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Statistical Optimization of Cellulase Production by *Breznakia pachnodae* T1-07 Isolated from Termite Gut

Piyanuch Niamsup, Saichol Kantawang , Wasukanya Rakthanyakam and Pairote Wongputtisin*

Faculty of Science, Maejo University, Chiang Mai, Thailand 50290
**Corresponding author: piyanuch@mju.ac.th*

Introduction

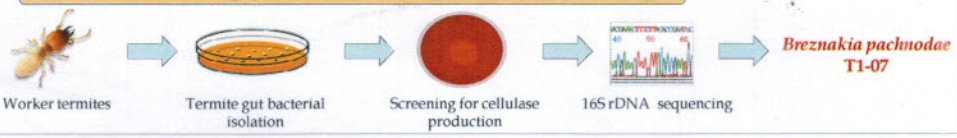
- Lignocellulose biomass is a renewable and abundant resource with great potential for bioconversion to value-added bioproducts.
- The biorefining process remains economically unfeasible due to a lack of biocatalysts that can overcome cost hurdles, cellulase play an important role in processing biomass through advanced biotechnological approaches.
- Termites are one of the most important soil insects that efficiently decompose lignocelluloses with the aid of their associated microbial symbionts.

Objectives

- This study aimed at screening for cellulase-producing bacteria from termite gut and to optimize the fermentation conditions for maximum cellulase production using the Plackett-Burman Design (PBD) and Central Composite Design (CCD)

Materials and Methods

Isolation and screening of cellulase-producing bacteria from termite gut



Optimization of cellulase production

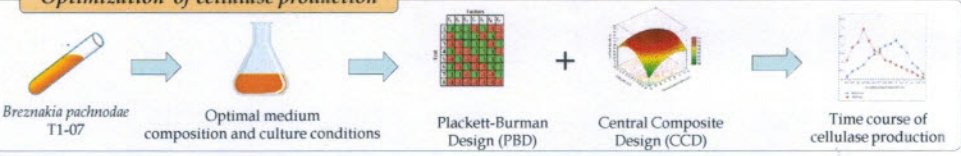


Table 1 Nutrient and conditions screening using Plackett-Burman Design

Factors	Low (-1)	High (+1)
A. Temperature (°C)	25	45
B. Skim milk (g)	0.1	1.0
C. Inoculum size (ml)	0.1	1.0
D. $NaNO_3$ (g)	0.05	0.5
E. pH	5	9
F. KCl (g)	0.05	0.5
G. K_2HPO_4 (g)	0.05	0.5
H. Wheat bran (g)	0.1	1.0
I. Agitation (rpm)	120	180
J. Time (days)	3	7
K. $MgSO_4$ (g)	0.025	0.25

Table 2 Plackett-Burman Design for screening of factors for cellulase production

	A	B	C	D	E	F	G	H	I	J	K	Cellulase activity (Unit/ml)
1	+	+	-	+	+	+	-	-	-	+	-	0.683
2	+	-	+	+	+	-	-	-	+	-	+	0.079
3	-	+	+	+	-	-	-	+	-	+	+	0.351
4	+	+	+	-	-	-	+	-	+	+	-	0541
5	+	+	-	-	-	+	-	+	+	-	+	0.579
6	+	-	-	-	+	-	+	+	-	+	+	0.020
7	-	-	-	+	-	+	+	-	+	+	+	0.172
8	-	-	+	-	+	+	-	+	+	+	-	0.261
9	-	+	-	+	+	-	+	+	+	-	-	0.117
10	+	-	+	+	-	+	+	+	-	-	-	0.071
11	-	+	+	-	+	+	+	-	-	-	+	0.702
12	-	-	-	-	-	-	-	-	-	-	-	0.086

Table 3 Statistical analysis of Plackett-Burman Design showing coefficient values, T- and P-value for each variable

