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RESEARCH ARTICLE

A study on Extracellular Cellulase enzyme synthesis from *Penicillium sp.* using Rice Husk as a Substrate

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Abstract: *The synthesis of extracellular cellulase enzymes by the fungus* Penicillium funiculosum *has garnered significant interest due to its potential applications in various industrial processes, particularly in the production of biofuels and waste management. This study explores the conditions that optimize the production of cellulase from* P. funiculosum, *highlighting the impact of various environmental and nutritional factors on enzyme yield and activity. The study of utilizing agricultural waste as a substrate for microbial growth is gaining significant attention due to its environmental and economic benefits. This research focuses on the utilization of rice husk, an abundant agricultural by-product, as a substrate for the growth and activity of Penicillium species, known for their enzymatic capabilities and secondary metabolite production.*

Keywords: *Extracellular cellulase, Rice Husk, Penicillium species. Fungi.*

1. INTRODUCTION

Enzymes are biocatalyst synthesized by living systems, which are important in synthetic as well as degradative processes. The study of enzymes is an important area, because it exists just on the borderline where the biological and physical sciences meet. Life depends on the complex network of chemical reactions carried out by specific enzymes may have far reaching consequences for the living organism. (Dixon and Web, 1964).

The primary objective of this study is to evaluate the efficacy of rice husk as a viable substrate for Penicillium sp. growth and to assess the potential production of valuable enzymes and metabolites. Secondary objectives include optimizing growth conditions to maximize yield and exploring the environmental implications of using rice husk.

The study demonstrates that rice husk is a sustainable and cost-effective substrate for Penicillium sp. cultivation. This approach not only provides a method for managing agricultural waste but also offers potential economic benefits through the production of high-value enzymes and metabolites. Future research will focus on scaling up the process and exploring other agricultural wastes as substrates.

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2. AIM AND OBJECTIVES OF THE STUDY

The present investigation was initiated to study about the activity of cellulase enzyme with the following objectives

To screen cellulase producing fungi from mangrove soil samples collected.

To observe the enzyme activity in various parameters like pH temperature, incubation periods, natural waste (Rice husk)

To compare the enzyme activity in wild type of Penicillium sp.

3. MATERIALS AND METHODS

3.1. Sample collection

The soil sample was collected from mangrove habitat. The salinity of the soil ranging from 40 ppt to 165 ppt.

3.2. Isolation of fungi

The soil sample collected from sampling spot was processed using serial dilutions and plating methods.

3.3. Screening of Cellulase production

The colonies observed on the PDA and RBA plates were transferred on to the growth medium amended with cellulose as sole carbon source. The growth medium used was as Modified Park's agar. The clear zone observed around the colony was the indication cellulase production.

3.4. Characterization of Fungi

Staining – lacto phenol cotton blue mounting of fungi.

- A drop of lacto phenol cotton blue was placed on a clean slide.
- A small tuft of fungus, usually with spores and spore bearing structure was placed on to the drop using a flamed, cooled needle.
- The material was gently teased using two mounting needle.
- The mold structure was gently mixed with stain.
- The cover slip was placed over the preparation taking care to avoid trapping air bubbles in the stain.

Observation

- This preparation was examined under low power and high power objectives.
- The hyphae, conidiophore, condiogenous cells, conidia and their arrangements on the conidiophores were observed.
- The representative microscopic field under low power and high power magnification were drawn.
- The mold was identified on the basis of characteristic features produced.

3.5. Substrates used

In the present study, the waste material like rice husk used as the carbon source in the fermentation medium. These substrates are good source of cellulose.

3.6. Fermentation

The fungi was inoculated into the fermentation medium modified Czapek's cellulose medium and incubated at room temperature for ten days and enzyme activity and protein production was determined.

3.7. Effect of various parameters on cellulase activity

Effect of pH on cellulase activity

The fungi was inoculated in to the fermentation medium containing the substrate rice husk used as carbon source. The pH of the medium was adjusted in to 5,7 and 9 and incubated and the enzyme activity and protein production was determined.

