Research article Available online www.ijsrr.org ISSN: 2279–0543

International Journal of Scientific Research and Reviews

Isolation and Morphological Identification of Fiber Degrading Bacteria from the Bovine Rumen

Satyanaga lakshmi Karri [1, 2], Sunil Kumar Sirohi[1], Talla Sridhar Goud[1, 2], Suneel Kumar Onteru[1] and Vijay A.K.B. Gundi[2]*

[1]ICAR-National Dairy Research Institute Karnal 132001, Haryana. INDIA
[2]Department of Biotechnology Vikrama Simhapuri University Nellore 524320, Andhra Pradesh, INDIA

ABSTRACT

In ruminants, the food is subjected to microbial fermentation in the rumen before it passes into intestine. This process is taking place by the microbes, which degrades carbohydrates such as cellulose and hemi cellulose into simple sugars. The ruminant health and productivity depends on feed digestion capacity and synthesis of volatile fatty acids and proteins in rumen by microbial activity. To understand metabolic function of microbes in rumen, isolation, cultivation and characterization are very important parameters. In this study, we aimed to isolate fibrolytic bacteria and their morphological characterization from Karan Fries cattle. We isolated 62 isolates from rumen liquor sample, among these 47 strains showed fiber degrading activity.

KEYWORDS: Fiber degrading bacteria; conventional culture methods; rumen, morphology.

***Corresponding author**

Vijay A.K.B. Gundi

Department of Biotechnology, Vikrama Simhapuri University

Nellore-524320, Andhra Pradesh, India

Email: gundi.vijay@gmail.com, sameer.satya@gmail.com

INTRODUCTION

Ruminants are herbivores, fed on plant derived biomass; cellulose is most abundant component of it (Heredia et al., 1995)[1]. Ruminants digestive system allows digesting and absorbing of these plant derived materials, converting the energy stored in plant biomass to digestible food products such as nutrients and volatile fatty acids (Flint et al., 2008)[2]. Microbial community present in rumen such as bacteria, fungi (Theodorou et al., 2007)[3] and protozoa (Coleman, 1992)[4] describes as most effective and elegant cellulose-digesting mechanism (Weimer et al., 2009)[5], among these microbial population bacteria is most predominant (Brulc et al., 2009)[6]. With breakthrough of hungate roll tube culture method (Hungate, 1969)[7] for strict anaerobic micro organisms, diverse microbes were cultured and identified. The conventional culture based methods have provided predominant diversity of rumen micro biota, therefore, more than 200 species of bacteria, and at least 100 species of protozoa and fungi inhabiting the rumen have been identified (Chaucheyras-Durand & Ossa, 2014)[8]. Most of the knowledge (diversity, metabolic function) we have obtained of rumen bacteria from traditional methods such as isolation and cultivation of pure strains (Bryant, 1959)[9]. Fiber degradation, significantly mediated by important micro organisms such as *Fibrobacter succinogenes* and cellulolytic fermitutes *Ruminococcus flavefaciens*, and *Ruminococcus albus* (Dassa et al., 2014)[10]; also found to be active cellulolytic bacteria of all mesophillic organisms from any habitats. Many Bacteroidetes were also reported as amylolytic bacteria, and genus *Prevotella* is also considered to be involved in digestion and utilization of starch, xylan, and pectin (Jami & Mizrahi, 2012)[11].

The Karan Fries cattle is a cross bred of Holstein (Friesian) and Tharparkar, an Indian milch breed. It is good milk yielder and is highly valued for their ability to withstand to variable environmental conditions, higher resistance to diseases and capacity to thrive on low feed resources.

In this study, we isolated fiber degrading bacteria by using conventional culture methods under strict anaerobic conditions and morphological characterization of these bacteria was done.

MATERIAL AND METHODS

Ethical Permission

All the animals were closely monitored and were provided similar managemental inputs during experimental period. The animals were treated following the compliance of the institute's norms for ethical treatment. The experiment was approved by the Institutional Animal Ethics Committee (IAEC) constituted as per the article number 13 of the CPCSEA rules laid down by

Government of India. Norms regarding the ethical treatment of animals during the whole operation were strictly followed.

