

ISOLATION AND SCREENING OF CELLULOSE DEGRADING MICROORGANISMS FROM FECAL MATTER OF HERBIVORES

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Abstract

Herbivores are the organisms that are adopted to eat plants. Herbivory is a form of predation in which organisms consume autotrophs. i.e. in this context Herbivory refers to animals eating plants. Cellulase due to its massive applicability has been used in various industrial processes such as biofuels like bioethanol, triphasic bioremediation; agricultural and plant waste management; chiral separation and ligand binding studies. The cellulase is used for many industrially important processes like commercial food processing in coffee, brewery, wineries, and textile and paper industries. Cellulases that catalyze the hydrolysis of cellulose, it is produced mainly by symbiotic bacteria in the rumen of ruminants. In present studies the cellulase producers isolated from fecal matter of deer and elephant by serial dilution technique and producers are screened on CMC (Carboxy Methyl Cellulose) media. The present work concentrates on the isolation of cellulose-degrading bacteria from herbivores excreta and assessment of their cellulolytic activity. The coculturing of cellulose-degrading bacteria and yeast was also carried out for simultaneous saccharification and fermentation of cellulose into ethanol.

Keywords: - Cellulase, cellulose, Herbivores.

Introduction

Cellulose is a linear polysaccharide of glucose residues with β -1, 4-glycosidic linkages. Abundant availability of cellulose makes it an attractive raw material for producing many industrially important commodity products. Sadly, much of the cellulosic waste is often disposed of by biomass burning, which is not restricted to developing countries alone, but is considered a global phenomenon. With the help of cellulolytic system, cellulose can be converted to glucose which is a multiutility product, in a much cheaper and biologically favourable process.

Cellulolysis is basically the biological process controlled and processed by the enzymes of cellulase system. Cellulase enzyme system comprises three classes of soluble extracellular enzymes: 1, 4- β -endoglucanase, 1, 4- β -exoglucanase, and β -glucosidase (β -D-glucoside glucohydrolase or cellobiase). Endoglucanase is responsible for random

cleavage of β -1, 4-glycosidic bonds along a cellulose chain. Exoglucanase is necessary for cleavage of the nonreducing end of a cellulose chain and splitting of the elementary fibrils from the crystalline cellulose, and β -1, 4-glycosidase hydrolyses cellobiose and water-soluble cellodextrin to glucose. Only the synergy of the above three enzymes makes the complete cellulose hydrolysis to glucose or a thorough mineralization to H₂O and CO₂ possible.

Source for cellulase system extraction is best suitable from microbial system found in the gut of organisms thriving on cellulosic biomasses as their major feed. Insects like termites (*Isoptera*), bookworm (*Lepidoptera*), and so forth, are found to have syntrophic symbiotic microflora in their guts responsible for cellulosic feed digestion. Many microorganisms have been reported with cellulosic activities including many bacterial and fungal strains both aerobic and anaerobic. *Chaetomium*, *Fusarium*, *Myrothecium*, *Trichoderma*, *Penicillium*, *Aspergillus*, and so forth, are some of the reported fungal species

responsible for cellulosic biomass hydrolysis. Cellulolytic bacterial species include *Trichonympha*, *Clostridium*, *Actinomycetes*, *Bacteroides succinogenes*, *Butyrivibrio fibrisolvens*, *Ruminococcus albus*, and *Methanobrevibacter ruminantium*.

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A ruminant is a mammal of the order Artiodactyls that digests plant-based food by initially softening it within the animal's first compartment of the stomach, principally through bacterial actions, then regurgitating the semi-digested mass, now known as cud, and chewing it again. The process of rechewing the cud to further break down plant matter and stimulate digestion is called "ruminating".

There are about 150 species of ruminants which include both domestic and wild species. Ruminating mammals include cattle, goats, sheep, giraffes, bison, moose, elk, yaks, water buffalo, deer, camels, alpacas, llamas, antelope, pronghorn, and nilgai. Taxonomically, the suborder Ruminantia includes all those species except the camels, llamas, and alpacas, which are Tylopoda. Therefore, the term 'ruminant' is not synonymous with Ruminantia. The word "ruminant" comes from the Latin *ruminare*, which means "to chew over again."

Technical Scheme of Project:

The project was carried out with the following scheme.

1. Collection of animal excreta sample from different places

2. Isolation of cellulose degrading microorganisms on Carboxymethyl cellulose medium at pH 7.2

3. Detection of cellulose utilizing ability of microorganism from isolates.

4. Identification and characterization of bacterial species by morphological and biochemical test.

Materials and methods:

All the chemicals used in this project were from Hi-Media Pvt. Ltd. Mumbai. The glassware's Used are of Borosil. Before use all the glassware were cleaned with chemical acid and rinsed with double distilled water.

