

Expression Level of *FUT1* Gene in Different Pig Populations and its Relationship with ETEC F18 Resistance

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Abstract

F18-fimbriated *Escherichia coli* are associated with porcine post weaning diarrhea and edema disease. Some pigs show inherent resistance to F18 ETEC infection and this is associated with a G/A mutation at position M307 of the alpha (1,2)- fucosyltransferase (*FUT1*) gene. Pigs with genotype AA are resistant to ETEC F18 and pigs with genotype GG or AG are susceptible to ETEC F18 infection. So the M307 of *FUT1* gene has been proposed as a genetic marker to distinguish the *E. coli* F18 resistant from susceptible phenotypes in some imported pigs. The objective of this study was to investigate the different expression levels of *FUT1* among Yorkshire, Meishan, and *E. coli* F18-resistant Sutai groups, especially in Meishan. The results showed that *FUT1* was expressed consistently in 11 tissues in the three populations, with relatively high level in the lungs, stomach and gastrointestinal tract. The expression was highest in *E. coli* F18-resistant Sutai pigs, followed by Yorkshire and Meishan. Considering that the biological processes and pathways *FUT1* gene involved in was related to glycosphingolipid biosynthesis, we can speculate that higher expression of *FUT1* in jejunum and duodenum is beneficial to the formation of receptors to *E. coli* F18. Besides, Meishan piglets may have its characteristic immune system for the infection of *E. coli* F18.

Keywords: *Escherichia coli* F18; *FUT1*; Gene expression; Weaning piglets

Introduction

F18- fimbriated *Escherichia coli* are associated with porcine post-weaning diarrhea. The presence of *E. coli* F18 receptor on small intestinal villi is the essential requirement for the adhesion and colonization. At present, there are a lot of researches on the receptor of *E. coli* F18, and the genetic locus for this receptor has been mapped to porcine chromosome 6 (SSC6), based on its close linkage to the S locus and other loci of the halothane (HAL) linkage group [1]. Coddens, et al. [2] found that F18-fimbriated *E. coli* selectively interact with glycosphingolipids having blood group ABH determinants on type 1 core, and blood group A type 4 heptaglycosylceramide. Alpha(1,2)-fucosyltransferases (FUTs) are key enzymes involved in the formation of blood group antigens of the porcine AO blood group system, which corresponds to the human ABO blood group system [3]. A pig

$\alpha(1,2)$ -fucosyltransferase exhibiting $\alpha(1,2)$ -fucosylation of glycolipid and glycoprotein acceptors has been purified from submaxillary gland mucin [4]. Thurin and Blaszczyk-Thurin M [5] identified this enzyme as the homologue of the human Secretor enzyme. Recent studies indicated that *FUT1* gene was important in the synthesis of the structure that was beneficial to the adhesion between *E. coli* F18 fimbriated bacteria and the small intestinal wall [6].

By linkage analysis, Meijerink, et al.[7] estimated that the *FUT1* gene polymorphism was less than 1 centimorgan from the S and *E. coli* F18 receptor loci. Therefore, *FUT1* gene was regarded as a good candidate for gene controlling the expression of the receptor for *E. coli* F18 bacteria. Sequencing of the *FUT1* gene revealed a polymorphism (G or A) at nucleotide 307 resulting in an amino acid Ala being substituted for Thr at position 103. The *E. coli* F18-resistant pigs showed presence of the a nucleotide on both alleles (AA genotype), whereas pigs susceptible to *E. coli* F18 had either the heterozygous AG genotype or the homozygous GG genotype [7,8].

However, previous investigations conducted by Chinese scientists have shown that the polymorphism of *FUT1* gene at nucleotide 307 only displays in foreign pig breeds and hybrid lines bred with foreign lineages such as the Sutai pig. There was no AA genotype or even the AG genotype in most Chinese domestic pig breeds, except the Lingao pig breed which carried a small proportion of AG genotype [9-11].

