J.Agric.Chem.and Biotechn., Mansoura Univ.Vol. 6 (10): 435 - 445, 2015

Enhancement of inulinase production from an *acetobacter* sp. In submerged cultures

Aida H. Afify; F.I.A. Hauka; M.M. Kassem and A.M. El-Sawah
Microbiol. Dept., Fac. Agric., Mansoura Univ., Egypt.

ABSTRACT

Recently, inulinases one of the most important enzymes which have gained much attention in industrial purposes. They can be applied for production of high fructose syrup from inulin and could reduce the process steps, resulting in cost reduction and increasing the amount of products. *Acetobacter* sp. produced extracellular inulinase *in vitro*. Maximum inulinase production was obtained at pH 7.0, 30^{0}C temperature, ammonium sulphate as nitrogen source, inoculum volume 5% (v/v), 1% sucrose as additional carbon source and 48 hr after inoculation. The enzyme showed maximum activity at pH 7 and 50 ^{0}C. The enzyme was stable more than 80% of its maximal activity at 30-50 ^{0}C after 30 min heat treatment.

Keywords: *Acetobacter* sp., inulinase, inulin, hydrolysis, high fructose syrup.

INTRODUCTION

Inulinases are industrial enzymes which have gained much attention recently (Tinrat *et al.*, 2010). They can be applied to produce of high fructose syrup and inulooligosaccharides from Inulin, which found in some plants as a reserve carbohydrate such as Jerusalem artichoke, dandelion, chicory, dahlia, and several other members of the family Compositae (Chi *et al.,* 2010) and composed of linear chains of β-(2,1) linked fructose residues with a terminal glucose molecule at the end of each fructose chain (Vandamme and Derycke, 1983).

Fructose as an alternative sweetener to sucrose in the food and allied industries has received so much attention due to the several health benefits associated with the consumption of fructose. It does not trigger the production of insulin by the pancreatic β cells hence ideal for diabetic patients. Fructose has also been found to increase iron absorption in children and also to stimulate calcium absorption in postmenopausal women. Furthermore, studies have shown fructose to prevent colon cancer due to the stimulation of the growth of Bifidogenic organisms in the small and large intestine. The current industrial production of fructose involves the combined action of three enzymes (α- amylase, amyloglucosidase and glucose isomerase) acting on starch in succession yielding about 55% fructose and 40% glucose. Fructose from this process is costly and practically not sufficient to meet the demand of the various fructose-utilizing industries (Heuvel *et al*., 2000; Durieux *et al.*, 2001; Sanchez-Lozada *et al*., 2008; Garuba *et al*., 2015)

Microbial inulinases can be divided into exo- and endo-acting enzymes according to their modes of action on inulin. Exoinulinases (β-d-fructan fructohydrolase; EC 3.2.1.80) successively split off terminal fructose units from the non-reducing end of inulin and also hydrolyse sucrose and raffinose, endoinulinases (2,1-β-d-fructan fructanohydrolase; EC 3.2.1.7) hydrolyse the

internal β-2,1-fructofuranosidic linkages to yield inulooligosaccharides as the main products, inulotriose, inulotetraose, and inulopentaose (Vandamme and Derycke ,1983; Ohta *et al*., 2002).

A number of fungal, yeast, and bacterial strains have been used for the production of inulinases. Very few studies have been devoted to bacterial inulinases, and very little information is available on their occurrence in nature and on their enzymatic properties (Allais *et al*., 1986 ; Selvakumar and pandey, 1999 ; Singh and Gill, 2006; Neagu and Bahrim, 2011). The present study was focused to study some factors affecting production and activity of inulinase by *Acetobacter* sp.

