Comparative Study of Cellulase Production by Aspergillus niger and Trichoderma viride Using Solid State Fermentation On Cellulosic Substrates Corncob, Cane Bagasse and Sawdust

1G. L. Bhoosreddy

Department of Microbiology, Sevadal Mahila Mahavidyalaya, Sakkardara Square, Umrer Road, Nagpur, (M.S), India

Abstract: The enzyme activities of Trichoderma viride and Aspergillus niger grown on various waste cellulosic materials such as corncob, saw dust and sugarcane bagasse were tested for a period of 192 hrs. The maximum activity of 0.33 mg/min/ml & 0.026 mg/min/ml of cellulase enzyme were found to be produced on saw dust after 144 hrs by Aspergillus niger & Trichoderma viride, respectively. Corncob showed the highest activity at 120 hrs of 0.22 mg/min/ml & at 96 hrs of 0.0246 mg/min/ml by Aspergillus niger & Trichoderma viride, followed by sugarcane with the highest activity of 0.026 mg/min/ml & 0.0256 mg/min/ml 120 hrs by Aspergillus niger & Trichoderma viride, respectively. From the above results, Aspergillus niger produced the highest amount of cellulase activity with sawdust as substrate followed by Trichoderma viride by solid state fermentation.

Keywords: A niger, T viride, SSF, Cellulase, cellulosic’s

1. Introduction

The recent thrust in bioconversion of agricultural and industrial wastes to chemical feedstock has led to extensive studies on cellulolytic enzymes produced by fungi and bacteria. Cellulases have a wide range of enormous potential applications in microbiology. Some of the most important applications of cellulases are in food, brewery & wine, biofuels, animal feed, textile & laundry, paper & pulp industry, as well as in agriculture & research purposes. (Y.M. Galante, A. Deconti, and R. Monteverdi. 1998)1, and (R. K. Sukumaran et. al. 2005)2. Cellulase belongs to a class of enzymes produced chiefly by fungi, bacteria, and protozoans. It is a synergistic enzyme that is used to break up cellulose into glucose or other oligosaccharide compounds. Cellulases hydrolyze cellulose (β-1,4-D-glucan linkages ) & produce as primary products glucose, cellobiose & cello oligosaccharide. There are three major types of cellulase enzymes. Several different kinds of cellulases are known, which differ structurally and mechanistically (Chellapandi and Jani, 2008)3.

Cellulase production on a commercial scale is induced by growing the fungus on solid cellulose or by culturing the organisms in the presence of a disaccharide inducer such as lactose. However, on an industrial scale, both methods of induction result in high costs. Since the enzymes are inducible by cellulose, it is possible to use cellulose containing media for production. The ability to secrete large amount of extracellular protein is characteristic of certain fungi & such strains are most suited for production of higher level of extracellular cellulases. One of the most extensively studied fungi is Trichoderma reesei, which converts native as well as derived cellulose to glucose. Most commonly studied cellulolytic organisms include fungal species -Trichoderma, Humicola, Penicillium, & Aspergillus. While several fungi can metabolize cellulose as an energy source, only few strains are capable of secreting a complex of cellulase enzyme, which could have practical application in the enzymatic hydrolysis of cellulose. Besides T. reesei, other fungi like Humicola, Penicillium & Aspergillus have the ability to yield high levels of extracellular cellulases. (Fan et al., 1987)4.

The selection of substrates for enzyme production in a solid state fermentation process upon several factors, mainly related to cost & availability of substrate, the substrates that provides all the needed nutrients to the microorganisms growing in it should be considered as the ideal substrate. Thus, the solid-state fermentation (SSF) offers a low-cost alternative for producing cellulases using natural polymers derived from agro industrial residues (Milala et al. 2005)5.

2. Materials and Methods

All chemicals, media and reagents were purchased from Hi-media (Mumbai) and were of analytical grade.

2.1. Fungal strains used in cellulase production

Strains of Aspergillus niger and Trichoderma viride maintained as laboratory stock cultures, on PDA slants at 4°C, subcultured every week were used in the present investigation.

2.2. Collection and Preparation of cellulosic waste

Corn cob waste and Sugarcane bagasse was collected as a throw away waste, and saw dust was obtained from saw mills during the period of investigation. The preparation of cellulosic substrate for production of cellulase enzyme was done according to D. S. Syawala et al (2013)6 with slight modification. The cellulosic material such as corn cob, and sugarcane bagasse was chopped and sundried for period of 48 hrs. For first delignification, corn cob and sugarcane bagasse was first grounded to yield a fine powder. This powder was stored at 5°C for further experiments. About 100 gm each of the above cellulosic powdered waste was soaked using 0.1N NaOH at room for five hours with a ratio of 1:8.
Absorbance at 540 nm pressure conditions make fungi efficient & competitive in filamentous fungi because the hyphal mode of fungal growth. Solid state fermentation is preferable for cultivation of fungi.

