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RESEARCH ARTICLE

YEASTS ISOLATION AND SELECTION FOR BIOETHANOL PRODUCTION FROM INULIN HYDROLYSATES

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Abstract

In many countries bioethanol is already an alternative or a complement to gasoline. The bioalcohol production from Jerusalem artichokes has been studied since the end of XIXth century. The ethanol production from Jerusalem artichoke consists in a saccharification and fermentation bioprocesses. In the saccharification step, the inulin, which is the most abundant carbohydrate in Jerusalem artichoke tubers, is hydrolyzed to fructose. Yeast strains for bioethanol production from Jerusalem artichoke have to have the ability to use fructose as substrate for alcoholic fermentation. The main objective of this study is isolation and selection of some yeast strains able for high yield bioethanol production from Jerusalem artichoke tubers. After selection a yeast strain able to production good yield of fermentation of fructose into ethanol was identified.

Key words: inulin hydrolysates, Saccharomyces, fermentation.

Introduction

Energy crops have a significant potential for contributing to the future energetic scenarios. Although more widely recognized now, the environmental, economic, strategic and infrastructure advantages offered by the production of ethanol from different unconventional materials, such as Jerusalem artichoke, were not appreciated in the past (Wyman, 2001).

The primary feedstock for ethanol production worldwide remains sugar or starch from agricultural crops, but other sources were also exploited, such as lignocellulose crops or oil crops (Smith, 2006).

Jerusalem artichoke is a well known subject for studying the metabolism and synthesis of inulin (Praznik, 1987). It contains nearly 20% of carbohydrates, 70-90% of which is inulin. Jerusalem artichoke has good potential for bioethanol production when fermented by suitable organisms (Xiang-Yang Ge, 2005). Jerusalem artichoke can grow well in poor land, shows a high tolerance to frost and various plant diseases (Szambelan, K., et al., 2004)

Inulin is a polyfructan, consisting of linear β-2, 1-linked polyfructose chains terminated by a glucose residue. Inulin is a reserve carbohydrate in roots and tubers of plants like Jerusalem artichoke, dahlia or chicory.

The direct fermentation of inulin extracts into ethanol by several inulinase producing yeasts has been studied, using *Kluyveromyces* and *Saccharomyces* yeast strains. Another approach involves a two-step process, in which the inulin extract is first hydrolyzed using bacteria or fungi for subsequent fermentation to ethanol (Ohta, K., et al., 1993).

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Saccharomyces cerevisiae is an important microorganism in bio-industry and its tolerance to ethanol is one of the main characteristics to decide whether it can be used for alcoholic fermentation. Thus, in industrial ethanol production, there are many important factors to be considered, such are ethanol or sugar tolerance of yeast strains, ability to do fermentation at higher temperatures (termotolerance) and enzymatic activities for certain transformations (Mobini-Derhkordi et al., 2007, Patrascu et al., 2009) Also, for fermentation of inulin hydrolysates substrates, yeasts require the ability to ferment fructose, the main carbon source of the medium after inulin hydrolysis.

In the present study, yeast strains for bioethanol production from inulin hydrolysates were isolated from different sources and characterized for their ability to growth in medium having fructose as carbon source and their ability to fermentation of this monosaccharide.

Materials and methods

Hydrolysis of the inulin

Commercial inulin was hydrolysed by using inulinase crude extract produced by the strain *Aspergillus niger* MIUG 1.15 which is preserved in the Laboratory of Industrial Microbiology Collection. In hydrolysate the content of fructose was established by Schaffer Somogyi method.

Yeast strains isolation

Several yeast strains were isolated from different sources; such are bees, honey, flowers, Jerusalem artichoke and soil of the Jerusalem artichoke tubers, beer, yoghurt, fruits (banana, strawberry) and also, two commercially yeast strains for ethanol were used. The isolation sources were divided into small pieces using a sterilized cutter and inoculated on liquid wort medium.

After two days, few milliliters of the liquid malt wort medium were passed in Petri dishes on wort agar medium. The Petri dishes were kept for 3 days at 26°C and were observed daily. T

he grown colonies were then isolated and characterized microscopically and cultural characters examination. For microscopic examination a Karl Zeiss Jena microscope was used.

