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Cellulase Enzyme Production By *Aspergillus Niger* And *Trichoderma Reesei* On Different Lignocellulosic Wastes

Seema J.Patel*[1], R.Onkarappa[2], B.Basavaraju[1], A.G.Pradeep[1], Pramodkumar Harvi[1], S.Mohan[1], Santhosh Shet[1]

[1]Department of Biotechnology, GM Institute of Technology, Davanagere, Karnataka, (INDIA)

[2]Department of Microbiology, Sahyadri Science College, Shimoga, Karnataka, (INDIA)

Phone : 08192233345 ; Fax : 08192233344

E-mail : sjp_kv@yahoo.co.in

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ABSTRACT

Wheat straw, rice straw and rice husk were used as lignocellulosic substrates for the production of cellulase enzyme using *Aspergillus niger* and *Trichoderma reesei*. From fermentation studies, rice straw gave the best results with a CMCase activity of 0.61IUml^{-1} for *T.reesei* and 0.60IUml^{-1} for *A.niger*, FPU of 0.42IUml^{-1} for *T.reesei* and 0.50IUml^{-1} for *A.niger* compared to wheat straw(0.47IUml^{-1} CMCase and 0.32IUml^{-1} FPU for *T.reesei* and 0.46IUml^{-1} CMCase and 0.45IUml^{-1} FPU for *A.niger*) and in rice husk(0.49IUml^{-1} CMCase and 0.38IUml^{-1} FPU for *T.reesei* and 0.40IUml^{-1} CMCase and 0.30IUml^{-1} FPU for *A.niger*). Optimum conditions like pH and temperature for enzyme from each fungi were studied, 6 pH and 30^{0}C temperature for *T.reesei* and 5.5 pH and 32^{0}C for *A.niger* were found to be optimum. Molecular weight of these enzymes were determined by SDS PAGE and it was found to be 30 kDa for *T.reesei* and 40 kDa for *A.niger*.

KEYWORDS

Cellulases;
CMCase;
FPU;
SDS PAGE;
Molecular weight.

INTRODUCTION

Agricultural wastes and in fact all lignocellulosics can be converted into products that are of commercial interest such as ethanol, glucose, single cell protein. Cellulase enzyme has been reported for the bioconversion of lignocellulosics to these useful products[1]. This enzyme is produced by several microorganisms, commonly by bacteria and fungi. Although a large number of microorganisms are capable of degrading cellulose, only a few of these produce significant quantities of cell free enzymes capable of completely hydrolyzing crystalline cellulose *in vitro*. Fungi are the main cellulase producing microorganisms, though a few bacteria and actinomycetes have also been reported to yield cellulase activity.

Fungal genera like Trichoderma and Aspergillus are taught to be cellulase producers and crude enzymes produced by these microorganisms are commercially available for agricultural use. In general, bacterial cellulases are constitutively produced, whereas fungal cellulase is produced only in the presence of cellulose[2].

Filamentous fungi particularly *Aspergillus* and *Trichoderma* spp. are well known efficient producers of cellulases[3].

Several studies were carried out to produce cellulolytic enzymes from biowaste degradation process by many microorganisms including fungi such as *Trichoderma, Penicillium, Aspergillus* spp. etc[4]. Since the production of cellulase enzyme is a major process and economically viable, much work has been done on the production of cellulase from lignocellulosics. This work focused at improving its yield by using various cheaper sources of lignocelluloses viz, wheat straw, rice straw and rice husk, thus accessing the effect of the various lignocellulosic sources on the yield of cellulase by *T.reesei* and *A.niger*.

MATERIALS AND METHODS

Substrates

Wheat straw, rice straw and rice husk were obtained from local mills and local fields of Davanagere district. Each raw material was powdered and sieved into a 1mm seiver and this was used as carbon source.

Isolation of fungi

Screening of fungi capable of degrading cellulose was done from the soil of local paddy and wheat fields[5] and fungi of interest that is *T.reesei* and *A.niger* were selected after proper identification through manuals and confirmation by NCIM Pune.

Pre-treatment of substrates

The substrates were soaked individually in 1% sodium hydroxide solution in the ratio 1:10(substrate: solution) for two hours at room temperature. After which, they were washed for free of chemicals and autoclaved at 121^{0}C for one hour. The treated substrates were then filtered and washed with sterile distilled water until the wash water become neutral[6].

Inoculum preparation

Fungal cultures were inoculated onto PDA medium in the Petri plate. After 4-5 days, culture is used for inoculation.

Fermentation conditions for enzyme production

10g l^{-1} of each residue was taken in conical flask containing 200 ml of Mandle's medium. Mandle's medium was prepared by adding(gl^{-1}): urea 0.3, $(NH_4)_2SO_4$ 1.4, KH_2PO_4 2, $CaCl_2$ 0.3, $MgSO_4 7H_2O$ 0.3, bacto peptone 0.75, and yeast extract 0.25. Trace elements were also added, using a 1%(v/v) solution of salts(ml l^{-1}): $FeSO_4 7H_2O$ 0.5, $MnSO_4$ 0.16, $ZnSO_4$ 0.14, $CoCl_2$ 2. pH was adjusted to 5.5-6.0 before sterilization[7]. The conical flasks were plugged with cotton and sterilized at 15lbs per sq.inch for 20 minutes. Each flask was inoculated with 4-5 discs of 4-5 days old cultures of different fungi. These flasks were incubated at room temperature for 5days on an orbital shaker. After five days mycelium was separated by filtration through Whatman filter paper No.1. The filtrate was used for further studies[8].

