Pretreatment Effects on the Rice Bran Saccharification with Newly Identified Fungal Enzymatic Activities

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Abstract
In order to develop an efficient microbial source of cellulase enzyme system, cellulolytic fungal strain was newly isolated from traditional Korean nuruk samples in the present study. The crude enzyme extract of this fungal strain, avicelase, CMCase and β-glucosidase activities reached the maximal points of 6.73, 3.22, and 5.64 units/mL, respectively and was used for the subsequent enzymatic saccharification on pretreated deproteinised and lipid-extracted rice bran. This strain was identified as *Penicillium* sp. determined by cellular fatty acid composition analysis. Three different pretreatment conditions were evaluated on the deproteinised and lipid-extracted rice bran at 121°C/1.5 psi for 5 different residence times: one with 0.1 N sulfuric acid, another with 0.1 N sodium hydroxide, and the last with distilled water. The greatest enzymatic saccharification yield increased up to 75.2% from acid-catalyzed autoclaving pretreatment for 30 min. The acid-catalyzed autoclaving pretreatment enhanced the saccharifying ability of the newly isolated cellulolytic fungal strain on the deproteinised and lipid-extracted rice bran.

Key words: cellulolytic fungal strain, cellulase, enzymatic saccharification, pretreatment

Introduction
Bioethanol can be produced by pretreating lignocellulosic materials followed by enzymatic saccharification and fermentation (Öhgren et al., 2007). Unlike cereal endosperm where the major carbohydrate is starch, lignocellulosic material is composed of cellulose, hemicelluloses, and lignin. The complexity of lignocellulosic material makes it much more difficult than starch to be enzymatically decomposed to fermentable sugars (Banerjee et al., 2009). The pretreatment is a prerequisite step to alter structural characteristics of lignocellulosic substrate prior to enzymatic saccharification. These structural modifications are highly dependent on the conditions of pretreatments employed (Kumar et al., 2009; Alvira et al., 2010). Deproteinised and lipid-extracted rice bran obtained from food processing industries has a potential to serve as a low cost feedstock for bioethanol production. Therefore, it is necessary to properly pretreat deproteinised and lipid-extracted rice bran before enzymatic saccharification step to enhance the subsequent fermentable sugar yield. For this purpose, the effect of different pretreatment strategies, such as 0.1 N acid and alkaline pretreatment on the saccharification of deproteinised and lipid-extracted rice bran were investigated in this study.

The widely accepted mechanism for enzymatic saccharification involves synergistically combined actions by endoglucanase (EC 3.2.1.4), exoglucanase (EC 3.2.1.91), and β-glucosidase (EC 3.2.1.21) (Ferreira et al., 2009). There has, therefore, been extensive research for an efficient microbial source of cellulase enzyme system. *Trichoderma* sp. has been considered to be the most powerful source of cellulose-active enzymes among fungi, but its β-glucosidase activity is low for achieving good cellulose hydrolysis (Pecauty, 2007). β-Glucosidase plays an important role in the complete saccharification of cellulose to glucose by catalyzing the hydrolysis of cellobiose (Ng et al., 2010). In this study, we report a newly isolated fungal strain from traditional Korean *nuruk* samples with higher β-glucosidase activity among wild-type strains. Traditional Korean *nuruk* is a kind of fermentation starter for rice wine. Various airborne microorganisms such as fungi, yeast, and bacteria are naturally inoculated during the traditional manufacturing process of *nuruk*. To our knowledge, the research regarding a newly isolated cellulolytic fungal strain from traditional Korean *nuruk* samples and its crude enzyme extract for the subsequent enzymatic saccharification on the pretreated deproteinised and lipid-extracted rice bran is being presented for the first time. The objective of this study was to investigate the pretreatment effects on saccharification.
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Materials and Method

Isolation of cellulosytic fungi

Five samples of traditional Korean nuruk, which were collected from different areas in South Korea, were used for isolating strong cellulosytic fungi. Ten grams of each sample were suspended in 200 mL of NaCl solution (1%, w/v) for 3 h. The suspension was diluted from $10^{-1}$ to $10^{-5}$ and then 100 $\mu$L of the dilutions were seeded on the isolation plates. Czapek-Dox medium was used as an isolation plate with addition of 1% (w/v) carboxymethyl cellulose (CMC, Sigma-Aldrich Chemical Co., St. Louis, MO, USA), 1% (w/v) microcrystalline cellulose (Avicel PH 101, Fluka, Buchs, Switzerland), and 1.5% (w/v) agar (Dae-Jung Chemical Co., Seoul, Korea). The inoculated plates were incubated at 30°C for 7 days. The aliquots (10 mL) of culture were taken on daily basis, which were centrifuged at 4°C and 11,000 $\times$ g for 15 min (Eppendorf 5804R, Germany). The clear supernatants were used as crude enzyme extracts for the subsequent enzyme assays.

Enzyme assays

Avicelase (crystalline cellulose hydrolase) activity was assayed by incubating 100 $\mu$L of the same enzyme extracts with 400 $\mu$L of 0.2% (w/v) Avicel PH 101 in 50 mM sodium acetate buffer (pH 4.8) at 45°C for 60 min (Yamanobe et al., 1987). CMCase (Carboxymethyl cellulase) activity was determined by incubating 100 $\mu$L of appropriately diluted enzyme extracts with 400 $\mu$L of 0.2% (w/v) CMC in 50 mM sodium acetate buffer (pH 4.8) at 45°C for 30 min (Yamanobe et al., 1987). $\beta$-Glucosidase activity was determined under the same conditions at 45°C for 30 min as described above, except for 10 mM salicin solution (D-(-)-salicin, Sigma-Aldrich Chemical Co., St. Louis, MO, USA) as a substrate (Yamanobe et al., 1987). After the incubation, the amount of released reducing sugars were determined using the 3,5-dinitrosalicylic acid (DNS) method (Miller, 1959; Tabka et al., 2006). One unit of each enzyme activity was defined as 1 $\mu$mol of...