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Isolation and characterization of two soil derived yeasts for bioethanol production on Cassava starch

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ABSTRACT

Two ethanol-producing yeast strains, CHY1011 and CHFY0901 were isolated from soil in South Korea using an enrichment technique in a yeast peptone dextrose medium supplemented with 5% (w v^{-1}) ethanol at 30 °C. The phenotypic and physiological characteristics, as well as molecular phylogenetic analysis based on the D1/D2 domains of the large subunit (26S) rRNA gene and the internally transcribed spacer (ITS) 1 + 2 regions suggested that they were novel strains of *Saccharomyces cerevisiae*. During shaking flask cultivation, the highest ethanol productivity and theoretical yield of *S. cerevisiae* CHY1011 in YPD media containing 9.5% total sugars was $1.06 \pm 0.02\ g\,l^{-1}\,h^{-1}$ and $95.5 \pm 1.2\%$, respectively, while those for *S. cerevisiae* CHFY0901 were $0.97 \pm 0.03\ g\,l^{-1}\,h^{-1}$ and $91.81 \pm 2.2\%$, respectively. Simultaneous saccharification and fermentation for ethanol production was carried out using liquefied cassava (*Manihot esculenta*) starch in a 5 l lab-scale jar fermenter at 32 °C for 66 h with an agitation speed of 2 Hz. Under these conditions, *S. cerevisiae* CHY1011 and CHFY0901 yielded a final ethanol concentration of $89.1 \pm 0.87\ g\,l^{-1}$ and $83.8 \pm 1.11\ g\,l^{-1}$, a maximum ethanol productivity of $2.10 \pm 0.02\ g\,l^{-1}\,h^{-1}$ and $1.88 \pm 0.01\ g\,l^{-1}\,h^{-1}$, and a theoretical yield of $93.5 \pm 1.4\%$ and $91.3 \pm 1.1\%$, respectively. These results suggest that *S. cerevisiae* CHY1011 and CHFY0901 have potential use in industrial bioethanol fermentation processes.

1. Introduction

The economics of ethanol production by fermentation are influenced by the cost of the raw materials, which accounts for more than half of the production costs [1]. For this reason, bioethanol production from renewable agricultural resources by several microorganisms (e.g. *Saccharomyces cerevisiae*, *Saccharomyces uvarum*, *Kluyveromyces marxianus*, and *Zymomonas mobilis*) has attracted considerable attention in recent years [2–7]. More recently, there is growing interest in finding alternate bioresources for commercial ethanol production apart from sugar cane/beet molasses and starchy crops such as sweet sorghum, cassava (*Manihot esculenta*) and sweet potato [8,9]. In particular, cassava starch is a cheap substrate that is easily available in tropical countries such as Southeast Asia and Africa [8,10,11]. There have been many reports of the potential

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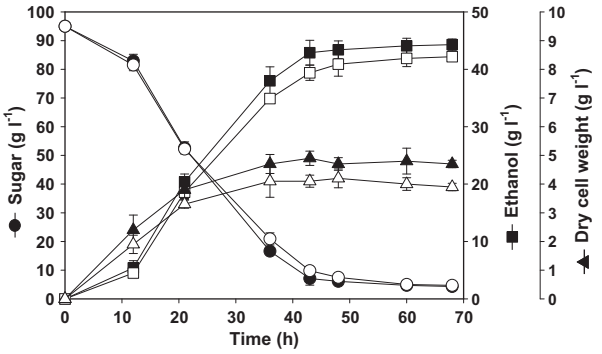

Fig. 1 – The time course profiles of ethanol production (square) and sugar concentration (circle) and dry cell weight (triangle) during flask cultivation by CHY1011 (black color) and CHFY0901 (white color).

