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Research Article

Production of Cellulase from *Aspergillus fumigatus* Under Submerged and Solid State Fermentation Using Agricultural Waste

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ABSTRACT

This study aimed to the production of Cellulase from *Aspergillus fumigatus* was isolated and identified from soil and used for the study. *Aspergillus fumigatus* were screened for their cellulase production ability. Cellulase production was analysed in agricultural waste such as rice bran, coconut coir pith, wheat bran and rice husk. Among the study *Aspergillus fumigatus* have high enzyme activity in rice bran.In the study, the optimum parameters for the isolated organism for cellulase production were studied under varying condition such as of pH, temperature and substrates constrations. The maximum production of cellulase was noticed at temperature 25^{o}C and pH 4, substrate concentration 5g for *Aspergillus fumigatus* Finally concluded that the *Aspergillus fumigatus* showed highest level of cellulase production which was recommended for industrial level cellulase production.

Keywords: Cellulase, Submerged fermentation, Solid state fermentation *A.fumigatus*.

INTRODUCTION

Cellulase is an important extracellular microbial enzyme, which hydrolyzes cellulose. It is also one of the most inexpensive sources of biomass utilized for the production of pressing in the fruit juice industry and other factories via enzyme bioconversion, which proves to have a high industrial value. A great number of microorganisms, mostly fungi are able to degrade cellulose for their growth and produced a complete set of cellulose for the hydrolysis of cellulose to soluble sugar[1 2]. The agro waste such as sugarcane Becgasse, Pineapple peels, Rice straw, Wheat bran, Rice bran, Maize bran etc can be used as the best substrate for bioconversion [3].

Cellulase is commonly degraded by an enzyme called cellulase. This enzyme is produced by several microorganisms, commonly by bacteria and fungi[4 5 6]. Fungal genera like *Trichoderma sp* and *Aspergillus sp* are taught to the cellulase producers and crude enzymes produced by these microorganisms are commercially available for agricultural use[7]. Commercial production of cellulose by solid state fermentation appears to be less productive. The production of these enzymes is particularly dependent on cultural conditions[8].

Submerged Fermentation

Industrially important enzymes have traditionally been obtained from submerged fermentation (SMF) because of the case of handling and greater control of environmental factors such as temperature and pH.

Solid state fermentation

However, solid state fermentation (SSF) technique can improve the yield and reduces the cost of enzyme production. Filamentous fungi are the most commonly used microorganisms in SSF because they are able to grow on solid materials with low water contents. They are several reports describing use of agro industrial residues for the production of cellulose such as wheat straw, wheat bran and rice straw as substrates[9].

MATERIALS AND METHODS

Sample collection

Soil Samples were collected from a cellulosic waste composed area at Thirumangalakkottai,

Thanjavur District. Tamilnadu. It was thoroughly mixed' through a sieve 2mm pore size sieve and placed in polyethylene bags closed tightly and then stored in specific container.

Collection of biowaste substrates

Coconut coir pith, rice husk, wheat bran, and rice bran was collected from Thirumangalakkottai, Thanjavur, Tamilnadu.

Isolation and identification of fungi from soil samples

After sample collection, serial dilution was performed for isolating microbial growth from the collected samples. Isolated fungi identified based on cultural and morphological characteristics using Lactophenol Cotton blue staining method.

Inoculum Preparation

Cultures of *Aspergillus fumigatus* were maintained by stock culture in Czapek-Dox agar slants. They were grown at 37^0C for 24 hours and stored at 4^0C for regular subculturing 100ml of inoculums was prepared for each culture using Czapek-Dox broth in 250ml flasks. The inoculums was Kept in shaker (200rpm) at 37^0C for 24 hrs before it was used the fermentation process.

