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Isolation, Characterization of *Lachnospira pectinoschiza* from Goat Rumen and Effect of Addition on *in vitro* Fermentation Parameters

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Abstract

Isolation of pectinophile bacteria Lachnospira pectinoschiza from goat rumen was carried out. Their molecular characterization was done after amplification of 16s rDNA on the basis of gene sequence using Ez Bio Cloud database. Isolated culture was evaluated in In vitro gas production test for their effect on gas production, methane production, in vitro true digestibility and volatile acid fraction on high (100% gram straw) and medium (50% Gram straw and 50% concentrate mixture) roughage ration. There was no significant effect of Lachnospira pectinoschiza culture on the in vitro gas production, methane production as well as in vitro true digestibility. Total volatile fatty acids and their fractions were also similar among all the groups. The effect was similar on both type of ration. However lower methane production and higher digestibility was reported with medium roughage ration. The present study concluded that Lachnospira pectinoschiza isolated from rumen of goat has no significant effect of on in vitro rumen fermentation metabolites on high and medium roughage ration.

Keywords: Goat, *In vitro* Methane, *Lachnospira pectinoschiza*, Rumen Bacteria, Volatile Fatty Acid.

Introduction

Rumen of goats is consortia of a number of microbe types that is bacteria, protozoa, fungi, Archea and virus. These ruminal microbes synergistically act on fibrous feedstuffs and convert to volatile fatty acids (VFAs) (Wang and McAllister, 2002; Kim *et al*., 2011). Rumen bacterial species varies depending upon feed resources and diet pattern. Isolation of novel rumen bacteria having some specific property along with their role in feed digestibility is in interest of rumen microbiologist and animal nutritionist. Pectin is a complex polysaccharide consisting mainly of methoxy esterified α, d-1, 4-galacturonic acid units. Pectin is the main component of all plants and makes up about two-thirds of the dry mass of prime cell walls of plants. It offers structural integrity, strength, and flexibility to the cell wall and acts as a barrier to the external environment. Pectin is a significant source of dietary fiber. Pectinophile bacteria *Lachnospira pectinoschiza* utilize pectin and only a few related compounds. Obligate anaerobic pectinophiles have been isolated from the intestinal tracts and gingivae of humans and from the rumen of cattle. Cornick *et al*. (1994) isolated three strains of pectinophilic bacteria from colonic contents of pigs. This isolated bacterium was evaluated for their fibre degrading enzyme. The effect of addition of this bacterium on fermentation pattern and digestibility was studied in *in vitro* gas production test.

Materials and Methods

The study was conducted at ICAR-Central Institute for Research on Goats, Makhdoom (Uttar Pradesh), India. Rumen liquor was collected from goats maintained under intensive system and was fed with concentrate pellet and *ad lib* gram straw supplemented with Pakad (*Ficus virens*). Isolation of rumen bacteria was carried out using serial dilution method.0.5 ml of inoculums from the test tube labeled 10^{-12} was inoculated in cellulose degrading agar medium by Roll tube technique. These inoculated cultures were allowed for growth of bacteria in strict anaerobic condition at 39°C for 3 days. Singlet colonies was picked up and transferred to cellulose degrading broth media. The transferred colony was again grown on agar media for purity check of the culture. Pure cultures of different isolates of bacteria were subjected for extraction of DNA. The DNA was isolated by tradition PCI method DNA samples was used for amplification of 16S rRNA gene using relevant primers (F-S*-univ-530a-S- 16 and R- S*-univ-1392-a-A-15) and amplified products were subjected for sequencing of desired genes. Characterization of rumen bacteria was done on the basis of gene sequence using Ez Bio Cloud database (https://www.ezbiocloud.net). Rumen bacteria was identified and characterized on the basis similarity of the sequences of the amplified product.

The culture of bacteria was evaluated for the major biochemical test for further characterization and its behaviour under different biochemical components. The API A20 strips were used for biochemical tests manufactured by Biomerieux, USA. This strip contains ready to use 21 biochemical test cupules which can produced color on reaction with the culture either negative or positive.

