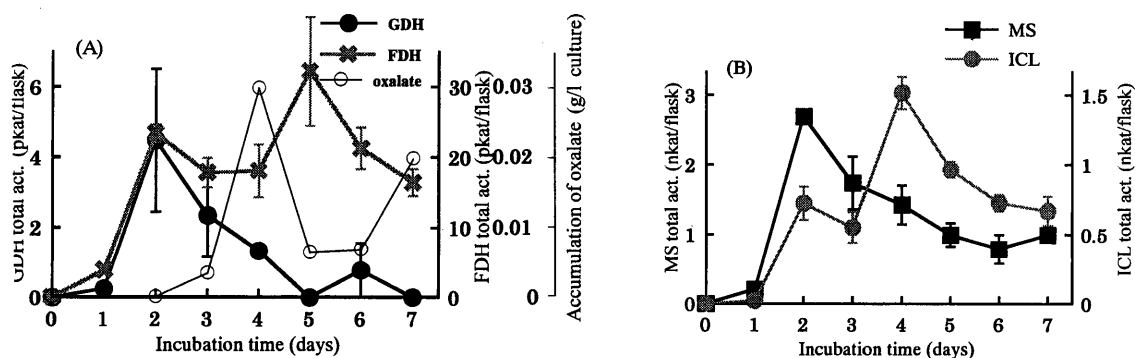


Fig. (A), (B). Changes in the accumulation of oxalate and activities of GDH, FDH, MS, and ICL during cultivation of *P. chrysosporium*.

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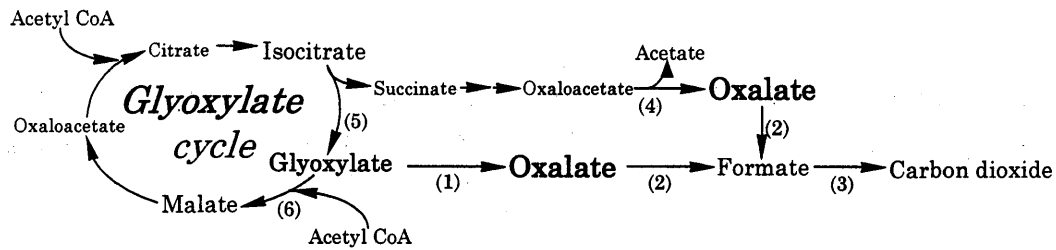


Fig. 2. Glyoxylate cycle and oxalate metabolism in white-rot fungus. (1) Glyoxylate dehydrogenase, (2) Oxalate decarboxylase, (3) Formate dehydrogenase, (4) Oxaloacetase, (5) Isocitrate lyase, (6) Malate synthase.

besides lignin peroxidase/veratryl alcohol and manganese peroxidase/Mn(II)-mediated degradation of oxalate as reported^{10,11)}, FDH together with oxalate decarboxylase might also be involved in the complete decomposition of oxalate. Because FDH activity increased around day 4 to day 5 while the amounts of accumulated oxalate decreased. However, oxalate decarboxylase was not assayed at this time. Alternatively, another enzyme, oxaloacetase might be involved in the production of oxalate, although oxaloacetase activity could not be detected from this fungus.

In conclusion, the glyoxylate cycle enzymes such as ICL and MS, and GDH and FDH are suggested to be interlinked with each other and function cooperatively in the production and decomposition of oxalate in the culture of *P. chrysosporium*, as shown in Fig. 2. Further research is needed to elucidate the pathway for the production of oxalate.

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