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Research article

Optimization of Fermentation Conditions for Trehalose Synthase Production by *Corynebacterium Glutamicum*

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Abstract

The maximum yield of trehalose synthase was obtained after optimizing the culture conditions by *Corynebacterium Glutamicum*. The medium adjusted to pH 7.0, incubated at 34℃ for 30h, inoculated with 1×10^8/mL, the velocity of rotary shaker was 200rpm and the culture volume was 70mL/250mL produced maximum trehalose synthase activity of 621U/mL.

Key words: trehalose synthase, trehalose, fermentation, optimization

Introduction

Trehalose, a disaccharide with non-reducing as metabolite of cell in hostile environment, was used in domains of

food, cosmetic, biological medicine and agricultural [1,2,3,4]. In nature, trehalose is synthesized by diverse organisms such as bacteria, fungi, invertebrates, algae, insects, and plants in response to stress. Trehalose accumulation in cells has been associated with improved tolerance to a number of stresses such extreme conditions as high temperature, salinity, drought and freezing[5,6,7,8,9]. Trehalose is found in cytoderm glycolipids of the genera *Nocardia, Rhodococcus, Mycobacterium and Corynebacterium*[10]. Trehalose synthase, one of important enzymes for tehalose production, converted reversibly maltose into trehalose by isomerizing a-1,4 bond to a-1,1 bond[11,12]. *Corynebacterium glutamicum* contains three pathways of trehalose synthesis, so it's considered to be an important strain which has potential values in producing trehalose.

There are many mathematic methods such as response surface methodology, orthogonal design and "one factor at a time" to optimize fermentation. This paper optimized the fermentation conditions and obtained maximum yield of trehalose synthase the using "one factor at a time" method because of none interaction among these factors. There are many parameters affecting trehalose synthase production by *C.glutamicum*. As we know, initial pH has effect on membrane permeability and the temperature has effect on both cell growth and enzyme activity. Factors such as velocity of rotary shaker and culture volume have effect on the O2 concentration of fermentation liquor. *C.glutamicum* was cultured in medium in rotary shaker for trehalose synthase production. We chose initial pH, incubation temperature, velocity of rotary shaker, culture volume, inoculum size and incubation time as main factors in fermentation to optimize for maximum yield of trehalose synthase.

Materials and methods

Organism and culture

C. glutamicum UP15 mutant was obtained from microbiology lab of Jiangxi agricultural university. The strain was cultured on LB slants at 30℃ and maintained at 4℃. DMCG I medium containing maltose was used to culture the bacterial strain and produce trehalose synthase.

Growth conditions

The growth parameters were optimized by varying one parameter at a time. The sequence of parameters optimized was: initial pH, incubation temperature, velocity of rotary shaker, culture volume, inoculum size and incubation time.

Assay of trehalose synthase activity

Trehalose synthase was extracted by centrifuging and the supernatant was used as an enzyme extract. The

reaction was carried out using buffered suspension (pH 7.0) with 2 ml enzyme extract in a tube at 30℃ for 30 min. The reaction was terminated by placing the tube in boiling water bath for 5 min. After cooling to room temperature, the reaction mixture was centrifuged (16000×g, 30min) and the trehalose production in the supernatant during the reaction was determined following the method described by Slade[13]. One unit(U/mL) corresponds to 1ug of trehalose per minute using maltose as substrate under standard conditions.

Optimization of parameters

The conditions were set different levels to cultivate strain for trehalose synthase production. Trehalose synthase activity was determined by determining the trehalose content under shake-flask culture for initial pH, incubation temperature, inoculum size, incubation time, culture volume, velocity of rotary shaker. All experiments were carried out in triplicate.

Results and discussion

Effect of initial pH

Initial pH of the culture plays a meaningful role in both cell growth and metabolite biosynthesis with affecting the permeability of biomembrane, cellular morphology, absorption of nutrients and the ability of product secretion. Different pH values (5.0-8.0) were examined in order to evaluate trehalose synthase yield. The maximum trehalose synthase production was 559U/mL at pH 7.0(Fig. 1). The optimum pH for trehalose synthase from *C.glutamicum* was pH 7.0, so it promoted the synthesis of trehalose synthase in cells under pH 7.0. It had an inhibitory effect on the synthesis of trehalose synthase in acidic or alkaline solutions because of the low activity in strong acid or alkaline condition. Trehalose synthase protein was easily to inactivate with low yield of enzyme in these conditions[14]. Controlling pH of fermentation liquor was important for trehalose synthase production by *C.glutamicum*.

