한국농화학회지 Vol. 27, No. 4, Dec. 1984
J. Korean Agricultural Chemical Society

Production of Inulase Using Jerusalem Artichoke Tuber Extract

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(Received Nov. 19, 1984)

돼지감자 추출물을 이용한 이눌라아제 생산

최원상 · 최용경 · 김수일* · 변시명

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초 록

Kluyveromyces fragilis No. 351을 이용하여 이눌라아제 생산을 최대로 할수있는 배지 조성 및 배양 조건을 조사하였다. 탄소원 및 inducer로는 돼지감자 추출액을 이용하였으며 전체 중량 3.5%가 가장 적합하였다. 일반적으로 많이 이용되는 유기 질소원인 yeast extract 보다 bactocasitone이 더 좋았고 2.0%에서 효소 생성이 최대로 되었다. 초기 pH 5.5 및 30°C가 알맞은 배양조건이었으며, $NH_4H_2PO_4$ 0.5% 첨가로 효소 생성이 증가되었다. 이눌라아제의 생성은 통기를 높여감에 따라 더욱 많이 생성되었고 intracellular inulase의 생성은 균체증식에 비례하여 증가되었다. Ultrafiltration과 에탄올 침전법을 이용하여 효소를 회수한 결과 72%의 수율을 얻을 수 있었다.

Introduction

Much works so far opened the room for potential utilization of Jerusalem artichoke tubers containing inulin, a fructose polymer, for production of fructose[1~5] or ethanol[6~10]. Hence inulase is required for this utilization of inulin or the artichoke tuber. There were some limitations, howerver, in the production of inulase, since inulases produced from the most of microorganism are inducible and inulin is required as inducer[6,11~15].

In this work, therefore, we aimed to determine the optimal conditions of the inulase production from *Kluyveromyces fragilis* using the Jerusalem artichoke tuber instead of using inulin as inducer.

Materials and Methods

1. Materials

The Jerusalem artichoke tuber and the other materials were the same as used in the previous works[1~3] and the artichoke tuber extract was also prepared as described previously[3].

2. Assays

Protein, sugar and inulin were determined by the methods of Lowry, *et al.*[16], Somogy-Nelson[17] and Kim[18], respectively. The determination of inulase activity and its preparation were carried out as described previously[11].

3. Determination of cell dry weight

Samples of 5 m*l* culture broth were contrifuged at 3,000×g for 15 min. After removal of the supernatant carefully, cells were washed with deionized water and dried in dry-oven at 105°C until constant weight was achieved.

4. Culture of microorganism

Kluyveromyces fragilis strain No. 351 was used as the source of inulase. Cultures were maintained on yeast-malt extract agar slants. The carbohydrates of the artichoke tubers which were extracted with boiling water were used as the carbon source instead of inulin. The seed culture was cultivated in 250 m*l* Erlenmeyer flask. The basal culture media contained 3.5% the artichoke tuber extract, 2.0% bactocasitone, and 1.0% $NH_4H_2PO_4$. The media was autoclaved at121°C for 20 min. The culture was carried out in 250 m*l* Erlenmeyer flask in a controlled Environment Incubator Shaker(Lab-Line Instruments, Inc.) for 36 hr at 30°C with shaking at 200 rpm. pH was adjusted to 5.5 and the inoculum size was 2.0 %. For examination of effects of various parameters on the production of the enzyme in terms of substrate, pH, temperature, N sources, and aeration, the basal culture media was modified in terms of corresponding parameters.

Batch culture was carried out in 500 m*l* glass vessel containing 350 m*l* medium with a New Burnswick Bio-Flo model C-30 chemostat. Incoming air was sterilized by passing through a glass wool filter. The inoculum size was 10 %.

5. Preparation of industrial grade enzyme

Culture broth containing inulase prepared from the batch culture described above was used. By ultrafiltration using a concentration unit (Amicon model 2,000) equipped with Diaflo-ultrafiltration membranes(type PM10), the volume of the enzyme solution was reduced to 1/3. The solution was clarified by the addition of ethanol cooled at −40°C (40 % final conc.) and operation was carried out at 0°C. The protein precipitated under these conditions was removed by centrifugation. Increasing the ethanol concentration to 80 %, the precipitated inulase was recovered by centrifugation at −4°C. The precipiate was resuspended in 0.1 M sodium acetate buffer, pH 5.0.