Animal feed and sampling process

The rumen liquor samples were collected from four fistulated crossbreed adult Karan Fries cattle with an average body weight (BW) of 450±20 kg in cattle yard, Indian Council of Agricultural Research–National Dairy Research Institute (ICAR-NDRI), Karnal, Haryana, India, located at latitude 29º N and longitude 76° E. For conducting this experiment, four animals were fed 21 days with wheat-straw-based standard diet (concentrate/roughage ration, 40:60). Approximately, 500ml of rumen liquor was collected into nitrogen passed thermos flask and carefully carried to the laboratory. The collected rumen fluid was filtered through a four layer cheese cloth to separate solid and liquid fractions (Chaudhary et al., 2012)[12].

Isolation of anaerobic Fiber Degrading Bacteria (FDB)

For isolation of Fiber Degrading Bacteria (FDB) from fistulated cattle, the rumen liquor was collected in thermos flask, strained through four layers of muslin cloth and centrifuged at 2000 x g for 10 min to remove protozoa and fungi. Plating on anaerobic culture media was carried out with 10^{-6} and 10^{-12} dilutions of rumen liquor in anaerobic diluents, incubated at 37°C for 24 h and different colonies selected based on their morphological characters. The anaerobic technique were used to culture the rumen bacteria (Hungate, 1969), with modifications to screen FDB (Das, 2012)[13]. In brief, to screen fibrolytic activity in the media, cellulose was used as a carbon source instead of glucose. Selectively diverse microbial strains were screened on basis of colony morphology and Gram's staining procedure (Kaplan, 1933)[14].

RESULTS AND DISCUSSION

From the rumen liquor sample, a total of 62 bacterial isolates were recovered from culture plates after 24 hrs of incubation under strict anaerobic conditions. Based on the morphological characters such as gram staining and colony characterization colony size (small/large), shape (oval/ round/ irregular/ irregular edge) colour (whitish/ yellow/ transparent/ mucoid/ creamy/ dark centred) 47 different morphological strains were isolated. 75% of the bacteria isolated from rumen are majorly fiber degrading bacteria are associated with feed stuff or free flouting in rumen. The positive correlation between fiber and non fiber degrading bacteria are important in maintaining and promoting fibrolytic activity Koike and Kobayashi, 2009)[15] such as *Ruminobacter anylophilus, Selenomonas ruminantium* and *Treponema bryanti* (Scheifinger and Wolin, 1973)[16]. The Co-culture of *R. flavefaciens* and *S. ruminantium* increased fiber digestibility and propionate production as

compared to mono-culture of *R. flavefaciens*. Hence, fibrolytic and non-fibrolytic bacteria should be monitor to estimate overall contribution of bacteria to ruminal fiber digestion. Among 47 isolates, 14 were gram negative, 32 isolates were rod shaped, 10 were cocci, and five were cocci with chain. The enzymatic activity and fermentation profile of these isolates will be studied.

