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# STUDIES ON CELLULOSE DEGRADATION BY *DRECHSLERA PUTTAPARTHII* SP.NOV, A LOCAL ISOLATE FROM PRASANTHI NILAYAM.

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## ABSTRACT

A study on the enzyme activity of *Drechslera puttaparthii* sp.nov, a new species growing as a pathogen on Royal Palm tree *Roystonia regia* from Prasanthi Nilayam, Puttaparthi was carried out. A Semi -Solid Fermentation technique with Lemon grass as a substrate was used for growing the fungi .The activity of the crude enzyme produced by the fungi for degrading the Lemon grass leaf in semi - solid fermentation process was detected using Benedict's test for reducing sugars. The present preliminary investigation revealed the presence of enzymes, whose activity was revealed on starch and cellulose. This present investigation unravels the mystery of spectrum of enzymes produced by the new Hyphomycete namely *Drechslera puttaparthii* sp.nov, isolated from semi-arid tropical forest area of Puttaparthi mandal, Anantapur District, Andhra Pradesh

**Key words:** *Drechslera puttaparthii sp.nov, Semi-Solid Fermentation, Lemon-grass.*

## INTRODUCTION

Fungi are ubiquitous organisms which grow on variety of substrates. some depend on the dead and decaying matter for source of carbon, others survive as pathogens on plants and animals. The fungi secrete various cell wall degrading enzymes which hydrolyse the cell wall, leading to pathogen penetration. Cellulose is the most abundant cell wall component in the biosphere with its estimated synthesis rate of 1010 tonnes per year (Lynd *et al.*, 2002). Cellulose-rich plant biomass is one of the foreseeable and sustainable sources of fuel, animal feed and feed stock for chemical synthesis (Bhat, 2000). The utilization of cellulosic biomass continues to be a subject of worldwide interest in view of fast depletion of our oil reserves and food shortages (Kuhad *et al.*, 1997). The conversion of cellulosic mass to fermentable sugars through biocatalyst cellulase derived from micro-organisms has been suggested as a feasible process and offers potential to reduce use of fossil fuels and reduce risk of environmental pollution (Dale, 1999; Lynd *et al.*, 1999). However, the high cost of production of these enzymes has hindered the industrial application of cellulose bioconversion. One of the different approaches to overcome this hindrance is to make continuous search for organisms with secretion of cellulase enzymes in copious amounts and to optimize enzyme production with them. In this paper the cellulase enzyme production by Drechslera

puttaparthii (see fig. 1.2), a local isolate, in semi-solid fermentation in a laboratory scale is studied.

## MATERIALS AND METHODS

Isolation & Identification of fungal species: The fungal species was first identified as a pathogen inhabiting the Royal Palm tree growing in the University campus (See Figures, 1.1 and 1.3). The characteristic feature of the pathogen is the presence of light brown necrotic patches on the dorsal surface of leaf lamina (see figure 1.1). The organism is later identified as a new species and named as *Drechslera puttaparthii* sp.nov. (This fungus is confirmed as new species confirmed by National Fungal Culture collection Centre (NFCC), Pune.)

## SEMI-SOLID FERMENTATION

**Pre-treatment of substrates:** The fungus is cultured using semi-solid fermentation technique (see figure 1.4). The substrate for fermentation, lemon-grass is sun dried individually to reduce the moisture content to make them more susceptible for culturing. Lemon-grass is soaked in 1% sodium hydroxide solution (NaOH) to remove surface pathogens and washed with distilled water until the wash water became neutral. This surface sterilized Lemon-grass substrate is cut into small pieces of uniform size (approximately 5-6 cm in length). The lemon grass pieces were then re-sterilized by autoclaving at 121$^0$C for 1hr. The lemon grass pieces were weighed using a common balance. 100 gms of this sterile lemon -grass substrate is placed in each of 500ml sterile Erlenmeyer flask. To this 50ml of distilled water is added.

**Inoculum preparation:** The isolated cultures of *Drechslera puttaparthii* were maintained as stock culture in Potato Dextrose Agar (PDA) flasks. The pure cultures were grown on PDA slants. They were grown at 30$^0$C for 5 days and stored at 4$^0$C. Conidial suspensions were prepared from slants by flooding the surface of the cultures with sterile water and gently rubbing with inoculation needle. The inoculum was kept in shaker for (150 rpm) before it was used for the fermentation process.

