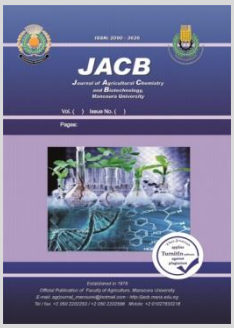
Journal of Agricultural Chemistry and Biotechnology

Journal homepage: www.jacb.mans.edu.eg
Available online at: www. jacb.journals.ekb.eg

Inulinase Production from Plant Materials by some Local Yeast Strains

Zeinab O. M. Othman[1*] ; A. E. I. Selim[1] ; Samia M. M. Bayoumy[1] and W. I. A. Saber[2]

[1]Department of Microbiology, Faculty of Agriculture, Mansoura University, Egypt.
[2]Department of Microbiology, Soils, Water and Environment Research Institute, Agricultural Research Center, Giza, Egypt.



ABSTRACT

Four inulinolytic yeast isolates were isolated and identified by molecular methods. They were belonged to *Candida catenulata, Sarocladium kiliense, Galactomyces candidum* and *Scopulariopsis brevicaulis.* The production of inulinase by the previous isolates through, 72h was done on two media using jerusalem artichoke tubers, garlic bulbs, dahlia tubers and chicory roots as natural sources of inulin. The highest values of the produced inulinase were obtained on Bharathi *et al.* (2011) medium after 48h of incubation. Jerusalem artichoke proved to be the best inulin source for enzyme production by *Scopulariopsis brevicaulis* (38.22 µg/ml), followed by dahlia (32.45 µg/ml). The best substrate for inulinase production by *Sarocladium kiliense* was dahlia (29.21 µg/ml) followed by garlic (28.65 µg/ml). As for *Galactomyces candidum* the maximum enzyme production was obtained using jerusalem artichoke (34.96 µg/ml) followed by garlic (31.84 µg/ml). The best material for inulinase production by *Candida catenulate* (34.15 µg/ml) was dahlia, followed by garlic (31.40 µg/ml). It can be concluded that inulin containing plant materials could be employed as a carbon source for inulinase production, it offer advantage in comparison to purified substrate because it have low cost and high productivity.

Keywords: Inulinase - Yeasts - plant materials - fructose production

INTRODUCTION

Inulin is a linear polymer of β (2, 1)-linked fructose that occurs in tubers and root of reserve carbohydrate in helianthus tuberosus, onion, garlic, dandelion, burdock, jerusalem artichoke, dahlia and chicory (Rocha *et al*., 2006 and Pandey *et al*.,1999). The production of inulin in the world is estimated to be about 350,000 tons. Inulin is considered as a major source for ultra-high fructose syrups production, with oligofructose syrups and D-fructose content over 95%, (Rocha *et al*., 2006). Cho *et al*. (2001) mentioned that inulin could be an efficient source for producing inulo-oligosaccharides.

Inulinase (β-2,1-D fructan fructanohydrolase, EC 3.2.1.7) hydrolases inulin into fructose. It acts on the β-2,1 linkages. According to their mode of action inulinases are classified into endo and exoinulinase. Endoinulinase (2,1-β-D fructan fructanohydrolase; EC 3.2.1.7) break the bonds between fructose units which located away from the end of the polymer of inulin. On the other hand, exoinulinase (β-D-fructohydrolase; EC 3.2.1.80), split the terminal units of fructose in inulin, raffinose and sucrose to give fructose as a main product (Rawat *et al*.,2015). Inulinase has received much attention because it can be widely used for produce high fructose syrup and fuel ethanol from inulin (Gao *et al*.,2007).

Inulin containing plant materials could be employed as a carbon source for inulinase production, it offer advantage in comparison to purified substrates because it have low cost and high productivity (Mazutii *et al*., 2010). Inulinases can be produced by some yeasts such as strains of *Pichia* sp, *Kluyveromyces* sp, *Candida* sp and *Sporotrichum* sp, also *K. marxianus* produced commercially acceptable yields of the inulinase (Pandey *et al*.,1999). More inulinase can be produced by yeast strains than fungal and bacterial strains (Gong *et al*., 2007 and Sheng *et al*., 2008).

The objective of this work is to (A) isolate and identify some inulinolytic yeasts, (B) study inulinase production by the isolates using some plant materials as inulin sources.

MATERIALS AND METHODS

Materials:

- **Microorganisms:**

Among twenty yeast isolates isolated from rhizospheric region of jerusalem artichoke plants, four isolates were identified by molecular identification methods. The identified yeast strains were used for inulinase production from plant materials contained inulin. The isolates were maintained on the second fermentation medium at 4°C after incubated at 30°C for 48h. Isolates were subcultured monthly.

- **Used substrates:**

Four plant materials that contained inulin such as chicory roots, dahlia tubers, jerusalem artichoke tubers and garlic bulbs were used for enzyme production.

- Media used:

(Gao *et al*., 2007)medium.

It is used for isolation of inulinolytic yeast strains, it is composed of (g/L) polypeptone 20, yeast extract 10, inulin 20 and agar 20. Chloramphenicol 0.05 % was added as antibacterial. pH was adjusted at 5.0.

(Bharathi *et al*., 2011)medium 1 :

It is used to determine inulinase production by yeast isolates through 120 h. The medium composed of (g/L): polypeptone 20, yeast extract 10 and plant material 20. pH was adjusted at 5.0.

* **Corresponding author.**
E-mail address: zeinab.husien@gmail.com
DOI: 10.21608/jacb.2020.87000

(Bharathi *et al*., 2011)medium 2:

It is also used to study time course of enzyme production by yeast isolates through 120 h using the substrates as carbon source. It is composed of (g/l): dipotassium hydrogen phosphate 3.0, yeast extract 5.0 and plant material 40 pH was adjusted at 5.0.

