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CELLULASE ENZYME ACTIVITY OF *Aspergillus fumigatus* FROM MANGROVE SOIL ON LIGNOCELLULOSIC SUBSTRATE

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Abstract

Sawdust was used as lignocellulosic substrates for the production of cellulase enzyme using *Aspergillus fumigatus* which was isolated from mangrove soil after pretreatment with 4% sodium hydroxide. Parameters like pH, temperature, nitrogen sources and inducers were optimized for the cellulase production.

Introduction

Lignocellulose biomass is the most abundant organic raw material in the world. It constitutes a major portion of agriculture and forest wastes which accumulate in the environment causing pollution problem. The bioconversion of cellulosic materials is now a subject of intensive research as a contribution to the development of large scale conversion process beneficial to mankind (Kumakura, 1997).

Cellulases are among the industrially important hydrolytic enzymes and are of great significance in present day biotechnology.Cellulases are widely used in the food, feed, textile and pulp industries (Nakari and Pentilla, 1996). Cellulase hydrolysis is accomplished with the aid of cellulase enzyme complex which is made up of three classes of enzymes namely exoglucanase, endoglucanase and β-glucosidase (Beguin, 1990). This study deals with the production and optimization of cellulase by *Aspergillus fumigatus* isolated from mangrove soil on sawdust as lignocellulosic substrate.

Materials and Methods

Isolation and identification

Soil samples were collected randomly under aseptic condition from the selected mangrove rich sites of Kunhimangalam, Kannur district, Kerala. Samples were cultured and purified on PDA medium prepared in half strength sea water. Fungal isolates were identified based on cultural, morphological and microscopic characteristics.

Preliminary screening for cellulase enzyme activity

Cellulose degrading activity of the isolated fungi was primarily tested by culturing on Czapeck-Dox medium supplemented with 1% Carboxymethyl cellulose (CMC). The plates were incubated at 28±2°C for 7days. After incubation the culture plates were stained with 2% Congo red reagent. The clear zone around the fungal colony was regarded positive for cellulase activity.

Lignocellulosic sources and pretreatment

Substrate used in the fermentation consists of sawdust which was obtained from Western India Plywoods, Valappatanam Kannur. The substrate was oven dried before use until attaining constant weight. 5gm of the sample was treated with 4% Sodium hydroxide. The sample was washed until neutrality and dried again at 65°C and autoclaved.

Production medium

Medium composition by Mandel and Weber was used for fermentation. The composition of media was (gL-1) KH_2PO_4 2.0g, $CaCl_2$ 0.3g, $MgSO_4.7H_2O$ 1g, NH_4NO_3 2g and (mgL^{-1}) of $FeSO_4.7H_2O$ 5mg, $MnSO_4.4H_2O$ 1.6 mg, $ZnSO_4.7H_2O$ 3.45 mg and $CoCl_2.6H_2O$ 2 mg and 5g substrate of pH 7.0. Cultivation was carried out for known incubation period at 30^0C with the inoculum concentration of 1×10^6 spores mL^{-1}.

Enzyme assay

The cellulase activity was analyzed by filter paper assay (FPase). 50mg dry Whatmann NO.1 filter paper strip was incubated with 0.5ml culture filtrate and 0.5ml sodium citrate buffer and incubated for 1hour at 50^0C. The glucose liberated was measured by DNS method. One unit of enzyme activity is defined as the amount of enzyme required to release 1µmol reducing sugars per ml under assay condition in both case. CMCase activity was assayed using culture filtrate.

Optimization of culture condition

The optimization for cellulase production was performed based on the modification of the physical parameters and the supplementation of additional nutrients. The effect of physical factors was determined by modification of pH of the moistening agent in the

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range of (4.5,5.0,6.0,6.5,7.0,7.5,8.0 and 9.0) and cultivation temperature in the range of (25,30,32,35,37°C. The effect of additional carbon sources, nitrogen sources and inducers were examined. Nitrogen sources were 4% of peptone, urea and yeast extract. Cellulose and carboxymethlycellulose were used as the inducer for optimization study.

Results and Discussion

The results show the extend of production of cellulase enzyme .Enzyme activity was optimized using various parameters

Cellulase production was determined at pH values 5.0,6.0,6.5,7.0,7.5,8.0 and 9.0 .FPase activity obtained were 0.144,0.166,0.246,0.173,0.163,0.133 and 0.131 IU/g respectively whereas CMCase activity was obtained as 1.65,2.53,4.24,3.03,2.42,1.40,1.11 IU/g as shown in Table 1.It was observed that Fpase and CMCase activity was highest at 6.5.Optimum temperature was found to be 32°C as in Table 2 with maximum activity of Fpase and CMCase activity 0.255 and 5.08 IU/g respectively. Among nitrogen sources yeast extract was the best to enhance the enzyme activity Fpase (0.281±0.13 IU/g) and CMCase (3.66±0.02 IU/g) indicated in Table 3. According to results in Table 4 Cellulose was found to be the best inducer which showed FPase activity of 0.288 IU/g and 5.84IU/g CMCase activity.

The media optimization is an important aspect to be considered in the development of fermentation technology. pH of the growth medium plays an important role by inducing morphological changes in microbes and in enzyme secretion. The pH change observed during the growth of microbes also effects product stability in the medium (Gupta et al., 2003).The optimum temperature was found to be 32°C.At higher temperature, the organism has to spend a lot of energy for maintenance and at lower temperature transport of nutrients is hindered (Pirt, 1975).The study shows cellulase activity of *Aspergillus fumigatus* from mangrove soil. And also that sawdust could be used as a substrate for maximum cellulase production.

Table1: Optimization of pH

pH	FPase (FPU g^{-1} substrate)	CMCase (U g^{-1} substrate)
5.0	0.144±0.01	1.65±0.07
6.0	0.166±0.02	2.53±0.10
6.5	0.246±0.04	4.24± 0.04
7.0	0.173 ±0.01	3.03±0.05
7.5	0.163±0.01	2.42±0.05
8.0	0.133 ±0.01	1.40±0.04
9.0	0.131 ±0.01	1.11±0.03

Table 2: Optimization of temperature

Temperature (0C)	FPase (FPU g^{-1} substrate) Mean ± SD	CMCase (Ug^{-1} substrate) Mean ± SD
25	0.170±1.3	3.23±0.04
30	0.198± 0.0	3.61±0.11
32	0.255± 1.3	5.08± 0.03
35	0.168± 1.2	2.22± 0.09
37	0.153± 0.04	1.09± 0.02

Table3: Optimization of nitrogen sources

Nitrogen source (% w/w)	FPase (FPU g^{-1}substrate) Mean ± SD	CMCase (Ug^{-1} substrate) Mean ± SD
Peptone	0.271 ±0.06	3.11 ± 0.01
Urea	0.265 ±0.05	2.25 ± 0.11
Yeast extract	0.281 ±0.13	3.66 ± 0.02
Sodium nitrate	0.248 ±0.05	2.15±0.08

Table4: Optimization of inducers

Inducers (% w/w)	Fpase (FPU g^{-1} substrate) Mean ±SD	CMCase (Ug^{-1} substrate) Mean ±SD
Cellulose	0.288±0.10	5.54±0.01
Carboxymethyl cellulose	0.252±0.05	5.10±0.01

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