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Isolation, Screening, and Characterisation of Cellulolytic Bacteria, Determination of Their Cellulolytic Potential

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Abstract: ***Cellulosic biomass is one of the most dominant waste materials from various industries. Cellulose degradation and its utilization are important for global carbon sources. The value of cellulose as a renewable source of energy has made its hydrolysis a subject of research and industrial interest. The present investigation is based on isolation, screening, and characterization of cellulolytic bacteria and determination of their cellulolytic potential. The work also concentrates on the production of cellulase enzyme by submerged fermentation and its partial purification by centrifugation. Enzyme activity of selected isolates was determined by DNS method and finally the application of potential isolates in degradation of various natural cellulosic substrates.***

Keywords: ***Cellulosic biomass, cellulolytic bacteria, cellulose degradation, cellulolytic potential, submerged fermentation, partial purification***

I. INTRODUCTION

Cellulose is the most abundant organic compound on the ecosystem and is the major component of plant biomass. It is the dominant waste from agricultural industry in the form of stalks, stems, and husk. The cellulose is composed of D-glucose units linked together to form linear chain via ß-1, 4-glycosidic linkages. Enzymes endoglucanase and exoglucanase catalyzes hydrolysis of cellulose. Mainly efficient cellulase activities are observed in fungi, but there is increasing interest in cellulase production by bacteria as they have higher growth rate as compared to fungi.

Cellulases have a great potentiality to be used in various industries such as textiles, wine, and brewing, detergent, paper and pulp, agriculture, food, and biorefinery. The final product of cellulose degradation by cellulase is glucose which is a simple sugar. Thus isolation and characterization of cellulolytic bacteria is an important aspect of biofuel research, biodegradation, and bioremediation. With the help of the cellulolytic system, cellulosic wastes which are often disposed of by biomass burning can be restricted.

The present study was attempted with these objectives: To isolate and screen cellulolytic bacteria from different natural cellulosic sources, Production of cellulose enzyme from potential isolated by submerged fermentation process, Partial purification of cellulase enzyme and determination of its enzyme activity and Application of potential isolates in biodegradation of cellulosic materials.

II. MATERIALS AND METHODS

A. Sample collection

Different samples rich in cellulose were used for the isolation of organisms. Soil samples (Here both agricultural as well as the non-agricultural soil was collected as samples and used for isolation), Cow dung, Kitchen wastes, Saw dust and Straw were used for the study.

B. Isolation of microbes present in the cellulosic samples

The organisms were isolated on CMC (carboxy methyl cellulose) media from different samples like soil, cow dung, kitchen wastes, saw dust and straw. The composition of CMC media: Tryptone 1%, CMC 1%, di-potassium hydrogen phosphate 0.2%, Magnesium sulphate 0.03%, Ammonium sulphate 0.25%, Gelatine 0.2%, Agar 1.5%.

C. Screening of cellulose degrading bacteria

Isolated bacterial strains were screened for cellulose degrading activity by Congo red test. After incubation, the media plates were flooded with 0.2% Congo red dye for 20 minutes. The dye was discarded and plates were flooded with 1M NaCl for 20 min. NaCl was discarded and then observed for the zone of clearance around the colony. Those colonies which show maximum clearance are used for the enzyme assay.

D. Morphological Characterisation of Selected Isolate

The cellulose degrading isolates were examined for its morphology by its colony characteristics and Gram staining.

E. Biochemical Characterization of the Selected Isolate

The culture was characterized by different biochemical tests as per Bergey's Manual of Systematic Bacteriology which included Indole, methyl red, Voges- Proskauer, citrate utilization test, catalase, oxidase, starch, gelatin hydrolysis, sugar fermentation and nitrate reduction test.

F. Submerged Fermentation process

Production medium [composition (g/l): Carboxymethylcellulose (CMC) 5; Tryptone 2g; KH2PO4 4g; Na2HPO4 4g; MgSO4.7H2O 0.2g; CaCl2.2H2O 0.001g; FeSO4.7H2O 0.004g and pH adjusted to 7 was inoculated with selected isolates individually and incubated at 37 ºC for 24 hrs.. Fermentation was carried out in 250ml flasks, each containing 100 ml sterile production medium and inoculated with 5% of standard inoculums. The flasks were incubated at 37^0C on a rotary shaker at 150rpm for 72hrs.

G. Preparation of crude enzyme

After incubation, the cultures were centrifuged at 1600 RPM for 20 min at 4°C and supernatant was used as a source of crude enzyme. The crude enzyme solution was used for determination of enzyme activities.

H. Enzyme assay

The supernatants were diluted with sterile distilled water and CMC solution pre-heated in a water bath at 37°C for 30 mins. The reaction mixtures containing 1ml each of the diluted supernatant and CMC solution and 2 ml of DNS reagent. It was then heated in a boiling water bath for 5 mins. After cooling, sterile distilled water was added to give a final volume of 25 ml. Absorbance was measured spectrophotometrically at 540nm. Enzyme activities are then found using standard glucose curve.

