The Optimum Conditions for the Production of Invertase by Local Isolate *Escherichia coli* from the Soil and Grown on Different Carbon Source Substrate

Dhafer Fakhri Al-Rawi **Harith Kamel Buniya**
Aymen Shehab Al-Rawi

University of Anbar/ College of Education for Pure Science – Dept. of Biology
Anbar – Iraq

E_mail: hkbuniya@uoanbar.edu.iq

Abstract

The present study aims to use a local isolate of *Escherichia coli* for the production of invertase enzyme and study the effects of some factors that may enhance the production of this enzyme. For this purpose, different nutrient requirements and different culture conditions such as pH, temperature, carbon source, carbon concentration, inoculum volumes and incubation period were used. The results revealed that the optimum pH, temperature, and incubation period were 7.0, 30 ℃ and 72 hours, respectively. The red carrot, as a substrate, was the best carbon source for Escherichia coli at the concentration of 2.5% to get highest enzyme production up to 2.4 U/ml

Keywords: *Escherichia coli*, Invertase, Optimization, and Carbon Sources

الظروف المثلى لإنتاج إنزيم الانفرتيز بواسطة عزلة محلية لبكتريا العصيات القولونية من التربة والمنماة في مصادر كاربونية مختلفة

ظافر فخري الراوي **حارث كامل بنية** **أيمن شهاب الراوي**

جامعة الانبار/ كلية التربية للعلوم الصرفة - قسم علوم الحياة
الانبار- العراق

الخلاصـــة

هدفت هذه الدراسة إلى استخدام بكتريا القولون المعزولة محليا في إنتاج أنزيم الانفرتيز ودراسة تأثير بعض العوامل التي تعمل على تحسين إنتاج هذا الأنزيم. استخدمت أوساط بديلة وظروف خارجية مختلفة لتنمية البكتريا كالرقم الهيدروجيني ودرجة الحرارة والمصدر الكاربوني وتركيز الكاربون وحجم اللقاح المستخدم وفترة الحضن لهذا الغرض. أظهرت النتائج أن الاس الهيدروجيني الأمثل ودرجة الحرارة المثلى وفترة الحضن المثلى كانت 7.0 و30 درجة مئوية و72 ساعة على التوالي. وكان الجزر الأحمر البديل للسكر أفضل مصدر كاربوني بديل لنمو بكتريا *E. coli* بتركيز 2.5% للحصول على اعلى انتاجية للانزيم بلغت 2,4 وحدة/ مل.

الكلمات المفتاحية: العصيات القولونية والانفرتيز والظروف المثلى ومصادر كربونية

Introduction

Invertase (β-D-fructofuranosidase, E.C 3.2.1.26) split the glycosidic bond between fructose and glucose in sucrose by hydrolysis and liberation mono-saccharides (Mobini-Dehkordi, *et al*.,2008). The enzyme, hydrolysis β-D-fructofuranoside (Sucrose, Raffinose, Stachyose, and Inulin) from the end of fructose (Gore, *et al*.,2009; Mohandesi, *et al*. 2016).

There are numerous manufacturing utilizations of invertase in candies, liquid refreshment, pastries and medical industries. It plays an important function in the production of invert syrup i.e., a blend of fructose and glucose and high fructose syrup (HFS) from sucrose. Invert syrup and HFS created by hydrolysis of the enzyme are favored over those syrups which are produced by acid hydrolysis of sucrose giving unfavorable by-products, absence of sugary savor, minimal diversion effectiveness, high ash contents and so are very expensive. On the other hand, the enzymatic hydrolysis gives altitude pureness products that superior in savor, constancy, non-crystallizable and free from any unwanted by-products (Oztop, *et al*., 2009; Celebi, *et al*., 2009). Invertase is used in some industries, like in the manufacture of lactic acid and glycerol (Sanchez, *et al*., 2001; Lincoln and More, 2017). The significant utilization of invertase in the composition of creams, jams, desserts, milk powder for infants, synthetic nectar, digestive aid tablets and plasticizing factors in beauty care products (Marquez, *et al*., 2008; Kotwal & Shankar, 2009).

