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RESEARCH ARTICLE

CELLULASE FROM ISOLATES OF BANANA FIELDS IN NANJANGUD AND BANNUR VILLAGES OF MYSORE DISTRICT

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ABSTRACT

Banana is one of the widely used fruit though out the world and it is grown almost in all seasons and places, so in the present study fungal species were isolated from banana fields and banana plantation dumping yards for cellulase which can be used for the bioconversion of banana plantation waste generated after the recovery of fruits. Samples of soil and leaf were obtained from villages of Nanjangud and Bannur villages the fungal isolated were tested to find their ability to produce cellulase(which can degrade cellulose, which is a homopolymer made of glucose units linked by β1, 4 glycosidic bonds) Out of 35 fungal colonies7 showed cellulytic activity and three fungal strains noticed to show maximum zone of hydrolysis of carboxyl-methyl cellulose and cellulase produced by the fungi were determined by carboxy methyl cellulose assay. Maximum cellulase production was obtained after 72 hr of incubation at 37°C in Czapek-Dox broth supplemented with 1% carboxymethyl cellulose medium and the pH was adjusted to 4.5-5.0. Fungal studies indicated *Aspergillus* and *Curvularia* and *Fusarium* were more frequent fungal strains found and banana fields and banaba plantation dumping yards. The purpose of the current study was to screen potential cellulytic fungi from the Banana fields in order to study the use of them for the bioconversion of the banana wastes into usable form.

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INTRODUCTION

Cellulase enzyme is used for the digestion of polysaccharide cellulose, Cellulose is the most common abundant, renewable polysaccharide on earth Cellulose is considered as one of the most important sources of carbon on earth and its annual biosynthesis by both land plants and marine occurs at a rate of 0.85×10^{11} tons per annum (Nowak *et al*., 2005). Cellulase degradation and its subsequent utilizations are important for global carbon sources. The value of cellulose as a renewable source of energy has made cellulose hydrolysis the subject of intense research and industrial interest (Bhat *et al.,* 2000). There has been much research aimed at obtaining new microorganisms producing cellulase enzymes with higher specific activities and greater efficiency (Subramaniyan and Prema, 2000). Over the years, a number of organisms, in particular fungi, possessing cellulose-degrading enzymes have been isolated and studied extensively (Bhat *et al.,* 1997). Cellulolytic enzymes play an important role in natural biodegradation processes in which plant lignocellulosic materials are efficiently degraded by cellulolytic fungi, bacteria, actinomycetes and protozoa. In industry, these enzymes have found novel applications in the production of fermentable sugars and ethanol, organic acids, detergents and other chemicals. Cellulases provide a key opportunity for achieving tremendous benefits of biomass utilization (Wen *et al.,* 2005).Cellulolytic enzymes are synthesized by a number of microorganisms. Fungi and bacteria are the main natural agents of cellulose degradation (Lederberg, 1992). The cellulose utilizing population includes aerobic and anaerobic mesophilic bacteria, filamentous fungi, thermophilic and alkaliphilic bacteria, actinomycetes and certain protozoa (Alexander, 1961). However, fungi are well known agents of decomposition of organic matter, in general, and of cellulosic substrate in particular (Lynd *et al*., 2002). Cellulose is the major component of plant biomass. Plants produce 4×10^{9} tons of cellulose annually. It is a polymer of β-1, 4 linked glucose units. Its crystalline structure and insoluble nature represents a big challenge for enzymatic hydrolysis. Microorganisms are important in conversion of lignocelluloses wastes into valuable products like biofuels produced by fermentation. Successful bioconversion of cellulosic materials mainly depends on the

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nature of cellulose, sources of cellulolytic enzyme and optimal conditions for catalytic activity and production of enzymes.

MATERIALS AND METHODS

Sample collection

Soil samples from banana growing fields were collected aseptically from in and around Mysore (Bannur and Nanjangud).

Isolation of cellulase producing fungi

Soil samples from banana growing fields were collected aseptically from in and around Mysore (Bannur and Nanjangud). Collected samples were serially diluted, pour and spread plating techniques were used later the plates are incubated for 5 to 7 days at 37ºC. Colonies were selected and sub cultured to obtain pure culture and maintained on potato dextrose agar medium.

Morphological identification of colonies

The isolated strains were identified by morphological characters including their growth patterns and reproductive structures by using cotton blue in lacto phenol stain

Primary screening of cellulolytic activity

Cellulase producing fungi were screened on selective Czapek-Dox medium used in this method contained (g/l): sucrose – 30, $NaNO_3$ – 2, K_2HPO_4 - 1, $MgSO_4$ – 0.05, Kcl – 0.5, $FeSO_4$ – 0.01, carboxy- methyl cellulose – 1%, Agar agar - 20. pH of the medium was adjusted to 5 (Teather and Wood 1982) The pure culture of the selected isolates were point inoculated on center of the plate with equal amounts and incubated at 35± 2°C until substantial growth was recorded. After around 50%of the growth of the colonies the plates were flooded with Congo red solution (1%) and stirred at 50 rev/ min for 15 min then the stain was discarded and de stained with 10 ml of 1 N NaOH and stirred for 15 min at 50 rev/min . Finally 1 N NaOH was also discarded and the stained plates were analyzed for the formation of clear or yellowish zones around the fungal colonies when the enzyme had utilized the cellulose. (Hankin and Anagnostaksis, 1975)

