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PRODUCTION OF FRUCTOOLIGOSACCHARIDES BY TWO NEW RECOMBINANT β-FRUCTOFURANOSIDASE ENZYMES FROM MEZCAL FERMENTING YEASTS

PRODUCCIÓN DE FRUCTOOLIGOSACÁRIDOS CON DOS NUEVAS ENZIMAS β-FRUCTOFURANOSIDASA RECOMBINANTES DE LEVADURAS AISLADAS DE LA FERMENTACIÓN DEL MEZCAL

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Abstract

The gene *Cap inv32* from *Candida apicola* and *Tde inv32* from *Torulaspora delbrueckii* were identified as β-fructofuranosidase belonging to glycoside hydrolases family 32, with an opening reading frame of 1593 base pairs and 1743 base pairs, and a molecular weight of 59 kDa and 65 kDa, for *Cap inv32* and *Tde inv32* correspondingly. These enzymes contained the motif characteristics of the GH32 and showed an identity of 40% (*Cap inv32*) and 52.4% (*Tde inv32*) with an invertase of *S. cerevisiae*. The fructosyltransferase activity was tasted obtaining for *Cap inv32* the quantity of FOS 12.4 gL^{-1} and 9.03 gL^{-1} with 420 gL^{-1} and 700 gL^{-1}, the production of FOS with *Tde inv32* was 14.61 gL^{-1} (420 gL^{-1}) and 13.3 gL^{-1} (700 gL^{-1}). *Cap inv32* and *Tde inv32* are β-fructofuranosidase with fructosyltransferase activity, with potential to industrial applications, focused in FOS production.

Keywords: *Candida apicola*, *Torulaspora delbrueckii*, β-fructofuranosidase, fructooligosaccharides.

Resumen

Los genes *Cap inv32* de *Candida apicola* y *Tde inv32* de *Torulaspora delbrueckii* fueron identificados como β-fructofuranosidasas pertenecientes a la familia 32 de las glicosil hidrolasas, con un marco de lectura abierto de 1593 pares de bases y 1743 pares de bases, y un peso molecular de 59 kDa y 65 kDa, para *Cap inv32* y *Tde inv32* según corresponde. Estas enzimas presentan los motivos característicos de las GH32 y muestran una identidad del 40% (*Cap inv32*) y 52.4% (*Tde inv32*) con una invertasa de *S. cerevisiae*. La actividad fructosiltransferasa fue probada obteniendo para *Cap inv32* la cantidad de FOS de 12.4 gL^{-1} y 9.03 gL^{-1} con 420 gL^{-1} y 700 gL^{-1}, la producción de FOS con *Tde inv32* fue de 14.61 gL^{-1} (420 gL^{-1}) y 13.3 gL^{-1} (700 gL^{-1}). *Cap inv32* y *Tde inv32* son β-fructofuranosidasas con actividad fructosiltransferasa, con el potencial de usos industriales, enfocados en la producción FOS.

Palabras clave: *Candida apicola*, *Torulaspora delbrueckii*, β-fructofuranosidasa, fructooligosacáridos.

1 Introduction

β-fructofuranosidase belongs to glycoside hydrolase family 32 (GH32), these enzymes present two domains, a N-terminal β-propeller domain where the active site is located, which contain three conserved regions (catalytic triad) in the active site involved in a double displacement mechanism for the hydrolysis of β fructosyl linkages, the first group corresponds to an aspartic acid that carried out the nucleophilic attack, the second one is a glutamic acid that function as acid/base, the third conserved region is composed of an aspartic acid responsible of the stabilization of the reaction (Jiang *et al.*, 2016; Ma *et al.*, 2016; Trollope *et al.*, 2015; van Wyk *et al.*, 2013). The second domain identified in β-fructofuranosidase is a C-terminal β-sandwich domain involved in protein stability.

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At high sucrose concentrations, some of these β-fructofuranosidases have a preference to the transfructosylation activity, by cleaving the β-(2→1) linkage releasing glucose and transferring the fructose to an acceptor different than water, obtaining fructooligosaccharide of short chain (FOS) (Arrizon *et al.*, 2012; Santos and Maugeri, 2007; van Wyk *et al.*, 2013).

These FOS, are non-digestible carbohydrates (humans) formed by fructosyl linkages with a molecule of glucose at the extreme or inside the fructose chain, the fructosyl linkage of these polymers depends of the source of the enzyme, they selectively activate the proliferation of non-pathogenic microbiota in the colon (probiotics), due to this property they can be used as prebiotics (Álvaro-Benito *et al.*, 2010; Sabater-Molina *et al.*, 2009; van Wyk *et al.*, 2013). As FOS have beneficial effects for the human health, they are useful for the formulation of functional foods (Sabater-Molina *et al.*, 2009). FOS can be produced industrially by three methods: extraction from fructan accumulating plants, by enzymatic synthesis from sucrose or by enzymatic hydrolysis of inulin (Singh *et al.*, 2016). In the case of industrial FOS synthesis from sucrose (transfructosylation), fungal commercial enzymes from *Aspergillus aculeatus* and *A. oryzae* and high substrate concentrations have been used 600 gL^{-1}, these reactions have been carried out at a pH varying from 4.5-6.5 and temperature from 45-60 °C (Arrizón *et al.*, 2014; Vega-Paulino and Zúñiga-Hansen, 2012).

