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ENZYME PROFILE OF AQUATIC HYPHOMYCETES ISOLATED FROM THE STREAMS OF KODAGU DISTRICT OF KARNATAKA

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Abstract

Aquatic hypomycetes, isolated from the streams of Bloomfields, Somwarpet of Kodagu district, are capable of secreting an array of wall degrading enzyme, which transform the leaf litter into more palatable food for the detrivores. The objectives of the present study was to check the ability of these fungi in producing wall degrading enzymes invitro. Only two of the fungi namely Acremonium sps. and a non sporulating unidentified white colony producing sps. were used for the Present study. These were grown on media containing cellulose, starch and pectin as substrate and their ability to produce cellulose, anylase ,invertase and pectinase studied. Depending upon the availability of the substrate these fungi were able to produce varying conc. of enzymes. It was very intresting to note that the non-sporulating sps. showed a nine fold increase in pectinase production compared to Acremonium sps.

Introduction

The major natural habitat of aquatic hyphomycetes appear to be the submerged decaying leaves of dicotyledonous plants. Leaves begin to shed in summer and find their way into streams and rivers, but the main leaf fall is in October. Probably as a consequence, these fungi reach their richest development in the last three or four months of the year. These fungi can be found throughout, but the Autumn months are recommended. Aquatic hyphomycetes are filamentous and mostly moniliaceous, conidia forming fungi. Their conidia are characteristically

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shaped –They are tetraradiate, sigmoid, coiled etc and often appendaged. Aquatic hyphomycetes bring about the degradation of leaf litter as they secrete wall degrading enzymes. Among the micro organisms aquatic hyphomycetes are the most active in degrading leaf tissue in a stream, (Kaushik and Hynes, 1917; Kostlos and Seymour, 1976; Barlocher and Kendrick, 1981), thus transforming the leaf litter into a more palatable food for the detrivores.

The present study deals with the collection, aeration, isolation and identification of aquatic hyphomycetes based on type of spores followed by the study of wall degrading enzyme secreted by them invitro . Nine sps. of aquatic hyphomycetes were isolated, of which two species – Acremonium sps. and a non-sporulating white colony producing fungi was used to study the enzyme profile.

Materials and Methods

Collection of leaf litter was preferably done from clean tree lined streams that are fast flowing with occasional rapids. Leaves are best gathered individually from the bed of the stream. Dark brown leaves that are softened and partially skeletonised are collected from the streams and used as samples, 5-10 such leaves or twigs are collected, rinsed in water and placed in clean fresh polythene bags and are labelled and transported in an ice pack container to the laboratory.

Aeration

Each leaf is placed in a clean wide mouth bottle with distilled water at room temperature for two to several days and a jet of air using an aquarium aerator is passed through to hasten sporulation. A drop of detergent is added to this water and shaken well. Spores that are floating get caught in the foam. The foam is scooped out onto a slide and is examined under a microscope. When the foam settled a collection of spores can be seen when the slide is dried and stained.

Isolation

In order to isolate the spores from the aerated litter and obtain a single spore culture the following method was employed:

1. The aeration was increased and foam scooped out with a clean slide.

2. After focussing the slide under the microscope single spores can be picked up with a help of a pointed needle.

3. The spores are then transferred onto PDA Petri plates.

Maintenance of Pure Culture

Cultures were maintained on PDA slants to which an antibiotic was added. The slants were maintained in a refrigerator at 10° C and sub-cultured every three months.

Sporulation

Generally freshwater hyphomycetes do not sporulate above water (Ingold, 1975). Therefore one cannot expect a sporulating colony in the media. However, in order to identify the isolate, the fungus was induced to sporulate. All freshwater hyphomycetes sporulate readily as soon as they get back to water. This was done as follows.

1. A strip of pure agar culture was cut and submerged in sterile distilled water under aseptic conditions.

2. In a few days conidia developed. In case conidia do not develop a stream of air has to be sent from a "fish aerator" and after several hours spores can be noticed emerging out from the fungal colony submerged in water.

Study of wall degrading enzymes invitro

In fungi many enzymes are known, whose appearance is dependent upon the presence of a substrate. These enzymes which are secreted make the insoluble substance available for growth by cleavage of complex organic compounds. Acremonium sps. and the non-sporulating white cottony colony forming fungi were capable of producing cellulase, pectinase, amylase, invertase and pectin trans – eliminase.

Media used to induce enzyme production

Three different liquid media were used to induce enzyme production – Asparagine glucose pectin medium, Czepek dox medium with 1% cellulose, Czepek dox medium with 1% starch. 100 ml of liquid medium was dispensed into 250 ml conical flasks. Flasks were plugged with cotton wool and capped with aluminium foil and autoclaved for 15mins. at 120°C.

Inoculation

Each of the flasks was inoculated with one disc of 1 cm diameter of agar with the mycelium from Petri plates using a sterile cork borer. One flask containing a disc of PDA served as control.

