Sains Malaysiana 48(3)(2019): 523–531
http://dx.doi.org/10.17576/jsm-2019-4803-04

Structural Properties, Production, and Commercialisation of Invertase

(Sifat Struktur, Pengeluaran dan Pengkomersialan Invertase)

WEI CHENG PANG, AIZI NOR MAZILA RAMLI* & NUR DINI JOHARI

ABSTRACT

The knowledge gained from yeast fermentation made invertase one of the earliest exploited enzymes in human history. Invertase functions as carbohydrases by hydrolysing sucrose into its simplest unit. Extensive studies on invertase have made it well-characterised through the discovery of its existence in a variety of living organisms. It is interesting to study the different types of invertase from either the same or different origins as they might have distinct properties and could possess unique characteristics. With the advancement in technology, the three-dimensional structure, catalytic domain, and mechanism of invertase action have been discovered. Furthermore, it is important to understand how this enzyme has been produced via fermentation or recombinant technology methods. Finally, invertase has been employed in several important industries and its future commercialisation is promising.

Keywords: Commercialisation; invertase; production; structural analysis

ABSTRAK

Pengetahuan yang diperoleh daripada penapaian ragi telah menjadikan invertase sebagai salah satu enzim yang paling awal dieksploitasi di dalam sejarah manusia. Invertase berfungsi sebagai karbohidrase dengan menghidrolisis sukrosa kepada glukosa dan fruktosa. Pelbagai kajian mengenai invertase dan penemuannya dalam pelbagai organisma telah mendalamkan pemahaman tentang invertase. Kajian mengenai invertase daripada punca yang sama atau berlainan adalah menarik kerana ia terdapat ciri-ciri yang berbeza dan unik. Dengan kemajuan bidang teknologi, struktur tiga dimensi, domain pemangkin dan mekanisme tindakan invertase telah dikaji. Tambahan pula, pengetahuan tentang bagaimana enzim ini dihasilkan melalui kaedah penapaian atau kaedah rekombinan juga sangat penting. Akhirnya, invertase telah digunakan dalam pelbagai sektor industri dan pengkomersilannya adalah sangat baik.

Kata kunci: Analisis struktur; invertase; pemasaran; pengeluaran

INTRODUCTION

Enzymes are biological molecules which are responsible for several biochemical metabolisms in living organisms (Gurung et al. 2013). Enzymes act as biological catalysts which can speed up biochemical reactions up to 10 million folds when introduced in minute quantities (Shinde et al. 2015). The industrial understanding of enzymes revolves around yeasts and malt, a terrain currently been explored by the baking and brewing industries (Kulshrestha et al. 2013). Early studies using *Saccharomyces* made yeast enzymes such as invertase a well-studied and commonly used model in enzyme kinetic analysis (Lincoln & More 2017; Sainz-Polo et al. 2013).

Invertase or β-fructofuranosidase (EC 3.2.1.26) is one of the simplest carbohydrases found in nature (Kotwal & Shankar 2009). The term 'invertase' is derived from the observed inversion of right handed rotation plane of polarised light through sucrose solution to the left handed rotation plane through invert sugar solution (Nadeem et al. 2015). Other than β-D-fructofuranoside, other synonym for invertase include β-D-fructofuranoside fructohydrolase, sucrase, invertin, and saccharase (Kotwal & Shankar 2009; Nadeem et al. 2015). Invertase is a highly polymorphic protein classified into the glycoside hydrolases (GH) families 32 (Benkeblia et al. 2004; Mohandesi et al. 2016). It catalyses the hydrolysis of sucrose, forming an equimolar of D-glucose and D-fructose irreversibly (Alves et al. 2013; Chandra et al. 2012). The product is termed invert sugar and is sweeter than sucrose due to their high degree sweetness of fructose (Shankar et al. 2014b). Interestingly, invertase can also catalyse the hydrolysis of oligosaccharides, raffinose and stachyose (Belcarz et al. 2002).

Invertase has been discovered from a wide range of living organisms such as microorganisms, plants, and animals (Kumar & Kesavapillai 2012). Extensive studies on invertase, including its biochemical properties (Lincoln & More 2017), production (Madhanasundareswari & Jeyachitra 2015; Raju et al. 2016), purification techniques (Marepally 2017; Zouaoui et al. 2016) and immobilisation (Kotwal & Shankar 2009) have boosted its potential for biotechnological applications. For instance, invertase has made its mark in the sugar market, such as in the beverage industries, baking, and confectionaries (Lincoln & More 2017) due to its ability to produce non-crystallisable sugar syrup from sucrose (Shankar et al. 2014b). Moreover,

the confectionary's preference for invert sugar hovers around its ability to keep the products fresh and soft for prolonged periods (Kumar & Kesavapillai 2012). In addition, invertase also play a major role in pharmaceutical applications, and in other industries. Therefore, the prospects of invertase is promising.

Further studies on invertase will provide the latest information on invertase that are necessary. Hence, the purpose of this review is to provide an in-depth summary of the current findings on invertase, in terms of its sources, types, sequence, and structural properties, as well as their production strategies, including the use of recombinant technology. Finally, a review of the literature on the commercialisation of invertase was also be conducted. It is hoped that this review will offer relevant and useful data for further studies on invertase.

SOURCES AND TYPES OF INVERTASE

Microbial invertase is isolated from a wide range of sources, including bacteria, yeasts, and fungi (i.e. *S. cerevisiae*, *Aspergillus niger*, *Arthrobacter* sp., and *Bacillus macerans*). Among all, extensive studies have made *S. cerevisiae* the best model for invertase study. Microbial invertase is classified into intracellular and extracellular; the latter is a glycosylated glycoprotein containing 50% carbohydrate, 5% mannose, and 3% glucosamine, while the former is a non-glycosylated protein without any carbohydrate content (Lincoln & More 2017).

