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Improve of indulines enzyme productivity from recombinant *Escherichia coli* BL21 (DE3)

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Abstract

In the present study, the effects of process parameters on inulinase production by recombinant Escherichia coli BL21 (DE3) have been studied by pH of medium, incubation time, shaking speed of incubation (rpm), hydrolysis of Inulin and effect of metal ion in activity of enzyme. The results indicated that increased of production inulinase enzyme by change in parameter of which the optimal incubation time was 8 h, the maximal activity and specific activity under that condition was 7.5 Unit/ ml and 19.7 Unit/ mg, respectively. pH of the medium of maximum inulinase activity 8.3 Unit/ ml and specific activity 20.6 Unit/ mg were obtained at pH 7. Increase in the enzyme activity was observed as the shaking speed increase from 50-250 rpm, with the maximum inulinase activity at 200 rpm. It was revealed that the enzyme had the efficiency to hydrolyze 85% of 5% inulin solution when treated at 40 ºC for 80 min.

Keywords: inulinase, recombinant, enzymes, Inulin

Introduction

Enzymes that catalyze biosynthesis and promote the hydrolysis of carbohydrate polymers into monosaccharide fuel molecules are very diverse [1]. Among the hydrolase enzyme, inulinase, an inulin-hydrolyzing enzyme, there are two types of inulinases, endoinulinase (EC 3.2.1.7) and exoinulinase (E.C.3.2.1.80), and each is distinguished from other by the mode of action on inulin [2],[3]. Endoinulinase hydrolyzes the internal β-2,1-fructofuranosidic linkages to yield inulooligosaccharide, whereas exoinulinase cleaves off terminal fructose units from the nonreducing end of the inulin, and also hydrolyzes sucrose and raffinose [4],[5]. Exoinulinase has attracted great research interest to ability of the enzyme to high-fructose syrup produce that can be used in industrial application [6]. Though the inulinase was first isolated from plants, it is very difficultly isolated from plant also produce inulinase in insufficient quantities, whereas chemically acid hydrolysis of inulin to fructose has several demerits therefore interest towards the microbial inulinases [7]. Yeasts usually produce higher of the enzyme than other fungi and bacteria, Kluyveromyces is especially [8],[9]. The commercially main source of inulinase is microorganisms due to the ease of large scale cultivation and hydrolysis activity of high inulin [10]. In recent years, inulinases have a potential to use for various applications in biofuel, food, fermentation, pharmaceutical and chemical industries [11]. Applications of inulinases in various fields therefore encourage researchers to search for new sources of inulinases, cloning of inulinase genes and optimization for the production of inulinases [12]. Wang et al. cloned and expressed the endoinulinase gene from Aspergillusficuum in Escherichia coli BL21 (DE3), and showed the highest enzyme

activity 75.22 U/mg [13]. In previous study cloned and overexpressed inuA1 gene of Penicilliumjanthinellum in Pichiapastoris [14]. Furthermore, Gao et al. cloned the gene encoding exoinulinase from Paenibacilluspolymyxa ZJ-9 in Escherichia coli BL21 (DE3) [15]. In this paper, we report the factors that influence the increase of inulinase production from recombinant E. coli BL21 (DE3).

Material and Methods:

Strain, Culture media

The recombinant strain of E. coli BL21 (DE3)/ PET-28a used for investigation of inulinase expression was obtained from a previous study in Izmir Institute of Technology/ Turkey.

Prepared 100 ml of Luria Bertani Broth (LB) was composition from as follows: 0.5 g peptone, 0.5 g yeast extract and 0.5 g NaCl, after cooling the media at 55 ºC was added to 0.01 ml of Kanamycin.

Overnight culture was transferred to 100 ml of Luria Bertani Broth; bacterial density was determined by measuring density at 600 nm, it reached to 0.8 then incubated for 4 h. culture were centrifugation at 12,000 × g for 10 min. The pallet was resuspension by mixed with 0.05 M of phosphate buffer (pH 7), the suspension was disrupted by sonicator to produce the crude enzyme.