A broad assortment of microbes, plants or animals' origins is applied for the production of commercial enzymes. Generality enzyme production procedure depends on the microbial origin. Even though invertase has been found in animals and higher plant origin, but the microorganisms are the good option being fast growth and simple in genetic manipulation (Gangadhara, *et al*., 2008; Bhalla, *et al*., 2017).

The aim of this study is to produce invertase from a local bacterial isolate and study the optimum conditions for its production.

Materials and Methods

1- Isolation and Screening of Invertase Production Bacteria

Bacterial strains were isolated from the agriculture soil in Al-Ramadi city, west of Iraq by using dilution plate method. The production of invertase enzyme was screened in the culture of bacterial isolates *Escherichia coli.*

2- Fermentation

The medium used for the production of the enzyme under immersed fermentation consists of gm\L: sucrose 10, yeast extract 6.0, KH_2PO_4 1.0, K_2HPO_4 1.0 and pH 7.2 (Yi, *et al*., 2006). Cultivation was done in 250 ml Erlenmeyer flasks each one has fifty ml of sterile media. When the inoculums reached (1×106 cfu/ml), after 48 hours incubation in the shaker incubator at 150 rpm. Cells were harvested by centrifugation in 6000 rpm for 20 min. The pellet was washed with 10 mM of Tris-HCL buffer (pH 7.0) containing 1 mM EDTA and re-suspended in 10 ml of the same buffer. Ultrasonic disruption of cells was carried out with a Branson Sonicator (3 Times, 15 Sec. at 40 Watt, with 45 sec. Intervals Between Each Run). The supernatant was used an enzyme source. The sucrose in the media was replaced with Dates, Red carrot and Wheat bran as substrate.

3- Processing of the Substrate Source

Different carbon sources (Dates and Red Carrot Waste) were obtained from the fruit market in Falluja city – Iraq, washed and cut into slices. The slices

were spread on the trays for drying and saved in the polyethylene bags at room temperature for using later as enzyme substrate.

4- **Assay of Enzyme**

Enzyme activity was determined by the procedure described by Whitaker & Berhard (1972) with variation by mixing 0.1 ml of enzyme solution with 0.9 ml of sucrose in 0.03 M acetate buffer (pH 5.5), ending the reaction done by added 1 ml of dinitro salicylic acid reagent and boiling for 5 min. at 100 ℃. Finally, the absorbance was measured in a spectrophotometer at 540 nm. 1 unit of enzyme (U) is defined as the amount of invertase which releases 1 µmole of glucose/minute/ml under the study status.

5- Optimization of Invertase Production

The effects of various factors on the production of enzyme like pH, temperature and incubation time (Hours) were studied by incubating culture flasks with different pH (6, 7, 8, 9 and 10) and temperature (25,30,35,40 and 45°C) for 24 – 120 hours and study the effect of some factors such as inoculums size (0.5, 1, 1.5, 2, 2.5 and 3%), type of carbon source (Sucrose, Dates, Red Carrot and Wheat Bran), and concentration.

Results and Discussion

Temperature and pH are the highest significant variables, which notably effect the invertase activity. High invertase activity was recorded at 30 °C. Furthermore, the increment in the incubating temperature revealed that the decreases in the enzyme activity as temperature increases and this clearly shown in Figure (1). The effect of pH on the activity of enzyme shows that the invertase had high activity at 7.0 and this is illustrated in Figure (2).

Different substrate such as Dates, Red carrot and Wheat bran were used for induction enzyme production as shown in Figure (3). In this study, three substrates evaluated and the red carrot was appeared to be a better substrate for the production of invertase. The maximum enzyme production occurred at 2.5% of red carrot concentration as represented in Figure (4).