MATERIALS AND METHODS

Bacterial strain and medium:

Acetobacter sp. was obtained from Agric. Microbiol. Dept., Fac. of Agric., Mansoura Univ., Mansoura, Egypt (El-sawah, 2013 ; Afify *et al*., 2014). Bacterial strain was grown in Allais *et al*. (1986) medium. The medium consists of (g/l): $(NH_4)_2SO_4$, 0.5; $MgSO_4$ $7H_20$, 0.2 ; KH_2PO_4, 3.0 ; Inulin, 2.0 ; Mineral salts solution 2 ml,(Atlas, 1995) pH of the medium was adjusted to 7.0.

Preparation of inoculum:

Agar slants were inoculated with *Acetobacter* sp. and incubated at 30 ^{0}C for 24 hr. The growth on the agar slants was scraped, using 5 ml sterilized distilled water, then transferred to a flask containing 50 ml of the fermentation medium and incubated for 24 hr on a rotary shaker operating at 150 rpm at 30 ^{0}C. The resulting cell suspension was used for inoculation of fermentation medium employed for inulinase production.

Fermentation technique:

Inulinase production was carried out by submerged fermentation using 250 ml Erlenmeyer flasks, each containing 50 ml of the production medium. After inoculation with 2.5 ml of *Acetobacter* sp. inoculum, the flasks were then incubated at 30 ^{0}C in a temperature controlled rotary incubator-shaker operated at 150 rpm. After an incubation time the contents of the flasks were centrifuged at 5000 rpm for 20 min at 4 ^{0}C. Culture supernatant was used as a source of crude enzyme for further studies.

Enzyme assay:

The reaction mixture containing 0.1 ml crude enzyme and 0.9 ml of (0.1 M acetate buffer, pH 5) containing 0.2% inulin was incubated at 50 ^{o}C for 20 min. The reaction was inactivated immediately by keeping the reaction mixture at 100 ^{o}C for 10 min. The amount of reducing sugar in the reaction mixture was assayed by the method of Nelson (1944) modified by Somogyi (1952). One inulinase unit (U) was defined as the amount of enzyme that produces 1 µg of fructose per minute under the assay conditions used in this study.

Bacterial growth measurement:

The bacterial growth was measured as optical density at wave length 620 nm (Allais *et al*., 1987). Before reading, the suspensions always were diluted to give turbidity reading lower than 1.0.

pH determenation:

Values of pH were determined using a pH meter, model JENWAY 3505.

Effect of time course on inulinase production:

The crude enzyme preparations were taken after 6, 12, 24, 36, 48, 60, 72, and 84 hours for assaying inulinase activity.

Effect of additional carbon and nitrogen sources on inulinase production:

Seven carbon sources, i.e, glucose; galactose; fructose; lactose; maltose; sucrose and starch were added to the basal production medium containing inulin as an inducer at concentration of 1 % (w/v). Also, initial nitrogen source was replaced with different nitrogen sources as a sole nitrogen source at the same nitrogen level to study their effect on inulinase production. These nitrogen sources are $(NH_4)_2HPO_4$, $NH_4H_2PO_4$, NH_4Cl, KNO_3, peptone, beef extract and yeast extract.

Effect of initial pH, inoculum volume and incubation temperature on inulinase production:

For the pH study, the medium was adjusted to pH ranging from 4 to 9 by using 1N NaOH and 1N HCL. Inoculation of the main cultures was performed with five different inoculum volumes (1, 2.5, 5, 7.5 and 10%). Four degrees of incubation temperatures (25°, 30°, 35° and 37°C) were tested to determine the optimum temperature for inulinase production.

Effect of the pH optimum on inulinase activity:

The effect of pH on inulinase activity was investigated by incubating the crude enzyme at pH range 4.0-9.0 with acetate buffer (0.1 M, pH 4.0-5.5), phosphate buffer (0.1 M. pH 6.0-8.0) and glycin-NaOH buffer (0.1 M, pH 8.5-9.0). The inulinase activity was measured after incubation at 50°C.

Temperature optimum and stability of on inulinase:

The effect of temperature on inulinase activity was investigated by incubating the enzyme reaction at different temperatures (30-70°C). The temperature stability of the crude enzyme was carried out by heating the crude enzyme at 30-70°C in the absence of substrate for 30 min. Then the residual activity was determined at optimum conditions.

