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REVIEW ARTICLE

INULINASES - A VERSATILE TOOL FOR BIOTECHNOLOGY

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Abstract

Inulinases are hydrolysis enzymes that act on the β-2,1 linkage of inulin, resulting fructose, glucose and inulo-oligosaccharides as reaction products. Microorganisms are the best sources for inulinases production, as they are easy to be cultivated and produce high yields of enzymes. Inulinases have a molecular weight over 50.0 kDa, an optimal pH between 4.5-7.0 and an optimal temperature between 30°C and 60°C, depending of their producers, moulds, bacteria or yeasts. Purified enzymes are activated in the presence of some ions (like Ca^{+2}, K^{+}, Na^{+}), while other metallic ions act like inhibitors (Mg^{+2} or Ag^{+}). Inulinases are widely used for ultra-high-fructose syrup and oligofructans production from inulin, for bioethanol production from inulin rich feedstock, citric acid, single cell protein and other chemicals obtaining. In this article, inulinases obtaining and their properties characterization, as well as their potential applications are reviewed.

Keywords: inulinases, applied biotechnology

Introduction

Inulinases (2,1-β-D-fructan fructanohydrolase, EC 3.2.1.7) catalyse the hydrolysis of inulin, producing inulo-oligosaccharides, fructose and glucose as main products.

Inulin consists of linear chains of β 2,1-D-fructofuranose molecules terminated with a glucose residue at the reducing end. It can be found as a reserve carbohydrate in plants such are Jerusalem artichoke, dahlia and chicory and in smaller amounts in garlic and onion (Chi *et al.*, 2011; Singh and Gill, 2006; Nagem *et al.*, 2004; Molina *et al.*, 2005). Inulins are different in their degree of polymerization, having also different functional properties (Molina *et al.*, 2005). The degree of polymerization depends upon plant source, climate and growing conditions, storage time after harvest etc. (Chi *et al.*, 2011).

Inulinases can be divided into exo-inulinases and endo-inulinases. The exo-inulinase removes the terminal fructose residues from the non-reducing end of inulin, whereas the endo-inulinase acts on the internal linkages of the inulin molecule but lacks invertase activity (Chi *et al.*, 2009; Chi *et al.*, 2011; Ertan *et al.*, 2003).

In the last decades a large number of fungal, yeast and bacterial strains were used for inulinase production. Among the various microbial strains, *Kluyveromyces marxianus* and *Aspergillus niger* are reported as the most common and preferred sources for inulinase production (Singh and Gill, 2006; Pandey *et al.*, 1999; Chi *et al.*, 2009). Inulinases have different catalytic properties (molecular weight, optimum pH, optimum temperature, stability), depending especially upon their provenience.

Generally, the inulinase activity (I) is accompanied by invertase activity (S) and the enzymatic complex is characterized by I/S ratio. When I/S ratio is higher than 10^{-2}, the enzyme complex has a preponderate inulinase activity, while for invertase activity the I/S ratio is lower than 10^{-4} (Sharma *et al.*, 2006).

Inulinases can be used in a wide range of industrial applications: for ultra-high fructose syrup obtaining from inulin, bioethanol production,

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inulo-oligosaccharide production, single-cell oil and single-cell protein production, some chemicals production, like citric acid, butanediol, alcohols and lactic acid (Chi *et al.,* 2011; Chi *et al.,* 2009; Pandey *et al.,* 1999; Liu *et al.,* 2010).

As in the past years a great progress was made in the inulinases obtaining process, the new knowledge concerning inulinase production, characterization of new inulinases and their applications are summarized in this review article.

Natural substrates for inulinase production

Inulinases have been produced using different substrates as carbon sources, from pure inulin to agro-industrial residues (Table 1). Naturally occurring inulin rich materials are the preferred substrates for inulinase obtaining but lately, agro-industrial residues have gained researchers attention.

