Simultaneous Saccharification and Fermentation (SSF) of lignocellulosic biomass to ethanol with Antarctic yeast *Mrakia blollopis*

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The biosphere consists of a number exists cold climate areas including Arctic, Siberia, Alps, and Antarctica. Since microbesl, adapted to these environments, can grow below 0 °C, they are expected to utilize as de novo, bioprocesses including cold-adapted enzymes. Previously, we isolated 305 fungal isolates (8 Ascomycetes and 6 Basidiomycetes) from Antarctica of which 75 belonged to the genus *Mrakia*, a psychrophilic basidiomycetous yeast. These results suggested that *Mrakia* spp. were dominant in Antarctica and highly adapted to the Antarctic environment. *Mrakia* spp. and *Mrakiella* spp. also have been found in cold climate areas worldwide such as the Arctic, Siberia, Central-Russia, the Alps and the Antarctica. *Mrakia blollopis* SK-4 was isolated from Naga-ike, Skarvsnes, East Antarctica. Optimal growth temperature of this yeast was 15 °C. This yeast had some unique characteristics t such as survival ability in the presence of organic acids, organic solvents and phenolic compounds, by virtue of which it can be used in ethanol fermentation from lignocellulosic biomass. Moreover, this yeast could ferment typical sugars and had cellulase and β-glucosidase enzymes.

Lignocellulosic biomass is mainly composed of heterogeneous complex of cellulose, hemicellulose and lignin, which are linked with cellulose and hemicellulose. These bounds impede enzymatic hydrolysis. Hitherto, various pretreatment were developed, such as mechanochemical comminution, dilute acid, alkali and organosolv process. However, end-product from lignocellulose has indicated to play a major role as a inhibitor against hydrolysis reaction. Glucose, cellobiose and ethanol have significantly inhibited ability for β-glucosidase and cellulase. Moreover, phenolic compounds, derived from lignin, were toxic to fermentability microorganisms.

Simultaneous Saccharification and Fermentation (SSF) were firstly reported by Takagi (1977). In this technique is enzymatic hydrolysis and ethanol fermentation is carried out at the same time. In the presence of high concentration of glucose, cellulase activity was considerably depressed. Although, when yeast is mixed with enzymatic hydrolysis reactor, glucose, formed by cellulase activity from cellulolytic biomass, was maintained at low concentration and quickly converted to ethanol via yeast. Therefore, this technique is expected to improve saccharification and ethanol fermentation rate. SSF using psychrophiles however has not yet been reported. In this study, we performed ethanol fermentation and simultaneous saccharification and fermentation (SSF) from lignocellilosic biomass via *Mrakia blollopis* SK-4 at low temperature.

As the results of ethanol fermentation of 4 (w/v) % and 6 (w/v) % glucose by *M. blollopis* SK-4 at 10°C, approximately 1.7 (v /v) % ethanol were obtained from 4 (w/v) % glucose and 2.1 (v/v) % ethanol were produced from 6 (w/v) % glucose after 96 h fermentation. Then, we performed to SSF from 5 (w/v) % filter paper, 10(w/v) % Japanese cider and eucalyptus with Acremonium cellulolyticus cellulase. In the case of filter paper, ethanol was gradually formed, according to fermentation time. However, when this yeast was applied to SSF of Japanese cider, ethanol fermentation was done about 80% reactions within first 48 h. This is the first report of simultaneous saccharification and fermentation using psychrophile at low temperature. We considered that *M. blollopis* SK-4 have a good potential for SSF at low temperature