



Research Article

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HYDROLYSIS OF PECTIN: STUDY OF PECTINOLYTIC FUNGI RESIDING IN MANDEEPKHOL CAVE, RAJNANDGAON DISTRICT OF CHHATTISGARH, INDIA

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ABSTRACT

Pectin is a complex colloidal acidic polysaccharide present in the primary cell wall and middle lamella of fruits and vegetables. Some species of fungi are an important microflora which help in degradation of pectin. For biomass utilization, waste management, organic farming and various other industrial purpose the pectin degrading-enzymes will help to achieve tremendous benefits. In present studies a total of 71 fungal species were isolated from Mandeepkhoh cave which is situated in Chhattisgarh state and is located in deep forest. The studies were performed to evaluate the degradative potency of fungal species for pectin digestion. The assessment was done on the basis of zone of digestion and mycelial dry weight. Further quantitative test was also performed. Results show that out of total species studied 25 species were found to produce pectin degrading enzyme in substantial tune. Out of 25 species, best grown species were *Alternaria alternata*, *Penicillium mealagrimum* var. *viridiflavum*, *Penicillium. citrinum*, *Aspergillus ochraceous*, *Aspergillus fumigatus*, *Aspergillus niger* and *Phanerochaete chrysosporium*.

Keywords: Degradation, Pectin, Biomass and Mandeepkhoh cave

INTRODUCTION

Biodegradation is the disintegration of materials by bacteria, fungi or other biological means. It is the nature's way of recycling wastes or breaking down organic matters into nutrients that can be used by other organisms. This is an often used in waste management and environmental remediation, also known as bioremediation. Waste materials are generally organic matters which is needed to be degraded. Fungi play important role in biodegradation as they break down tough organic materials like lignin and cellulose enabling bacteria to continue the decomposition process once most of the cellulose and lignin have been exhausted. Pectin is a complex colloidal acidic polysaccharide present in the primary cell wall and middle lamella of fruits and vegetables. They are the only polysaccharides responsible for cell cohesion. Pectinases are enzyme which helps to break pectin and convert into simple compound. Pectinases are group of enzymes which are produced by plants and microorganisms. Fungi plays major role in degradation of pectin. The present studies deal with study of diversity and degradation potency of fungi isolated from Mandeepkhoh cave. Cave is situated in Salewara hills situated in Rajnandgaon district, Chhattisgarh. It is totally dark cave. As the temperature of most of the caves remain constant between 26°C-28°C throughout the year, it is confirmed that the fungi present in that environment are basically mesophilic. The present studies aim towards identification of these cave fungi, assaying of their degradative potency and to get their genetic makeup so that in future these microbes may be used for fast degradation of waste materials in ambient temperature to get rid of soil waste pollution.

MATERIALS AND METHOD

Samples were collected from Mandeepkhoh cave from different deposits. The isolation of fungi was done using potato dextrose agar media. The samples were serially diluted using serial dilution method in sterilized distilled water. The petriplates were kept for incubation at 26°C. After incubation fungal mycelium were purely cultured in sterile test tube containing media to get axenic culture. The axenic cultured fungi were then allowed to

grow in hankin media where citrus pectin were taken as sole carbon source for testing their growth and were examined for pectinase activity by fungal. mycelia dry weight method and by digestion zone method to ascertain the fungal growth.

Culture Media:

Citrus pectin - 5gm,
(NH₄)₂ SO₄ - 2 gm,
KH₂PO₄ - 4gm,
Na₂HPO₄ - 6 gm,
FeSO₄ · 7H₂O - 0.2 gm,
CaCl₂ - 1mg,
H₃BO₃ - 10 mg,
ZnSO₄ - 10 mg,
MoO₃ - 10 mg,
ZnSO₄ - 70 mg,
Yeast extract - 1 gm,
Agar - 15 gm and Distilled water - 1000 ml

20 ml of prepared media was then poured in sterile petriplate and isolated fungi were inoculated to test their pectinase activity. It was then allowed to grow in 26°C ± 2°C for 3 days. After incubation fungal plates were recorded at different intervals up to 3 days. To measure mycelia dry weight isolates were grown in citrus pectin broth.

RESULTS AND DISCUSSION

A total of 71 species belonging to 24 genera and two sterile mycelia were isolated. Among them Deuteromycotina fungi dominated over the member of Ascomycotina and Zygomycotina. Out of total isolates 8 species (6 genera) belong to Zygomycotina, 4 species (4 genera) belong to Ascomycotina and 59 species (14 genera) belong to Deuteromycotina.

Evaluation of Pectin Degradation Potency of Fungal Isolates

Results revealed that 25 species were found to produce pectin degrading enzyme in substantial tune. Out of 25 species, best grown species were *Alternaria alternata*, *Penicillium*

mealagrinum var. viridiflavum, *Penicillium citrinum*, *Aspergillus ochraceus*, *Aspergillus fumigatus*, *Aspergillus niger* and *Phanerochaete chrysosporium*. They showed greater zone of digestion and highest mycelial weight, as compared to other fungi. Dube and gour¹ in their study observed that extracellular pectic enzymes were produced by *Macrophomina phaseolina* on Richard's solution containing sucrose, Richard's solution containing pectin and potato pectin medium and later on divulged that they produced maximum in pectin containing media. Phutela² and his coworker in their work isolated 120 fungal species for pectinase and polygalacturonase production they observed that *Aspergillus fumigatus* was best for pectinase and polygalacturonase production on the other side Akbar³ and his coworker also studied in which *Aspergillus carbonarius* showed maximum pectolytic activity. Benoit⁴ with their team studied degradation of different pectins by fungi and has correlated and differentiated the pectinolytic enzyme. They have isolated 12 best fungi for pectin degradation. Patil and Choudhary⁵ in their research work has identified that *Pencillium* sp are potent polygalacturonase producer and are potent degraders and this study is supported by findings of Banu⁶ and coworkers who has