In Vitro Gas Production Test

Two sets of *In vitro* gas production test (IVGPT) were carried out for the evaluation of rumen bacteria culture on fermentation parameters (Menke and Steingass, 1988). In one set (GS) the substrate was 0.2g gram straw (100% roughage) and in second set (GS+CM) substrate was 0.1 g gram straw and 0.1g concentrate mixture (50% roughage+50% concentrate). Accurately weighed substrate was taken in the graduated 100 ml calibrated glass syringe with the help of weighing boat with removable stem, so that the sample was put at the bottom of the syringes without sticking to its wall. The piston was greased with paraffin soft while LR (Hi media laboratories Pvt. Ltd. 39-56^{0}C) up to the mark on it and pushed into the barrel of the syringes. In every set culture was tested at 0.0 (control), 0.5ml and 1.0 ml/30ml of fermentation medium. For each test there were sixteen syringes (4 syringes each for no media, 0.5ml media, 1.0ml media, 0.5ml media+ culture, 1.0ml media+ culture).There was two syringes of substrate blank (without substrate), two syringes as standard. Syringes were incubated at 39^{o}C for 24 hours in water bath. Rumen liquor was collected from the three adult male Barbari goats of 1-2 years of age (Body weight 36.53 ± 1.4 Kg) by stomach tube method. These animals were fed with 400 g concentrate pellet feed and *ad lib* gram straw. Water was available free choice. The rumen liquor was sampled just before feeding (0 h) from all three these animals and transported in insulated flasks under anaerobic conditions to the laboratory, pooled in equal proportions and used as a source of inoculum.

Estimation of Fermentation Metabolites

After 24 h of incubation at 39ºC in water bath, displacement of the syringe piston indicated gas production. The reading of blank was subtracted to calculate gas and methane production from the substrate. From the head space of each syringe 100µl gas was collected by purging the silicon tube and injected in gas chromatograph for the estimation of methane. It was estimated in Clarus 580 Gas chromatograph from Perkin Elmer equipped with stainless steel column packed with porapak-Q and Flame ionization detector. The standard calibration gas (Sigma gas and Services, New Delhi) consisted of 30.50% carbon dioxide, 31.16% methane and rest is hydrogen. The flow rates for nitrogen, hydrogen and air were 30, 30 and 320 ml/min. respectively. Temperatures of injector, oven and detector were 50 º C, 40ºC and 50 º C respectively (Agarwal *et al*., 2009). After that content of the test syringes were used pH measurement and VFA analyses. For volatile fatty acid 1 ml of the supernatant was mixed with 0.2 ml of 25% meta phosphoric acid and after 2 h, centrifuged at 5000x*g* for 10 min. From the clear supernatant 1 µl was injected into Clarus 580 Gas chromatograph from Perkin Elmer equipped with FID and capillary column. Different VFAs of thesamples were identified on the basis of their retention time and their concentration (mM) was calculated by comparing the retention time as well as the peak area of standards (Cottyn and Boucque, 1968).

The content of other set of syringes was transferred to spout less beaker by repeated washing with 100 ml neutral detergent solution. The flask contents were refluxed for 1h and filtered through pre-weighed Gooch crucibles (Grade G1). The dry matter content of the residue was weighed and in *vitro* true digestibility of feed was calculated as follows (Van soest and Robertson, 1988).

True digestibility (TD) = (Initial DM of feed taken for incubation - NDF residue)/ (Initial DM of feed taken for incubation) X100.

Feed Analysis and Statistical Analysis

The concentrate mixture and gram straw were dried, ground and passed through 1mm screen and analysed for proximate analysis as per AOAC (2006). The NDF and ADF contents were analyzed as per Van Soest *et al*. (1991).

The data were analysed using Generalized Linear model multivariate and means were compared using Duncan's Multiple Range Test at a significance level of 95 % as per Snedecor and Cochran (1994).

Results and Discussion

Chemical Composition of Feed Used in In Vitro Gas Production Test

The per cent organic matter (OM), crude protein (CP) and ether extract (EE) in concentrate were 93.16, 17.32 and 4.51 respectively and for gram straw it was 88.95, 6.29 and 0.94 respectively. The content of total ash (%) in concentrate and gram straw were 6.83 and 11.04, respectively. Neutral detergent fibre (NDF), acid detergent fibre (ADF), and cellulose per cent in concentrate were 26.96, 10.06, 1.82 respectively and that for gram straw were 55.89, 36.48 and 13.46, respectively.

Biochemical Test

The isolated bacteria *Lachnospira pectinoschiza* has shown positive reaction with Glucose, Lactose, Saccharose, Maltose, Salicin, Arabinose, Gelatin, Esculin, Cellobiose, Mannose, Raffinose and Trehalose. The fermentation reaction was negative for Indole, Urea, Mannitol, Xylose, Glycerol, Melezitose, Sorbitol, Rhamnose and Catalase. These biochemical test of bacterial culture isolated from goat was done using API 20A KIT. Biochemical tests are among the most important methods for microbial identification. Microbial biochemistry tests shorten the time required to identify microbes, reduce costs, and ensure or enhance the accuracy of identification of an unknown sample (Zhou and Li, 2015).

In Vitro Fermentation Metabolites

Effect of addition of *L. pectinolytica* in high (GS) roughage ration is presented in table 1. There was no significant effect of media and culture on the *in vitro* gas production. The methane production (mg/g DM) was 28.77 in control group while 29.23 and 30.13 in 0.5 and 1.0 ml culture inoculated group. Similarly, *In vitro* true digestibility (IVTD) was 58.12 in control group while 57.49 and 56.65 in 0.5 and 1.0 ml culture inoculated group. There was no

significant effect on methane production as well as *in vitro* true digestibility. Total volatile fatty acids and their fractions were also similar among all the groups.