In the nature there are different sources of fructosyltransferase and β-fructofuranosidase for FOS synthesis, including plants, fungi, yeast and bacteria, some of them have been cloned and expressed for biochemical characterization as well as for the improvement of their catalytic capacities (Álvaro-Benito *et al.*, 2010; Arrizon *et al.*, 2012; Santos and Maugeri, 2007; Singh and Singh, 2010; Trollope *et al.*, 2015; Trujillo *et al.*, 2001; van Wyk *et al.*, 2013). In the particular case of microbial enzymes from yeasts, different β-fructofuranosidase have been studied for the FOS synthesis such as *Xantophyllomyces dendrorhous* (Linde *et al.*, 2012; Gimeno-Pérez *et al.*, 2014), *Saccharomyces cerevisiae* (Lafraya *et al.*, 2011), *Schwanniomyces occidentalis* (Álvaro-Benito *et al.*, 2010), *Aureobasidium pullulans* (Trollope *et al.*, 2015), *Kluyveromyces marxianus* (Santos and Maugeri, 2007), *Rhodotorula* sp. (Hernalsteens and Maugeri, 2008a), *Candida* sp. (Hernalsteens and Maugeri, 2010) and *Cryptococcus* sp. (Hernalsteens and Maugeri, 2008b).

Recently, new sources of β-fructofuranosidase with fructosyltransferase activity have been found from non-*Saccharomyces* yeasts isolated from the fermentation process of mezcal, such as the case of *Candida apicola* and *Torulaspora delbrueckii* microbial sources (Arrizon *et al.*, 2012; Rivera-Noriega *et al.*, 2017). *C. apicola* is a high osmotolerant yeast capable to synthesized biosurfactants (sophorolipids) of commercial interest as well as proteases (Bednarski *et al.*, 2004; Girhard *et al.*, 2013; Reid *et al.*, 2012).This yeast has been found in wine and cachaca must (Oliveira *et al.*, 2005; Tofalo *et al.*, 2009).

T. delbrueckii is a non-*Saccharomyces* yeast, it has high freeze tolerance and osmotolerance that make it suitable for fermentative processes in the wine, baker and dairy industry (García *et al.*, 2017; Hernández-López *et al.*, 2007; Lu *et al.*, 2017). There are some studies regarding enzyme production with *T. delbrueckii*, nevertheless only few have been characterized and not for the FOS synthesis (García *et al.*, 2017; Maturano *et al.*, 2012).

In this work, for the first time, the cloning and expression of two novel β-fructofuranosidase from *C. apicola* and *T. delbrueckii* for fructooligosaccharide production, which could have potential for prebiotic synthesis with applications in the production of functional syrups for food industry.

2 Materials and methods

2.1 In silico analysis of two β-fructofuranosidase genes

An alignment analysis of nucleotide sequences was carried out using the Basic Local Alignment Search Tool (BLAST) service provided by the National Center for Biotechnology Information (NCBI) website (www.ncbi.nlm.nih.gov/BLAST). Multiple sequence alignments on transcriptomes of *C. apicola* and *T. delbrueckii* were carried out as well as comparisons of protein sequences using the programs of ClustalW alignment software provided by the European Biotechnology Institute (EBI) (www.ebi.ac.uk/clustalw). The nucleotide sequences were translated into amino acid sequences and alignments were performed using Transeq and Show align from the EMBOSS software suite (http://www.ebi.ac.uk). The secretion signal peptide of the putative protein was detected by the use

of the software (http://cbs.dtu.dk/services/SignalP) (version 4.0) on the CBS website. For the 3D structure modeling and molecular weights of the putative proteins, the software of the Expasy website was utilized (https://www.expasy.org/). For the phylogenetic study, the software was used CLC Main Workbench version 7.6.4 (Qiagen, Venlo, The Netherlands).

2.2 Organisms, transformation and culture conditions

Pichia pastoris X33 wt competent cells were prepared, stored and transformed following the supplier's instructions (Invitrogen Catalog No. V20020). *P. pastoris* was grown at 30 °C on a rotatory shaker at 250 rpm on YPGS medium (10 gL^{-1} yeast extract, 20 gL^{-1} bacto peptone, 10 gL^{-1} glycerol, 20 gL^{-1} sucrose).