Plant invertase (classified based on their subcellular localisation, solubility, isoelectric point, and optimum pH), are grouped into soluble acid invertase, insoluble acid invertase, and insoluble alkaline/neutral invertase (Hsiao et al. 2002; Kulshrestha et al. 2013). Both soluble and insoluble invertase are glycosylated proteins usually found in vacuoles and cell wall, respectively. In addition, alkaline/neutral invertase is a non-glycosylated protein mostly present in cytosols, mitochondria, and/or plastids (Chandra et al. 2012; Fotopoulos 2005; Tauzin & Giardina 2014). These isoforms of invertase are involved in osmoregulation, sugar metabolism, sucrose allocation, development, and defence action in plants (Kulshrestha et al. 2013). Moreover, alkaline/neutral invertase is the only invertase classified into the GH family 100 (Lammens et al. 2009) due to its catalytic ability in limited substrate (which is only sucrose) (Kulshrestha et al. 2013), and its possession of different structural components compared to the acid invertase. These differences will be subsequently explained in detail.

Although invertase has been characterised extensively among microorganisms and plants, there are few studies on invertase from animal sources. This might be due to the higher active carbohydrate metabolism in animals, causing difficulty in the quantification of animal-sourced invertase (Heil et al. 2005; Marepally 2017). The earliest research on animal-sourced invertase was reported by Carnie and Porteous (1962). They reported an invertase with an α-glucosidase property and optimum pH at 6.7. This invertase which was isolated from the small intestine of a rabbit exhibited kinetic parameters of *Km* and *Vmax* in the range of 6.2 mM and 3.2 μmoles/hr/mg, respectively. Other animal-sourced invertase studies, including those of ants (Ayre 1967; Ricks & Vinson 1972), birds (Carlos Martínez 1990), cockroach (Zhang et al. 1993) and larvae of *Anthereae mylitta* Drury (Marepally 2017) have been reported.

Until now, three types of invertase, namely, α-glucosidase, β-fructosidase, and fructosyltransferase have been discovered with differences in their modes of action. It was found that yeast produce β-fructosidase type of invertase while fungal/mold invertase is classified as α-glucosidase (EC 3.2.1.20) (Kotwal & Shankar 2009; Nadeem et al. 2015). The α-glucosidase catalyse the hydrolysis of sucrose by cleaving α-1,4-glycosidic linkages (Shankar et al. 2014a) compared to β-fructosidase which act on the β-1,2- glycosidic bond of sucrose. In addition, certain invertases from wheat (Schroeven et al. 2008), *Aspergillus* (Kurakake et al. 2010) and *Saccharomyces* (Khandekar et al. 2014) possess transfructosylating activity which can serve as an alternative to fructosyltransferase (E.C.2.4.1.9). This ability allows invertase to produce fructooligosaccharides (FOS) through the process of transfructosilation (Rashad & Nooman 2009) by cleaving β-(2→1) bonds and transferring the resulting fructosyl group from one sucrose unit to another (Alméciga-Díaz et al. 2011). FOS are fructose oligomers such as 1-kestose, 1-nystose, and 1-β-fructofuranosyl nystose (Michel et al. 2016).

SEQUENCE AND STRUCTURAL ANALYSIS OF INVERTASE

Multiple sequence alignment showed that amino acid sequences of homologous invertase contain three conserved amino acid regions, each consisting of NDPN, EC and RDP, which are highly conserved among the invertase and may constitute their catalytic domain (Hsieh et al. 2006; Niu et al. 2014; Yao et al. 2014). These three conserved motifs are responsible for the catalytic action of invertase. It was found that the NDPN residues are near to N-terminus while EC residues are near to C-terminus region (Kulshrestha et al. 2013).

On the other hand, the three-dimensional (3D) structural information of invertase has been obtained through different experimental procedures such as X-ray diffraction, electron microscopy, and nuclear magnetic resonance (NMR) (Dorn et al. 2014). The 3D structure of invertase from bacteria (Alberto et al. 2004; Bujacz et al. 2011) and plants (Chen et al. 2009; Yao et al. 2014) depicted that the N-terminus region of invertase consists of five bladed β-propeller module, while the C-terminus is formed by two six-stranded β-sheets known as β-sandwich module. Three major conserved motifs are located in the active site of the β-propeller domain. Besides, each of the β-propeller blades consist of four antiparallel β-strands assembly with 'W' topology around the central axis, which is the negatively charged cavity of the active

site. Thus, invertase is grouped into GH-J clan with other GH-32 families which share this bimodular arrangement (Lammens et al. 2009; Sainz-Polo et al. 2013). A 1.8 Å crystal structure of the β-fructofuranosidase complex with β-D-fructose from *Bifidobacterium longum* KN29.1 (PDB code: 3PIJ) is shown in Figure 1.

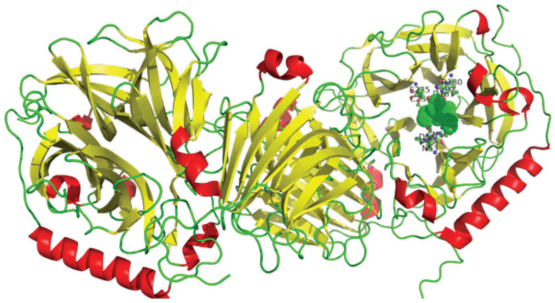

FIGURE 1. Crystal structure of the β-fructofuranosidase complex with β-D-fructose from *Bifidobacterium longum* KN29 (PDB code: 3PIJ) .1. All the three conserved amino acid regions of NDPN, EC and RDP are represented as balls and sticks

Recently, Xie et al. (2016) demonstrated that alkaline/neutral invertase which belongs to GH-100 family exhibits different structural properties compared to GH-32 invertase. In this study, they reported the first structure of alkaline invertase. The alkaline invertase obtained from *Anabaena*, a genus of cyanobacteria with nitrogen-fixing ability, was found to be acting on the α-1,2-glycosidic bond in sucrose. The crystal structure of alkaline/neutral invertase exists as a hexamer with (α/α) 6 barrel core structure in addition to an insertion of three helices. The core (α/α) 6 barrel structure is mainly composed of 12 α-helices and arranged in two concentric layers to form six helical hairpins. They also proposed that Asp188 and Glu414 are putative catalytic residues of the invertase in this family.