Inulinase assay

Inulin was added to 0.1 M acetate buffer pH 5 (0.3% w/v). 0.9 ml of Inulin solution was added to 0.1 ml crude enzyme and the tubes were kept in a boiling water bath for 10 min to inactivate the enzyme and then cooled to room temperature and added to 10 ml of distill water. Inulinase activity was determined using spectrophotometer at 450 nm by measuring the initial rate of fructose production. The reaction mixture was assayed for fructose content by the dinitrosalicylic acid DNS method [16]. Blank were run simultaneously with the enzyme and substrate solution. The inulinase activity was calculated from a standard curve of fructose solution. One unit of inulinase activity was defined as that catalyzes produce 1 µmol of fructose under the assay conditions. All the experiments were carried out in triplicate.

Optimization studies

Optimization of the production parameters were investigated for the production of inulinase as (a) incubation time, (b) different initial pH values of medium (5-8), (c) shaking speed of incubation (rpm).

Effect of metal ions on enzyme activity

Effect of metal ions on enzyme activity was investigated such as Mercury chloride (HgCl2), Potassium chloride (KCl), Cupric sulfate (CuSO4) and magnesium sulfate (MgSO4)

Hydrolysis of inulin

Hydrolysis of inulin was carried out under experimental conditions previous. 1 ml of recombinant enzyme was added to 10 ml of 5% inulin in 0.1 M sodium acetate buffer, and then incubated at 40 ºC, as followed hydrolyze of inulin by measuring in different time intervals of

20, 40, 60, 80 and 100 minutes, before measure the hydrolyze of inulin was stopped by heating the reaction in a boiling water bath for 10 minutes.

Results and Discussion.

The production of inulinase from the recombinant *Escherichia coli* BL21 (DE3) in different incubation periods were employed, maximuminulinase activity 5.8Unit/ ml and specific activity16.2Unit/ mgwere obtained at 8 h, after that decreased of enzyme productionas shown in Figure (1).Kumar*et al.* (2005) reported that theinulinaseactivity of maximum 79 Unit/ ml for a*Aspergillusniger* AUP19 at 72 h [17].

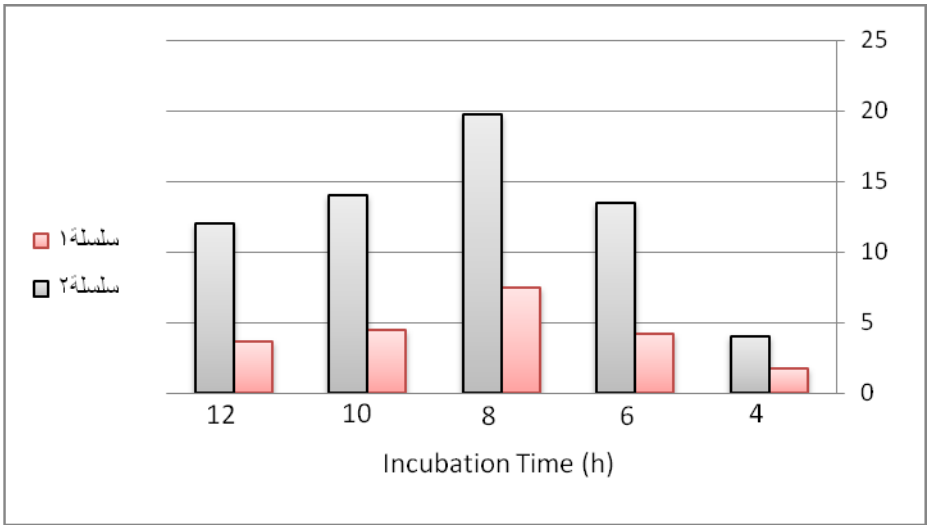

Figure (1): The Effect of incubation time on inulinase production from recombinant*Escherichia coli* BL21 (DE3)

Different initial pH values was investigated,therecombinant *Escherichia coli* BL21 (DE3)had ability to inulinase production with different pH values (5-8), the activity of enzymes related tocell growth was lower at low pH, which resulted inreduced growth rate of the strain.The enzyme production increased by increasing in pH of the medium to reachedmaximuminulinase activity 6.1Unit/ ml and specific activity17.3 Unit/ mgwere obtained at pH 7, as shown in figure (2). In previous studies, the inulinase gene cloned from*Paenibacilluspolymyxa* ZJ-9 in *Escherichia coli* BL21 (DE3), the recombinant enzyme showed maximum activity at optimum pH 6 [18]. In other study, the recombinant (from *E. coli* HB101) and native (from *Geobacillusstearothermophilus* KP1289)exo-inulinases were between pH 4.5-8.6, with maximum optimum atapproximately pH 6 [6].