2.3 Cloning, expression and protein purification

The genes from *C. apicola* and *T. delbrueckii* were synthetized by Gene Script, these genes were cloned into pGAPZB into E. coli DH5α, using zeocin as selection method for positive cloned colonies. The enzyme expression was carried out into competent cells of *P. pastoris* X33 wt. The positive clones with the highest β-fructofuranosidase activity were cultivated on YPGS at 30 °C and 250 rpm during 36 h, they were harvested at an optical density (DO) of approximately 1.4, then the enzymatic extract (extracellular enzymes) was separated by centrifugation and storage at 4 °C for purification. The purification of the enzymes was carried out as follows; the enzymatic extract (500 mL) was filtered and dialyzed on equilibrium buffer pH 7.5 (20 mM sodium phosphate, 10 mM imidazole, 150 mM sodium chloride) [buffer A]. This enzymatic solution was passed through a chromatography column (Ni-Sepharose, GE Healthcare) balanced with Buffer A. The protein was eluted in a second buffer at pH 7.5 (50 mM sodium phosphate, 500 mM imidazole, 150 mM sodium chloride) [buffer B] with an imidazole lineal gradient (10-400 mM) with a flux of 0.5 $mLmin^{-1}$. The pool was separated in different fractions, those with β-fructofuranosidase activity were pooled and dialyzed on 100 mM acetate buffer pH 5.5 and it was storage at 8 °C for biochemical characterization. The purity of the enzyme was verified by SDS-PAGE.

2.4 SDS-PAGE and Zymogram analysis

The presence of the proteins was verified by 12% SDS-PAGE, using the MiniProtean III system (Bio-Rad), the molecular weight of the proteins was estimated employing standard broad range (Bio-Rad), the staining technique was Coomassie brilliant blue R-250 (Bio-Rad). Protein concentration was measured using Bradford method with bovine serum albumin as standard (Bradford *et al.*, 1976). β-fructofuranosidase zymogram analysis was performed on 12% polyacrylamide gels. After the electrophoresis, the gel was treated twice with 100 mM sodium acetate (pH 5.5) and was incubated by 30 minutes on 2% sucrose solution. Then the gels were washed twice with distilled water and incubated on room temperature by 15 min in 1 N sodium hydroxide, 0.1% TTC (2,3,5-Triphenyltetrazolium chloride) without light. The standard employed was Precision Plus Protein Kaleidoscope (Bio-Rad).

2.5 Enzyme assay

The β-fructofuranosidase activity was determined with sucrose as substrate, the enzyme assay was performed as follows; 50 μL pure enzyme with 50 μl of substrate (1% w/v 100 mM acetates buffer), at pH 6 by 10 min and 55 °C. The reaction was stopped by the addition of 100 μL DNS (3,5-dinitrosalicylic acid) and 5 min boiling, followed by 2 min on ice.

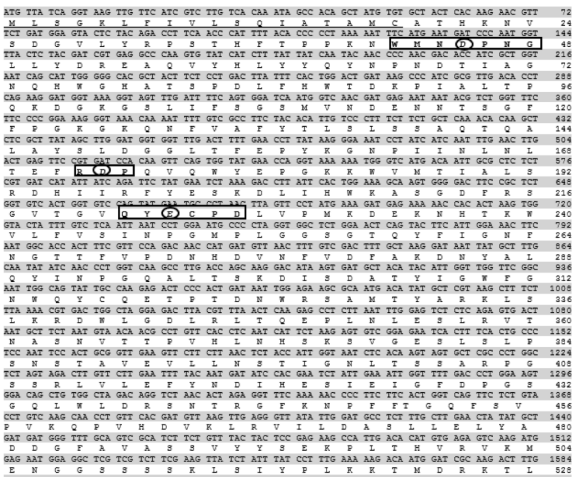

Fig. 1. Nucleotide sequence of *Cap inv32* gene and its respective predicted amino acid sequence. Signal peptide underlined, β-fructofuranosidases motif inside the square and the catalytic triade are inside circle.

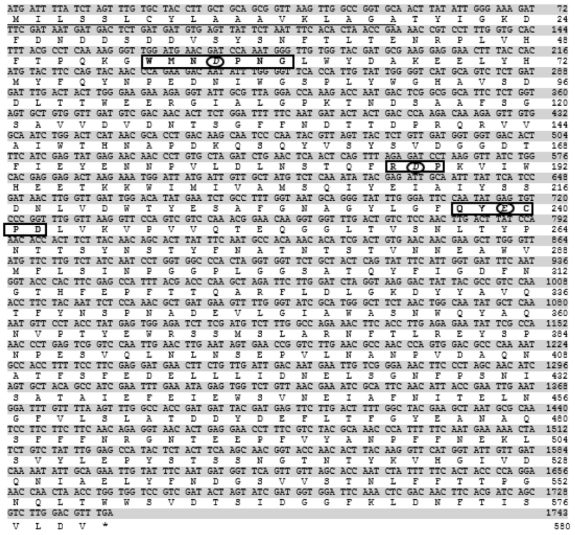

Fig. 2. Nucleotide sequence of *Tde inv32* gene and its respective predicted amino acid sequence. Signal peptide underlined, β-fructofuranosidases motif inside the square and the catalytic triade are inside circle.