Methods:

Isolation was done using serial dilution methods. Serial dilutions of soil samples were done in 0.9 % sodium chloride and plated on isolation medium agar plates. Plates were incubated at 30°C for 72 h and then plates were flooded with lugol's iodine solution for 2 min. Isolates that formed clear halo around the colonies were picked up and were considered as inulinolytic yeasts.

- **Preparation of inoculum:**

Isolates were grown on the second fermentation agar medium slants for 48 days at 30°C. To each slant 2 ml of sterilized distilled water were added and the growth was crushed and transferred into conical flask (250 ml) contains 50 ml of fermentation medium. Each 1ml of inoculum contains 3 X 10^6 cells.

- **Preparation of the used plant materials:**

Plant materials of jerusalem artichoke tubers, garlic bulbs, dahlia tubers and chicory roots were cleaned with tap water and dried completely at 80°C for 96 h. After grinded; the resulted powders were used as substrates for enzyme production.

Enzyme production using the two fermentative media through 120 h:

Each flask of erlenmeyer flasks (250 ml) was filled with 50 ml of the used fermentative medium supplemented with plant material. Flasks were inoculated with 2% of each inoculum and incubated for 120 h at 30°C. Flasks were withdrawn at 48, 72, 96 and 120 h. After centrifugation at 5000 rpm, the cell-free extract was obtained and used as a crude enzyme.

Determination of enzyme activity :

The inulinase activity was measured by the technique described by Gao *et al*. (2007). The liberated reducing sugars were determined by DNS method according to Miller (1959). The reaction mixture, containing 0.1 ml of cell-free extract and 0.9 ml of 2.0% inulin acetate buffer (pH 5), was incubated at 40°C for 30 min. The reaction was stopped by adding 1 ml of DNS (The reagent consisted of 1% dinitrosalcylic acid, 0.2 % phenol, 0.05 % of sodium sulphite, 1 % sodium Hydroxide and 20 % Rochell salt) and heated at 100°C for 10 min, then cooled in cold water. The color produced was measured at 575 nm against the reagent blank. Standard curve was made using glucose. One enzyme unit was defined as the amount of fructose released from inulin (µmol) per ml per min under the optimum conditions (Yuan *et al*., 2013).

All the experiments were performed triplicate and mean values were reported.

RESULTS AND DISCUSSION

Molecular identification of the selected yeast isolates:

In order to identify the selected yeast isolates, molecular identification was done by Sigma Scientific Services Co. The resulted nucleotide sequences were blasted in National Center for Biotechnology Information database (NCBI) (www.ncbi.nlm.nih.gov/blast) to identify the DNA sequence. Results of Molecular identification are illustrated in Fig.1 (a, b. c and d).

Scopulariopsis brevicaulis strain CBS 398.54 5.8S ribosomal RNA gene, partial sequence; internal transcribed spacer 2, complete sequence; and large subunit ribosomal RNA gene, partial sequence

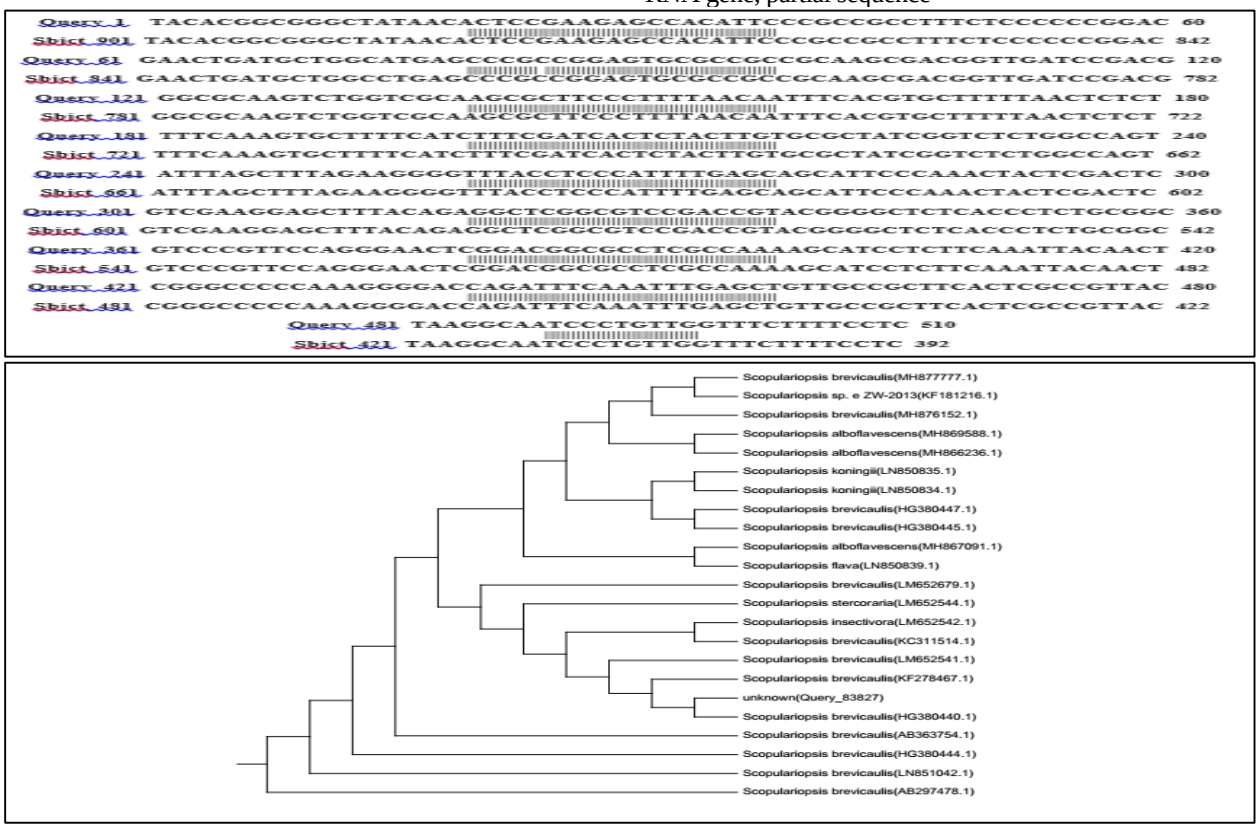
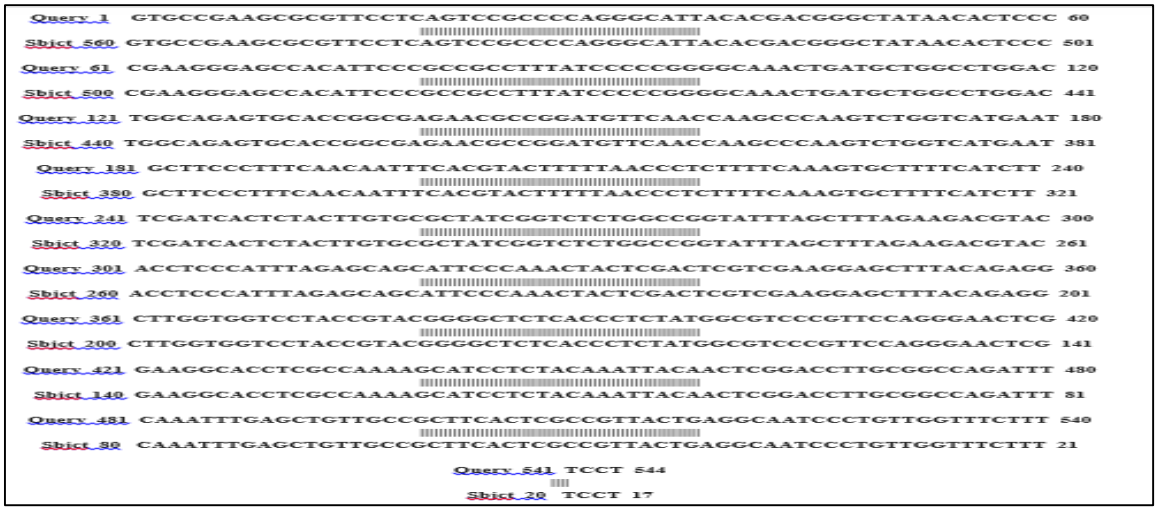


