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RESEARCH ARTICLE

ISOLATION AND SCREENING OF NEW MOULD STRAINS ABLE FOR INULINASE BIOSYNTHESIS AND INULIN FROM JERUSALEM ARTICHOKE HYDROLYSIS

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Abstract

Inulin, a widespread polyfructan in plants, is of great interest as it is a relatively inexpensive and abundant substrate for the production of fructose syrups and bioethanol. The aim of this work was to isolate some inulinase producing moulds and to investigate their ability to produce extracellular inulinases and to hydrolyze the Jerusalem artichoke substrate. Five moulds were isolated from different sources and tested for inulinase production. Three of them had inulinase activity, ranging from 0.01 to 1.76 UI. Three of the isolated moulds were tested for Jerusalem artichoke hydrolysis.

Key words: inulinase, DNS, Jerusalem artichoke, hydrolysis

Introduction

Inulin is one of the numerous polysaccharides found in plants, consisting of linear chains of β (2,1)-linked fructose residues with a terminal glucose molecule. Inulin sources, like Jerusalem artichoke, chicory and dahlia, have recently attracted scientists' attention because of their availability, inexpensive production and as substrate for the production of fructose syrups and bioethanol. Current research in the development of alternative fuels area has led to the investigation of a wide variety of carbohydrate sources from which ethanol may be derived (Bajpai and Margaritis, 1982).

Microbial inulinases, the enzymes that hydrolyze inulin, have been proposed as the most promising approach to obtain fructose syrups from inulin rich feedstock. Although inulin-hydrolyzing activity has been reported from various microbial strains, yeasts (*Kluyveromyces spp.)* and *Aspergillus spp.* have proved the best inulinase activity (Singh and Gill, 2006). Other inulinase producing microorganisms are *Penicillium spp.*, *Alternaria alternata*, *Rhizopus spp.*, and also *Bacillus spp*., *Clostridium spp*. and *Xanthomonas spp*. (Singh and Gill, 2006). Inulinases are enzymes that degrade the β-(2,1) linkages of β-fructans, like inulin. They act using two mechanism: exo-inulinases sequentially split-off the terminal β-(2,1) fructofuranosidic bonds, while endo-inulinases

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hydrolyze the internal linkages in inulin and release inulooligosaccharides (Ertan *et. al*., 2003).

Jerusalem artichoke is considered one of the best sources for ethanol production, as it commonly produces high levels of fermentable sugars (Rodrigues *et al*, 2007). Tubers contain 24-31% dry matter, consisting mostly from carbohydrates. The most important carbohydrate in Jerusalem artichoke tubers is water-soluble inulin, which reaches 50-60% of dry matter (Danilcenko *et. al*., 2008, Bekers *et. al*., 2008).

In the present work, the inulinase biosynthesis potential of some fungi strains isolated from different sources was evaluated. Then, the moulds with extracellular inulinase activity were tested for their ability to hydrolyse inulin from Jerusalem artichoke tubers.

Materials and methods

Microorganisms and media

Aspergillus niger MIUG 1.15 strain, with proved high inulinase activity, from Collection of microorganism of Bioaliment Platform (acronym MIUG) was used as reference. Six other moulds were isolated from different sources, as Jerusalem artichoke tubers, soil, dahlia roots and plant, chicory plant, *Colocasia esculenta* corms, using specific methods of isolation. The isolated strains were maintained on slants with wort agar medium.

The isolated microorganisms were tested for the ability to grow on inulin as carbon source using the medium with the following composition (% w/v): inulin 2, $NaNO_3$ 0.2, KCl 0.05, $MgSO_4$ 0.05, $FeSO_4$ 0.001, K_3PO_4 0.1, the medium pH was 7.3. The microorganisms were cultivated on Petri dishes for 4 days at 25°C.

The YPD medium for inoculum preparation consisted of 2% glucose, 2% yeast extract and 1% peptone with pH 6.5.

The fermentative medium for inulinase production had the following composition (% w/v): inulin 2, $NaNO_3$ 0.2, KCl 0.05, $MgSO_4$ 0.05, $FeSO_4$ 0.001, K_2HPO_4 0.1, the medium pH was 6.8.

