

Short Communication

Isolation and characterization of cellulose hydrolysing microorganism from the rumen of ruminants

Oyeleke, S. B.* and Okusanmi, T. A.

Department of Microbiology, Federal University of Technology, Minna, Nigeria.

Accepted 23 August, 2016

Microorganisms that were isolated from the rumen of three different ruminants (cow, sheep, and goat) include *Pseudomonas aeruginosa* (9.0%), *Bacillus* (37.8%), *Micrococcus* (8.1%) and *Streptococcus* (44.3%) species for bacteria, while the fungi isolated were species of *Fusarium* (21.2%), *Penicillium* (23.4%), *Aspergillus* (14.7%) and *Mucor* (40.6%). These organisms were later examined for their ability to hydrolyze cellulose. The results revealed that *P. aeruginosa*, *Streptococcus*, *Bacillus*, *Penicillin*, *Aspergillus*, *Mucor* and *Fusarium* species were able to hydrolyze cellulose. The study suggests that the rumen of ruminants harbors various microorganisms that are active in cellulose breakdown.

Key words: Ruminants, rumen, cellulose, microorganisms.

INTRODUCTION

A ruminant is any animal that digests its food in two steps, first by eating the raw material and regurgitating a semi-digested form known as cud, then eating (chewing) the cud, a process called ruminating (Madigan et al., 2000). Ruminants have a fore-stomach with four chambers. These are the rumen, reticulum, omasum, and abomasum. The rumen is a special digestive vessel, within which the digestion of cellulose and other plant polysaccharides occurs, through microbial activity. In the first two chambers, the rumen and the reticulum, the food is mixed with saliva and separates into layers of solid and liquid material. Solids clump together to form the cud (or bolus). The cud is then regurgitated, chewed slowly to completely mix it with saliva and to break down the particle size. Fiber, especially cellulose and hemicellulose, is primarily broken down into the three volatile fatty acids, acetic acid, propionic acid and butyric acid in these chambers by microbes (bacteria, protozoa, and fungi). Even though the rumen and reticulum have different names, they represent the same functional space as digesta can move back and forth between them. Microbes produced in the reticulo-rumen are also digested in the small intestine. Fermentation continues in the large intestine in the same way as in the reticulo-rumen (Schwarz, 2001).

Almost all the glucose produced by the breaking down of cellulose and hemicellulose is used by microbes in the rumen, and as such ruminants usually absorb little glucose from the small intestine. Rather, ruminants' requirement for glucose (for brain function and lactation if appropriate) is made by the liver from propionate, one of the volatile fatty acids made in the rumen (Hays, 2004). The bacteria *Fibrobacter succinogenes*, *Bacteroides succinogenes*, *Ruminococcus albus*, *Ruminococcus flavafacens*, *Clostridium ochtheadii*, *Bacillus Licheniformis*, and *Streptococcus Anaerobius* are generally regarded as the predominate cellulolytic microbes in the rumen (Findlay, 1998).

The objectives of this study are to isolate and characterize microorganisms from the rumen and to test the ability of these organisms to hydrolyze cellulose.

MATERIALS AND METHODS

Collection and analysis of samples

Samples of faeces were collected from cut rumen of ruminant slaughtered at the abattoir in Minna, Niger State. The collection was carried out by taking 5 representative samples each from three different ruminants (goats, sheep and cow). The rumen of the ruminants was swabbed using a swab stick. The samples were then cultured onto nutrient agar, MacConkey agar, Sabouraud dextrose agar, and incubated aerobically and anaerobically. The nutrient and MacConkey agar plates were incubated at 37°C for 24 h while the SDA plates were incubated at room temperature (28 ± 2°C) for 72 h

*Corresponding author. E-mail: droyeleke@yahoo.com.

Table 1. Microorganisms isolated from the rumen of three different ruminants.

Microorganism	Goat	Cow	Sheep
Bacteria			
<i>Bacillus</i> sp.	5 (38.5)	7 (35.0)	6 (40.0)
<i>Streptococcus</i> sp.	6 (46.2)	8 (40.0)	7 (46.7)
<i>Micrococcus</i> sp.	1 (7.7)	2 (10.0)	1 (6.7)
<i>Pseudomonas</i> sp.	1 (7.7)	3 (15.0)	1 (6.7)
Fungi			
<i>Fusarium</i> sp.	4 (22.2)	6 (21.4)	4 (20.0)
<i>Penicillium</i> sp.	6 (33.3)	2 (7.1)	6 (30.0)
<i>Aspergillus flavus</i>	1 (5.6)	8 (28.6)	2 (10.0)
<i>Mucor</i> sp.	7 (38.9)	12 (42.9)	8 (40.0)

Number in the parenthesis represents the percentage of occurrence.

