Available online at www.derpharmachemica.com

ISSN 0975-413X
CODEN (USA): PCHHAX

Der Pharma Chemica, 2016, 8(12):157-161
(http://derpharmachemica.com/archive.html)

Production of inulinase by *Pichia caribbica* using artichoke extract and pure chicory Inulin

Mounira Kara Ali[1], Serge Hiligsmann[2] and Noreddine Kacem Chaouche[1]

[1]*Laboratory of Mycology, Biotechnology and Microbial Activity (LaMyBAM), Nature and Life Sciences Faculty, Brothers Mentouri University, Constantine 25000-Algeria*
[2]*Unit of Biotechnology and Bioprocess (3BIO-BioTech). Free University of Brusselles.*

ABSTRACT

Inulinases constitute an important class of enzymes for producing fructose and fructooligosaccharides, which are extensively used in pharmaceutical and food industry. In this study, two carbon sources were evaluated for their production of inulinase by yeast, Pichia caribbica (KC977491), i.e., pure chicory inulin and artichoke extract. The highest inulinase activity (70 IU/ml) and the good growth cell (DO 38.5 at 600nm) were observed in artichoke extract as a sole carbon and nitrogen source at 37°C within 48 h. Furthermore, the optimal conditions such as pH and temperature, to obtain the maximum stability and activity of the enzyme were identified. The enzyme activity was found to be the highest at pH 3,4 and at 65°C. Results suggested that the artichoke extract induced exoinulinase synthesis in P. caribbica (KC977491) and can be utilized as a potential substrate for inulinase production. Thus, P. caribbica (KC977491) may play an important role in biotechnological domain.

Keywords: Inulinase, *Pichia caribbica*, Artichoke, Inulin

INTRODUCTION

Enzymes are the key tools of biotechnology because they offer many possibilities for applications in various industries, i.e., food and agricultural industries, chemical, pharmaceutical and for renewable energy product as bio-ethanol [1, 2, 3]. Inulinases are a group of enzymes which hydrolyse the inulin, a linear β-(2,1) linked fructose polymer terminated by a glucose residue, through a sucrose-type linkage at the reducing ends [4,5]. This polymer is considered as an interesting carbohydrate reserve in roots and tubers of several plants such as Jerusalem artichoke (*Helianthus tuberosus*), chicory (*Cichorium intibus*), dahlia (*Dahlia pintana*) and dandelion (*Taraxacum officinal*) [6,7]. Inulinases are classified into endo and exo-inulinases, depending on their mode of action. Endo-inulinases (2,1-b-D-fructanfructanohydrolase; EC 3.2.1.7) are specific for inulin and hydrolyze it by breaking bonds between fructose units that are located away from the ends of the polymer network, to produce oligosaccharides [8] and exo-inulinases sequentially split-off the terminal β-(2,1) fructofuranosidic bonds to liberate fructose [9].

It is very important to signal that the inulinases have caught the attention of many researchers for their ability to produce many products, i.e., high fructose syrups, fructooligosaccharides, inulooligosaccharides, single cell oil, sorbitol, ethanol, etc. [10, 11, 12]. Several microbe species are known for their capacity to produce inulinase [13, 14], but the highest activity was obtained from limited number of them [15, 16].

P. caribbica, is one of these strains, firstly, was selected for its ability to produce ethanol from inulin (our results [17]; executing the two steps; the saccharification of inulin to fructose and the fermentation of the fructose to ethanol, in contrast with several studies which showed that the same process was ensured by two types of microorganisms [18, 19].

The present work is, to our knowledge, the first to characterize the strain *Pichia carribica* (KC977491) capable to produce a large quantity of inulinase from artichoke extract. The main objectives are to compare the production of inulinase by *P. caribbica* on media containing pure chicory inulin and artichoke extract, followed by determination of the optimal conditions of the highest enzyme activity.

MATERIALS AND METHODS

Microorganism

The yeast *P. caribbica* (KC977491) used in this study was previously isolated and identified (our result) [17]. Reactivated by plating on YEPD medium (composed g/l: yeast extract 10, Bacto-Peptone 10, glucose 20, agar 20. The pH medium was adjusted to 5), followed by incubation at 30°C for 48 hours.

Substrates and chemicals

The pure inulin (from chicory) was obtained from Cosucra Groupe Warcoing S.A (Belgium), and artichoke was obtained from locally farms (Setif, Algeria).

Preparation of artichoke extract

The method of Singh et al. [20] was utilized for obtaining the artichoke extract with minor modifications**.** The leaves and stems of artichokes (200 g) were washed, peeled and placed in 1000 mL of distilled water at 100°C for one hour. The slurry obtained was allowed to stand for sedimentation of particulate matter. Afterwards, it was filtered through muslin cloth and the filtrate was centrifuged at 8000 rpm for 10 min, the supernatant was used as the crude inulin extract.