The modelling of the 3D structure of invertase assisted in discovering its catalytic mechanism. The available literature suggests that the mechanism of action of invertase involves acting as a retaining enzyme (which is via a two-step reaction known as double-displacement mechanism) (Alberto et al. 2006; Sainz-Polo et al. 2013). This mechanism uses aspartate from NDPN motif as a nucleophile, while glutamate is used as an acid/base catalyst. Furthermore, aspartate residue from RDP motif is used as a transition state stabiliser (Van Wyk et al. 2013). This process involves the protonation of the glycosidic oxygen by the acid/base catalyst, followed by a nucleophile attack on the anomeric carbon of the sugar substrate by a carboxylate group (Figure 2(a)). This subsequently leads to the formation of a covalent fructose–enzyme intermediate (Figure 2(b)). Subsequently, the fructose-enzyme intermediate is hydrolysed which will release fructose and the free invertase (Altenbach & Ritsema 2007; Lammens et al. 2008) (Figure 2(c) & 2(d)). Due to the different 3D structures, it was found that heavy metals can inhibit acid invertases but not alkaline/neutral invertases. However, both acid and alkaline/neutral invertases are inhibited by their own end products (Kulshrestha et al. 2013).

PRODUCTION OF INVERTASE

Nowadays, microorganisms are preferable for invertase production compared to plants and animals due to the less harmful materials released during the production process (Zouaoui et al. 2016). Currently, most invertase production processes are performed via submerged state fermentation

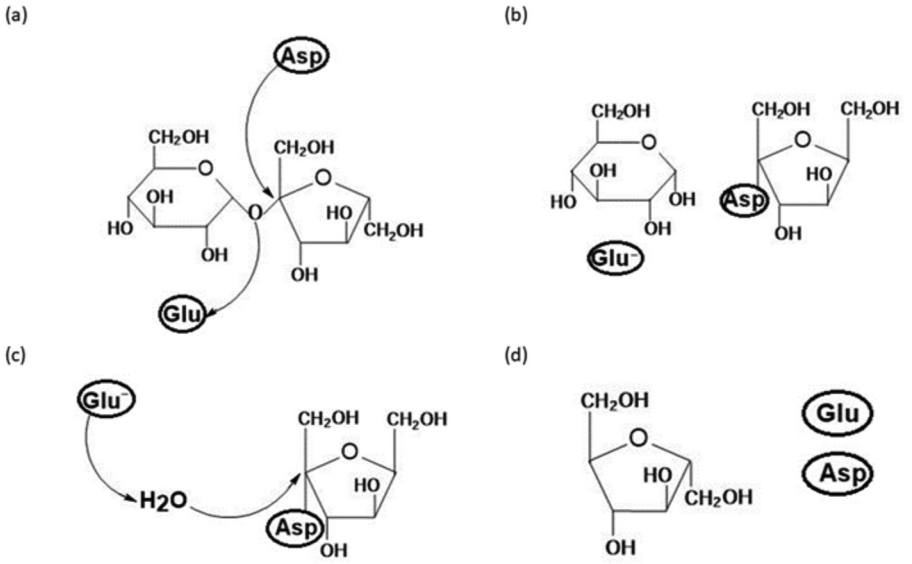

FIGURE 2. Catalytic mechanism of invertase: (a) Protonation and nucleophilic attack on sucrose by invertase, (b) Formation of glucose and fructose-invertase intermediate, (c. & d.) Hydrolysis of fructose-invertase intermediate resulting fructose as the end product

(SmF), followed by solid-state fermentation (SSF) (Kumar & Kesavapillai 2012; Lincoln & More 2017; Rashad & Nooman 2009).

SmF is a traditional fermentation strategy (Sundarram & Murthy 2014) for a large-scale production of invertase (Ravindran & Jaiswal 2016). It is a cost-effective technology with high productivity per reactor volume, and can be easily subjected to culture control and down-stream processing (Nadeem et al. 2015). In SmF production, the selected microorganisms are grown in closed vessels containing nutrients broth and oxygen. These invertase producers utilise the nutrients to produce invertase and release into the fermentation medium (Renge et al. 2012). The advantages of using SmF production are the suitability for the use of genetically modified organisms, as well as the ease of sterilising the medium and purifying the produced invertase (Sundarram & Murthy 2014).

On the other hand, SSF cultivate microorganisms on a solid substrate rather than a liquid medium (Renge et al. 2012). This approach provides an environment which is more similar to the microbe's native environment (Guimarães 2012) and suitable for the growth of organisms that require less moisture (Sundarram & Murthy 2014). SSF is not used in large-scale production of invertase due to its limitations, including difficulty to standardise the processes, as well as being less reproducible (Ravindran & Jaiswal 2016). However, SSF has been proposed to replace SmF as it has several advantages over SmF such as high volumetric productivity, relatively higher concentration of products, less effluent generated, and use of simple fermentation equipment (Renge et al. 2012).

The increasing concern on pollution and the search for cost effectiveness have increased the interest on using waste materials for invertase production (Qureshi et al. 2017). Different waste materials and fermentation strategies have been reported for use in the production of invertase, as shown in Table 1. However, it is difficult to determine the type of waste material or the fermentation approach suitable for commercial production of invertase since these studies are only conducted in lab scale using bioreactors. The use of the same/similar approach in future will provide more necessary information.

RECOMBINANT INVERTASE

Recombinant technology or heterologous expression is referred to a series of processes in the identification of genes of interest and their transfer into a desired host for the synthesis of targeted proteins (Yesilirmak & Sayers 2009). It is an efficient strategy towards meeting market commercialisation requirements such as high production efficiency and low cost of final products (Palomares et al. 2004). The advancements in recombinant technology have enable enzyme production across species carriers since there is no single biological system that can meet all the ideal criteria for the synthesis of proteins, including the production of the required protein at the right conformation, high productivity, ease of handling and maintenance, safe and economic, and ease of downstream processing (Desai et al. 2010).

