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Potential of bioethanol production from olive mill solid wastes

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HIGHLIGHTS

- Two yeasts were isolated from OMSW, *I. orientalis*, and *P. galeiformis/manshurica*.
- *I. orientalis* showed better kinetics to xylose compared to the other strains.
- *I. orientalis* on hydrolysate supplemented with glucose showed best ethanol production.
- Using SSF process following pretreatment, the ethanol yield was 3 g/100 g dry OMSW.

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ABSTRACT

The main objective of this study was to screen endogenous microorganisms grown on olive mill solid wastes (OMSW) with the potential to ferment pentoses and produce ethanol. Two yeasts were isolated and identified as *Issatchenkia orientalis*, and *Pichia galeiformis/manshurica*. The adaptation of the strains displayed a positive impact on the fermentation process. In terms of xylose utilization and ethanol production, all strains were able to utilize xylose and produce xylitol but no ethanol was detected. Separate hydrolysis and fermentation process on hydrolysate undergo detoxification, strain *I. orientalis* showed the best efficiency in producing of ethanol when supplemented with glucose. Using simultaneous saccharification and fermentation process following pretreatment of OMSW, the average ethanol yield was 3 g/100 g dry OMSW. Bioethanol production from OMSW is not economic despite the raw material is cheap.

1. Introduction

In recent decades renewable energy sources have considerable interest worldwide. Biomass energy is one of the oldest and most promising sources and includes organic waste, sewage, and energy crops, agricultural and industrial residues that can be utilized to produce bio-fuel. Biomass can be converted biologically to liquid or gaseous fuels, such as ethanol, methanol, methane and hydrogen by fermentation processes (Haagensen et al., 2009).

Ethanol has attracted interest as alternative liquid fuel, especially for transportation. It has immense importance for countries which depends heavily on import of crude oil, spending a huge amount of its annual budget. Currently, bioethanol is mainly produced from biomass containing sugar or starch as sugar cane and corn as raw material (first generation) (Patni et al., 2013). Because these products are used as food and feed for humans and animals, there is competition of using biomass as a food or fuel. Due to this competition, bioethanol production from lignicellulose (second generation) enthusiastically have been investigated worldwide (Lin et al., 2012). Lignocellulosic biomass, such as trees and agricultural residues is an attractive raw material for bioethanol production because of the large amount of potential sugar for fermentation and bioenergy production (Bellido et al., 2013; Choi et al., 2010; Cuevas et al., 2010; Kumar et al., 2009; Olsson and Hahn-Hägerdal, 1996).

Production of olive oil symbolizes one of the most important economic agro-food sectors in the Mediterranean basin. The traditional oil industry generates two byproducts at the end of the process, olive cake (residue) and olive mill wastewater, which can cause serious environmental pollution problems (Ballesteros et al., 2001). Crude olive cake, the leftover solids following the pressing of olives, is a mixture of skin, pulp, and seeds. It comprises approximately 35% of the beginning olive weight, is rich in carbohydrates, and is available in appreciable quantities in the

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Mediterranean area (Banat et al., 2013; El Asli and Qatibi, 2009). Global annual production of olive cake has been estimated to approach 4×10^8 kg of dry matter (El Asli and Qatibi, 2009).

The main structural components of lignocellulosic biomass are cellulose, hemicellulose, and lignin. Of these, only cellulose and hemicellulose can be used as raw materials to produce ethanol by fermentation of carbohydrates obtained through chemical or enzymatic hydrolysis (saccharification). The biological process for converting lignocellulose to fuel ethanol requires: (1) delignification to liberate cellulose and hemicellulose; (2) depolymerization of carbohydrate polymers to produce free sugars; and (3) fermentation of mixed hexose and pentose sugars to produce ethanol (Cuevas et al., 2010; Kumar et al., 2009; Lin et al., 2012; Matsushika et al., 2009). Hydrolysis of these polysaccharides (cellulose and hemicellulose) is usually accomplished by acid and/or enzymatic treatment. The utilization of both cellulose and hemicellulosic sugars like hexose, pentose and others present in a typical biomass hydrolysate is essential for the economical production of ethanol (Kumar et al., 2009; Olsson and Hahn-Hägerdal, 1996).

The production of ethanol from pretreated material may be accomplished either by sequential hydrolysis and fermentation or by a simultaneous saccharification and fermentation (SSF) process (Ahmed et al., 2013; Cuevas et al., 2010; Oberoi et al., 2012). During the SSF process, end product inhibition can be overcome as glucose is simultaneously fermented as it is formed; another advantage is that a single reactor is used for both saccharification and fermentation processes. However, the enzymatic reaction in SSF process is operated at a temperature lower than its optimum level owing to the mismatch in optimum temperatures for hydrolysis (approx. 50 °C) and fermentation (approx. 30 °C) (Cuevas et al., 2010; Ruiz et al., 2006).

