Journal of Research in Biology

FicusPublishers

OPEN ACCESS

Original Research Paper

An International Online Open Access Publication group

Identification of prokaryotic organisms in goat rumen based on metagenomic DNA sequences

Authors:
Jing Zhou, Brian Copeland, Cheng Zhang, Zong Liu, Sarabjit Bhatti, Roger Sauve, Suping Zhou.

Institution:
Department of Agricultural Sciences, School of Agriculture and Consumer Sciences, Tennessee State University, 3500 John A Merritt Blvd, Nashville, TN 37209.
Charles Lee, USDA-ARS, 800 Buchanan St., Albany, CA 94710.
Ryszard Puchala School of Agriculture and Applied Sciences, Langston University, Langston, OK 73050.

ABSTRACT:

A protocol for isolating pure DNA from goat ruminal fluid was developed. DNA was partially digested using ApoI enzymes and ligated onto Lambda ZAP II Predigested EcoR I/CIAP-treated vector to make a metagenomic DNA library. DNA sequence analyses showed that positive clones carried complete or partial sequences for structural genes in different strains of *Escherichia coli*. Several clones did not share significant similarity with nucleotide in the databases. Research results indicate that the population of prokaryotes in the goat rumen contained *E. coli*, and possibly other organisms that have not been characterized. This is the first report of the use of molecular approaches to characterize microbial genes or organisms from goat rumens.

Keywords:
Goat, rumen, microbes, metagenome, *Escherichia coli*.

Corresponding author:
Suping Zhou

Email:
suping@tnstate.edu

Phone No:
6159632146.

Fax:
615-9631557.

Web Address:
http://jresearchbiology.com/Documents/RA0077.pdf.

Article Citation:
Jing Zhou, Brian Copeland, Cheng Zhang, Zong Liu, Sarabjit Bhatti, Roger Sauve, Suping Zhou.
Identification of prokaryotic organisms in goat rumen based on metagenomic DNA sequences.
Journal of research in Biology (2011) 6: 451-455

Dates:
Received: 06 Aug 2011 /**Accepted:** 11 Aug 2011 /**Published:** 18 Oct 2011

Journal of Research in Biology

INTRODUCTION

Goats are known for having an extremely varied diet (Stoneberg 1989). These animals feed on the tips of woody shrubs and trees, lignocellulosic agricultural by-products such as cereal straws and stovers. There have been claims that these animals can feed on newspapers. Symbiont microbes in the rumen of these herbivores play key roles in providing the hosts with various nutrients (Hungate 1966). Enzymes secreted by ruminal microbes are needed for the conversion of cellulose and hemi-cellulose into simple sugars. These sugars serve as a source of energy for these animals (Miyagi et al.1995). Ruminants also obtain the majority of their essential nitrogen needs from the microbial degradation of dietary protein within the rumen (Brooker et al. 1995). Moreover, the microbial activity is responsible for detoxification and synthesis of essential substances for the host animals (Wroblewski et al. 2001; Bas et al. 2003; Kronberg et al. 2006). The composition of the microbial population differs with goat species as well as with their food (Shi et al., 2008).

Analysis of microorganisms in the ruminal fluid of different herbivores revealed the presence of bacteria (1010–1011 cells/ml, representing more than 50 genera), ciliate protozoa (104–106/ml, from 25 genera), anaerobic fungi (103–105 zoospores/ml, representing five genera) and bacteriophages (108–109/ml) (Hobson, 1989; Sajiad et al. 2008). These numbers show only a small fraction of the microbial species found in rumens of animals on fiber based diets since less than 10-20% of microbial populations these microbes are cultivable on synthetic media (Amman et al. 1992; McSweeney et al. 1999).

A recently developed molecular approach to obtain microbial genes in a microenvironment allows for the identification of the entire genome present. This method involves cloning genomic materials into bacterial chromosome (BAC), or plasmid cloning vectors hosted in a cultivable bacterium such as *Escherichia coli* (Brady et al., 2004; Rhee et al., 2005). Subsequent functional screening of this cultivable bacterium has resulted in the isolation of target genes encoding for the antibiotic turbomycin (Gillespie et al. 2002), or the enzyme xylanase (xyn8) (Lee et al. 2002) and novel lipases (Lee et al. 2006) and forage specific glycoside hydrolases in the bovine rumen microbiome (Brulca et al., 2009) and many others. Moreover, individual organisms can be identified from a mixed population communities based on DNA segments of the bulk metagenomic DNA sequences, such as the virus strain responsible for Honey Bee Colony Collapse disorder (Cox-Foster et al., 2007), the virus for several human diseases (Victoria et al., 2009) and other pathogenic organism (Nakamura et al., 2008). DNA sequences in a metagenomic DNA library can reveal the genetic diversity under various environments (Biddle et al. 2008; Woyke et al. 2006).

