



Integrated delignification and simultaneous saccharification and fermentation of hard wood by a white-rot fungus, *Phlebia* sp. MG-60

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HIGHLIGHTS

- ▶ *Phlebia* sp. MG-60 is able to selectively degrade lignin of oak wood.
- ▶ MG-60 is able to produce ethanol from oak wood after biological delignification.
- ▶ MG-60 enabled the fermentation of oak wood by solely biological processes.
- ▶ Integrated Fungal Fermentation (IFF) is proposed.

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ABSTRACT

We propose a new process of unified aerobic delignification and anaerobic saccharification and fermentation of wood by a single microorganism, the white-rot fungus *Phlebia* sp. MG-60. This fungus is able to selectively degrade lignin under aerobic solid state fermentation conditions, and to produce ethanol directly from delignified oak wood under semi-aerobic liquid culture conditions. After 56 d aerobic incubation, 40.7% of initial lignin and negligible glucan were degraded. Then under semi-aerobic conditions without the addition of cellulase, 43.9% of theoretical maximum ethanol was produced after 20 d. Changing from aerobic conditions (biological delignification pretreatment) to semi-aerobic conditions (saccharification and fermentation) enabled the fermentation of wood by solely biological processes. This is the first report of ethanol production from woody biomass using a single microorganism without addition of chemicals or enzymes.

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1. Introduction

Ethanol is the most widely utilized liquid biofuel alternative to fossil fuels. Ethanol produced from cellulosic biomass has been widely regarded as a readily available sugar source to replace starch materials in fermentation. To reduce the ethanol production costs from lignocellulose biomass, a single microorganism with the ability to degrade lignin, hydrolyze cellulose, and ferment glucose and xylose to ethanol, is an attractive candidate. Basidiomycetes play important roles in the carbon cycle as decomposers in forest ecosystems. Lignin-degrading basidiomycetes, referred to as white-rot fungi, are the microbes responsible for degrading all the components of plant cell wall polymers including polysaccharides (cellulose and hemicelluloses) and the recalcitrant aromatic polymer, lignin (Kirk and Fenn, 1982). Three major classes of extracellular enzymes, lignin peroxidases, manganese peroxidases, and laccases, are believed to be important

in the fungal degradation of lignin (Lundell et al., 2010). Many researchers have therefore reported that biological delignification using white-rot fungi is useful for pretreatment before enzymatic saccharification of lignocellulosic biomass (Bak et al., 2009; Taniguchi et al., 2005; Wan and Li, 2011; Yamagishi et al., 2011). White-rot fungi which have the ability to ferment cellulose directly may therefore be potentially applicable for the one-step fermentation of lignocellulosic biomass.

Lignin degradation is an oxidative process, and replacing air with an oxygen atmosphere stimulates lignin degradation by many white-rot fungi (Isroi et al., 2011). On the other hand, it has been reported that some white-rot fungi have the ability to produce ethanol under aeration-limited conditions (Okamoto et al., 2010). We have recently revealed that the white-rot fungus *Phlebia* sp. MG-60 shows an ability to convert lignocellulose to ethanol directly, under semi-aerobic conditions (Kamei et al., 2012). When this fungus was cultured with 20 g/L of unbleached hardwood kraft pulp (UHKP), 8.4 g/L ethanol was produced after 168 h incubation, giving ethanol yields of 0.42 g/g UHKP; 71.8% of the theoretical maximum. When this fungus was cultured with waste newspaper, 4.2 g/L of

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