The main component of lignocellulosic hydrolysates is glucose, a hexose sugar derived from cellulose and hemicellulose. Although the proportion of mono-saccharides in hemicellulose hydrolysates varies depending on the raw material and the hydrolysis procedure, they all contain both pentose sugars, such as D-xylose and L-arabinose and hexose sugars as well. D-Xylose is the second most abundant carbohydrate and its content is particularly high in grass and hardwood (Hendriks and Zeeman, 2009; Matsushika et al., 2009).

One of the most effective ethanol-producing microorganisms for hexose sugars including glucose, mannose and galactose is *Saccharomyces cerevisiae* yeast with high ethanol productivity, high tolerance to ethanol, and tolerance to inhibitory compounds present in the hydrolysate of lignocellulosic biomass (Matsushika et al., 2009). However, this strain is unable to utilize xylose for growth or fermentation in order to produce ethanol. Instead, this strain metabolizes D-xylulose, an isomerization product of D-xylose. Some yeast strains have been reported to ferment xylose into ethanol, but the rate and yield of ethanol production from xylose in these xylose-utilizing yeast strains are considerably low compared to their glucose fermentation process (Ferreira et al., 2011; Lin et al., 2012). Therefore, genetic engineering and/or adaptation may be promising methods to develop sufficient xylose fermentation in *S. cerevisiae*.

To date, numerous studies regarding the metabolic engineering of *S. cerevisiae* for xylose utilization have been reported, and many reviews have already addressed the current advancement in metabolic engineering of xylose-fermenting strains and factors which affect xylose metabolism in yeasts (Matsushika et al., 2009).

The objectives of the current study were to isolate yeast strains from olive mill solid wastes (OMSW) with the potential to utilize xylose and produce ethanol to be compared with other commercial known yeast strains. In addition to adapt the different yeast isolates to produce bioethanol from the OMSW hydrolysate.

2. Methods

2.1. Raw material

Olive mill solid waste (OMSW), the solid residue from the traditional olive oil production process, was obtained from Dabburia, Galilee region of Israel after the harvest months of the year 2009/2010. OMSW was dried at 45 °C, milled to particle size between 0.25–1 mm, and stored at room temperature until being used.

2.2. Content test

Content test was performed to quantify the potential of a feedstock (raw material) and thus test the effectiveness of pretreatment to the total content of cellulose and hemicellulose. Wall sugar content was tested after water extraction, testing the solid percentage and the ash content was performed according to standard analytical procedures as described in protocols of the NREL. The composition of the OMSW used throughout the experiments included: extractive, lignin, acid insoluble, cellulose, hemicellulose, xylose and galactose, arabinose, acetyl, ash, with dry matter percentage of 18.3 ± 1.7, 40.2 ± 5.1, 39.5 ± 5.1, 18.4 ± 0.2, 15.9 ± 0.1, 14.1 ± 0.1, 1.8 ± 0.0, 2.7 ± 0.1, 4.5 ± 1.2, respectively.

2.3. Pretreatment of OMSW

The biomass at a solid loading of 7.5% (w/v) was mixed with diluted sulfuric acid (final concentration of 2% (v/v)) at 100 °C, with the residence time of 2 h. After treatment, the reactor was removed from heating jacket and cooling to room temperature. The pretreated material was separated by filtration into two fractions, i.e., the solid insoluble residue and the liquid fraction (hydrolysate). The composition of the hydrolysate regarding main sugars (glucose and xylose) and inhibitors compounds (furfural, acetic acid, and formatic acid), is shown in Table 1. Other sugars, like galactose, arabinose and mannose, as well as hydroxymethylfurfural (HMF), were also present in hydrolysates at lower concentration (Cara et al., 2008).

2.4. Enzymatic hydrolysis

After dilute acid pretreatment, enzymatic hydrolysis was performed using cellulases (Cellulase Complex-Ecostone L900 and β-glucosidase (Novozyme 188S from *Trichoderma reesei* were purchased from Sigma) at 50 °C and 100 rpm for 24 h in a water bath shaker. In order to maintain the pH at 4.8 in the mixture, 0.05 M sodium citrate buffer (pH 4.5) was added.

2.5. Detoxification of diluted sulfuric acid hydrolysate with activated carbon

Detoxification of diluted sulfuric hydrolysate was carried out using 5% (w/v) activated carbon in an incubated water bath shaker at 50 °C for 30 min, according to the methodology described by Díaz et al. (2009). Then the mixture was cooled to room temperature, sodium hydroxide was added until the desired pH (4.5) was achieved. Afterward was centrifuged to remove the solid material and kept below 4 °C for further analysis.

2.6. Yeast strains

Three strains of yeast were used in this study; one was purchased from ATCC, *S. cervisiae* (24860 ATCC, designated as S.C.),