In nature, inulin can be found in plant species from mono- and dicotyledonous families, such are *Liliaceae, Amaryllidaceae, Gramineae* and *Compositae* (Chi *et al.,* 2011). Excepting *Gramineae* plants, inulins are usually stored in bulbs, tubers and roots. Jerusalem artichoke and chicory, which belong to Compositae family, are the most used carbon sources for inulinase production as they contain over 50% (dry matter) inulin (Chi *et al.,* 2011, Pandey *et al.,* 1999, Danilcenko *et. al*., 2008, Bekers *et. al*., 2008). Jerusalem artichoke (*Helianthus tuberosus*) attracted scientists' attention because of their availability, they present cold and drought tolerance, saline tolerance, wind and sand resistance, they have strong fecundity and they are resistant to pests and diseases (Chi *et al.,* 2011).

Table 1. *Substrates used for inulinase production*

Substrates	Microorganisms	References
Pure substrates		
Inulin	*Pichia guilliermondii*	Yu *et al.,* 2009
	K. lactis, K. marxianus spp.	Guerrero *et al.,* 2006
	Streptomyces spp.	Sharma *et al.,* 2006
Sucrose	*Kluyveromyces marxianus*	Kalil *et al.,* 2001, Santisteban *et al.,* 2009
	A. niger, A. oryzae, A. ficuum	Ge and Zhang, 2005
		Santisteban *et al.,* 2009
Fructose	*Kluyveromyces marxianus*	
	K. lactis, K. marxianus spp.	Guerrero *et al.,* 2006
Glucose	*Kluyveromyces marxianus*	Santisteban *et al.,* 2009
Inulin containing plant materials	*Streptomyces* spp.	Sharma *et al.,* 2006
(rye, barley, banana, garlic, onion,	*A. niger*	Kango, 2008
wheat, chicory, dahlia, dandelion,	*Kluyveromyces marxianus* YS-1	Singh *et al.,* 2006
Asparagus roots, Jerusalem artichoke)	*Rhizoctonia solanis*	Singh and Bhermi, 2008
		Ertan *et al.,* 2003a
Agro-industrial residues (cassava	*Aspergillus ochraceus*	Guimaraes *et al.,* 2007
flour, corncob, oat meal, rice straw,	*Streptomyces* spp.	Dilipkumar *et al.,* 2011
sugar cane bagasse, wheat bran,	*Kluyveromyces marxianus* NRRL	Mazutti *et al.,* 2007, Bender *et al.,* 2006,
pressmud)	Y-7571	Mazutti *et al.,* 2010, Sguarezi *et al.,*
	Aspergillus ficuum JNSP5-06	2009, Treichel *et al.,* 2009
		Chen *et al.,* 2011

Among the pure substrates used for inulinase production, inulin and sucrose were the preferred carbon sources. Additional pure substrates that supplement the production medium could have a positive impact on inulinase production. Kumar *et al.* (2005) supplemented the medium for inulinase production with sucrose, glucose, fructose, galactose, maltose and dextrose and observed that galactose supplemented medium gave maximum inulinase yield, followed by maltose.

Agro-industrial residues and vegetal extracts appear to be a good source for inulinase production. Cassava flour, corncob, oat meal, rice straw, sugar cane bagasse, wheat bran, glucose and sucrose were used as carbon sources to establish the influence of carbon source on the production of inulinase by *Aspergillus ochraceus* (Guimaraes *et al.,* 2007). The highest level of extracellular inulinase activity was obtained when sugar cane was used as carbon source (108 activity units). *A. ochraceus* inulinase activity was stimulated by the supplementation with glucose of the reaction medium (Guimaraes *et al.,* 2007). Sharma *et al.* (2006) used also various substrates for inulinase production (rye, barley, banana, garlic, pure inulin, wheat, chicory, onion and dahlia). The highest inulinase activity was observed when garlic was used as carbon source.

It was previously reported that inulinase production is usually inducible. The inductive effect of some octadecanoylsucrose esters was demonstrated by Ge and Zhang (2005). The sucrose ester is attached on the surface of the fungal cell and acts as a persistent signal to initiate response in the cell, enhancing inulinase production near 7-fold higher, at a concentration of 6g/L (Ge and Zhang, 2005).

Inulinase - expressing microorganisms

A large number of bacteria, fungi and yeasts have been used for inulinase production (Table 2). Among them, the strains belong to *Aspergillus* and *Kluyveromyces* genus were the most common and preferred choice for inulinase production.