Fig. (1a). showed that isolate No (3) belonged to *Scopulariopsis brevicaulis*.



Sarocladium kiliense strain CBS 145.62 large subunit ribosomal RNA gene, partial sequence

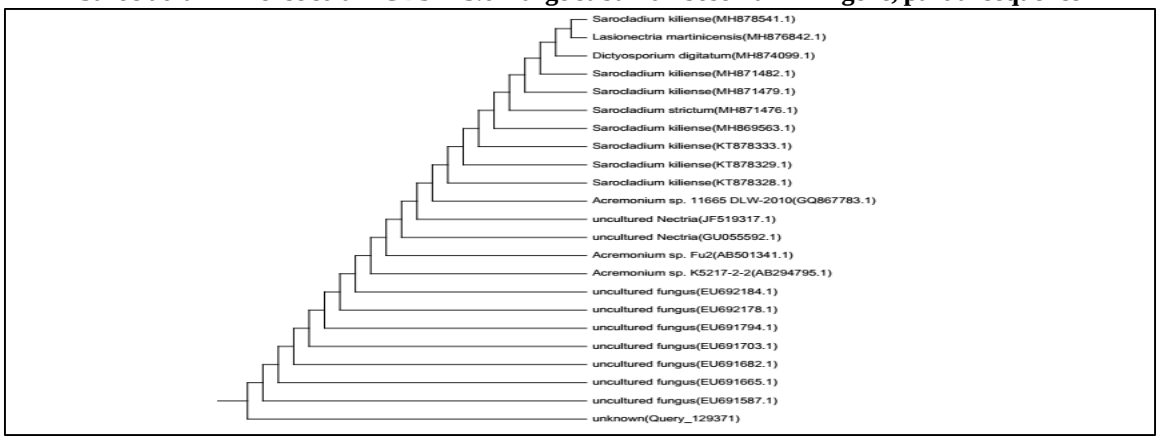
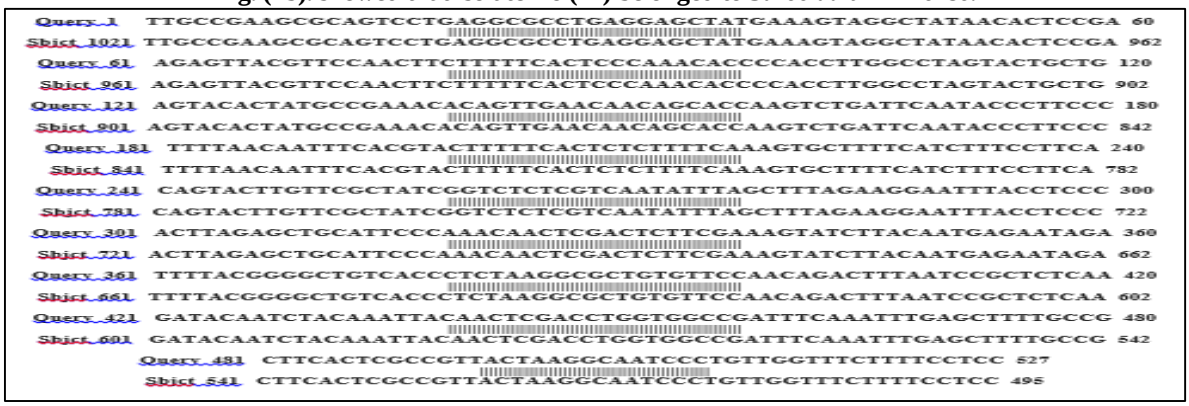


Fig. (1b). showed that isolate No (11) belonged to *Sarocladium kiliense*.