Table 1: Effect of *in vitro* addition of *L. pectinolytica* culture on fermentation parameters gram straw

Group	Total gas (ml/gDM)	Methane (ml/gDM)	IVTD (%)	TVFA (mmol/100ml)	Acetate (%)	Propionate (%)	Butyrate (%)	Ac: Pr
Con	153.85±4.22	28.77±1.37	58.12±1.29	3.55±0.13	72.46±0.36	18.73±0.12	8.9±.24	3.86±0.04
0.5	143.41±3.31	26.13±2.33	56.47±0.80	3.45±0.21	73.35±0.17	18.79±0.08	7.85±0.08	3.90±0.02
1	159.43±1.77	29.27±0.88	55.84±0.65	4.14±0.20	71.56±0.43	19.12±0.24	9.30±0.22	3.74±0.06
0.5 CUL	156.22±6.8	29.23±1.28	57.49±2.09	4.06±0.19	71.33±0.52	19.67±0.44	8.99±0.22	3.63±0.10
1.0 CUL	161.38±3.78	30.13±0.71	56.56±1.42	4.21±0.27	71.71±0.53	19.40±0.20	8.88±0.33	3.69±0.06
Mean	155.32±2.72	28.82±0.62	5.91±0.61	3.91±0.11	72.01±0.25	19.19±0.14	8.78±0.15	3.75±0.03
Sig	0.3	0.361	0.855	0.103	0.06	0.158	0.28	0.108

Similar effect was observed on the addition of *L. pectinolytica* in medium (50% GS and 50% CM) ration (Table 2) on all the parameters studied.

Table 2: Effect of *in vitro* addition of *L. pectinolytica* culture on fermentation parameters gram straw+ concentrate mixture

Group	Total gas (ml/g DM)	Methane (ml/g DM)	IVTD (%)	TVFA (mmol/100ml)	Acetate (%)	Propionate (%)	Butyrate (%)	Ac: Pr
Con	138.22±1.55	25.91±1.58	69.57±0.91	3.04±0.08	64.81±0.01	18.71±0.38	16.47±0.38	3.46±0.06
0.5	141.45±2.61	25.69±1.12	67.95±1.21	3.60±0.55	65.80±0.16	19.15±0.37	15.03±0.26	3.43±0.07
1.0	143.61±9.26	26.30±1.39	66.70±0.97	3.52±0.08	68.26±0.33	20.01±0.68	11.71±0.05	3.40±0.10
0.5 CUL	152.38±4.22	28.58±1.12	68.30±1.18	3.01±0.11	65.02±0.71	19.53v0.23	15.43±0.57	3.33±0.07
1.0 CUL	158.55±10.69	29.65±1.64	65.60±0.44	3.83±0.48	64.22±0.33	19.6±0.28	16.71±0.21	3.37±0.06
Mean	147.86±2.68	27.45±0.68	67.54±0.51	3.40±0.15	65.50±0.74	19.29±0.18	15.19±0.87	3.39±0.03
Sig	0.058	0.229	0.098	0.377	0.549	0.262	0.457	0.739

However lower methane production and higher digestibility was reported with medium roughage ration. Doto and Liu (2011) evaluated *Bacillus licheniformis* (Bl) and *Clostridium butyricum* (Cb) and their combinations with yeast culture on *in vitro* rumen fermentation and reported that the in *vitro* organic matter digestibility (IVOMD) was influenced ($P<0.05$) by addition with Bl, Cb or yeast culture, with highest IVOMD observed when Bl or Cb was combined with 60 mg yeast culture. Total volatile fatty acids were affected by Bl and yeast culture ($P<0.01$), but not by Cb ($P>0.05$). There were significant interaction effects on pH, acetate to propionate ratio and Ammonia-N between yeast culture and Bl or Cb. This reflects that the effect on fermentation metabolites varied depending on the culture tested. Sachan *et al*. (2014) reported that *in vitro* addition of herbs can also affect the digestibility of feed. Lee *et al*. (2004) reported that addition of rumen anaerobic fungus, *Piromyces communis* strain 22 culture significantly ($P<0.05$) increased gas production, numbers of total bacteria, cellulolytic bacteria and anaerobic fungi along with enzyme activities of avicelase, carboxymethyl cellulase (CMCase) and xylanase compared with the control.

Conclusion

The present study concluded that *Lachnospira pectinoschiza* isolated from goat's rumen had no significant effect of on *in vitro* rumen fermentation metabolites on high and medium roughage ration.

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Contribution by Authors

Each co-author contributes equally.

Conflict of Interests

There is no conflict of interest.

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