Production of cellulase

Out of 31 cultures isolated only 5 showed cellulytic activity. The 5 cultures were grown in 100 ml of Czapek-Dox broth supplemented with 1% carboxymethyl cellulose medium and pH was adjusted to the 4.5 to 5 and transferred to 250 ml conical flasks and autoclaved, then the flasks were inoculated with fungal cultures and incubated at 35ºC. Growth of mycelia is observed after 7 days the contents of flasks were centrifuge to separate mycelia from culture broth. Then the supernatant is used as crude enzyme.

Quantitative analysis of Cellulase enzyme

The crude enzyme was used for estimation of total protein content and total activity of cellulase by whatman filter paper-1 activity test as prescribed by International Union of Pure and Applied Chemistry (IUPAC) guide line.

Cellulase assay was determined according to Mandels *et al*. (1976) method. The amount of reducing sugar released from whatman filter paper digestion by cellulase isolated from different fungal strains, following formula is used to calculate enzyme activity.

RESULT AND DISCUSSION

Sample collection

Soil samples and infected banana leaves were aseptically collected from fields and serial dilution was carried out for the isolation of fungi with cellulytic activity.

Soil sample in zipped cover

Banana plant

Waste leaf

Isolation of fungi

Samples were serially diluted and followed by spread and pore plate plating technique using PDA media and the pH is adjusted to 4.5 - 5.5 after 48 to 72 hours of incubation at 37°C and CFU (colony forming units) was counted.

Screening of cellulose producing fungi

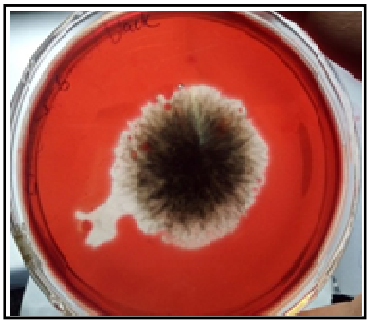
The isolated fungal colonies were checked for the production of cellulose enzyme by culturing them in czapek-Dox broth medium with 1% carboxy methyl cellulose and the pH was adjusted to 5 and transferred to the 500 ml conical flasks and autoclaved at 121°C at 15lbpresure, after cooling the media is transferred to petriplates in laminar air flow chamber and then inoculated with isolated fungi to identify cellulolytic fungi. After 3 days of incubation the cellulytic activity was identified by adding Congo red stain to the culture plates, appearance of the clear zone around the colony was strong evidence that the fungi produced cellulase in order to degrade cellulose (Wood and Bhat, 1988). Out of 10 fungal isolates, only 3 fungal colonies produced zones of hydrolysis in CMC agar plates, the fungi which shown maximum activity were selected and used for further analysis.

Morphological identification of colonies

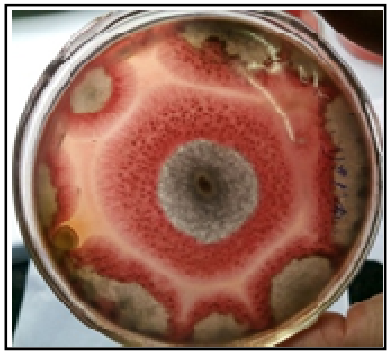
Isolated fungal colonies were identified with microscopic observation of mycelia and reproductive spores by using cotton blue in lacto phenol stain

Production of cellulose

The selected colonies were grown in 100 ml of Czapek-Dox broth supplemented with 1% carboxymethyl cellulose medium and pH was adjusted to the 4.5 to 5 and transferred to 250 ml conical flasks and autoclaved, then the flasks were inoculated with fungal cultures and incubated at 35ºC. After 3 to 5 days the fungal mycelia were filtered and the filtrate is used as a crude enzyme to identify the presence of cellulase.