Galactomyces candidum strain OMON-1 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene and internal transcribed spacer 2, complete sequence; and large subunit ribosomal RNA gene, partial sequence

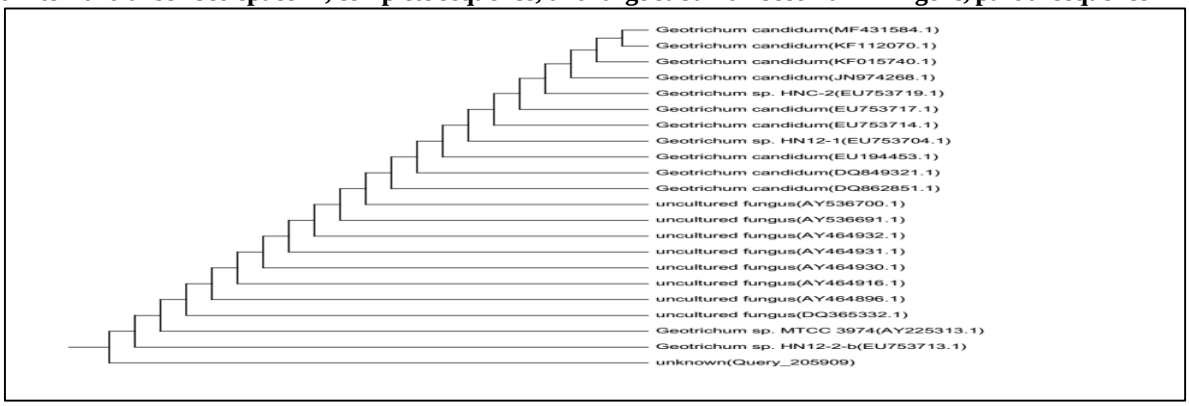
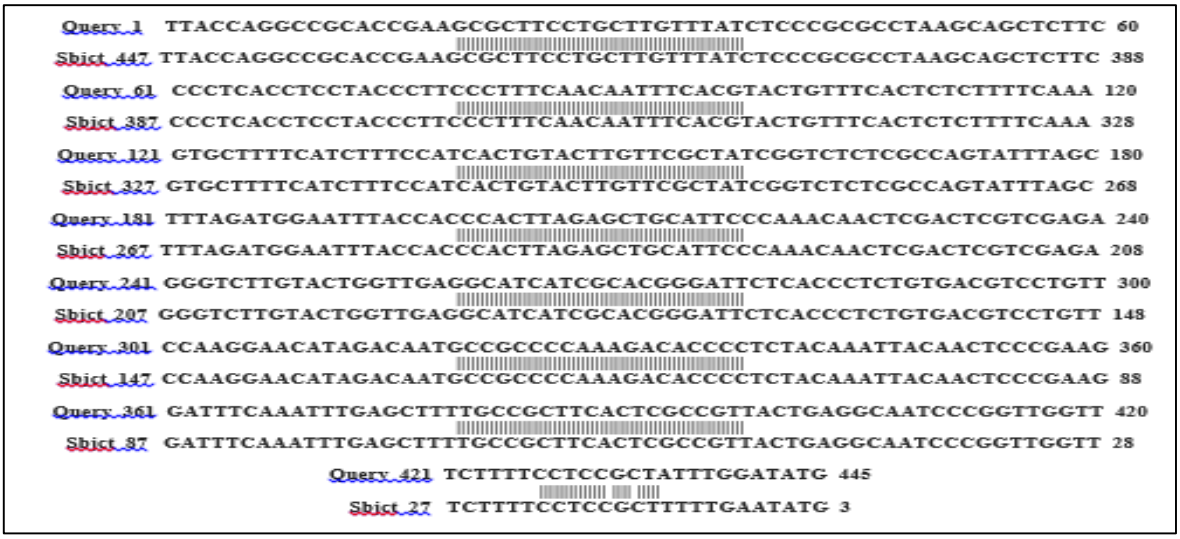


Fig. (1c). showed that isolate No (12) belonged to *Galactomyces candidum*.



