

## Jejunal Expression of Mucin4 Gene in Pigs Differentially Adhesive to Diarrhoeagenic *E. coli*

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**Abstract** The *E. coli* mediated piglet diarrhoea is the major cause of the piggery industry which causes severe economic hardship to piggery sector. *E. coli* binds to the brush border of the epithelial cells of the intestine on this basis of receptors and fimbriae adhesion pattern. Among the putative candidate genes associated with adhesion pattern, mucin4 gene was localized on targeted region of SSC13 and considered as a putative candidate gene due to its biochemical property as well as physical location. The present

investigation was conducted to study mucin4 gene expression profile in different adhesion phenotypes. A total of 4 types of adhesion pattern were observed with different frequencies. RT-PCR analysis revealed that mucin4 mRNA expression level was different across the differentially adhesive phenotypes.

**Keywords** Mucin4, Jejunum expression profiling, Desi pig.

### Introduction

Pigs are mostly prone to various diseases. Among all diseases piglet diarrhoea is most important disease. *Escherichia coli* are the major pathogenic bacteria causing diarrhoea in swine, which accounts for 56.2% of the incidence of piglet diarrhoea and 24.7% of the mortality from diarrhoea [1]. This organism can adhere and colonize at the brush border membrane of the epithelial cells of a piglet's small intestine through its fimbriae and secrete enterotoxins. An enterotoxin stimulates the small intestine for secreting massive fluid and electrolyte into the gut lumen resulting diarrhoea. However, not all piglets are equally susceptible to *E. coli*. The adhesion difference happens because of the presence or absence of specific bacterial adhesion receptors in the small intestine epithelial cells of the host. However, the exact/specific genes that encode for the receptor/susceptibility are not yet known. Linkage analyses have shown that the loci encoding for the receptors for the two most frequent variants F4ab

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and F4ac were mapped to 13<sup>th</sup> chromosome (SSC13) of the pig. Mucin4 is one among the putative positional candidate genes found in this region. Hence, the study was designed with the objectives to study the jejunal expression profile of mucin4 in different adhesive phenotypes.

## Materials and Methods

### Sample collection and microscopic adhesion test

A total of 150 desi pigs slaughtered in different places of Bareilly, U. P. were screened for *E. coli* adhesion pattern. Jejunum tissue samples were collected within 30 min of slaughter and brought to laboratory in ice maintaining sterile condition. After cleaning it properly with cold PBS, a small part (250 mg) of the sample was stored in 1 ml RNA later solution at -20°C for RNA isolation. The part of jejunum (approximately 2 cm) was kept at 4°C for the Microscopic Adhesion Test (MAT) on the same day. Samples were screened for adhesion pattern of the porcine brush border epithelial cells with Indian isolate of diarrhoea genic *E. coli* through MAT as described by Li et al. [2]. Animals were classified as strongly adhesive, adhesive, non-adhesive and weakly adhesive as per Li et al. [2].

### Isolation of total RNA and first strand cDNA synthesis

The animals with different adhesion patterns (non adhesive, weak adhesive, adhesive and strong adhesive) 6 samples each were subjected to jejunal expression profiling of mucin4 gene. Total RNA was isolated from jejunum tissue using Trizol reagent (Thermo Scientific, USA) and chloroform according to manufacturer's protocol [3]. For synthesis of first strand cDNA, reverse transcription was carried out in 20 µl reaction mixtures using Revert Aid H Minus First Strand cDNA Synthesis Kit (Thermo Scientific, USA) as per manufacturer's protocol.

### Real time PCR

Quantitative real-time PCR was performed with SSO Fast Eva Green® qPCR kit (Biorad) using Agilent

**Table 1.** Primers information of mucin4 and GAPDH gene under investigation.

Target gene	Primer name	Sequence of nucleotide (5'-3')	Frag. size (bp)
MUCIN4	RTMUC4_F	AGCCAGCTGGACACTAAAGG	93
	RTMUC4_R	GAAAGGATGGCCTGGTAGGT	
GAPDH	GAPDH_F	F:ACACTCACTCTTCTACCTTTG	90
	GAPDH_R	R:CAAATTCATTGTCGTACCAG	

Mx3005P QPCR System (USA) operated by MxPro QPCR software. The Master Mix was prepared using 8.0 µl of nuclease free water, 10 µM of forward and reverse primers each and 10 µl of Eva green mix (Biorad) and 1 µl of cDNA was added. A qPCR amplification program was used (One cycle of Hot start PCR at 95°C for 20 sec followed by 40 cycles of denaturation at 95°C for 3 sec and annealing/extension at 58°C-60°C for 30 sec).

### Quantification of candidate gene expression

Once the  $C_T$  value is collected for each reaction, it can be used to generate a relative expression level. In our experiment, there were four conditions (Non-adhesive, Weak-adhesive, Adhesive and Strong adhesive), where we measured the expression levels of mucin4 gene compared to an endogenous control gene (GAPDH) using the method described by Livak et al [4]. (Table 1). Non-adhesive samples were taken as control. The statistical significance of differences ( $p < 0.05$ ) in mRNA expressions was assessed by using one way ANOVA with Tukey's Multiple Comparison Test as post hoc was performed to determine the significant differences between dCTs of the analyzed groups.

## Results and Discussion

The present study was undertaken to explore *E. coli* adhesion patterns and their frequencies. A total of 34 animals (22.66%) were found to be negative for adhesive (i.e non-adhesive), 87 animals (58%) were adhesive, 23 (15.33%) were weakly adhesive and 6 (4%) strongly adhesive. The result indicates that

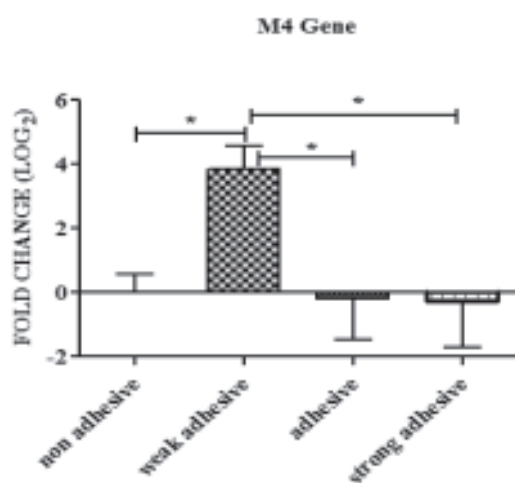


Fig. 1. Expression profiling of mucin4 gene.

enough starting material (non-adhesive phenotypes) was available within the desi pig to start with breeding program against diarrhoea. The mRNA expression study of mucin4 gene revealed that 3.84, -0.19 and -0.27 log<sub>2</sub> fold difference of mucin4 mRNA expression in weak adhesive, adhesive and strong adhesive groups respectively, as compared to non adhesive groups (Fig. 1). Jacobsen et al. [5] did not find mucin4 expression differences between the

F4ab/acR<sup>+</sup> Yorkshire piglets and F4ab/acR<sup>-</sup> Yorkshire piglets. Down regulated mRNA expression in mucin4 was also observed by Zhou et al. [6]. The mucin4 gene expression is also affected if there is intense inflation in intestine. The weak adhesion has been considered to arise from either the influence of epistatic genes on receptors expression or from post-expression inhibition or modification of gene receptors side.

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