The heterologous expression of invertase is usually performed using a yeast/fungal as the host-producing organism due to the glycosylated structure of invertase. For instance, *S. cerevisiae* is used in the recombinant expression of the invertase gene isolated from *Ceratocystis moniliformis* (Van Wyk et al. 2013). The advantages of using *S. cerevisiae* as an invertase production system includes the ease of characterisation due to the lower number of extracellular proteins produced throughout the production process. As a result, additional purification method is not necessary for subsequent characterisation of the recombinant invertase. Besides, it satisfies the economic efficiency and biosafety regulations for human application (Gomes et al. 2016). However, several problems like hyperglycosylation, less secretion of recombinant proteins and a low level of expression reduce the potential of *S. cerevisiae* in biotechnological industries (Mohandesi et al. 2016). Thus, several efforts have been made in searching for new host organism as an alternative to *S. cerevisiae* for recombinant invertase production.

In addition, *Schizosaccharomyces pombe* has been earlier used for the heterologous expression of invertase. *S. pombe* is a fission yeast which share many molecular, genetic, and biochemical features with multicellular organisms, making it a suitable expression host for proteins with complex structures (Takegawa et al. 2009). In a study reported by Zarate and Belda (1996), a heterologous invertase gene isolated from *S. cerevisiae* was cloned and expressed in *S. pombe* at an optimum pH of 4.5 (stabilised at a pH range of 4.0-7.0). This recombinant invertase was also found to be stable at temperature less than 50°C and a *Km* of 0.026 mol/L. This study further signified the capability of *S. pombe* to hyperglycosylate the gene product due to the high carbohydrates content (65%) of the recombinant invertase.

Recently, several studies have used *Pichia pastoris* for the production of recombinant invertase from plants, *Elsholtzia haichowensis*, and baker yeast *(S. cerevisiae)* (Liu et al. 2015; Mohandesi et al. 2016). The advantages of using *P. pastoris* include a high production yield (Yesilirmak & Sayers 2009), good glycosylation capability (Gomes et al. 2016), and a superior performance for the expression of secreted proteins (Voegele et al. 2006). Comparing the heterologous invertases of *E. haichowensis* and *S. cerevisiae*, the latter has a slightly higher optimum pH and temperature (4.8 and 60°C, respectively) compared to the former (pH4.0 and 50°C, respectively).

Among all the invertase cloning studies, two interesting studies reported the expression of invertase gene from *S. cerevisiae* in a higher eukaryotic organism (mouse fibroblasts) (Bergh et al. 1987) and a plant (*Solanum tuberosum*) (Deryabin et al. 2014). Both studies showed positive recombinant invertase production outcomes. It was found that the mouse fibroblasts produced glycosylated invertase which was secreted efficiently and enzymatically active. On the other hand, the recombinant

TABLE 1. Overview of invertase production through submerged and solid state fermentation in different studies

Submerged state fermentation				
Source	Substrate	Optimum production condition	Yield	Reference
Fusarium solani	Molasses	pH5.0 at 30°C for four days	9.90 U/mL	Bhatti et al. (2006)
Cladosporium cladosporioides	Pomegranate peel	pH4.0 at 30ºC for four days	~45.0 IU/mL	Uma et al. (2012)
A. niger	Fruits peel	pH5.0 at 30ºC for four days	16.25±0.60 µM	Mehta and Duhan (2014)
Unknown bacteria isolated from sugarcane juice	Waste jiggery	pH6.0 at 37°C	2.63 U	Shah et al. (2016)
A. nidulans and *Emericela nidulans*	Rye flour	pH4.8 - 5.6 at 54°C - 62°C	927.0±35.3 U	Alves et al. (2013)
A. flavus	Agriculture-based by-products	pH6.5 at 40°C for two days	7.41 U/mL	Ahmed et al. (2015)
A. niger	Agro-industrial wastes	pH6.5 at 25°C for six days	15.9±2.44 u/g	Al-Hagar et al. (2015)
Solid state fermentation				
Source	Substrate	Production Condition	Yield/Enzyme activity	Reference
S. cerevisiae	Red carrot residue	30°C for 72 h	272.5 U/g	Rashad and Nooman (2009)
S. cerevisiae	Sugarcane press mud	pH5.0 at 40°C for three days	430 U/mg	Kumar and Kesavapillai (2012)
A. niger	Wheat bran	pH5.5 at 30°C for three days	194.71 U/g	Esawy et al. (2014)
Aspergillus sp.	Carrot peel	pH7.0 at room temperature for five days	6.2 U/mL/min	Madhanasundareswari and Jeyachitra (2015)
A. niger	Agro-industrial wastes	30°C and 50% moisture for three days	154.27±9.38 μg^{-1}	Ohara et al. (2015)
A. niger	Fruits peels	pH5.0 at 30°C for four days	51 U/mL	Raju et al. (2016)

invertase produced by the potato plant was found in the extracellular space in a soluble form where it was weakly adsorbed to the cell wall. These results provide an insight on the alternative sources for invertase production other than microorganisms. However, there is a need for more studies on recombinant invertase production using higher eukaryotes since the available information in this regard is still limited.

COMMERCIALISATION OF INVERTASE

Enzymes have been largely employed in industrial processes due to their mild operating conditions such as pH and temperature (Nisha et al. 2012), non-toxicity, readily degradable with less formation of by-products, as well as requiring a simple purification process to be separated from the final products (Kulshrestha et al. 2013). Today, almost 75% of industrial enzymes are hydrolytic enzymes in which carbohydrases, proteases, and lipases account for more than 70% of the enzyme market share (Chaudhary et al. 2015; Li et al. 2012). Nowadays, invertase has found application in several industries such as food, beverages, pharmaceuticals and biosensor.

INVERTASE IN THE FOOD AND BEVERAGE INDUSTRIES

In the food and beverage industries, invertase has been largely employed in the production of invert syrup and high fructose syrup (Addezio et al. 2014; Ashraf & Bilal 2015). Invertase is used as an alternative to the traditional acid hydrolysis method which might produce undesirable by-products, unfavourable colour, and bitter taste; could also result in low conversion efficiencies and high ash contents (Aburigal et al. 2014). Due to the hygroscopic nature and low crystallisable properties of invert sugar, it is largely used as a humectant in the production of soft-centered candies, fondants (Kotwal & Shankar 2009), artificial honey (Kumar & Kesavapillai 2012), calf feed preparation, jams, and chocolates (Lincoln & More 2017).