Candida catenulata strain QD16.1 26S ribosomal RNA gene, partial sequence

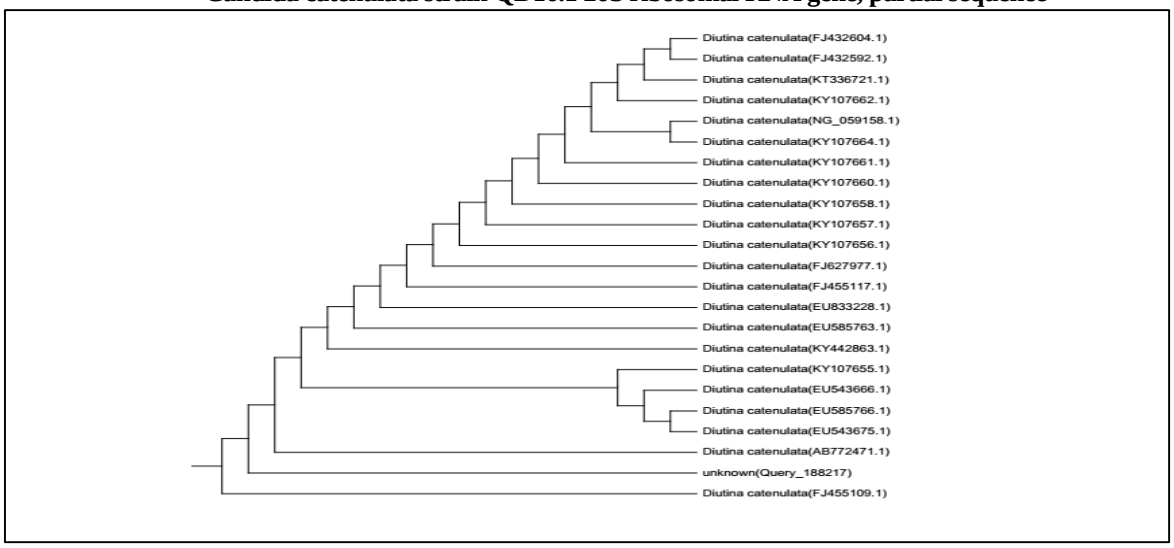


Fig. (1d). showed that isolate No (15) belonged to *Candida catenulata*.

Results presented in Figs. (2 & 3) showed inulinase production by *Scopulariopsis brevicaulis* using different substrates through 120 h. On the first fermentative medium results (Fig. 2) showed that dahlia is the best substrate (26.31 µg/1ml) at 96 h followed by dahlia after 72h (25 µg/1ml), garlic after 72h (24.38 µg/1ml), jerusalem artichoke (23.47 µg/1ml).

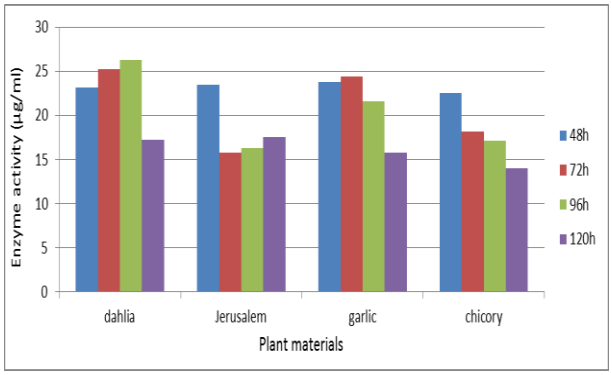


Fig. 2. Production of inulinase by *Scopulariopsis brevicaulis* medium (1).

While by using the second fermentative medium (Fig. 3), the best source for enzyme production was jerusalem artichoke (38.22 µg/1 ml) after 48h of incubation followed by the same plant substrate after 72h.

It is clearly showed that the production of inulinase by the two media was decreased by increasing incubation period and the highest values of production were obtained after 48 h with the four substrates. Also, the second medium proved to be the best for enzyme production by *Scopulariopsis brevicaulis*.

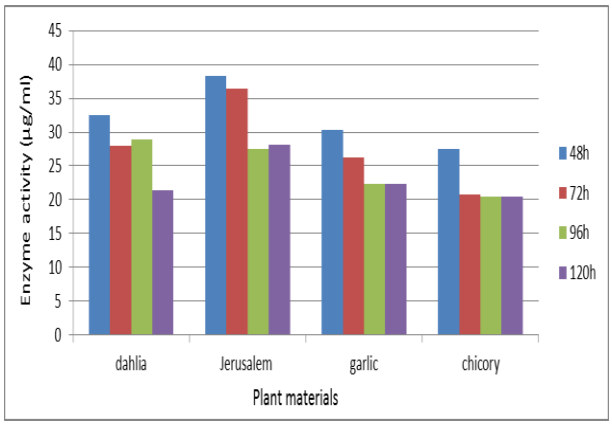


Fig. 3. Production of inulinase by *Scopulariopsis brevicaulis* on medium (2).

Results presented in Fig. 4 and 5 showed inulinase production by *Sarocladium kiliense* using different substrates through 120 h. On the first fermentative medium results (Fig. 4) showed that the highest value was obtained with garlic (25.96 µg/ml) after 48 h followed by chicory at 48 h (23.97 µg/ml). Enzyme production decreased by increasing incubation period except with jerusalem plant.

While on the second fermentative medium, the best value of inulinase by *Sarocladium kiliense* was obtained on medium supplemented with dahlia after 48 h (29.21 µg/ml) followed by garlic (28.65 µg/ml), chicory after 72 h and 48 h(26.33 µg/ml,25.00 µg/ml,respectively).

Results presented in Figs. (5&6) showed inulinase production by *Galactomyces candidum* using different substrates through 120 h. On the first fermentative medium results showed that the highest value of inulinase produced was obtained after 96 h with garlic (29.98 µg/ml), dahlia after 96 h (29.48 µg/ml).

Results in Fig. (6) showed inulinase produced by *Galactomyces candidum* grown on the second fermentative medium. The maximum enzyme production was obtained after 72 h where medium was supplemented with jerusalem artichoke (34.96 µg/ml) followed by the same plant after 48 h.

Data showed that by increasing time of incubation enzyme production increased with using either first or second fermentative medium.

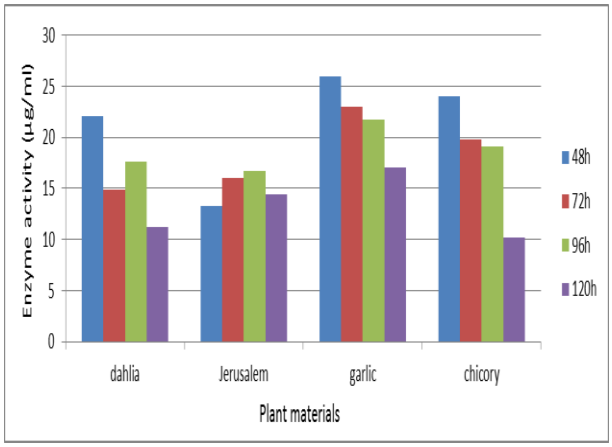


Fig. 4. Production of inulinase by *Sarocladium kiliense* on medium (1).