Enzyme Source

The inoculated flasks were incubated at room temperature. Cultures were harvested every third day till the tenth day and thereafter every alternate day till the twentieth day. Cultures were harvested by filtering through pre-weighed Whatmann filter paper discs using suction. The resulting filtrate served as the enzyme source. Enzymes were stored at 4° C till further use.

Growth and mycelial mat weight

The filter paper along with the mycelium was dried at 50° C for 2 – 3 days and weighed everyday till the weight was stable.

Proteins

Proteins were estimated by the method of Lowry et al, 1951, using BSA as standard. Samples were taken in duplicates.

Pectinase activity

Was measured by Viscometer method using 0.5% Pectin in Tris – HCl buffer pH 8.7, as substrate containing 0.2 M CaCl and incubated at 34 +/- 1° C. Pectinase activity was calculated as follows:-

$$\frac{To - T1}{To - Tw} \times 100$$

Where To = flow time in seconds (zero time)

T1 = flow time of reaction mixture at time T

Tw = flow time of distilled water.

Pectin Trans-eliminase

Assay was by the spectrophotometric method using 0.5% Pectin and $CaCl_2$ and incubated at 34 +/- 1° C for 1 hour. The OD was read at 550 nm.

Cellulase

Was assayed by the Viscometric method using 0.5% Carboxy methyl cellulose in 0.2M Sodium Acetate buffer pH 5.2 and incubated at 44 +/- 1° C. Cellulase activity was calculated as for Pectinase.

Amylase

Reaction mixture contained 1% soluble starch, Sodium acetate buffer and enzyme, this was incubated at 37° C for 24 hours. Reducing sugars released was measured using DNS reagent and absorbance read at 575 nm.

Invertase

Reaction mixture contained 2.5% sucrose. Reducing sugars released was measured at 575 nm using glucose as standard.

pH

pH of the culture filtrate was measured using a pH meter.

Results

Acremonium sps. and the non – sporulating white colony producing fungi in this study produced Pectinase, Pectin transeliminase, Cellulase, Amylase, and Invertase. The peak activity of pectinase by Acremonium sps. was on the 14th day and that of the non – sporulating white colony producer was on the 19th day (fig.1&2).

It was interesting to note that the non–sporulating fungi showed a ninefold increase in pectinase activity when compared to Acremonium sps. PTE was maximum on the 15th day with Acremonium sps. and on the 21st day with the non-sporulating white colony producer (fig. 3&4). Amylase and Invertase production was on the 15th and 12th day for Acremonium sps. (fig. 5&6) and in the case of non-sporulating white colony producer the maximum activitywas on the 17th and 11th day (fig. 7&8).

Cellulase activity was maximum on the 18th day for Acremonium sps. and 16th day for the non-sporulating sps. (fig. 9&10).During cellulase production, the pH became basic and later acidic with the non-sporulating sps. (fig. 11). With Acremonium sps. the pH turned acidic during pectinase production (fig. 12). The protein concentration was maximum on the 14th and16th day for the fungi with cellulose medium (fig. 13&14), whereas with pectin medium maximum protein was on the 15th and 19th day respectively (fig. 15&16).

