Int. Journal of Universal Science and Technology
ISSN: 2454-7263, Volume: 05 No: 01 Published: Jan., 2019
Paper ID: IJUP3107, Web: www.universalprint.org
Title Key: Evaluation of Cellulase Enzyme …

Evaluation of Cellulase Enzyme Activity on Selected Fungi by Cup Plate Method

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ABSTRACT:

Soil samples from different soyabean fields located in Kalamb Dist Osmanabad tehsil area were collected .Fungi were isolated from rhizosphere soil of soyabean field by using PDA medium supplemented with different antibiotic by serial dilution ,spore suspension of pure culture of isolated fungal species were inoculated in broth medium and incubated for 6 days at room temperature on 7^{th} day inoculated flask were harvested with the help of wattmen filter paper and filtrate were collected as crude enzyme .PDA supplemented with antibiotic were prepared and cavities made in center with sterile cork borer and filled with crude enzyme and plates were incubated at 29^0C for 24 hours in incubator .After incubation activity of enzyme cellulase was checked by calculating measuring zone of inhibition . Zone of inhibition the activity were recorded either stimuli or inhibit. It plays an important role in controlling fungal diseases as well as eco-friendly management of diseases causing pathogens. It is coast reducing practice in the field of agriculture.

KEY WORDS **:** PDA, Cellulase, Enzyme activity, Soyabean

INTRODUCTION:

Cellulase are enzymes largely focused by researchers and industrial sector as these enzymes are used in various economically relevant process .Cellulase and other enzymes are excellent microbial products in solid state cultivation , when produced by filamentous fungi hyphae have a natural ability to cover solid nutritive surface of the substrate. Enzymes play a important role in biodeterioation. Fungi are main cellulase producing microorganisms some bacteria and actinomycetes have also been reported to produce cellulase .In present work cellulase activity of selected fungi *Rhizoctonia solani*, *Aspergillus flavus*, *Aspergillus niger*, *Penicillium notatum* have been studied by cup plate method species of Aspergillus are reported

As cellulase producers and crude enzyme produced by these species are commercially available for agriculture use cellulase is an inducible enzyme .It is most abundant renewable biological resource with low cost energy.

MATERIALS AND METHODS:

- **Collection of soil samples:**

The initial and post harvest soil samples were collected from soyabean field of four random regions. Composite soil samples were used for the experiment.

- **Preparation of medium:**

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Potato were peeled and cut into small pieces and washed with water. Pieces of potato were added in 500 ml distilled water and boiled for 30 min. It is filtered with muslin cloth. dextrose and agar was add and kept for boil to dissolve. The medium was autoclaved. Ph of medium was maintained as 5.6.after autoclaving 20 ml of medium was poured into sterilized petri dishes .

- **Isolation of fungi from Rhizospheric soil by serial dilution method:**

In this method known amount (1gm in 10 ml) suspended.1gm is suspended in known volume 10 ml to make suspension label serial dilution as $10^{-1,}$ 10^{-2}, 10^{-3},10^{-4},10^{-5}. 1 ml of various dilutions are added to 1ml of sterile Petri dishes containing solid PDA .Upon solidification of the media, incubate all the Petri plates in an inverted position at 29^{0}c for 3-4 days in incubator. Observe the plates for number and distribution of colonies of fungi from each dilution.

- **To study production of cellulase:**

For the production of cellulase liquid medium containing CMC10gm was used. Out of which 25ml of medium was poured in 100ml conical flasks and autoclaved at 151 lbs pressure for 15 minutes. After cooling the flasks the spore suspension of test fungi inoculated in flasks. The flasks were incubated for 6 days at 290c in incubator. On 7th day the flasks were harvested by filter contents through whatmann filter paper the filters were collected in sterilized conical flasks and termed as crude enzyme.

PREPARATION OF CRUDE ENZYME

- **Assay for cellulase enzyme by cup plate method:**

The assay medium contains 1% CMC and 2% agar was poured in petri dish & allowed to solidified, with the help of sterile cork borer make cavities in the center of petri plate. The cup was filled with 0.1ml culture filtrate and incubated at 290c for 24 hours. The activity zone was developed in the plates.Milky white coloured zones were clearly seen at the edges of cavity. The diameter zone was measured in mm.

RESULTS AND DISCUSSION:

1. In the present study, the four fungi of rhizosphere such as Rhizoctonia solani, Aspergillus flavus, Aspergillus niger, Penicillium notatum were isolated from rhizospheric soil of soyabean .These isolated fungal organism were exploited for production of cellulase **(**Hanne R. Johnsenand Kirsten Krause. et.al 2014) .With the help of whatman filter paper for total cellulase activity for the filtrate was determined according to standard method. prepared extract of four fungi which

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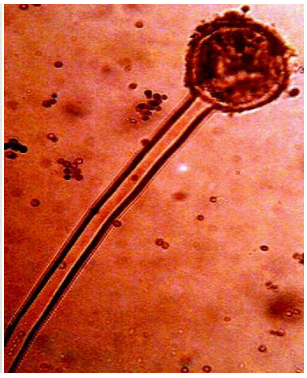
termed as crude enzyme, With the help of syringe 0.1 ml to 0.4 ml crude enzyme of each fungus injected into well(cup plate method) of PDA plate. **(T**. K. Ghose et.al 2009-01-01). All the plates were incubated for 24 hours. After incubation period, enzyme activity was detected by the appearance of zones either by substrates clearance or coloration and discoloration. *A .niger and P. notatum* were showed the highest zone of inhibition.

