Phenotypic Detection of Cellulose Hydrolysing Bacteria from the Rumen of Cow and Goat in Bauchi Metropolis

ABSTRACT

Background of Study: Rumen microorganisms are involved in the fermentation of substrates contained in the diet of the animals. Research on the isolation and identification of cellulase enzyme-producing by bacteria is still widespread. Biodegradation by cellulolytic bacteria found in rumen can be used as a source of cellulolytic bacteria which will function to degrade fibrous feed material so as to increase quality of nutrition and digestibility of ingredients at a cheaper price than the use of commercial cellulase production.
Aim: To isolate and characterize bacteria from animals' rumen for their ability to hydrolyze cellulose.
Place and Duration of Study: Conducted at the Microbiology Laboratory of Abubakar Tafawa Balewa University Bauchi, Bauchi state, Nigeria, between April to July, 2021.
Methods: Rumen of cows and goats was sliced and randomly swabbed with a swab stick. They were then inoculated on Nutrient and MacConkey agar media. The plates were then incubated aerobically and anaerobically for 24 hours at 37^{o}C. The resulting colonies that developed after the incubation period was purified and maintained on agar slants for further characterization. Pure isolates were then sub-cultured on carboxymethyl cellulose (CMC) plates and then incubated aerobically and anaerobically for seven days to test their ability to hydrolyze cellulose which was indicated by the appearance of clear zones around the colonies of the organisms. Secreted cellulase by the bacteria was further observed by colouring of the medium using Congo-red 0.3%.
Results: A total of 95 bacterial species isolated and characterized from rumen of cow and goat to test their ability to hydrolyze cellulose out of which 52 hydrolyzed celluloses after growing them on cellulose as seen by zone of clearance around the isolates. The isolates include *Bacillus subtilis* (28.8%), *Bacillus licheniformis* (27.0%), *Yersinia enterocolitica* (9.6%), *Micrococcus* sp. (5.8%), *Salmonella* sp. (11.5%), *Pseudomonas* sp. (3.8%) and *Streptococcus* sp. (13.5%).
Conclusion: This study demonstrates the potentiality of local strains of bacteria isolated from ruminants to hydrolyze cellulose. Proofs based on zone of clearance in cellulose led to the conclusion that the rumen of ruminants contains various microorganisms that can breakdown cellulose.

Keywords: Bacteria; Cellulase; *Bacillus subtilis*; Cellulose; Rumen

1. INTRODUCTION

Cellulose, the most abundantly available polymer, found in both plant and animal cells as a structural material, is a branched polymer with β-D-glycosidic linkages [9]. One of the key players in cellulosic degradation is the cellulolytic microorganisms [29], which can biologically convert cellulosic materials to volatile fatty acids (VFA) and gases such as hydrogen and carbon dioxide [13]. The different cellulolytic microorganisms, those that are naturally occurring in environment, such as the rumen has shown to be markedly more efficient in degrading cellulose to produce VFAs [13, 11]. In addition to its superior cellulose degrading capabilities, studies have indicated that the rumen is also a rich source of different enzymes with different activities which will eliminate the need for separate enzymatic hydrolysis as often found in bio refineries [35]. Fermentation using some mixed microbial

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rumen consortia, being nonsterile, can further significantly decrease process costs [35]. There are a number of forms of cellulose that are used to assay cellulases; carboxymethyl cellulose (CMC) is a soluble form that is an excellent substrate for endocellulases.

