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Methane fermentation of Japanese cedar wood pretreated with a white rot fungus, *Ceriporiopsis subvermispora*

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Abstract

Methane fermentation of Japanese cedar wood was carried out after pretreatment with four strains of white rot fungi, *Ceriporiopsis subvermispora* ATCC 90467, CZ-3, CBS 347.63 and *Pleurocybella porrigens* K-2855. These fungi were cultivated on wood chip media with and without wheat bran for 4–8 weeks. The pretreated wood chip was fermented anaerobically with sludge from a sewage treatment plant. Pretreatments with *C. subvermispora* ATCC 90467, CZ-3 and CBS 347.63 in the presence of wheat bran for 8 weeks decreased 74–76% of β-O-4 aryl ether linkages in the lignin to accelerate production of methane. After fungal treatments with *C. subvermispora* ATCC 90467 and subsequent 30-days methane fermentation, the methane yield reached 35 and 25% of the theoretical yield based on the holocellulose contents of the decayed and original wood, respectively. In contrast, treatment with the three strains of *C. subvermispora* without wheat bran cleaved 15–26% of the linkage and produced 6–9% of methane. There were no significant accelerating effects in wood chips treated with *P. porrigens* which has a lower ability to decompose the lignin. Thus, it was found that *C. subvermispora*, with a high ability to decompose aryl ether bonds of lignin, promoted methane fermentation of softwood in the presence of wheat bran.
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Keywords: White rot fungi; Methane fermentation; Lignin; Softwood; Japanese cedar

1. Introduction

The forestry and wood industries supply renewable biomass that provides electrical/heat energy, transport fuel or chemical feedstock. Residual wood in the forest area, and wood waste are abundant lignocellulosic materials that could be fermented to methane, ethanol and other chemical products (Delgenes et al., 1996; Kaygusuz and Türker, 2002; Palm and Zacchi, 2003). Generation of methane in landfills is facilitated by microbial decomposition of organic compounds under anaerobic condition, with carbon partitioned into

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approximately equal amounts of methane and carbon dioxide (Bingemer and Crutzen, 1988; Nozhevnikova et al., 1993). In general, fermentation of lignocellulosic material requires several steps: firstly, delignification to liberate cellulose and hemicellulose from their complex with lignin; secondly, depolymerization of the carbohydrate polymers (cellulose and hemicelluloses) to monosaccharides and fermentation (Aristidou and Penttilä, 2000). Lignin is the major factor determining the extent of organic substrate degradation in anaerobic conditions (Chandler et al., 1980). Due to the heterogeneity, lignin is resistant to biological attack by many kinds of microorganisms. However, basidiomycetes called white rot fungi are known as an aggressive lignin degrader (Kirk and Farrell, 1987).

Pretreatments of wood include mechanical size reduction, steaming, steam explosion, autohydrolysis, alkali treatments, chemical pulping, solvent extraction, fungal degradation and their combinations. The pretreatment is an essential step to decrease the amount of lignin, maximize subsequent bioconversion yields and minimize the formation of inhibitory compounds (Chynoweth and Jerger, 1985; Young and Frazer, 1987). The pretreatment increases the specific surface area of cell wall polysaccharides to accelerate enzymatic hydrolysis (Gharpuray et al., 1983).

Enhanced production of methane on pretreatment of *Eucalyptus globulus* wood chip by using steam, NaOH and steam explosion was reported (Nakamura and Godliving, 2003). The amount of methane gas produced depended on a decrease in Klason lignin of the treated wood chip (Nakamura and Godliving, 2003). However, there have been few reports demonstrating effectiveness of fungal pretreatments of wood for methane fermentation. We were interested to apply white rot fungi to the pretreatments for methane fermentation of wood. Methane fermentation is advantageous for on-site energy supply. Methane gas can be converted to electricity using fuel cells or turbine systems, or combusted directly. Development of bioconversion system from wood to methane should accelerate the establishment of bioenergy-based societies that use wood and forestry wastes to make electricity, heat and fuels.

In the fungal pretreatments of woody biomass for enzymatic saccharification and microbial fermentation, the network of lignin must be degraded with minimum loss of cell wall polysaccharides. Among the numerous wood rot fungi so far isolated, a white rot fungus, *Ceriporiopsis subvermispora*, is known as the best biopulping fungus that can degrade lignin without intensive damage of cellulose (Akhtar et al., 1992, 1998; Messner and Srebotnik, 1994). This fungus is unique in its ability to cleave β-O-4 aryl ether linkages between lignin units without intensive weight loss of cellulose in early stage of wood decay (Guerra et al., 2002). In the present paper, fungal pretreatments with several strains of *C. subvermispora* and *Pleurocybella porrigens* on methane fermentation of Japanese cedar (*Cryptomeria japonica* (Lf) D.Don) were studied. Japanese cedar is the most important softwood species in the Japanese forest industry, but it is difficult to decompose its cell wall structures by chemical and biological treatments. We herein report that pretreatment of Japanese cedar wood with a selective white rot fungus, *C. subvermispora*, in the presence of wheat bran increased production of methane. Correlation between the fungal pretreatment effects and changes in wood components including cleavage of β-O-4 aryl ether linkages in lignin is discussed.

2. Materials and methods

2.1. Reagents

Acetic anhydrate, tetracosane and pyridine were obtained from Nacalai Tesque (Kyoto, Japan). Acetyl bromide (AcBr), zinc dust and *trans*-cinnamyl alcohol were purchased from Wako Chemical Industries (Tokyo, Japan). All of the reagents used were of analytical grade.

2.2. Fungal pretreatments

C. subvermispora ATCC 90467, CZ-3 (ATCC 96608), CBS 347.63 and *P. porrigens* K-2855 (a strain obtained from Takara Shuzo Co. Ltd., Kyoto, Japan) were cultured on a PDA plate at 28 °C for 1 week. Four pellets of inocula from the precultures were added to 10 g of Japanese cedar wood chip media containing 30 ml Milli-Q^{TM} water and 1 g of wheat bran (Nisshin Flour Milling Inc., Tokyo) in a 300-ml Erlenmeyer flask. The flasks were incubated at 28 °C with 70% relative humidity for 4–8 weeks. The wood chips were used without pre-extraction. Wood media without the

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