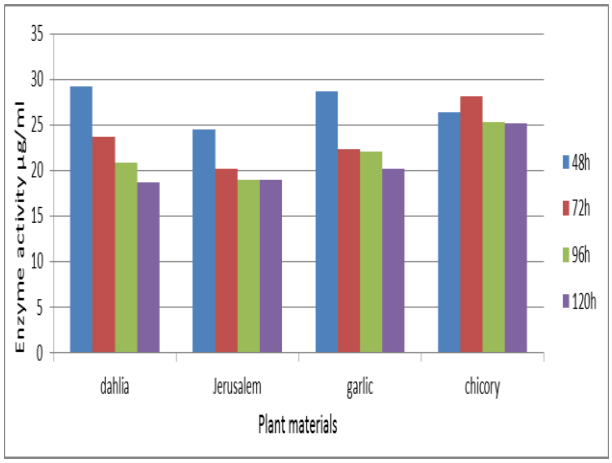


Fig.5. Production of inulinase by *Sarocladium kiliense* on medium (2).

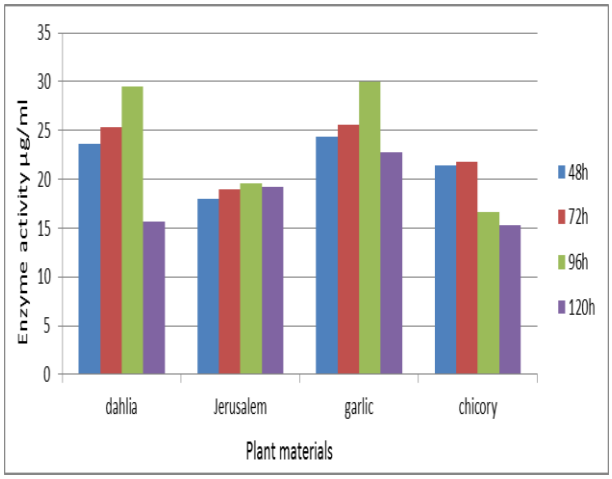


Fig. 6. Production of inulinase by *Galactomyces candidum* on medium (1)

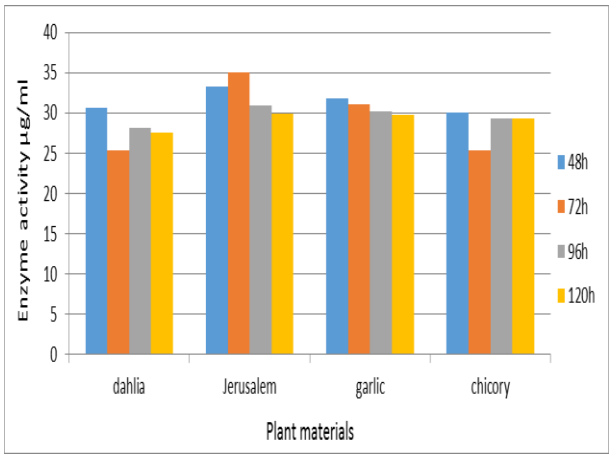


Fig. 7. Production of inulinase by *Galactomyces candidum* on medium (2).

Results presented in Figs. (8&9) showed inulinase production by *Candida catenulata* using different plant materials through 120 h. On the first fermentative medium results showed that dahlia (22.03 µg/ml) is the best source for inulinase production after 48h of incubation, it is followed by jerusalem artichoke (19.89 µg/ml), chicory (19.80 µg/ml) after 48 h. The production decreased through incubation period by increasing time of incubation.

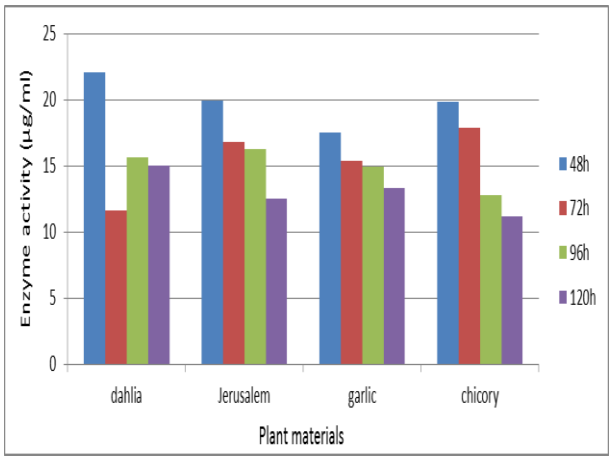


Fig. 8. Production of inulinase by *Candida catenulata* on medium (1).

Results in Fig. (9) showed that by using the second fermentation medium, dahlia was the best source for inulinase production. The highest enzyme production was obtained after 48 h of incubation with the four substrates. Dahlia is followed by garlic, jerusalem artichoke and chicory, the values were 34.15, 31.40, 31.07 and 28.00 µg/ml, respectively.

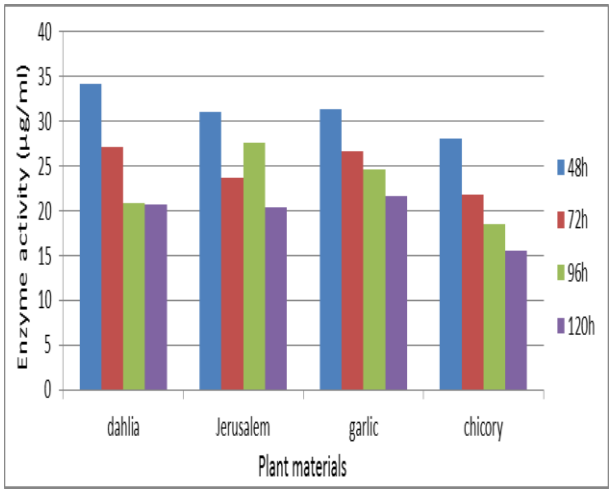


Fig. 9. Production of inulinase by *Candida catenulata* on medium (2).