Effect of culture temperature

Temperature is an important parameter which should be considered in optimizing fermentation conditions for trehalose synthase production[15]. To find the effect of culture temperature on the trehalose synthase production, cells were harvested at the late exponential growth phase and the temperature was set from 26℃-38℃. Maximum yield of trehalose synthase was 562U/mL at 34℃(Fig.2).

The amount of Trehalose synthase was higher in the range 32℃-34℃ than at temperatures below or over this range(Fig.2). Temperature not only affects the growth and metabolism of bacteria, but also affects the catalytic

activity of trehalose synthase. Higher temperature can cause the depravation of fermentation conditions to stimulate cells to accelerate the speed of trehalose synthase synthesis for high trehalose concentration to protect cytoarchitecture[16]. *C.glutamicum* didn't belong to thermophilic bacteria and may be hurt in metabolic pathways under megathermal condition with the decreased of trehalose synthase production. There were three pathways for trehalose production in *C.glutamicum* and TreS wasn't main pathway for trehalose synthesis under high temperature, that's good for the trehalose accumulation with high trehalose concentration because of reversible of trehalose synthase as suggested by Tae-Kyun Kim[17]. On the other hand, trehalose synthase concentration was decreased in cells at relative low temperature with decreasing of trehsalose in cell plasma.

Effect of velocity of rotary shaker

The effect of velocity of rotary shaker on fermentation was the control of O_2 supply. We evaluated the effect of different velocity (170rpm~230rpm) on trehalose synthase production and the results revealed that the maximum enzyme activity was 587U/mL at the velocity of 200rpm (Fig.3).

Effect of culture volume

The effect of culture volume on trehalose synthase production by shaking flask fermentation was reflected in the O_2 concentration of conical flask[18]. *C.glutamicum* belong to aerobic bacteria. Protein synthesis and other metabolite biosynthesis in cells were going well under adequate supply of O_2. This experiment examined the trehalose synthase yield with volume range of 40-130mL/250mL conical flask. Maximum enzyme activity was 601U/mL at the volume of 70mL/250mL conical flask(Fig.4).

Effect of inoculum size

Inoculum size was another important factor in affecting the outcome of the fermentation period[19,20]. Inoculum size played an important role in affecting the utilization rate of the nutrients in medium. The nutrients could be used up in the condition of large inoculum size. The trehalose synthase yield was insufficient with small inoculum size. The suitable inoculum size could effectively balance the cell number and the use of nutritional material in the culture. The maximum yield of trehalose synthase was 612U/mL at 10^8 /mL of inoculum size （Fig.5）. Thus, inoculum size was set to 10^8 /mL of the final medium volume.

Effect of incubation time

The reason of incubation time affecting trehalose synthase production was the different metabolic intensity in different culture period with the difference of accumulation of product. Time of trehalose synthase production

was recorded for 40h. Maximum trehalose synthase activity was 621U/mL observed at 30h, after which it decreased(Fig.6). Strain cells with short incubation had strong vitality with low yield because of the low number of cells. Although cells with long incubation time had the larger size, cells were ageing with a decline in meabolism to cause the low trehalose synthase yield. Some reported maximum trehalose synthase production were reported at exponential phase in *Corynebacterium sp.*.

Conclusion

The fermenting conditions such as initial PH, volume, incubation time etc. were studied, and the optimum conditions have been found. Our finding suggests that under the optimized conditions such as the volume of 70mL/250mL conical flask, the velocity of 200rpm, initial pH 7.0, culture temperature of 34℃, inoculum size of 10^8 /mL, incubation time of 30h. Maximum trehalose synthase production was 621U/mL after the optimization. It's higher than the former optimization of medium composition.

