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Research Article

Production and Detection of Cellulase by isolation *Trichoderma species* using agriculture waste

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ABSTRACT

Recent demands for the production of biofuel from lignocelluloses led to an increased interests in engineered cellulases from T richoderma spp or other fungal spores. Cellulase production by the fungus Trichoderma was studied using agricultural wastes.Trichoderma is isolated from soil samples of different areas o fKota.PDA media is used for the isolaton of trichoderma.The subculture medium was a salt solution consisting of monoxol.O.T.(Diactyl ester of sodium sulpho succinic acid).Fungal cells were sub culturedin an orbital shaker at 28c for 1-2 generation and were then used as an inoculum.Exponential cells were inoculated into medium containing wheat bran and other biomass. TO detect the presence of cellulase enzyme gel diffusion method is used. The effect of different agricultural waste on cellulose production was investigated.Data showed that cellulose in wheat has potential to be used as substrate to produce cellulose.

Keywords: *Trichoderma. Cellulase. wheatbran. biofuel.*

INTRODUCTION

India is an agricultural country has a lot of recyclable residues, obtained from wheat, rice, sugarcane and cotton. These crops yield wheat straw, wheat bran, rice husk, rice bran and sugarcane bagasse as by products and are abundantly available for utilization as subsrates for the production of bio-chemical such as cellulases. More than 14,000 species of fungi have been found to be active in degrading cellulose. The development of microbial strains, media composition and process control all contribute in the accumulation of high level of extra cellulose. [1]

The present study is concerned with exploitation agriculture by products of industrially important enzyme cellulases by the SSF (Solid State Fermentation) using locallyisolated *Trichoderma Spp*. Solid-state Fermentation (SSF) offers some advantages over liquid fermentation, as there is higher productivity, reduced energy requirements, lower capital investment, and low waste water output and low downstream processing cost. [2]

In the present era occurrence of energy is one of the major problems which humanity is facing. All the waste cellulose is a source of food and is also a potential source of energy. [3]

Cellulase is a carbohydrate that is the essential and characteristics structural substance of the plant world [Goldstein,1975].Due to the energy and food storages , which are likely to become more acute in near future , the need to reuse the waste cellulase has become more important.

Trichoderma is a filamentous fungus that is widely distributed in the soil, plant material, Decaying Vegetation and wood. Colonies of *Trichoderma* grow rapidly and mature in 5 days at 25ºC to 30° C will not grow at 35°C on PDA (Potato Dextrose Agar). The colonies are wooly and becomes compact in time from the front the colour is white as the conidia are formed

scattered blue green or yellow green patches become visible. *Trichoderma* spp. Produces different enzymes (Cellulase,Hemicellulase, xylanase, Chitinase, etc.) as secondary metabolites. [4]

MATERIAL AND METHODS

Soil sample from various places for isolation of *Trichoderma* for cellulase extraction. PDA media is used for the isolation of *Trichoderma.* PDA is prepared and sterilizes it at 121^0C, 15 lbs pressure. Then incubate the culture for 48 hrs. after the incubation period fungal colonies appears on the media surface then this *trichoderma* is used for isolation of Cellulase.

Isolation of cellulose decomposing fungi

Soil from different areas of Kota was screened for the isolation of cellulose decomposing fungi. Sample were collected from a depth of 2 cm Soil dilution and plate method was employed in the present work to isolate cellulose degrading fungi.

In the soil dilution plate method, standard soil suspension was prepared by dissolving 1 gm of soil in 10ml of sterilized distilled water cotained in sterilized test tube.

This was the stock suspension different dilution such as 10^{-1} to 10^{-} were prepared from the stock solution.One ml of soil suspension, from 10^{-} , 10^{-} & 10^{-} , dilution were spread on the solidified surface of mineral salt cellulase Agar medium containing cellulose powder as a carbon source.

Therefore, only those fungi which have the capability to degrade cellulose to yield glucose were able to able to grow on the medium.

Identification of fungal Isolation

Each isolate was inoculated on to potato dextrose agar medium. After the growth of fungi staining is done for the screening of pure strain.

Preparation of Inoculums

The spores from 5-7 days old culture were wetted by adding 10.0 ml of 0.005% monoxol. O.T. (Diactyl ester of sodium sulpho succinic acid) to each slant. The spores were scratched with a sterilized inoculating needle and the tubes shaken gently. The supernatant containing spores were decanted off aseptically and the suspension was then used as an inoculums for solid and shake flask fermentation. Vegetative inoculums were developed in the shake flask using basal medium for the production of cellulase in the stirred fermentator. The size of the inoculum developed at 28°C for 48 hours, was 5% v/v. The inoculum was aseptically transferred to the fermentor containing sterile production medium. [5]

Fermentation procedure: Solid State Fermentation

10 gm of solid substrate such as wheat bran or others biomass was transferred to a 250ml of flask. It was moistened by distilled water or other buffers such phosphate buffer. The flasks, plugged with cotton wool, were sterilized in an autoclave at 121°C for 20 minutes. The collected substrate was then inoculated with 1 ml of spore suspension and the cultures were incubated at 37°C for 72 hours. The flask was shaken twice daily.

Preparation of enzymed extrat from solid state fermentation

The enzyme was extracted by adding 100ml water (or buffer) to the fermented mash in each flask. The flask was shaken on a rotary shaker for 1 hour at 30ºC the fermented substrate Suspension was filtered using whatman filtrate was analyzed for cellulolytic activity.

Detection of the presence of cellulose enzyme in enzyme extract

Prepare an agar gel containing 1.7% agar and 0.5% CMC (carboxy methyl cellulose). Pour gel into Petri dishes and allow it to set. After the gel has set, use a narrow cork-borer to punch Small cylinders in the gel.Then, using a mounted needle, remove each of these cylinders to create a series of similar sized wells in the agar. Four or more wells can be put in single dish, provided they are spaced qapart. Place similar volumes of extracts of fruits in the each of the Wells. In one well, place some distilled water, as a control.Incubate the dishes for at least 24 hours at 30ºC. After the incubation period is finished, use tap water to rinse out the contents of the wells, and then flood the dishes with Congo red solution for 15 minutes. 1 M NaCl solution for least 10-15 minutes. Wells containing cellulose should have a clear zone around them.

OBSERVATION AND RESULT

To detect the presence of Cellulase enzyme gel Diffusion method is used. In this method the wells containing enzyme substrate shows a clear

zone of cellulolytic activity was observed. In this study, wheat bran was found to be a good substrate for the production for cellulases because the wheat bran contains adequate amount of nutrients. The production of cellulases was found to be optimal when the initial pH at the moistening agent was kept 6.5 as the production of enzyme by mould culture is very specific to pH.

Observation table: 1

Plate No.	**Wells No.1 10 ul** (enzyme extract)	**Wells No.2 20 ul** (enzyme extract)	**Wells No.3 30 ul** (enzyme extract)	**Wells No.4 Control (D.W)**
1.	Smaller zone	Medium zone	Larger zone	No zone
2.	Smaller zone	Medium zone	Larger zone	No zone
3.	Smaller zone	Medium zone	Larger zone	No zone

CONCLUSION

Cellulosic biomass such as agricultural by-products provides a low cost feedstock for biological production of cellulases. In present study *Trichoderma* was isolated from soil. Wheat bran was selected as the best agricultural by-product for cellulases production of optimal temperature 28°C and pH 6.5 and 72 hours after inoculation.

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