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A New Biological Pretreatment Method for Enhancing Cellulase Performance

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Abstract

Introduction: Only a few bacteria capable of degrading lignin have been reported. Of these bacteria, *Bacillus* sp. is very useful because this soil bacterium can divide asymmetrically, producing an endospore that is resistant to environmental factors such as heat, acid, and salt for long periods of time. This manuscript suggests an effective biological pretreatment method for enhancing cellulase performance.

Materials and methods: An alkali lignin-degrading bacterium was isolated from forest soils and named CS-1. 16S rDNA sequence analysis indicated that CS-1 from Hokkaido from Okinawa was *Bacillus* sp. (100% identity with HQ727971.1).

Results: Strains CS-1 displayed alkali lignin degradation capability. With initial concentrations of 0.05–2.0 g l^{-1}, at least 61% alkali lignin could be degraded within 48 h. The maximum lignin-degrading rate of CS-1 was estimated to be 99.5% at a concentration of 0.05 g l^{-1}. High laccase activities were observed in crude enzyme extracts from the isolated strain. Very low (negligible) lignin peroxidase and low manganese peroxidase activities were observed. This result indicated that alkali lignin degradation was correlated with laccase activities.

Discussion: Judging from the net yields of sugars after enzymatic hydrolysis, the most effective pretreatment method for enhancing cellulase performance was a two-step processing procedure [pretreatment using *Bacillus* sp. CS-1 followed by lactic acid bacteria (*Lactobacillus bulgaricus*(NBRC13953) and *Streptococcus thermophiles* (NBRC13957)] at 68.6%. These results suggest that the two-step pretreatment procedure is effective at accelerating cellulase performance.

Keywords: Lignin degradation; *Bacillus* sp; Laccase; Lactic acid bacteria; Cellulase performance

Introduction

To date, the most extensively studied lignin-metabolizing microorganisms are the white- and brown-rot Basidiomycete fungi [1]. In contrast to fungal lignin degradation, the enzymology of bacterial lignin breakdown is currently not well understood. The advantage of taking a lignin-degrading enzyme from bacteria rather than fungi is that bacteria are much more amenable to genetic modification [2].

Removal of lignin from biomass before biological processing improves cellulose digestibility, reduces downstream agitation power requirements, provides less sites for nonproductive cellulase adsorption, reduces dissolved lignin compounds that are toxic to fermentations, facilitates cell and enzyme recovery and recycle, and simplifies the distillation step [3].

Recently, Rollin et al. [4] reported that improving the surface area accessible to cellulose is a more important factor for achieving a high sugar yield rather than attempt to improve the enzymatic digestibility of biomass by removing lignin. Organic acids which did not produce inhibitor such as furfural (from pentoses) and 5-hydroxymethylfurfural (HMF; from hexoses) may increase porosity and improves enzymatic digestibility, resulting in hemicellulose removal [5,6]. However, this sophisticated method also needs heating process at 130-170°C. Thus, although hemicellulose can be eventually removed from substrates but high energy requirement will remain problematic.

Recently, we collected many of forest soil samples throughout Japan from Hokkaido to Okinawa in order to better understand the diversity of lignin-decomposing bacteria. After isolation process, an isolated strain (*Bacillus* sp. CS-1) was further studied to evaluate their alkali lignin-degrading ability. In addition, their application in lignin degradation was examined using rice straw. Meanwhile, we optimized biological pretreatment method focusing on the development of environmentally-friendly and low energy requirement method for the removal of lignin and enhancing cellulase performance. To increase the surface area accessible to cellulose resulting in hemicellulose elimination, from two lactic acid bacteria (*Lactobacillus bulgaricus* and *Streptococcus thermophilus*) was examined [7]. Moreover, application of *Bacillus* sp. strain in combination with lactic acid bacteria for lignin degradation and enhancing cellulase performance was studied.Until a few years ago, strains of *Bacillus* have not been well studied as lignin-degrading bacteria, but lately several lignin-degrading strains of *Bacillus* sp. have been isolated in the world wide and studied their lignin-degrading/or decolorizing ability [8]. In general, the rate of biological pretreatment is too slow for industrial purposes. The residence time of 10-14 days is the disadvantage that makes this method of pretreatment less attractive on an industrial scale. However, the isolated *Bcillus* sp. CS-1 possessed high lignolytic enzyme activities (laccase activities).

To increase the surface area accessible to cellulose resulting in hemicellulose elimination, two lactic acid bacteria (*Lactobacillus bulgaricus and Streptococcus thermophilus*) was examined [9].

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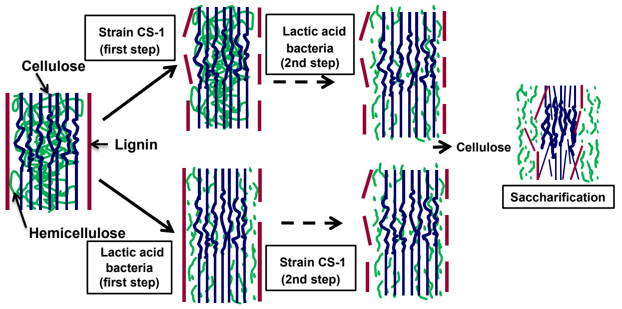

Figure 1: A conceptual scheme of biological pretreatment methods for enhancing cellulase performance.

Experiments were divided into four different processing procedures as follows: (1) single pretreatment of rice straw by *Bacillus* sp. CS-1; (2) single pretreatment by two lactic acid bacteria; (3) two-step processing procedure (sequential pretreatment using lactic acid bacteria following by *Bacillus* sp. CS-1; (4) two-step processing procedure (pretreatment using *Bacillus* sp. CS-1 following by lactic acid bacteria) (Figure 1). After each pretreatment processing procedure, the contents of holocellulose (hemicellulose and cellulose), cellulose, and lignin in rice straw were determined.

The results indicate that single pretreatment using lactic acid bacteria was more effective compared with those of *Bacillus* sp. CS-1 on the basis of the net yields of sugars even though lignin degradation ratio was slightly lower than those of *Bacillus* sp. CS-1. These results present that hemicellulose elimination is also an important factor for enhancing cellulase performance as previously reported [4].

Furthermore, the pretreated substrate (rice straw) was found to have greatly increased cellulase performance in accordance with the decrease of amount of lignin and hemicellulose when the two-step pretreatment procedure using the strain CS-1 and lactic acid bacteria (*Lactobacillus bulgaricus* and *Streptococcus thermophilus*) was implemented. Judging from the net yields of sugars after enzymatic hydrolysis, the most effective pretreatment method for enhancing cellulase performance was a two-step processing procedure (sequential pretreatment using *Bacillus* sp. CS-1 following by lactic acid bacteria) at 68.6%. This two-step pretreatment procedure is comparable to some novel fungal strains which have shown a high capacity of lignin degradation on the basis of lignin degradation ratio [10]. These results suggest that the two-step pretreatment procedure is effective in accelerating of cellulase performance.

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