Results and Discussion

1. Effect of the artichoke tuber concentration of medium on the production of inulase

The composition of the artichoke tubers used for this experiment was shown in Table 1. Inulin content was 12.9 % of the fresh artichoke tubers and 53.1 % on the dry weight base.

Table 1. General compositions of Jerusalem artichoke tuber used for experiment

Artichoke tuber / Composition	Fresh (%)	Dry (%)
Moisture	80.7	17.0
Total sugar	14.3	59.0
Inulin	12.87	53.1
Free reducing sugar	1.18	3.3
Non reducing sugar	13.12	55.7
Crude protein	2.5	12.5
Fiber	0.83	3.75
Ash	1.3	6.3

Since inulase from *K. fragilis* is an inducible enzyme, the inulase activity was not detected considerably when the yeast is cultured with the medium containing glucose instead of inulin.[11,12,13,19]. Hence, the artichoke tuber extract was used as not only inducer but also carbon source of the medium. Fig. 1 shows the effect of the concentration of the artichoke tuber extract of the medium on growth and inulase

production. The optimal concentration was 3.5 % in the medium on the dry weight base. A slight inhibitory effect was noted above this concentration of the extract. This was similar to the values of others reported[11~13].

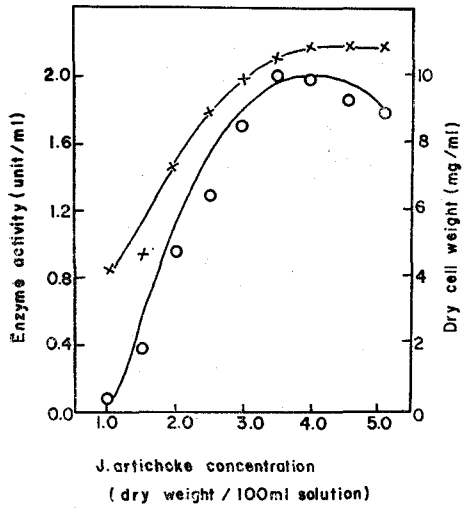

Fig. 1. Effect of the concentration of the Jerusalem artichoke tuber on the production of inulase
The medium composition was 2.0 % bactocasitone and 0.5 % $NH_4H_2PO_4$. ○—○ : extracellular enzyme activity and ×—× : dry cell weight.

2. Effect of initial pH of the medium

Fig. 2 shows the effect of initial pH of the medium on the growth and the production of inulase. The initial pH of the media varied between 4.0 to 7.0 before autoclave. Cell growth and enzyme production were greatly inhibited above pH 6.0. The pH between 5.0~5.5 was suitable for the cell growth and the enzyme production. The medium showed strong buffer capacity in this range.

3. Effect of temperature

The effect of temperature is depicted in Fig. 3. The temperature of media varied between 26°C and 38°C. Although there was no marked effect on the production of the intracellular inulase, extracellular activity was significantly affected by temperature. The suitable temperature for the growth and the enzyme production of the mesophile yeast, *K. fragilis*, was 30°C.

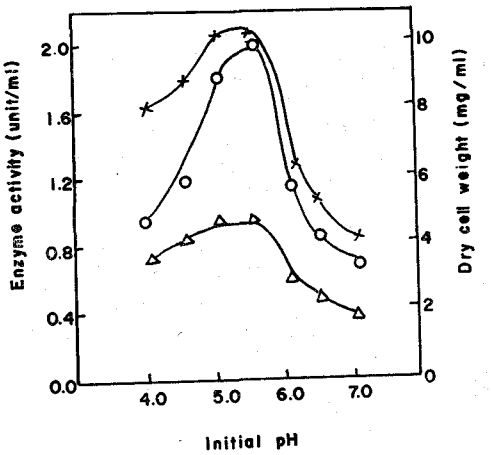

Fig. 2. Effect of initial pH of medium on the production of inulase
The medium composition was 3.5 % artichoke tuber, 2.0% bactocasitone and 0.5% $NH_4H_2PO_4$. ○—○ : extrac ellular enzyme activity, △—△ : intracellular enzyme activity and ×—× : dry cell weight.

