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ISOLATION AND SCREENING OF BACTERIA FOR ENZYME AVICELLASE ACTIVITY FROM THE BOVINE RUMEN

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ABSTRACT

The ruminants are herbivores, fed with feed stuff contains cellulose, hemicellulose and lignin. The microbial population present in rumen degrades and converts feed into utilizable form such as volatile fatty acids and proteins. Hence, the type of microbial population in rumen is very important to maintain animal health and productivity. To measure the enzymatic activity such as avicellase of bacteria in rumen, we performed conventional method to isolate fiber-degrading bacteria from rumen under strict anaerobic conditions. In this study, we isolated 36 isolates, of which 10 bacterial isolates such as b4, b6, b11, b18, c8, a1, a16, b8, b2 and c10 were found to be more potent avicellase producing isolates.

KEY WORDS

Enzyme activity; rumen; fiber-degrading bacteria; conventional culture method

INTRODUCTION

Rumen is an excellent ecosystem for fiber-degrading bacterial communities. Fiber degradation and fermentation is a unique process, efficient plant material degradation depends on the assistance between the fibrolytic enzymes produced by microorganisms and the host animal that provide anaerobic environment for microorganisms (1). The fiber-degrading bacteria are found to be important to maintain balance in rumen ecosystem. These bacteria degrade complex lignocellulosic biomass into soluble and simple sugars to utilize by other microbes and ruminants. The feed fibre consists of cellulose fibrils that are cross linked with complex hemicelluloses and lignin. The cellulose is a polymer of β-1,4-linked glucose residues, whereas hemi cellulose mainly consists of xylan, β-1,4-linked xylose residues lignin is a polymer of phenolic compounds. To degrade the cellulose, its essential to disrupt plant cell wall, as non-fiber degrading bacteria have limited ability to degrade this material (2). To manipulate plant fiber, synchronous and different types of hydrolytic enzymes are required for bacteria. However, in ruminants bacteria has been subject of intensive studies over the past 50 years, and several studies illustrated the isolation and characterization of bacterial communities from ruminants (3). Most of the rumen bacterial characterization and metabolic functions were known by conventional culture method only. In this study, to understand the fiber-degrading efficiency, we carried out our work in conventional culture-based method in anaerobic conditions and avicellase activity was measured.

MATERIAL AND METHODS

Ethical permission

The experiment conducted was approved by Institutional Animal Ethics Committee (IAEC) constituted as per the article number 13 of the CPCSEA rules laid down by Government of India.

Maintenance of animals

Four Karan fries cattle were fed with standard diet (concentrate/roughage ration, 40:60) for 21 days at Indian Council of Agricultural Research–National Dairy

Research Institute (ICAR-NDRI), Karnal. Rumen liquor sample was collected from four fistulated animals, approximately 500 ml of rumen liquor was taken into nitrogen passed thermos flask and carefully carried to the laboratory. The liquid part was separated from solid feed particles by squeezing through a four-layer cheese cloth (4).

Screening for avicellase enzyme activity

The rumen liquor was centrifuged at 2000 x g for 10 min to remove protozoa and fungi. The dilution was carried out using anaerobic diluents up to 10^{-12} and plated on media containing avicel as a carbon source and incubated at 37°C for 24 hrs under strict anaerobic conditions (5). After 24 hrs of incubation, colonies were selected to screen avicellase activity. Selected isolates were cultured on wheat straw and 0.3% cellobiose (w/v) as carbon source and incubated at 39°C, strict anaerobic conditions were maintained during entire process of experiment. The cultured medium was centrifuged at 5000 rpm for 10 min and the supernatant used as a source of crude enzyme (6). Assay was carried out in triplicate at 39°C in a water bath using the dinitrosalicylic acid method of (7).The enzyme activity was measure at different time intervals such as 24, 48, 72 and 96 hours and activity was calculated in U/ml.

RESULTS AND DISCUSSION

In this study, thirty-six bacterial isolates were isolated and all were shown to be having avicellase activity. Of which, 10 bacterial isolates including b4, b6, b11, b18, c8, a1, a16, b8, b2 and c10 were found to be more potent avicellase producing isolates. Among these, b4, b6 isolates were found to be more potent bacteria in avicellase activity. All isolates showed maximum avicellase activity at 48 h except b4 and b18 isolate. Isolates b4 and b18 have produced maximum enzyme with 15.823U/ml and 15.674U/ml, respectively at 4 hrs and the activity started decreasing with 13.539 U/ml and 14.83U/ml, respectively after 48 hrs. The b6 isolate had shown maximum production of avicellase with 15.641U/ml at 24h and optimum production at 48 hrs was 19.828U/ml and then enzyme production started to decline after 48 hrs. The isolates b11 and c8 showed 15.037U/ml and 13.051U/ml at 24h and 17.494U/ml and 19.306U/ml at 48 h enzyme activity, respectively. The isolates a1, a16 and b8 showed maximum activity at 48 h with 15.66U/ml, 14.673U/ml and 14.524U/ml enzyme activity, respectively. Among isolates, b2 showed maximum activity with 14.325U/ml followed by c10 (9.195U/ml) at 48 h.

