



5-Methylcytosine DNA Methylation Patterns among Gut Predominate Commensal *Escherichia coli* and *Lactobacilli* from the Balbas and Mazekh Domestic Sheep Breeds

Pepoyan AZ^{1,2*}, Balayan MA^{1,2}, Bezhanyan T², Badalyan M^{1,2}, Tsaturyan V², Melnikov V³, Kamiya S⁴, Netrebov⁵ and Chikindas M⁶

¹Armenian National Agrarian University, Armenia

²International Association for Human and Animals Health Improvement, Armenia

³Central Research Institute of Epidemiology, Russia;

⁴Kyorin University School of Medicine, Japan

⁵School of Environmental and Biological Sciences, USA

⁶Center for Digestive Health, NJ Institute for Food, USA

*Corresponding author: Astghik Pepoyan, Food Safety and Biotechnology department, 74 Teryan, Yerevan 0009, Armenia

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Abstract

The aim of current investigation was to compare the 5-methylcytosine level in genomic DNA of commensal *Escherichia coli* and *Lactobacilli* from the widely bred in Armenia sheep breeds-Mazekh and Balbas. The results revealed statistically confirmed differences in 5-methylcytosine methylation patterns of these bacteria in the bacterial isolates from Mazekh and Balbas sheep. The percentage of 5-methylcytosine of the genomic DNA was statistically higher for isolates from the Mazekh sheep in comparison with those of Balbas sheep (34.5 ± 7.57 vs. 5.05 ± 2.4 , $P < 0.05$). The Scatter Plot of relationship between cell surface hydrophobicity and genomic DNA's 5-methylcytosine levels shows a moderately strong negative relationship between these characteristics of sheep gut isolates (Pearson R is -0.75). These results may be important for veterinary medicine and breeding programs.

Keywords: Commensals; *Escherichia coli*; Gut; *Lactobacilli*; Sheep

Introduction

At present, significant changes have occurred in the natural habitat of animals and humans. Ecologically unfavorable territories have appeared where a large number of people live and thousands of farm animals and birds are held. Maintaining the quality of life in such areas is the most important task of veterinary medicine. Microbial biocenosis, and, especially, the biocenosis of the gastrointestinal tract is an important part in the well-being of animals and humans. It refers to the qualitative and quantitative composition of bacteria specific to each species. It is known that the formation and state of the gut microbiota is influenced by the breed characteristics of animals [1-3]. On the other hand it became apparent that the traditional view on bacterial population as clonal is not correct, and that phenotypic heterogeneity is common in bacteria. Divergent bacterial subpopulations are formed during adaptation to different environmental conditions, including bacterial colonization of animals' gut by commensals and pathogens. Epigenetic mechanisms can often be a cause of such heterogeneity.

Epigenetic refers to biochemical modifications of chromatin that do not change the sequence but regulate gene expression and may be inherited. Several types of epigenetic modifications exist, such as DNA methylation, histone modification, and nucleosome positioning, and they can regulate a variety of processes, including transcription and protein binding to DNA. In most cases, DNA methylation is associated with gene silencing and is mediated by a family of enzymes called DNA methyltransferases [4,5]. The aim of current investigations was to compare the 5mC (5-methylcytosine) methylation levels of genomic DNA in commensal *E. coli* and *Lactobacilli* from the widely bred in Armenia breeds of sheep – Mazekh and Balbas.

Materials and Methods

The study included 2-3 years old 15 Mazekh and 15 Balbas sheep from the Armenian farms. 30 *E. coli* and 30 *Lactobacilli* isolates from the fecal microbiota of each sheep breed were investigated. Fecal

materials were collected and analyzed as previously described [6]. Four predominant isolates found in the most diluted samples were grown and investigated. Bacteria were grown anaerobically according to Stepanyan and co-authors [7,8]. de-Man, Rogosa and Sharpe (MRS) Broth, Difco, (Fisher scientific) was used to propagate both *Lactobacillus spp.* and *E. coli* cells.

gDNA were extracted from bacterial cultures grown overnight using QIAamp® DNA Mini kit (Qiagen, Hilden, Germany) and the 5-mC methylation were identified by 5-mC DNA ELISA kit (Zymoresearch, California, USA) according to manufacturers' protocols. The absorbance was measured at 450 nm on Stat Fax® 3300 (Awareness Technology, Inc, USA). The relationship between CSH and 5-mC methylation levels of isolates' DNA was evaluated by the Pearson test (Excel 2010).