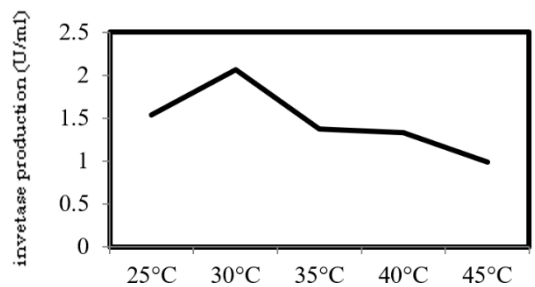

Figure (1) Effect of Temperature on Invertase

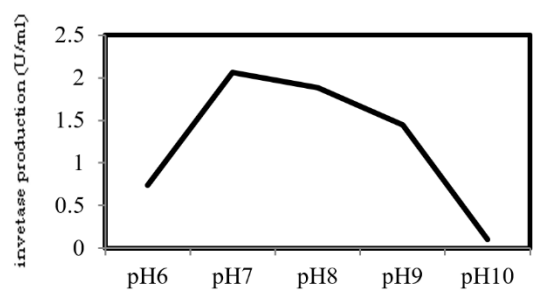

Figure 2 Effect of pH on Invertase Production

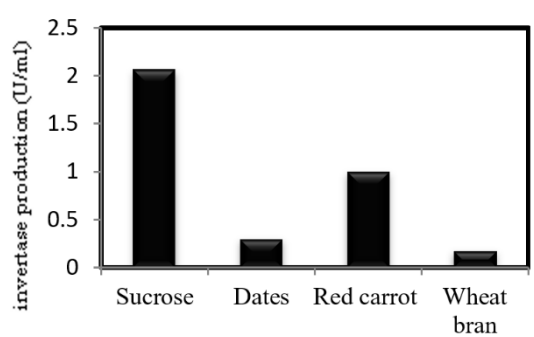

Figure (3) Effect of Carbon Source on Invertase production

To estimate the influence of inoculum volumes on enzyme production, different bacterial cell concentrations (0.5 - 3%) were added to several flasks and then fermentation was accomplished. In the case of *Escherichia coli*, it was obvious from the Figure (5) that the highest enzyme production happened at 2.5% inoculum volumes for red carrot as the substrate with the production of invertase

enzyme (2.5U/ml). The medium was inoculated with the bacterial isolate of *Escherichia coli*. and incubated for different periods (1-5 Days). The highest activity was seen after 72 hrs. incubation (2.4 U/ml) (Figure 6).