The metagenome method provides the investigator with the global microbial gene pool without the need to culture these microorganisms (Yun et al., 2004). This method has been widely used to map microbiomes in ruminants such as the cow (Morrison et al. 2005). For this study, a metagenomic DNA library was constructed using the prokaryotic genomic DNA from goat's ruminal fluid. Based on sequence analyses of cloned DNA fragments, gene identities and the classification of microbes present were predicted. The determination of microbial populations inhabiting goat rumens is very important for understanding nutrient absorption from high-fiber diets and consequently enhancing productivity of such animals (Onodera et al. 1998; Sajiad et al. 2008).

MATERIALS AND METHODS

Collection of goat ruminal fluid

Eight mature goats were ruminally cannulated following the procedure developed by the American Institute for Goat Research. After recovery from the surgery, these goats were fed a diet that consisted of fiber supplemented with minerals and vitamins. Goats were fed at 0900 h and were given free access to water. After being on this diet for 14 days, collection of ruminal fluid samples were initiated. Samples were collected at 4, 6, 8 and 10 h after feeding for 4 days. These samples were pooled before DNA extraction.

Isolation of metagenomic DNA

The ruminal fluid composite sample was centrifuged at 13,000 rpm for 10 min. After discarding the supernatant, pellet was used to extract bacterial and prokaryotic microbial DNA using the CLS-TC buffer in FastDNA Spin Kit (Cat#6540-600, MP Biomedicals, LLC, Solon, Ohio). Extracted DNA was further purified utilizing Geneclean Kit (MP Biomedicals).

Partial digestion of purified genomic DNA was carried out at 50^{o}C for 50 min with FastDigest ApoI (Fermentas, Maryland). Digestion products were separated in 0.8% agarose gels. Bands

between 4-10 kb and those > 10kB, <4kb were separately collected from the gel. DNA was recovered from gel slices using Qiagen Gel Extraction Kit (Qiagen Sciences, Maryland).

Construction of metagenomic library

The recovered DNA was ligated onto Lambda ZAP II Predigested EcoR l/CIAP-treated Vector and packaged into the Gigapack III Gold Packaging Extracts (Stratagene, CA). Titering of the packaging reaction was on NZY agar plates. The background of the genomic DNA library was determined by the Blue and White color selection method on NZY plates supplemented with 3.8 mM IPTG (isopropyl β-D-1-thiogalactopyranoside) and 50 mg/ml final concentration X-gal (bromo-chloro-indolyl-galactopyranoside), both chemicals were obtained from Sigma (Missouri, USA). The color of background plaques was blue, while recombinant plaques were white. The white plaques were randomly selected for single–clone excision to isolate the phagemid (pBluescript SK-).

Identification of genes and microbial species

Single stranded DNA were recovered, processed and sequenced using M13 forward (-20) and M13 reverse (20mer) primers from both directions using the Bigdye Terminator version 3 on a Avant 3100 DNA Analyzer (Applied Biosystems, CA) following a previously described procedure (Zhou et al. 2006). The identity of cloned gene fragments were determined by BLASTing against NCBI nucleotide database, (others excluding human and mouse databases. In addition, DNA sequences were also translated into protein using the web-based translation tool (http:// www.expasy.ch/tools/dna.html). Longest peptide sequences were searched for conserved domains and protein identities (Marchler-Bauer et al., 2004, 2009). All clones were submitted to the NCBI databank at http://www.ncbi.nlm.nih.gov.

RESULTS AND DISCUSSIONS

The following clones contained full-length sequences that encode for structural genes in *E. coli*: GQ343311 for L-arabinose isomerase in *E. coli* str. K12 substrain; W3110. GQ343312 for tyramine oxidase, copper-requiring in *E. coli* BL21 (DE3); GQ343313 for dehydrogenase in *E. coli;* BW2952 and other strains; GQ343314 for cysteine synthase B (O-acetylserine sulfhydrolase B) in *E. coli* BW2952; GQ343309 for a conserved protein in *E coli* BW2952; GQ343315 for citrate lyase, citrate-ACP transferase (alpha) subunit in *E. coli* BW2952; GQ343316 for tRNA(Ile)-lysidine synthetase in *E. coli* BW2952; GQ343317 for a predicted lactam utilization protein in *E. coli* BW2952; GQ343318 for fused DNA-binding transcriptional regulator/proline dehydrogenase in *E. coli* BW2952; GQ343319 for Cpn60 chaperonin GroEL, large subunit of GroESL in *E. coli* BW2952; GQ343320 for glyceraldehyde-3-phosphate dehydrogenase C (pseudogene) in *E. coli* str. K12 substrain DH10B; GQ343321 for predicted methyltransferase in *E. coli* BL21(DE3); GQ343324 for Cytochrome b in *E. coli*; GQ343326 for fused predicted transporter subunits of ABC superfamily in *E. coli* BL21(DE3); GQ343327 for protein associated with replication fork in *E. coli* BW2952; GQ343328 for ferrochelatase acetyl esterase in *E. coli* BW2952; GQ343329 for the predicted pyruvate formate lyase in *E. coli* BW2952; GQ343331 for D-alanyl-D-alanine carboxypeptidase in *E. coli* BW2952; GQ343332 for fused ribonuclease E: endoribonuclease/RNA-binding protein in *E. coli* BL21(DE3); and GQ343333 for periplasmic sensory protein associated with the TorRS two - component regulatory system in *E. coli* O157:H7 strain TW14359.