The enzymatic activity was calculated with the absorbance on microplate at 540 nm. A hydrolytic activity unit is defined as reduced sugars micromoles released per minute. The fructosyltransferase activity was determined by HPLC. Sucrose, glucose and fructose concentrations were used to calculate the difference between them to determine the micromoles of fructose transferred for transfrucosylation. One unit of activity (U) was defined as the quantity of the enzyme needed to transfer a micromole of fructose per minute.

2.6 *Fructooligosaccharide production*

For the FOS production, the enzymes (Cap Inv32 or Tde Inv32) were added to a sucrose solution with a concentration of 420 gL^{-1} and 700 gL^{-1} (100 mM sodium acetate), at pH 5 and 45 °C for Cap Inv32 and pH 6 and 40 °C for Tde Inv32, in a total reaction volume of 2 mL. It was a kinetic of 24 h, employing 6 IU of enzyme per gram of sucrose.

2.7 *High Performance Liquid Chromatography*

The fructose, glucose and sucrose concentration was determined by HPLC with BioRad HPLC column for carbohydrate analyses (Aminex HPX-87C column, 300X7.8 mm; BioRad), at 65 °C and water elution with a flux of 0.2 mL min^{-1}.

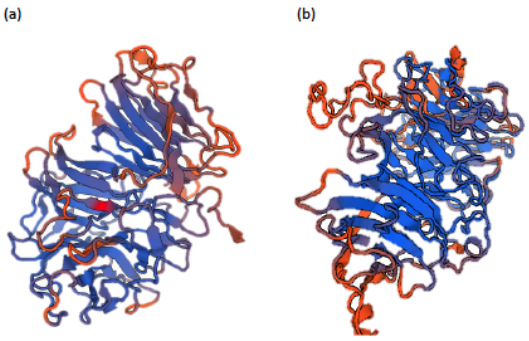

Fig. 3. Predicted 3D structure of (a)*Cap Inv32* and (b) *Tde Inv32*. The structures were predicted with Swiss-Model Server (ExPASy) and modelling using as reference a β-fructofuranosidase of *Schwanniomyces occidentalis* (E5D0X5) for *C. apicola* and an invertase 2 of *Saccharomyces cerevisiase* (P00724) for *T. delbrueckii*.

The samples were diluted on distilled water to approximately 10 g L^{-1} and were filtered before the analysis.

3 Results and discussion

3.1 *In silico analysis of β-fructofuranosidase from Candida apicola and Torulaspora delbrueckii*

The codifying genes for β-fructofuranosidase showed an open reading frame (ORF) of 1593 bp for *Cap inv32* (*Candida apicola*) (Fig. 1) and 1743 bp for *Tde inv32* (*Torulaspora delbrueckii*) (Fig.2). The genes were named employing the nomenclature suggested by Henrissat *et al.*, 1998. The molecular weight for the proteins was predicted, they were 59.31 and 65.25 for Cap Inv32 and Tde Inv32 respectively. Which was similar to other β-fructofuranosidase reported (Jiang *et al.*, 2016; van Wyk *et al.*, 2013).

It was predicted the peptide signal for the β-fructofuranosidase, which were founded at the first 18 amino acids (Fig. 1-2) of the polypeptides of *Cap inv32* (530 amino acids) and *Tde inv32* (580 amino acids), these segments of the structures indicate that the enzymes were extracellular. Similar characteristics are reported for a β-fructofuranosidase from *Ceratocystis moniliformis* (van Wyk *et al.*, 2013).