Methodology:

Isolation and screening of microorganism:

A) Sample Collection: The sample was collected from the Siddharth Garden of AMC, Aurangabad.

Sample "A" = Elephant "B" = Elephant and Sample "C" = Deer

B) Isolation of Sample:

The two samples were serially diluted in 10 ml tube separately. Like 10⁻¹, to 10⁻¹⁰ and inoculated on the respective CMC agar plates, the plates were incubated at 37°C for 24 to 72 hrs & colonies were observed.

C) Enrichment of Sample:

The three colonies were selected and the colonies were inoculated in enrichment medium and incubated at 37°C for 24 hrs.

D) Slant Culture:

The isolates were preserved on Carboxy-methyl cellulose agar slants.

Morphological & Biochemical Examination of Isolates:

A) Morphological Examination: The isolates was examined for morphological characteristics like, Colony size, shape, Color, Margin, Surface, Elevation, Opacity, Consistency, Gram's Nature, Motility, Endospore staining, Capsule staining.

B) Biochemical Examination: The isolates were subjected to IMViC test, Starch hydrolysis Gelatin liquefaction and sugar fermentation and result was interpreted.

A) Morphological Examination:

Table: 1: Morphological Characteristics “Colony- A” and “Colony- B” Colony- C
Preliminary examination shows that the isolated organism belongs to Bacillus Sp.

Sr.No	Characteristics	Elephant A	Elephant B	Deer
1	Size	0.5-1.0 0.6-6.0	0.6-1.0 1.2-3.0	0.5x1.2
2	Shape	Short rod	Long rod	Rod
3	Colour	White	Cream colour	White
4	Margin	Oval	Oval	Oval
5	Elevation	Elevated	Elevated	Convex
6	Opacity	Opaque	Opaque	Opaque
7	Consistency	Mucous	Mucous	Butyrous
8	Gram's Nature	-ve	-ve	+ve
9	Motility	Non Motile	Motile	Motile

Table: 2: Biochemical characteristics of selected microorganism.

Test Sample	Sample A		Sample B		Sample Deer	
Starch Hydrolysis	Negative		Negative		Positive	
Gelatin Liquefaction	Negative		Negative		Positive	
Indole Production	Negative		Negative		Negative	
Methyl Red Test	Negative		Negative		Negative	
Voges Proskauer	+/-		Positive		Negative	
Citrate Utilization	Positive		Positive		Positive	
Sugar Fermentation	Acid Formation	Gas Formation	Acid Formation	Gas Formation	Acid Formation	Gas Formation
Lactose	Positive	Positive	Positive	Positive	Negative	Negative
Dextrose	Positive	Positive	Positive	Positive	Positive	Negative
Sucrose	Positive	Positive	Positive	Positive	Positive	Negative
Casein utilization test	Positive		Positive		Positive	
Catalase test	Positive		Positive		Positive	

Conclusion:

The Cellulose utilizing organism isolated and studied for the cellulolytic activity, the observation and result shows that the isolates are potent cellulose degraders, the isolate has peculiar morphological properties. sample of Elephant "A" were Casein positive but gelatin & Starch negative. Whereas Deer sample is Starch, Gelatin & Casein positive. The further identification is needed from the national laboratories for confirmation of genus and species. From the preliminary studies of college lab shows that the organism is potential cellulose degrader and can be used industrially

From observations it can be concluded that

Colony A = *Klebsiella* genus.

Colony B = *Enterobacter* genus.

Colony C = *Bacillus* genus.

Applications:

1. Pulp and Paper Industry
2. Textile Industry
3. Bioethanol Industry
4. Wine and Brewery Industry
5. Food Processing Industry
6. Animal Feed Industry
7. Agricultural Industries
8. Olive Oil Extraction
9. Carotenoid Extraction
10. Detergent Industry
11. Waste Management

Inference

The biological aspects of processing of cellulosic biomass become the crux of future research involving cellulases and cellulolytic microorganisms. Cellulases are being commercially produced by several industries globally and are widely being used in food, animal feed, fermentation, agriculture, pulp and paper, and textile applications. With modern biotechnology tools, especially in the area of microbial genetics, novel enzymes and new enzyme applications will become available for the various industries. Improvements in cellulase activities or imparting of desired features to enzymes by protein engineering are probably other areas where cellulase research has to advantages.

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