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Screening and Identification of Cellulase- Producing Bacteria Isolated From Rumen of Camel in Sokoto Main Abattoir

Nafi'u, A., [1] ***M. H. Usman*** [1]***, B. Y., Abdullahi*** [2]***, Mustapha G*** [1] ***and H. M. Maiturare*** [1]

[1]Department of Microbiology Sokoto State University, Sokoto Nigeria
[2]Federal college of Forestry Mechanization, Afaka
*Corresponding author: Nafi'u Abdulkadir

Abstract

Cellulose is the most abundant polymers on the earth and was extensively used for many industrial purposes. The aim of the study was screening and isolation of cellulase producing bacteria from rumen of camel in slaughter house of Sokoto. A numbers of rumen content samples were collected from four different camels for analysis. Rumen cellulase producing bacteria were Isolated and characterized in this experiment following serial dilution up to six fold and inoculated into Nutrient agar. Biochemical test such citrate utilization, indole, catalase, urease, methyl red, vogues proskauer, motility, and sugar fermentation, starch hydrolysis, hydrogen sulphide production (H_2S) from triple sugar iron (TSI) agar and sugar fermentation were adopt in this study. Screening of the isolates was performed in an enriched media containing Carboxymethyl cellulose (CMC) flooded with Congo red and washed with NaCl. The result revealed the colonial characterization such as shape, color, size, elevation margin, opacity, and gram reaction were determined for each of the isolates. A total of seven isolates were identified, six were identified as gram positive while one as gram negative. Bergy's manual of determinative bacteriology confirmed the isolates to be genus of *Bacillus spp*. The mean total count of bacteria are 2.5 x 10^6 cfu/g, 2.8 x 10^6 cfu/g, 3.5 x 10^6 cfu/g, 3.6 x 10^6 cfu/g, 2.7 x 10^6 cfu/g, 3.5 x 10^6 cfu/g and 3.0 x 10^6 cfu/g. Five out of seven show CMCase activity after flooding with Congo red and are considered as RC3, RC4, RC5, RC6, and RC7 while two isolates RC1 and RC2 lack ability to produce cellulase RC7 show highest ability to produce cellulase with highest diameter of 6.0 mm. It has concluded that rumen content of camel is good source of carbon sole and cellulase producing bacteria.

Key words: Rumen content, Camel, Carboxymethyl cellulose Bacillus spp

Introduction

Cellulase is an inducible enzyme used for the bioconversion of cellulosic and lignocellulosic residues (Shankar and Isaiarasu, 2011) This enzyme act sequentially in the synergistic system and subsequently convert cellulose into an utilizable energy source and glucose, hence, cellulases provide a key role in biomass utilization (Shankar and Isaiarasu, 2011). Rumen is a special organ in the ruminant animal such as camel within which the digestion of cellulose and other plant polysaccharides occurs through the activity of specific microbial populations. A large numbers of microorganisms mainly bacteria and fungi in rumen break down plant material (polysaccharides, partially degrades lignin, hemicellulose and pectin) ingested by

the host animal and provide the animal with protein, vitamins and assimilable carbon and energy yielding substrates (Neeru and Manjula, 2007). Bacteria and fungi have been found to produce and secrete these enzymes freely in solution; however, some microorganisms have also been found to produce cell-bound enzymes and multi-protein complexes expressing cellulases and hemicellulases called cellulosomes (Maki *et al*., 2011)

Shankar and Isaiarasu (2011) reported that the cellulolytic activity shown by the isolated bacterial species was reported to depend on the source of occurrence in various natural environments enables them to be responsible for the degradation of cellulose that occurs in various amount of bio-waste. Cellulases can be produced by groups of microorganisms such fungi, bacteria or actinomycetes during their growth on cellulosic materials (Ibrahim and El-diwany, 2007), but the most common producer is fungi (Lee and koo 2001; Ariffin *et al.,* 2006).