INVERTASE IN THE PHARMACEUTICAL INDUSTRY

On the other hand, invertases are important in the pharmaceutical industry for the formulation of drugs, cough syrup, digestive aid tablets, nutraceuticals, and infant foods (Lincoln & More 2017). Besides, the commercial value of invertase in the pharmaceutical industry is further increased by the discovery of invertase with

transfructosylating activity which produces FOS. FOS is oligosaccharides compose of short fructose chains which is less sweet than sucrose, and low in calories, making them preferable for diabetic patients (Dominguez et al. 2013). In addition, FOS have been reported to have beneficial effects on human health as they serve as prebiotics, involved in the production of short-chain fatty acids, prevent constipation and colon cancer, help in mineral absorption, as well as regulate sugar and lipid metabolism (Maiorano et al. 2008).

INVERTASE AS A BIOSENSOR

The commercial potential of invertase is further expanded as it serves as a biosensor for the detection of sucrose (Shankar et al. 2014a) in an easy and rapid way compared to the conventional methods such as chromatography and electrophoresis (Galant et al. 2015). Bagal-Kestwal et al. (2015) reported the fabrication of invertase-mediated nano-gold clusters synthesised on onion membranes. The proposed design can be used as fluorescence-based sucrose biosensors. They suggested that this invention can be used for glucose detection with some modifications in the future.

INVERTASE IN OTHER INDUSTRIES

Invertase also plays a major role in the fermentation processes of ethanol, glycerol, and lactic acid production (Kotwal & Shankar 2009). Invertase aids in hydrolysing the sucrose from the provided carbon source such as cane molasses into glucose and fructose, thereby, increasing the yield of the final products (Aksu & Kutsal 1986; Ueno et al. 2003; Uma et al. 2010). Finally, invertase has potential application in the cosmetic and paper industries (Kotwal & Shankar 2009; Kulshrestha et al. 2013).

CONCLUSION

Invertase, an enzyme found in a wide range of organisms, possesses a high commercial value, especially in the food and pharmaceutical industries. Various studies have previously provided a full characterisation of this enzyme but the current studies which focus on using different waste materials as production media can reduce the cost of invertase production. Furthermore, structural studies have exploited the catalytic mechanism of invertase and this might be useful in artificial enzymes development in the future. Through recombinant technology, the potential of invertase in the biotechnology industries can be further enhanced through a search for more suitable host organisms that will serve as alternatives to the current producers. In the future, the screening for potential invertase producers from extremophiles might lead to the production of invertase under extreme industrial conditions. It is also expected that invertase will be isolated from more different sources and might have unique characteristics such as transfructosylating activities that will boost the commercial value of invertase.

ACKNOWLEDGEMENTS

This research was supported by Universiti Malaysia Pahang through research grant RDU180345 and PGRS180362.

REFERENCES

Aburigal, A.A.A., Elkhalifa, E.A., Sulieman, A.M.E. & Elamin, H.B. 2014. Extraction and partial kinetic properties of invertase from *Schizosaccharomyces pombe*. *International Journal of Food Science and Nutrition Engineering* 4(3): 80-85.

Addezio, F.D., Yoriyaz, E.J., Maria, C. & Vitolo, M. 2014. Sucrose hydrolysis by invertase using a membrane reactor: Effect of membrane cut-off on enzyme performance. *Brazilian Journal of Pharmaceutical Sciences* 50(2): 257-259.

Ahmed, K., Valeem, E.E., Mahmood, T., Mahmood, I. & Haq, Q.U. 2015. Optimal cultural conditions for industrial enzyme production by using shaken flask technique of submerged fermentation. *FUUAST Journal of Biology* 5(1): 21-26.

Aksu, K. & Kutsal, T. 1986. Lactic acid production from molasses utilizing *Lactobacillus delbrueckii* and invertase together. *Biotechnology Letters* 8(3): 157-160.

Al-Hagar, O.E.A., Ahmed, A.S. & Hassan, I. 2015. Invertase production by irradiated *Aspergillus niger* OSH5 using agricultural wastes as carbon source. *British Microbiology Research Journal* 6(3): 135-146.

Alberto, F., Jordi, E., Henrissat, B. & Czjzek, M. 2006. Crystal structure of inactivated *Thermotoga maritima* invertase in complex with the trisaccharide substrate raffinose. *Biochemical Journal* 395(3): 457-462.

Alberto, F., Bignon, C., Sulzenbacher, G., Henrissat, B. & Czjzek, M. 2004. The three-dimensional structure of invertase (β-fructosidase) from *Thermotoga maritima* reveals a bimodular arrangement and an evolutionary relationship between retaining and inverting glycosidases. *Journal of Biological Chemistry* 279(18): 18903-18910.

Alméciga-Díaz, C.J., Gutierrez, A.M., Bahamon, I., Rodríguez, A., Rodríguez, A.M. & Sánchez, O.F. 2011. Computational analysis of the fructosyltransferase enzymes in plants, fungi and bacteria. *Gene* 484(1-2): 26-34.

Altenbach, D. & Ritsema, T. 2007. Structure-function relations and evolution of fructosyltransferases. In *Recent Advances in Fructooligosaccharides Research,* edited by Shiomi, N., Benkeblia, N. & Onodera, N. Kerala, India: Signpost. pp. 135-156.

Alves, J.N.O., Jorge, J.A. & Guimarães, L.H.S. 2013. Production of invertases by anamorphic (*Aspergillus nidulans*) and teleomorphic (*Emericela nidulans*) fungi under submerged fermentation using rye flour as carbon source. *Advances in Microbiology* 3: 421-429.

Ashraf, H. & Bilal, Z.E.H. 2015. Biosynthesis, partial purification and characterization of invertase through carrot (*Daucus carota* L.) peels. *Journal of Biochemical Technology* 6(1): 867-874.

Ayre, G.L. 1967. The relationships between food and digestive enzymes in five species of ants (Hymenoptera: Formicidae). *The Canadian Entomologist* 99(4): 408-411.

Bagal-Kestwal, D., Kestwal, R.M. & Chiang, B.H. 2015. Invertase-nanogold clusters decorated plant membranes for fluorescence-based sucrose sensor. *Journal of Nanobiotechnology* 13: 30.

