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Extracellular Production of Thermostable Endoglucanase by Thermophilous Fungi

Ashok K. Shukla

Department of Microbiology & Biotechnology, Holy Cross Women's College, Ambikapur Chhattisgarh, India

Abstract: ***Fungi that can degrade cellulosic materials produce the cellulase complex. According to present concepts its components are endoglucanase, exoglucanase and B-glucosidase. It is established that the concerted action of all the three components is necessary for the complete hydrolysis of pretreated cellulose to glucose. It has been suggested that the endoglucanase is a random acting enzyme on the polysaccharide chain which results in the formation of cellulose oligomers and it has been considered to be the one which initiates the hydrolysis. Endoglucanase activity is of prime importance in cellulose degradation and hence in the present investigation extracellular production of this enzyme by thirty eight thermophilous fungi has been studied under two culture conditions.***

Keywords: Endoglucanase enzyme, Thermophilous fungi, Biodegradation, Cellulosic waste

1. Introduction

A prominent carbonaceous constituent of higher plants and probably the most abundant organic compound in nature is cellulose. It is a major constituent of plant cell wall in addition to other poly saccharides. In living plant tissue, it provides resistance against microbial attack. Meanwhile, it is hard to decompose quickly, when it become a component of litter. The microbial degradation of cellulose waste is of paramount importance from its indispensable role in the recycling of carbon element in biosphere. Cellulose is a linear polymer of glucose units bond together by B-1, 4-linkages.The nature of cellulosic substrates and their physical state, crystallinity index etc are important factor in enzymatic hydrolysis especially from the point of adsorption and desorption of cellulose enzyme during their saccharification (Fan et. al. 1980; Lee et. al.1983).

The group of micro-organism which has received the most attention from the point of cellulose degradation and formation of cellulolytic enzyme are the fungi. Among them the fungi *imperfectii* and *basidiomycetes* have yielded promising cultures for cellulose biotechnology. One of the most extensively studied cultures is *Trichoderma reesei* on which pioneering work was carried out by Reese 1956.Other fungi besides T. reesei which have also the ability to secrete high level of extracellular cellulose(s) enzyme (Mishra et. al.1983, 1984 and Rao and Mishra 1989). In recent years many advances have been made in developing acid hydrolysis processes for cellulose biotechnology. Problems of corrosion as well as non-specific nature of acid hydrolysis are not enough and are operative under relatively mild conditions. Cellulolytic microbes are well known to hydrolyze this substrate to produce microbial biomass by virtue of cellulose enzyme (Eriksson and Larsson 1975, Johri 1980, Nwagu and Okolo 2011).

Today's scenario, there is an urgent need in the developing world to search alternate energy sources. Cellulose is produced in millions of tons annually through photosynthesis in all over the world and notably in the tropical region of the world which have only scanty fossil fuel reserves. The realization that the limited nature of fossil fuel reserves which the civilized world has been depleting at an alarming rate emphasizes the need for utilizing renewable polysaccharide(s) resources for meeting mankind's future needs of food and fuel. Hence, cellulose bioconversion through enzymatic hydrolysis to obtain sugars which can be in turn fermented to produce liquid fuels has received global attention (Mishra and Maheshwari 1996, Wood, 1975).

2. Materials and Methods

Extracellular endoglucanase activity of the test fungi was determined by the following methods (Shukla 2014 a):

2.1 Preparation of spore suspension

Test organisms were grown on malt salt agar medium and the spores were harvested to prepare the inoculums. For this, spore suspension having $2X10^6$ Spores/ml in 0.1 per cent Tween-80 were prepared and considered as spore suspension.

2.2 Medium

For enzyme production, two media of different composition were used for the production of extracellular enzyme. With minor modifications the media were prepared consisting of the following composition, as suggested by Chapman *et al* (1975).First medium was having (K_2HPO_4-01.00 g,$(NH_4)_2HPO_4$-01.20 g, Urea-01.40 g, $MgSO_4.7H_2O$-00.30 g, Yeast extract-00.10 g, Peptone-01.10 g). Microelement solution (Fe-01 mg, Zn-01 mg, Mn-0.5 mg, Cu-0.08 mg, Co-0.1 mg) of one ml and CMC-Na-10.00 mg in one Liter distilled water. While second medium consist of the same composition except that CMC-Na was deleted in order to know the constitutive or adoptive/indusive nature of enzyme (Shukla 2014 b).