However, cellulases produced by bacteria are often more effective catalysts. They may also be less inhibited by the presence of material that has already been hydrolyzed. The greatest potential importance is the ease with which bacteria can be genetically engineered. This is needed especially in order to enhance cellulase production (Ariffin *et al.,* 2006). Cellulose is the most abundant polymer found on the earth. Cellulases are a group of fibrolytic enzymes which cooperatively hydrolyze plant cell wall fibers into glucose, cellobiose or oligosaccharides (Murad and Azzaz, 2010; Chinedu *et al*., 2010).

Cellulases are among the industrially important hydrolytic enzymes and are of great significance in present day biotechnology (Haight, 2005; Azzaz, 2009). These enzymes are used in textile, detergent, pulp paper industries and in animal feed as well as in production of biofuel. Over long period of time, the microbiology of rumen of cattle and sheep has been widely studied and reviewed by many researchers but studies on rumen microbes of camels are limited. In view of the above, the present study aimed at screening and isolation of cellulase producing bacteria from rumen content of camel.

Materials and methods

Sample collection and processing

Samples were collected from rumen of camel in the morning after slaughtering at the main abattoir in Sokoto metropolis, in a clean sterile polythene bag. All samples were labeled and immediately transported to the laboratory. A number of samples of camel rumen content were collected from four different camels for screening of cellulolytic activity. One gram (1g) of solid rumen sample was weighed and dispensed into 9ml of sterile distilled water and was heat shocked by heating in water bath at 80^{0} C for 15minutes.

Isolation of cellulase producing bacteria

One gram (1g) of rumen sample was weight and transferred to 10ml of sterile distilled water and was serially diluted up to seven times to (10^{-5}). An aliquot of 0.1ml from each test tube was transferred using

sterile pipette into sterile molten nutrient agar plate, spread using sterile bent glass rod and incubated at 37^0 C for 24h.

Maintenance of colonies

The colonies that developed after 24h of incubation were continually sub cultured unto nutrient agar plate until a pure culture was obtained. The pure cultures were then sub cultured on nutrient agar slant until when required.

Identification and characterization of Colonies

Bacterial isolates were identified and characterized after staining using Gram reaction. Other tests performed were citrate utilization, indole, catalase, urease, methyl red, vogues proskauer, motility, and sugar fermentation, starch hydrolysis, hydrogen sulphide production (H_2S) from triple sugar iron (TSI) agar and sugar fermentation. The tests were carried according to the methods described by Cheesbrough (2006). The isolates were confirmed using Bergey's Manual of deterministic bacteriology 2^{nd} edition.

Screening of cellulase producing bacteria

Carboxymethylcellulose (CMC) agar plates were prepared by enriching nutrient agar with 1%carboxymethylcellulose using method of (Ariffin *et al.,* 2006). The isolate was stabbed on the solidified agar and allowed to incubate for 48h to express cellulose depolymerization through cellulase producing bacteria into its surrounding medium. To visualize the hydrolysis zone of inhibition, the plates were flooded with an aqueous solution of 0.1% Congo red for 15minutes. These were washed with 1m of sodium chloride. To indicate the cellulase activity of the organism, diameter of cleared zone around the colonies on carboxymethylcellulose agar were measured.

Statistical data Analysis

A descriptive statistics through simple tables and charts were used to present data obtained during the course of study using statistical package of social science SPSS version 20.

Results and Discussion

From the results of the research conducted, seven bacterial strains were isolated from parent plates. Six out of seven isolates were gram positive and one was gram negative rods. The results of the colonial characterization carried out for isolation of bacteria from sample were presented in Table 1. The mean total count of the bacteria in each of rumen samples collected was presented in table 2. The strains were isolated and identified. This indicates that gram positive bacteria are more predominant than gram negative in rumen of camels in the sampled area. Neeru and Manjula (2007) state that Rumen of ruminant animal such as

camel composed of microbial population that are able to carry out digestion of cellulose and other plant polysaccharides to occurs through the production of enzyme cellulases.

