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The isolation of pentose-assimilating yeasts and their xylose fermentation potential

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ABSTRACT

For the implementation of cellulosic ethanol technology, the maximum use of lignocellulosic materials is important to increase efficiency and to reduce costs. In this context, appropriate use of the pentose released by hemicellulose hydrolysis could improve de economic viability of this process. Since the *Saccharomyces cerevisiae* is unable to ferment the pentose, the search for pentose-fermenting microorganisms could be an alternative. In this work, the isolation of yeast strains from decaying vegetal materials, flowers, fruits and insects and their application for assimilation and alcoholic fermentation of xylose were carried out. From a total of 30 isolated strains, 12 were able to assimilate 30 g L^{-1} of xylose in 120 h. The strain *Candida tropicalis* S4 produced 6 g L^{-1} of ethanol from 56 g L^{-1} of xylose, while the strain *C. tropicalis* E2 produced 22 g L^{-1} of xylitol. The strains *Candida oleophila* G10.1 and *Metschnikowia koreensis* G18 consumed significant amount of xylose in aerobic cultivation releasing non-identified metabolites. The different materials in environment were source for pentose-assimilating yeast with variable metabolic profile.

Introduction

Bioethanol or first generation ethanol in Brazil is obtained by fermenting glucose from sugar cane juice and molasses (a residue from sugar making) using *Saccharomyces cerevisiae*. While this technology is a consolidated industrial process, production of second generation ethanol remains a challenge. One of the bottlenecks is the utilization of pentoses released by hydrolysis of hemicellulose that correspond to around 30% of the lignocellulosic feedstock. Yeasts able to convert xylose into ethanol have been described like as *Scheffersomyces (Pichia) stipitis*, *S. (Candida) shehatae* and *Pachysolen tannophilus*.[1,2] However the yield has not reached a satisfactory level for industrial

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applications On the other hand, pentose-assimilating yeasts can be used for obtainment of high aggregate value products. The pentose-assimilating capacity of yeasts *Pseudozyma antarctica* PYCC 5048^T, *P. aphidis* PYCC 5535^T and *P. rugulosa* PYCC 5537^T and production of a glycolipid biosurfactant was demonstrate[3] as well as the production of xylitol and arabitol by *Debaryomyces hansenii* from pentose.[4]

A number of recent studies have been focused on the genetic engineering of *S. cerevisiae*, aimed at making it able to produce ethanol from glucose and xylose.[5] Nevertheless, the yield achieved resembles the xylose-fermenting species, the xylose utilization is slow and occurs only after glucose exhaustion.[3,4,6,7] The search for genetically modified strains is the focus of several studies. Thus, the study of microorganisms pentose-fermenting and isolation of genes involved in pentose fermentation can afford support for these studies.

Another research tendency is the use of pentose to produce alternative compounds like organic acids,[8–12] xylitol,[13] lipids for biodiesel production,[14–16] isobutanol[17] and hydrogen[18] all using mainly yeast and bacteria. Besides compounds already cited, new alternative products with high aggregate value, are still appearing, increasing the options for the utilization of these sugars in biorefineries system.[19]

Considering the importance of the use of pentoses from lignocellulosic materials, the present work aimed at isolating yeast strains with the ability to assimilate and/or ferment xylose for future use for obtainment of products with biotechnological interest. Thus, this study contributes by increasing the information on xylose-fermenting strains and offers genomes for future studies of the metabolism of microorganisms.

Materials and methods

Isolation and identification of strains

The strains were isolated from fruits, flowers, insects and grape residues. About 0.5 g of material was suspended in 4 mL of YEPX medium (1% of yeast extract, 2% of peptone and of 2% xylose) and incubated at 30 °C. A loop of the homogenized culture was then streaked on the solid YEPX medium in Petri dishes to isolate the colonies. The strain identification was made using sequences of the D1/D2 domains of the rDNA.[20]

Xylose assimilation assay

To evaluate the potential of xylose assimilation, the strains were pre-cultivated in a YEP medium (1% yeast extract, 2% peptone and 2% glucose) and the biomass was centrifuged, watched twice with sterile distilled water and a suspension of them used as inoculum at 1 g of dry biomass per liter of culture medium composed of KH_2PO_4 (2.0 g L^{-1}), $(NH_4)_2SO_4$ (2.0 g L^{-1}), $MgSO_4 \cdot 7H_2O$ (1.0 g L^{-1}), urea (0.3 g L^{-1}), $CaCl_2$ (0.3 g L^{-1}) and xylose (30.0 g L^{-1}). The incubation was carried out at 30 °C and 150 rpm. The assays were done in duplicate and with three repetitions.

Ethanol production assay

The alcoholic fermentation was assayed in 125 mL Erlenmeyer flasks adapted for alcoholic fermentation, closed with a valve containing sodium metabisulfite solution at 1 g L^{-1} to ensure that no oxygen got, and containing 60 mL of medium described above but with yeast extract at 10 g L^{-1} and xylose at 100.0 g L^{-1}. The incubation was at 30 °C. The assays were done in duplicate with three repetitions.

Analytical methods

To evaluate the dry cell mass, samples of fermented medium were centrifuged at 10,000 × *g*, by 15 min, the supernatant was discarded and the precipitated cells were dried at 60 °C until they reached constant weight.

The reducing sugar concentration was determined using the dinitrosalicylic (DNS) acid method, based on Miller (1959).[21]

The ethanol concentration was measured by a gas chromatograph (HP 5890) with an FFAP capillary column (polyethylene glycol – 30 mm × 0.22 mm × 0.3 μm) and a flame ionization detector, one split/splitless injector. Nitrogen was utilized as a carrier gas at 30 mL min^{-1}. Temperatures at injector and detector were 250 °C.

The xylitol quantification was performed by high-performance anion-exchange chromatography with pulsed amperometric detection (HPAEC-PAD). All samples were filtered (0.22 μm membrane) and injected (20 μL) in HPAEC-PAD System (ICS, Dionex Corporation, USA) equipped with automatic sampler AS40. The form of wave pattern used was the standard quadruple with the following potential pulse and durations: E1 = 0.10 V (*t*1 = 0.40 s); E2 = −2.00 V (*t*2 = 0.02 s); E3 = 0.60 V (*t*3 = 0.01 s); E4 = −0.10 V (*t*4 = 0.06 s). An Isocratic run was performed with 10 mM NaOH at a flow rate of 1 mL min^{-1} and 35 °C.

Results

Isolation, identification of yeasts and evaluation of xylose assimilation

A total of 30 strains were isolated and able to grow on the liquid YEPX medium, but, among those, four were not able to grow in liquid medium with xylose as the sole carbon source (Table 1). In aerobic cultivation, nine strains consumed all the xylose present in the medium (30 g L^{-1}) in 120 h.

The strain *Pichia guilliermondii* G1.2 was the most efficient in the xylose consumption (maximum at 48 h) while strains *Candida tropicalis* FP, *C. tropicalis* S4 and *P. guilliermondii* G4.2 exhausted the sugar in 72 h.

Species or genera isolated in this work, such as *Aureobasidium pullulans*, *Issatchenkia terricola*, *I. orientalis*, *Metschnikowia bicuspidata*, *M. chrysoperlae*, *M. zobellii*, *P. guilliermondii*, *P. stipitis*, *Rhodotorula minuta* and *R. mucilaginosa* are mentioned in the literature as being able to produce ethanol from xylose.[22–29] The genera Hanseniaspora and the specie *I. terricola* are glucose fermenting, but there is contradicting information about

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