2.3 Preparation of flask

Thirty ml of the liquid medium was dispensed in each 150 ml Erlenmeyer flask and then autoclaved. Test organism were inoculated with 1 ml of spore suspension ($2x10^6$ spore/ml) in duplicate and a set of uninoculated flasks for each medium run as control. Flasks were then incubated at

45^0C temperature for seven days under stationary culture condition. After incubation flasks were taken out and the contents were filtered. Cell free filtrates so obtained were used as enzyme sample for detection of endoglucanases activity. Dry mycelial growth of each test organism was also determined.

2.4 Enzyme assays

CMCase activity in enzyme sample was determined following method described by Shukla (1991).For this, 1ml of enzyme sample was incubated with 9 ml of 0.55% CMC in 0.055 M citrate buffer pH 5.0 for 1 hr at $45^0 \pm 1^0$C. One milliliter of uninoculated medium was also inoculated in the similar manner which served as control (Miller 1972). The enzyme activity was assayed in term of amount of reducing sugar formed by the hydrolysis of CMC. For this, 1 ml of above reaction mixture was combined with 3 ml of DNS reagent. After adding the DNS reagent the mixture was boiled for 3 min, immediate cooled and observed absorbance at 550 nm was noted with a Remi C4 UV-Visual spectrophotometer. The reaction mixture of uninoculated control was used to set absorbance at zero. The amount of D-glucose formed due to enzyme hydrolysis in each case was determined by using a standard method (Shukla 1991).

3. Result and Discussion

Thirty eight fungal strains belonging to 14 species of 10 genera were tested for extracellular production of endoglucanase enzyme. For this, fungi were grown on two different media i.e. with CMC (Medium 1) and without CMC (Medium 2). All the strain of test species were found to produce this enzyme in medium 1, while some strains of different species failed to produce enzyme when grown on Medium 2. These include three strain of *Aspergillus nidulans* (TH 13, TH 21 and TH23), one of *Aspergillus terreus* (TH22) and two of *Emericella nidulans* (TH 15 and TH 24), two of *Emericella quadrilineata*(TH 37, TH 69), *Rhizopus oryzae* (TH 27) and both the test strain of *Thermomyces lanuginosus* (TH14and TH 16). However, other test strains were found to synthesize this enzyme in medium 2, and showing their constitutive nature (Cochrane, 1958 & Shukla 2010). Most endoglucanases are known as inducible enzyme when subjected to catabolic repression (Horton and Keen 1966, Muller and Batman 1971, Westermark and Eriksson 1975). However, constitutive production of this group of enzyme has also been reported. Induced endoglucanases synthesis by fungi in culture media containing cellulose is well known but, there is an optimum concentration above which endoglucanase synthesis is also repressed. In present investigation, activity of endoglucanases was recorded at $45^0 \pm 1^0$ C temperature. At this temperature many of the test thermophilous fungi showed greater endoglucanases activity. Endoglucanases from other sources are also reported to refer higher temperature for their optimum activity (Chapman et al. 1975, Doshi 1982). Mathur and dube (1982) reported activity of endoglucanases at a wide range of higher temperature, i.e., from 40^0 C to 90^0 C temperatures. In the present study, the test fungi have produced endoglucanases having its activity at 45^0 C $\pm 1^0$ C. This suggested their possible role in the cellulose degradation by these moulds in heated environments such as compost piles, grain storage etc. With an aim to compare the efficiency of test fungal species they have been classified in to four categories on the basis of the amount of glucose liberated due to the activity of enzyme produced by one gram of fungal mycelia in per hour. The test fungi are further classified as weak, moderate, good and strong producer of endoglucanase enzyme, in the medium. On the basis of above criteria a brief summary of the results was prepared and presented in table. *Aspergillus fumigatus* was found to the strong producer of endoglucanases. In addition to this, some strain of *Aspergillus nidulans*, *Corynascus sepedonium* and *Emericella nidulans* were found with strong potency to produce this enzyme. However, one strain of *Corunascus sepedonium* was found to be weak producer of endoglucanases. Some of the strains of *Aspergillus nidulans* failed to produce this enzyme in cultures when grown on Medium 2.