**Fermentation process:** To the sterilized Lemon-grass substrate inoculum is added.1 ml of dense spore suspension of Drechslera puttapartii was used as inoculum for each 500ml Erlenmeyer flask. The inoculated cultures were incubated at 25$^0$C on rotary shaker at 140 rpm. Flasks were withdrawn after 7-day incubation period and fungal culture is filtered through Whatman No.1 filter paper to separate mycelial mat and culture filtrate.

**Measurement of cellulase activity:** The amount of enzymes secreted by the pathogen to degrade the cell wall is measured. The enzymatic activity of the cellulases produced by the fungus is measured by using Benedict test for reducing sugars. 5ml of the culture filtrate containing the enzyme is added to 5ml each of 1% and 5% starch and Cellulose solutions in 4 different test tubes and incubated for 5 min, for the enzymes to act on substrates (1% and 5% starch and cellulose). One test

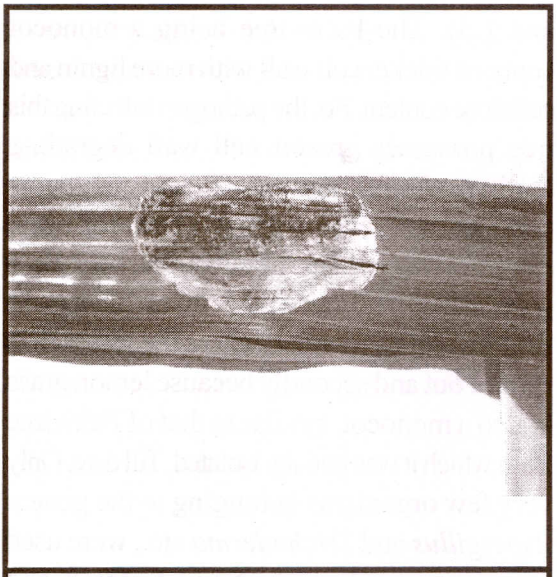

**Fig. 1.1**
**Royal Palm tree leaf infested by pathogen**

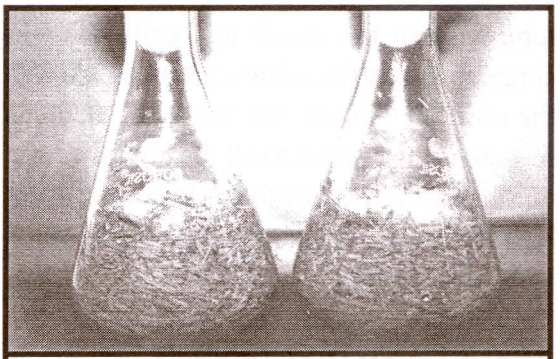

**Fig. 1.4**
**Culturing of *Drechslera puttaparthii* on Lemon- grass Substrate**

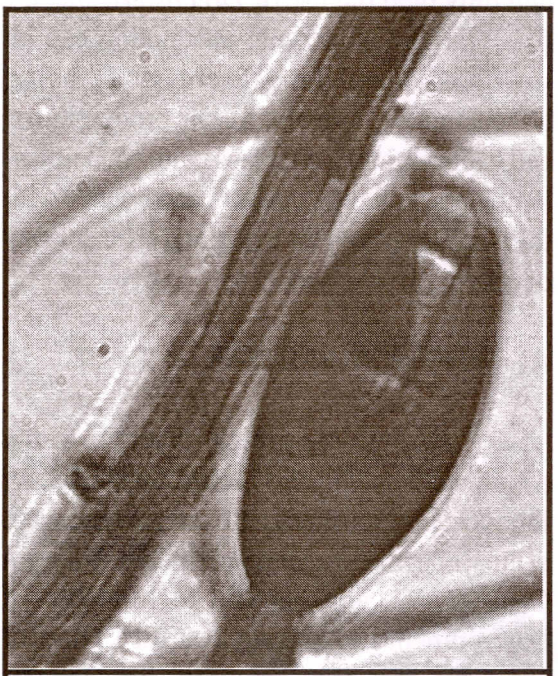

**Fig. 1.2**
**100 X magnification of *Drechslera puttaparthii***

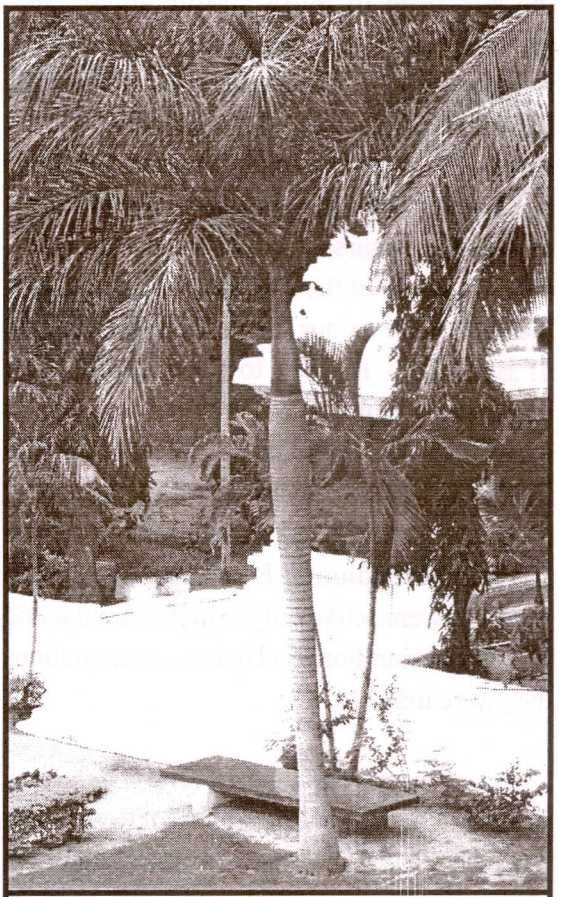

**Fig. 1.3**
**Palm tree *Roystonia regia* growing in the University**

tube without the starch and cellulose and containing only the culture filtrate is taken as the control. Equal volume of Benedict's reagent is then added to all the test tubes and this mixture is then incubated in boiling water bath for 25 min. Entire experiment is repeated with three replicas

## RESULT AND DISCUSSION

The change of colour is the proof of the presence of reducing sugars. The enzymes from filtrate convert the starch and cellulose of the substrate to more simple sugars like glucose (reducing sugars). Formation of the reducing sugars is visualized by treating with Benedict's reagent. The activity of the cellulolytic enzymes present in the filtrate