Fermentation Process

Submerged Fermentation[8]

Submerged fermentation was carried out in 250ml Erlenmeyer flasks containing 100ml of fermentation medium. The composition of the medium contained the following g/l of distilled water KH_2 PO_4-0.2, $(NH_4)_2$ SO_4-0.14, Urea-0.03, $Mgso_4$-0.03, $Cacl_2$-0.03, $Feso_4$-0.5, $Mnso_4$-0.16, $Znso_4$-0.14, $Cacl_2$-0.2, Coconut coir pith, rice husk, wheat bran, and rice bran. The medium was sterilized by autoclaving at 121^0C for 15 min. Each flask was inoculated with 1ml of the above set inoculum. The cultures were incubated on a rotary shaker (120rpm) at 30^0C for 72h.

Solid State Fermentation[3]

Solid state fermentation was caused out in 250ml Erlenmeyer flasks that contained 10g of coconut coir pith, rice husk, wheat bran, rice barn and 15ml of distilled water (Moistening agent). The flask were Sterilized at 121^0 C for15 min and cooled to room temperature. About 1ml of inoculums was added, mixed well and incubated at 30^0 in a humidified incubator for 96h. The flask were periodically mixed by gentle shaking.

Enzyme extraction[10]

At the end of the fermentation the culture broth from submerged fermentation was centrifuged at 6000 rpm for 15min and the supernatant was used as a source of extracellular enzyme. In solid state fermentation the enzyme was extracted from the boasted by mixing homogenously the entire waste with (1:10 w/v) distilled water and agitated on a rotary shaker (120rpm) at 30^0C with a contact time of 1 hours. Dampened cheese cloth was used to filter the extract and pooled extracts were centrifuged at 6000rpm for 15min and the clear supernatant was used as a source of extracellular enzyme.

Cellulase activity determination

The cellulase activity was determined by streaking the identified fungal cultures individually on the carboxy methyl cellulose agar plates and was incubated at 30^0C. After 5 days of growth, the zone was identified around the culture by treating the plate with congo red and NaOH.

Determination of reducing sugars and proteins cellulase activity

The total amount of reducing sugars in 1.0 ml supernatant was determined by modified Dinitro Salicylic method (DNS) [11] and Proteins[12].

Partial purification of cellulase

Enzyme source preparation

The fungal organisms *Aspergillus flavus* were grown in optimized media (100 ml of individual carbon source) at 30^0C for 24 hours individually. Then after growth, the culture filtrates were collected separately by centrification process[10].

Alcohol precipitation of cellulase

100 ml of crude enzyme (it is the culture filtrate of the respective organisms grown individually) from each source (both organisms and substrates) were taken individually and along with that 500 ml each of ethyl alcohol was added. They were allowed for precipitation for an hour and then centrifuged at 5000 rpm for 10 min. The precipitated enzymes from each source were refrigerated (4^0C) until further analysis.

Dialysis

The precipitate collected from each source was dissolved individually in 30 ml of sodium acetate buffer (0.2m) at pH 5.5 and were dialyzed against the same buffer overnight at 4^0C.

Effect of Supplements (Carbon & Nitrogen Sources)

Optimization of cellulase production

Various carbon source (sucrose, glucose, carboxy methyl cellulose, maltose, starch) and nitrogen sources (peptone, yeast extract, sodium nitrate, ammonium sulphate and ammonium nitrate) at concentration of 5% w/v were supplemented as individual components to the fermentation medium containing coir waste as substrate. The medium was inoculateand incubated at 30^0C for 3 days in an orbital shaker incubator (120rpm). The broth was

centrifuged and the enzyme assay was carried out. Care was taken to see that monosaccharide's were prepared a: l0x solution and sterilized separately by autoclaving at 101bs for 10min and required concentration was added to the medium before inoculation.