Figure 1

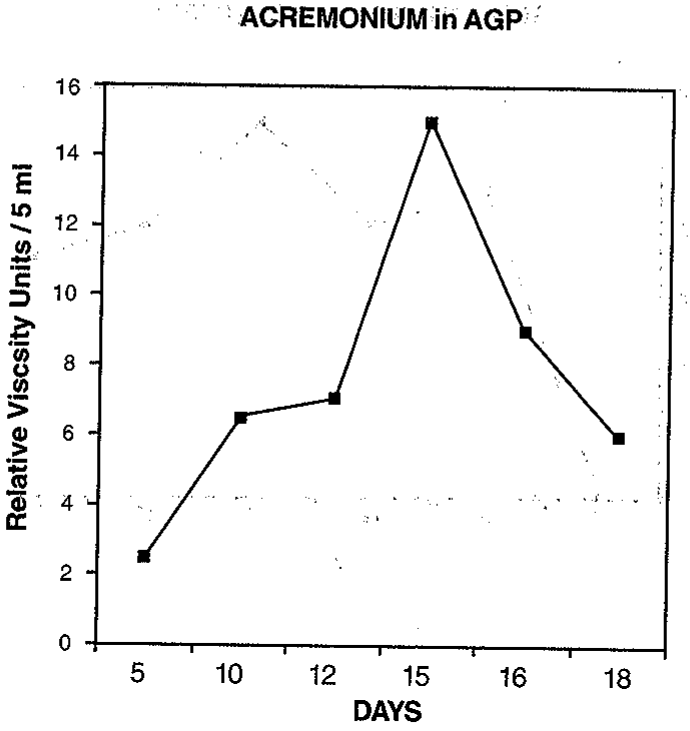


Figure 2

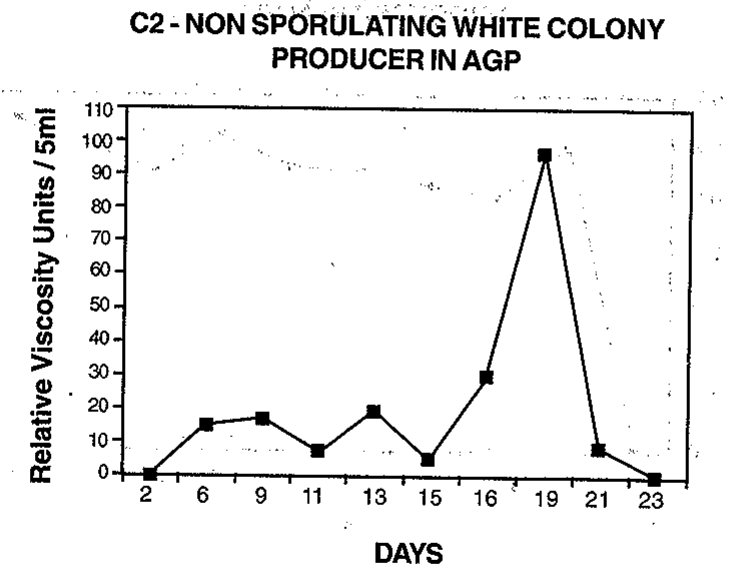


Figure 3

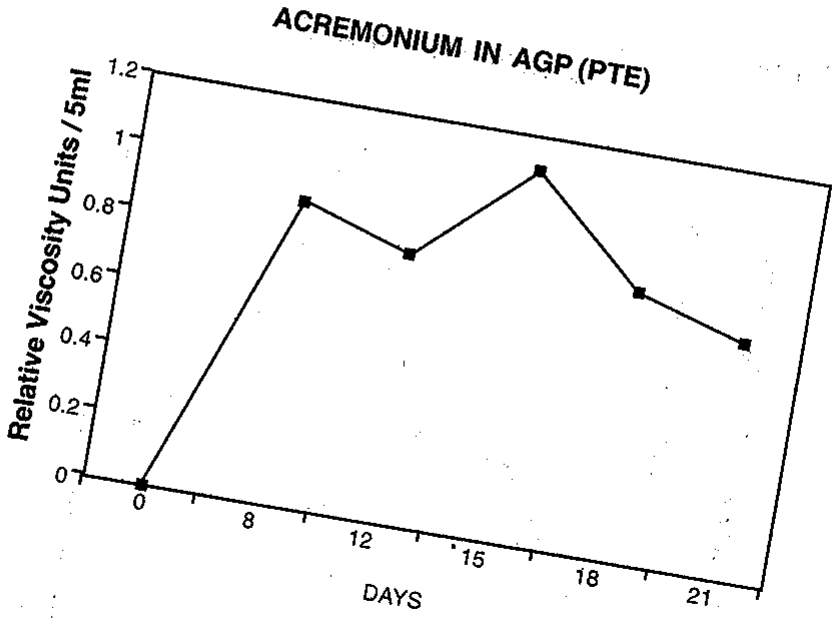


Figure 4

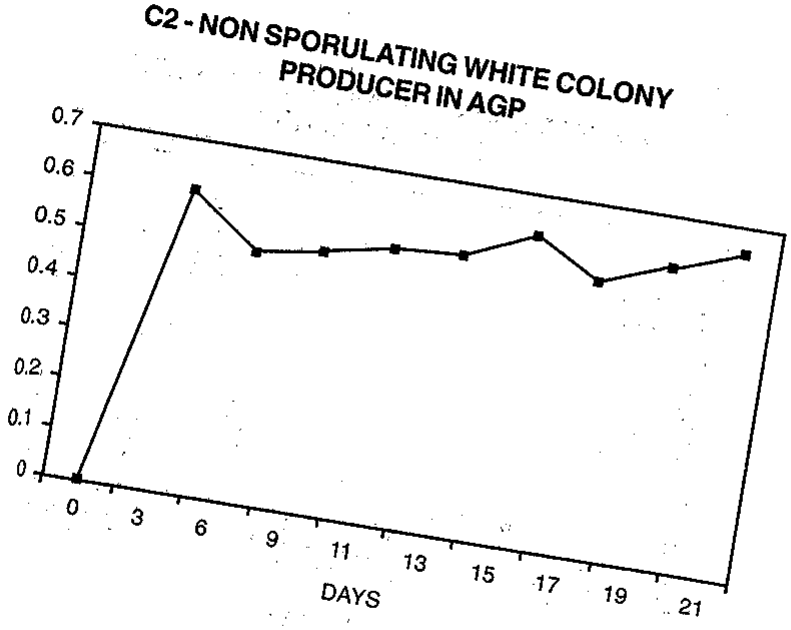


FIG. 5
ACREMONIUM IN CZ (STARCH) - AMYLASE ACTIVITY

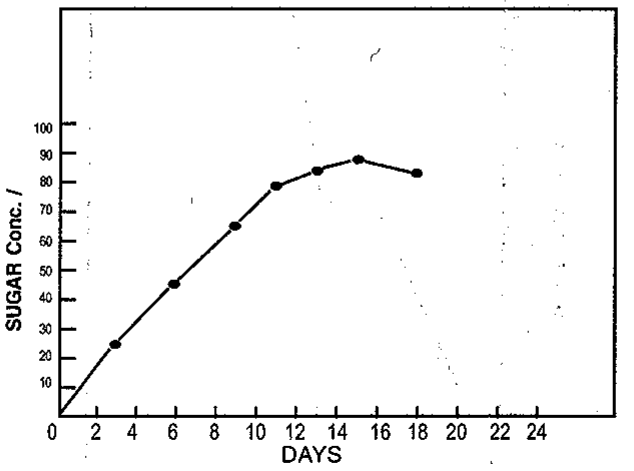


FIG. 6
ACREMONIUM IN CZ (STARCH) - INVERTASE ACTIVITY