is measured indirectly by measuring the formation of reducing sugars. It is observed by the visual colour change of the Benedict's reagent from blue to Orange colour, 25 min after the addition of Benedict's reagent to the mixture. The presence of maximum enzyme activity is observed in the 5% cellulose medium followed by the 5% starch. There was no measurable enzymatic activity in 1% cellulose as well as 1% starch mediums. It is thought that the enzymes were active only in high cellulose and starch concentrations and in low concentrations they were inactive.

This new species of *Drechslera* was isolated from the necrotic regions of Royal Palm tree *Roystonia regia,* grown as the ornamental plant in the Prasanthi Nilayam campus of Sri Sathya Sai University. (See figures 1.1, 1.2, and 1.3). The Palm tree being a monocot contains thicker cell wall with more lignin and cellulose content. So, the pathogen infecting this tree possesses greater cell wall degrading ability and must possess efficient enzymatic secretions. Lemon grass *(Cymbopogon citratus)* was used as the substrate, because it grows abundantly as wild plant on hill tops in the Puttaparthi mandal, where the research is carried out and secondly because lemon grass is also a monocot, similar to that of Palm tree from which it was initially isolated. Till date, Only very few organisms belonging to the genera *Aspergillus* and *Trichoderma* etc., were used for commercial production of starch and cellulose degrading enzymes . The production of the enzymes by *Drechslera puttaparthii* has to be compared with already commercially exploited species like *Aspergillus* niger etc., and only then the use of *Drechslera species* for commercial enzyme production can be validated. And the present study using Drechslrea *puttaparthii* for the production of cell wall degrading enzymes, with economical lemon grass as substrate is in accordance with the ongoing search for better and efficient way of enzyme production.

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