Statistical analysis:

The obtained experimental data were statistically analyzed using software of COSTAT.

RESULTS AND DISCUSSION

Effect of time course on inulinase production:

Maximal yield of enzyme was attained after 48 hours of incubation (Fig.1), then inulinase productivity decreased.

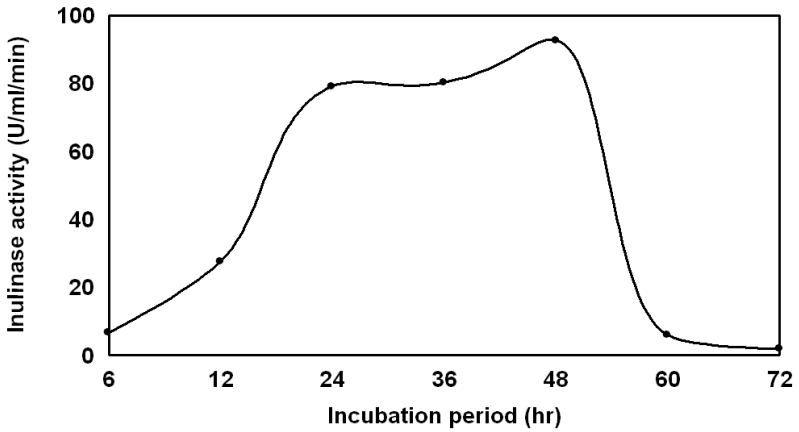

Fig. 1. Time course profile for inulinase activity.

Effect of additional carbon sources on inulinase production:

The results indicated in (Table.1) showed the effect of different carbon sources on inulinase production by *Acetobacter* sp. All carbon sources tested were added to the production medium containing inulin as an inducer at concentration of 1 % (w/v). The obtained results showed that the kind of used additional carbon sources greatly affected the yield of inulinase produced in culture fluids of the producer microorganism. Addition of sucrose promoted the highest inulinase production (105.61 U/ml/min), the inulinase production was increased by (12.96 U/ml/min) over the value of the control. On the other hand, other additional carbon sources inhibit inulinase production. In this connection, Gill *et al*. (2003) reported that maximal inulinase production by *streptomyces* sp. GNDU 1 was found in the presence of inulin while, the presence of simple sugars inhibits inulinase production. Naidoo *et al*. (2009) reported that the highest inulinase activity was found in sucrose grown culture by *Xanthomonas campestris* pv. Phaseoli. Zherebtsov *et al*. (2002) found that *Bacillus polymyxa* 722 and *Bacillus polymyxa* 29 displayed the maximal activity on a starch-containing culture medium, while the maximal activity of *Bacillus subtilis* 68 was observed in the presence of sucrose.

Table.1. Effect of additional carbon sources on inulinase production by *Acetobacter* sp.

Carbon Sources (1% w/v)	Final culture pH	Growth (O.D.)	Inulinase activity (U/ml/min)
Control (inulin 2g/L)	5.97	2.725	92.65
Glucose	5.19	1.570	1.67
Galactose	5.82	1.950	3.27
Fructose	5.29	0.985	1.90
Lactose	5.86	2.395	0.25
Maltose	5.73	2.505	3.96
Sucrose	4.97	1.900	105.61
Starch	6.14	1.765	0.73
LSD at 5%		0.027	0.321

Effect of nitrogen sources on inulinase production:

The original nitrogen source in the culture medium was replaced by different nitrogen sources at the same N-level. The results Table.(2) clearly indicated that the source of nitrogen greatly affected the yield of inulinase, it is obvious that none of substituted nitrogen sources yielded more inulinase in comparison with control source. The highest values of inulinase production by *Acetobacter* sp. were 105.38 U/ml/min in case of using $(NH_4)_2SO_4$. Zherebtsov *et al*. (2002) reported $(NH_4)_2HPO_4$ as the best source for inulinase production by *Bacillus polymyxa* 29, *Bacillus polymyxa* 722 and *Bacillus subtilis* 68. Naidoo *et al*. (2009) reported tryptone as the best source for inulinase production by *Xanthomonas campestris* pv. phaseoli KM 24. Gao *et al*. (2009) reported $(NH_4)H_2PO_4$ as the best source for inulinase production by *Bacillus Smithii* T7. Li *et al*. (2011) reported peptone as the best source for inulinase production by *Marinimicrobium* sp. LS-A18.