Acknowledgements

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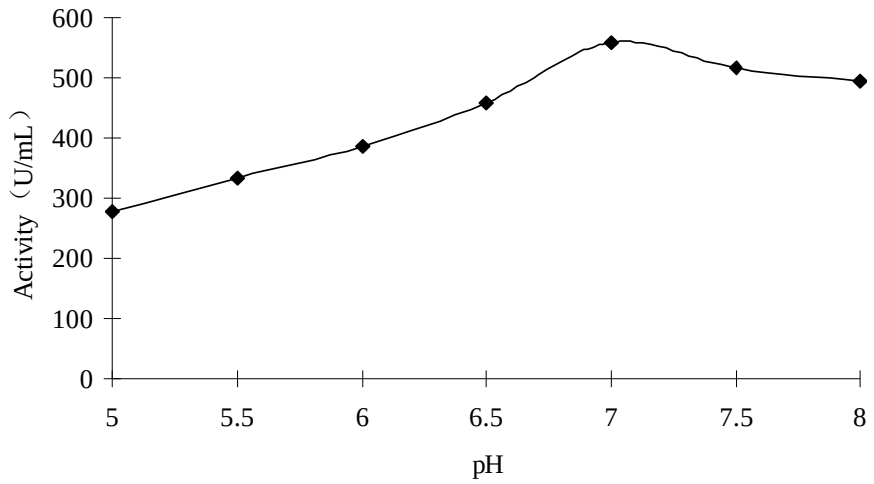