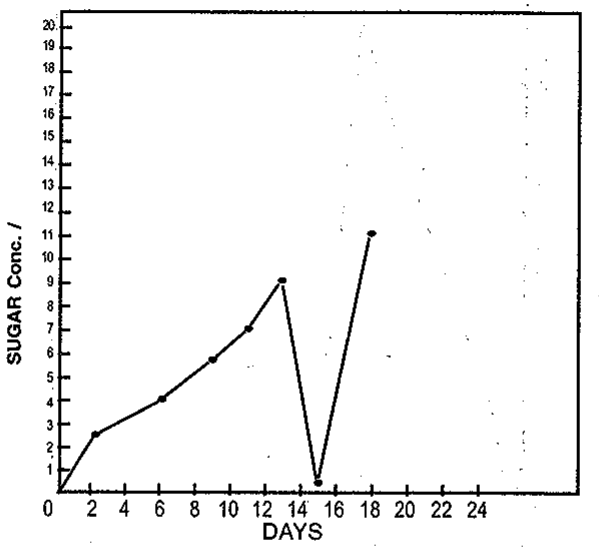


FIG. 7
C2 - NON SPORULATING WHITE COLONY PRODUCER - AMYLASE IN CZ (STARCH) ACTIVITY

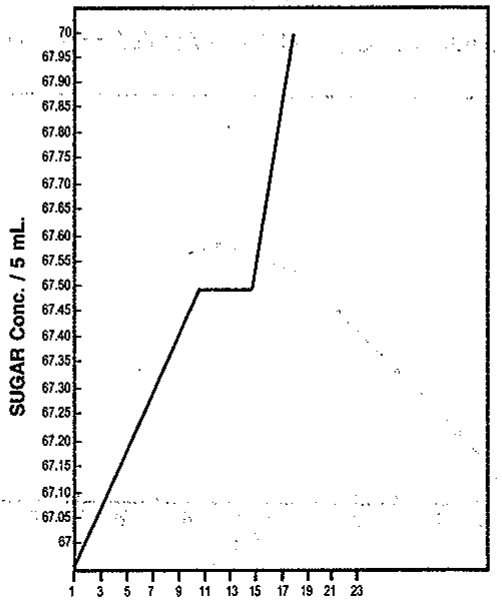


FIG. 8
C2 - NON SPORULATING WHITE COLONY PRODUCER - INVER IN CZ (STARCH) ACTIVITY

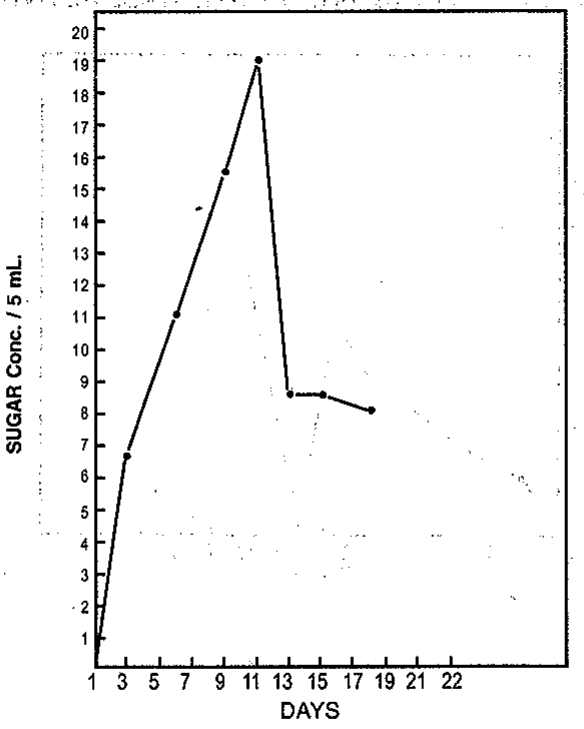


FIG. 9
ACREMONIUM IN CZ (CELLULOSE) - CELLULASE ACTIVITY