Effect of pH and Temperature

The optimized media were prepared using pH range such as 3,4,5,6 respectively by adding 1% NaOH and concentrated Hcl. Then the media were autoclaved. Later they were inoculated with broth culture and were placed in a shaker (150rpm) at 37^0C for 2 days. Simultaneously, for both the organism and both substrate, assay was carried out separately. The optimized media were prepared individually by using the substrates and autoclaved. Later it was inoculated with broth culture and was act at different temperatures 25,30,35,45 and 50^0C respectively. The effect of temperature on the production of cellulolytic enzyme was determined by growing the organisms at the above temperatures. Simultaneously for the organisms and both the substrates, separate assay was carried out. The enzyme solution obtained from there two (pH and temperature) experiments was individually activity method as described earlier.

Statistical Analysis

The results obtained in the present investigation were subject to statistical analysis like mean (x) and Standard Deviation (SD)[13].

RESULTS

In the present study fungal species were isolated from the soil sample. This identified fungal species *Aspergillus fumigatus* was used for the production of cellulase by Submerged and Solid state fermentation using various substrates such as rice bran, wheat bran, rice husk, coconut coir pith.

Isolation and identification of fungal species

In this study, fungal species were isolated from soil sample in Thirumangalakkottai, Thanjavur District. From the soil sample to dominant fungal strains were noticed and cultural characteristics two fungal strains of *Aspergillus fumigatus* were green in colour.

Identification of isolated colonies were identified by lactophenol cotton blue staining technique. The microscopic result was compared with standard fungal identification manual.

Cellulase production from various substrates

In solid state fermentation of *A. fumigatus*, the substrates like rice bran, wheat bran, rice husk and coconut coir pith were used for the production of the enzyme (0.37± 0.05 IU/ml), (0.36± 0.071 IU/ml), (0.30± 0.06 IU/ml) and (0.38± 0.07 IU/ml). In submerged fermentation of *A. fumigatus*, were used for the production of enzyme (0.96± 0.007 IU/ml), (0.54± 0.05 IU/ml), (0.52± 0.05 IU/ml) and (0.51± 0.51 IU/ml) respectively (Table 1).

Optimization of cellulase production

P^H Vs cellulase production

The cellulase production was optimized by supplementation using different p^H range medium from 4 to 9. In solid state fermentation maximum cellulase production was *A.fumigatus* noticed at p^H 6 in rice bran (0.84±0.041 IU/ml), and submerged fermentation maximum cellulase production was noticed at P^H 8 in rice bran (0.29±0.32 IU/ml) respectively. (Table 2)

Temperature Vs cellulase production

The cellulase production was optimized for rice bran using different temperature range of medium from 25°C to 45°C. In solid state fermentation maximum cellulase production was noticed *A.fumigatus* at 35°C (0.36±0.05 IU/ml), and submerged fermentation maximum cellulase production *A.fumigatus was* noticed at 35°C (0.56±0.06 IU/ml) respectively. (Table 2)

Carbon sourceVs Cellulase production

The Cellulase production was optimized using different supplementation medium such as Sucrose, Glucose, Carboxyl methyl cellulose,Maltose and starch. In solid state fermentation maximum cellulase production was noticed *A.fumigatus* in rice bran for CMC (0.13±0.05 IU/ml) and submerged fermentation maximum cellulase production was noticed in rice bran for CMC (0.11±0.04IU/ml) respectively. (Table 3)

Nitrogen source Vs cellulase production

The cellulase productivity was optimized using different nitrogen supplementation medium such as Peptone, yeastextract, Sodium nitrate, Ammonium sulphate and Ammonium nitrate. In solid state fermentation maximum cellulase production was noticed *A.fumigatus* in rice bran for nitrogen source in Ammonium nitrate (0.19±0.05 IU/ml). and submerged fermentation maximum cellulase production was noticed in rice bran for nitrogen source in Ammonium nitrate (0.17±0.04 IU/ml) respectively.(Table 3)

DISCUSSION

In the present study cellulase productivity were estimated in the isolated fungal strains of *A.flavus, A.fumigatus* optimized the enzyme productivity using various physicochemical parameters. Bioconversion of renewable lignocellulosic biomass to ethanol as an alternative to liquid fuels has attracted an intensive attention of researchers since oil crisis broke out. Cellulases provide a key opportunity for achieving tremendous benefits of biomass utilization. The enzymatic degradation of

cellulosic materials by fungal enzyme systems has been suggested as a feasible alternative to produce fermentable sugars and fuel ethanol from lignocellulosics[15]. Therefore, our study was correlated with cellulases producted by fungi, especially by *T.reesei* and *T.viride* have been extensively studied and many progresses have been made. Inspite of present succeses, the task of finding new highly active cellulases or efficient producers of cellulases remains topical.