Effect of Temperature on cellulase activity

The fungi was inoculated in to fermentation medium and incubated at different temperature like 30, 40 and 50 and the enzyme activity and protein production was determined.

Effect of incubation period on cellulase activity

The fungi was inoculated into the fermentation medium and incubated at different periods like. 3, 6 and 9 days and the enzyme activity and protein productions were determined.

3.8. Mutational studies

The fungi was subjected to mutation by UV for 5 minutes and the mutated fungi was inoculated into the fermentation medium which contain the carbon source rice husk, of cellulose.

The mutated strain was inoculated in to fermentation medium which contain the

rice incubated at different pH, temperature and incubation periods and the enzyme activity and protein production were also studied.

3.9. Enzyme assay: Determiation of enzyme activity

In the cell free filtrate of each culture medium the extra cellular cellulase activity was determined as follows.

One ml of the cell free filtrate was mixed with 9 ml of one percent carboxy methyl cellulose in 55 mM citrate buffer (pH5) for 30 min at 40°C. At the end of the reaction time the reducing sugar liberated was determined

3.10. Estimation of cellulase activity

One unit of cellulase enzyme is defined as the amount of enzyme that liberates 1 mg of glucose from carboxy methyl cellulose.

4. RESULTS AND DISCUSSION

In the present investigation, different colonies of *Aspergillus* sp. and *Penicillium* sp. were isolated from mangrove environment. Among these *Penicillium sp.* was isolated, identified and analysed for cellulase enzyme activity confirmed by screening methods. From the isolated fungi, enzyme activity and specific activity were also studied (Tables 1-5).

5. SUMMARY

Most of the enzymes are of microbial origin and the microorganism can be easily and rapidly cultivated, thus forming an unlimited enzyme source. Commercial enzymes are also available but they are expensive, can not be recycled and also laborious. So, the best alternative is the enzyme of biological origin. Though these enzymes are produced by bacteria, fungi and actinomycetes, for our present study, we have taken one such fungi ***Penicillium sp*** which is reported to be the best source of cellulase enzyme.

Penicillium sp. was isolated from from mangrove habitat and we have studied its enzyme activity on different natural wastes (rice husk) by altering the parameters like, temperature, pH and incubation periods. Rice husk was found to be more effectively degraded by the organism and hence, can be the best source of enzyme.

The organism was exposed to UV radiation and the mutational effect on enzyme activity was also studied.

Table 1: Cellulase activity in Fermentation medium.

Incubation	Reducing sugar	Enzyme activity	Soluble Protein	Specific Activity
3 Days	30	28	18	1.55
6 Days	49	46	27	1.70
9 Days	103	96	40	2.40

Table 2 (a): Effect of pH on cellulase activity of *Penicillium funiculosum* (Wild type) on Bagasse, Coir pith and Rice husk on three day incubation.

Substrate	5				7				9			
	R.S	E.A	S.P	S.A	R.S	E.A	S.P	S.A	R.S	E.A	S.P	S.A
Bagasse	30	27	21	1.28	31	28	21	1.13	41	37	19	1.05
Rice husk	32	29	55	0.53	30	27	56	0.48	35	32	47	0.68
Coir Pith	27	24	30	0.80	22	19	47	0.40	31	28	56	0.50

***R.S** = Reducing sugar**; E.A** = Enzyme activity**; S.P** = Soluble protein**; S.A** = Specific Activity.

Table 2 (b): Effect of pH on cellulase activity of *Penicillium funiculosum* (Wild type) on Bagasse, Coir pith and Rice husk on six day incubation.