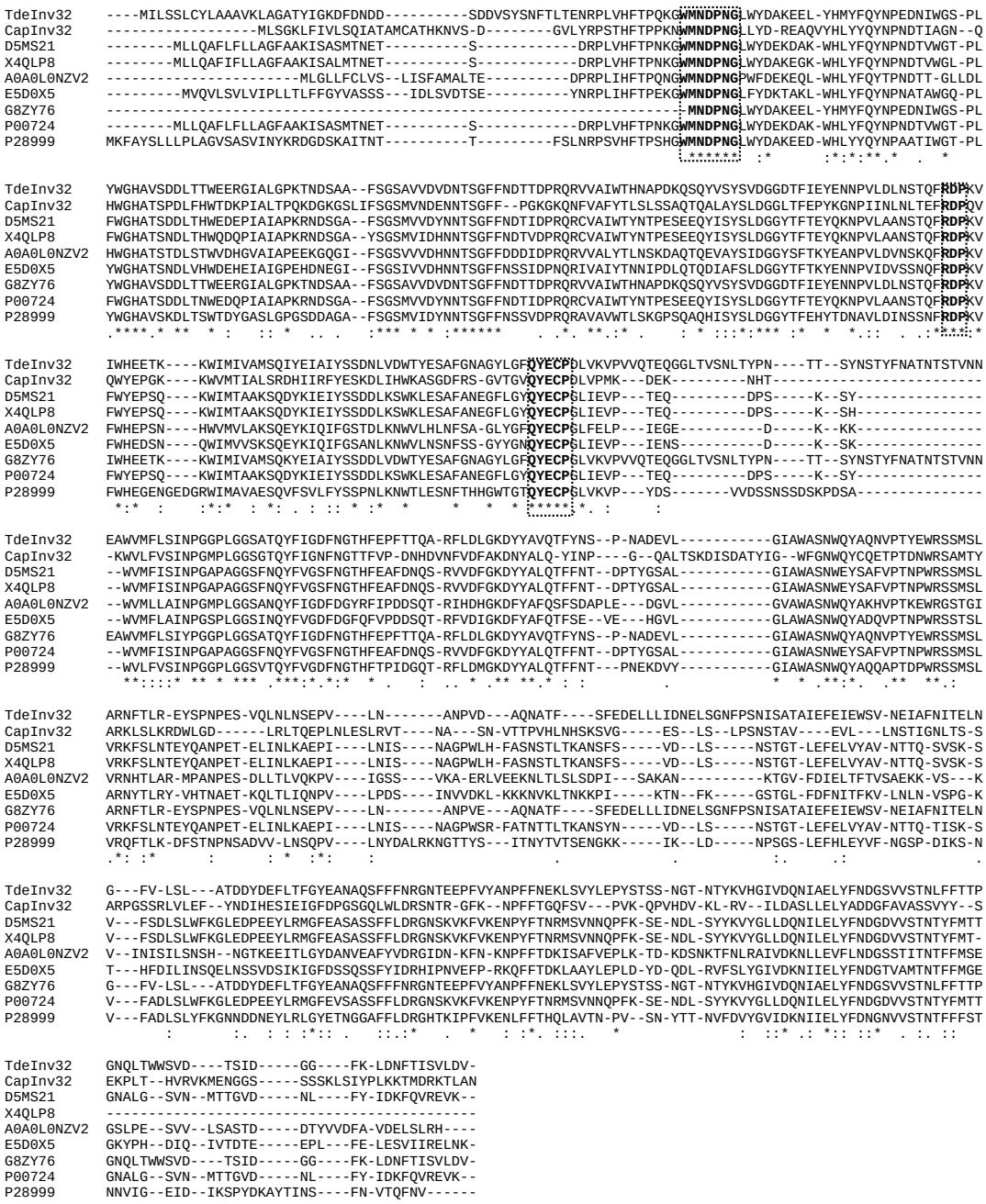

Fig. 4. Multiple alignment of the amino acid sequences of fructanases from different microorganisms performed using CLUSTALW. *C. apicola* (*Cap Inv32*, this work), *T. delbrueckii* (*Tde Inv32*, this work), *Saccharomyces cerevisiae* (D5MS21, X4QCP8, P00724), *Candida auris* (A0A0L0NV2), *Schwanniomyces occidentalis* (E5D0X5), *Torulaspora delbrueckii* (G82Y76) and *Kluyveromyces marxianus* (P28499). Conserved amino acids are indicated with asterisk (*) and identical with points (.). β-fructofuranosidases motif inside the squares.

Table 1. Purification steps for β-fructofuranosidases of *C. apicola* and *T. delbrueckii*.

Candida apicola					
Purification Step	Total Activity (IU)	Total Protein (mg)	Specific Activity ($IUmg^{-1}$)	Purification (fold)	Yield (%)
Crude Extract	1510.19 ± 0.85	98.96 ± 0.25	15.26	1	100
Ni-sepharose	770.48 ± 0.15	25.86 ± 0.18	29.79	1.9	51.02
Torulaspora delbrueckii					
Purification Step	Total Activity (IU)	Total Protein (mg)	Specific Activity ($IUmg^{-1}$)	Purification (fold)	Yield (%)
Crude Extract	1211.57 ± 0.55	95.26 ± 0.95	12.72	1	100
Ni-sepharose	845.76 ± 0.45	24.26 ± 0.88	34.86	2.74	69.81