Dehority and Grubb (1977) isolated 44 different bacterial strains from the rumen contents of goats. These strains were grouped based on their morphology, Gram stain reaction, anaerobiosis, motility, fermentation end products, and 16S ribosomal DNA-restriction fragment length polymorphism analysis. Members of *E. coli* strains are phenotypically diverse but have similar chromosomal organizations (Welch et al. 2002). Bacterial clones identified in this study were (99-100%) identical to the DNA sequences reported in the database. This suggests that these sequences were cloned from *E. coli* strains present in goat's ruminal fluid.

In rumens of different herbivores, bacteroids (obligate anaerobic organisms) are responsible for the degradation of cellulosic polysaccharides from plant sources (Ramirez and Dixon, 2003; Franklund Glass, 1987). These organisms could play a similar role in goats. In this study, several clones had no significant similarity when searched in the DNA database. However one of the six frame translation-predicted peptide sequence from one clone matched a fragment of a glucanase homologs from bacteroids, suggesting that cellulosic microbes are presenting in goat rumen. Other clones had no

matching sequences (at neither peptide nor DNA level), these clones could be genomic sequences from organisms that have not been characterized previously. These clones will be deposited in the database after the full-length gene sequences become available.

CONCLUSION

Gene sequences that encode for structural and functional proteins have been identified in the goat rumen. This study provides molecular evidence for the presence of microbial organisms that have not previously been observed in the goat rumen and demonstrates that functional genes can be cloned directly from bulky DNA extracts from goat ruminal fluid.

ACKNOWLEDGEMENT

This research was financially supported by National Institute of Food and Agriculture, Capacity Building Research Grant Award No. 2010 -38821-21598, and Evan Allens funds from the United States Department of Agriculture. The authors wish to thank Dr. Johnson Terrace for assistance in conducting the research.

REFERENCES

Amann RI, Lin C, Key R, Montgomery L, Stahl DA. 1992. Diversity among *Fibrobacter* isolates: towards a phylogenetic classification. Syst Appl Microbiol., 15:23-31.

BAS P, Archimende H, Rouzeaua, Sauvant D. 2003. Fatty acid composition of mixed-rumen bacteria: Effect of concentration and type of forage. J Dairy Sci., 86:2940-2948.

Biddle JF, Fitz-Gibbon S, Schuster SC, Brenchley JE, House CH. 2008. Metagenomic signatures of the Peru Margin subseafloor biosphere show a genetically distinct environment. *PNAS* 105:10583-10588.

Brady S, Chao C, Clardy J. 2004. Long-chain N-acyltyrosine synthases from environmental DNA. Appl Environ Microbiol., 70:6865-6870.

Brooker JD, Lum DK, Miller S, Skene I, O'Donovan L. 1995. Rumen microorganisms as providers of high quality protein. Livestock Research for Rural Development. On-line access 09-09-2009: http://www.lrrd.org/lrrd6/3/1.htm.

Brulca JM, Antonopoulosb DA, Millera MEB. 2009. Gene-centric metagenomics of the fiber-adherent bovine rumen microbiome reveals forage specific glycoside hydrolases. PNAS 106:1948-1953.

Cox-Foster DL, Conlan S, Holmes EC. 2007 A metagenomic survey of microbes in honey bee colony collapse disorder. Science 318:283-287.

Dehority BA, Grubb JA. 1977. Characterization of the predominant bacteria occurring in the rumen of goats (*Capra hircus*). Appl Environ Microbiol., 33:1030-1036.

Franklund CV, Glass TL. 1987. Glucose uptake by the cellulolytic ruminal anaerobe *Bacteroides succinogenes*. J Bacteriol 169:500-506.

Gillespie DE, Brady SF, Bettermann AD, Cianciotto NP, Liles MR, Rondon MR, Clardy J, Goodman RM, Handelsman J. 2002. Isolation of antibiotics turbomycin A and B from a metagenomic library of soil microbial DNA. Appl Environ Microbiol., 68:4301-4306.

Hungate RE. 1966. The rumen and its microbes. Academic Press, New York.

