O-BB-08

Invertase Production from *Saccharomyces cerevisiae* DB-KKU-Y-53 Using Low Cost Medium

Kankanok Charoenpunthuwong[1], Chanika Somsila[1] and Weera Piyatheerawong[1, 2*]

[1]*Department of Biotechnology, Faculty of Technology, Khon Kaen University, Khon Kaen 40002, Thailand*

[2]*Fermentation Research Center for Value Added Agricultural Products, Khon Kaen University, Khon Kaen 40002, Thailand*

*****Corresponding author. E-mail: weera@kku.ac.th*

DOI:

ABSTRACT

Invertase (β-fructofuranosidase, EC 3.2.1.26) catalyzes the hydrolysis of sucrose into glucose and fructose. Since these invert sugars have desirable properties such as a source of instant energy, high osmotic pressure and solubility, they are widely used in food, pharmaceutical, cosmetic, medicine and beverage industries. Saccharomyces cerevisiae, a non-pathogenic microorganism, is easily to cultivate and handle during enzyme production. This yeast strain is normally used as the main source of invertase for food industry. In this study, production of invertase fro m S. cerevisiae DB-KKUY-53 using low cost medium has been investigated. To achieve the optimal composition of medium for invertase production, response surface methodology was employed. Screening of the significant variables for enzyme production was conducted using Plackett-Burman Design. The results suggested that concentrations of ammonium sulfate, magnesium sulfate and sucrose were found to be the statistically significant variables for invertase production ($p < 0.05$). Then, the optimal composition of medium was studied through central composite design. The results indicated that the specific activity of invertase increased to 56.70% (5.14 U/mg) when this yeast was cultivated with the medium containing ammonium sulfate concentration of 3.5 g/l, magnesium sulfate concentration of 1.0 g/l and sucrose concentration of 13.2 g/l, respectively. Furthermore, statistical analysis implied that the mathematical model was statistically significant ($p < 0.01$). The predicted

enzyme activities were in agreement with the experimental results and the difference was less than 10%.

Keywords: Invertase production, *Saccharomyces cerevisiae*, mineral salt medium, response surface methodology, central composite design

INTRODUCTION

Invertase (β-fructofuranosidase, EC 3.2.1.26) is one of the glycoside hydrolases that catalyzes sucrose into glucose and fructose. The complete or partial hydrolysis of sucrose provides sweet syrup that is more stable than pure sucrose syrup. These sugars are normally applied in several products such as food, pharmaceutical, cosmetic, medicine and beverage because they have desirable properties such as source of instant energy, high osmotic pressure and solubility (Kotwal & Shankar, 2009). Due to the importance of invertase in sugar syrup production, it has been widely characterized and commercial sources of the purified invertase are readily available. Normally, invertase can be produced from various species of plant and microorganism such as Bambusa edulis (Bamboo), Aspergillus niger, and Saccharomyces cerevisiae. Yeast, a non-pathogenic and a non-toxicogenic microorganism, is usually used as the main source of invertase for food industry because it is easily to cultivate and handle during enzyme production (Kotwal & Shankar, 2009; Lincoln & More, 2017). Productivity of fermentation is typically controlled by several parameters such as the composition of medium, agitation rate, pH value, inoculum size, and temperature. The optimal design of medium composition is one of important parameters to be considered in the improvement of fermentation. For large-scale production, formulation of the cost-effective medium is usually required. Initially, formulation of the medium in batch fermentation is frequently employed to obtain the optimal amount of carbon and nitrogen sources as well as the trace elements (Guo et al., 2018; Venkateswarulu, Prabhakar, & Kumar, 2017). Response surface methodology (RSM), an experimental design, evaluates relationship between the responses and the independent variables into the mathematical model. This design describes the influence of the independent variables to the responses through statistical analysis. Optimal levels of the independent variables to achieve the desirable responses could be predicted according to the model (Box & Wilson, 1951). The aim of this study was the production of invetase from S. cerevisiae DB-KKU-Y-53 using low cost medium. To achieve the optimal composition of medium for invertase production, RSM was employed.