Belcarz, A., Ginalska, G., Lobarzewski, J. & Penel, C. 2002. The novel non-glycosylated invertase from *Candida utilis* (the

properties and the conditions of production and purification). *Biochimica et Biophysica Acta - Protein Structure and Molecular Enzymology* 1594(1): 40-53.

Benkeblia, N., Onodera, S., Yoshihira, T., Kosaka, S. & Shiomi, N. 2004. Effect of temperature on soluble invertase activity, and glucose, fructose and sucrose status of onion bulbs (*Allium cepa*) in store. *International Journal of Food Sciences and Nutrition* 55(4): 325-331.

Bergh, M.L., Cepko, C.L., Wolf, D. & Robbins, P.W. 1987. Expression of the *Saccharomyces cerevisiae* glycoprotein invertase in mouse fibroblasts: Glycosylation, secretion, and enzymatic activity. In *Proceedings of the National Academy of Sciences of the United States of America* 84: 3570-3574.

Bhatti, H.N., Asgher, M., Abbas, A., Nawaz, R. & Sheikh, M.A. 2006. Studies on kinetics and thermostability of a novel acid invertase from *Fusarium solani*. *Journal of Agricultural and Food Chemistry* 54(13): 4617-4623.

Bujacz, A., Jedrzejczak-Krzepkowska, M., Bielecki, S., Redzynia, I. & Bujacz, G. 2011. Crystal structures of the apo form of β-fructofuranosidase from *Bifidobacterium longum* and its complex with fructose. *FEBS Journal* 278(10): 1728-1744.

Carlos Martínez, d.R. 1990. Dietary, phylogenetic, and ecological correlates of intestinal sucrase and maltase activity in birds. *Physiological Zoology* 63(5): 987-1011.

Carnie, J.A. & Porteous, J.W. 1962. The invertase activity of rabbit small intestine. *Biochemical Journal* 85: 450-456.

Chandra, A., Jain, R. & Solomon, S. 2012. Complexities of invertases controlling sucrose accumulation and retention in sugarcane. *Current Science* 102(6): 857-866.

Chaudhary, S., Sagar, S., Kumar, M., Sengar, R.S. & Tomar, A. 2015. The use of enzymes in food processing: A review. *South Asian Journal of Food Technology and Environment* 1(3&4): 190-210.

Chen, T.H., Huang, Y.C., Yang, C.S., Yang, C.C., Wang, A.Y. & Sung, H.Y. 2009. Insights into the catalytic properties of bamboo vacuolar invertase through mutational analysis of active site residues. *Phytochemistry* 70(1): 25-31.

Deryabin, A.N., Berdichevets, I.N., Burakhanova, E.A. & Trunova, T.I. 2014. Characteristics of extracellular invertase of *Saccharomyces cerevisiae* in heterologous expression of the Suc2 gene in *Solanum tuberosum* plants. *Biology Bulletin* 41(1): 24-30.

Desai, P.N., Shrivastava, N. & Padh, H. 2010. Production of heterologous proteins in plants: Strategies for optimal expression. *Biotechnology Advances* 28(4): 427-435.

Dominguez, A.L., Rodrigues, L.R., Lima, N.M. & Teixeira, J.A. 2013. An overview of the recent developments on fructooligosaccharide production and applications. *Food and Bioprocess Technology* 6(12): 1-14.

Dorn, M.E., Silva, M.B., Buriol, L.S. & Lamb, L.C. 2014. Three-dimensional protein structure prediction: Methods and computational strategies. *Computational Biology and Chemistry* 53: 251-276.

Esawy, M.A., Kansoh, A.L., Kheiralla, Z.H., Ahmed, H.E., Kahil, T.A.K. & El-Hameed, E.K. 2014. Production and immobilization of halophilic invertase produced from honey isolate *Aspergillus niger* EM77 (KF774181). *International Journal of Biotechnology for Wellness Industries* 3: 36-45.

Fotopoulos, V. 2005. Plant invertases: Structure, function and regulation of a diverse enzyme family. *Journal of Biological Research* 4: 127-137.

Galant, A.L., Kaufman, R.C. & Wilson, J.D. 2015. Glucose: Detection and analysis. *Food Chemistry* 188: 149-160.

Gomes, A.R., Byregowda, S.M., Veeregowda, B.M. & Balamurugan, V. 2016. An overview of heterologous expression host systems for the production of recombinant proteins. *Advances in Animal and Veterinary Sciences* 4(7): 346-356.

Guimarães, L.H.S. 2012. Carbohydrates from biomass: Sources and transformation by microbial enzymes. In *Carbohydrates - Comprehensive Studies on Glycobiology and Glycotechnology,* edited by Chang, C.F. Belgium: Intech. pp. 441-458.

Gurung, N., Ray, S., Bose, S. & Rai, V. 2013. A broader view: Microbial enzymes and their relevance in industries, medicine, and beyond. *BioMed Research International* 2013: 329121.

Heil, M., Büchler, R. & Boland, W. 2005. Quantification of invertase activity in ants under field conditions. *Journal of Chemical Ecology* 31(2): 431-437.

Hsiao, C.C., Fu, R.H. & Sung, H.Y. 2002. A novel bound form of plant invertase in rice suspension cells. *Botanical Bulletin of Academia Sinica* 43: 115-122.

Hsieh, C.W., Liu, L.K., Yeh, S.H., Chen, C.F., Lin, H.I., Sung, H.Y. & Wang, A.Y. 2006. Molecular cloning and functional identification of invertase isozymes from green bamboo *Bambusa oldhamii*. *Journal of Agricultural and Food Chemistry* 54: 3101-3107.

Khandekar, D.C., Palai, T., Agarwal, A. & Bhattacharya, P.K. 2014. Kinetics of sucrose conversion to fructo-oligosaccharides using enzyme (invertase) under free condition. *Bioprocess and Biosystems Engineering* 37(12): 2529-2537.

Kotwal, S.M. & Shankar, V. 2009. Immobilized invertase. *Biotechnology Advances* 27(4): 311-322.

Kulshrestha, S., Tyagi, P., Sindhi, V. & Yadavilli, K.S. 2013. Invertase and its applications - a brief review. *Journal of Pharmacy Research* 7(9): 792-797.