For both enzymes it was identified the conserved motif of the amino acid sequences WMNDPNG, RDP and QYECPD, corresponding to the 32 glycosyl hydrolase family (GH32) (Fig 1-2) with the typical structure of β-propeller and β-sandwich type (Fig. 3), and this conserved motif contained the catalytic triad formed by the two Asp and Glu residues reported for the β-fructofuranosidase (Jiang *et al.*, 2016; Ma *et al.*, 2016; van Wyk *et al.*, 2013). A basic local alignment analysis of the *C. apicola* amino acid sequence showed a low identity with respect to β-fructofuranosidase from different microorganisms (less than 40% of identity), for the invertase from *S. cerevisiae* (D5MS21) the identity was 39.4% (Oda *et al.*, 2010), 37.5% of identity for a β-fructosidase from *S. cerevisiae* (X4QLP8) (Naumova *et al.*, 2014), a 36.02% of identity for an inulinase from *Candida auris* (A0A0L0NZV2) (Chatterjee *et al.*, 2015), and a 36.02% of identity with respect to a β-fructofuranosidase from *Sc. occidentalis* (E5D0X5) (Álvaro-Benito *et al.*, 2010)(Fig. 4).

While in the case of the amino acid sequence of *T. delbrueckii*, it showed a higher homology with respect to other amino acid sequences, 57.89% of identity for an invertase 2 from *S. cerevisiae* (P00724) (Lafraya *et al.*, 2011), a 52.4% of identity with another invertase from *S. cerevisiae* (D5MS21) (Oda *et al.*, 2010) and 49.0% of identity with an inulinase from *K. marxianus* (P28499) (Laloux *et al.*, 1991). Nevertheless, the highest similarity was the uncharacterized protein from *T. delbrueckii* (G8ZY76) with a 99.2% of identity (Gordon *et al.*, 2011) (Fig. 4).

3.2 *Expression and purification of the Cap Inv32 and Tde Inv32*

The β-fructofuranosidase was produced in liquid media, samples were taken at different times to measure the enzymatic activity and the maximum was observed at an optical density (OD) of 1.4 (36 h) for both recombinant enzymes with 57.14 UI mL^{-1} and 57.8 UI mL^{-1} for Cap Inv32 and Tde Inv32 respectively (Fig. 5a and 5b).

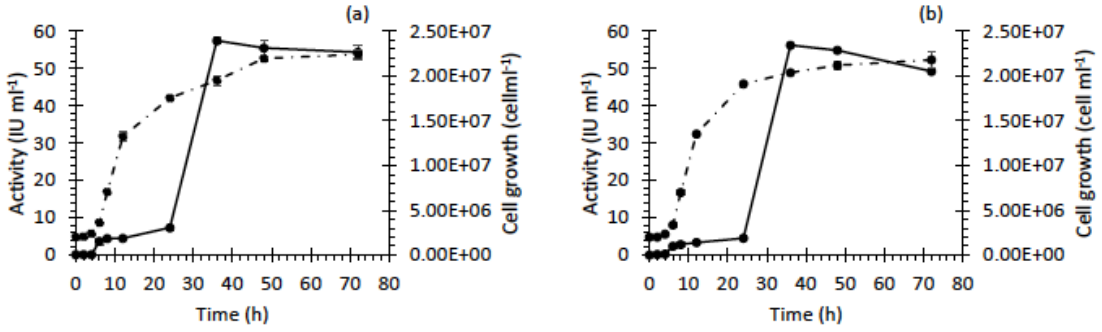

Fig. 5. Production of the recombinant enzymes (a) *Cap Inv32*; (b) *Tde Inv32*. -●- Extracellular β-fructofuranosidase activity; -.-●-.- Cellular growth.

Table 2. Nucleotide sequence of *Tde inv32* gene and its respective predicted amino acid sequence. Signal peptide underlined, β-fructofuranosidases motif inside the square and the catalytic triade are inside circle.

Enzyme	Number accession	Organism	Enzymatic activity	Sucrose concentration (gL^{-1})	FOS (gL^{-1}) (gL^{-1})	References
β-Fructofuranosidase	WR	*Candida apicola* expressed	6 U/g	420	12.4	
		in *P. pastoris*	6 U/g	700	9.03	This work
β-Fructofuranosidase	WR	*Torulaspora delbrueckii* expressed	6 U/g	420	14.61	
		in P. pastoris	6 U/g	700	13.3	
β-Fructofuranosidase	ADN34605	*Schwanniomyces occidentalis*	0.3 U/ml	342	70.3	(Álvaro-Benito *et al.*, 2010)
Invertase	NP_012104.1	*Saccharomyces cerevisiae*	0.6 U/ml	599	108	(Lafraya *et al.*, 2011)
β-Fructofuranosidase	ACL79833.1	*Xanthophyllomyces dendrorhous*	0.5-1 U/ml	600	180	(Gimeno-Pérez et al., 2014)
Fructosyltransferase	NR	*Aspergillus terreis* expressed in *Kluyveromyces lactis*	NR	600	217.3	(Spohner and Czermak, 2016)
β-Fructofuranosidase	NR	*Xanthophyllomyces dendrorhous*	0.5 U/ml	420-600	65.9	(Linde *et al.*, 2012)
β-Fructofuranosidase	ADN34605	*Schwanniomyces occidentalis*	0.3 U/ml	600	101	(Abreu *et al.*, 2007)
Fructofuranosidase	NR	*Candida sp.*	55.2 U/ml	500	220	(Hernalsteens and Maugeri, 2010)

NR, Not Reported; WR, Without Register.