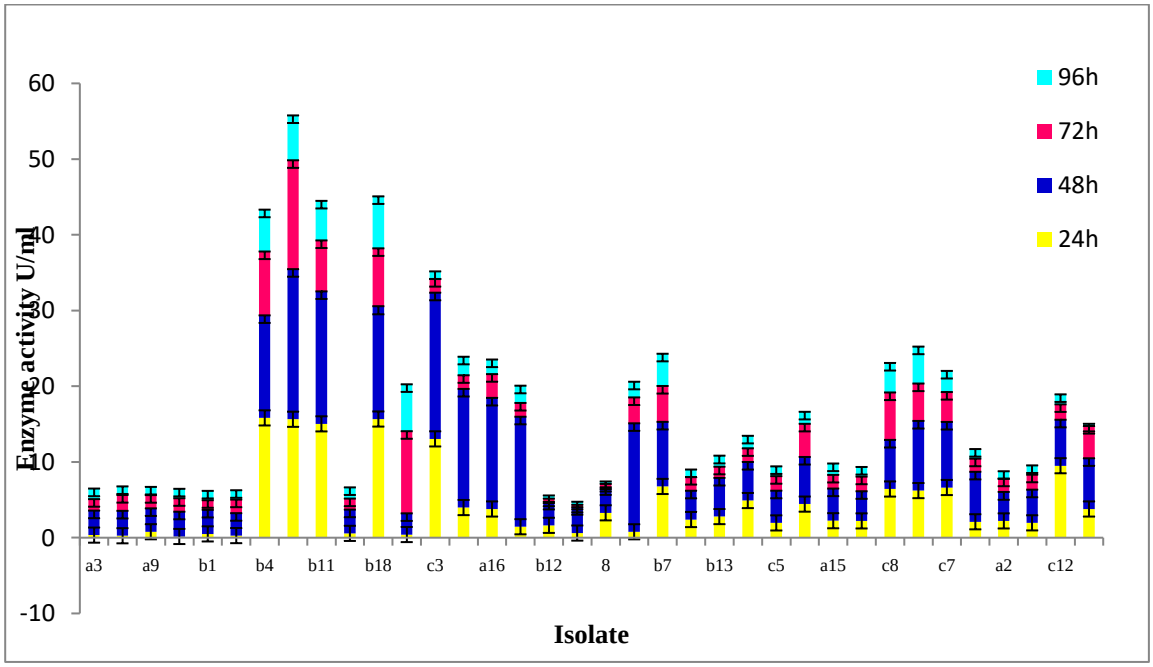

Figure 1. Enzyme activity of avicellase at different time interval times.

Based on the preliminary screening 47 isolates were selected, but only 36 isolates showed fiber degrading efficiency. All the bacteria started degrading complex sugars at 24 h and optimal at 48 h, after 48 h all bacterial enzyme activity was decreased. Previous studies suggest that among major rumen bacteria, the species of *Fibrobacter succinogenes, Ruminococcus albus, Ruminococcus flavefaciens* were found to be most abundant cellulolytic bacteria (8), *Butyrivibrio fibrisolvens*, *Prevotella ruminicola*, *Eubacterium cellulosolvens* and *Eubacterium ruminantium* were also recognized as fibrolytic bacterial species (1, 9). Though the bacteria are capable of degrade fiber, no significant efficiency was detected. This may be due to the feedback inhibition of glucose. For the better efficiency of fiber-degrading bacteria, association of non fiber-degrading bacteria may be required. Few reports suggested that co-culture of *Ruminococcus flavefacience* with *Prevotella ruminicola* increased the fiber-degrading bacteria efficiency than monoculture (10).

CONCLUSION

Based on the results, fiber degrading enzyme avicellase activity was found to be active at 48hr of incubation time. Most of the isolates showed avicellase activity. Further these isolated will be screened for *In vitro* fermentation profile such as volatile fatty acids production, feed digestibility.

CONFLICT OF INTEREST

All authors declared no conflict of interest.

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