Table 1: Amount of glucose liberated due to the activity of enzyme produced by mg/hr/g dry weight of fungal mycelium.

S no.	Name of organism	Isolate No.	Medium I			Medium II		
			Dry weight (mg)	Amount of glucose librated due to activity of enzyme mg/hr/ml	Liberation of glucose due to CMCase activity mg/hr/g dry wt. basis	Dry weight (mg)	Amount of glucose liberated due to activity of enzyme mg/hr/ml	Liberation of glucose due to CMCase activity mg/hr/g dry wt. basis
1	*Acrophialophora fusispora*	TH 40	36	0.024±0.000	20.000±0.007	49	0.047±0.000	28.770±0.020
2	*Aspergillus fumigates*	*TH 59*	*24*	*0.036±0.000*	*45.000±0.000*	*44*	*0.144±0.000*	*98.180±0.000*
3	*A. nidulans*	*TH 13*	*24*	*0.016±0.000*	*20.000±0.000*	*30*	*0.000±0.000*	*00.000±0.000*
4	*A. nidulans*	*TH*	*44*	*0.018±0.000*	*12.270±0.020*	*30*	*0.031±0.000*	*31.000±0.000*
5	*A. nidulans*	*TH21*	*42*	*0.113±0.000*	*80.710±0.000*	*40*	*0.000±0.000*	*00.000±0.000*
6	*A.nidulans*	*TH 23*	*91*	*0.065±0.000*	*21.420±0.000*	*43*	*0.000±0.000*	*00.000±0.000*
7	*A.nidulans*	*TH 35*	*30*	*0.074±0.000*	*71.000±0.000*	*36*	*0.068±0.000*	*56.660±0.000*
8	*A. nidulans*	*TH 38*	*50*	*0.047±0.000*	*28.200±0.004*	*82*	*0.026±0.000*	*09.510±0.021*
9	*A .nidulans*	*TH 82*	*32*	*0.060±0.000*	*56.250±0.001*	*72*	*0.039±0.000*	*16.250±0.000*
10	*A.nidulans*	*TH 129*	*39*	*0.037±0.000*	*28.460±0.007*	*52*	*0.062±0.000*	*35.760±0.001*
11	*A.nidulans*	*TH 133*	*62*	*0.039±0.000*	*18.870±0.000*	*48*	*0.072±0.000*	*45.000±0.000*
12	*A.terreus*	*TH 19*	*36*	*0.031±0.000*	*25.830±0.000*	*70*	*0.014±0.000*	*06.000±0.004*
13	*A.terreus*	*TH 22*	*31*	*0.028±0.000*	*27.090±0.005*	*37*	*0.000±0.000*	*00.000±0.000*
14	*Corynascus sepedonium*	*TH 127*	*60*	*0.055±0.000*	*27.500±0.002*	*44*	*0.131±0.000*	*89.31±0.001*
15	*C. sepedonium*	*TH 130*	*60*	*0.044±0.000*	*22.000±0.000*	*61*	*0.118±0.000*	*58.030±0.000*
16	*Emericella nidulans*	*TH02*	*38*	*0.059±0.000*	*46.570±0.000*	*85*	*0.143±0.002*	*21.170±0.000*
17	*E. nidulans*	*TH 15*	*45*	*0.015±0.000*	*10.000±0.000*	*50*	*0.000±0.000*	*00.000±0.000*
18	*E. nidulans*	*TH 24*	*35*	*0.051±0.000*	*43.710±0.000*	*55*	*0.000±0.000*	*00.000±0.000*
19	*E. nidulans*	*TH 80*	*36*	*0.029±0.000*	*24.160±0.000*	*40*	*0.160±0.000*	*120.00±0.002*
20	*E. quadrilineata*	*TH 6B*	*67*	*0.034±0.000*	*18.210±0.000*	*58*	*0.031±0.000*	*16.030±0.001*
21	*E.quadrilineata*	*TH 12*	*44*	*0.043±0.000*	*18.210±0.000*	*48*	*0.094±0.000*	*58.750±0.000*
22	*E.quadrilineata*	*TH 37*	*39*	*0.044±0.000*	*33-840±0.000*	*41*	*0.000±0.000*	*00.000±0.000*
23	*E.quadrilineata*	*TH 69*	*44*	*0.048±0.000*	*32.720±0.004*	*56*	*0.000±0.000*	*00.000±0.000*
