CSCanada
Energy Science and Technology
Vol. 5, No. 1, 2013, pp. 54-57
DOI:10.3968/j.est.1923847920130501.658

ISSN 1923-8460[PRINT]
ISSN 1923-8479[ONLINE]
www.cscanada.net
www.cscanada.org

Study of Mutagenesis on the Strain Producing Cellulase

LIU Junhong[a],*; WANG Fumei[a]; ZHAO Qi[a]; CHENG Hui[a]

[a] Bio–engineering Department of Henan University of Urban Construction, Pingdingshan, Henan, China.
* Corresponding author.
Supported by Henan Science and Technology Bureau (The item number: 092300410083).

Received 13 December 2012; accepted 21 February 2013

Abstract
Cellulose is insoluble in water, dilute acid and dilute alkali at room temperature, which is slowly decomposed under natural conditions. Microbial plays an important role in the degradation of cellulose. This article, taking the strain producing cellulase as the objective, dealed with the effect of mutagenesis on the activity of cellulase. The strain producing cellulase was activated and experienced mutagenesis by ultra violet, then fermentation experiment for enzyme production was carried out, DNS method was taken to calculate the activity of cellulase, hoping to find out optimum conditions for mutagenesis. It came to the conclusion that the activity of cellulase arrived at 107.75μg/mL·min on conditions of UV exposure for 4 min, accompanied by 34,600/mL growth concentration of strain.
Key words: Cellulase; Enzyme Activity; Mutagenesis; Strain

Liu J. H., Wang, F. M., Zhao, Q., & Cheng, H. (2013). Study of mutagenesis on the Strain Producing Cellulase. *Energy Science and Technology*, *5*(1), 54-57. Available from: URL: http://www.cscanada.net/index.php/est/article/view/10.3968/j.est.1923847920130501.658
DOI: http://dx.doi.org/10.3968/j.est.1923847920130501.658

INTRODUCTION
Biomass energy, belonging to renewable energy, is in close connection with human being's life, its comprehensive utilization with high efficiency has been standing out in recent years (Wang *et al.*, 2005). Bioethanol ranks among biomass energy, materials, rich in starch and cellulose, are degraded to glucose, which can be fermented to bioethanol. Utilization of starch on big scale leads to grain shortage, cellulose is widespread in nature, the technology of ethanol production from cellulose has attracted great attention (Liu *et al.*, 2007). The utilization of cellulose is of significance to relieve energy crisis, grain shortage and environment protection. Cellulose is hard to be degraded, therefore, cellulase is introduced to degrade cellulose for ethanol production, which results in the high cost. Increasing study focused on screening strain which is able to release cellulase during the course of growth, up to now, activity of cellulase, produced by microorganism, is low, for another, its activity gets to the uttermost only under harsh conditions (Liu *et al.*, 2007). Consequently, how to obtain strain with high cellulase activity is the backbone to improve ethanol production. Mutagenesis is one of the effective way to heighten the activity of cellulase, among which ultraviolet (UV) mutagenesis is the common practice.

Considering the strain, screened from the soil by research group, as original strain, this paper analyzed effects of mutagenesis time and different dilution ratios of strain on the activity of cellulase.

1. EXPERIMENT MATERIAL

1.1 Strain
Strain producing cellulase screened from the soil.

1.2 Main Reagent
0.9% normal saline, DNS reagent, CMC-Na, Citric acid buffer solution.

1.3 Culture Medium
(1) Activation culture medium
Patato 6 g, agar 5 g, glucose 0.6 g, distilled water 300 mL.

(2) Congo red culture medium

Agar 30 g, CMC-Na 20 g, Congo red 0.4 g, $(NH_4)_2SO_4$ 6 g, $MgSO_4 \cdot 7H_2O$ 1 g, K_2HPO_4 3 g, distilled water 2000 mL.

(3) Fermentation culture medium for cellulase production

CMC-Na 30 g, $(NH_4)_2SO_4$ 6 g, K_2HPO_4 6 g, $MgSO_4 \cdot 7H_2O$ 0.8 g, peptone 4 g, bran 20 g, yeast extract 2g,$CaCl_2$ 0.6 g,$ZnCl_2$ 0.0034 g, Twain 0.4 g, distilled water 2000 mL.

1.4 Appratus

Thermostat water bath, magnetic heating blender, ultraviolet spectrophotometer, steam sterilization pot, oscillation incubator, electronic balance, refrigerated centrifuge with high speed, thermostatic drying oven, asepsis room, hemacytometer.