3.1. Fungal strains used in cellulase production

3. Result and Discussions

3.2. Cellulase assay

2.4. SSF for production of cellulose

2.3. Inoculum preparation

2.5. Cellulase assay

Calculation

EA = absorbance of enzyme solution x (mg/ml/min) Time of incubation (min)

Whereas, standard factor (SF)

SF = Concentration (mg/ml) of standard glucose x Absorbance at 540 nm

3.  Result and Discussions

3.1. Fungal strains used in cellulase production

natural microflora for bioconversion of solid substrates. (Milala et al. 2005). The ability to secrete large amount of extracellular protein is characteristic of certain fungi & such strains are most suited for production of higher level of extracellular cellulases. It has been reported that fungal cellulases are well-studied enzymes and are used in various industrial processes. The enzymatic depolymerization of cellulose material has come from Trichoderma cellulase system. Species of Trichoderma can produce substantial amounts of endoglucanase and exoglucanase but very low levels of β-glucosidase. This deficiency necessitates screening of fungi for cellulolytic potential. (Fan et al.1987) and (Sohail M., R. Siddiqi. 2009). The present investigation is therefore an attempt using the laboratory maintained strains for production of higher yields of cellulase enzyme by Aspergillus niger & Trichoderma viride using cheap and available cellulolytic substrates such as Corn cob, saw dust, & sugarcane bagasse as major waste substrates using solid state fermentation. SSF of using A. niger and T. viride on corn cob, sugarcane bagasse and saw dust is depicted in photolates 1 to 6.

Plates 1: Production of cellulase by using Aspergillus niger with corn cob.
Plates 2: Production of cellulase by using Aspergillus niger with bagasse.
Plates 3: Production of cellulase by using Aspergillus niger with sawdust.

Plates 4: Production of cellulase by using Trichoderma viride with corn cob.
Plates 5: Production of cellulase by using Trichoderma viride with bagasse.
Plates 6: Production of cellulase by using Trichoderma viride with sugarcane bagasse.

3.2. Cellulase assay

The cell free supernatants subjected to enzyme assay yielded the following results. Figures 1 and 2 shows the comparison of the enzyme activities of Trichoderma viride and Aspergillus niger grown on various waste cellulolytic materials such as corn cobs, saw dust and sugarcane bagasse for a period of 192 hrs. The organisms have different periods for optimal cellulase yield. Depression in cellulase activities was observed after the 196 hours for both the organisms. This is as expected for enzymatic reactions that may be
prone to post-reaction accumulation of hydrolytic by-products (Howell, 1978).

It was found that the maximum activity 0.33 mg/min/ml & 0.026 mg/min/ml of cellulase enzyme were found to be produced on saw dust as substrate after 144 hrs by Aspergillus niger & Trichoderma viride, respectively. Corncob showed the highest activity at 120 hrs of 0.22 mg/min/ml & at 96 hrs of 0.0246 mg/min/ml by Aspergillus niger & Trichoderma viride, respectively. From the above results, Aspergillus niger produced the highest amount of cellulase activity with sawdust as substrate followed by Trichoderma viride by solid state fermentation. Our results are consistent with those reported by (Juwaied, et al. 2008).

Figure 1: Comparison of enzyme activity between different substrates by using Trichoderma viride.

Figure 2: Comparison of enzyme activity between different substrates by using Aspergillus niger

4. Summary

The aim of present investigation “Comparative studies on cellulase production by Aspergillus niger & Trichoderma viride” was carried out using different cellulosic waste material. The cellulase production was demonstrated from various waste materials such as corncob, sugarcane, sawdust using fungi like Aspergillus niger & Trichoderma viride by solid state fermentation. From the observation it was found that the enzyme activity was most from Aspergillus niger using sawdust. The present study suggests that sawdust, the by-product of saw-mill operations, is available in large amount and therefore could be a suitable low-cost substrate for cellulase production using strains of Aspergillus niger & Trichoderma viride.

References


Authors Profile

G. L. Bhoosreddy received his M. Sc (Microbiology) and Ph. D degree from Rashtrasant Tukadoji Maharaj Nagpur University, Nagpur in 1984 and 1988, respectively. He is affiliated as an Associate Professor, Department of Microbiology, Sevadal Mahila Mahvaidyalaya, Nagpur with a teaching experience of about 28 years at undergraduate level. He has to his credit a number of Research papers published in International and National journals of repute and in International and National conference proceedings. He is an author of three National Textbook and two local textbooks. Students under him have completed their Ph.D and M.Phil degrees.