Inulinase production

Two media cultures were used to produce inulinase by *P. caribbica*. The first medium containing pure inulin as essentially carbon source was composed per liter of: inulin 40 g, yeast extract 4g, peptone 4g. However, the second medium was composed only of artichoke extract (as sole carbon and nitrogen source). Erlenmeyer flasks (250 mL) containing 100 mL aliquots of both media were autoclaved (20 min, 121°C) and inoculated with two loops from 48 h old culture of *P. caribbica*. Flasks were incubated at 37°C on a rotary shaker (170 rpm) for 120h. All the experiments were carried out in duplicate.

Enzyme assay

After the fermentation period, the biomass was separated from medium using centrifugation technique at 5,000 rpm under 4°C for 5 min. The supernatant was taken as a crude enzyme. The reaction mixture containing 0,1ml of the crude enzyme and 0,4ml of inulin (5% w/v dissolved in 50mM sodium acetate buffer, pH 5) was incubated at 37°C for 15 min. The reaction was stopped immediately by keeping the reaction mixture at 100°C for 5 min. The amount of reducing sugar in the reaction mixture was assayed by the method of Miller [21]. One inulinase unit (IU) was defined as the amount of enzyme that produces one micromole of reducing sugar per minute under the assay conditions.

Characterization studies on inulinase activity

Optimization of pH for inulinase activity

The optimal pH of inulinase activity was determined by incubating the reaction mixture in a buffer at 37°C and values of pH varying from 3, 3 to 7 for 15 min. The inulinase activity was measured using the assay method cited above.

Optimization of temperature for inulinase activity

The effect of temperature on inulinase activity was determined by incubating the reaction mixture in optimal pH 3,4 at temperatures values ranging between 37 °C and 70°C. The inulinase activity was measured as previously described.

RESULTS AND DISCUSSION

Among various substrates that were employed as a carbon source for inulinase production, inulin-containing plant materials offer advantage in comparison to purified substrates in terms of lower cost and high productivity [12**,** 22]. Artichoke (*Cynara cardunculus* var.scolymus) is a variety of thistle specie which was cultivated as a food [23]. This variety is very abundant in several countries as Algeria; its production was estimated at 83.675 tons for 2013. Sheets, external bracts and stems which represent about 80% of the plant biomass could be a promising source of a cheap inulin compound [24, 25]. In the present study *P. caribbica* was able to utilize pure chicory inulin and artichoke contains inulin in its growth. However, maximum growth cell production (DO 38.5) and the highest inulinase activity (70 IU/ml) were obtained at 48 h of culture (Figure 1) when artichoke extract was used as sole

carbone and nitrogen source. In contrast, when a pure chicory inulin was used as a sole carbon source, the maximum of biomass value (DO=8) was reached at 120 h and the maximum inulinase activity was 54,27UI/mL after 96h (Figure 1). So, the use of artichocke extracts to produce inulinase was more efficient. The same results were obtained previously using other strains. In fact, Cruz et al. [26] used various carbon sources had obtained higher enzyme activity (3.68 UI /mL) with dahlia extract versus pure inulin (2.92 UI/mL). In addition, Singh and Bhermi, [27] found that a high inulinase activity (8.42 IU/ml) was established by *Kluyveromyces marxianus* yeast YSA, on *Asparagus officinalis* roots tubers versus pure chicory inulin (2.7 IU /mL). Furthermore, Jain et al. [28] observed a high inulinase activity reaching 1,49 UI/ mL by *Kluyveromyces marxianus* MTCC 39951 on Dahlia extract, which was higher compared to pure inulin (1,05 UI/ mL). The inulinase activity value which was found in our work (70 IU/ml) after only 48h of fermentation when using artichock extract as a sole carbon and nitrogen source made *P. caribbica* yeast strain an interesting microbial tool to produce inulinase. In the other hand, the importance of our work consists on the use of a relatively cheap and easily available substrate (artichocke) which can serve as a feedstock for large-scale fermentation, whereas, pure inulin is only available in limited quantities with very high cost.