Table 2. *Inulinase-expressing microorganisms*

Microorganisms	Maximal activities	References
Moulds		
Aspergillus niger	1.75 g/L	Gern *et al.,* 2001
	100 U[a]/mL	Ge and Zhag, 2005
	52.5 IU[a]/mL	Kango, 2008
	176 U/mL	Kumar *et al.,* 2005
Aspergillus fumigatus	Not available	Gill *et al.,* 2006
Aspergillus awamori	Not available	Nagem *et al.,* 2004
Aspergillus ochraceus	108 Total U	Guimaraes *et al.,* 2007
Aspergillus ficuum	193.6 U/gds[b]	Chen *et al.,* 2011
Aspergillus parasiticus	2.9 U/mL	Ertan *et al.,* 2003b
Geotrichum candidum	45.65 IU/mL	Mughal *et al.,* 2009
Rhizoctonia solani	1.792 U/mL	Ertan *et al.,* 2003a
Chrysosporium pannorum	115 U/mL	Xiao *et al.,* 1988
Bacteria		
Paenibacillus spp.	2.48 g/L	Gern *et al.,* 2001
Streptomyces spp.	524 IU/L	Sharma *et al.,* 2006
	89 U/gds	Dilipkumar *et al.,* 2011
Bacillus spp.	42.36 U/mL	Zherebtsov *et al.,* 2002
Pseudomonas spp.	Not available	Kim *et al.,* 1997
Arthrobacter spp.	Not available	Kang *et al.,* 1998
Yeasts		
Pichia guilliermondii	39.56 U/mL	Gao *et al.,* 2007
	61.5 U/mL	Chi *et al.,* 2009
	130.38 U/mL	Yu *et al.,* 2009
	60.1 U/mL	Gong *et al.,* 2007
Cryptococcus aureus	52.37 U/mL	Gao *et al.,* 2007
	85 U/mL	Chi *et al.,* 2009
	436.2 U/gds	Chi *et al.,* 2009

Yarrowia lipolitica	62.85 U/mL	Gao *et al.*, 2007
	22.5 U/mg	Liu *et al.*, 2010
Debaryomyces hansenii	52.53 U/mL	Gao *et al.*, 2007
Candida kefyr	40 U/mL	Pessoa and Vitolo, 1998
Kluyveromyces marxianus	194.1 U/mL	Kalil *et al.*, 2010
	127 U/mL	Kalil *et al.*, 2001
	176 IU/mL	Santisteban *et al.*, 2005
	208 IU/mL	Santisteban *et al.*, 2009
	262.9 U/mg	Golunski *et al.*, 2011
	1294 U/mL	Treichel *et al.*, 2009
	18743 U/mL	Kushi *et al.*, 2000
	50.2 IU/mL	Singh and Bhermi, 2008
	47.1 IU/mL	Singh *et al.*, 2006
	250 U/gds	Mazutti *et al.*, 2007
	47.2 U/mL	Mazutti *et al.*, 2010
	1317 U/mL	Treichel *et al.*, 2009
	1139 U/mL	Sguarezi *et al.*, 2009

U[a], IU[a] – inulinase activity expressed as international activity

gds[b] – gram of dry substrate

Bacteria

Bacterial strains are used for inulinase production, mainly because of their thermostability. Data on inulinases biosynthesis using bacterial strains are scarce and mainly concern endo-inulinases. *Streptomyces* spp. was found as a good producer of inulinases; Sharma *et al.* (2006) obtained 524 IU/L inulinase activity with *Streptomyces* spp. using garlic as substrate and Dilipkumar *et al*. (2011) had a maximum inulinase activity of 89 U/gds by *Streptomyces* spp. and using pressmud as carbon source.

Bacteria of genus *Bacillus* are also active producers of extracellular inulinase – 42.36 U/mL inulinase activities were obtained on sucrose as substrate (Zherebtsov *et al.*, 2002). *Pseudomonas* spp. (Kim *et al.*, 1997) and *Arthrobacter* spp. (Kang *et al.*, 1998) were also tested for the ability to produce inulinases; data concerning their inulinase activities are not available.