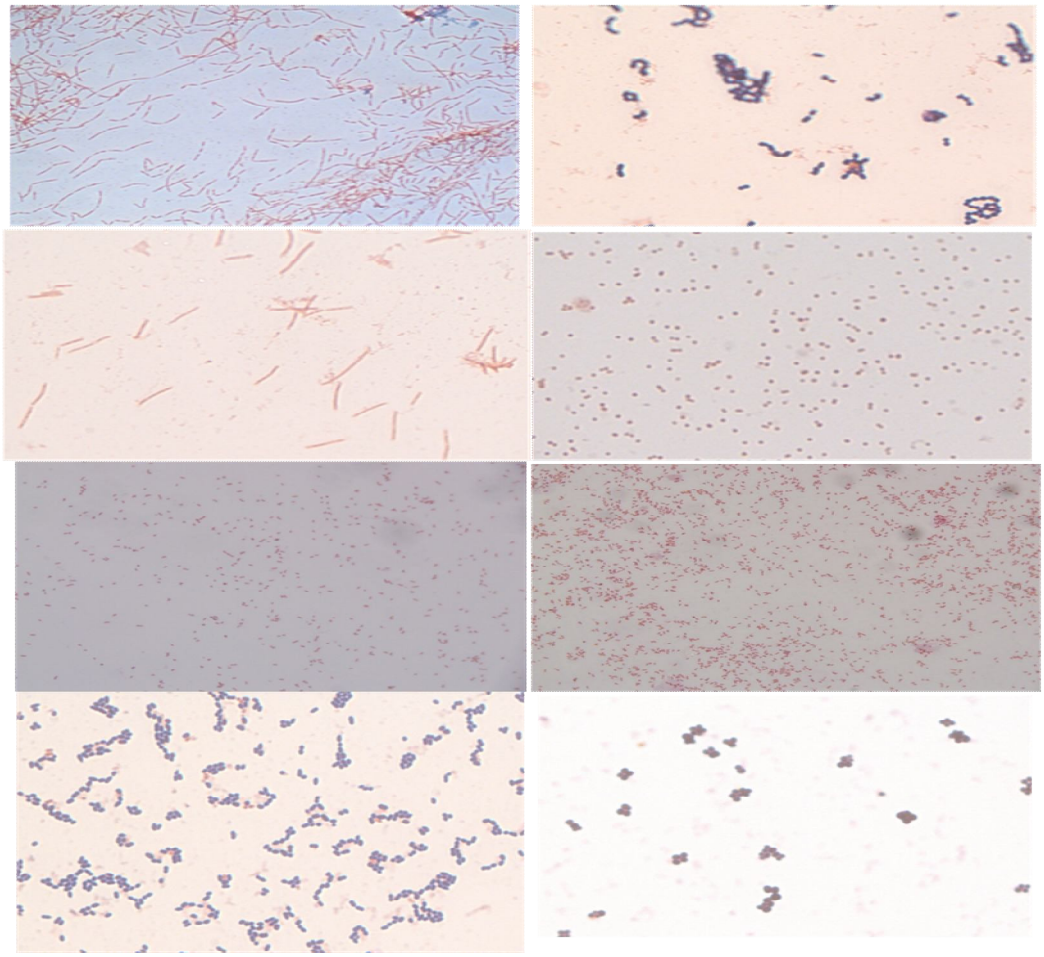

Figure no1: Gram staining of the isolated bacterial species from rumen liquor sample.

Table no 1: The morphological and Gram staining of different isolates from rumen liquor sample.

Screening of bacterial colonies based on their morphology and staining			
S.No	**Colonial Morphology**	**Gram staining**	**Cell morphology**
1.	Small/oval/whitish/smooth	-ve	Rod
2.	Large/round/smooth	+ve	Cocci
3.	Dark/whitish/smooth	+ve	Cocci ,chain
4.	Large/white/dark centered	+ve	Cocci
5.	Small/round/white	-ve	Cocci
6.	White/round	+ve	Cocci
7.	Small/mucoid	-ve	Rod
8.	Small/white/round	-ve	Rod
9.	Circular/small/yellow	+ve	Rod
10.	Small/yellowish	-ve	Rod
11.	Small/mucoid/yellowish	+ve	Rod
12.	Very Small/transparent/round	+ve	Rod
13.	Small/white/circular	+ve	Cocci chain
14.	Small/irregular	+ve	Cocci chain
15.	Very small/oval/white	+ve	Cocci
16.	Large/white	+ve	Cocci
17.	Medium/white/circular	+ve	Cocci
18.	Small/white/mucoid	+ve	Cocci
19.	Small/mucoid/round	-ve	Rod
20.	Small/mucoid/irregular	-ve	Rod
21.	Very small/irregular/round	-ve	Rod
22.	Small/mucoid/round	+ve	Rod
23.	Small/mucoid/irregular	+ve	Rod

24.	Very/small/irregular	-ve	Rod
25.	Small/yellowish/mucoid	+ve	Rod
26.	Small/yellowish	+ve	Rod
27.	Large /irregular edge	+ve	Rod
28.	Small/irregular edge/white	+ve	Cocci chain
29.	Small/white/creamy	-ve	Rod
30.	Small/mucoid/round	-ve	Rod
31.	Circular/white/creamy	+ve	Rod
32.	Large/ creamy/mucoid	+ve	Rod
33.	Oval/yellowish/ irregular	+ve	Rod
34.	White/ irregular edge	+ve	Rod
35.	Small/ yellowish	+ve	Rod
36.	Yellowish/ irregular	+ve	Cocci chain
37.	Small/round	+ve	Rod
38.	Small/ yellowish/ round	-ve	Rod
39.	Oval/white/creamy	+ve	Rod
40.	White/small /irregular edge	+ve	Rod
41.	yellowish/ irregular	+ve	Rod
42.	Small/white/creamy	+ve	Rod
43.	Oval/yellowish/ irregular	-ve	Cocci
44.	Circular/white/irregular	+ve	Cocci
45.	Small/white/creamy	+ve	Rod
46.	Large/white/dark centered	+ve	Rod
47.	white/dark centered	-ve	Rod