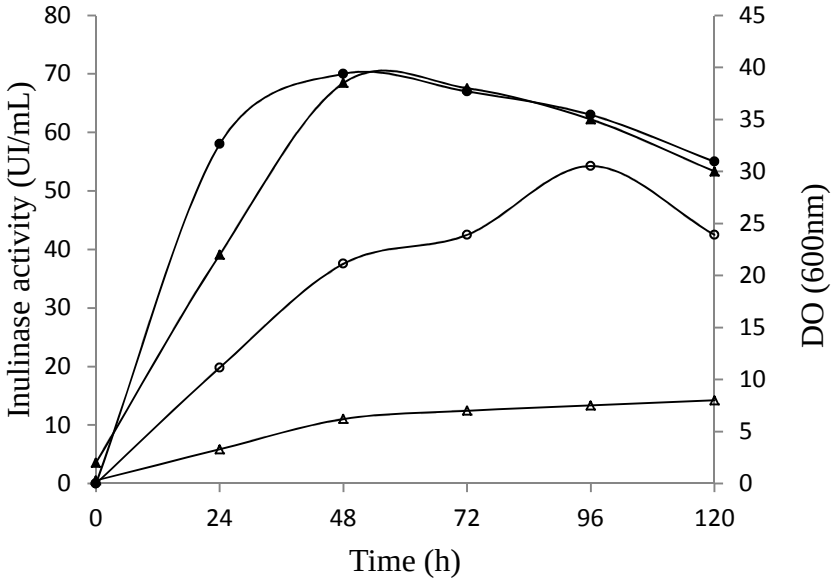

Figure 1: Production of inulinase by P. caribbica: (●) on Artichoke extract medium within (o) its DO, and (▲) On pure chicory inulin within (∆) its DO.

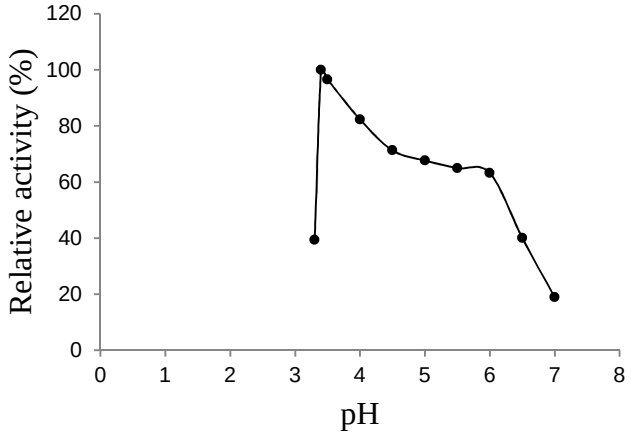

Figure 2: Effect of different pH on inulinase activity of *P. caribbica*

In this report, the optimal conditions such as pH and temperature to obtain the maximum activity of the enzyme were identified. The optimal pH and temperature for inulinase activity of *P. caribbica* were 3,4 and 65°C respectively (Figure 2 and Figure 3). Approximate results were obtained in several researches using other strains. In fact, the optimal temperature and pH of enzyme activity were respectively 60°C and 4,5 [29], 55°C and 4.4 [30], 60°C and 5 [31], 60 °C and 6 [32], 50°C and 4 [28]. Whereas, inulinases from *Aspergilus niger* mutant 817 and

Bacillus polymyxa where more active at 40°C and 35°C respectively near neutral pH [33,34]. The inulinase that was obtained in this study showed a considerable activity at moderately high temperature and wide range of pH may find strong interesting industrial applications.

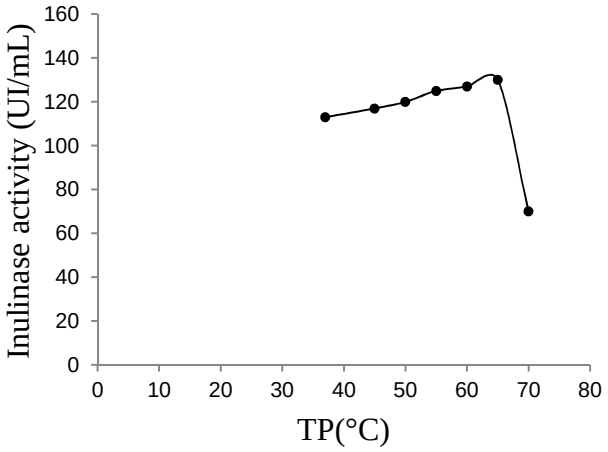

Figure 3: Effect of different temperature on inulinase activity of *P. caribbica*

CONCLUSION

In this study, *P. caribbica* was investigated for its ability to produce a large quantity of inulinase on the media containing, separately, chicory pure inulin and artichoke inulin. However, the amount of enzyme production using artichoke inulin was better comparatively to the same enzyme production on pure chicory inulin. This result let concluded that the artichoke could be, effectively, a cheaper carbon source. On other hand, the partial characterization of the enzyme considering pH and temperature of the optimal activity showed that the obtained enzyme was active at moderately high temperature and pH converging to acids values. This investigation confers inulinase, that was produced in this work, diverse industrial applications. In addition to that, the use of *P. caribbica* in the field of biotechnology, agriculture and in the food industry is unknown. Therefore, these results can be considered as an important advance in various fields.