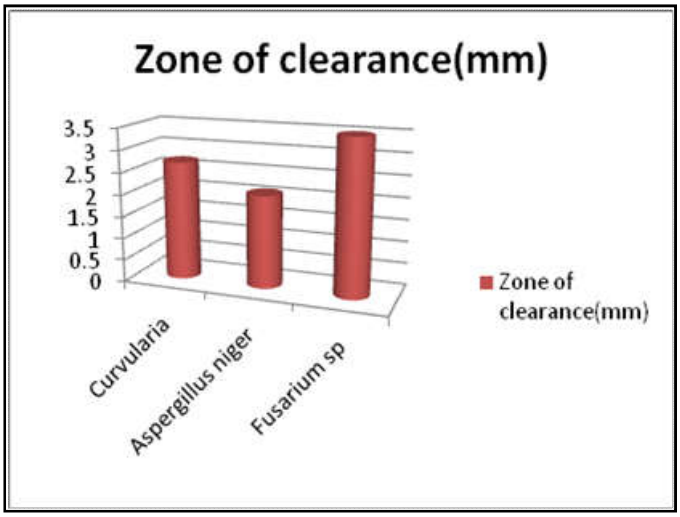
Curvularia

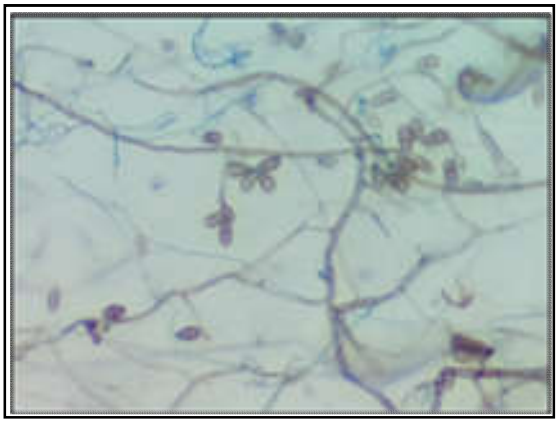


Aspergillus niger

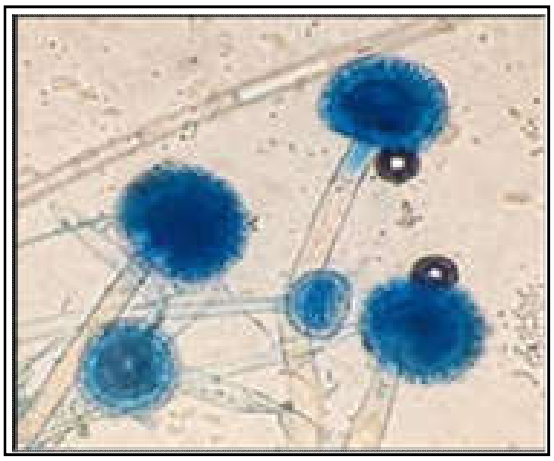
Fusarium sp

Fig. 2. Fungal colonies showing clear zone which indicate cellulase activity

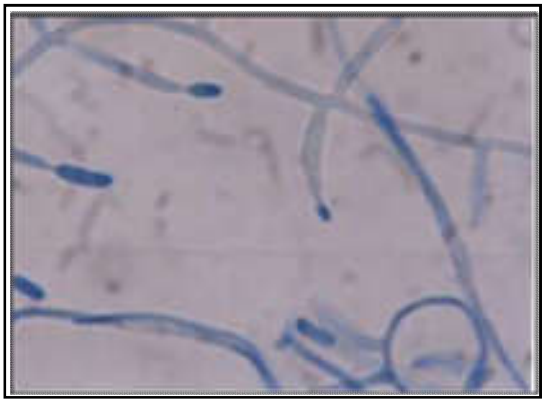




Curvularia



Aspergillus niger



Fusarium sp

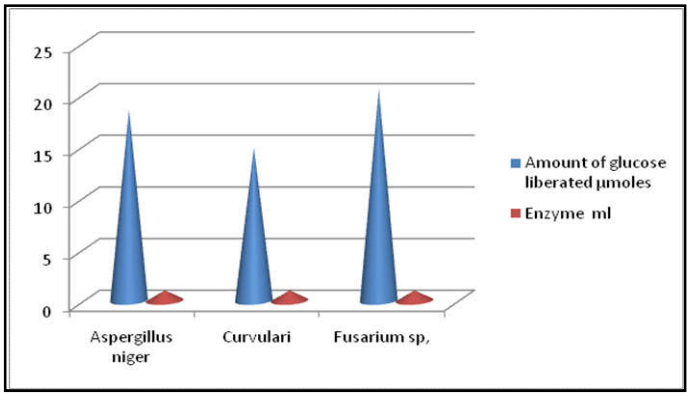
Fig. 4. Conical flasks with fungal cultures

Estimation of total activity of cellulase

Total Cellulase activity was checked by DNS method (dinitro salicylic acid) by the estimation of reducing sugars released from the sample. Sugars liberated were determined by measuring absorbance at 540 nm. Cellulase production was estimated by using glucose standard curve. The activities of enzyme cellulases were determined by using the following formula:

Enzyme activity = amount of glucose liberated/mg enzyme /minute at 45°C **(Ghose *et al.*, 1987)**

Enzyme activity



Conclusion

In the study cellulose enzyme is isolated from three fungal stains and their culture conditions were studied. It is found that the cellulase enzyme was almost equally produced from all three isolated at 72 h of incubation at 45°C and pH of 4.5-5.0. Further work to the present study includes isolation of many more fungal strains from other fields around Mysore and purification of the enzyme to get higher cellulytic activity by Ammoniumsulphate precipitation, Dialysis, PAGE, Zymogram and chromatography. Thus the study reveals that banana field waste contains many cellulytic fungal stains can be produced in bulk for bioconversion of cellulose to other important producs

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