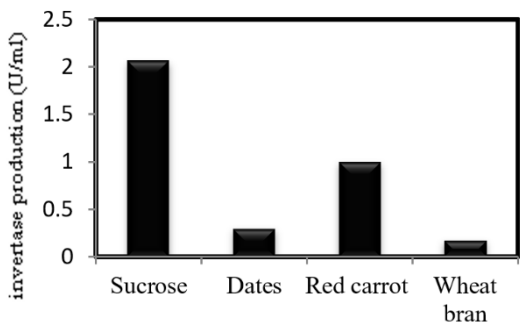

Figure (4) Effect of Substrates Concentration on Invertase Production

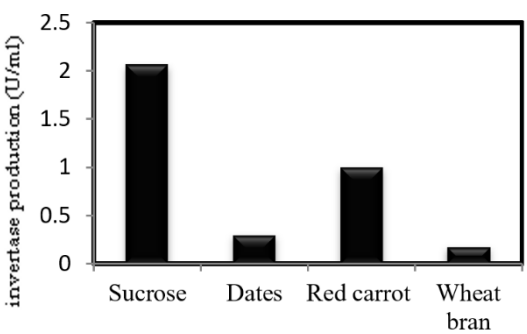

Figure (5) Effect of Inoculum Size on Invertase Production

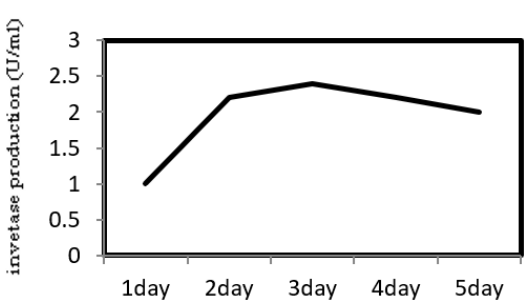

Figure (6) Effect of Incubation Time on Invertase Production

The optimization of the culture's environment is a serious part to be think in the evaluation of fermentation technology. However, there are some reports about the optimization of medium formation particularly for bacterial strains in the production of invertase. The incubation periods differ with enzyme productions. The current study was accomplished to estimate the influence of incubation time on invertase production by *Escherichia coli* where the highest yield of the enzyme was obtained after 5 days incubation. moreover, increase the incubation time gave minimal amount of enzyme. It might be due to a reduction in the availability of nutrients in medium making ability of yeast or consumption of sugar substance (Berka and Cherry, 2006; Shah, *et al*., 2016). The result was supported by Qureshi, *et al*., (2012) who showed that the invertase production was ultimate on the 2 days of cultivation by *Mucorgeophillus* EFRL 03. Guimaraes, *et al*., (2007) revealed that for *Aspergillus ochraceus*, invertase achieved its highest scale when supplemented with sugarcane at 3 days at 40 ºC under the irritation of 100 rpm.

The temperature degree is a basic parameter that must be controlled and it different from organism to other one and it limits the success of optimization of bio-system. The temperature has highly impacted on the production of invertase directly or indirectly. The high enzyme production was recorded at 30°C. Similar behavior was notified for extracellular invertase produced by *Aspergillus niger* and *Lactobacillus reuteri* (Gomez, *et al*., 2000). Among the physical parameters, the pH of the culture media assumes a significant function by stimulate morphological variation in microorganisms and secretion of enzyme. The pH differs saw through the growth of microorganism likewise influences product constancy in the medium (Persike, *et al*., 2002). Optimum pH for invertase was showed to be in a range from 6.0 to 8.0 for the bacteria (Yoon, *et al*., 2007) and the current study recorded 7.0 as an optimum, which correspond with previous findings. For the carbon sources, red carrot gave the best result.

The results were confirmed by the results of Rashad and Nooman (2009). Further, like invertase of *Bacillus macerans*, invertase of *Streptomyces* sp. ALKC8 also shows to be regulated by a double mechanism of substrate induction and product inhibition. Catabolic repression is further supported with a decrease in invertase production at an increase in sucrose concentration (> 2.5%), which might be because of increment in the degrees of reducing sugars (Kaur and Sharma, 2005; Ahmed, 2008).

Conclusion

The invertase enzyme produced by the local bacterial isolate using cheap carbon source available in the local market and found the typical condition for enzyme production.

References

Ahmed, S. A. (2008). Invertase Production by *Bacillus macerans* Immobilized on Calcium Alginate Beads. J of Appl. Sci. Res. 4(12), 1777-1781.

Bhalla, T. C.; Thakur, N. and Thakur N. (2017). Invertase of Saccharomyces Cerevisiae SAA-612 Production, Characterization and Application in Synthesis of Fructo - oligosaccharides. LWT Food Sci. Technol. 77, 178–185.

Berka, R. M. and Cherry, J. R. (2006). Enzyme Biotechnology. In Basic Biotechnology, 3rd: Cambridge University. Landon.

Celebi, S. E.; Ibibican, S. K.; Kayahan, B. and Toppare, L. (2009). Immobilization of Invertase in Copolymer of 2,5-Di (Thiophen-2-yl)-1-p-tolyl-1H-pyrrole with Pyrrole. J. Macromolecular Sci. 46, 739-744.

Gangadhara; Kumar, P. R. and Prakash, V. (2008). Influence of Polyols on the Stability and Kinetic Parameters of Invertase from Candida Utilis: Correlation with the Conformational Stability and Activity. J Protein. 27, 440-449.

Gomez, S.; Augur, C. and Gonzalez, G. (2000). Invertase Production by *Aspergillus niger* in Submerged and Solid-state Fermentation. Biotechnology Letters. 22(15),1255-1258.

