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STUDIES ON THE ENHANCEMENT IN PRODUCTION OF CELLULASE FROM BACTERIAL SPECIES

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ABSTRACT

For the production of energy from cellulose, the enzyme - cellulase is used. The cellulase can be produced by various organisms such as bacteria, fungi, protozoans, animals and plants. The sample was collected from cellulose rich areas, after screening culture 2 from sample 1 was selected on the basis of screening. During the optimization of production media it was found that the peptone, mannitol and $MgSO_4$ as best carbon nitrogen and metal ion sources for the cellulase production. After the purification of cellulase we got enzyme activity (u/ml/min) 0.036 for crude and 0.0792 for purified one.

Keywords: Enzyme Activity, Cellulase, Lignocellulosic, Optimization, Carbohydrate-Binding Modules

INTRODUCTION

The most lavish renewable biological resource with low-cost energy source is cellulose which is based on energy content approx. $3–4/GJ **[1,2].** According to Zhnag et al. 2008, lignocellulosic products would be beneficial to the environment, local economy and national energy security due its cost effective nature for bioenergy production **[3].** For the production of energy from cellulose, the enzyme - cellulase is used. It hydrolyze the β - 1,4 linkages present in cellulose chains. The cellulase can be produced by various organisms such as bacteria, fungi, protozoans, animals and plants. Based on the amino acid sequences and crystal structures the catalytic property of cellulases has been divided into many families **[4].**

Cellulases also comprise of noncatalytic carbohydrate-binding modules (CBMs) and additional known or unknown functional modules, which is situated at C or N terminus of a catalytic module. Naturally, three types of cellulase mediate the hydrolysis of cellulose are (1) endoglucanases (EC 3.2.1.4), (2) exoglucanases, including cellobiohydrolases (CBHs) (EC 3.2.1.91), and (3) β -glucosidase (BG) (EC 3.2.1.21). Microorganisms secrete cellulases in the form of free or cell surface bound to metabolize or hydrolyze the insoluble cellulose **[5].** Cellulases are used in numbers of varieties for industrial purposes such as in pulp and paper industry, food industry, in the textile industry, as well as an additive in improving digestibility of animal feeds and detergents. Cellulases had become a major share of the world's industrial enzyme market. It can also be used for the production of bioethanol from lignocellulose, through enzymatic hydrolysis of lignocelluloses materials **[6,7,8].** For making the process economically feasible, the catalystic acivity must be increase and the cost of hydrolysis of pretreated lignocellulosic materials should be decreased **[9,10].** For production it more cost effective we are producing the cellulase from bacterial sources by providing cheap nutrients.

METHODOLOGY

Collection of samples:

The samples were collected from different areas, which are rich in the source of cellulose content **[11]** such as: 1) cow dung 2) soil sample near to wood wastes from park 3) soil sample from timber house.

Isolation of cellulase producing bacteria from samples:

1 gram samples were serially diluted in 0.85% NaCl solution and then spread on nutrient agar plates **[12].** The colonies were selected on basis of different morphological parameters after 24 hours of incubation at 37°C. Selected colonies purified from mixed cultures by streaking **[13].** These pure cultures were screened for cellulase production by congo red test after incubation at 37°C for 48 hours of re-streak in minimal salt agar media supplemented with 1% carboxy methyl cellulose **[14].**

Enzyme estimation:

DNS test was followed for the estimation of enzyme by using 1% carboxy methyl cellulose as substrate for cellulase enzyme **[15].**

Optimization of the production media:

For the fermentation we prefer the production media which present the best condition of the growth for culture. Thus the production media is modified to meet the best condition for our culture. Different modification was done like changes in the nitrogen sources, concentration of the nitrogen source etc. this will help us to get the best result of the enzyme activity for our culture **[16].**

Production and purification of the cellulase:

The production was performed by using shake flask fermentation [17]. The culture was inoculated in optimized production and media and incubated at 37°C for 72 hours. The purification was done by executing salt precipitation (40% ammonium sulphate) and dialysis **[18].** Further the concentration and enzyme activity was calculated by using Lowry's test **[19]** and DNS test **[20]**.

RESULTS

Sample collection and isolation of cellulase producing bacteria:

Samples were collected from different location which possible has the cellulolytic activity. Three different samples were examined for the cellulolytic activity. The bacterial colonies were obtained in mixed culture plate after serial dilution and spreading as shown in figure 1a. The colonies were selected on the basis of morphology (table 1) and streaked on nutrient agar plates for making pure cultures (figure 1b).

Zone of hydrolysis was measured for the selection of cellulase producing culture in primary and secondary screening as indicates in figure 1c, and table 2. After screening it was found that the culture 2, 3 of sample 1 (cow dung) and culture 4 of sample 3 (soil sample) is able to produce the cellulase. Hence, DNS test was performed for the estimation of enzyme during the selection of best culture and as a result it was found that culture 2 from sample 1 (cow dung) is best among others.

a: bacterial colonies in the culture plates

b: Pure bacterial culture plates

c: clear zone indicates the zone of hydrolysis which represent the positive culture for cellulase production.