Moulds

Inulinase activity has been obtained using different mould strains, *Aspergillus* spp. being the favourite specie for inulinase production. Sixteen fungal strains and three bacterial strains, reported as inulinase producers, were investigated by Gern *et al.* (2001) for endo-inulinase production. The best endo-inulinase producer was the strain coded CDB 003, identified as *Paenibacillus* spp. From the fungal strains, *Aspergillus niger* DSM 2466 was selected as the best endo-inulinase producer by Gern *et al.* (2001). Ge and Zhang (2005) used also an *Aspergillus niger* strain and obtained a maximum inulinase activity of 100 U/mL in the presence of S-770 sucrose ester as nutritive substrate added into the fermentative medium at a concentration of 6 g/L. Using an infusion prepared of tap roots of dandelion, Kango (2008) obtained 52.5 IU/mL inulinase activity after 96h of cultivation with a selected *A. niger* strain. Kumar *et al.* (2005) obtained a maximum inulinase activity of 176 U/mL at a 5% (w/v) inulin concentration in the medium, using a soil isolated fungal strain identified as *A. niger*.

Other strains of *Aspergillus* species were reported in literature for inulinase production and characterization, such are *A. fumigatus* (Gill *et al.*, 2006), *A. awamori* (Nagem *et al.*, 2004), *A. ochraceus* (Guimaraes *et al.*, 2007), *A. ficuum* (Chen *et al.*, 2011) and *A. parasiticus* (Ertan *et al.*, 2003b).

In the research of Mughal *et al.* (2009), eight strains of *Geotrichum candidum* were isolated from soil samples and tested for inulinase activity. The activities of these strains ranged from 0.12 to 1.38 IU/mL. After improvement through induced

mutagenesis using methyl methane sulphonate, ethyl methane sulphonate and UV exposure, the culture gave a 50-fold improved inulinase activity (45.65 IU/mL) compared to wild isolate (Mughal *et al.,* 2009).

Rhizoctonia solani, isolated from soil, had the maximum inulinase activity of 1.792 U/mL in the second day of cultivation using Jerusalem artichoke powder as carbon source (Ertan *et al.,* 2003a). *Chrysosporium pannorum*, isolated from soil, was found also as a very active inulinase producer; its highest activity was 115 U/mL (Xiao *et al.,* 1988).

Yeasts

Yeasts have been used in enzyme production for ages, as they are easier to grow and handle in comparison with bacteria. Among the yeasts that can produce inulinases, *Kluyveromyces* spp., *Pichia* spp. and *Candida* spp. have high potential for producing high yields of inulinase activity.

Gao *et al.* (2007) screened over 400 marine yeasts and found that some of marine yeasts strains have the ability to secrete large quantities of inulinase. Among them, *Pichia guilliermondii*, *Cryptococcus aureus*, *Yarrowia lipolitica* and *Debaryomyces hansenii* can secret over 40 U/mL of extracellular inulinase (Gao *et al.,* 2007, Chi *et al.,* 2009, Gong *et al.,* 2008, Gong *et al.,* 2007, Sheng *et al.,* 2008). Using mutagenesis, ultraviolet exposure combined with LiCl treatment of the cells of *Pichia guilliermondii*, Yu *et al.* (2009) obtained a maximum inulinase activity of 130.38 U/mL in the medium optimization studies. Surface-engineered *Yarrowia lipolitica* yeast can produce 22.5 U/mg inulinase activities within 96 h (Liu *et al.,* 2010).

Kluyveromyces spp. is, by far, the most wide used yeast for inulinase production. Kalil *et al.* (2010) obtained a maximum inulinase activity of 194.1 U/mL when investigated the main parameters affecting the inulinase purification obtained from *Kluyveromyces marxianus* var. *bulgaricus*, using an ion exchange fixed bed column. In his previous study of optimization of inulinase production, the maximum inulinase activity obtained was 127 U/mL in the optimized medium (Kalil *et al.,* 2001). Santisteban *et al.*(2005) investigated some key factors in inulinase production (agitation, aeration and shear stress) by *Kluyveromyces marxianus* and found the best fermentation conditions of 1 vvm aeration, 450 rpm agitation, with pitched blade up impeller in a stirred reactor, with a production of 176 IU/mL. In a latter work, Santisteban *et al.* (2009) investigated the effects of carbon and nitrogen sources and oxygenation on the inulinase production and 208 IU/mL inulinase activity was obtained when 20 g/L sucrose was used as carbon source. In their trials for ethanol precipitation and ultrafiltration of inulinases from *Kluyveromyces marxianus*, Golunski *et al.* (2011) obtained a maximum inulinase specific activity of 262.9 U/mg. Treichel *et al.* (2009) obtained a maximum inulinase activity of 1294 U/mL in their studies on the production, partial purification and characterization of inulinase from *K. marxianus*, using agro-industrial residues as substrate, while Kushi *et al.* (2000) obtained 18743 U/mL after dialysis and lyophilisation of extracellular inulinase from *K. marxianus*.