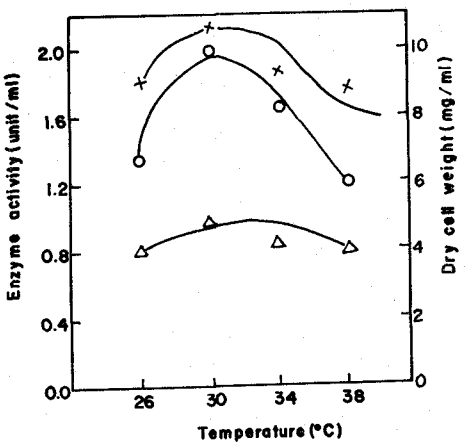

Fig. 3. Effect of temperature on the production of inulase
The medium composition was 3.5 % artichoke tuber, 2.0 % bactocasitone and 0.5 % $NH_4H_2PO_4$. ○—○ : extra cellular enzyme activity, △—△ : intracellular enzyme activity and × —× : dry cell weight.

4. Effect of nitrogen source

The nitrogen sources which can be utilized by microorganism probably include most, if not all, of the organic, and inorganic forms of nitrogen.

Table 2. Effect of organic nitrogens on the production of inulase

Nitrogen source	Enzyme activity (unit/m*l*)			Dry weight (mg/m*l*)	
	1%	2%		1%	2%
	Extracellular	Extracellular	Intracellular		
None	0.130	0.130	0.342	7.286	7.286
Yeast extract	1.214	0.821	0.542	9.402	10.842
Peptone	0.411	0.531	0.211	7.564	10.370
Isoelect. casein	0.865	1.166	—	—	—
Caseinric(milk)	0.212	0.840	—	—	—
Bactocasitone	1.940	1.989	0.956	9.758	10.476
Casamino acids	0.488	1.404	0.774	8.020	9.038
Malt extract	0.267	0.309	0.169	8.334	8.668
Urea	1.003	1.456	0.765	6.204	6.996
Bactotryptone	1.711	1.850	0.876	7.814	10.600
Neopeptone	0.258	0.630	—	—	—

Fermentation condition: initial pH 5.5, 36 hr and culture medium; 3.5 % artichoke tuber and 1.0% $NH_4H_2PO_4$.

Ten kinds of organic nitrogen wese tested (Table 2).

As shown in Table 2, bactocasitone, bactotryptone, casamino acids, and urea were effective on the production of inulase. The effect of bactocasitone on the production of inulase is shown in Fig. 4. The concentration of bactocasitone between 1.0 and 2.0% in the medium was suitable for inulase production.

The addition of $NH_4H_2PO_4$ was the most effective among the tested inorganic nitrogens (Table 3). It was also used as phosphorus requirement. NH_4NO_3, $NaNO_2$ and $NaNO_3$ exerted inhibitory effects on the growth and the production of the enzyme. The optimal concentration

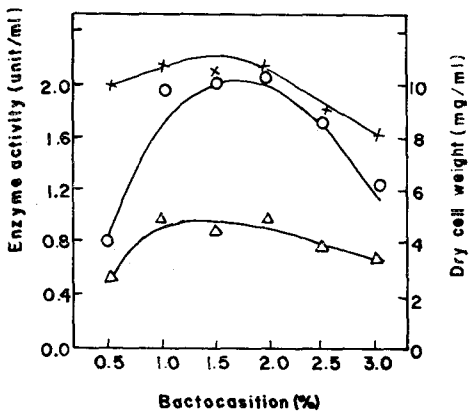

Fig. 4. Effect of bactocasitone on the production of inulase
The medium composition was 3.5 % artichoke tuber and 0.5 % $NH_4H_2PO_4$. ○—○ : extracellular enzyme activity, △—△ : intracellular enzyme activity and ×—× : dry cell weight.