2. METHODS

2.1 Activation of the Strain Producing Cellulase

Inoculate the screened strain producing cellulase to plates with activation culture medium, then purify by crossed separation, incubate for 7 days at 30 ℃, choose plates growing better for UV mutagenesis.

2.2 UV Mutagenesis

(1) Preparation of spore suspension

① Add sterilized normal saline to chosen plates and scrape gently to get spore suspension, then shift the spore suspension to centrifuge tube, stir for 5 min by magnetic stirrers to be well-distributed.

② Centrifuge spore suspension for 10 min at 3500 r/min, remove supernatant, add normal saline, then centrifuge again, repeat 3 times.

③ Add normal saline in sediment from the above operation, stir for 10 min to get well-distributed sample.

④ Count the number of spore by microscope with hemacytometer.

⑤ Write down the number of spore in suspension before mutagenesis, shown in table 1.

Table 1
The Number of Spore in Medium Square

The number of spore in the Nth medium square					The number of spore in 5 medium square	The number of spore in each small square
1	2	3	4	5		
110	81	105	115	93	504	6.3

(2) Mutagenesis

① Add 0.5 mL spore suspension to centrifuge tube of matched group, dilute with 4.5 mL normal saline, the following operation is carried out to get various dilution ratios of 10^{-2},10^{-3},10^{-4},10^{-5} and 10^{-6}. (It is essential to shake spore suspension uniformly).

② Take out the centrifuge tube with the dilution ratio of 10^{-4}, shake uniformly and take out 0.1 mL to a plate with Congo red culture, spread uniformly, 3 plates need to be prepared.

③ The same operation is carried out according to the step ② as to the dilution ratio of 10^{-5}, 10^{-6}, then pack these plates with newspaper.

④ Thereafter, prepare the test group for UV mutagenesis, add 1mL spore suspension to centrifuge tube, other operations are the same as the matched group.

⑤ Time gradients of UV exposure are 30 s, 1 min, 2 min, 4 min and 6 min respectively.

⑥ Turn on UV light for 2 min, when the UV tends to stable, turn off it.

⑦ Open the plate, put magnetic rotor sterilized into the plate with time gradients of 30 seconds, set the plate to the center of magnetic stirrer in the black box, make it exposed in the red light for 1 min.

⑧ Open magnetic rotor and the plate, when the velocity of the rotor remains steady, close the black box, turn on UV light for 30 s, after which turn on the red light for 1 min.

⑨ Shift 0.5mL spore suspension from ⑧ to centrifuge tube, add 4.5mL normal saline and shake uniformly, thereafter, dilute it to gradients of 10^{-2}, 10^{-3}, 10^{-4}, 10^{-5} and 10^{-6} with 0.9% normal saline gradually.

⑩ Take out centrifuge tube with 10^{-4}, add 0.1mL to Congo red plate, spread uniformly, 3 plates need to be prepared.

⑪ As to the time gradient of 1 min, 2 min, 4 min and 6 min, operate according to ⑦,⑧,⑨ and ⑩. The above operations are carried out in asepsis room.

⑫ Pack all the plates with newspaper and incubate at 30 ℃ for 7 days.

⑬ After a week, observe the result and count the number of colony in plate. The number of colony after mutagenesis is in table 2

Survival rate= the number of colony after mutagenesis / the number of colony before mutagenesis ×100%

Death rate= the number of colony before mutagenesis-the number of colony after mutagenesis / the number of colony before mutagenesis ×100%

Table 2
The Number of Colony

Colony / Dilution ratio / Time	10^{-4}	10^{-5}	10^{-6}	Survival rate	Death rate
30 s	391	174	33	68.31%	31.69%
1 min	307	91	10	27.88%	72.12%
2 min	387	89	12	31.15%	68.85%
4 min	346	42	4	14.66%	85.34%
6 min	135	37	4	11.38%	88.62%
0 s	641	91	64	---------	---------

2.3 Fermentation Experiment for Cellulase-Production

After 6 days' incubation, take out 3 single colony and incubate at 30 ℃ and 160 r/min in shake flask with 100mL fermentation culture medium.

2.4 Measurement of CMC Enzyme Activity

(1) Standard curve of glucose (Liu and Yin, 2010)
y=6.934x-0.1403（y：OD value；x：concentration of glucose, mg/mL）

(2) Preparation of rough enzyme solution
Take out 5mL spore suspension from the fermentation culture medium and put it to centrifuge tube, after which centrifuge is operated for 15 min at 4 ℃ and 4000 r/min.