Table 1: Colony characterization (Macroscopically and microscopically)

S/No	Morphological characterization	RC 1	RC 2	RC 3	RC 4	RC 5	RC 6	RC 7
1	Shape	Large rod	Small rod	Medium rod	Large rod	Large rod	Large rod	Large rod
2	Size	0.3-0.4	0.1-0.2	0.2-0.3	0.3-0.4	0.3-0.4	0.3-0.4	0.3-0.4
3	Color	Milky	Milkish	Milkish	Milkish	Milkish	Red	Milkish
4	Elevation	Raised	Lowered	lifted	lowered	lifted	Lifted	lifted
5	Margin	Smooth circular	Smooth circular	Rough slender	Smooth slender	Smooth circular	Smooth circular	Smooth round
6	Gram's rxn	+ve	+ve	+ve	+ve	+ve	+ve	-ve
7	Opacity	Opaque	Cloudy	Cloudy	Opaque	Opaque	Opaque	Opaque

Key: + (Positive), - (Negative)

Table 2: Bacterial count of rumen sample from camel

Rumen sample	Bacterial count in (x 10^{-6}CFU/g)
RC1	2.5
RC2	2.8
RC3	3.5
RC4	3.6
RC5	2.7
RC6	3.5
RC7	3.0

The result of biochemical characterization carried out on isolate of camel rumen was presented in Table 3. The biochemical profile through Bergy's Manual of Determinative Bacteriology (2^{nd} edition) confirmed the isolates i.e gram positive as RC1= *Bacillus firmus,* RC2= *Bacillus megaterium,* RC3= *Bacillus lentus,* RC4=*Bacillus pumilus* RC5= *Bacillus alvei,* RC6= *Bacillus circulans* and gram negative as RC7= *Bacillus macerans.*

The results of screening of cellulose producing bacteria using carboxymethylcellulose (CMC) media after flooding with Congo red and sodium chloride on the isolates was presented in Table 4. The isolates that have CMCase activity were presented in figure 1. The highest CMCase activity on the culture surface of

6mm was recorded for RC7 and the least activity was 2mm for RC3 and RC5 respectively. This indicates that RC7 can degrade cellulolytic materials from plants and serve as CMC degrading enzymes producer. This might happen due to available carbon sources in the ruminant animal. While RC1 and RC2 showed no clearing zones. The result was supported with the findings of Fouad *et al.,* (2014), who found out that Both *Bacillus spp* and *Acrodictys fimicola* were mainly isolated organisms and given positive test on Carboxyl Methyl Cellulose (CMC) media due to producing cellulase enzyme

Screening of cellulase production by the isolated strains showed no clearing zone after staining with Congo red, around *Bacillus firmus* and *Bacillus megatarium*. This might indicate that they are not cellulase producers (due to the absence of CMCase activity). As for *Bacillus lentus, Bacillus pumilus, Bacillus alvei, Bacillus circulans* and Bacillus macerans that showed a clearing zone when placed on carboxymethylcellulose (CMC) media in all Petri dishes prepared indicates the ability of producing cellulase enzymes. The findings of this study is comparable to the work of Arun *et al.,* (2007) who isolate *Bacillus circulans* and from Fish gut for the production of cellulase. The same organisms were also identified by Ariffin *et al.,* (2006), who used local *Bacillus pumilus* to produce cellulase. This study is in line to that of Shanker (2011).screened as cellulolytic bacteria from mid-gut of the popular composting *Earth worm*.

Kamra (2005) found out that the efficiency of ruminants to utilize such a wide variety of feeds is due to highly diversified rumen microbial ecosystem consisting of bacteria (10^{10}–10^{11} cells/ml, representing more than 50 genera), ciliate protozoa (10^{4}–10^{6}/ml, from 25 genera), anaerobic fungi (10^{3}–10^{5} zoospores/ml, representing five genera) and bacteriophages (10^{8}–10^{9}/ml). These numbers might even be larger as majority of them are non-culturable

Similarly, microbial cellulases find tremendous application in various industries and constitute a major group of the industrial enzymes, and there is resurgence in utilization of biomass for fuel production employing cellulose recently and hence forth in obtaining better yield. Improving the economics of such processes will involve cost reduction in cellulases production which may achieve better bioprocesses and genetic enzymes. However, these enzymes can be further engineered using available knowledge of enzymes structure and function through rational design and or they can improve using random mutagenesis techniques (Ranjeev *et al.,* 2007).