Determination of yeasts ability to fructose fermentation

Yeasts ability for fructose fermentation was determined using Wickerham medium where the carbon source was fructose and Durham fermentation tube. The Wickerham liquid medium had the following composition (g/dm^3): peptone 10, yeast extract 5, bromthymol blue 0.005. pH was adjusted to 7.2 and the medium was distributed 9 mL in test tubes with Durham tube and sterilized at 121°C for 15 minutes. 1 mL of sterile 20% fructose solution was added to each test tube and then the yeasts were inoculated in Wickerham medium and stored at 26°C for 5 days. The CO_2 accumulation was then observed in Durham tube and this was the first criteria for yeast strain selection.

Determination of yeast ability to ferment inulin hydrolysates

Inulin hydrolysate was obtained using the synthetic medium with the following composition (g%): inulin 10, yeast extract 0.65, $(NH_4)_2SO_4$ 0.26, KH_2PO_4 0.272, $MgSO_4$ 0.05, $CaCl_2$ 0.05, $ZnCl_2$ 0.000042, citric acid 0.15 and sodium citrate 0.6.

The synthetic medium with inulin as the sole carbon source was inoculated with *Aspergillus niger* spores and incubated stationary, for 3 days at 37°C for hydrolysis. After 3 days, yeast inoculum of 1.5x10^7 cells/mL was added and fermentation dynamics was measured.

Selection of yeast strains for bioethanol production from inulin hydrolysates

For yeast strains selection yeast inocula were obtained for each strain isolated. Yeast biomass needed for pitching was obtained by streaking on synthetic medium with 2% agar at temperature of 28°C for 4 days. The cells were counted using Thoma cytometer. The synthetic medium used had the following composition (g%): fructose 8, yeast extract 0.65, $(NH_4)_2SO_4$ 0.26, KH_2PO_4 0.272, $MgSO_4$ 0.05, $CaCl_2$ 0.05, $ZnCl_2$ 0.000042, citric acid 0.15 and sodium citrate 0.6. The medium was distributed in Erlenmeyer flasks and sterilized at 121°C for 15 minutes. The fructose solution was sterilized separately avoiding Maillard reaction.

The yeast inoculum of $1.5x10^7$ cells/mL was added after cooling in aseptic conditions.

The samples obtained were incubated at constant temperature of 20°C and weighted daily until their weight remained constant.

Yield of ethanol determination

The formed ethanol content, expressed in % (v/v), was calculated using the following equation:

$$\text{Alcohol} = (G_1 - G_2) \times 1.045 \qquad \text{(Eq. 1)}$$

Where:

$G_1 - G_2 = CO_2$ liberated during fermentation, g/100 mL

1.045 = coefficient for CO_2 transformation in the ethanol, resulted from Gay-Lussac equation

Results and discussion

Twelve yeast strains were isolated from different sources and were characterized macro and microscopically. The main characteristics are presented in Table 1.

Table 1. Morphological characteristics of the isolated yeasts

Yeast strain code	Source of isolation	Macroscopic characteristics	Microscopic characteristics
N1	Jerusalem artichoke	colony diameter=4.33mm, circular form, convex profile, smooth shiny surface	rounded cells, linked in associations
P1	soil from Jerusalem artichoke	colony diameter =5.66mm, circular form, bellied profile, smooth shiny surface	single rounded cells
N2	Yeast for alcohol production	colony diameter =1.66mm, circular form, bellied profile, smooth shiny surface	single rounded cells
P2	soil from Jerusalem artichoke	colony diameter =3.83mm, circular form, bellied profile, smooth shiny surface	rounded cells, linked in associations
P2'	soil from Jerusalem artichoke	colony diameter =2.83mm, curly form, shiny surface	rounded cells
M2	honey	colony diameter =2.33mm, circular form, bellied profile, smooth shiny surface	rounded cells, linked in associations
B2	beer	colony diameter =3.16mm, circular form, bellied profile, smooth shiny surface	oval and rounded cells, linked in associations
ER	Yeast for wine production	colony diameter =3.16mm, circular form, bellied profile, smooth shiny surface	oval and rounded cells, linked in associations
A1	Bee	colony diameter =2mm, circular form, bellied profile, smooth shiny surface	oval and rounded cells
BN	banana	colony diameter 2.14 mm, circular form, smooth shiny surface	oval and rounded cells
F2	flowers	colony diameter 1.8 mm, circular form, smooth shiny surface	oval and rounded cells
I1	yoghurt	colony diameter 2.65 mm, circular form, smooth shiny surface	oval and rounded cells

The isolated yeasts were then inoculated in Wickerham medium with fructose as the only carbon source, containing Durham fermentation tube, to observe yeasts ability to ferment fructose. Thus, all the yeasts excepting those isolated from Jerusalem artichoke tubers have the ability to ferment fructose, and those yeasts liberated CO_2 visible in Durham tube (Table 2).