I. Estimation of Degradation of natural cellulosic substrates by the Isolates

10 mL of Stanier's Basal broth with 100 mg of different cellulosic substrates as sole carbon source viz. Vegetables peel, filet paper, cotton, and straw and saw dust were prepared separately. The broths were autoclaved and inoculated with the selected strains. The tubes were incubated in the shaker incubator at 37 °C for a week. After the incubation period, the culture broths were filtered through previously weighed filter papers. The filter papers with the residues were dried in the hot air oven for 15 min and re-weighed. The difference between the initial and the final weights gave a number of cellulosic substrates degraded by the isolate.

III. RESULTS AND DISCUSSION

Investigations were conducted at the Department of Microbiology RCASC, Bangalore, to isolate and characterize cellulolytic bacteria and determine their cellulolytic potential. The results of this study indicate the effectiveness of different cellulolytic strains in degrading natural cellulosic substrates.

A. Sample collection

Table 1: DIFFERENT CELLULOSIC SUBSTRATES

Serial no.	Samples collected
1	Agricultural soil
2	Non-agricultural soil

3	Fresh cow dung
4	Kitchen wastes
5	Saw dust
6	Straw

Further,

Biochemical tests	AG1	AG2	AG3	AG4	AG5	NAG1	CD1	CD2	KW1	KW2	SD1	SD2	ST1	ST2	ST3	ST4
Catalase	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-
Oxidase	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Indole	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Methyl red	+	+	-	+	-	+	+	-	+	+	-	+	-	+	+	-
Voges-Proskauer	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-
Citrate utilization	+	-	+	-	-	+	+	-	+	+	-	+	+	+	+	-
Motility test	+	+	-	+	+	+	-	-	+	+	+	+	+	-	-	-
Starch hydrolysis	-	+	-	-	+	+	-	-	-	+	+	+	+	+	+	+
Gelatine liquefaction	+	+	+	-	-	+	+	-	-	+	+	+	+	-	+	+
Nitrate test	+	+	-	+	+	+	-	+	+	+	+	+	+	+	+	+
Fermentation test of : maltose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
glucose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
cellulose	+	+	-	+	+	+	+	+	+	+	+	+	-	+	+	+

cellulolytic isolates were characterized for their morphological features and Gram reactions. The colonies were further characterized by various biochemical tests and results are tabulated in table 2.

Table 2: BIOCHEMICAL CHARACTERIZATION OF THE CELLULOSE DEGRADING ISOLATES

The isolates were further used for determining the cellulase activity and to find the most efficient cellulose degrading microorganism out of the several cellulolytic microbes isolated.

Table 3: CELLULASE ENZYME ACTIVITY OF DIFFERENT ISOLATES FROM VARIOUS SUBSTRATES

The isolates were further checked for their

Isolate no.	OD at 540nm	Enzyme activity (µg/ml/min)
AG1	0.34	31.5
AG4	0.22	22.02
AG5	0.20	20.52
NAG	0.23	23.01
CD1	0.18	19.5
CD2	0.22	22.02
KW1	0.17	18.51
SD1	0.26	25.02
ST2	0.17	18.51
ST3	0.19	20.01

degradation capacity on various natural cellulosic sources such as saw dust, straw, filter paper, cotton and finely grated vegetable peels. The degradation efficiency is measured by the growth of the cellulolytic microbes in particular substrates. More the growth of the organisms, higher the degradation efficiency. Because the sole carbon source added in the medium is the natural cellulosic substrate. The results for the degradation efficiency is tabulated in terms of dry weight in milligram in table 4.

Table 4: DEGRADATION EFFICIENCY OF DIFFERENT ISOLATES ON VARIOUS SUBSTRATES

Isolates	**Dry weight obtained in milligrams**				
	straw	**Saw dust**	**Vegetable peels**	**cotton**	**Filter paper**
AG1	400	490	50	310	440
AG4	400	430	70	270	430
AG5	210	490	70	340	430
NAG	350	390	200	180	450
CD1	320	400	300	290	380
CD2	260	470	310	280	430
KW1	350	420	330	390	340
SD1	320	330	460	230	360

ST2	240	430	420	290	280
ST3	240	420	90	400	350

From the determination of enzyme activity, the isolate AG1 appear to be a most efficient organism in producing cellulase enzyme and also it has been shown its ability to degrade all cellulosic substrates used in the biodegradation study. The least enzyme activity was shown by isolates KW1 and ST2. From the different cellulosic substrates used for the study, several of the isolates were obtained and ten of them were found to be efficient cellulose degraders having higher enzyme activity. These efficient microbes utilized natural cellulosic substrates as their sole carbon source which was noticed by their maximum growth. The most efficient cellulose degrading microorganism identified can be further studied and the same can be utilized for large scale applications. Further improvement in the performance of cellulase can be imparted by mutagenesis and protein engineering techniques for the industrial applications

CONCLUSION

Cellulases are one of the most widely used enzymes in various industries. The present study focused on isolation, screening, and characterization of cellulolytic bacteria and determination of their cellulolytic potential. From different cellulosic samples used for the study, several isolates were obtained, out of which ten of them were found to be better cellulose degraders having higher enzyme activities. By the selection of efficient cellulolytic organisms and cost-effective operational techniques, production of useful end products from cellulose degradation can be very beneficial. Further improvement in cellulase performance can be imparted using various techniques for industrial applications.

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