isolated ten fungi from municipal waste soil samples and screened for pectinase production and observed that maximum pectinase activity by *Penicillium chrysogenum*. Gacura⁷ et al in their work compared pectin-degrading fungal communities in temperate forests using glycosyl hydrolase family 28 pectinase primers targeting Ascomycete fungi. Arunima⁸ and coworker isolated 32 pectin degrading bacteria from Mandeepkhol cave and out of which 3 were potent degraders. Adeleke⁹ and coworker has also examined pectinase and cellulase activity. According to them *Penicillium atroventum*, *Aspergillus Flavus* and *Aspergillus oryzae* were highest pectinase and cellulase producer where as Okafor¹⁰ with his team demonstrated pectolytic activity on *Aspergillus clavatus*, *Aspergillus niger*, *Fusarium sp.*, *Penicillium chrysogenum* and *Trichoderma sp.* In which they used citrus pectin as sole carbon source. They observed maximum pectinase production by *Aspergillus niger* and *Penicillium chrysogenum*. Arunima and coworkers¹¹⁻¹⁴ explored Mandeepkhol cave and isolated 72 different fungal species and 33 bacterial species. They studied ligninolytic activities of bacteria and fungi. Later on, they studied fungal diversity in Borra Cave.

Table 1: Zone of digestion and mycelial dry weight in pectin media

Isolated fungi	Zone of digestion(diameter)	Mycelial dry weight per 50ml	Mycelial dry weight per 1000ml
<i>Alternaria alternata</i>	40.26mm	0.34gm	6.8gm
<i>Alternaria radicina</i>	28mm	0.12gm	2.4gm
<i>Aspergillus awamoori</i>	26.74mm	0.09gm	1.8gm
<i>Aspergillus japonicas</i>	28.6mm	0.11gm	2.2gm
<i>Aspergillus fumigatus</i>	31.76mm	0.18gm	3.6gm
<i>Aspergillus luchensis</i>	30.16mm	0.14gm	2.8gm
<i>Aspergillus niger</i>	28.7mm	0.12gm	2.4gm
<i>Aspergillus ochraceus</i>	36.76mm	0.23gm	4.6gm
<i>Aspergillus parasiticus</i>	27.5mm	0.10gm	2.0gm
<i>Aspergillus stellatus</i>	28.51mm	0.11gm	2.2gm
<i>Aspergillus versicolor</i>	32.5mm	0.16gm	3.2gm
<i>Curvularia clavata</i>	25.4mm	0.07gm	1.4gm
<i>Curvularia lunata</i>	26.12mm	0.08gm	1.6gm
<i>Curvularia pallesence</i>	25.4mm	0.06gm	1.2gm
<i>Penicillium citrinum</i>	36.26mm	0.26gm	5.2gm
<i>Penicillium digitatum</i>	27.43mm	0.12gm	2.4gm
<i>Penicillium meleagrinum var.viridiflavum</i>	37.5mm	0.29gm	5.8m
<i>Penicillium multicolor</i>	29.19mm	0.14gm	2.8gm
<i>Penicillium notatum</i>	27.2mm	0.10gm	2.0gm
<i>Penicillium oryzae</i>	21.1mm	0.05gm	1.0gm
<i>Penicillium rugulosum</i>	20.46mm	0.04gm	0.08gm
<i>Phoma shorgina</i>	21.51mm	0.05gm	1.0gm
<i>Phoma exigua</i>	22.44mm	0.06gm	1.2gm
<i>Phanerochaete chrysosporium</i>	28.4mm	0.09gm	1.8gm
<i>Trichoderma viridae</i>	31.22mm	0.10gm	2.0gm

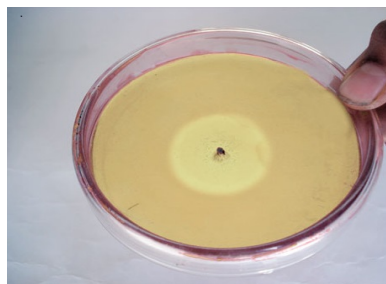


Fig 1: Pectin digestion- *Alternaria alternata*



Fig 2: Pectin digestion- *Penicillium meleagrinum Var Viridiflavum*

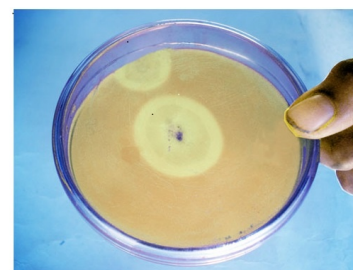


Fig 3: Pectin digestion- *Penicillium citrinum*

CONCLUSION

Speleology is most attractive area in research and that is attracting many researchers for findings. Mandeepkhol cave is situated in deep forest and it is unexplored yet. It is been noticed that the bat guano and other organic materials are decomposed in very fast rate which gives light on fact that the microorganism thriving in that area must have potential to degrade them. Moreover, the temperature in the cave is maintained throughout year which is 28°C giving impression that degradation is on mesophilic temperature. The present work would help in exploiting those potent organisms in waste management.

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