Table 2. The ability of the isolates yeast strains to ferment fructose

Yeast strain code	Yeast ability to ferment fructose
N1	+--
P1	++-
N2	+++
P2	+--
P2'	+++
M2	+--
B2	+++
ER	+--
A1	+++
BN	++-
F2	---
I1	+--

All yeasts which had the ability to ferment fructose were used for fermentation dynamics determination. The yeasts were inoculated in Erlenmeyer flasks containing synthetic medium with fructose as the sole carbon source. The flasks were weighted daily to observe the CO_2 elimination. The results are presented in Figure 1.

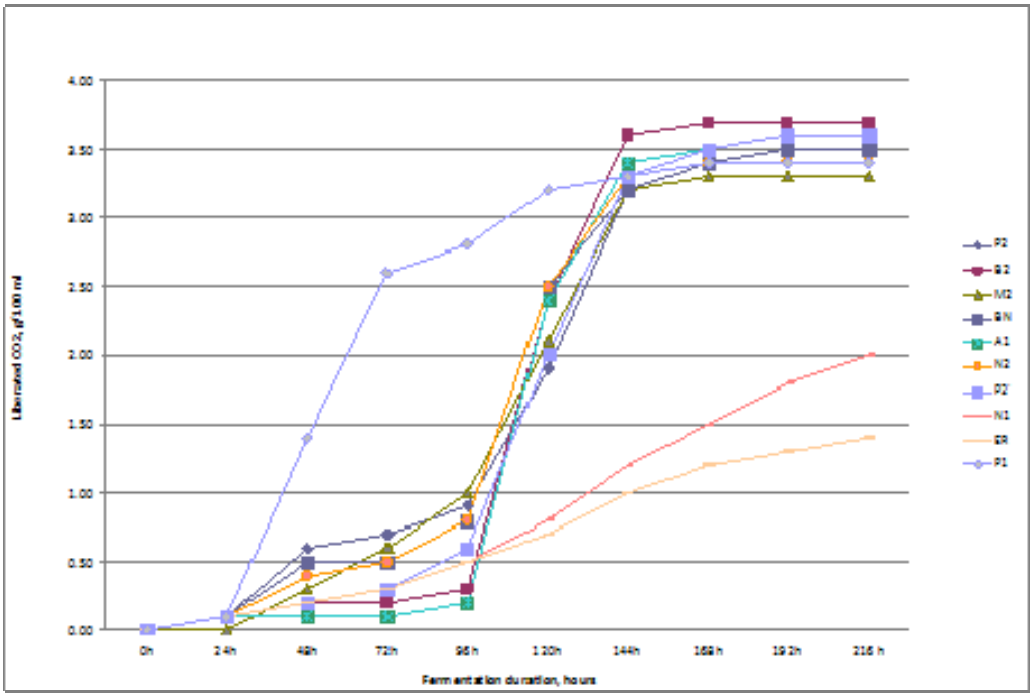

Figure 1. The fructose fermentation dynamics by selected yeast strains

As it can be observed from figure 1, yeast noted B2 eliminated the highest CO_2 amount, 3.7 g/100 mL respectively. Also, all yeasts except ER and N1 liberated similar CO_2 amounts, between 3.4 and 3.6 g/100 mL. The yeast coded B2 was selected for further experiments concerning inulin hydrolysates fermentation.

The ethanol content of the samples was calculated using the equation 1. The results obtained are presented in figure 2.