Predictor variables	Coefficient	STD error	T-value	P-value	VIF
Constant	0.30517	0.00924	33.03	0.0009	
Inoculum	0.02900	0.00924	3.14	0.0883	1.0
K_2HPO_4	-0.03467	0.00924	-3.75	0.0642	1.0
KCl	0.10617	0.00924	11.49	0.0075	1.0
$NaNO_3$	-0.05967	0.00924	-6.46	0.0231	1.0
Agitation	-0.01367	0.00924	-1.48	0.2772	1.0
Skim milk	0.19033	0.00924	20.60	0.0023	1.0
Temperature	0.02367	0.00924	2.56	0.1245	1.0
Time	0.3283	0.00924	3.55	0.0709	1.0
Wheat bran	-0.07200	0.00924	-7.79	0.0161	1.0
Standard	R-squared	Resid.mean	Adjusted		
0.03200	0.9972	0.00102	0.9844		

Table 4 CCD of experiments with evaluated cellulase enzyme activity

Run CCD	A (skim milk)	B (wheat bran)	Observed	Predicted
1	-1	-1	1.608	1.314419
2	1	-1	1.824	1.788401
3	-1	1	1.939	1.611849
4	1	1	1.838	1.768831
5	-1.4142	0	1.122	1.485796
6	1.4142	0	1.933	1.931954
7	0	-1.4142	1.277	1.434637
8	0	1.4142	1.426	1.631113
9	0	0	2.054	2.054
10	0	0	2.054	2.054
11	0	0	2.054	2.054
12	0	0	2.054	2.054
13	0	0	2.054	2.054

Table 5 Statistical analysis of CCD with Design-Expert software

Source	Coefficient	DF	Adj SS	Adj MS	F-value	P-value
Model		5	0.870981	0.174196	3.059614	0.0886
A	0.16	1	0.199058	0.199058	3.496283	0.1037
B	0.069	1	0.038603	0.038603	0.678026	0.4374
AB	-0.079	1	0.025122	0.025122	0.441252	0.5278
A*A	-0.17	1	0.207150	0.207150	3.638421	0.0981
B*B	-0.26	1	0.472298	0.472298	8.295526	0.0236
Error		7	0.398538	0.056934		
Lack-of-Fit		3	0.398538	0.132846	*	*
Pure error		4	0.00000	0.00000		
Total		12	1.269519			
Model summary						
S		R-sq	R-sq (adj)	R-sq (pred)		
0.24		0.6861	0.4618	-1.2324		

The equation for cellulase activity is given by:

Cellulase (U/ml) = 2.05 + 0.16A + 0.069B - 0.079AB - 0.17 A*A - 0.26 B*B

(A = skim milk, B = wheat bran)

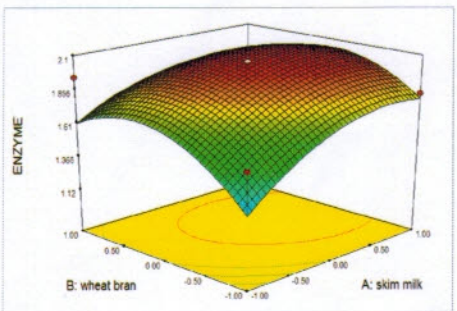


Figure 1 Three-dimentional response surface plot for cellulase production showing the effects of wheat bran and skim milk

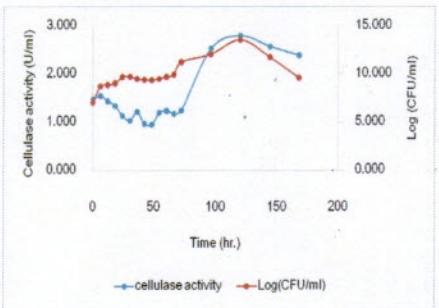


Figure 2 Time course of cellulase enzyme production

Conclusion

- A cellulase-producing bacterium was isolated from termite gut, *Breznakia pachnodae* T1-07, was found to produced highest cellulase activity.
- PBD showed that wheat bran, skim milk, sodium nitrate ($NaNO_3$) and potassium chloride (KCl) were found to exert a significant effect on cellulase productivity
- From CCD, response surface methodology showed the highest cellulase activity at 2.79 U/ml was achieved in 250 ml erlenmeyer flask using optimal conditions compared to the basal medium (32 mU/mL) under the following optimum level of these medium components; 13.7 g/L wheat bran, 15.2 g/L skim milk, 0.5 g/L $NaNO_3$ and 5.0 g/L KCl

Acknowledgements

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