All the media were sterilized at 121°C for 15 minutes before use.

Inulinase production

One loop of moulds spores/mycelia was transferred to 50 mL of YPD media in 150 mL Erlenmeyer flasks and cultivated aerobically for 24 hours at 25˚C, for vegetative inoculum production. 5 mL of the vegetative inoculum cell culture was transferred then to 45 mL of the fermentative medium having the composition described above. The cultures were grown by shaking at 150 rpm, 28˚C for 3 days. The fermented broth was centrifugated at 10000 rpm, 4˚C for 10 minutes and the supernatant was used as the crude enzyme extract. The enzyme extract was kept at -70˚C prior to use.

Inulinase assay

For the determination of the inulinase activity, 2 mL of inulin solution (0.2% w/v) were mixed with 2 mL acetate buffer (pH=5.0, 0.1M) and 0.5 mL of crude enzyme extract. The mixture was incubated at 30˚C for 20 minutes. The reaction was inactivated immediately by keeping the reaction mixture in boiling water for 10 minutes. The same mixture, with inactivated crude enzyme extract was used as the control. The amount of the reducing sugars in the reaction mixture was assayed using the DNS method. One inulinase unit (U.I..) was defined as the amount of the reducing sugar, in mg, produced by 1 ml of enzymatic extract per hour, at 30˚C, under the assay conditions used in this study.

Jerusalem artichoke hydrolysis ability

For Jerusalem artichoke hydrolysis ability testing of the isolated moulds, 15 g of milled Jerusalem artichoke and 5 mL of water were mixed in Petri dishes and sterilized at 121˚C, 15 minutes. The Petri dishes were inoculated with 3 mL of sterile inoculum and thermostated at 25˚C for 5 days. Every 24 hours, 2 samples were analyzed for reducing sugars content with DNS method and water content using the thermobalance (model AND-MF50). The reducing sugars content was expressed as mg fructose/g dry matter.

Results and discussions

Moulds isolation

Six moulds were isolated from different sources: Jerusalem artichoke, chicory, dahlia, taro corms and soil, using specific isolation techniques. The *Aspergillus niger* MIUG 1.15 selected strain, from MIUG collection, with proved inulinase activity, was used as control.

The isolated strains and the control strain were then inoculated on synthetic medium with inulin as the sole carbon source for selection. Five of the seven mould strains were able to grow on synthetic medium using inulin as carbon sources. The moulds able to grow on synthetic medium with inulin were coded as it follows:

Table 1. *Codes, isolation source and genus for the moulds used in experiments*

Mould code	Isolation source	Genus
Aspergillus niger MIUG 1.15	MIUG Collection 1.15	*Aspergillus niger*
Rhizopus	Taro corms	*Rhizopus sp.*
Aspergillus niger	Jerusalem artichoke tubers	*Aspergillus niger*
PN	Soil from Jerusalem artichoke tubers	Could not be identified
Aspergillus oryzae	Dahlia flower	*Aspergillus oryzae*

Extracellular inulinase biosynthesis

After moulds selection, the five strains able to grow on synthetic medium with inulin were tested for inulinase activity using the method described above. Three of the five moulds had extracellular inulinase activity, expressed as milligrams of reducing sugar per hour, at 30˚C, under the assay conditions used in this study, as shown in Figure 1.

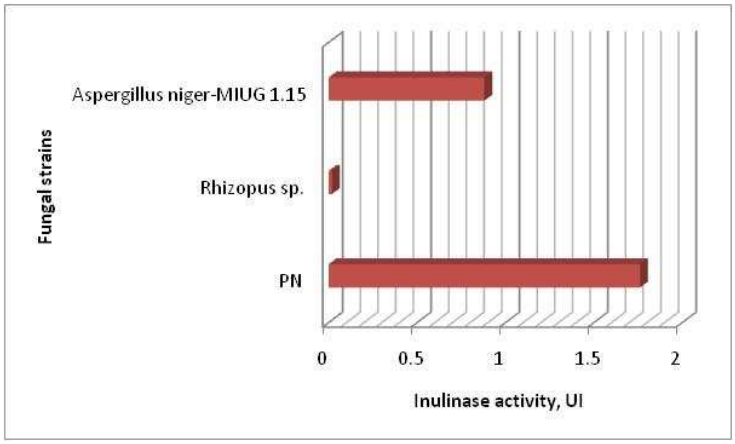

Figure 1. *Inulinase biosynthesis potential of new isolated mould strains comparing with the selected strain Aspergillus niger MIUG 1.15*