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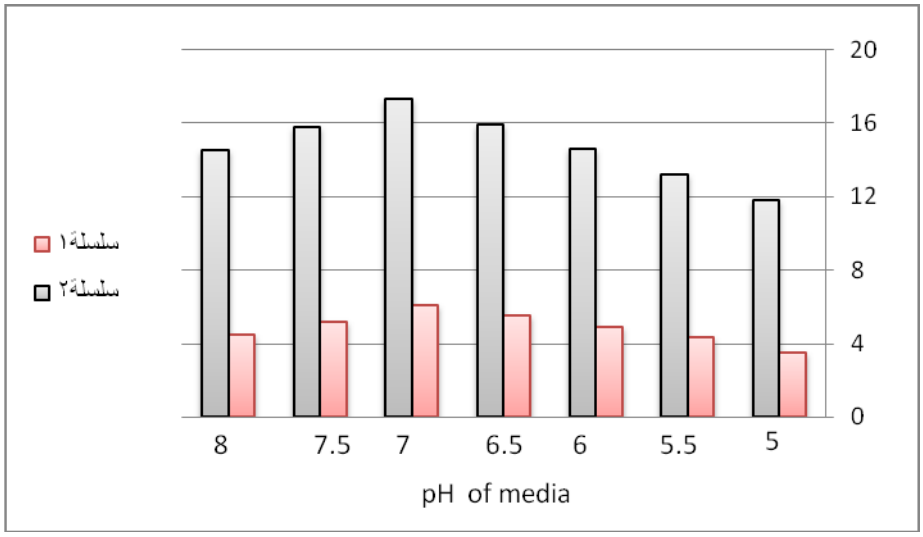

Figure (2): The Effect of pH on inulinase production from recombinant *Escherichia coli* BL21 (DE3)

Increase in the enzyme activity with increasing speed of shaking incubator to reach maximum inulinase activity at speed of incubator shaking (200 rpm) then it was observed decrease in activity at 250 rpm, shown in figure (3).Decrease inenzyme activity may be due to the shear stresscaused bythe impeller blades which effecton the microorganism's physiology and consequently, biomass formation and enzyme production [19]. The maximum inulinase activity was 47.3 Unit/ml at 200 rpm [20].

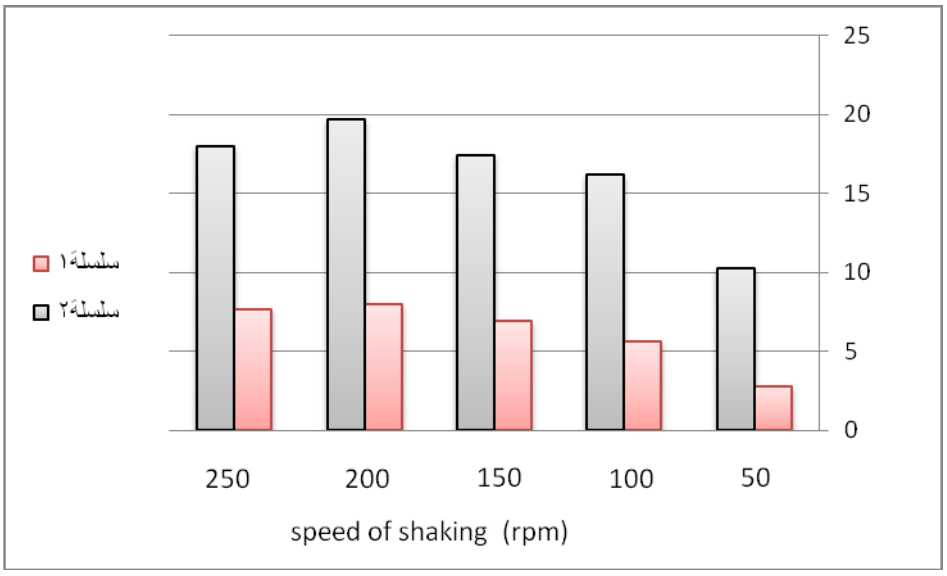

Figure (3): The Effect of speed of shaking incubator on inulinase production from recombinant *Escherichia coli* BL21 (DE3)