Table. 2. Effect of nitrogen sources on inulinase production by *Acetobacter* sp.

Nitrogen sources	Final culture pH	Growth (O.D.)	Inulinase activity (U/ml/min)
Control $(NH_4)_2SO_4$)	4.97	1.900	105.38
NH_4Cl	4.73	1.815	78.82
KNO_3	5.83	6.350	56.80
$(NH_4)_2HPO_4$	4.93	1.490	67.60
$NH_4H_2PO_4$	5.49	1.563	68.82
Peptone	6.65	3.950	70.54
Beef extract	6.76	4.036	68.40
Yeast extract	6.75	6.256	60.51
LSD at 5%		0.075	0.509

Effect of initial pH on inulinase production

The maintenance and control of pH is necessary for optimal enzyme formation. It is obvious from the results illustrated in (Fig.2) that pH of the growth medium greatly affected the inulinase production by *Acetobacter* sp..

The optimum pH for inulinase production seemed to be in the pH range 7.0-8.0. The maximum yield of inulinase was found at pH 7.0 (110.30 U/ml/min). The results showed also that there is gradual increase in enzyme production up to pH 7, then inulinase activity was decreased. There was negligible growth and enzyme production below pH 6. The obtained results are, indeed, in close agreement with the findings of Zherebtsov *et al*. (2002), Ayyachamy *et al*. (2007), Gao *et al*. (2009), Naidoo *et al*. (2009) and Li *et al*. (2011).

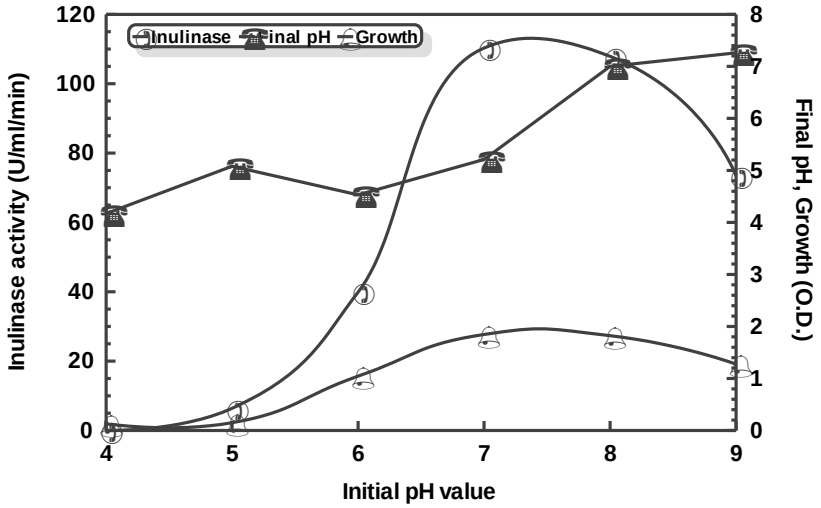

Fig. 2. Effect of medium initial pH on inulinase production by *Acetobacter* sp.