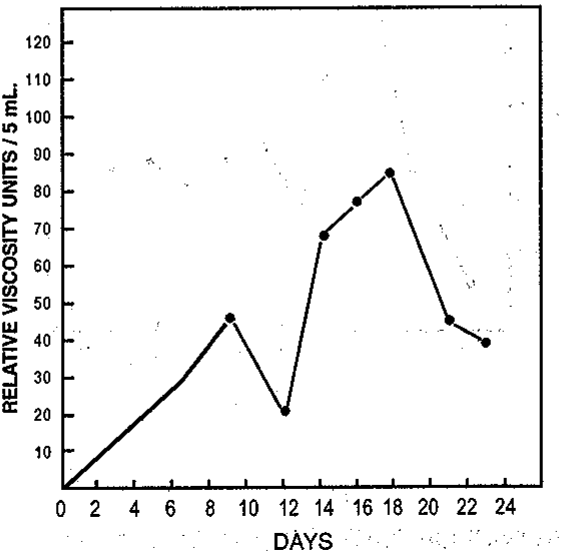


FIG. 10
C2 - NON SPORULATING WHITE COLONY PRODUCER - CELLULASE IN CZ (CELLULOSE) ACTIVITY

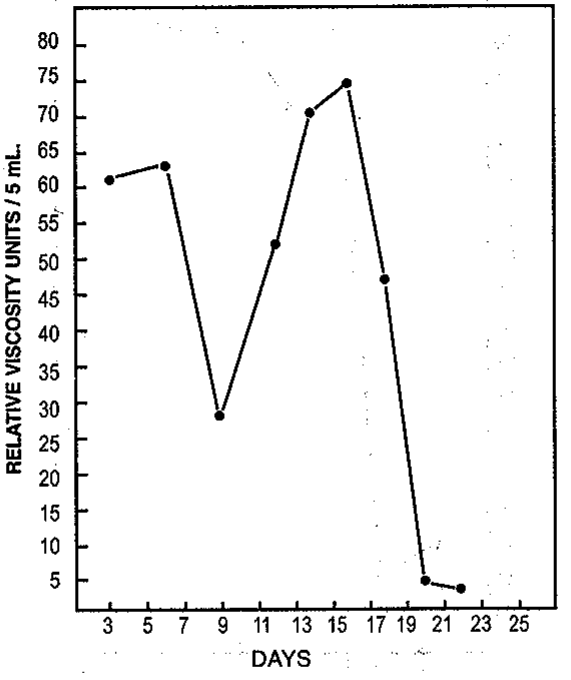


FIG. 11
NON-SPORULATING WHITE COLONY - pH
PRODUCER IN CZ (CELLULOSE)

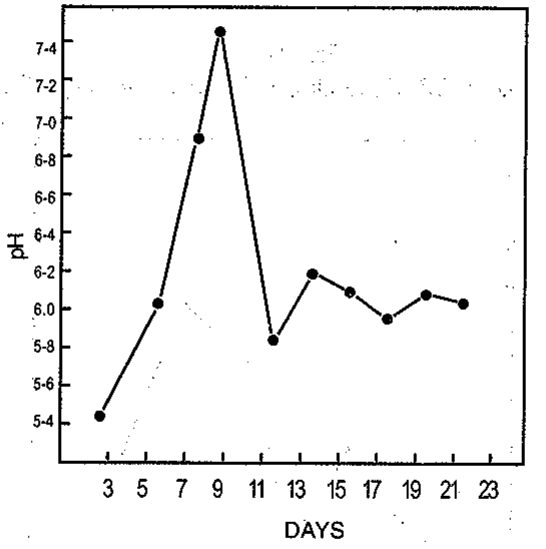


FIG. 11a
NON-SPORULATING WHITE COLONY - pH
PRODUCER IN AGP (PECTIN)