Kumar, R. & Kesavapillai, B. 2012. Stimulation of extracellular invertase production from spent yeast when sugarcane pressmud used as substrate through solid state fermentation. *SpringerPlus* 1: 81.

Kurakake, M., Masumoto, R., Maguma, R., Kamata, A., Saito, E., Ukita, N. & Komaki, T. 2010. Production of fructooligosaccharides by β-fructofuranosidases from *Aspergillus oryzae* KB. *Journal of Agricultural and Food Chemistry* 58(1): 488-492.

Lammens, W., Le Roy, K., Schroeven, L., Van Laere, A., Rabijns, A. & Van den Ende, W. 2009. Structural insights into glycoside hydrolase family 32 and 68 enzymes: Functional implications. *Journal of Experimental Botany* 60(3): 727-740.

Lammens, W., Le Roy, K., Van Laere, A., Rabijns, A. & Van den Ende, W. 2008. Crystal structures of *Arabidopsis thaliana* cell-wall invertase mutants in complex with sucrose. *Journal of Molecular Biology* 377(2): 378-385.

Li, S., Yang, X., Yang, S., Zhu, M. & Wang, X. 2012. Technology prospecting on enzymes: Application, marketing and engineering. *Computational and Structural Biotechnology Journal* 2(3): e201209017.

Lincoln, L. & More, S.S. 2017. Bacterial invertases: Occurrence, production, biochemical characterization, and significance of transfructosylation. *Journal of Basic Microbiology* 57(10): 803-813.

Liu, C., Xu, Z., Cai, S. & Xiong, Z. 2015. CDna cloning, heterologous expression and characterization of a cell wall invertase from copper tolerant population of *Elsholtzia haichowensis*. *Biologia (Poland)* 70(8): 1063-1069.

Madhanasundareswari, K. & Jeyachitra, K. 2015. Production and optimization of growth conditions for invertase enzyme by *Aspergillus* in solid state fermentation (SSF) using carrot peel as substrate. *SIRJ-APBBP* 2(1): 16-22.

Maiorano, A.E., Piccoli, R.M., da Silva, E.S. & de Andrade Rodrigues, M.F. 2008. Microbial production of fructosyltransferases for synthesis of pre-biotics. *Biotechnology Letters* 30(11): 1867-1877.

Marepally, L. 2017. Purification and characterization of invertase from the midgut of fifth instar larvae of *Anthereae mylitta* Drury (Daba TV). *International Journal of Recent Scientific Research* 8(6): 17330-17334.

Mehta, K. & Duhan, J.S. 2014. Production of invertase from *Aspergillus niger* using fruit peel waste as a substrate. *International Journal of Pharma and Bio Sciences* 5(2): 353-360.

Michel, M.R., Rodríguez-Jasso, R.M., Aguilar, C.N., Gonzalez-Herrera, S.M., Flores- Gallegos, A.C. & Rodríguez-Herrera, R. 2016. Fructosyltransferase sources, production, and applications for prebiotics. In *Production Probiotics and Prebiotics in Human Nutrition and Health*, edited by Rao, V. Belgium: Intech. pp. 169-189.

Mohandesi, N., Siadat, S.O.R., Haghbeen, K. & Hesampour, A. 2016. Cloning and expression of *Saccharomyces cerevisiae* SUC2 gene in yeast platform and characterization of recombinant enzyme biochemical properties. *3 Biotech* 6: 128-138.

Nadeem, H., Rashid, M.H., Siddique, M.H., Azeem, F., Muzammil, S., Javed, M.R., Ali, M.A., Rasul, I. & Riaz, M. 2015. Microbial invertases: A review on kinetics, thermodynamics, physiochemical properties. *Process Biochemistry* 50(8): 1202-1210.

Nisha, S., Karthick, A. & Gobi, N. 2012. A review on methods, application and properties of immobilized enzyme. *Chemical Science Review and Letters* 1(3): 148-155.

Niu, J.Q., Wang, A.Q., Huang, J.L., Yang, L.T. & Li, Y.R. 2014. Isolation, characterization and promoter analysis of cell wall invertase gene SoCIN1 from sugarcane (*Saccharum* Spp.). *Sugar Tech* 17(1): 65-76.

Ohara, A., de Castro, R.J.S., Nishide, T.G., Dias, F.F.G., Bagagli, M.P. & Sato, H.H. 2015. Invertase production by *Aspergillus niger* under solid state fermentation: Focus on physical-chemical parameters, synergistic and antagonistic effects using agro-industrial wastes. *Biocatalysis and Agricultural Biotechnology* 4(4): 645-652.

Palomares, L.A., Estrada-Mondaca, S. & Ramírez, O.T. 2004. Production of recombinant proteins: Challenges and solutions. In *Recombinant Gene Expression,* edited by Balbás, P. & Lorence, A. *Methods in Molecular Biology* 267: 15-52.

Qureshi, A.S., Khushk, I., Ali, C.H., Majeed, H. & Ahmad, A. 2017. Production of invertase from *Saccharomyces cerevisiae* Angel using date syrup as a cost effective carbon source. *African Journal of Biotechnology* 16(15): 777-781.

Raju, A.I.C.H., Pulipati, K. & Jetti, A. 2016. Production of invertase by *Aspergillus niger* under solid state fermentation using orange fruit peel as substrate. *Advances in Crop Science and Technology* 4: 247.

Rashad, M.M. & Nooman, M.U. 2009. Production, purification and characterization of extracellular invertase from *Saccharomyses cerevisiae* NRRL Y-12632 by solid-state fermentation of red carrot residue. *Australian Journal of Basic and Applied Sciences* 3(3): 1910-1919.

Ravindran, R. & Jaiswal, A. 2016. Microbial enzyme production using lignocellulosic food industry wastes as feedstock: A review. *Bioengineering* 3(4): 30.

Renge, V.C., Khedkar, S.V. & Nandurkar, N.R. 2012. Enzyme synthesis by fermentation method: A review. *Scientific Reviews and Chemical Communications* 2(4): 585-590.

Ricks, B.L. & Vinson, S.B. 1972. Digestive enzymes of the imported fire ant, *Solenopsis richteri* (Hymenoptera: Formicidae). *Entomologia Experimentalis et Applicata* 15: 329-334.