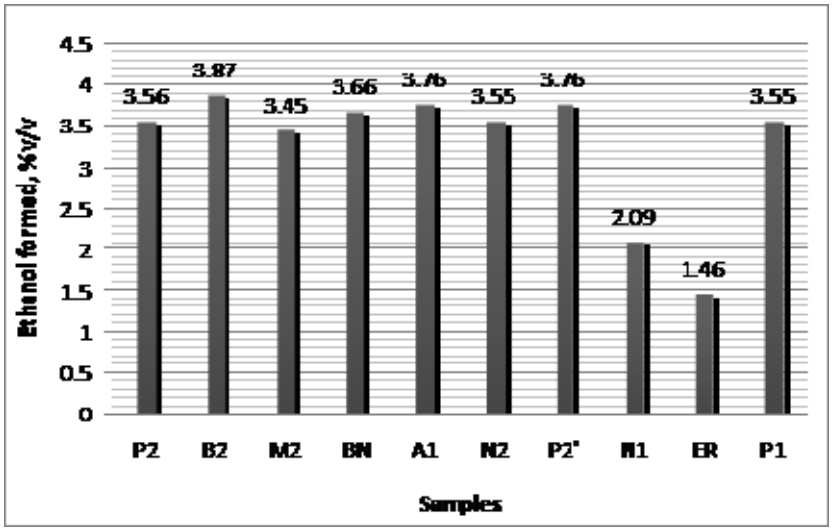

Figure 2. The yield of the ethanol formed during fructose fermentation

The yeast coded B2, with the highest ability to ferment fructose, was tested for the ability to ferment inulin hydrolysate, obtained from synthetic medium with 10% inulin as the sole carbon source inoculated with *Aspergillus niger* spores (Figure 3). After 3 days of hydrolysis, the fructose content of the medium was 100.7 mg/g.

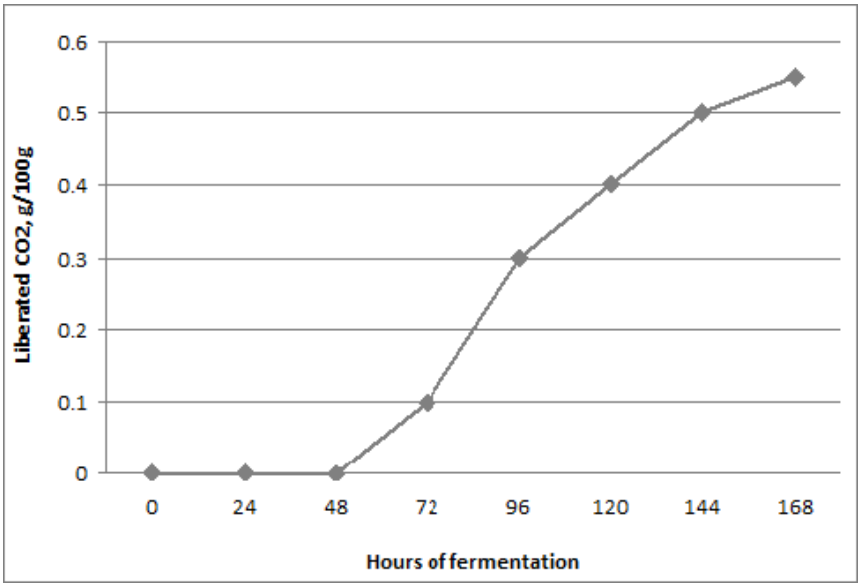

Figure 3. The fermentation dynamics for inulin hydrolysate using B2 coded yeast strain

As it can be observed from figure 3, after 168 hours of fermentation, 5.5 g of CO_2 were liberated in 100 mL of medium, corresponding to 5.75 % v/v alcohol, calculated using equation 1.

Conclusions

Twelve yeast strains were isolated from different sources (bees, honey, flowers, Jerusalem artichoke and soil of the Jerusalem artichoke tubers, beer, yoghurt, fruits (banana, strawberry) and also, two commercially yeast strains for ethanol production) and tested for their ability to ferment inulin hydrolysates. Ten of the twelve isolated yeast strains had the ability to ferment fructose, as the test on Wickerham medium showed.

These yeasts were then inoculated on synthetic medium with the fructose at the same inoculation rate, to select the yeast with the higher ability to ferment fructose.

The yeast isolated from beer sample, coded B2, had the highest ability to ferment fructose expressed by the amount of the ethanol formed. Also, yeast strain coded B2 has the ability to ferment inulin hydrolysates, obtaining 5.75% v/v alcohol after 168 hours of fermentation.

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