It is observed that all plant materials used proved to be good source for inulinase production. Several investigators (Sguarezi *et al*., 2009; Ayyachamy *et al*., 2007 and Sharma *et al*., 2006) used various plant materials that contain inulin such as onion, garlic, corn steep liquor sugarcane molasses, rice bran, wheat bran, corn flour and coconut oil cake for inulinase production. From our results, it is noticed that, all the used inulin-containing plant materials showed interesting result for inulinase production, data is in agreement with those obtained by *Ongen-Baysal et al. (1994) & Park and Yun. (2001)*. Singh and Behermi (2008) & Singh *et al*. (2007a) used dahlia roots for producing extracellular inulinase from *Kluyveromyces marxianus* YS-1. Jain *et al*. (2012) obtained the highest inulinase activity by *Kluyveromyces marxianus* with dahlia extract as a carbon source, the enzyme was higher than that found in media contained pure chicory inulin. Sharma *et al*. (2006) used garlic bulbs for inulinase production from *Streptomyces* sp. Sumat *et al*. (2012) used dahlia extract for inulinase production by *Kluyveromyces marxianus* MTCC. Cruz *et al*. (1998) obtained higher activity of inulinase produced by *Asperginllus niger* – 245 with dahlia extract in compared to pure inulin. Abd El Aty *et al*. (2014) used chicory roots, jerusalem artichoke tubers, garlic peel, sugarcane bagasse, orange rinds, banana leaves as low cost substrates for inulinase production. Wenling *et al*. (1998) used extract of jerusalem artichoke for inulinase production by *Kluyveromyces* sp Y-85. The cost of the substrate has an important contribution to the overall inulinase production cost and it can be minimized by using cheaper plant materials.

As for fermentation periods, all isolates secreted high amounts of enzyme after 48h. Results are in agreement with those obtained by Vranesic *et al*. (2002), who found the highest values of inulinase produced by *Kluyveromyces bulgaricus* after 48 h of incubation. Gong *et al*. (2007) found that the maximum value of inulinase was produced by *Pichia guilliermondii* within 48h of fermentation. Among different substrates, maximum inulinase production was observed after 48 h. On the other hand, Singh *et al*. (2007b) showed that the highest value of inulinase produced by *Kluyveromyces marxianus* YS-1 was obtained after 60 h. Jain *et al*. (2012) found that the maximum production of inulinase by *Kluyveromyces marxianus* MTCC 3995 obtained on the fourth day of incubation using dahlia tuber extract.

REFERENCES

Abd El Aty A.A. , Wehaidy H.R. , Mostafa F.A. (2014). Optimization of inulinase production from low cost substrates using Plackett–Burman and Taguchi methods. Carbohydrate Polymers ,102: 261–268.

Ayyachamy M. , Khelawan k. , Pillay D. , Permaul K. , Singh S. (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.

Bharathi S. , Saravanan D. , Radhakrishnan M, Balagurunathan R. (2011). Bioprospecting of Marine Yeast with special reference to Inulinase production. International Journal of Chem Tech Research, 3(3): 1514-1519.

Cho, Sinha J. , Park J.P., Yun J.W. (2001). Production of inulooligosaccharides from chicory extract by endoinulinase from *Xanthomonas oryzae* No. 5. , Enzyme Microb. Technol; 28: 439–445.

Cruz V.A. , Belote J.G. , Belline M.Z. , Cruz R. (1998). Production and action pattern of inulinase from *Aspergillus niger*-245: Hydrolysis of inulin from several sources. Revista de Microbiol; 29: 301-306.

Gao L , Chi Z. , Sheng J. , Wang L. , Li J. and Gong F. (2007). Inulinase-producing marine yeasts: Evaluation of their diversity and inulin hydrolysis by their crude enzymes. Microbial Ecology; 1-16.

Gong F. , Sheng J. , Chi Z. and Li J. (2007). Inulinase production by a marine yeast *Pichia guilliermondii* and inulin hydrolysis by the crude inulinase. J. Ind Microbiol Biotechnol; 34:179–185.

Jain S.C. , Jain P.C. , Kango N. (2012). Production of inulinase from *Kluyveromyces marxianus* using dahlia tuber extract. Braz J Microbiol; 43 (1):62–69.

Mazutti M.A. , Zabot G. , Boni G. , Skovronski A. , de Oliveira D. , Luccio M.D, Rodrigues MI, Treichel H, Maugeri F (2010). Optimization of inulinase production by solid-state fermentation in a packed-bed bioreactor. J. Chem. Technol. Biotechnol; 85:109–114.

Miller G.L. (1959). Use of dinitrosalicylic acid reagent for determination of reducing sugars. Anal Chem; 31 (3): 426-429.

Ongen-Baysal, G. , Sukan, S.S. and Vassilev N. (1994). Production and properties of inulinase from *Aspergillus niger*. Biotechnology Letters; 16 (3): 275–280.

Pandey A. , Soccol C.R. , Selvakumar P. , Soccol V.T. , Krieger N. , Jose D. Fontana J.D. (1999). Recent developments in microbial inulinases, its production, properties and industrial applications. Appl. Biochem Biotechnol; 81:35–52.

Park J.P. and Yun J.W. (2001). Utilization of chicory roots for microbial endoinulinase production. Lett. Appl. Microbiol; 33: 183-187.