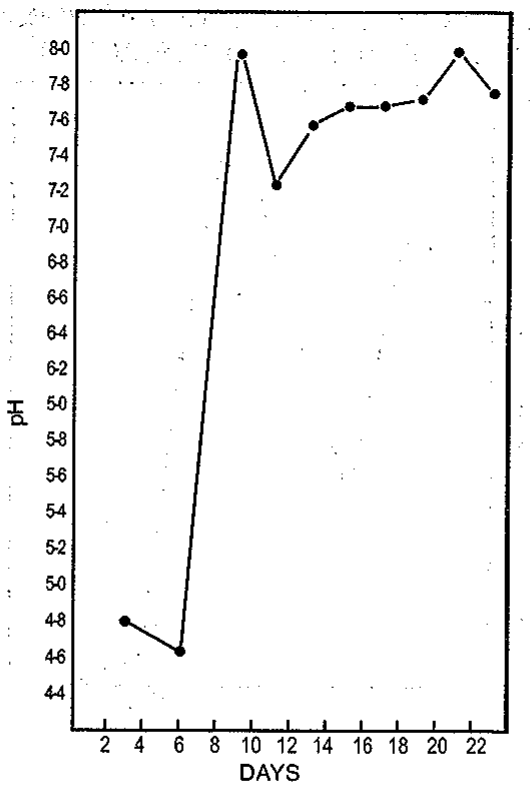


FIG. 12
ACREMONIUM IN CZ (CELLULOSE) - pH

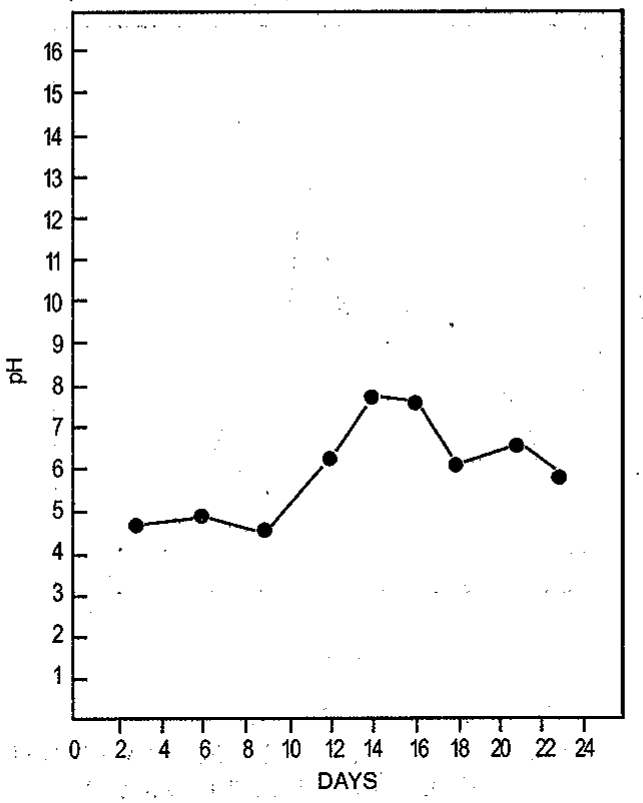


FIG. 12a
ACREMONIUM IN AGP (PECTIN) - pH

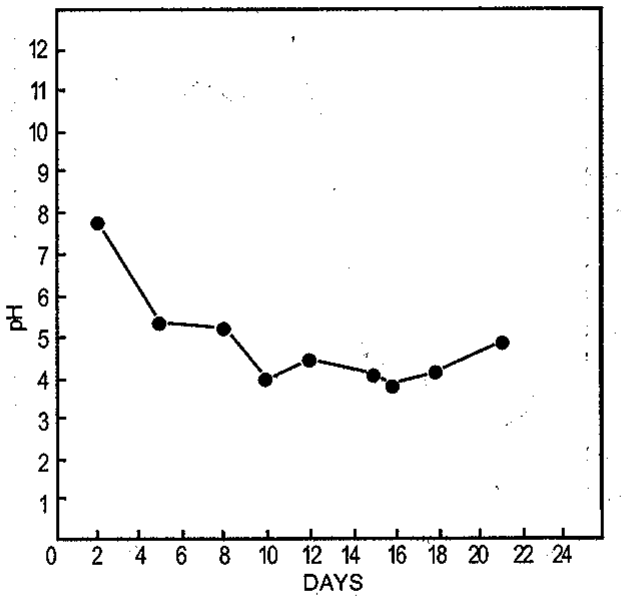


FIG. 13
ACREMONIUM IN CZ (CELLULOSE) - PROTEIN

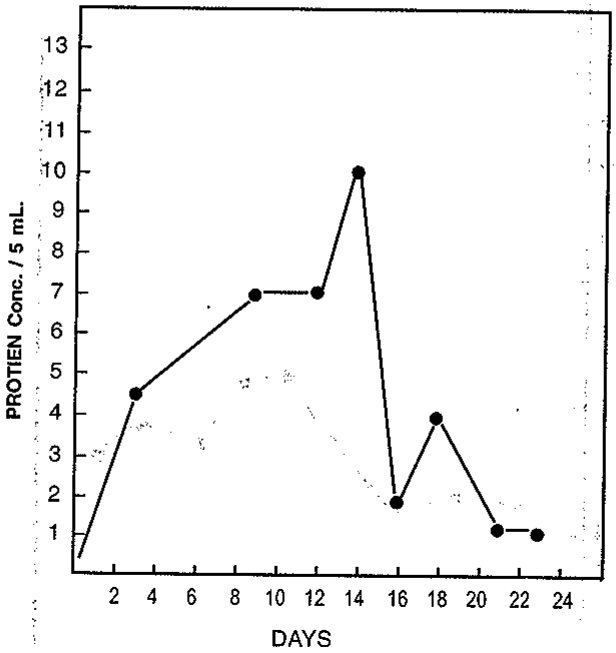


FIG. 14
NON-SPORULATING WHITE COLONY-PROTEIN
PRODUCER IN CZ (CELLULOSE)