Sainz-Polo, M.A., Ramírez-Escudero, M., Lafraya, A., González, B., Marín-Navarro, J., Polaina, J. & Sanz-Aparicio, J. 2013. Three-dimensional structure of *Saccharomyces* invertase: Role of a non-catalytic domain in oligomerization and substrate specificity. *Journal of Biological Chemistry* 288(14): 9755-9766.

Schroeven, L., Lammens, L., Van Laere, A. & Van den Ende, W. 2008. Transforming wheat vacuolar invertase into a high affinity sucrose: Sucrose 1-fructosyltransferase. *New Phytologist* 180(4): 822-831.

Shah, H.S., Patel, C.M. & Parikh, S.C. 2016. Production of invertase from bacteria by using waste jaggery. *The Microbes* 3: 19-23.

Shankar, T., Thangamathi, P., Rama, R. & Sivakumar, T. 2014a. Characterization of invertase from *Saccharomyces cerevisiae* MK obtained from toddy sample. *Journal of Bioprocessing and Chemical Engineering* 2(1): 1-6.

Shankar, T., Thangamathi, P., Rama, R. & Sivakumar, T. 2014b. Characterization of invertase from *Saccharomyces crevisiae* MTCC 170. *African Journal of Microbiology Research* 8(13): 1385-1393.

Shinde, V., Deshmukh, S. & Bhoyar, M.G. 2015. Applications of major enzymes in food industry. *Indian Farmer* 2(6): 497-502.

Sundarram, A. & Murthy, T.P.K. 2014. α -Amylase production and applications: A review. *Journal of Applied & Environmental Microbiology* 2(4): 166-175.

Takegawa, K., Tohda, H., Sasaki, M., Idiris, A., Ohashi, T., Mukaiyama, H., Giga-Hama, Y. & Kumagai, H. 2009. Production of heterologous proteins using the fission-yeast (*Schizosaccharomyces pombe*) expression system. *Biotechnology and Applied Biochemistry* 53(4): 227-235.

Tauzin, A.S. & Giardina, T. 2014. Sucrose and invertases, a part of the plant defense response to the biotic stresses. *Frontiers in Plant Science* 5: 1-8.

Ueno, T., Ozawa, Y., Ishikawa, M., Nakanishi, K. & Kimura, T. 2003. Lactic acid production using two food processing wastes, canned pineapple syrup and grape invertase, as substrate and enzyme. *Biotechnology Letters* 25(7): 573-577.

Uma, C., Gomathi, D., Ravikumar, G., Kalaiselvi, M. & Palaniswamy, M. 2012. Production and properties of invertase from a *Cladosporium cladosporioides* in SmF using pomegranate peel waste as substrate. *Asian Pacific Journal of Tropical Biomedicine* 2: 605-611.

Uma, C., Gomathi, D. & Gopalakrishnan, V.K. 2010. Fungal invertase as aid for production of ethanol from sugarcane bagasse. *Research Journal of Microbiology* 5(10): 980-985.

Voegele, R.T., Wirsel, S., Möll, U., Lechner, M. & Mendgen, K. 2006. Cloning and characterization of a novel invertase from the obligate biotroph *Uromyces fabae* and analysis of expression patterns of host and pathogen invertases in the course of infection. *Molecular Plant-Microbe Interactions* 19(6): 625-634.

Van Wyk, N., Trollope, K.M., Steenkamp, E.T., Wingfield, B.D. & Volschenk, H. 2013. Identification of the gene for β-fructofuranosidase from *Ceratocystis moniliformis* CMW 10134 and characterization of the enzyme expressed in *Saccharomyces cerevisiae*. *BMC Biotechnology* 13: 100.

Xie, J., Cai, K., Hu, H.X., Jiang, Y.L., Yang, F., Hu, P.F., Cao, D.D., Li, W.F., Chen, Y. & Zhou, C.Z. 2016. Structural analysis of the catalytic mechanism and substrate specificity of *Anabaena* alkaline invertase InvA reveals a novel glucosidase. *Journal of Biological Chemistry* 291(49): 25667-25677.

Yao, Y., Wu, X.H., Geng, M.T., Li, R.M., Liu, J., Hu, X.W. & Guo, J.C. 2014. Cloning, 3D modeling and expression analysis of three vacuolar invertase genes from cassava (*Manihot esculenta* Crantz). *Molecules* 19(5): 6228-6245.

Yesilirmak, F. & Sayers, Z. 2009. Heterelogous expression of plant genes. *International Journal of Plant Genomics* 2009: 296482.

Zárate, V. & Belda, F. 1996. Characterization of the heterologous invertase produced by *Schizosaccharomyces pombe* from the SUC2 gene of *Saccharomyces cerevisiae*. *The Journal of Applied Bacteriology* 80(1): 45-52.

Zhang, J., Scrivener, A.M., Slaytor, M. & Rose, H.A. 1993. Diet and carbohydrase activities in three cockroaches, *Calolampra elegans* Roth and Princis, *Geoscapheus dilatatus* Saussure and *Panesthia cribrata* Saussure. *Comparative Biochemistry and Physiology - Part A: Physiology* 104(1): 155-161.

Zouaoui, B., Ghalem, B.R., Djillali, B. & Fatima, S. 2016. Characterization of partially purified extracellular thermostable invertase by *Streptococcus* Sp. isolated from the date. *Bulletin of Environment, Pharmacology and Life Sciences* 5(9): 65-72.

Wei Cheng Pang, Aizi Nor Mazila Ramli* & Nur Dini Johari
Faculty of Industrial Science & Technology
Universiti Malaysia Pahang
Lebuhraya Tun Razak
26300 Gambang, Kuantan, Pahang Darul Makmur
Malaysia

Aizi Nor Mazila Ramli*
Bio Aromatic Research Centre of Excellence
Universiti Malaysia Pahang, Lebuhraya Tun Razak
26300 Gambang, Kuantan, Pahang Darul Makmur
Malaysia

*Corresponding author; email: aizinor@ump.edu.my

Received: 30 April 2018
Accepted: 8 January 2019