Effect of inoculum volume on inulinase production

Fig.3 showed the effect of inoculum volume on inulinase production by *Acetobacter* sp. Five different inoculum volumes (1, 2.5, 5, 7.5 and 10%) were testeded. The results showed that inulinase production reached its maximum value of 105.45 U/ml/min with 5% (v/v) inoculum. the inulinase production was gradually increased with the increase of inoculum volume up to 5% (v/v) and then decreased. Similary, Selvakumar and Pandey, (1999) found 3% inoculum volume to be sufficient for maximal extra-cellular inulinase production from *Staphylococcus* sp. While Ayyachamy *et al*. (2007) found 10% inoculum volume to be optimum for inulinase production from *Xanthomonas campestris* pv *phaseoli.*

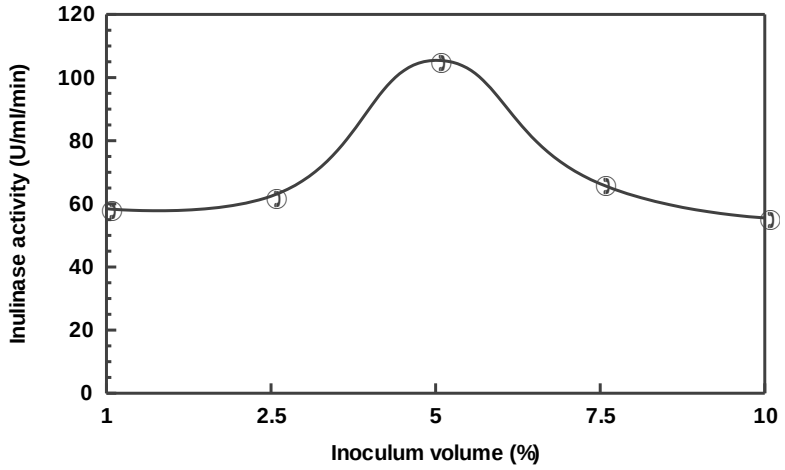

Fig. 3. Effect of inoculum volume on inulinase production by *Acetobacter* sp.

Effect of incubation temperature on inulinase production

The obtained data illustrated by Fig (4) show that the highest inulinase activity (103.20 U/ml/min) was reached at incubation temperature of 30°C. At higher or lower temperature a noteable decrease in inulinase production was observed. *Acetobacter* sp. was able to grow at 25–37 °C with maximum growth at 35 °C. These results are in harmony with those obtained by Selvakumar and Pandey (1999), Zherebtsov *et al*. (2002), Gao *et al*. (2009), Naidoo *et al*. (2009) and Li *et al*. (2011).

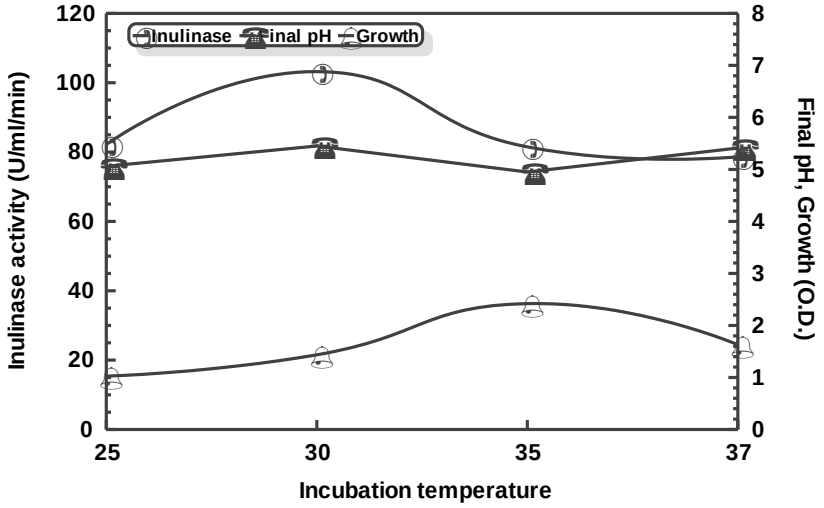

Fig. 4. Effect of incubation temperature on inulinase production by *Acetobacter* sp.