- **Table 1: Assay of cellulase enzyme shows that**

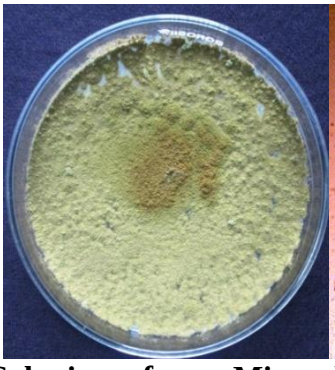
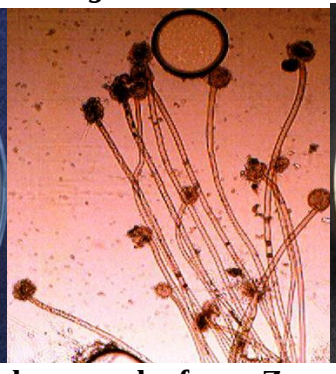
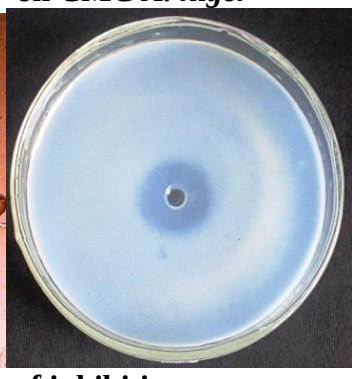
Sr. no.	**Fungi (crude enzyme) 0.1 ml**	**Diameter(mm)**
1.	*A. niger*	1.4
2.	*A. flavus*	1.0
3.	*P. notatum*	1.2
4.	*R. solani*	1.1

Activity of cellulase enzyme expressed in mm

- **PHOTO PLATE OF FUNGI**


Colonies of *A. niger* **Microphotograph of *A. niger*** **Zone of inhibition on CMC *A. niger***

Colonies of *A. flavus* **Microphotograph of *A. flavus*** **Zone of inhibition *on* CMC *A. flavus***

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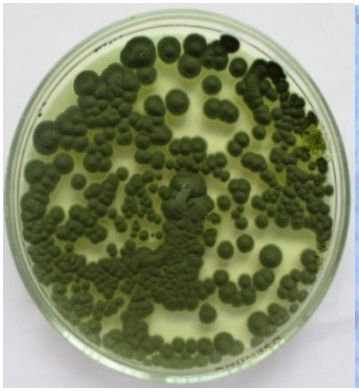
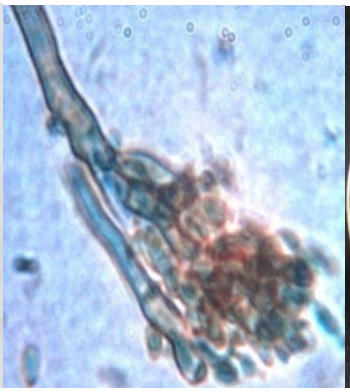
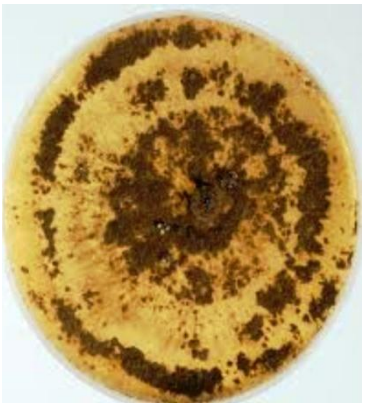
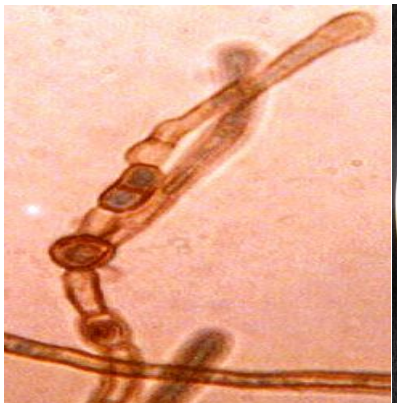
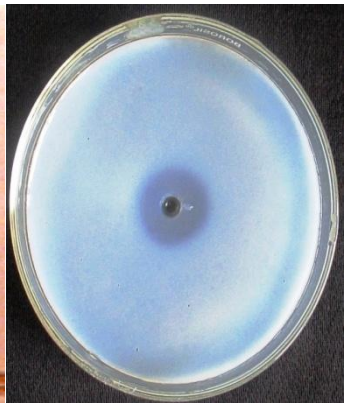




Colonies of *P. notatum* **Microphotograph of *P. notatum*** **Zone of inhibition on CMC *P. notatum***

Colonies of *R. solani* **Microphotograph of *R. solani*** **Zone of inhibition on CMC *R. solani***

References:

1. M.K.Bhat -Cellulases and related enzymes in biotechnology(13 September 2000)
2. T. K. Ghose- Measurement of cellulase activities (DE GRUYTER VOL NO 5,2009-01-01)
3. Lakshmi, A., Narasimha, G. 2012- Production of cellulases by fungal cultures isolated from forest litter soil. *Annals of Forest Research 55*(1): 85-92.
4. *DF Oliveros, N Guarnizo, EM Perea, WM Arango* - Cellulase activity of filamentous fungi induced by rice husk. (*African Journal of Biotechnology*, Vol 13(45) 4236-4245)
5. Narasimha, A Sridevi, Viswanath Buddolla, Chandra M. Subhosh, Reddy B. Rajasekhar- Nutrient effects on production of cellulolytic enzymes by *Aspergillus niger (African Journal of Biotechnology*, Vol 5,No5)
6. Hanne R. Johnsenand Kirsten Krause -Cellulase Activity Screening Using Pure Carboxymethylcellulose: Application to Soluble Cellulolytic Samples and to Plant Tissue Prints *nt. J. Mol. Sci.* 2014, *15*(1), 830-838.