The rumen is a complex ecosystem where nutrients consumed by the microorganisms such as bacteria, protozoa, and fungi are digested anaerobically. The main end products of fermentation are volatile fatty acids (VFAs) and microbial biomass, which are used by the host ruminant. The interaction between microorganisms and the host animal results in a symbiotic relationship that allows ruminants to digest diets rich in fiber and low in protein. In the rumen the environment favors the microorganisms to provide the enzymes necessary to digest the nutrients. Ruminants have the ability to convert the low-quality fibrous materials into products such as meat, milk and fibers, which are useful to humans. The ability of ruminants' microorganisms to produce the enzymes necessary for fermentation processes allows ruminants to efficiently obtain the energy contained in forages [3]. However, the ruminal fermentation process is not completely efficient because it produces some final products such as methane gas [15] and excess ammonia [30]. Ruminants such as cattle, sheep, and goats have evolved to use fibrous food efficiently [25]. The anatomical adaptation of their digestive system allows them to use cellulose as an energy source without requiring external sources of vitamin B complex [30] or essential amino acids because ruminal microorganisms are able to produce such products [5].

The ruminant digestive system is composed of reticulum, rumen, omasum, and abomasum. The rumen is mainly where the major fermentation processes are held [33]. Enzymes present in the rumen are produced by microorganisms. These enzymes are used to digest and ferment food eaten by ruminants; thus, the rumen is considered as a fermentation vat [1]. The main factors influencing the growth and activity of ruminal microbial populations are temperature, pH, buffering capacity, osmotic pressure and redox potential (reference). These factors are determined by rumen environmental conditions. The rumen temperature is maintained in the range of 39 to 39.5 °C [34] and may increase up to 41 °C immediately after the animal eats because the fermentation process generates heat [2]. The pH depends on the production of saliva, the generation and absorption of short-chain fatty acids (SCFA), the type and level of feed intake, and the exchange of bicarbonates and phosphates through the ruminal epithelium [1]. Thus, these factors determine both pH and buffering capacity in the reticule ruminal environment. The pH constantly changes [30], but it usually remains in the range of 5.5 to 7.0 [16], depending on the diet and buffering capacity of saliva, because saliva production is a constant process that provides bicarbonates and phosphates into the rumen.

In the rumen, there are various types of bacteria, fungi, and protozoa [20] which each has different functions so that complex carbohydrates can be converted into organic acids that can be utilized by livestock. Rumen microbes that have been identified in producing cellulase include bacteria, fungi, and actinomycetes. The bacteria *Fibrobacter succinogenes*, *Bacteroides succinogenes*, *Ruminococcus albus*, *Ruminoccus flavafaceins*, *Clostridium ochheadii*, *Bacillus licheniformis*, and *Streptococcus anaerobius* are generally regarded as the predominate cellulolytic microbes in the rumen [21]. Some of the cellulase-producing bacteria such as *Bacillus*, *Paenibacillus*, *Pseudomonas*, *Clostridium*, *Cellulomonas*, *Thermomonospora*, *Ruminococcus*, *Bacteroides*, *Erwinia*, *Serratia*, *Acetivibrio*, *Methanobrevibacter*, *Gluconoacetobacter*, and *Rhodobacter*, *Enterobacter* [32,10,23,18,12, ,24]; Some species of Bacillus produce extracellular enzymes such as proteases, lipases, amylases, and cellulases that can ~~aid~~ digestion of different nutrients in the animal body. *Bacillus* species (*B. cereus, B. clausii and B. pumilus*) are included in five commercial probiotic products consisting of bacterial spores that have been characterized and potentially for colonization, immune-stimulation, and antimicrobial activity [7].

Cellulases, a complex group of enzymes which are secreted by a broad range of microorganisms including bacteria, fungi, and actinomycetes. Cellolytic microorganisms such as those secreted from the rumen play an important role in the hydrolysis of lignocellulosic polymer which can be used in biofuel production. Cellulases have also shown potentials in agriculture to increase quality of nutrition and digestibility of ingredients, textiles and paper and pulp industry among others.