Figure 1: Isolation of cellulase producing bacteria from samples.

Table 1: Primary and Secondary screening of cultures for the production of cellulase

Culture	primary screening	secondary screening
S101	**+**	**-**
S102	**+++**	**+++++**
S103	**++**	**+++**
S204	**-**	**-**
S201	**+**	**-**
S202	**+**	**+**
S203	**+**	**-**
S301	**-**	**-**
S302	**+**	**+**
S303	**+**	**-**
S304	**++**	**+++**

Table 2: Selection of bacterial cultures on the basis of morphology

culture name	Shape	Margin	Elevation	Pigmentation	Surface	Texture	Opacity
S101	Circular	Entire	Flat	White	Rough	Hard	Opaque
S102	Spindle	Discrete	Raised	Off-white	Smooth	Soft	Translucent
S103	filamentous	Curled	Convex	Green	Smooth	Soft	Opaque
S204	Punctiform	Lobate	Pulvonate	Yellowish	Rough	Hard	Translucent
S201	Circular	Discrete	Flat	Off-white	Smooth	Soft	Opaque
S202	Rhizoidal	Curled	Convex	White	Rough	Hard	Opaque
S203	Rhizoidal	Lobate	Convex	White	Rough	Hard	Opaque
S301	Circular	Entire	Raised	Off-white	Rough	Hard	Opaque
S302	Rhizoidal	Discrete	Flat	Off-white	Smooth	Soft	Opaque
S303	Circular	Entire	Convex	Off-white	Smooth	Soft	Opaque
S304	Irregular	Curled	Flat	Off-white	Smooth	Soft	Opaque

Optimization of production media:

The production media we used was optimized according to the need of our culture so as to get the best result of the growth and the enzyme activity. The different physical and chemical factors of the production were changed such as the nitrogen source, pH and the substrate concentration, were varied and after every 48 hours, the DNS test was performed and the O.D. was compared with standard graph and from the result we got best nitrogen sources, pH and the substrate concentration for our bacterial strain.

Effects of different nitrogen, carbon and metal ion sources:

We varied the production media by testing the nitrogen and carbon sources and the result which we obtained after the DNS assay. In which we had seen that Peptone as a nitrogen, mannitol as a carbon source and $MgSO_4$ (table 3 and figure 2) as metal ion (table 4 and figure 3), enzyme activity got maximum.

Table 3: Effect of the different nitrogen and carbon sources varied in the production media

S no.	Sources	O.D. (540nm)	Concentration of enzyme (µg/ml)	Enzyme activity (unit/ml/min)
A.	**Nitrogen sources**			
1.	Yeast(MM1)	0.52	0.7	0.0336
2.	Peptone(MM2)	0.81	1.01	0.04848
3.	Tryptone(MM3)	0.46	0.65	0.0312
B.	**Carbon sources**			
4.	Dextrose(MM4)	0.46	0.6	0.0288
5.	Sucrose(MM5)	0.50	0.65	0.0312
6.	Mannitol(MM6)	1.01	1.35	0.0648
7.	Maltose(MM7)	0.63	0.885	0.04248

Table 4: Effect of the different metal ion varied in the production media

S no.	Metal ion source	O.D. (540nm)	Concentration of enzyme ((µg/ml)	Enzyme activity (unit/ml/min)
1.	$MgSO_4$(MM8)	0.37	0.495	0.02376
2.	$Cacl_2$(MM9)	0.02	0.02	0.00096
3.	$FeSo_4$(MM10)	0.07	0.035	0.00168
4.	$CuSo_4$(MM11)	0.02	0.02	0.00096

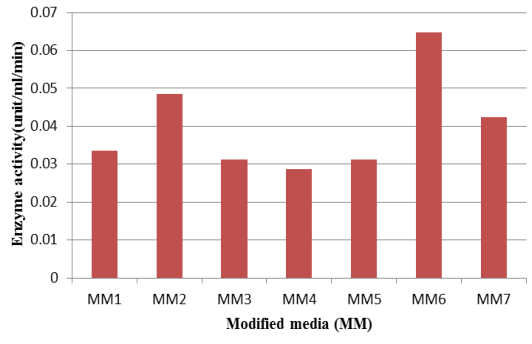

Figure 2: Activity of cellulase enzyme produce by culture at different nitrogen and carbon sources.

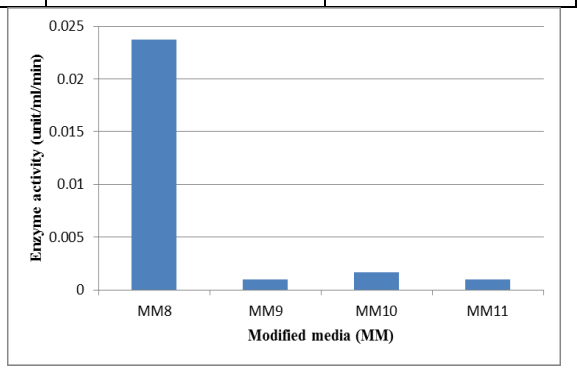

Figure 3: Activity of cellulase enzyme produce by culture at different metal ion sources.