In their studies concerning the inulinase production using roots of *Asparagus* spp. and *Kluyveromyces marxianus* yeast, Singh and Bhermi (2008) and Singh *et al.* (2006) obtained 50.2 IU/mL and 47.1 IU/mL respectively, showing that inulinase yield is six times higher when produced in bioreactor comparative with the shake flask trials.

Kluyveromyces marxianus was used in many optimization studies for inulinase production, when different yields of inulinase activity were obtained. Mazutti *et al.* (2007) obtained a maximum inulinase activity of 250 U/gds by solid-state fermentation of sugar cane bagasse and 47.2 U/mL in submerged liquid fermentation (Mazutti *et al.,* 2010), Bender *et al.* (2006) obtained 444.8 U/g inulinase activity from agro-industrial residues using solid-state fermentation, Treichel *et al.* (2009) obtained 1317 U/mL using agro-industrial residues as substrate, while Sguarezi *et al.* (2009) obtained 1139 U/mL by using similar substrates.

Characteristics of inulinases

Molecular weight of inulinases

It was reported that microbial inulinases have over 50kDa molecular weight (Chi *et al.,* 2009). For

example, Sheng *et al.* (2008) purified and characterized the inulinase from *Cryptococcus aureus* and its molecular weight was estimated to 60.0 kDa. For *Pichia guilliermondii* derived inulinases, molecular weight of 50 kDa was reported by Gong *et al.* (2008) and 54 kDa by Chi *et al.* (2009). Inulinases with molecular weight of 250 kDa were produced by *Kluyveromyces fragilis* (Pandey *et al.,* 1999).

Molecular weight of inulinases produced by bacterial strains was similar to those produced by yeasts. Kang *et al.* (1998) characterized the endo-inulinases produced by *Arthrobacter* spp. and estimated its molecular weight at 75 kDa.

For inulinases produced by moulds strains molecular weights between 50 kDa and 300 kDa were reported: *Aspergillus ochraceus* – 79 kDa (Guimaraes *et al.,* 2007), *Penicillium* spp. – 68 kDa (Chi *et al.,* 2009), *F.oxysporum* – 300 kDa (Pandey *et al.,* 1999).

Table 3. *Properties of some microbial inulinases*

Sources	Molecular weight, kDa	Optimum pH	Optimum temperature, ˚C	pH stability range	Temperature stability, ˚C	References
K. marxianus	-	3.5	60	-	-	Mazutti *et al.,* 2007
K. marxianus	-	4.4	50	-	-	Mazutti *et al.,* 2010
K. marxianus	-	4.75	55	-	40	Kushi, 2000
K. fragilis	250	-	55	-	-	Pandey *et al.,* 1999
Yarrowia lipolitica	-	4.5	50	3-7	50	Liu *et al.,* 2010
Pichia guilliermondii	50	6.0	60	6-7	60	Gong *et al.,* 2008
Cryptococcus aureus	60	5.0	50	4.0-6.5	65	Sheng *et al.,* 2008
Arthrobacter spp.	75	7.5	50	5-10.5	30…40	Kang *et al.,* 1998
Bacillus spp.	-	7.0	-	6-8	25…40	Zherebtsov, 2002
Streptomyces spp.	-	6.0	60	-	60…70	Sharma *et al.,* 2006
A. niger	-	4.4	-	-	-	Sharma *et al.,* 2006
A. niger	70	5.0	40	-	-	Pandey *et al.,* 1999
Penicillium janczewskii	-	4.8-5.0	35…45	-	-	Sharma *et al.,* 2006
A. ochraceus	79	4.5	60	-	60	Guimaraes *et al.,* 2007
F. oxysporum	300	5.8-6.2	30…37	-	-	Pandey *et al.,* 1999