CONCLUSION:

A total of 62 strains were isolated. Based on the colony morphology, size, shape and colour of colony, gram staining 47 isolates were conformed for fiber degradation. Further, these isolates will be screened for fiber degrading efficiency and identification.

REFERECES

1. Heredia A, Jimenez A & Guillen R Composition of plant cell walls. Zeitschrift fur Lebensmittel-Untersuchung und –Forschung 1995; 200: 24-31.
2. Flint HJ, Bayer EA, Rincon MT, Lamed R & White BA Polysaccharide utilization by gut bacteria: potential for new insights from genomic analysis. Nat Rev Microbiol 2008; 6: 121-131.
3. Theodorou MK, Mennim G, Davies DR, Zhu W-Y, Trinci APJ & Brookman JL Anaerobic fungi in the digestive tract of mammalian herbivores and their potential for exploitation. Proceedings of the Nutrition Society 2007; 55: 913-926.
4. Coleman GS The rate of uptake and metabolism of starch grains and cellulose particles byEntodiniumspecies,Eudiplodinium maggii, some other entodiniomorphid protozoa and natural protozoal populations taken from the ovine rumen. Journal of Applied Bacteriology 1992; 73: 507-513.
5. Weimer PJ, Russell JB & Muck RE Lessons from the cow: what the ruminant animal can teach us about consolidated bioprocessing of cellulosic biomass. Bioresour Technol 100: 2009; 5323-5331.
6. Brulc JM, Antonopoulos DA, Miller MEB, et al. (2009) Gene-centric metagenomics of the fiber-adherent bovine rumen microbiome reveals forage specific glycoside hydrolases. Proc Natl Acad Sci USA 106.
7. Hungate RE Chapter IV A Roll Tube Method for Cultivation of Strict Anaerobes. Methods in Microbiology, Vol. Volume 3, Part B (Norris JR & Ribbons DW, eds.), p.^pp. 1969; 117-132. Academic Press.
8. Chaucheyras-Durand F & Ossa F REVIEW: The rumen microbiome: Composition, abundance, diversity, and new investigative tools. The Professional Animal Scientist 2014; 30: 1-12.
9. Marvin P. Bryant bacterial species of the rumen. Bacteriol. Rev. September 1959; 23(3): 125-153.
10. Dassa B, Borovok I, Ruimy-Israeli V, et al. Rumen cellulosomics: divergent fiber-degrading strategies revealed by comparative genome-wide analysis of six ruminococcal strains. PLoS One2014; 9: e99221.
11. Jami E & Mizrahi I Composition and Similarity of Bovine Rumen Microbiota across Individual Animals. PLOS ONE 2012; 7: e33306.

12. Chaudhary PP, Sirohi SK and Saxena J Diversity analysis of methanogens in rumen of Bubalus bubalis by 16S riboprinting and sequence analysis. Gene 2012; 493 (1):13-7
13. Das KC & Qin W (2012) Isolation and characterization of superior rumen bacteria of cattle (<i>Bos taurus</i>) and potential application in animal feedstuff. Open Journal of Animal Sciences 2012; 02.(4): 6.
14. Kaplan ML & Kaplan LThe Gram Stain and Differential Staining. Journal of Bacteriology 1933; 25: 309-321.
15. Koike, S. and Y. Kobayashi.. Fibrolytic Rumen Bacteria: Their Ecology and Functions. Asian-Australas J Anim Sci 2009; 22(1):131-138.
16. Scheifinger, C. C. and M. J. Wolin.. Propionate formation from cellulose and soluble sugars by combined cultures of Bacteroides *succinogenes* and *Selenomonas ruminantium*. Applied microbiology 1973; 26(5):789-795.