Effect of the pH of the reaction mixture on inulinase activity:

The effect of pH on inulinase activity was investigated by varying the reaction of the buffer solution in the experiment, acetate, phosphate and glycine-NaOH buffer were used to provide the pH range 4.0-9.0. The enzyme reaction mixture was incubated at 50°C. Data illustrated in Table (3) showed that the optimum pH for *Acetobacter* sp. inulinase was found to be 7.0. The enzyme was active in the pH range 5.0-7.5. The enzyme retained 95.13 and 96.62% of its relative activity at pH 6.5 and 7.5 respectively. However, the enzyme activity decreases to about 60.00 % at pH 4.5. The enzyme retained 75.16 and 71.89% of its activity at pH 8.0 and 8.5, respectively. This values decreases to be 64.23% at pH 9.0. The obtained results are in agreement with those obtained by Kang *et al*. (1998) reported that the enzyme produced by *Arthrobacter* sp. exhibited high activity at range of pH 5.0 – 10.5 and optimum was recorded at pH 7.5. Cho and Yun (2002) found that the enzyme produced from *Xanthomonas oryzae* No.5. reached the highest activity at pH 7.5, while Haraguchi (2010) found that the maximum activity of the enzyme produced by *Arthrobacter ureafaciens* D13-3 was obtained at pH 5.5.

Table.3. Effect of pH of the reaction mixture on the activity of inulinase *Acetobacter* sp.

pH of reaction mixture	Inulinase activity (U/ml/min)	Relative activity (%)
4.0	60.69	51.03
4.5	71.36	60.00
5.0	103.48	87.00
5.5	103.62	87.12
6.0	106.57	89.60
6.5	113.14	95.13
7.0	118.94	100.0
7.5	114.54	96.62
8.0	89.40	75.16
8.5	85.51	71.89
9.0	76.40	64.23

Effect of the temperature on inulinase activity:

The inulinase activities were examined in the temperature ranged from 30°C to 70°C using 0.1 M phosphate buffer pH 7.5. Data showed condesiderable enzymes activities at wide range of temperatures from 30°C to 70°C with optimum at 50°C. Data illustrated in (Table.4) show that enzyme activity increased with temperature within the range of 30°C - 50°C. A reduction in enzyme activity was observed at temperatures above 50°C. Inulinase activity showed the maximum value at 50°C this value increased by 31.32%, 25.63%, 25.59% and 2.72% over that value obtained at 30°C, 35°C, 40°C and 45°C, respectively. The activity was then decreased up 70°C to record the lowest value with decrease percent equal 39.16%. The obtained results are in agreement with those obtained by Kang *et al*. (1998) found that the optimum temperature for the enzyme produced by *Arthrobacter* sp. was 50°C. Cho and Yun (2002) found that the enzyme produced from

Xanthomonas oryzae No.5. reached the highest activity at 50°C also, Haraguchi (2010) found that 50°C was the optimum temperature for enzyme activity produced by *Arthrobacter ureafaciens* D13-3.

Table. 4. Effect of temperature on the activity of inulinase produced by *Acetobacter* sp.

Temperature of reaction (°C)	Inulinase activity (U/ml/min)	Relative activity (%)
30	81.68	68.68
35	88.46	74.37
40	88.50	74.41
45	115.70	97.28
50	118.93	100.0
55	117.33	98.65
60	95.51	80.30
65	86.83	73.01
70	72.36	60.84

Effect of heat on inulinase stability:

The crude enzyme was heated at 30-70°C in the absence of substrate for 30 min. After cooling, the remaining activity was assayed in optimum assay conditions as mentioned before. The results in Table (5) showed that the enzyme did not affect by heating at 30°C for 30 min comparing with control. The enzyme was stable at 30–50°C more than 80% of their maximal activities, the enzyme retained 86.24% and 81.68% of the original activity after being heated at 40°C and 50°C for 30 min, in the absence of substrate, respectively. The activity was then decreased to record the lowest value with decrease percent equal to 60.35% at 90°C heat treatment. The obtained results are in agreement with those obtained by Kang *et al*. (1998).