These activities were higher than other reports, for example, a recombinant enzyme of *Aureobasidium* sp P6 produced 31 UI mL^{-1} in 108 h (Jiang *et al.*, 2016); two enzymes of *X. dendrorhous*, a recombinant protein produced 15 UI mL^{-1} and a native protein produced 4 UI mL^{-1}, in 24 and 80 h (Gimeno-Pérez *et al.*, 2014; Linde *et al.*, 2012) and an enzyme of *S. occidentalis* produced 12 UI mL^{-1} in 16 h (Abreu *et al.*, 2007). The results of the purification of the proteins *Cap inv32* and *Tde inv32* are shown in Table 1. The purification yield was 51.02% and 69.81%, the purification fold was 1.9 and 2.74 and the specific activity was 29.79 IU mg^{-1} and 34.86 IU mg^{-1} for the *Cap inv32* and *Tde inv32* respectively. Similar results were reported for purified enzymes of other yeasts, 49.5 UI mg^{-1} for a β-fructofuranosidase from *K. marxianus* and 88.72 UI mg^{-1} for *K. cicerisporus* (Arrizon *et al.*, 2011; Ma *et al.*, 2016), *K. marxianus* has also a high secretion capacity for other enzymes (Lara-Hidalgo *et al.*, 2017). A SDS-PAGE was used to determine the purity of the enzymes, it was detected a single band in 59 kDa corresponding to the *Cap inv32* and other in 65 kDa for *Tde inv32* supporting the previous predicted molecular weight (Fig. 6), these molecular weights were similar to others reported as was indicated in the section 3.1. A β-fructofuranosidase zymogram was carried out for both enzymes and a characteristic pink band on sucrose was detected for both proteins (Figure 6), demonstrating the hydrolytic capacity of enzymes.

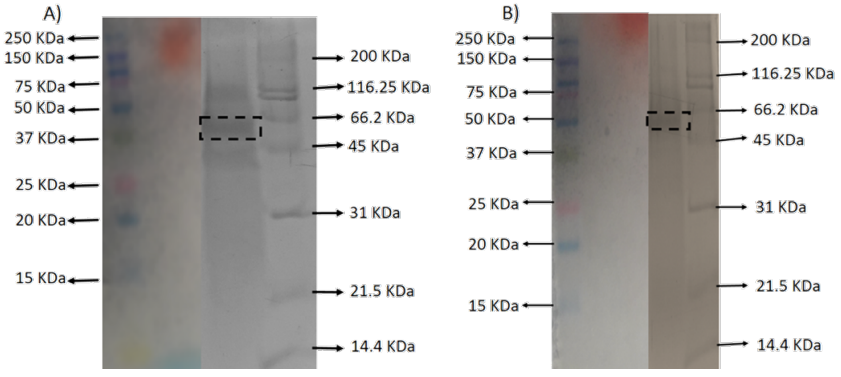

Fig. 6. Protein analysis (a) Zymogram and SDS-PAGE for *C. apicola* recombinant enzyme, Cap Inv32; (b) Zymogram and SDS-PAGE for *T. delbrueckii* recombinant enzyme, Tde Inv32.