(3) Enzyme activity measurement by DNS method

①Take out 0.5 mL supernate, add 1.5 mL citric acid buffer solution with CMC-Na, shake uniformly, put it to the water bath with 50 ℃ for 30 min.

② Add 2 mL DNS reagent and 4mL distilled water, shake uniformly, after which put it to boiling water bath for 7 min, the operation is shown in table 3.

③ Cool the solution, measure the OD value at the wavelength of 550 nm.

Table 3
Measurement of Enzyme Activity

Time	blank	0 s	30 s	1 min	2 min	4 min	6 min
Rough enzyme solution/mL	---------	0.5	0.5	0.5	0.5	0.5	*0.5*
Buffer solution /mL	1.5	1.5	1.5	1.5	1.5	1.5	*1.5*
				50 ℃, 30 min			
Distilled water /mL	4.5	4	4	4	4	4	*4*
DNS/mL	2	2	2	2	2	2	*2*
				100 ℃, 7 min, cool			

3. RESULTS AND ANALYSIS

The cellulase activity is set as follows. At 25 ℃ and pH4.4, 1μg glucose from cellulose by cellulase in 1 min is defined as one unit (Bao and Wang, 2007).

Cellulase activity unit (μg/mL·min) = NxG/(TxV)

N: Dilution ratios of rough enzyme solution;
T: Reaction time;
V: The volume of rough enzyme solution;
G The amount of glucose according to OD value.

Table 4
Enzyme Activity

Enzyme activity / Dilution ratio / Time	10^{-4}	10^{-5}	10^{-6}
30 s	30.08	23.58	31.89
1min	26.76	24.83	30.36
2 min	96.26	37.01	43.80
4 min	107.75	31.75	24.68
6 min	56.26	46.28	26.62
0 s	28.56	26.62	31.89

In table 4, as to dilution ratio of 10^{-4}, enzyme activity after mutagenesis were higher than the matched ones. About 10^{-5}, except for UV exposure for 30s, enzyme activity of the others were all higher than the matched group. The series of 10^{-6} suggested that enzyme activity were lower than the matched ones except for UV exposure for 2min and 4min, which resulted from contamination, for when the shake flask was taken out, the tampon was wet and there existed gray bacteria circle.

Compared with the matched group, increase ratio of enzyme activity was shown in table 5.

Table 5
Increase Ratio of Enzyme Activity

Increase ratio / Dilution ratio / Time	10^{-4}	10^{-5}	10^{-6}
30 s	4.34%	-2.00%	-23.66%
1 min	3.65%	1.32%	-11.73%
2 min	197.76%	77.09%	40.08%
4 min	256.23%	70.21%	8.62%
6 min	79.41%	74.26%	-26.88%

Generally, the more the yield of glucose was, the higher the enzyme activity came. Therefore, as shown in table 4, UV exposure for 4 min was suitable, and the most

suitable growth concentration of strain for mutagenesis was 34600/mL.

By the same time of UV mutagenesis, table 5 indicated that the lower the bacteria concentration was, the lower the enzyme activity got to. The choice of growth concentration of strain after UV mutagenesis was the key for the utilization of material and the accumulation of colony. It was difficult to count the number of surviving colony in shake flask, so it was hard to estimate the most suitable growth concentration.

ACKNOWLEDGEMENT

Thanks a lot for the great support from Henan Science and Technology Bureau and Bio-engineering Department of Henan University of Urban Construction.

REFERENCES

[1] Wang, G. H., Tian, Y. L., & Yuan, J. T. (2005). Energy and Sustainable Development. Beijing: Chemical Industrial Press.

[2] Liu, B. Q., Li, X. F., Gao, W., & Yang, X. S. (2007). Study on Bioethanol Strain from Ignocellulose. *Rewable Energy*, *4*, 53-55.

[3] Liu, J. H., & Yin, X. Z. (2010). Study on Optimized Conditions of Cellulose Degradation by Dilute Sulfuric Acid of Bermuda Grass. *Rewable Energy*, *8*(4), 66-68.

[4] Bao, K., & Wang, X. H. (2007). Screening of Strain Producing Cellulase and Determination of Cellulase Activity. *Journal of Biology*, *24*(2), 56-58.