MATERIAL AND METHODS

Inoculum preparation

S. cerevisiae DBKKU-Y-53 was kindly obtained from Assoc. Prof. Dr. Pornthap Thanonkeo, Faculty of Technology, Khon Kaen University, Khon Kaen, Thailand. The yeast stain was inoculated to 50 ml of yeast and malt extract (YM) broth supplemented with 1% (w/v) sucrose. Then, it was incubated at 35 °C with shanking rate of 150 rpm for 18 h. Viable cell concentration was analyzed using a haemocytometer. Then, yeast cells were centrifuged at 10,000 rpm, 4 °C for 15 min and were washed with 10 ml of sterilized 0.85% NaCl.

Invertase production from *S. cerevisiae* DBKKU-Y-53

Inoculum was cultured in mineral salt broth supplemented with sucrose as the carbon source. The composition of the salt medium included 20.0 g/l of sucrose, 1.5 g/l of $(NH_4)_2SO_4$, 1.0 g/l of KH_2PO_4, 0.5 g/l of $MgSO_4$, 0.2 g/l of NaCl, 0.2 g/l of $CaCl_2$, 0.03 g/l of $FeSO_4$, respectively. Then, it was incubated at 35 °C with shanking rate of 150 rpm for 24 h. Yeast cells were harvested by centrifugation. The cells were washed with 50 ml of sterilized 0.85% NaCl and were resuspended in 10 ml of 0.2 M sodium acetate buffer (pH 5.0). Then, the suspension was sonicated at 4 °C for 10 min. Crude extract containing invertase was harvested by centrifugation.

Determination of invertase activity and protein concentration

The invertase activity was determined by using sucrose as substrate. The reaction mixture was incubated at 40 °C. The concentration of reducing sugar was evaluated by dinitrosalicylic acid solution. One unit of invertase activity was defined as the amount of enzyme that released 1 µmol of glucose equivalent per minute under the assay condition. Protein concentration was determined by Lowry method, using bovine serum albumin as the standard.

Experimental design to optimize the medium composition

In this study, evaluation of the optimal composition of salt medium for invertase production from *S. cerevisiae* DBKKU-Y-53 was conducted in two steps. Initially, Plackett-Burman design was employed to investigate influence of parameters on the production of invertase. These parameters were the amount of sucrose, $(NH_4)_2SO_4$, KH_2PO_4, $MgSO_4$, NaCl, $CaCl_2$, $FeSO_4$, respectively (Taskin et al., 2016). This design investigates high and low levels of different experimental parameters and it has been used to screen and identify

the important medium components for enzyme production as shown in Table 1. Plackett-Burman design is based on the first order model as presented in Equation 1 (Plackett & Burman, 1946).

$$Y = \beta_0 + \sum \beta_i X_i \quad [1]$$

Where Y is the response (enzyme activity), β_0 is the model intercept and β_i is the linear coefficient, and X_i is the level of the independent variable. This model does not describe interaction among factors. In this study, seven experimental variables were screened in twelve experimental treatments. The invertase activity was selected as response (Table 2).

The next step in medium optimization was to evaluate the optimum level of each significant variable. RSM was used to determine the optimal level of medium composition for enzyme production. Central composite design (CCD) was used to fit a second order response surface model. To predict the optimal point, a quadratic model is expressed according to Equation 2.

$$Y = \beta_0 + \sum \beta_i X_i + \sum \beta_{ii} X_i^2 + \sum \beta_{ij} X_i X_j \quad [2]$$

Where Y is the response variable, β_0 is intercept, β_i are linear coefficients, β_{ii} are squared coefficients, β_{ij} are interaction coefficients and X is the coded levels of the independent variable.

RESULTS

Effect of medium component on the production of invertase from *S. cerevisiae DBKKU-Y-53*

In this study, evaluation of the optimal composition of salt medium for invertase production from *S. cerevisiae* DBKKU-Y-53 was conducted in two steps. Initially, Plackett-Burman design was employed to investigate influence of parameters on the production of invertase. Seven experimental variables were screened in twelve experimental treatments. The invertase activity was selected as response (Table 2). The results suggested that concentrations of ammonium sulfate, magnesium sulfate and sucrose were found to be the statistically significant variables for invertase production ($p < 0.05$) as shown in Table 3.