Table.5. Effect of heat on the stability of inulinase produced by *Acetobacter* sp.

Temperature (°C)	Residual activity (%)
Control (without heating)	100.0
30	100.0
40	86.24
50	81.68
60	74.51
70	52.81
80	46.18
90	39.65

REFERENCES

Afify, Aida H.; M.M. Kassem,.; F.I.A. Hauka and A.M. El-Sawah (2014). Isolation and identification of inulinolytic bacteria. The 1st Mans.Inter.Food Con.November.

Allais, J ; G.H. Lopez ; S. Kammoun and J.C. Baratti (1987). Isolation and characterization of thermophilic bacterial strains with inulinase activity. Appl. Environ. Microbiol. 53 (5): 942-945.

Allais, J; S. kammoun; P. Blanc; C. Girard, and J. C. Baratti (1986). Isolation and characterization of bacterial strains with inulinase activity . Appl. Environ. Microbiol. 52:1086-1090.

Atlas, R. (1995). Handbook of media for environmental microbiology. CRC Press, Inc., Florida , USA.

Ayyachamy, M.; K. Khelawan ; D. Pillay ; K. Permaul and S. Singh (2007). Production of inulinase by *Xanthomonas campestris* pv phaseoli using onion (*Allium cepa*) and garlic (*Allium sativum*) peels in solid state cultivation. Lett. Appl. Microbiol. 45: 439-444.

Chi, Z.M ; T. Zhang ; T.S Cao ; X.Y Liu ; W. Cui and C.H. Zhao (2010). Biotechnological potential of inulin for bioprocesses. Bioresource Technol. 102: 4295–4303.

Cho, Y.J. and J.W. Yun (2002)..Purification and characterization of an endoinulinase from *Xanthomonas oryzae* No.5. Process Biochem. 37: 1325-1331.

Durieux A. ; C. Fougnies ; H. Jacobs ; J.P. Simon (2001). Metabolism of chicory fructooligosaccharides by biofidobacteria, Biotechnol Lett. 23: 1523– 1527.

El-sawah, A.M. (2013). Studies on inulinase producing bacteria. M.Sc. Thesis, Fac. Agric., Mansoura Univ., Mansoura, Egypt.

Gao, W ; Y. Bao ; Y. Liu ; X. Zhang ; J. Wang and L. An (2009). Characterization of thermo-stable endoinulinase from a new strain *Bacillus smithii* T7. Appl. Biochem. Biotechnol. 157: 498-506.

Garuba O.E. ; A.A. Onilude and A.K. Oyinlola (2015). Isolation and molecular characterization of inulinaseproducing thermophilic fungi from waste dumpsite located at apete-awotan in Ibadan, South-Western Nigeria. Innov. Rom. Food Biotechnol. 16: 31 – 36.

Gill, P.K.; A.D. Sharma; R.K. Harchand and P. Singh (2003). Effect of media supplements and culture conditions on inulinase production by an actinomycetes strain. Biores. Technol., 87: 359-362.

Haraguchi, K. (2010). Inulin fructotransferase (DFAIII) producing from *Arthrobacter ureafaciens* D13-3. Carbohydr. Polym. 82: 742-746.

Heuvel E.G. ; M.H.C. Schoterman ; T. Muijis (2000). Transgalactooligosaccharides stimulate calcium absorption in postmenopausal women, Journal of Nutrition. 130: 2938–2942.

Kang, S.I ; Y.J. Chang ; S.J. Oh and S.I. Kim (1998). Purification and properties of an endo-inulinase from an *Arthrobacter* sp. Biotechnol. Lett. 20: 983–986.

Li, A. X ; L. Z. Guo and W. D. Lu (2011). Alkaline inulinase production by a newly isolated bacterium *Marinimicrobium* sp. LS–A18 and inulin hydrolysis by the enzyme. World J Microbiol Biotechnol. 28: 81-89.