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Table 3: Result of biochemical characterization of isolate from rumen of camel

Isolate	Catalase	Citrate	Glucose	Lactose	Sucrose	Indole	Urease	MR	VP	H_2S	Motility	Starch	Gas
1	+	-	+	-	+	-	-	+	-	-	+	+	-
2	+	-	+	+	+	+	+	+		+	+	-	-
3	+	-	+	-	+	-	+	+	-	-	+	+	-
4	+	-	+	-	+	+	-	-	+	-	+	-	-
5	+	-	+	+	+	+	+	+	-	+	+	+	-
6	+	-	+	-	+	-	-	-	+	+	+	+	-
7	+	-	+	-	-	-	-	-	+	-	+	+	-

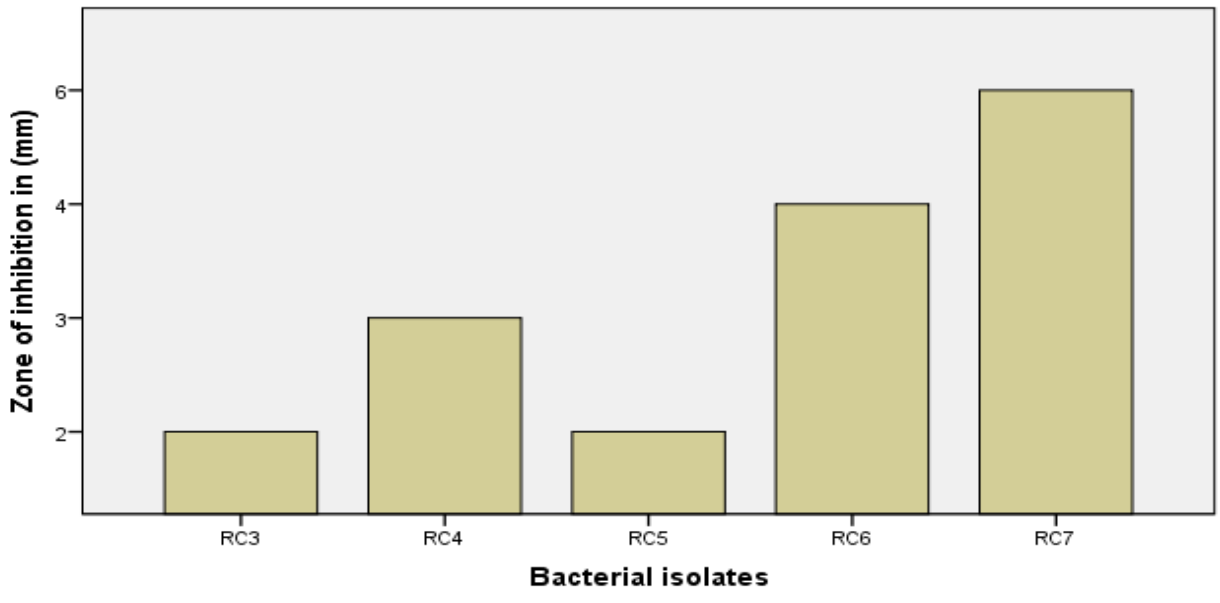
Key: + (Positive), - (Negative)

Table 3: Result for screening of cellulase activity

S/N	Bacterial isolates	Zone/cellulase activity(mm)
1	RC1	Nil
2	RC2	Nil
3	RC3	2
4	RC4	3
5	RC5	2
6	RC6	4
7	RC7	6

Key: RC= rumen content of camel

Figure 1: Cellulase depolymerization clearance zone



Conclusion

Based on the result of the research the study shows that the samples of rumen material of camels are populated by a large number of *Bacillus spp*, gram positive organisms are superior and have potential capability to be used in production of cellulase. Therefore, it can be concluded that camel rumen can be good sources for the isolation of cellulase producing bacteria.

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