Inulinase Production from Plant Materials by some Local Yeast Strains

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ABSTRACT

Four inulinolytic yeast isolates were isolated and identified by molecular methods. They were belonged to Candida catenulata, Sarocladium kilense, Galactomyces candidum and Scopulariopsis brevicatulis. 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 Jerusalem artichoke (38.22 µg/ml), followed by dahlia (32.45 µg/ml). The best substrate for inulinase production by Sarocladium kilense 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 catenulata (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 roots (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 inulosaccharides. Inulinase (β-2,1-D fructan fructanohydrolase, EC 3.2.1.7) hydrolyzes inulin into fructose. It acts on the β-2,1 linkages. According to their mode of action inulinas are classified into endo and exoinulinas. 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). Inulinas 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 inulinas 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.
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(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 g/l. 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 1 ml 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 erlemeyer 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% dinitrosalicylic 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).

**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

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).

While by using the second fermentative medium (Fig. 3), the best source for enzyme production was jerusalem artichoke (38.22 µg/1ml) 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*.
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).](image)

![Fig. 5. Production of inulinase by *Sarocladium kiliense* on medium (2).](image)

![Fig. 6. Production of inulinase by *Galactomyces candidum* on medium (1).](image)

![Fig. 7. Production of inulinase by *Galactomyces candidum* on medium (2).](image)

![Fig. 8. Production of inulinase by *Candida catenulata* on medium (1).](image)

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 48 h 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.
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 et al. (1994) & Park and Yun. (2001). Singh and Behemi (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 Aspergillus 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 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


الإنتاج الإنزيمي من بعض المواد النباتية باستخدام بعض سلالات الخميرة المختارة

البحث يتم عن طريق استخدام المواد النباتية المحتوية على الإنزيمات، وذلك باستخدام بعض أنواع الخنافس ومشتقاتها.

-. Scopulariopsis brevicaulis
-. Candida catenulata
-. Galactomyces candidum
-. Galactomyces candidum
-. Sarocladium kiliense
-. Candida catenulata

10، 239 ميكروجرام / مل باستخدام Scopulariopsis brevicaulis تلاحظاً ميدالية في الإنتاجات الإجمالية في تركيبات ميكروجرام / مل مع Galltomyces candidum

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