Optimum pH and temperature activity of inulinases

The optimum pHs activity of inulinases from moulds and yeasts vary, in general, in the range of 4.5-6.0 (Table 3). Inulinases from *A. niger* were found to have an optimum pH at 4.4, inulinases produced by *A. versicolor* have the optimal pH at 5.5 and *Penicillium janczewskii* produces inulinases with optimal pH at 4.8-5.0 (Sharma *et al.,* 2006). Inulinases produced by *A. ochraceus* are optimal at pH 4.5 (Guimaraes *et al.,* 2007). There are inulinases with lower optimal pH: *K. marxianus* produces inulinases which have the highest activity at 3.5 (Mazutti *et al.,* 2007) and *Pichia guilliermondii* at 3.4 (Gao *et al.,* 2007).

In contrast, the inulinases produced by some bacterial strains hydrolyze inulin optimally at pH 7.0-7.5, like *Arthrobacter* spp. (Kang *et al.,* 1998) and *Bacillus polymyxa* (Zherebtsov *et al.,* 2002, Chi *et al.,* 2009).

Usually, the purified inulinases are optimal activity at 50…60˚C. *Kluyveromyces* spp. strains produce inulinases with maximum activity at 50…55˚C (Mazutti *et al.,* 2010, Pandey *et al.,* 1999, Kushi, 2000), while inulinases derived from *Pichia guilliermondii* (Gong *et al.,* 2008, Chi *et al.,* 2009), *A. ochraceus* (Guimaraes *et al.,* 2007) and *Streptomyces* spp. (Sharma *et al.,* 2006) have optimum activity at 60˚C and present very good stability to this temperature. *F. oxysporum* (Pandey *et al.,* 1999), *Penicillium janczewskii* (Sharma *et al.,* 2006) and *A. niger* (Pandey *et al.,* 1999) produce inulinases optimal at 30…40˚C, indicating that the optimal temperatures of inulinases from different species of microorganisms are significantly different.

Effect of metal ions and protein inhibitors on inulinase activity

Some metal ions added to the reaction medium can promote the inulinase activity, while others have inhibitory effect on the enzyme. *Kluyveromyces marxianus* inulinase is activated by Co^{+2}, Mn^{+2}, Mg^{+2} and low concentrations of SDS (0.001%) and is inactivated by Cu^{+2}, Fe^{+3}, Zn^{+2}, Tween 20, Tween 80 and Brij-35 (Singh and Bhermi, 2008) and also by Ca^{+2}, Ba^{+2}, Zn^{+2}, Na^{+} (Kushi, 2000). Inulinase derived from *Cryptococcus aureus* is activated by low concentrations of Ca^{+2}, K^{+}, Na^{+}, Zn^{+2} and Cu^{+2} and inhibited by Mg^{+2}, Hg^{+2} and Ag^{+} (Sheng *et al.,* 2008). Similar results were observed for *Pichia guilliermondii* inulinase also (Gong *et al.,* 2008). Both yeast strains were isolated from marine environment. Hg^{+2} and Ag^{+} have inhibitory effect on inulinase activity produced by moulds and bacteria also, suggesting the importance of thiol-containing amino acid residues in the enzymes function. *Arthrobacter* spp. (Kang *et al.,* 1998), *Streptomyces* spp. (Sharma *et al.,* 2006) and *Aspergillus ochraceus* (Guimaraes *et al.,* 2007) inulinases are inhibited by the presence of these metals into the reaction medium. The effect of metal ions on inulinase activity is relevant especially when high salt containing feedstock is used as substrate. Also, pepstatin, EDTA, 1,10-phenenthroline have inhibitory effects on some inulinase activity (Sheng *et al.,* 2008; Gong *et al.,* 2008, Kang *et al.,* 1998), demonstrating that the characterized enzymes were metalloenzymes (Gong *et al.,* 2008).

Applications of inulinases

Inulinases can be used in a large spectrum of applications, ranging from food industry to bioethanol production and pharmacology.