Results and Discussion

Gut infections and inflammation are frequently go together with dysbiosis. Inflammation induces a cascade of pro-inflammatory and anti-inflammatory molecules. The balance between these two groups of regulators controls cell death and repair of tissue damage. In recent years it has become apparent that gut bacteria produce many molecules which can counteract pro-inflammatory and anti-inflammatory pathways leading to activation or repression of immunity. Commensal bacteria can control the inflammatory process by altering the gut environment, changing the permeability barrier of the intestine, and by degradation of enteral antigens. Recent experiments have identified bacterial DNA, and unmethylated CpG motifs in particular, as another microbial stimulus which can be sensed by cells of the innate immune system, and induce the synthesis of an array of cytokines by immune cells [4,9]. DNA methylation plays a significant role in regulating gene expression, DNA replication and repair. Bacterial strains differ in DNA-methylation patterns. Approximately 35% of the bacterial DNAs contained N4-methylcytosine (4-mC), about 60% contained 5-mC, and about 90% had N6-methyladenine (6-mA) [10]. These modifications are performed for double-stranded DNA using methyltransferases or other methyl transfer machinery. Methylation of adenines may have broad roles in gene regulation and DNA replication in bacteria [11]. in comparison with eukaryotic cells: chromosome replication, nucleoid segregation, DNA repair, transposition of insertion elements [12], and transcription of specific genes is regulated by DNA adenine methylation (Dam), moreover, Dam methylation is required for virulence in pathogenic *Enterobacteriaceae spp.*, including pathogenic *E. Coli* (Wion and Casadesús, 2006).

At the same time, cytosine can undergo modifications, forming 5-mC and its oxidized products 5-hydroxymethylcytosine (5-hmC), 5-formylcytosine (5-fC) and 5-carboxylcytosine (5-caC). 5-mC reduces and 5-hmC enhances DNA flexibility, and 5-caC does not have a measurable effect [13]. In bacteria, there are no known type I-III restriction-modification systems involving 5hmC or ghmC, though, several type IV systems that restrict DNA containing 5hmC and/ or ghmC have been described [14-16]. In *E. coli* 6-mA and 5-mCbases are products of reactions catalyzed by three enzymes

that, besides Dam, are specified by the host specificity (*hsd*) and DNA cytosine methylation (*dcm*) genes. DNA methylases isolated from *E. coli* are site specific, they do not methylate DNA from *E. coli in vitro*, but they act on DNA from unrelated organisms.

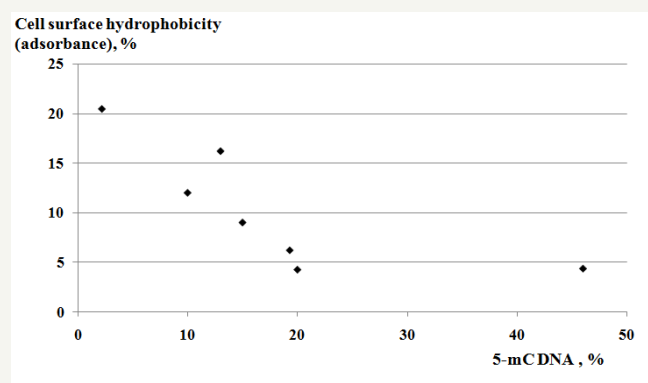


Figure 1: Scatter plot of cell surface hydrophobicity - 5-methylcytosine relationship in sheep breeds of Mazekh and Balbas.

The results of our investigations revealed statistically confirmed differences between the 5-mC DNA methylation patterns of Mazekh and Balbas breeds' bacterial isolates. The percentage of 5-mC DNA was statistically higher in investigated isolates from the Mazekh sheep in comparison with those of Balbas sheep (34.5 ± 7.57 vs. 5.05 ± 2.4 , $P < 0.05$). The Scatter Plot picture of relationship between cell surface hydrophobicity and genomic DNA's 5-mC levels is presented in Figure 1. A moderately strong negative relationship between the 5-mC levels and CSH percentage were found for the seven randomly chosen isolates (Pearson R is -0.75) (Figure 1).

More research is needed to understand the role of these differences in the DNA methylation of gut commensals for animals' immune status. Taking into account the above mentioned literature data, we hypothesized that the association of N6-methyladenine in eukaryotes and 5-mC- in bacteria might be important in host-bacteria interactions. At the same time these results may be important for the specific sheep husbandry/breeding programs.

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