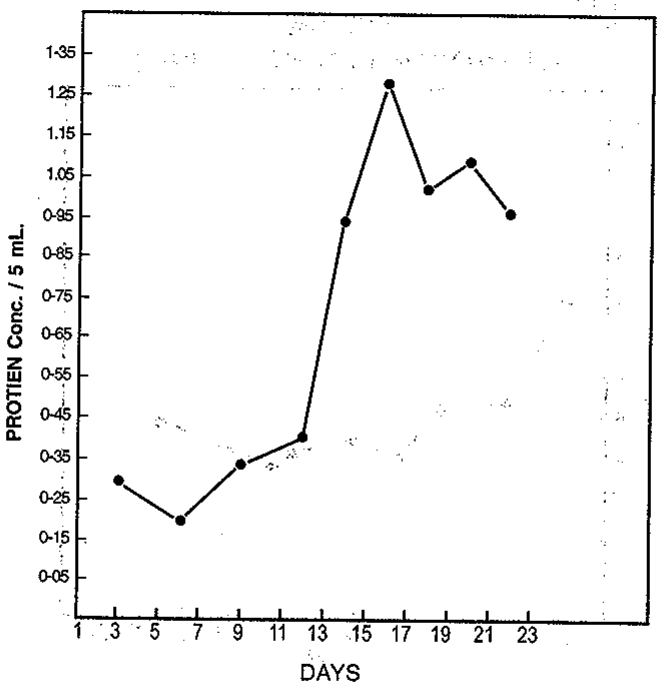


FIG. 15
ACREMONIUM IN AGP (PECTIN) - PROTEIN

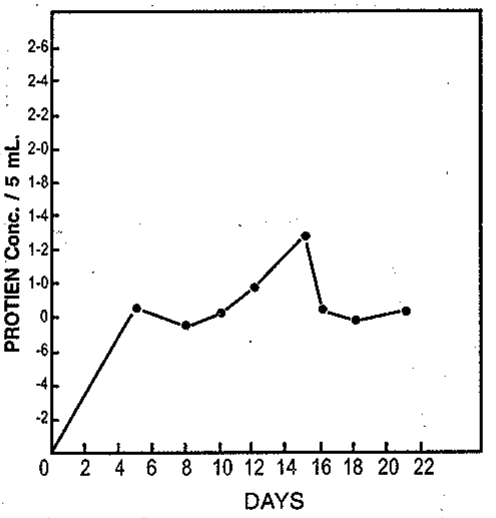


FIG. 16
NON-SPORULATING WHITE COLONY-PROTEIN
PRODUCER IN AGP (PROTIN)

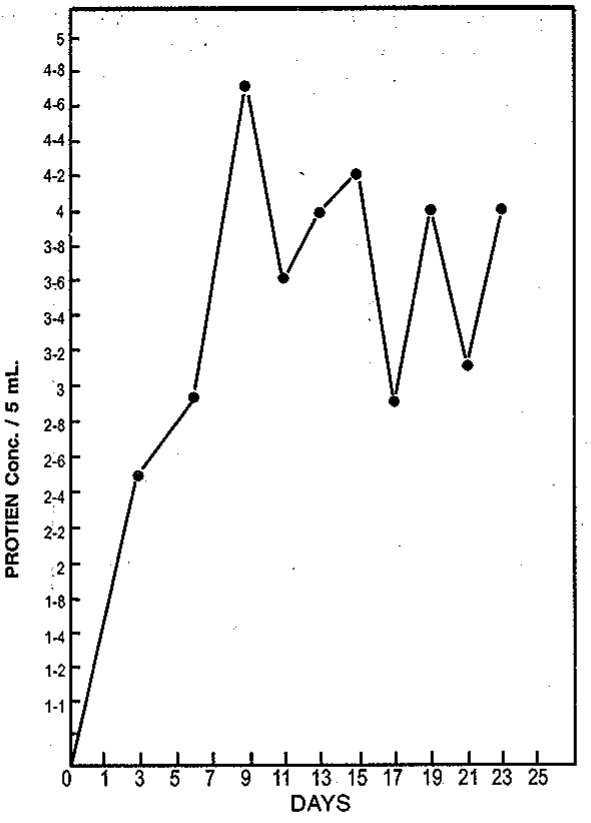


FIG. 17
ACREMONIUM IN CZ (CELLULASE) - MAT WEIGHT

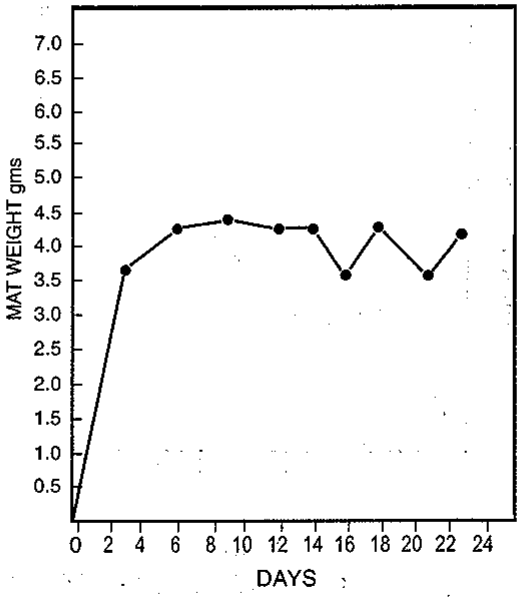


FIG. 18
NON-SPORULATING WHITE COLONY - MAT WEIGHT PRODUCER IN CZ (CELLULOSE)