High fructose syrup

Industry uses a large amount of natural polysaccharides and in the last years researchers' attention has been directed toward producing these polysaccharides using microbial fermentation. Fructose is the sweetest of all naturally occurring carbohydrates and is often produced by enzymatic process from starch. Conversion of starch to fructose involves the use of three different enzymes and the maximum yield is 45% (Pandey *et al.,* 1999). A simple and high productivity method to obtain high fructose syrup is the enzymatic hydrolysis of inulin, a single step process that uses inulinases and yields 95% pure fructose (Chi *et al.,* 2009, Ricca *et al.,* 2009). Fructose has beneficial effects in diabetics, obesity, stimulates calcium absorption, stimulates grow of bifidobacteria, increases the iron absorption in children and prevents colon cancer (Chi *et al.,* 2009). Furthermore, fructose metabolism bypasses the known metabolic pathway of glucose and does not require insulin (Rocha *et al.,* 2006, Gong *et al.,* 2007). It is widely used in food industry, pharmaceutics and beverages (Chi *et al.,* 2009, Rocha *et al.,* 2006).

Inulo-oligosaccharide production

The endo-inulinases are responsible for inulo-oligosaccharides (IOS) production. Many microorganisms have been reported as endo-inulinases producers: *Yarrowia lipolitica*, *Cryptococcus aureus* (Gao *et al.,* 2007), *Arthrobacter* spp. (Kang *et al.,* 1998), *Pseudomonas* spp. (Chi *et al.,* 2009), *Paenibacillus* spp. (Gern *et al.,* 2001). IOS have wide applications in food industry: confectionery, milk desserts, yoghurt and cheese production, bakery, chocolate, ice-cream and sauces (Chi *et al.,* 2011). It was found that the major IOS obtained after inulin hydrolysis with endo-inulinases have a

degree of polymerization of 3 and 4. Inulo-oligosaccharides are prebiotics; their positive effect on human health has been widely acknowledged (Rocha *et al.,* 2006, Chi *et al.,* 2009).

Bioethanol production

Biochemical and thermo-chemical conversion technologies can convert biomass into carbon-containing biofuels such as biodiesel and other liquids. The primary feedstock for ethanol production worldwide remains sugar or starch from agricultural crops, and its primary use is as a blend with gasoline (at 5-90% blend). Nowadays, studies concerning bioethanol production from various unconventional feedstock, such are lignocellulose materials or kitchen refuse, are increasing. Alcohol production from inulin rich feedstock has been studied since the end of 19th century.

Although more widely recognized now, the dramatic environmental, economic, strategic and infrastructure advantages offered by the production of ethanol were not appreciated in the past.

Inulin rich raw materials gained researchers attention for bioethanol production. The microbial exo-inulinases can remove the terminal fructose residues from the non-reducing end of inulin molecule, producing fructose and glucose, which can easily be fermented to ethanol by *Saccharomyces* spp. yeast strains (Chi *et al.,* 2011). Some yeast strains can perform simultaneous hydrolysis and fermentation of the inulin: *Kluyveromyces marxianus* and some *Saccharomyces* spp. yeasts can produce both active inulinase and ethanol (Chi *et al.,* 2011, Rosa *et al.,* 1986, Kim *et al.,* 1998, Lim *et al.,* 2011).

Other applications of inulinases

Inulinases have also found their application for inulin substrates hydrolysis for single-cell oil and single-cell protein production (Chi *et al.,* 2011). The marine yeast *Cryptococcus aureus* can be used for single cell protein production by cultivation on inulin hydrolysates from Jerusalem artichoke tubers. The same applications are important to produce citric acid, 2,3 butanediol, lactic acid and sugar alcohols, like mannitol (Chi *et al.,* 2011; Saha, 2006; Liu *et al.,* 2010).

Conclusions

Inulin and inulin containing plants represent a renewable, inexpensive and abundant raw material for industry. After the inulin hydrolysis to fructose and inulo-oligosaccharides by exo- and endo-inulinases, these raw materials can be used for many practical applications as bioethanol production, in food industry, single-cell oil and single-cell protein obtaining, citric acid and other chemical production. It is clear that many advances in microbial inulinase production, purification and characterization have been made in the last years. Genetic engineering has been used for over-producing of inulinases, but the microbial inulinase production can be further greatly improved.

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