Hobson PN. 1989. The Rumen Microbial Eco-system. Elsevier Applied Science, London.

Kronberg SL, Halaweish FT, Hubert MB, Weimer PJ. 2006. Interactions between euphorbia esula toxins and bovine ruminal microbes. J Chem Ecology 32:15-28.

Lee CC, Kibblewhite-Accinelli RE, Wagschal K, Robertson GH, Wong DWS. 2006. Cloning and characterization of a cold-active xylanase enzyme from an environmental DNA library. Extremophiles 10:295-300.

Lee MH, Lee CH, Oh TK, Song JK, Yoon JH. 2006. Isolation and characterization of a novel lipase from a metagenomic library of tidal flat sediments: evidence for a new family of bacterial lipases. Appl Environ Microbiol., 72:7406-7409.

Marchler-Bauer A, Bryant SH. 2004. CD-Search: protein domain annotations on the fly. Nucleic Acids Res. 1:32(Web Server issue):W327-31.

Marchler-Bauer A, Anderson JB, Chitsaz F, Derbyshire MK. 2009. CDD: specific functional annotation with the Conserved Domain Database. Nucleic Acids Res 37 (Database issue):D205-10.

McSweeney CS, Dalrymple BP, Gobius PM, Kennedy PM, Krause DO, Mackie RI, Xue GP. 1999. The application of rumen biotechnology to improve the nutritive value of fibrous feedstuffs: pre and post-ingestion. Liv Prod Sci., 59:265-283.

Miyagi T, Kaneichi K, Aminov Rim Kobayashi Y, Sakka K, Hoshino S, Ohmiya K. 1995. Enumeration of transconjugated *Ruminococcus albus* and its survival in the goat rumen microcosm. Appl Environ Microbiol., 61:2030-2032.

Morrison M, Adams SE, Nelson KE, Attwood GT. 2005. Metagenomic analysis of the microbiomes in ruminants and other herbivores. I n Harinder P.S. Makkar and Christopher S. McSweeney Ed. Methods in Gut Microbial Ecology for Ruminants Springer Netherlands. 209-220.

Nakamura S, Maeda N, Miron IM. 2008. Metagenomic diagnosis of bacterial infections. Emerg Infect Dis., 14:1784-1786.

Onodera R, Murakami K, Ogawa K. 1998. Cellulose-degrading enzyme activities of mixed rumen ciliate protozoa from goats. Agri. Biol. Chem., 52:2639-2640.

Ramirez RF, Dixon BA. 2003. Enzyme production by obligate intestinal anaerobic bacteria isolated from oscars (*Astronotus ocellatus*), angelfish (*Pterophyllum scalare*) and southern flounder (*Paralichthys lethostigma*). Aquaculture 227:417-426.

Rhee JK, Ahn DG, Kim YG, Oh JW. 2005. New thermophilic and thermostable esterase with sequence similarity to the hormone-sensitive lipase family, cloned from a metagenomic library. Appl Environ Microbiol., 71:817-825.

Sajjad M, Andrabi SMH, Akhter S, Afzal M. 2008. Application of biotechnology to improve post -ingestion forage quality in the rumen. Pakistan J Nutr., 7:70-74.

Shi PJ, Meng K, Zhou ZG, Wang YR, Diao QY, Yao B. 2008. The host species affects the microbial community in the goat rumen. Lett Appl Microbiol., 46:132-135.

Stoneberg S. 1989. Goats make “cents” out of the scourge of leafy spurge. Rangelands 11:264-265.

Victoria JG, Kapoor A, Li L, Blinkova O, Slikas B, Wang C, Naeem A, Zaidi S, Delwart E. 2009. Metagenomic analyses of viruses in stool samples from children with acute flaccid paralysis. J Virol., 83:4642-4651.

Welch RA, Burland V, Plunkett G, Redford P. 2002. Extensive mosaic structure revealed by the complete genome sequence of uropathogenic *Escherichia coli*. Proc Natl Acad Sci USA 99:17020-17024.

Woyke T, Teeling H, Ivanova NN. 2006. Symbiosis insights through metagenomic analysis of a microbial consortium. Nature 443:950-955.

Wroblewski K, Muhandiram R, Chakrabartty A, Bennick A. 2001. The molecular interaction of human salivary histatins with phenolic compounds.Eur J Biochem., 268:4384-4397.

Yun J, Kang S, Park S, Yoon H, Kim MJ, Heu S, Ryu S. 2004. Characterization of a novel amylolytic enzyme encoded by a gene from a soil-derived metagenomic library. Appl Environ Microbiol., 70:7229-7235.

Zhou S, Chen FC, Nahashon S. 2006. Cloning and characterization of glycolate oxidase and NADH-dependent hydropyruvate reductase genes in *Pachysandra terminals.* Hortscience 132:1-5.