In this present study Cellulase productivity was estimated among the three substrate. The maximum cellulose productivity noted is saw dust (0.088 U/ml/min). In this study maximum cellulose productivity noted in pH 5. The similar results were reported[14]. The maximum cellulose production was obtained between pH 5 and 6. In this study optimization using different temperature upto 30-50°C. Among this study highest productivity noted 25°C.

It was concluded that the nature of the substrate incubation time, temperature, pH etc, all influence the production of cellulase in submerged fermentation and soild state fermentation of rice bran. The results provide valuable information for the production of cellulase by *Aspergillus using* relativity inexpensive carried out in this study proved to be fruitful in enhancing programs for enzyme of biotechnological important.

Table 1: Cellulase enzyme production by solid state fermentation and submerged fermentation various substrates using *A.fumigatus*

Substrate	Solid State Fermentation IU/ml	Submerged Fermentation IU/ ml
	A.fumigatus	***A.fumigatus***
Rice Bran	0.37 ± 0.05	0.96 ± 0.007
Wheat bran	0.36 ± 0.071	0.54 ± 0.05
Rice husk	0.30 ± 0.06	0.54± 0.03
Coconut coir pith	0.38 ± 0.07	0.51 ± 0.025

Values are represented as Mean ± Standard Deviation

Table 2: Cellulase productivity of *Aspergillus fumigatus* various pH and temperature

Substrate	pH	Solidstate Fermentation IU/ml	Submerged Fermentation IU/ml	Temperature	Solidstate Fermentation IU/ml	Submerged Fermentation IU/ml
Ricebran	4	1.16 ± 0.76	0.19 ± 0.031	25°c	0.26 ± 0.05	0.43 ± 0.081
	5	0892 ± 0.051	0.20 ± 0.05	30°c	0.32 ± 0.05	0.56 ± 0.06
	6	0.84 ± 0.041	0.24 ± 0.021	35°c	0.36 ± 0.05	0.51 ± 0.06
	7	0.83 ± 0.021	0.25 ± 0.41	40°c	0.33 ± 0.05	0.48 ± 0.06
	8	0.089 ± 0.31	0.29 ± 0.32	45°c	0.30 ± 0.05	0.51 ± 0.06

Values are represented as Mean ± Standard Deviation

Table 3: Cellulase productivity *Aspergillus fumigatus* at various Carbon and Nitrogen source

Substrate	Nitrogen Source	Solidstate Fermentation IU/ml	Submerged Fermentation IU/ml	Carbon Source	Solidstate Fermenation IU/ml	Submerged Fermentation IU/ml
Ricebran	Peptone	0.10± 0.03	0.11±0.03	Sucrose	0.12 ± 0.05	0.10 ± 0.02
	Beef Extract	0.07 ±0.003	0.09 ± 0.003	Glucose	0.9± 0.03	0.7 ± 0.03
	Sodium Nitrate	0.07 ±0.003	0.11±0.002	CMC	0.13 ± 0.05	0.11 ±0.04
	Ammonium Sulphate	0.07±0.003	0.12 ±0.04	Maltose	0.6 ± 0.04	0.03 ±0.08
	Ammonium Nitrate	0.19 ± 0.05	0.17 ± 0.04	Starch	0.10 ±0.02	0.05 ± 0.01

Values are represented as Mean ± Standard Deviation

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