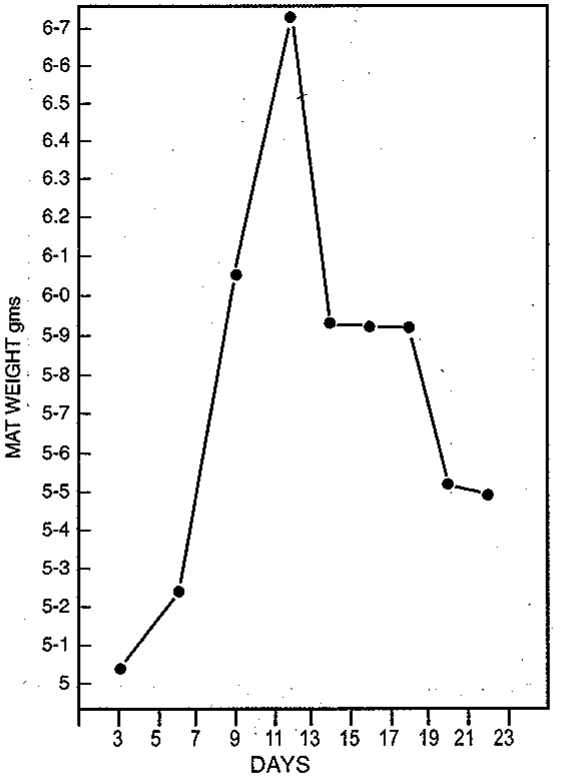


FIG. 19
ACREMONIUM IN AGP (PECTIN) - MAT WEIGHT

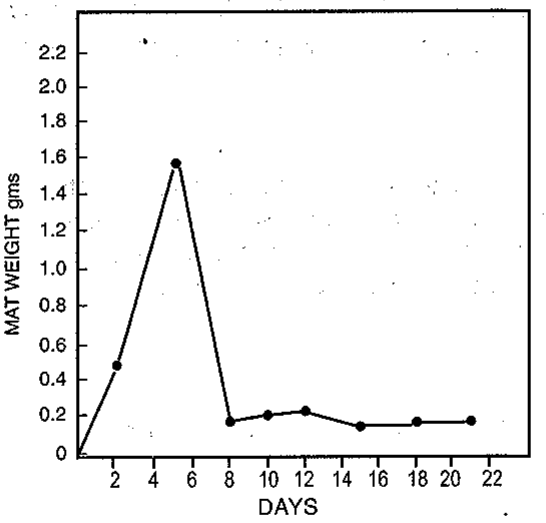
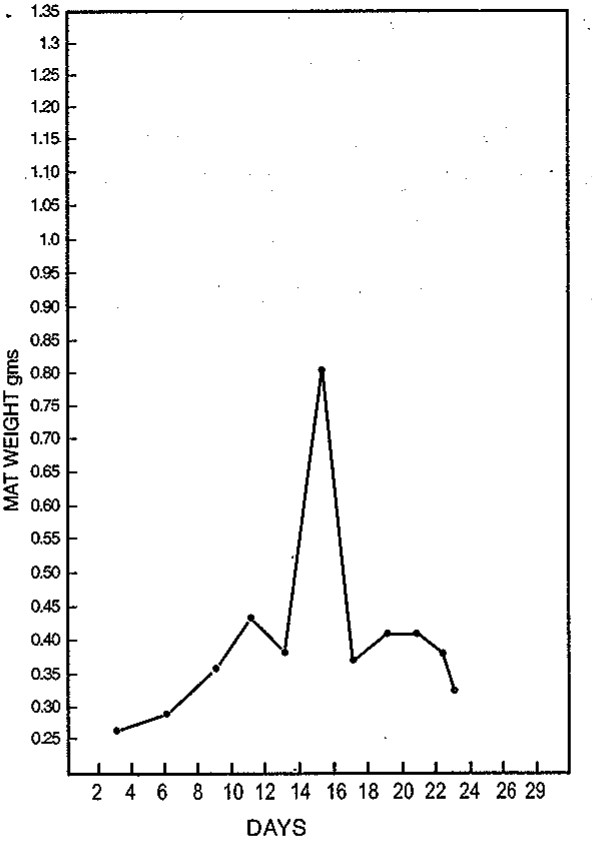


FIG. 20
NON-SPORULATING WHITE COLONY - MAT WEIGHT PRODUCER IN AGP (PECTIN)



Growth and mat weight

Pectinase production was maximum when the pH was basic with both the fungi. On the 18th day Acremonium sps. showed maximum growth and on the 11th day the non-sporulating sps. (fig. 17&18). On AGP medium the maximum growth was on the 5th and 15th day respectively (fig. 19&20).

Conclusion

From the results it is clear that the fungi colonizing coffee litter produced a galacturonase like pectic enzyme which degrade the pectin present in the cell wall separating the cells so that they became more vulnerable to the action of other wall degrading enzymes.

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