2. MATERIALS AND METHODS

Description of study area

This study was conducted ……

Isolation procedures

The cellulolytic microorganisms were isolated in accordance with the method described by Oyeleke and Okusanmi [26]. Rumen was bought from a local abattoir in Yelwa area of Bauchi metropolis and placed in a sterile bowl and transported immediately to the Microbiology laboratory of Abubakar Tafawa Balewa University, Bauchi. The rumen of the ruminant animals (cow and goat) was sliced and randomly swabbed with a swab stick at 10 different parts of the rumen for both cow and goat and the parts were name A, B, C, D, E, F, G, H, I and J (A = part 1, B = Part 2, C = part 3, D = part 4, E = part 5, F = part 6, G = part 7, H = part 8, I = part 9 and J = part 10 for both cow and goat respectively) respectively.

Animal management

Five each of the swabbed part from cow and goat were then inoculated on Nutrient agar and MacConkey agar (Oxoid, UK) plates. The plates were then incubated aerobically and anaerobically using candle jar for 24 hours at 37^{o}C.

Laboratory procedures

Colonies that developed after the incubation period were purified and maintained on agar slants for further characterization and identification. The pure isolates were then sub-cultured on carboxymethyl cellulose (CMC) plates and then incubated aerobically and anaerobically for seven days to test their ability to hydrolyze cellulose. Hydrolysis of cellulose was indicated by the appearance of clear zones around the colonies of the organisms. The bacterial isolates were characterized and identified using standard methods as described by Cheesebrough [4] and Oyeleke and Manga [27].

Microorganisms identification and management

Secreted cellulase of the bacteria was further observed by coloring the medium using Congo-red 0.3%. Coloring process was done using CMC medium that was incubated for 24 hours. In this interval time, Congo red detects the hydrolysis zone which is done by cellulase. It diffuses into the agar medium and is absorbed by a long chain polysaccharide that have β-D-glucan linkage [36]. Excess Congo red was rinsed with 1% NaCl salt solution as a wash. Congo red which did not interact with the polysaccharide chain is rinsed by 1% NaCl solution that make the cellulase hydrolysis zone appear clearly.

3. RESULTS AND DISCUSSION

3.1 Microorganisms Isolated from Cow and Goat

The result of the bacteria isolated from cow and goat are presented in table 1 below. From the result it can be seen that cow have the highest number of isolates 54 (56.8%) while goat had 41(43.2) which is agreement with the findings of Oyeleke and Okusanmi (2008) who isolated microorganisms from cow, goat and sheep and found out that cow have the highest number of isolates.

This study (table 1) shows that samples from cow have the highest number of organisms, this

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could be as a result of cattle grazing on hard stalk pastures, which contains a higher concentration of cellulose than those in soft leafy diets. Samples from goat contained an average high population of cellulolytic organisms because they could graze on hard stalk pastures and soft leafy diets as opined by Oyeleke and Okusanmi [26].

Table 1: Distribution of Microorganisms isolated from cow and goat rumen

Sampling Parts	Frequency (%) of Isolates in Cow rumen (n=54)	Frequency (%) of Isolates in Goat rumen (n=41)
A	07(13.0)	05(12.2)
B	08(18.8)	06(14.6)
C	05(9.3)	04(9.8)
D	07(13.0)	03(7.3)
E	06(11.1)	05(12.2)
F	04(7.4)	03(7.3)
G	05(9.3)	04(9.8)
H	04(7.4)	04(9.8)
I	03(5.6)	03(7.3)
J	05(9.3)	04(9.8)

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3.2 Diversity of Bacterial Flora Isolated in the Sample Source

This study (table 2) shows the diversity of bacteria isolated from the rumen of cow and goat. They include: *Bacillus subtilis, Bacillus licheniformis, Yersinia enterocolitica, Micrococcus* sp, *Salmonella* sp, *Pseudomonas* sp and *Streptococcus* sp. 52 isolates hydrolyzed cellulose, with cow having the highest number of isolates that are able to hydrolyze cellulose with 29 (55.8%) from cow whereas goat have the least number of isolates 23(44.2%) that are able to hydrolyze cellulose.