As it can be seen from figure 1, mould coded PN, isolated from soil from Jerusalem artichoke tubers, had the highest inulinase activity, 1.76 UI. The control strain of *Aspergillus niger* MIUG 1.15 had an extracellular inulinase activity of 0.88 UI. The mould isolated from taro corms had the lowest inulinase activity. The strains coded FPN and FD did not prove extracellular inulinase activity.

The strain *Aspergillus niger* MIUG 1.15 and new isolated strain coded PN were used for the

determination of their ability to hydrolyse inulin from Jerusalem artichoke. Also, *Aspergillus oryzae* strain was considered for this determination. Spores and hyphae fragments of moulds were inoculated on milled Jerusalem artichoke tubers and incubated for 5 days at 25 degrees. Every day samples were analyzed for fructose content and dry matter content in substrate in order to establish fungal inulin hydrolysis potential. As it can be seen in Figure 2, the strain coded PN, isolated from soil, produced the highest fructose amount, comparative with *Aspergillus* strains. Although the PN coded mould produced the highest amount of fructose in the fourth day, it produced almost twice amount of hydrolysis products as selected strain *Aspergillus niger* MIUG 1.15.

The strain PN coded mould will be further analysed for identification and other enzymatic activities, and it will be used in further experiments related to inulin hydrolysis.

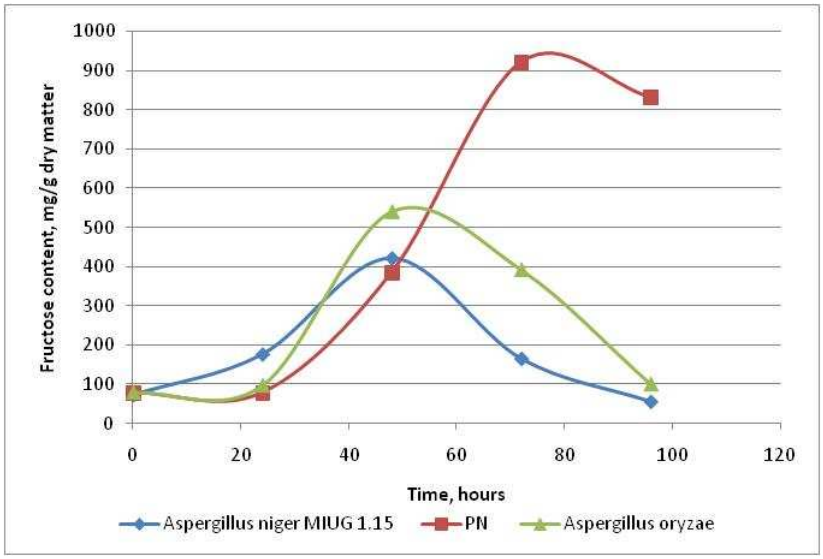

Figure 2. *Fructose producing dynamics during selected moulds cultivation for 5 days at 25C*

Conclusions

Six mould strains were isolated from different sources and tested for inulin hydrolysis ability. Only four mould strains proven their ability to grow on inulin as sole carbon sources, and only two strains, isolated from soil from Jerusalem artichoke tubers, proved extracellular inulinase activity, comparing with a selected strain from MIUG Collection (*Aspergillus niger* MIUG 1.15). The strain coded PN and isolated from soil had the highest inulinase activity and also the highest ability to hydrolyse inulin from Jerusalem artichoke tubers. In the fourth day of incubation it produced 922.14 mg of fructose/g dry matter. This new selected strain will be further analysed for proper characterisation and used for inulin hydrolysis in further experiments for bioethanol production.

Acknowledgements

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