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Optimization of production conditions of cellulase enzyme from micro-fungi *Aspergillus fumigatus* for Agriculture Application

Abstract

Cellulase enzymes are belonging to the hydrolytic group of enzymes facilitates the sugar release and its bioconversion into different valuable industrial products. Isolated microfungi from rice straw by dilution plating pouring method studied for playing a various role in industries as well as in agriculture application. Various micro-fungi show enzymatic degradation of lignocellulosic material. The present study optimized the growing conditions for cellulase enzymes production from *Aspergillus fumigatus*. Optimization of various growth conditions such as temperature, different pH level and nitrogen source were studied for the production of enzyme carboxymethyl cellulase during this study. The results showed that *Aspergillus fumigatus* produced highest cellulase activity (6.19 IU/ml) at pH 7.0 and temperature 30^0C with yeast extract and Fpase activity (0.921IU/ml) through solid state fermentation. In future agriculture applications and in industries the cellulase enzyme production attains a crucial role to acquire biodegradable yield.

Key word: Dilution plating method, Lignocellulosic, solid state fermentation, carboxymethyl cellulase, Fpase

Introduction

The practice of burning leftover rice straw is widespread throughout the Asia-pacific area. The negative result of burning rice straw on the environment, such as air pollution also contribute to changes in temperature, loss fertility of soil on agriculture land. Lignin, hemicelluloses and cellulose make up the majority of lignocellulosic biomass.

Cellulose is the most prevalent lignocellulosic biomass which accounts for 40-60% of its weight (Sharma et al., 2019). Cellulose has a polyacetal form of cellobiose(4-0-D glucopyranosyl-D-glucose) (Harmsen et al.,2010). According to Lynd et al. (2002), cellulose makes up the majority of plant biomass and is only present in the cell wall of plants.strong influences on lignocellulosic biomass recalcitrance can be found. Therefore, the cellulose was digested by an bio-enyme like cellulase to create glucose, which was then utilized in many businesses was then utilized in many

businesses. (Koomnok,2005). The biopolymer of cellulose can be converted into reducing sugars by cellulase enzyme which have various biotechnological uses(Bhat,2000).various microfungi,bacteria,actinomycetes generate this enzymes actinomycetes generate this enzyme (Jagdish and Pawandeep,2012)

Micro-fungi, which can be easily exploited to produce commercial cellulases and are found in nature, are natural agents for cellulase degradation. For the synthesis of cellulase,*Trichoderma* and *Aspergillus* are thoroughly investigated (Lee et al.,2002). Aspergillus species are present in almost all situation with high oxygen levels. The Aspergillus species has a number of qualities that make them exceptional organisms for use in agriculture and industries, including satisfactory fermentation proficiency, high levels of protein secretion, high sporulation capacity and ability to acclimate to various organic substrates. In addition, they are involved in the synthesis of enzymes that aid in the breakdown of plant cell wall components like lipids, starch and protein. (Rodrigues BSS. 2011)

A perfect environment is needed for multiplication of micro fungi stain and increase the production of the cellulase enzyme. The yield of the enzyme often depends on a complex relation between numerous variables, including inoculums size, pH, temperature the presence of inducers, growth period, moisture and medium of cultivation (Polyanna *et al*., 2011, Robson L.M *et al.*, 1989).The optimal pH,solubility and amino acid content of the majority of cellulase investigated are comparable. The substrate's specificity and thermal stability can change. Therefore the objective of the current study is to investigate high level cellulase enzyme produced by *Aspergillus fumigatus* and optimize the parameter to hasten the production of cellulase.

2. Material and methods

2.1 Isolation of fungus

Isolated *Aspergillus* fungus from rice straw by Dilution plating pouring method. The potato dextrose agar (Hi-media GMH09-India) was prepared according to instructions with pH 5.6 after that sterilized at 121^{0}C temperature and 15 lbs for 30 minutes and poured into petriplates,the plates were leave to solidify at room temperature.

Weigh one gram rice straw and taken into test tube with 9 ml distilled water and shaken at constant speed for 5 minutes. The rice straw suspension of 1µl from each dilution (upto10^{-3}) pours into petriplates and spread with the help of sterilized spreader. The plates were incubated fro 5-7 days at 28 ^{0}C. Further fungus morphologically and structurally identify as describe by text books. (Gilman1957; Barnettand hunter 1972). Identified *A. fumigatus* was stored at 4^{0}C in refrigerator for further uses.

2.2 Cellulase Enzyme Production

Cellulase production was performed by using rice straw as the sole carbon source in a 500 ml an Erlenmeyer flask containing broth media. The composition of the medium was in (g/l in distilled water yeast powder (2g/l), jaggery (5g/l) and urea (1g/l).

In this study Solid state fermentation (SSF) was used for the production of cellulase enzyme. Spore suspension were prepared with the same media for 4-5 days old culture of *A. fumigatus* and scratched with sterilized plastic loop under the aseptic conditions in laminar air flow. Then, 5ml of spore suspension were inoculated into the rice straw flasks media and gradually mixed. The flasks were then placed in static condition at incubator for different incubation period. The temperature of incubator was fixed at 30^0C. After selected time of incubation and growth times, the flask were filtered off (Whatman filter paper No.1) and transferred into falcon tube for centrifugation (Eppendorf) at 12,000 rpm for 15 minutes to remove all cell debris. The supernatants were used to measure the cellulolytic activity by the standard test method Ghose (1987).