Effect of pH:

The culture was inoculated in media with different pH as 5, 7, 9, 11. After the completion of the incubation period, the DNS assay was performed and as a result it was found that pH 9 is best for the production of enzyme as shown in table 5 and figure 4.

Table 5: Effect of different pH

pH	O.D. 540nm	Concentration enzyme (µg/ml)	Enzyme activity unit/ml /min
5(MM13)	0	0	0
7(MM14)	0.50	0.7	0.0336
9(MM15)	0.52	0.72	0.0340
11(MM16)	0.2	0.265	0.01272

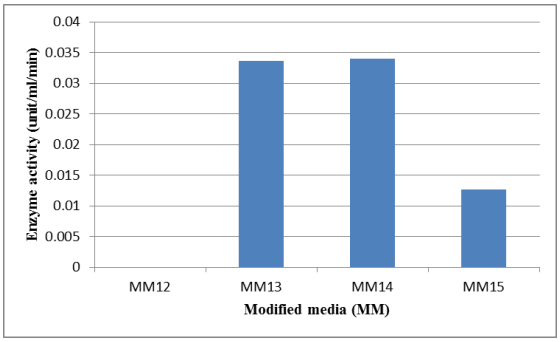

Figure 4: Graphical represent the activity of cellulase enzyme produce by culture at different pH.

Production and purification of cellulase

Fermentation was done by using shake flask method. The bacteria strain was fermented in the optimized media under optimum conditions. The crude enzyme we obtained after the centrifugation was salt precipitated under chilled conditions (40% Ammonium sulphate). After the completion of the salt precipitation enzyme was centrifuged and the pellet was dissolved in Tris buffer. Dialysis was done in order to remove the salt present in the salt enzyme.

DNS and Lowry's assay:

The DNS assay and Lowry's assay was done in order to find out the activity and concentration of the enzyme like crude salt precipitate and the pure enzyme. The reading was compared with the standard graph by which activity and concentration was calculated.

Table 6: DNS assay of crude and pure enzyme

ENZYME	O.D. at 540nm	Enzyme activity (u/ml/min)
Crude enzyme	0.56	0.036
Pure enzyme	1.23	0.0792

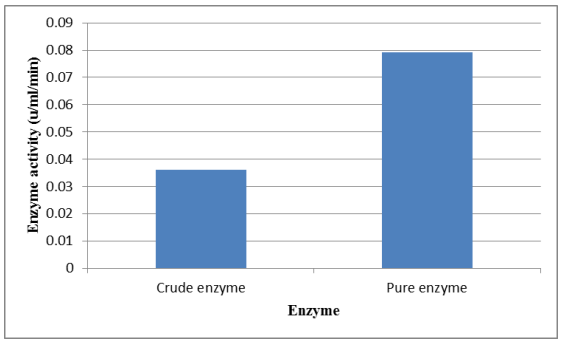

Figure 5: Graphical represent the activity of crude and pure cellulase enzyme

ENZYME	O.D. at 680nm	Concentration (µg/ml)
Crude enzyme	0.46	0.026
Pure enzyme	1.03	0.07

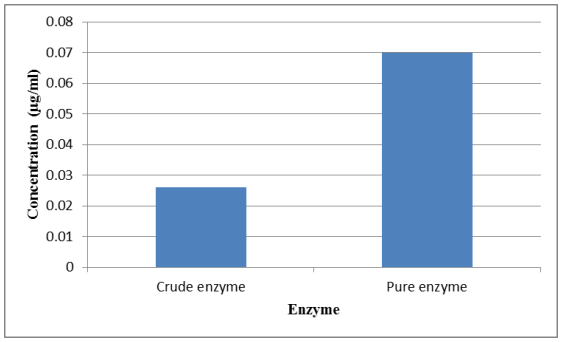

Figure 6: Above graph represent the concentration of crude and pure cellulase enzyme

DISCUSSION

The sample was collected from cellulose rich areas, then after serially dilution the samples were spread on nutrient agar plates an incubated for 24 hours at 37°C. Total 11 cultures were shortlisted on the basis of colony morphology from mixed culture plates and these colonies were purified by streaking on nutrient agar media. After screening culture 2 from sample 1 was selected on the basis of screening.

Further the media optimization was done on minimal salt media supplemented with 1% carboxy methyl cellulose. In optimization it was found that the peptone as a nitrogen, mannitol as a carbon source and $MgSO_4$ (table 3 and figure 2) as metal ion (table 4 and figure 3) and pH 9 (table 5 and figure 4) got maximum enzyme activity. The culture was inoculated in optimized media and incubated at optimum condition for shake flask fermentation. After fermentation the salt precipitation and dialysis was carried out for the purification of cellulase and we got enzyme activity (u/ml/min) 0.036 for crude and 0.0792 for purified one.

CONCLUSION

The bacterial culture isolated from the sample was capable for the production of cellulase. The pure cellulase was showing maximum enzyme activity as compare to crude one.

As the strain was soil isolate its isolation, culturing and industrial exploitation aspects will be very easy to control. We suggest the strain improvement by the mutagenic agents may enhance the activity.

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