Colonies that developed on the plates were subculture repeatedly to obtain pure culture, which were maintained on agar slants for further characterization and identification. The pure isolates were then subcultured onto cellulose agar plates and were incubated aerobically and anaerobically for seven days.

Characterization and identification of bacterial and fungal isolates

The characterization and identification of the bacterial isolates were based on cell morphology, biochemical test and their ability to hydrolyze cellulose. The characterization and identification of the fungal isolates were identified by microscope and macroscopic techniques as described by Cheesbough (2005).

RESULTS AND DISCUSSION

The study revealed the presence of the following cellulose hydrolyzing organisms, *Pseudomonas aeruginosa*, *Bacillus*, *Micrococcus* and *Streptococcus* species for bacteria (Table 1). This agrees with Lynd et al. (2002) who isolated these organisms from the rumen and were implicated in the hydrolysis of cellulose. Evidences based on zones of clearing in cellulose agar led to the conclusion that *Bacillus*, and *Pseudomonas*, possesses firmly bound cellulase, whereas that of *Streptococcus* is released from the cell. This is in agreement with Colombatto et al. (2000) who describe the cell morphology of *Streptococcus* species that has a thick cell coat and adheres loosely to the plant cells wall, while *Pseudomonas* and *Bacillus* species have a thin cell coat and adheres tightly to the plant cell wall.

Samples from cow had the highest number of organism with cellulolytic capabilities; this 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 sheep and goat contained an average high population of cellulolytic organisms because they could graze on hard stalk pastures and soft leafy

diets.

Streptococcus species are the predominant cellulolytic micro-organism that are associated with the possession of complex cellulose enzyme systems as reported by Schwarz (2001). The cellulosome is thought to allow concerted enzyme activity in close proximity to the bacterial cell, enabling optimum synergism between the cellulases presented on the cellulosome. Concomitantly, the cellulosome also minimizes the distance over which cellulose hydrolysis products must diffuse, allowing efficient uptake of these oligosaccharides by the host cell.

The fungi species isolated (Table 1) are related to the previous study of Mosoni et al. (1997). *Mucor* sp. are the predominant cellulolytic fungi and has the ability to proliferate faster. Cellulolytic filamentous fungi have the ability to penetrate cellulosic substrates through hyphal extensions, thus often presenting their cellulose systems in confined cavities within cellulosic particles. Carlie and Watkinson (1997) in his study observed that *Aspergillus*, *Penicillium* and *Fusarium* species have cellulolytic enzymes and wood degrading capability. The rumen represents a dynamic habitat, where the hydrolysis of cellulose takes place. Miron et al. (2001) suggested that the activities of rumen micro-organisms enable ruminants to convert pastures and agro industrial by-products into products for human use. These organisms can be explored for bio-fuel production.

REFERENCES

- Carlie MJ, Watkinson SC (1997). The fungi. Academic Press, New York, , pp. 269-275.
- Colombatto D, Mould FL, Bhat MK, Owen E (2000). The effect of fibrolytic enzyme application on the rate and extent of Alfalfa stems fermentation, assessed *in vitro*. World J. Microbiol. 83: 115-122.
- Findlay ALR (1998). Fermentation in the ruminant stomach, University of Cambridge, Cambridge, pp 12-15.
- Hays SM (2004). Rumen Bacteria rob cattle of nutrients. Department of Microbiology. Churchill Living Stone, London, pp. 51-57.
- Lynd R, Weimer J, William H, Isak S (2002). Microbial cellulose. Utilization: Fundamentals of Biotechnology. Microbiol. Mol. Biol. Rev., 66: 506-577.
- Madigan MT, Martinko JM, Parker J (2000). Biology of Microorganisms. Pentice- Hall International, London, pp. 681-685.
- Mosoni P, Fonty G, Govet P (1997). Competition between ruminal cellulolytic bacteria for adhesion to cellulose, J. Appl. Biotechnol. 66: 15-26.
- Miron J, Ben-Ghedalia D, Morrison M (2001). Adhesion mechanisms of rumen cellulolytic bacteria. J. Dairy Sci., 84: 1294-1309.
- Schwarz WH (2001). The cellulosome and cellulose degradation by anaerobic bacteria. Appl. Microbiol. Biotechnol. 56: 634-649.