# *Lactobacillus animalis* NP51 reduces *Escherichia coli* O157:H7 Virulence

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**Abstract – This study aimed to elucidate the gene expression profile of *Escherichia coli* O157:H7 when grown in the presence of the direct-fed microbial (DFM) *Lactobacillus animalis* NP51. In this study, three biological replicates of each treatment and controls were analyzed. Bacterial strains were grown at 39°C in media known to support co-culture of both DFM and pathogen. Total RNA was extracted and samples were rRNA depleted followed by bar-coding of individual samples. RNA-Seq libraries were sequenced on a MiSeq instrument; differentially expressed genes were annotated using Blast2go software. A total of 707 genes were differentially expressed at 2-fold change. Motility and virulence-related genes including those encoding for O-antigen production were found downregulated, suggesting that NP51 interferes with this pathway used for pathogen's virulence. This study provides important insight into DFM-pathogen interactions and mechanisms by which NP51 prevents *E. coli* O157:H7 from colonizing the host.**

**Key Words – Direct Fed Microbials (DFM), Gene Expression Regulation, Virulence**

## I. INTRODUCTION

*Escherichia coli* O157:H7 is one of the major zoonotic pathogens of public health concern due to its ability to produce a powerful toxin that causes bloody diarrhea, and hemolytic uremic syndrome [1], [2]. Many cases are linked to the consumption of contaminated food of animal origin such as beef [2], [3]. Contamination of beef carcasses has been correlated with fecal samples testing positive for the pathogen on the hides at slaughter [2], [3], [4]. Reduction of fecal shedding can decrease the risk of human exposure to this pathogen [1], [2]. *L. animalis* NP51 is a DFM widely used as a pre-harvest intervention due to its effectiveness reducing *E. coli* O157:H7 prevalence [2], [3], [4], [5], [6]. Studies have reported that the probability of *E. coli* O157:H7 recovery from feces was 49 to 58% lower in cattle receiving NP51 compared to controls [4], [6]. However, the molecular basis of DFM mode of action on *E. coli* O157:H7 are not completely understood, but it is known to be strain specific and multi-dimensional. In this study, RNA-Sequencing (RNA-Seq) was used to determine the transcriptome profiling of *E. coli* O157:H7 when grown in the presence of NP51, to provide insight into mechanisms by which NP51 exert antagonistic effect against this pathogen.

## II. MATERIALS AND METHODS

*E. coli* O157:H7 ATCC 43985 was isolated from raw hamburger meat implicated in hemorrhagic colitis outbreak in the United States. Bacterial cultures were grown overnight at 37°C in media shown to support co-culture of both *E. coli* O157:H7 and *Lactobacillus*; overnight cultures were diluted into fresh media contained in dialysis tubes. Tubes were then placed in falcon tubes containing media with or without NP51 (control). Samples were incubated at 39°C until mid-logarithmic phase was reached. Total RNA was extracted from three biological replicates; treatment and control samples were rRNA depleted followed by bar-coding of individual samples. RNA-Seq libraries were prepared and sequenced on a MiSeq instrument. Raw data sets were assembled *de novo*; DNAStar Array Star was used to analyze gene expression profiles, and Blast2go software was used to annotate differentially expressed genes.

## III. RESULTS AND DISCUSSION

A total of 707 genes were found differentially expressed at a 2-fold change; 61.81% ($n$=437) showed reduced expression while 38.19% ($n$= 270) increased their expression. Virulence-related genes that were differentially expressed are illustrated in Table 1. Curli production depends on two operons, *csgBA* and *csgDEFG*, which regulation is controlled by several two-component systems and transcriptional regulators, in this study the genes encoding proteins CsgE, CsgF, CsgG were found downregulated (Table 1). Curli are aggregative fimbriae with a key role in surface attachment, biofilm formation, and protecting bacterial cells from toxic compounds [7]. Results of this study suggest *E. coli* is diverging its energy to survive by decreasing growth rate and protecting itself from the stress produced by the presence of NP51 and its biomolecules.

**Table 1.** Virulence factors of *E. coli* O157:H7 differentially expressed by *Lactobacillus animalis* NP51

| Sequence | Regulation | Gene | Function |
|---|---|---|---|
| Seq851 | Downregulated | *csgG* | Curli production assembly/transport component |
| Seq852 | Downregulated | *csgF* | Curli assembly protein CsgF |
| Seq853 | Downregulated | *csgE* | Curli assembly protein CsgE |
| Seq1511 | Downregulated | *ibeC* | Hypothetical protein (Invasion of brain endothelial cells) |
| Seq1771 | Downregulated | *glrR* | Hypothetical protein (LEE encoded T3SS) |
| Seq2595 | Downregulated | *fimB* | Tyrosine recombinase (Type I fimbriae) |
| Seq2712 | Downregulated | *fliC* | Flagellin (peritrichous flagella) |
| Seq754 | Upregulated | *nleA/espI* | Type III secretion system effector NleA |
| Seq1294 | Upregulated | *rpoS* | Sigma S (sigma 38), major sigma factor in stationary phase |
| Seq1891 | Upregulated | *focD* | Outer membrane F1C fimbrial usher protein SfaF |

The O antigen is made of repeating oligosaccharide subunits, it is a component of LPS, major component of outer membrane in Gram-negative bacteria. The O antigens are synthesized by a wzx /wzy-dependent pathway, the process initiates by the addition of a first sugar to a membrane-bound molecule, undecaprenyl-phosphate (Und-P). Once O-antigen is completed, it is translocated across the membrane, newly produced O-antigens are transferred to lipid A-core, then LPS is exported to the outer membrane. In this study, genes encoding for all the proteins required for O-antigen production were downregulated suggesting NP51 interferes with this important pathway used for *E. coli* O157:H7 virulence.

## IV. CONCLUSION

*Lactobacillus animalis* NP51 downregulates the expression of *E. coli* O157:H7 virulence-related genes. Important transcriptional pathways used by *E. coli* O157:H7 were elucidated in this study, allowing a better understanding on the mechanisms used *in vivo* by NP51 to reduce pathogen's ability to colonize gastrointestinal tract, invade epithelial cells and replicate intracellularly in feedlot cattle.

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