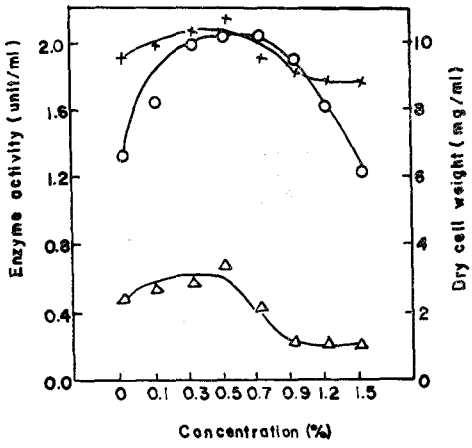

Fig. 5. Effect of ammonium phosphate monobasic on the production of inulase
The medium composition was 3.5 % the artichoke tuber and 2.0% bactocasitone. ○—○ : extracellular activity. △—△ : intracellular enzyme activity, and ×—× : dry cell weight.

Table 3. Effect of inorganic nitrogens on the production of inulase

Nitrogen source	Enzyme activity (unit/ml)			Dry weight	
	1%	0.5%		1%	0.5%
	Extracellular	Extracellular	Intracellular		
Control	1.333	1.333	0.596	9.980	9.740
$(NH_4)_2SO_4$	1.698	1.768	0.598	8.698	10.582
NH_4Cl	1.653	1.963	0.858	8.331	10.040
NH_4NO_3	0.293	—	0.833	8.782	9.496
$NaNO_2$	—	0.029	0.067	1.758	8.800
$(NH_4)_2HPO_4$	1.333	1.777	0.901	9.986	9.956
$NH_4H_2PO_4$	1.989	2.185	0.956	10.476	10.692
$NaNO_3$	0.326	—	0.817	8.816	9.418

Fermentation condition: initial pH 5.5, 36 hr and culture medium: 3.5 % artichoke tuber and 2.0 % bactocasitone.

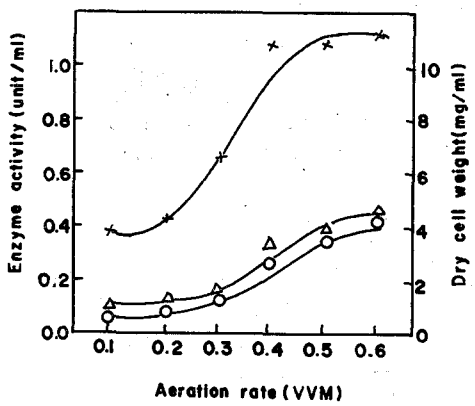

Fig. 6. Effect of aeration on the production of inulase
The medium composition was 3.5 % artichoke tuber, 0.5 % $NH_4H_2PO_4$, 2.0 % bactocasitone and 800 ppm PPG at 30°C for 8 hr culture. ○—○ : intracellular enzyme activity and ×—× : dry cell weight.

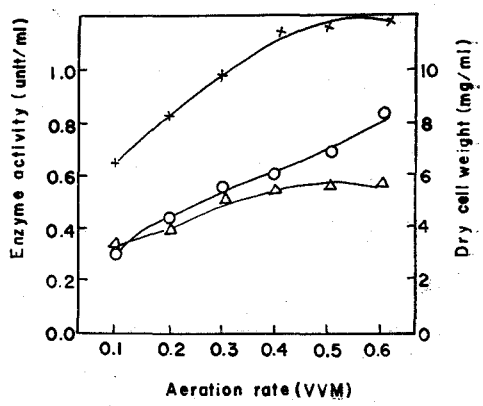

Fig. 7. Effect of aeration on the production of inulase.
The medium composition was 3.5% the artichoke tuber, 0.5% NH H PO, 2.0% bactocasitone, and 800ppm PPG at 30°C for 12 hr culture. ○—○ : the extracellular enzyme activity, △—△ : the intracellular enzyme activity and ×—× : dry cell weight.

of $NH_4H_2PO_4$ was 0.5 % as shown in Fig. 5 and a slight inhibitory effect was noted above this concentration.

5. Effect of aeration rate

Fig. 6 and. 7 show the effect of aeration rate in the fermentaor. Greater yields of the enzyme were obtained as the aeration rate increased. Nevertheless, we were not able to increase the aeration rate any more, since too much foam was produced, resulting over-flow and clogging of the airfilter. Because antifoaming agent (PPG) decreased the enzyme production, mechanical foam breaker or other antifoaming chemicals have been recommended to use. Eight hr culture showed the greater intracellular inulase activity than the extracellular one. But twelve hr culture, however, showed the opposite result. It reflects that the intracellular en-

zyme production proceeded to the cell growth and the extracellular inulase began to accumulate in the medium a little behind the cell growth.