Table 1. The parameters and levels used in Plackett-Burman design for screening medium components affecting invertase production

Parameters	Symbols	Levels of parameters (g/l)	
		Low value (-1)	High value (+1)
$(NH_4)_2SO_4$	A	1.5	3.0
Sucrose	B	20	40
KH_2PO_4	C	1.0	2.0
NaCl	D	0.2	0.4
$CaCl_2$	E	0.2	0.4
$FeSO_4$	F	0.03	0.06
$MgSO_4$	G	0.5	1.0
Dummy 1	H	-1	1
Dummy 2	I	-1	1
Dummy 3	J	-1	1
Dummy 4	K	-1	1

Table 2. Effect of parameters on invertase production from *S. Cerevisiae* DBKKU-Y-53 using Plackett-Burman Design

Std.	Levels of parameters											Specific Activity (U/mg)
	A	B	C	D	E	F	G	H	J	K	L	
1	+	+	-	+	+	+	-	-	-	+	-	1.23
2	-	+	+	-	+	+	+	-	-	-	+	0.92
3	+	-	+	+	-	+	+	+	-	-	-	4.55
4	-	+	-	+	+	-	+	+	+	-	-	1.07
5	-	-	+	-	+	+	-	+	+	+	-	2.80
6	-	-	-	+	-	+	+	-	+	+	+	2.62
7	+	-	-	-	+	-	+	+	-	+	+	4.61
8	+	+	-	-	-	+	-	+	+	-	+	1.28
9	+	+	+	-	-	-	+	-	+	+	-	0.75
10	-	+	+	+	-	-	-	+	-	+	+	0.54
11	+	-	+	+	+	-	-	-	+	-	+	2.22
12	-	-	-	-	-	-	-	-	-	-	-	1.60

Table 3. Analysis of variance for parameters used in Plackett-Burman Design

Source	DF	Mean square	F-value	*p*-value
Model	9	2.36	65.44	0.0151
$(NH_4)_2SO_4$	1	2.18	60.24	0.0162
Sucrose	1	13.22	365.92	0.0027
KH_2PO_4	1	0.033	0.93	0.4372
$CaCl_2$	1	5.89×10^{-3}	0.16	0.72
NaCl	1	0.19	5.33	0.1473
$FeSO_4$	1	0.56	15.60	0.0585
$MgSO_4$	1	1.95	.91	0.0180
Error	2	0.036		
Total	11			

Optimization of medium components for invertase Production from *S. cerevisiae DBKKU-Y-53*

The next step in medium optimization was to evaluate the optimal level of each significant variable. CCD was used to determine the optimal level of medium composition for invertase production. The results indicated that the specific activity of invertase improved to 56.70% (5.14 U/mg) when this yeast was cultivated with the medium containing ammonium sulfate concentration of 3.5 g/l, magnesium sulfate concentration of 1.0 g/l and sucrose concentration of 13.2 g/l, respectively (Table 4). Furthermore, statistical analysis implied that the mathematical model was statistically significant ($p < 0.01$) as displayed in Table 5. The predicted enzyme activities were in agreement with the experimental results and the difference was less than 10%.

Table 4. Effect of parameters on invertase production from *S. cerevisiae* DBKKU-Y-53 using central composite design

Std.	Level of parameters (g/l)			Specific activity (U/mg)
	$(NH_4)_2SO_4$	Sucrose	$MgSO_4$	
1	1.5	20.0	0.5	3.01
2	5.5	20.0	0.5	3.01
3	1.5	40.0	0.5	0.86
4	5.5	40.0	0.5	0.62
5	1.5	20.0	1.5	2.43
6	5.5	20.0	1.5	1.06
7	1.5	40.0	1.5	1.01
8	5.5	40.0	1.5	0.84

Table 4. Effect of parameters on invertase production from *S. cerevisiae* DBKKU-Y-53 using central composite design (Cont.)

Std.	Level of parameters (g/l)			Specific activity (U/mg)
	$(NH_4)_2SO_4$	Sucrose	$MgSO_4$	
9	0.14	30.0	1.0	2.28
10	6.86	30.0	1.0	1.16
11	3.5	13.18	1.0	5.14
12	3.5	46.82	1.0	0.75
13	3.5	30.0	0.16	1.20
14	3.5	30.0	1.84	0.87
15	3.5	30.0	1.0	1.37
16	3.5	30.0	1.0	1.06
17	3.5	30.0	1.0	0.85
18	3.5	30.0	1.0	0.78
19	3.5	30.0	1.0	0.85