Bacterial isolation and screening aim to determine the presence or absence of cellulolytic enzyme activity of isolates obtained from rumen of cow and goat on Nutrient agar, MacConkey agar and carboxymethyl cellulose (CMC) agar medium. CMC is an anionic polymer commonly used in cellulase activity testing [17]. The CMC is a polymer with high molecular weight that cannot be transported into cells of microorganisms [14]. The weight of the CMC molecule varies from 90-250 kDa. This causes the degradation enzyme CMC to be retained on the surface of the cell wall or released onto outside of the cell and makes the secreted cellulase diffuse into the surface of the agar medium [8,32].

In the rumen, there are various types of bacteria, fungi, and protozoa [20]. This study shows the diversity of bacteria isolated from the rumen of cow and goat which is in agreement with the work of Rabah *et al.* [28] who isolated *Bacillus* sp., *Yersinia* sp. and *Salmonella* sp. from rumen of cow, goat, sheep and camel and also in agreement with the work of Oyeleke and Okusanmi [26] who isolated *Bacillus* sp., *Streptococcus* sp., *Micrococcus* sp. and *Pseudomonas* sp. from the rumen of cow, goat and sheep. *Bacillus* species account for 27.9%

of the total isolates as seen in table 2 above. Rabah *et al.* [28] reported the isolation of 58% *Bacillus* species from rumen of goat, cow, sheep and camel. Oyeleke and Okusanmi [26] also reported the isolation of 37.8% of *Bacillus* species from rumen of cow, goat and sheep.

Table 2: Distribution of Cellolytic Bacteria Isolated from Rumen of Cow and Goat

Bacterial Isolates	No (%) Isolates in Cow (n=29)	No (%) Isolates in Goat (n=23)	Total (%) of Isolates (n=52)
Bacillus subtilis	09(31.0)	06(26.0)	015(28.8)
Bacillus licheniformis	07(24.1)	07(30.4)	014(27.0)
Yersinia enterocolitica	03(10.3)	02(8.7)	05(9.6)
Micrococcus sp	02(6.9)	01(4.3)	03(5.8)
Salmonella sp	03(10.3)	03(13.0)	06(11.5)
Pseudomonas sp	01(3.4)	01(4.3)	02(3.8)
Streptococcus sp	04(13.8)	03(13.0)	07(13.5)

The isolates were tested for the ability to hydrolyze cellulose and only 52 isolates were able to hydrolyzed cellulose after growing them on cellulose as seen by zone of clearance around the isolates, which did not agree with the work of reference [19] who isolated microorganisms from the rumen and were implicated to hydrolysis of cellulose and they all hydrolyzed cellulose. Proofs based on zone of clearance in cellulose led to the conclusion that *Bacillus and Pseudomonas* firmly bond cellulase, whereas *Streptococcus* is released from the cell which is in agreement with the findings of Commombattoo [6] who describe the cell morphology of *Bacillus* species as having a thin cell coat and which adhere tightly to plant cell wall and cell morphology of *Streptococcus* species has a thick cell coat and adheres loosely to the plant cells wall. *Streptococcus* species are the predominant cellulolytic micro-organism that are associated with the possession of complex cellulose enzyme systems as reported by Schwarz [31].

The cellulosome (multienzyme complexes) is thought to allow concerted enzyme activity in close proximity to the bacterial cell, thereby enabling optimum synergism between the cellulases presented on the cellulosome. Again, the cellulosome also minimizes the distance over which cellulose hydrolysis products must diffuse, allowing efficient uptake of these carbohydrates by the host cell [26].

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4. CONCLUSION

Based on the results of this study it can be concluded that cellulolytic bacteria have been isolated from the rumen of cow and goat and some potential cellulase-producing bacteria have been identified with *Bacillus* sp. as the most frequent. These enzymes can be harnessed and used in bio-refineries and the production of other valued-added products in the industries such as to function in the degradation of fibrous feed material in other to increase quality of nutrition and digestibility of ingredients at a cheaper price than the use of commercial cellulase enzymes.

Comment [Ma8]: The author is not clearly concluded at which animal the production of cellulose enzyme was high and why and what is the recommendation based on the finding

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