Gore, J.; Youk, H. and Oudenaarden, H. (2009). Snowdrift Game Dynamics and Facultative Cheating in Yeast. Nature. 459, 253-256.

Guimaraes, L. H. S.; Terenzi H. F.; Polizeli M. L. and Jorge J. A. (2007). Production and Characterization of a Thermostable Extracellular Beta-d-fructofuranosidase Produced by *Aspergillu sochraceus* with Agro Industrial Residues as Carbon Sources. Enzyme Microbial Technol. 42, 52-57.

Kaur, N. and Sharma, A. D. (2005). Production, Optimization and Characterization of Extracellular Invertase by an Actinomycete Strain. J. Sci. Ind. Res. 64, 515-519

Kotwal, S. M. and Shankar, V. (2009). Immobilized Invertase. Biotechnol. Adv. 27, 311-322.

Lincoln, L. and More, S. S. (2017). Bacterial Invertases: Occurrence, Production, Biochemical Characterization, and Significance of Transfructosylation. Journal of Basic Microbiology 57(10), 803-813.

Marquez, L. D. S.; Cabral, B.V.; Freitas, F.; Cardoso, V. L. and Ribeiro, E. J. (2008). Optimization of Invertase Immobilization by Adsorption in Ionic Exchange Resin for Sucrose Hydrolysis. J. Mol. Catalysis. 51(3-4), 86-92.

Mobini-Dehkordi, M.; Nahvi, I.; Zarkesh-Esfahani, H.; Ghaedi, K.; Tavassoli, M. and Akada, R. (2008). Isolation of a Novel Mutantstrain of *Saccharomyces cerevisiae* by an Ethyl Methane Sulfonate-induced Mutagenesis Approach as a High Producer of

Bioethanol. J Biosci. Bioeng. 105, 403-408.

Mohandesi, N., Siadat, S. O. R., Haghbeen, K. and 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.

Oztop, H. N.; Hepokur, C. and Saraydin. D. (2009). Hydrogels for Immobilization of Invertase. J. Food Sci. 74(7), 45-49.

Persike, D. S.; Bonfim, T. B.; Santos, M. H. R.; Lyng, S. M. O.; Chiarello, M. D. (2002). Invertase and Urease Activities in the Carotenogenic Yeast *Xanthophyllo mycesdendrorhous* (formerly *Phaffiarhodozyma*). Bioresource Technol. 82(1), 79-85.

Qureshi, A. S.; Khushk, I.; Bhutto, M. A.; Dahot, M.U.; ul-Haq, I.; Bano, S. and Iqbal, H. (2012). Production and Partial Characterization of Invertase from *Mucorgeophillus* EFRL 03. African Journal of Biotechnol. 11(47),10736-10743.

Rashad, M. M. and 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 of Basic and Applied Sciences. 3(3), 1910-1919.

Shah, H. S., Patel, C. M. and Parikh, S. C. (2016). Production of Invertase from Bacteria by Using Waste Jaggery. The Microbes. 3, 19-23.

Sanchez, M. P.; Huidobro, J. F.; Mato, I.; Munigategui S. and Sancho, M.T. (2001). Evolution of Invertase Activity in Honey Over Two Years. J. Agric. Food Chem. 49(1), 416-422.

Whitaker, J. R. and Berhard, B. A. (1972). Experiments for an Introduction to Enzymology. The Whiber Press. Davis, Calif.

Yi, S. H.; Lee, D. H.; No, J. D.; Lee, J. W.; Lee, D.H. and Lee, J. S. (2006). Production of Intracellular Invertase from Alkalophilic and Thermophilc *Bacillus sp*. TA-11 in the Recombinant *E. coli*. Kor J Microbiol Biotechnol. 34, 318-322.

Yoon, M. H.; Choi, W.Y.; Kwon, S.J.; Yi, S. H.; Lee, D. H. and Lee, J. S. (2007). Purification and Properties of Intracellular Invertase from Alkalophilic and Thermophilic *Bacillus cereus* TA-11J. Appl. Biol. Chem. 50(4), 196-201.