DISCUSSION

In this study, Plackett-Burman design was used to investigate effect of parameters on the production of invertase (Table 2). Specific activities fluctuated according to the content of each medium component from 0.54 to 4.61 U/mg. The results indicated that the 7th treatment gave the highest enzyme yield, followed by the 3th treatment. According to these results, this variation reflects the importance of each medium constituent to obtain higher efficiency. According to statistical analysis, concentrations of ammonium sulfate, magnesium sulfate and sucrose were found to be the significant variables for invertase production ($p < 0.05$) as shown in Table 3. Application of appropriate contents of carbon and nitrogen sources was very important for optimal production of invertase because sucrose metabolism shows a specific physiological response to the presence of nitrogen source (Shafiq et al., 2003). Once the significant parameters were identified, medium optimization was employed to examine the optimal level of each significant variable. CCD was used to determine the optimal level of medium composition for invertase production. The results indicated that the specific activity of invertase increased 56.70% (5.14 U/mg) when the yeast was cultivated with the medium containing ammonium sulfate concentration of 3.5 g/l, magnesium sulfate concentration of 1.0 g/l and sucrose concentration of 13.2 g/l, respectively (Table 4). The high levels of sucrose in the medium could deteriorate the production of invertase due to substrate inhibition.

Table 5. Analysis of variance for parameters used in central composite design

Source	DF	Mean Square	F-value	*p*-value
Model	9	2.43	11.09	0.0004
$(NH_4)_2SO_4$ (A)	1	0.99	4.51	0.0595
Sucrose (B)	1	13.48	61.	<0.0001
$MgSO_4$ (C)	1	0.54	2.49	0.1459
AB	1	0.11	0.52	0.4872
AC	1	0.21	0.97	0.3468
BC	1	1.05	4.81	0.01
A^2	1	0.35	1.62	0.2320
B^2	1	4.99	22.78	0.0008
C^2	1	0.11	0.48	0.5039
Residual	10	0.22		
Lack of Fit	5	0.34	3.54	0.0956
Error	5	0.096		
Total	19			

CONCLUSION

In this study, production of invetase from S. cerevisiae DB-KKU-Y53 using low cost medium has been investigated. To achieve the optimal composition of medium for invertase production, RSM was employed. The results showed that the specific activity of invertase enhanced to 56.70% (5.14 U/mg) when this yeast was cultivated with the medium containing ammonium sulfate concentration of 3.5 g/l, magnesium sulfate concentration of 1.0 g/l and sucrose concentration of 13.2 g/l, respectively.

ACKNOWLEDGEMENTS

This research project was under the research program “Improvement of ethanol production efficiency from sweet sorghum by thermotolerant yeast *Saccharomyces cerevisiae*”, which was financially supported by Khon Kaen University and National Research Council of Thailand.

REFERENCES

Box, G. E. P. and Wilson, K. B. 1951. On the Experimental Attainment of Optimum Conditions. Journal of the Royal Statistical Society: Series B (Methodological). 13(1): 1–38.

Guo, F., Zheng, H., Cheng, Y., Song, S., Zheng, Z., and Jia, S. 2018. Medium optimization for ε- poly- L- lysine production by *Streptomyces diastatochromogenes* using response surface methodology. Letters in Applied Microbiology. 66(2): 124–131.

Kotwal, S. M. and Shankar, V. 2009. Immobilized invertase. Biotechnology Advances. 27(4): 311–322.

Lincoln, L. and More, S. S. 2017. Bacterial invertases: Occurrence, production, biochemical characterization, and significance of transfructosylation. Journal of Basic Microbiology. 57(10): 803–813.

Plackett, R. L. and Burman, J. P. 1946. The Design of Optimum Multifactorial Experiments. Biometrika. 33(4): 305–325.

Shafiq, K., Ali, S. and Ul-Haq, I. 2003. Time Course Study for Yeast Invertase Production by Submerged Fermentation. Journal of Biological Sciences. 3(11): 984–988.

Taskin, M., Ortucu, S., Unver, Y., Tasar, O. C., Ozdemir, M., and Kaymak, H. C. 2016. Invertase production and molasses decolourization by cold-adapted filamentous fungus *Cladosporium herbarum* ER-25 in non-sterile molasses medium. Process Safety and Environmental Protection. 103: 136–143.

Venkateswarulu, T. C., Prabhakar, K. V. and Kumar, R. B. 2017. Optimization of nutritional components of medium by response surface methodology for enhanced production of lactase. 3 Biotech. 7(3): 202.