Substrate	5				7				9			
	R.S	**E.A**	**S.P**	**S.A**	**R.S**	**E.A**	**S.P**	**S.A**	**R.S**	**E.A**	**S.P**	**S.A**
Bagasse	57	53	21	2.52	53	49	30	1.63	55	51	42	1.21
Rice husk	65	60	57	1.05	62	58	64	0.91	67	62	56	1.10
Coir Pith	54	50	53	0.94	53	49	56	0.87	56	52	65	0.80

***R.S** = Reducing sugar**; E.A** = Enzyme activity**; S.P** = Soluble protein**; S.A** = Specific Activity.

Table 2 (c): Effect of pH on cellulase activity of *Penicillium funiculosum* (Wild type) on Bagasse, Coir pith and Rice husk on nine day incubation.

Substrate	5				7				9			
	R.S	**E.A**	**S.P**	**S.A**	**R.S**	**E.A**	**S.P**	**S.A**	**R.S**	**E.A**	**S.P**	**S.A**
Bagasse	107	103	28	4.21	115	110	43	2.55	121	118	45	2.51
Rice husk	131	122	86	1.41	141	134	71	1.90	141	135	96	1.40
Coir Pith	98	92	50	1.84	117	109	59	1.84	120	117	67	1.74

***R.S** = Reducing sugar**; E.A** = Enzyme activity**; S.P** = Soluble protein; **S.A** = Specific Activity.

Table 3 (a): Effect of temperature on cellulase activity of *Penicillium funiculosum* (Wild type) on Bagasse, Coir pith and Rice husk on three day incubation.

Substrate	30				40				50			
	R.S	**E.A**	**S.P**	**S.A**	**R.S**	**E.A**	**S.P**	**S.A**	**R.S**	**E.A**	**S.P**	**S.A**
Bagasse	31	28	22	1.27	38	35	17	2.05	23	19	12	1.58
Rice husk	30	27	57	0.47	34	31	65	0.47	17	16	20	0.80
Coir Pith	22	19	46	0.41	33	30	62	0.48	9	8	18	0.44

***R.S** = Reducing sugar**; E.A** = Enzyme activity**; S.P** = Soluble protein**; S.A** = Specific Activity.

Table 3 (b): Effect of temperature on cellulase activity of *Penicillium funiculosum* (Wild type) on Bagasse, Coir pith and Rice husk on six day incubation.

Substrate	30				40				50			
	R.S	**E.A**	**S.P**	**S.A**	**R.S**	**E.A**	**S.P**	**S.A**	**R.S**	**E.A**	**S.P**	**S.A**
Bagasse	53	49	23	2.13	55	50	22	2.27	28	25	18	1.38
Rice husk	62	57	65	0.87	61	56	73	0.76	19	17	26	0.65
Coir Pith	53	49	55	0.89	56	51	65	0.78	25	23	27	0.85

***R.S** = Reducing sugar**; E.A** = Enzyme activity**; S.P** = Soluble protein**; S.A** = Specific Activity.

Table 3 (c): Effect of temperature on cellulase activity of *Penicillium funiculosum* (Wild Type) on Bagasse, Coir pith and Rice husk on nine day incubation.

	30				40				50			
Substrate	**R.S**	**E.A**	**S.P**	**S.A**	**R.S**	**E.A**	**S.P**	**S.A**	**R.S**	**E.A**	**S.P**	**S.A**
Bagasse	98	91	42	2.16	115	100	42	2.38	39	35	20	1.75
Rice husk	138	130	71	1.83	142	135	91	1.48	62	58	35	1.65
Coir Pith	117	109	59	1.81	138	129	71	1.84	58	54	28	1.02

***R.S** = Reducing sugar**; E.A** = Enzyme activity**; S.P** = Soluble protein**; S.A** = Specific Activity.

Table 4 (a): Effect of pH on cellulase activity of *Penicillium funiculosum* (Mutant type) on Bagasse, Coir pith and Rice husk on three day incubation.