Cellulase assay

Cellulase enzyme production was studied by FPU assay and CMCase assay[9]

Effect of pH on the production of cellulase

The optimised media were prepared using the individual substrates and the pH was set at different level such as 5,5.5,6,6.5 and 7 respectively by adding 1% NaOH and concentrated HCl. Then the media were autoclaved. Later they were inoculated with a piece of mycelia and were placed in a shaker at 30^{0}C for 5days. Simultaneously, for both the organisms and both the substrates, assay was carried out separately.

Effect of temperature on the production of cellulase

The optimized media were prepared individually by using the substrates and autoclaved. Later it was inoculated with a piece of mycelia and was set at different temperatures 25,28, 30,32 and 35^{0}C respectively. The effect of temperature on the production of cellulolytic enzyme was determined by growing the organisms at the above temperatures[10]. Simultaneously for both the organisms and each substrate, separate assay was carried out. The enzyme solution obtained from these two (pH and temperature) experiments was individually optimized based on filter paper activity and CMCase assay.

Partial purification of cellulase

Enzyme source preparation

The fungal organisms were grown in optimized media(Madle's media with CMC as cellulose source)

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at 30^{0}C for 5days individually. Then after growth, the culture filtrates were collected separately by centrifugation process. Then alcohol precipitation of cellulase was done and the precipitate collected from each source was dissolved individually in 30ml of sodium acetate buffer(0.2 M) at pH 5.5 and were dialyzed against the same buffer overnight at 4^{0}C.

Analysis of enzyme fractions by SDS PAGE

The dialyzed enzyme samples from each source was analyzed through PAGE using specific standard molecular marker. The gel obtained was photographed and scanned using a gel documentation system. Then the molecular weight of individual enzyme fractions was determined by referring the molecular weight of the marker.

RESULTS AND DISCUSSION

Cellulase production by *T.reesei*

When effect of pH and temperature was studied (TABLE 1), pH 6 and 30^{0}C were found to be optimum.

Under optimum conditions the cellulase activity of *T.reesei* was found to be higher with rice straw as substrate(0.42IUml^{-1} FPU, 0.61IUml^{-1} CMCase) compared to other substrates. Both FPU and CMCase activity of cellulase produced from *T. reesei* are given in the TABLE 2.

Cellulase production by *A.niger*

When effect of pH and temperature on cellulase activity was studied (TABLE 3), 5.5 pH and 32^{0}C were found to be optimum for *A. niger*.

Under optimum conditions the cellulase activity of *A.niger* was found to be higher with rice straw as substrate(0.50IUml^{-1}FPU, 0.60IUml^{-1}CMCase) compared to other substrates. Both FPU and CMCase activity of cellulase produced from *A.niger* are given in the TABLE 4.

Molecular weight determination

Gel with molecular weight marker and samples is shown in figure 1. *A.niger* shows a band corresponding to 40kDa and *T.reesei* shows band corresponding to 30kDa.

Under optimum conditions rice straw was found to be good substrate for both the fungi we have taken in this work. Rice straw can be one o the cheapest source for a good yield of cellulases. The molecular weight of cellulase from *T.reesei* was found to be 30kDA and

TABLE 1 : FPU of *T.reesei* cellulase at different pH and temperature on different substrates

Substrate	pH					Temperature in ^{0}C				
	5	5.5	6	6.5	7	25	28	30	32	35
Wheat straw	0.25	0.26	0.32	0.30	0.27	0.22	0.24	0.32	0.29	0.22
Rice straw	0.34	0.36	0.42	0.37	0.35	0.32	0.34	0.42	0.37	0.34
Rice husk	0.29	0.31	0.38	0.35	0.33	0.27	0.30	0.38	0.32	0.30

TABLE 2 : Activity of cellulase from *T.reesei* under optimum conditions

Sl.No.	Substrate	FPU (IUml^{-1})	CMCase activity (IU/ml^{-1})
1	Wheat straw	0.32	0.47
2	Rice straw	0.42	0.61
3	Rice husk	0.38	0.49

TABLE 3 : FPU of *A.niger* cellulase at different pH and temperature on different substrates

Substrate	pH					Temperature in ^{0}C				
	5	5.5	6	6.5	7	25	28	30	32	35
Wheat straw	0.39	0.45	0.40	0.36	0.30	0.26	0.30	0.38	0.45	0.39
Rice straw	0.42	0.50	0.45	0.37	0.35	0.35	0.37	0.45	0.50	0.44
Rice husk	0.26	0.30	0.28	0.25	0.22	0.24	0.27	0.28	0.30	0.28

TABLE 4 : Activity of cellulase from *A. niger* under optimum conditions

Sl.No.	Substrate	FPU (IUml^{-1})	CMCase activity (IU/ml^{-1})
1	Wheat straw	0.45	0.46
2	Rice straw	0.50	0.60
3	Rice husk	0.30	0.40

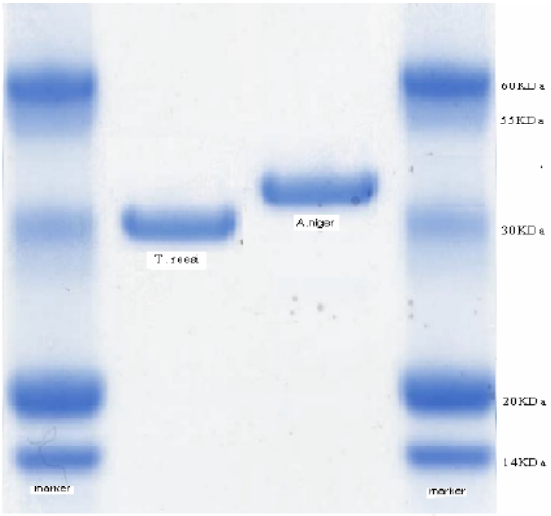

Figure 1

from *A.niger* was 40kDa.

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