6. Time course of the production of inulase

The time course of enzyme production, cell growth, and pH were investigated (Fig. 8). The intracellular inulase production proceeded parallel to the cell growth and diminished with the autolysis of cells, while the extracellular inulase began to accumulate in the medium a little behind cell growth and increased in the death phase. The pH increased and reached 6.0 at the stationary phase.

7. Preparation of industrial grade inulase

The procedure outlined above is simple and suitable for scale-up enzyme production. The overall recovery was as high as 72 % (Table 4). Although the specific activity increased only 4.5- and 3.3-fold for the extracellular and the intracellular enzymes, respectively, a relatively pure enzyme preparation was obtained. The inulase preparation was considered to be suitable for the industrial application.

8. Optimal culture condition

The optimized medium composition considered as the artichoke tuber 3.5 % (dry weight base), bactocasitone 2.0%, $NH_4H_2PO_4$ 0.5%. Using the composition of this medium, the maxmal activity 2.28 unit of extracellular enzyme activity and 1.20 unit of intracellular enzyme activity per ml were obtained. Compared with the other published results, the enzyme productivity increased significantly by the optmiization of the media. The dry cell weight per unit broth volume increased also about two-fold[1,13,20] The higher activities of inulase were primarily as a result of bactocasitone. Although intracellular enzyme activity using this media was lower than other media[20], the total activity was similar. It was not clear whether the difference is due to the extraction method or not because the temperature of enzyme assay is different.

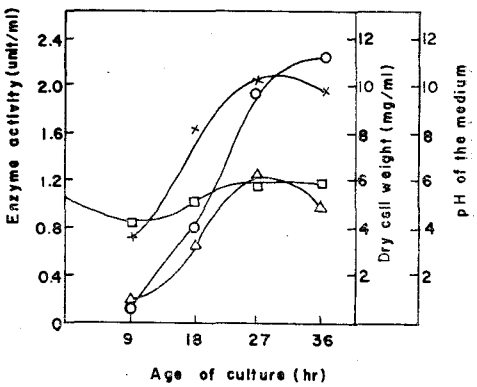

Fig. 8. Time course of enzyme production, cell growth, and pH
The medium composition was 3.5 % artichoke tuber, 0.5% $NH_4H_2PO_4$ and 2.0% bactocasitone. ○—○ : extracellular enzyme activity, △—△ : intracellar enzyme activity, ×—× : dry cell weight and □—□ : pH of the medium.

It is expected that the enzyme prcductivity will be greatly enhanced by control of pH, maintaining the constant level of the artichoke tuber extract, aeration and agitation using mechanical foam breaker or other antifoaming chemicals.

Table 4. Preparation of industrial grade inulase from *Kluyveromyces fragilis* No. 351

Purification step	Volume (m*l*)	Total protein (mg)	Total activity (unit)	Yield (%)	Specific activity (unit/mg protein)
Culture broth (cell harvested)	500(500)	2535.75(603)	783.5(483.5)	100	0.309(0.802)
Ultrafltration	150(150)	866.7(195.15)	665.9(433.5)	85(89)	0.768(2.221)
Ethanol precipitation	30(30)	406.7(128.2)	501.9(343.2)	72.9(71)	1.406(2.677)

The data in parenthesis are for the intracellular enzyme.

Abstract

To produce inulase from *Kluyveromyces fragilis* No. 351 using Jerusalem artichoke tuber extract, optimization of the condition was conducted. As results, the optimal concentration of artichoke tuber extract was 3.5 % and bactocasitone showed better production than yeast extract. The optimal temperature and pH were 30°C and 5.5, respectively. The addition of $NH_4H_2PO_4$ increased the enzyme production. Inulase synthesis was growth-associated and the enzyme production increased as con centration of dissolved oxygen increased. A higher quantity of industrial grade inulase was prepared by the combination of ultrafiltration and ethanol precipitation with 72% recovery.

Acknowledgements

This work was supported by the research fund of Korean Science and Engineering Foundation (KOSEF).

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