The effects of metal ions on purified ofinulinase activity was investigated, the results showed in table (1) the metal ions have stimulatory and inhibitory activity on inulinase enzyme, as Mg and K was stimulate enzyme activity whereas Hg and Cu inhibited enzyme activity. In previous studies, the inulinase gene cloned from*Paenibacilluspolymyxa* ZJ-9 in *Escherichia coli* BL21 (DE3), the recombinant enzyme showed that Cu^{+2}inhibited enzyme activitywhereasMg^{+2} stimulated the activity of the purified enzyme

[18]. Kwon *et al*. (2003) that suggests thiol group are essential for the catalytic activity of the enzyme [21].

Table (1): Effect of Various Metal Ions on Enzyme Activity

Metal salt	Concentration (mM)	Relative activity%
$HgCl_2$	5	3.54
	10	0.00
KCl	5	80.36
	10	88.02
$CuSO_4$	5	58.56
	10	74.31
$MgSO_4$	5	100.23
	10	117.9

Hydrolysis of inulin

Recombinant inulinase enzyme reactions were carried outat different time, which the results in figure (4) showed that hydrolysis of inulin in 20 minute about 29.9% to reached 90.41% in 100 minutes. In previous study, the maximum yield 79 % of inulooligosaccharides(IOS) was reached after8 h of hydrolysis of 400 g/ linulin [22], whereas 94.5% Hydrolysis of inulin have been previouslyreported after 24h using inulinase from*A.niger* [23].

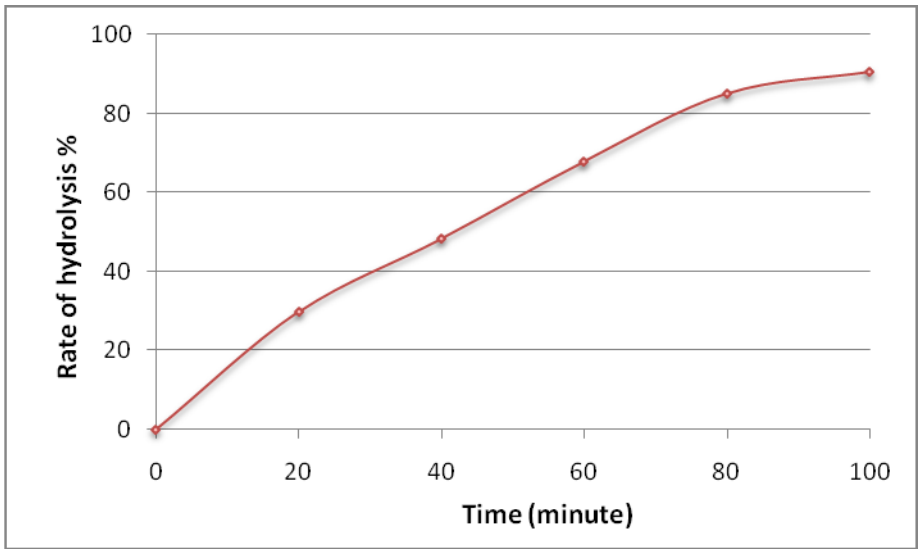

Figure (4): hydrolysis of inulin by inulinase produced from recombinant *Escherichia coli* BL21 (DE3)

Conclusion

Higher inulinase activity from recombinant *Escherichia coli* BL21 (DE3) was produced by change of some parameter as pH, incubation time and speed of shaking incubator. In other hand, some metal have stimulatory and inhibitory activity on inulinase enzyme activity

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