Naidoo, K ; M. Ayyachamy ; K. Permaul and S. Singh (2009).Enhanced fructooligosaccharides and inulinase production by a *Xanthomonas campestris* pv. phaseoli KM 24 mutant Bioprocess Biosyst Eng. 32: 689-695.

Neagu, C and G. Bahrim (2011). Inulinases - A versatile tool for biotechnology. Innov. Rom. Food Biotechnol. 9: 1-11.

Nelson, N. (1944) A photometric adaptation of the somogyi method for the determination of glucose. J. Biol. Chem. 153: 375-380.

Ohta K ; N. Norio and T. Nakamura (2002). Purification and properties of an extracellular inulinases from *Rhizopus* sp. strain TN−96. J. Biosci. Bioeng. 94 :78–80.

Sanchez-Lozada L.G. ; M.M. Segal ; R. J. Johnson (2008). How safe is fructose for persons with or without diabetes?. Am. J. Clin. Nutr. 88: 1189–90.

Selvakumar, P and A. Pandey (1999). Solid state fermentation for the synthesis of inulinase from *Staphylococcus* sp. and *Kluyveromyces marxianus*. Process Biochem. 34: 851–855.

Singh, P. and P.K. Gill (2006), Production of Inulinases. Food Technol. Biotechnol. 44 (2) 151-162.

Somogyi, M (1952) Notes on sugar determination J. Biol. Chem. 195*:* 19-23.

Tinrat, S ; K. Wongsariya and M.T. Chomnawang (2010)..Isolation of a novel inulinase producing bacteria from soil. The 8th International Symposium on Biocontrol and Biotechnology.

Vandamme, J.E and G.D. Derycke (1983). Microbial inulinases fermentation process, properties and applications. Adv. Appl. Microbiol. 29: 140-174.

Zherebtsov, N. A.; S. A. Shelamova and I. N. Abramova (2002). Biosynthesis of Inulinases by *Bacillus* Bacteria. Appl. Biochem. Microbiol. 38: 544–548.

تحسين إنتاجية إنزيم الإنيولينيز من بكتريا الأسيتوباكتر فى المزارع المغمورة.

عايدة حافظ عفيفى ، فتحى اسماعيل على حوقه ، محمد منصور قاسم و أحمد محمود السواح.

قسم الميكروبيولوجيا – كلية الزراعة – جامعة المنصورة – المنصورة – مصر .

جذبت إنزيمات الإنيولينيز انتباه الكثيرين ، نظراً لاستخدامها فى الأغراض الصناعية كإنتاج شراب الفركتوز ، حيث أمكن باستخدامها تقليل خطوات العملية الإنتاجية مما أدى لخفض التكلفة وزيادة كمية المنتج. استهدفت هذه الدراسة تعظيم إنتاج إنزيم الإنيولينيز من بكتريا الأسيتوباكتر ، وكذلك دراسة بعض خصائص الإنزيم المنتج ، وقد أوضحت الدراسة الأتى : تم الحصول على أعلى كمية من الإنزيم بعد ٤٨ ساعة من التحضين ، لوحظ ارتفاع إنتاج إنزيم الإنيولينيز عندما استخدم السكروز كمصدر إضافى للكربون ، كانت كبريتات الأمونيوم هى أفضل مصادر النيتروجين حثاً على إنتاج الإنزيم.، تم الحصول على أعلى إنتاج للإنزيم باستخدام حجم لقاح ٥%(ح/ح) ، تركيز أيون هيدروجين ٧ ،عند درجة حرارة ٣٠^{0}م ، كما تم دراسة خصائص الإنزيم المنتج حيث أظهر الإنزيم أقصى نشاط عند تركيز أيون هيدروجين ٧ ،ودرجة حرارة ٥٠ ^{0}م ، كما احتفظ الإنزيم المنتج بأكثر من ٨٠% من نشاطه بعد تسخينه لمدة ٣٠ دقيقة على درجات حرارة من ٣٠- ٥٠^{0}م.