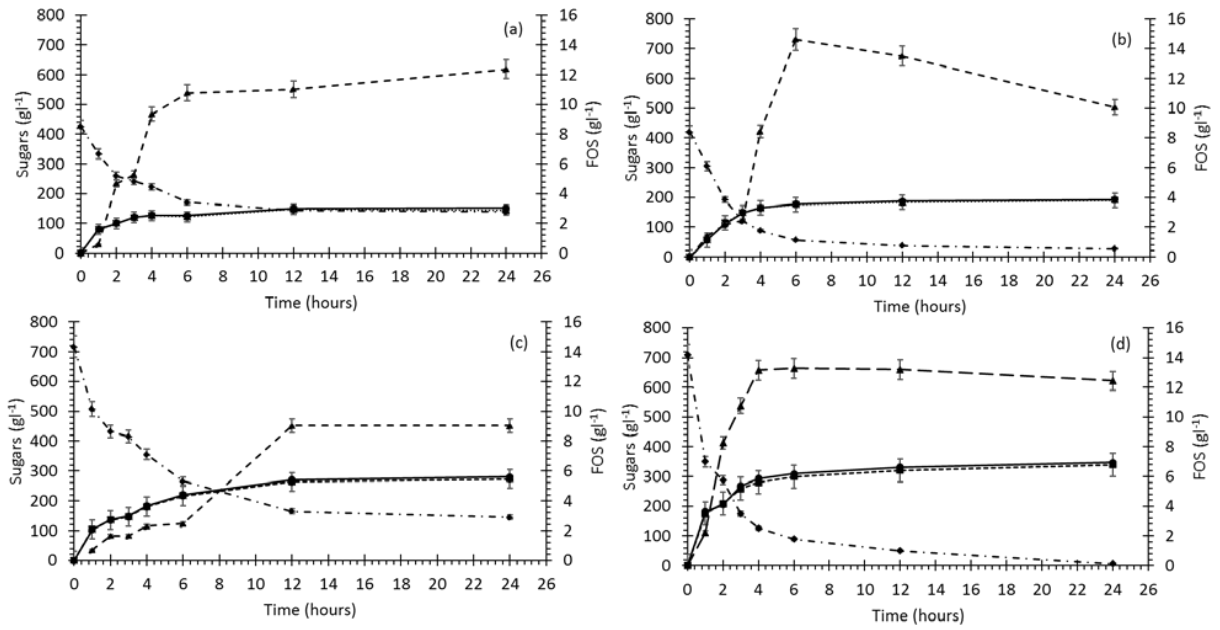

Fig. 7. Kinetic of FOS production catalyzed by β-fructofuranosidases (a) Cap Inv32, 420 gL^{-1}; (b) Tde Inv32, 420 gL^{-1}; (c) Cap Inv32, 700 gL^{-1};and (d) Tde Inv32, 700 gL^{-1}; Sucrose (♦), Fructose (■), Glucose (●), FOS (▲).

3.3 Fructooligosaccharides production

The enzymes *Cap inv32* and Tde Inv 32 were tested for fructooligosaccharides production by enzymatic transfructosylation employing sucrose as substrate, two concentrations were applied, 420 gL^{-1} and 700 gL^{-1}, during 24 hours. There were differences in the time of the maximum productivity in transfructosylation reaction. For *Cap inv32* at 420 gL^{-1}, the maximal FOS production was achieved at 6 h (12.4 gL^{-1}), then the FOS concentration remained constant until 24 h of reaction (Figure 7a).

This production was minor than other enzymes with similar concentrations of sucrose (Table 2), 70.3 gL^{-1} for a β-fructofuranosidase from *Sc. occidentalis* (Álvaro-Benito *et al.*, 2010), and 65.9 gL^{-1} for a β-fructofuranosidase from *Xantophyllomyces dendrorhous* at 420 - 600 gL^{-1} (Linde *et al.*, 2012). In the case of *Tde inv32* with 420 gL^{-1} of sucrose, the maximal FOS production was reached at 6 hours (14.6 gL^{-1}) (Figure 7b); in this case the FOS concentration decreased. For the enzyme *Cap inv32* with 700 gL^{-1}, the production was 9.03 gL^{-1} at 12 h and the FOS concentration remained constant until 24 h (Figure 7c). The production was lower than other reports, 50 gL^{-1} for an inulinase from *K. marxianus* with (Santos and Maugeri, 2007) and 217.3 gL^{-1} for a fructosyltransferase from *A. terreis* (Spohner and Czermak, 2016) (Table 2).

In the case of *Cap inv32* in both substrate concentration, when the maximum of transfructosylation was reached, the production and consumption kept constant, this behavior indicated a possible inhibition by substrate, this is confirmed by the unconverted sucrose 34% (420 gL^{-1}) and 23% (700 gL^{-1}). A similar relationship was reported by Antošová and Polakovič (2001) for fructosyltransferases in presence of 30% of glucose. While for *Tde inv32* at 700 gL^{-1} of sucrose the production was 13.3 gL^{-1} at 4 h and the FOS concentration remained constant until 24 h, (Figure 7d). Which were also lower than other reports (Santos and Maugeri, 2007; Spohner and Czermak, 2016).

Conclusions

In this study, it is reported for the first time the expression on *P. pastoris* of the genes *Cap inv32* and *Tde inv32* from *Candida apicola* and *Torulaspora delbrueckii* encoding for β-fructofuranosidase with fructosyltransferase activity. Both enzymes exhibit a high hydrolytic activity, nevertheless they were

capable to produce low levels of FOS at different sucrose concentrations, thus they can be used to produce syrups with low content of FOS as alternative sweeteners to the actual fructose or glucose syrups, which could add an economic value to the actual products of the sugar cane industry.

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