Figure 1 Effect of initial pH on enzyme activity

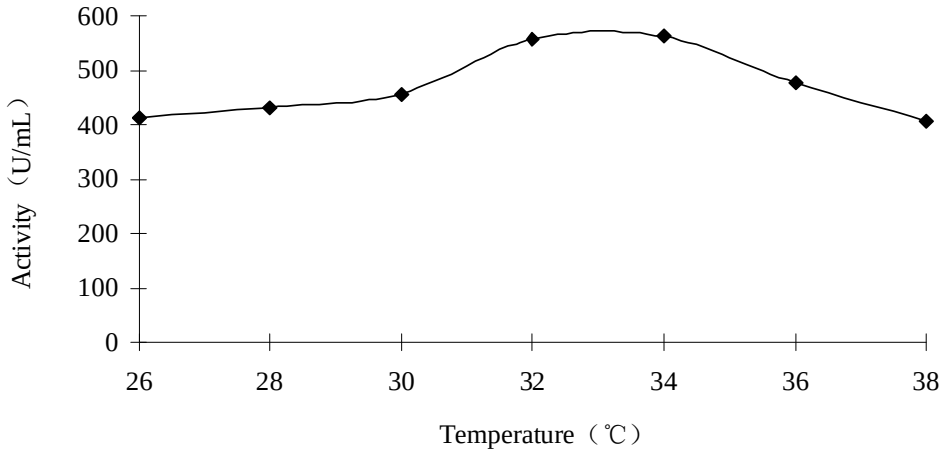

Figure 2 Effect of temperature on activity

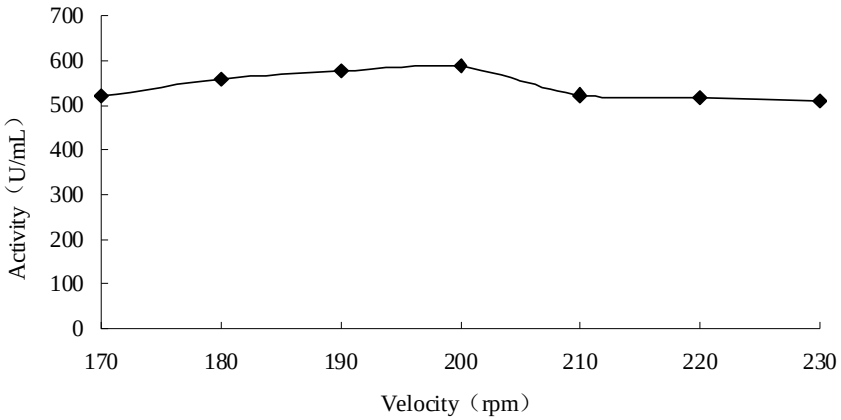

Figure 3 Effect of velocity of rotary shaker on activity

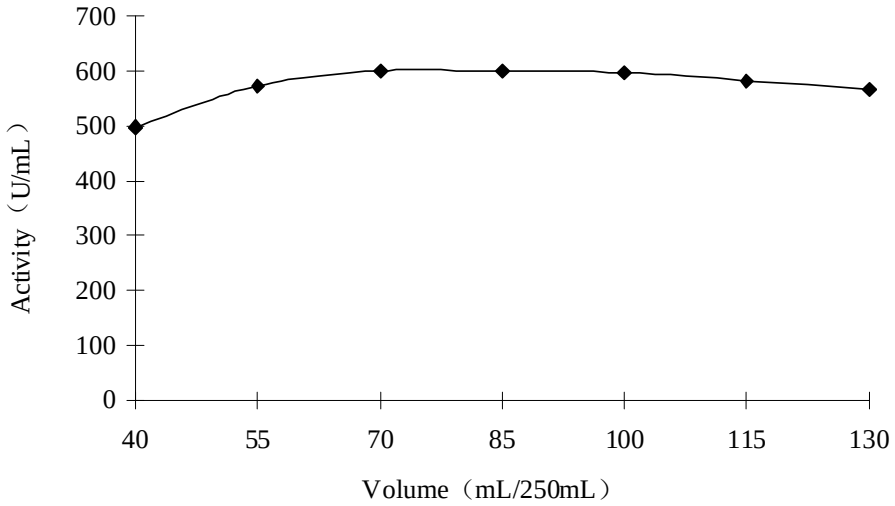

Figure 4 Effect of volume on activity

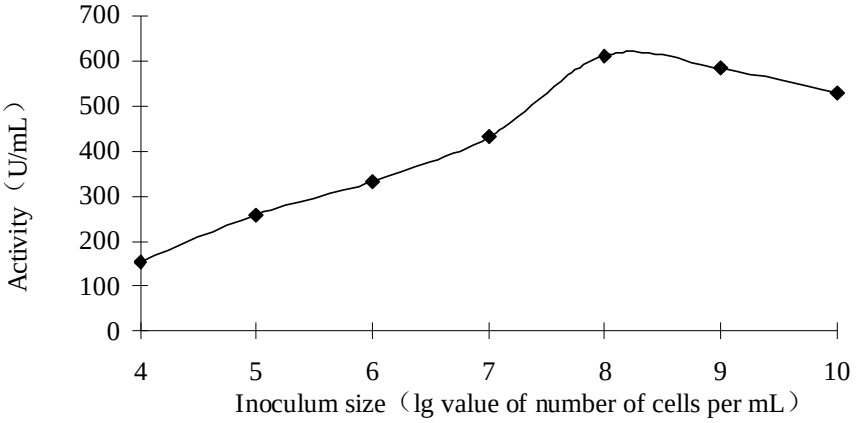

Figure 5 Effect of inoculum size on activity

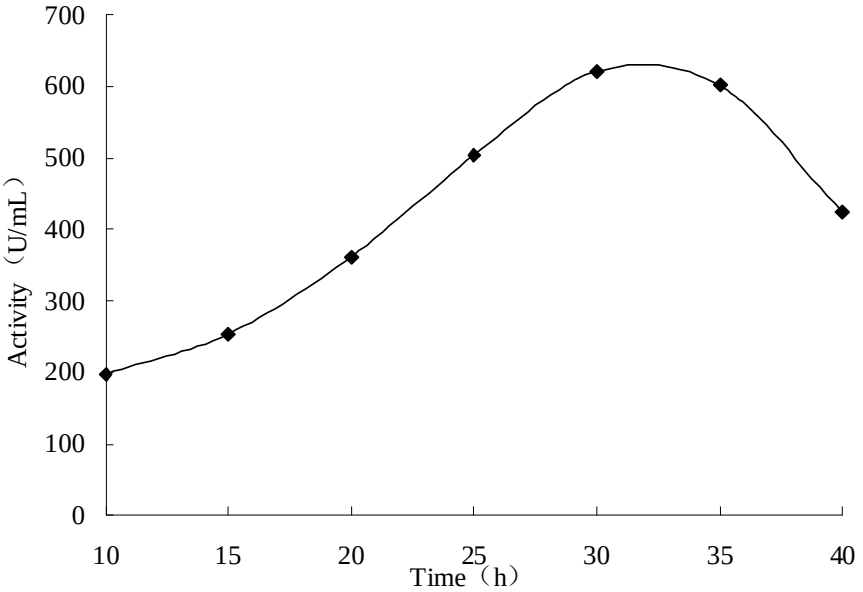

Figure 6 Effect of incubation time on activity

Table 1: Effect of parameters of fermentation on trehalose synthase activity

Factor	Level	Activity（U/mL）
Initial pH	5	277
	6	386
	7	559
	8	495
Temperature（℃）	26	413
	30	457
	34	562
	38	407
Velocity of rotary shaker（rpm）	180	559
	190	576
	200	587
	210	523
	220	517
Volume（mL/250mL）	40	498
	70	601
	100	598
	130	567

Inoculum size（lg value of number of cells per mL）	4	155
	6	333
	8	612
	10	528
Incubation time（h）	10	198
	20	362
	30	621
	40	424