24	*E. quadrilineata*	*TH81*	*44*	*0.053±0.000*	*36.130±0.003*	*47*	*0.058±0.000*	*37.020±0.008*
25	*E. quadrilineata*	*TH 131*	*45*	*0.044±0.000*	*29.330±0.002*	*57*	*0.066±0.000*	*34.700±0.011*
26	*E. quadrilineata*	*TH 132*	*43*	*0.033±0.000*	*23.020±0.000*	*54*	*0.089±0.000*	*49.440±0.002*
27	*E. quadrilineata*	*TH 134*	*54*	*0.050±0.000*	*27.770±0.008*	*54*	*0.087±0.000*	*43.330±0.016*
28	*E. quadrilineata*	*TH 135*	*52*	*0.040±0.000*	*23.070±0.001*	*50*	*0.003±0.000*	*01.80±0.001*
29	*E. rugulosa*	*TH 128*	*31*	*0.042±0.000*	*48.640±0.003*	*32*	*0.066±0.000*	*61.870±0.027*
30	*Myceliophthora fergusii*	*TH 01*	*39*	*0.066±0.000*	*50.760±0.002*	*79*	*0.043±0.000*	*16.320±0.000*
31	*M. fergusii*	*TH 03*	*51*	*0.006±0.000*	*03.520±0.000*	*64*	*0.038±0.000*	*17.810±0.000*
32	*Neosartoria fischeri*	*TH 39*	*36*	*0.037±0.000*	*30.830±0.017*	*28*	*0.031±0.000*	*33.210±0.000*
33	*Rhizomucor pusillus*	*TH 28*	*42*	*0.036±0.000*	*25.710±0.000*	*41*	*0.039±0.000*	*28.530±0.000*
34	*R .pusillus*	*TH 41*	*30*	*0.032±0.000*	*23.410±0.006*	*49*	*0.067±0.000*	*41.020±0.000*
35	*Rhizopus oryzae*	*TH 27*	*26*	*0.029±0.000*	*33.46±0.002*	*29*	*0.000±0.000*	*00.000±0.000*
36	*Thielavia terricola*	*TH 48*	*49*	*0.039±0.000*	*23.870±0.001*	*59*	*0.039±0.000*	*19.830±0.008*
37	*Thermomyces lanuginosus*	*TH 14*	*73*	*0.028±0.000*	*11.500±0.000*	*27*	*0.000±0.000*	*00.000±0.000*
38	*T .lanuginosus*	*TH 16*	*34*	*0.033±0.000*	*29.110±0.002*	*31*	*0.000±0.000*	*00.000±0.000*

4. Conclusion

The results of the present investigation can't be directly compared with other results of other workers because of variability allied to choice of methods adopted and representation of data using criteria of their own choice. The results presented in table represent the amount of glucose released in one hour due to the activity of enzyme produced by one gram of fungal mycelia (on dry weight basis). The data thus presented is a comparative account of test fungi for estimating the economic viability of enzymes produced by 1 gram of fungal mycelia. However, representation of data in the present form provide significant acceptable basis for the role of these fungi in grain deterioration under storage condition and in their degradative activities occurring in nature where fungistatic, antagonistic and many more types of interaction between microbes are occurring. Overall, under limiting environmental condition, these test fungi showing strong activity of endoglucanases enzyme, may play an important role in biodegradation and biodeterioration processes and helpful in modern biotechnology.

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