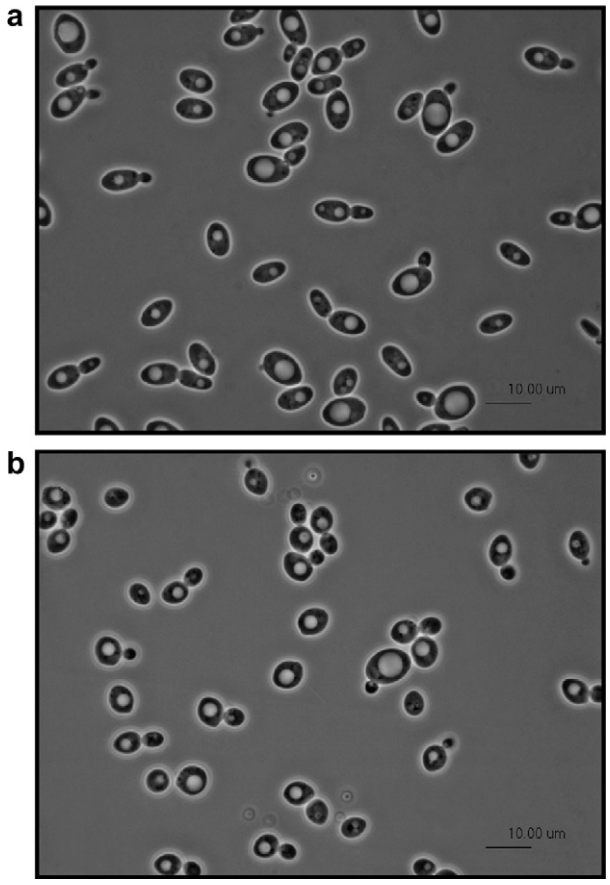

Fig. 2 – Phase contrast light microscopy images showing the cell morphology of CHY1011 (a) and CHFY0901 (b) when grown in a YPD broth for 1 days at 30 °C. Bars, 10 μm.

applications of cassava starch and its hydrolysate as an alternative bioresource in simultaneous saccharification and fermentation [12–14].

On the other hand, the most important aspect of bioethanol fermentation is the ethanol yield, or more specifically the industrial yield [15]. The yield is dependent on many factors, such as the fermentative capacity of the cell population, the microbial activity, and the resistance of those industrial cells to stress conditions, particularly those of yeasts [15,16]. Since *S. cerevisiae* can produce a high concentration of ethanol, it is commonly used in industrial ethanol production [17]. Although many studies are needed to improve the stain and fermentation process to produce more ethanol, *S. cerevisiae*, an efficient ethanol producer, has many advantages such as a well studied genetic and physiological background, faster fermentation rates with the ability to grow under both aerobic and anaerobic conditions, a high tolerance to ethanol and osmotic tolerance, easy to manipulate and safety for foods [16,18–20]. Hence, *S. cerevisiae* has significant potential in environmentally friendly ethanol fermentation processes. Therefore, this novel high ethanol-fermenting yeast requires isolation and study.

This study reports the screening and isolation of ethanol-resistant yeast cultures, which resulted in the isolation of two novel yeast strains (CHY1011 and CHFY0901) capable of ethanol fermentation. The isolates were identified by their morphological and physiological characteristics, as well as by phylogenetic analysis of the internal transcribed spacer (ITS1 + 2) regions and the D1/D2 domains of the large subunit (26S) rDNA coding gene. The analyses showed that both newly isolated yeasts are novel strains of *S. cerevisiae*. The suitability of the cassava root for ethanol production through enzymatic liquefaction and simultaneous saccharification and fermentation was also investigated. The results show that *S. cerevisiae* CHY1011 and CHFY0901 are capable of ethanol production from cassava powder hydrolysate through batch fermentation. Therefore, these novel strains have potential industrial applications to bioethanol production.

Table 1 – Ethanol production from CHY1011 and CHFY0901 in a yeast peptone dextrose (YPD) medium by flask cultivation for 68 h at 30 °C.

Yeast	Initial sugar concentration ($g l^{-1}$)	Final ethanol %($w v^{-1}$)	Ethanol yield[a] ($g g^{-1}$)	Volumetric product productivity[b] ($g l^{-1} h^{-1}$)	% of Theoretical yield
CHY1011	~95	4.43 ± 0.01 (68 h)	0.47 ± 0.03	0.65 ± 0.02	95.5 ± 1.2
CHFY0901	~95	4.22 ± 0.06 (68 h)	0.44 ± 0.01	0.62 ± 0.01	91.8 ± 2.2

a Mass of ethanol formed per mass of total sugar consumed.
b Ethanol formed per liter of YPD medium per hour.