2.3 Cellulase assay

The extracellular carboxymethyl cellulase enzyme assay, as well as fpase-enzyme assay was performed. For Carboxymethyl cellulase assay among three tubes, 1st tube was for substrate blank containing 1.6 ml sodium citrate buffers, 0.4 ml carboxymethly cellulose and for enzyme blank 1.6 ml enzyme of desired fungus, 0.4 ml sodium citrate buffer while 3rd tube was for test sample, containing 1.6 ml fungus enzyme, 0.4 ml Carboxymethyl cellulose. Then all the test tube and control tubes were kept in water bath at 45°C for 15 min and cool under running tap water after that we have taken 1 ml sample from another same set of test tube and added 1 ml dinitrosalicylic acid (DNS) and were boiled for 5 min. The optical density (OD) of the mixture was checked by spectrophotometer at 540 nm wavelength.

2.4 Filter paper activity (FPase) production

Filter paper assays were determined by standard methods (Eveleigh *et al.*, 2009).The filtrate of enzyme sample is collected in tube and added a whatman no. 1 filter paper strip (1×60 cm, 50 mg) and 1 milliliter of 0.05M sodium citrate buffer of 5.0 pH.Incubate all the tubes at 50 °C into water bath for 1 hour and cool down the tubes. Reducing sugars released were estimated by dinitrosalicyclic acid (DNS) method (Ghose, 1987). One unit of filter paper (FPU) activity was defined as the amount of enzyme required to liberate 1 µ mole reducing sugars from the filter paper per ml per minute under standard assay conditions (Gilna and Khaleel, 2011).

3. Optimization of Culture Conditions for Cellulose Enzyme Production under solid State Fermentation (SSF*)*

3.1 pH effect on cellulase production

During this experiment different pH (5, 6, 7, 9) were tested for enhance production of cellulase and Fpase activity. The pH level will affect the yield of cellulase production which will later use for further study.

3.2 Temperature effect on cellulase production

Incubation temperature influences various metabolic activities such as enzyme production. Therefore in this study different temperature (25 ^{o}C, 30 ^{o}C , 35 ^{o}C, 40 ^{o}C) were used for optimize cellulase production.

3.3 Nitrogen source effect on cellulase production

For cellulase production nitrogen is most important factor. Thus in this study different nitrogen source like [illegible], ammonium nitrate, yeast extract and diamonium phosphate (DAP) were used for hasten the cellulase production. So abundant nitrogen source was used for further studies.

4. Result and discussion

[illegible]les of *Aspergillus fumigatus* were prepared for morphological identification under compound microscope (Leica EC 4 10 X 40X). (Fig1)

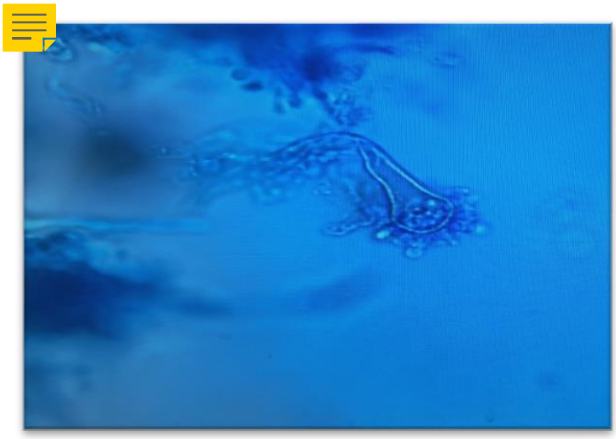

Figure1.Microscopic views of *A.fumigatus* under10X x 40X

The study shows an increase in production of cellulase enzyme.Cellulase enzyme activity was determined using various parameters.

At various pH value of 5.0,6.0,7.0 and 9.0 CMase activity were obtained as 2.47,2.59,4.96 and 1.58 IU/ml and Fpase activity obtain were 0.129,0.147,0.182 and 0.110 IU/ml as shown in Table 1.The result shows that CMase and Fpase activity are highest at 7.0 pH and the optimum temperature for highest CMase (6.19 IU/ml)and FPase enzyme activity (0.921 IU/ml) is at 30 ^{0}C respectively which is shown in Table 2.P[illegible]iously studied shows difference in temperature for cellulase production by *Aspergillius sp*. and *Tricoderma sp*. depends on different strain of micro-organism (Lu *et al*., 2003).The result are similar to Pothiraj and Eyini (2007) who reported that yeast extract was optimum nitrogen source for cellulase production similarly our result shows Optimum nitrogen source is yeast extract which enhance the CMase activity (4.11IU/ml) and Fpase(0.299 IU/ml) activity during degradation process indicated in Table 3.

Table-1 Optimization of pH

pH	CMase(IU/ml)	Fpase(IU/ml)
5.0	2.47	0.129
6.0	2.59	0.147
7.0	4.96	0.182
9.0	1.58	0.110

Table-2 Optimization of temperature

Temperature ^{0}C	CMase(IU/ml)	Fpase(IU/ml)
25	3.51	0.150
30	6.19	0.921
35	2.45	0.140
40	1.95	0.100

Table -3 Optimization of nitrogen

Nitrogen source %(w/w)	CMase(IU/ml)	Fpase(IU/ml)
Ammonium nitrate	2.19	0.254
Urea	3.25	0.291
Yeast extract	4.11	0.299
Di ammonium phosphate	2.92	0.275

5. Conclusion

In world lignocellulosic material degradation is a major problem. India and China are producing 90% of rice amongst Asian countries. As a result burning of rice straw is done by farmers. The cellulase enzymes play a vital role in degradation of lignocellulosic materials as well as used as alternative energy resources. In future micro-fungi strain are used for cellulase production. So the optimization of media parameters is important for fermentation. The growth of fungus depends on media pH and effect the stability of product. The present study shows highest cellulase and Fpase acivity by *Aspergillus Fumigatus*.

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