Substrate	5				7				9			
	R.S	**E.A**	**S.P**	**S.A**	**R.S**	**E.A**	**S.P**	**S.A**	**R.S**	**E.A**	**S.P**	**S.A**
Bagasse	37	34	24	1.42	38	35	17	2.05	46	42	20	2.1
Rice husk	34	31	46	0.67	34	31	66	0.47	37	34	51	0.66
Coir Pith	34	31	31	1.1	33	30	60	0.50	34	32	62	0.48

***R.S** = Reducing sugar**; E.A** = Enzyme activity**; S.P** = Soluble protein**; S.A** = Specific Activity.

Table 4 (b): Effect of pH on cellulase activity of *Penicillium funiculosum* (Mutant type) on Bagasse, Coir pith and Rice husk on six day incubation.

Substrate	5				7				9			
	R.S	**E.A**	**S.P**	**S.A**	**R.S**	**E.A**	**S.P**	**S.A**	**R.S**	**E.A**	**S.P**	**S.A**
Bagasse	61	54	34	1.7	55	50	22	2.27	60	58	28	1.93
Rice husk	63	58	50	1.16	61	56	73	0.76	68	62	54	1.15
Coir Pith	62	57	56	1.01	56	51	65	0.78	60	58	67	0.82

***R.S** = Reducing sugar**; E.A** = Enzyme activity**; S.P** = Soluble protein**; S.A** = Specific Activity.

Table 4 (c): Effect of pH on cellulase activity of *Penicillium funiculosum* (Mutant type) on Bagasse, Coir pith and Rice husk on nine day incubation.

Substrate	5				7				9			
	R.S	**E.A**	**S.P**	**S.A**	**R.S**	**E.A**	**S.P**	**S.A**	**R.S**	**E.A**	**S.P**	**S.A**
Bagasse	125	119	62	2.06	123	115	44	2.61	138	128	51	2.33
Rice husk	139	129	90	1.43	147	136	80	1.46	144	137	92	1.47
Coir Pith	111	104	67	1.55	126	117	71	1.64	138	128	73	1.75

***R.S** = Reducing sugar**; E.A** = Enzyme activity**; S.P** = Soluble protein**; S.A** = Specific Activity.

Table 5 (a): Effect of temperature on cellulase activity of *Penicillium funiculosum* (Mutant type) on Bagasse, Coir pith and Rice husk on three day incubation..

Substrate	30				40				50			
	R.S	**E.A**	**S.P**	**S.A**	**R.S**	**E.A**	**S.P**	**S.A**	**R.S**	**E.A**	**S.P**	**S.A**
Bagasse	41	38	19	2.0	46	42	20	2.1	20	18	10	1.8
Rice husk	35	32	46	0.69	37	34	54	0.63	15	14	27	0.52
Coir Pith	31	28	55	0.51	33	30	60	0.50	14	13	24	0.54

***R.S** = Reducing sugar**; E.A** = Enzyme activity**; S.P** = Soluble protein**; S.A** = Specific Activity.

Table 5 (b): Effect of temperature on cellulase activity of *Penicillium funiculosum* (Mutant type) on Bagasse, Coir pith and Rice husk on six day incubation.

Substrate	30				40				50			
	R.S	E.A	S.P	S.A	R.S	E.A	S.P	S.A	R.S	E.A	S.P	S.A
Rice husk	67	61	56	1.08	68	62	54	1.15	19	17	32	0.53

***R.S** = Reducing sugar**; E.A** = Enzyme activity**; S.P** = Soluble protein**; S.A** = Specific Activity.

Table 5 (c): Effect of temperature on cellulase activity of *Penicillium funiculosum* (Mutant type) on Bagasse, Coir pith and Rice husk on nine day incubation.

Substrate	30				40				50			
	R.S	E.A	S.P	S.A	R.S	E.A	S.P	S.A	R.S	E.A	S.P	S.A
Rice husk	141	134	92	1.46	142	136	98	1.38	62	57	36	1.58

***R.S** = Reducing sugar**; E.A** = Enzyme activity**; S.P** = Soluble protein**; S.A** = Specific Activity.

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