REFERENCES

[1] P Tiwari, M K U Padhyay, N Silawat, H N Verma, *Der Pharma Chemica*, **2011**, 3, 501-508.
[2] C Martin, M Galbe, C F Wahlbom, B Hahn-Hagerdal, L F Jonsson, *Enzyme Microb Technol*, **2002**, 31, 274-282.
[3] M S Selim, S S Mohamed, M G Mahmoud, M M Asker, O H El Sayed, *Der Pharma Chemica*, **2016**, 8, 150-159.
[4] P P Rutherford, A C Deacon, *Biochemical J*, **1972**, 129, 511-512.
[5] E J Vandamme, D G Derycke, *Adv Appl Microbiol*, **1983**, 29,139-176.
[6]A K Gupta, N Kaur, *J Sci Ind Res*, **1997**, 56, 447- 452.
[7] V Trojanova, L Rada, V Kokoska, E lkova, *Fi- toterapia*, **2004**, 75, 760-763.
[8] F Ertan, T Aktac, A C Kaboglu, F Ekinci, E Bakar, *Pakistan J Biol Sci*, **2003**, 6 ,1386 1388.
[9] K Ohta, N Norio, T Nakamura, *J Biosci Bioeng*, **2002**, 94, 78-80.
[10]A Pandey, C R Soccol, P Selvakumar, VT Soccol, N Krieger, J D Fontana, *Appl Biochem Biotechnol*, **1999**, 81, 35-52.
[11]C H Zhao, T Zhang, M Li, Z M Chi, *Process Biochem*, **2010**, 45, 1121-1126.
[12]M A Mazutti, G Zabot, G Boni, A Skovronski, D de Oliveira, M D Luccio, M I Rodrigues, H Treichel, F Maugeri, *J Chem Technol Biotechnol*, **2010**, 85, 109-114.
[13] L Zhang, J Wang, Y Ohta, Y Wang, *Proc Biochem*, **2003**, 38, 1209-1212.
[14] D Jose, *Appl Biochem Biotechnol*, **1999**, 8, 35-52.
[15]W J Yuan, X Q Zhao, X M Ge , FW Bai, *J Appl Microbiol*, **2008**, 105, 2076-2083.
[16] R S Singh, R Dhaliwal, M Puri, *Process Biochem* , **2006a**, 41,1703-1707.
[17]M Kara Ali, N Kacem Chaouche, A Ait Kaki, I Bataiche, M Youcef-Ali, F Delvigne, P Thonart, *Scholarly Journal of Agricultural Science*, **2013**, 3, 462-467.
[18] K Ohta, S Hamada, T Nakamura, *Appl Envtl Microbiol*, **1993,** 59, 729-733.
[19] T Nakamura, Y Ogata, S Hamada, K Ohta, *J Ferment Bioeng*, **1996**, 81, 564-566.
[20] R S Singh, B S Sooch, M Puri, *Biores Technol*, **2006b**, 98, 2518-2525.

[21] G L Miller, *Anal Chem*, **1959**, 31, 426-8.
[22] J P Park, J W Yun, *Lett Appl Microbiol*, **2001**, 33, 183-187.
[23] A Rottenberg, D Zohary, *Genet Resour Crop Ev*, **1996**, 43, 53-58.
[24] V Lattanzio, P A Kroon, V Linsalata, A Cardinali, *J Funct Foods*, **2009**, 1, 131-144.
[25] G Pandino, S Lombardo, G Mauromicale, G Williamson, *Food Chem*, **2011**, 126,417-422.
[26] VA Cruz, J G Belote, M Z Belline, R Cruz, *Rev Microbiol*, **1998**, 29, 301-306.
[27] R S Singh, H K Bhermi, *Biores Technol*, **2008**, 99, 7418-7423.
[28] S C Jain, P C Jain, N Kango, *Braz J Microbiol*, **2012**, 43, 1517-8382.
[29] W Wenling, WW L Huiying, W Shiyuan, *Process Biochem*, **1999**, 34, 643-646.
[30] R T Kushi, R Monti, J Contiero, *J Ind Microbiol Biotechol*, **2000**, 25, 63-69.
[31] M L Cazetta, P M M Martins, R Monti J Contiero, *J Food Eng*, **2005**, 66, 301-305.
[32] F Gong, J Sheng, Z Chi, J Li, *J Ind Microbiol Biotechnol*, **2007**, 34,179-185.
[33] T Nakamura, Y Nagatomo, S Hamada, Y Nishino, K Ohta, *J Ferment Bioengineer*, **1994**,78, 134-139.
[34] H J Kwon, S J Jeon, D J You, K H Kim, Y K Jeong, Y H Kim, Y M Kim, B W Kim, *Biotechnol Lett*, **2003**, 25,155-159.