Rawat H.K. , Ganaie M.A. , Kango N. (2015). Production of inulinase, fructosyltransferase and sucrose from fungi on low-value inulinrich substrates and their use in generation of fructose and fructooligosaccharides. Antonie Van Leeuwenhoek doi:10.1007/ s10482-014-0373-3.

Rocha J.R. , Catana R. , Ferreira B.S. , Cabral J.M.S. , Fernandes P. (2006). Design and characterization of an enzyme system for inulin hydrolysis. Food Chem: 95:77–82.

Sguarezi C. , Longo C. , Boni G. , Silva M.F. , Luccio M.D. , Mazutti M.A. , Maugeri F. , Rodrigues M.I. , Treichel H. (2009). Inulinase Production by Agro-Industrial Residues: Optimization of Pretreatment of Substrates and Production Medium. Food Bioprocess Tech., 2: 409–414

Sharma A.D., Kainth S., Gill P.K. (2006). Inulinase production using garlic (*Allium sativum*) powder as a potential substrate in *Streptomyces* sp. J. Food Eng., 77: 486-491.

Sheng J., Chi Z.M., Gong F., Li J. (2008). Purification and characterization of extracellular inulinase from a marine yeast *Cryptococcus aureus* G7a and inulin hydrolysis by the purified inulinase. Applied Biochemistry and Biotechnology., 144: 111–121.

Singh R.S., Bhermi H.K. (2008). Production of extracellular exoinulinase from *Kluyveromyces marxianus* YS-1 using root tubers of Asparagus of cinalis. Bioresour Technol., 99 (15):7418–7423.

Singh R.S., Dhaliwal R., Puri M. (2007a). Partial purification and characterization of exoinulinase from *Kluyveromyces marxianus* YS-1 for preparation of high fructose syrup. Journal of Microbiology Biotechnology., 17(5):733–738.

Singh R.S., Sooch B.S., Puri M. (2007b). Optimization of medium and process parameters for the production of inulinase from a newly isolated *Kluyveromyces marxianus* YS-1. Biores. Technol., 98: 2518-2525.

Sumat C.J., Jain P.C., Kango N. (2012). Production of inulinase from *Kluyveromyces marxianus* using dahlia tuber extract. Brazilian Journal of Microbiology .,43: 1517- 8382.

Vranesic D., Kurtanjek Z., Santos A.M.P., Maugeri F. (2002). Optimisation of inulinase production by *Kluyveromyces bulgaricus*. Food Technol Biotechnol., 40:67–73.

Wenling W., Zhonghui Z., Yueying L., Xinsheng Z. (1998). Optimizing the culture conditions for higher inulinase production by *Kluyveromyces* sp. Y-85 and scaling-up fermentation. J. Ferment. Bioeng., 86: 395-399.

Yuan W. , Zhao X. , Chen L. , Bai F. (2013). Improved ethanol production in jerusalem artichoke tubers by overexpression of inulinase gene in *Kluyveromyces marxianus*. Biotechnology and Bioprocess Engineering ,18: 721-727.

انتاج الإنيولينيز من بعض المواد النباتيه باستخدام بعض سلالات الخميرة المحلية

زينب عثمان محمد عثمان[١] ، عبدالله العوضى ابراهيم سليم[١] ، ساميه محمد مرسى بيومى[١] و وسام الدين اسماعيل على صابر[٢]

[١] قسم الميكروبيولوجي – كلية الزراعة –جامعة المنصورة - مصر

[٢] قسم الميكروبيولوجي – معهد بحوث الأراضى والمياه والبيئة – مركز البحوث الزراعية – جيزة - مصر

تم عزل٢٠ سلالة خميرة واختيار أربعة عزلات منها منتجة للإنيولينيز وصُنفت باستخدام الطرق الجزيئية على أنها *Scopulariopsis brevicaulis* , *Sarocladium kiliense* , *Galactomyces candidum* , *Galactomyces candidum* , *Candida catenulate*. كما تم استخدام بعض الأجزاء النباتية المحتوية على الإنيولين والمتمثلة فى درنات نباتى الطرطوفة و الداليا و جذور السريس وبصلات الثوم فى إنتاج الإنزيم بواسطة العزلات خلال ١٢٠ ساعة على بيئتين مختلفتين وكانت أعلى معدلات إنتاج للميكروبات باستخدام المواد النباتية على البيئة الثانية وقد خُلصت نتائج البحث للآتى: كانت أعلى قيمة لإنتاج الإنزيم بواسطة ميكروب *Scopulariopsis brevicaulis* بعد٤٨ ساعة من التحضين (٣٨,٢٢ ميكروجرام / مل) باستخدام jerusalem artichoke تلاها (٣٢,٤٥ ميكروجرام / مل) باستخدام chicory dahlia. كانت أعلى قيمة للإنتاج الإنزيمى بواسطة ميكروب *Sarocladium kiliense* بعد ٤٨ ساعة وهى (٢٩,٢١ ميكروجرام / مل) مع dahlia و (٢٨,٦٥ ميكروجرام / مل) مع garlic. كذلك أعطى ميكروب *Galactomyces candidum* أعلى قيمة من الإنزيم (٣٤,٩٦ ميكروجرام / مل) مع jerusalem artichoke وذلك بعد ٩٦ ساعة و (٣١,٨٤ ميكروجرام / مل) مع garlic. كما كانت الكمية المنتجة من الإنزيم بواسطة ميكروب *Candida catenulate* أفضلها (٣٤,١٥ ميكروجرام / مل) مع dahlia تلاها (٣١,٤١ ميكروجرام / مل) مع garlic.وقد أوضحت الدراسة إمكانية استخدام المواد النباتية المحتوية على الإنيولين كمصدر رخيص للإنتاج الإنزيمى بدلاً من الإنيولين النقى مما يقلل من تكلفة انتاج الإنزيم اقتصادياً.