The Sutai pig is a new hybrid between the Duroc and Taihu breeds that produces high-quality lean meat. In previous studies, we identified a few *FUT1* AG animals (9.2%) in a Sutai pig population and selectively bred them to generate the prized Sutai *FUT1* AA individuals (ETEC F18 resistant). After five years of continuous selection and breeding, the *E. coli* F18-resistant resource population with AA genotype was established [12]. Simultaneously, we also constructed a type V secretion system to express ETEC F18 adhesin. The display of functional adhesin through the type V secretion system was combined with receptor binding experiments to further analyze and verify the resistance

to the ETEC F18 strain among this *E. coli* F18-resistant resource population [13].

Following the initial studies on the *FUT1* gene, examination on the expression of *FUT1* gene in imported, hybrid and Chinese domestic breeds (Yorkshire, Sutai and Meishan) is an attractive route for the analysis of differences in genetic resistance and mechanism to *E. coli* F18. And it can be also be helpful in the development of research on the *E. coli* F18 receptor and the mechanism of resistance to *E. coli* F18 in Chinese native breeds.

Material and Methods

Experimental materials and sample collection

Yorkshire, Sutai and Meishan pig were collected from Engineering Research Centre for Molecular Breeding of Pig in Changzhou City, Jiangsu Province, *E. coli* F18-resistant population in Suzhou Taihu Pig Breeding Center and Meishan Pig Conservation Breeding Company, respectively. Each group included eight weaning piglets aged 35 days old from different litters, which were healthy and the growth characteristics are basically identical. After sacrifice, the following organs, heart, liver, spleen, lung, kidney, stomach, thymus, lymph node, jejunum, duodenum and muscle, were collected in 1.5 ml Eppendorf nuclease-free tubes and stored immediately in liquid nitrogen and then placed in a low temperature freezer (-80°C) until further study.

Primer sequences

Real-time PCR primers sequences of *FUT1* gene were designed as P1: 5'- CAGATAAGCGAGGCCGTCATT-3' and P2: 5'-TTGCAGCCACAAAAAGCA-3' using Primer Express 2.0 Software. For specificity, sequences of primers were aligned using the GenBank BLAST program, available online (www.ncbi.nlm.nih.gov). Each primer was designed to span the exon boundaries in order to avoid genomic DNA contamination and designed to produce amplification fragment of 100 bp in length. In this study, GAPDH was used as a housekeeping gene to normalize the threshold cycle (Ct) values of other tissue products with primer sequences as P1: 5'- ACATCATCCCTGCTTCTACTGG-3' and P2: 5'- CTCGGACGCCTGCTTAC -3'.

RNA extraction

Total RNA was extracted from homogenized tissues (50–100 mg) using Trizol reagent (TaKaRa Biotechnology Dalian Co., Ltd). Precipitated RNA was resuspended in 20 µL of RNase-free H₂O and then stored at -80°C. RNA quality and quantity were assessed by 2.2 M denatural agarose electrophoresis and UV spectrophotometry, respectively. The total RNA with A260/A280 value should be about 2.0.

Real-time PCR

Total RNA (500 ng) was reverse transcribed in a final reaction volume of 10 µL using Primer Script RT reagent Kit (TaKaRa Biotechnology Dalian Co., Ltd) containing 5 µL 5 × Primer Script Buffer, 0.5 µL Primer Script RT Enzyme Mix I, 0.5 µL Oligo (dT), 0.5 µL random 6-mers and RNase-free H₂O. The cDNA was

synthesized at 37°C for 15 min followed by a termination step at 85°C for 5 s and then stored at -20°C.

Real-time PCR amplification was performed in 20 µL reaction mixtures containing 1 µL cDNA, 0.4 µL 50 × ROX Reference Dye II, 10 µL 2 × SYBR Green Real-time PCR Master Mix, 7.8 µL dd H₂O, 0.4 µL (10 µM) of each gene specific primers and GAPDH primers. PCR reactions were performed on the ABI 7500 Real-time PCR System. PCR cycling parameters were initially started at 95°C for 15 s, and then 95°C for 5 s followed by 62°C for 30 s for 40 cycles. Dissociation curve analysis was performed at the end of 40 cycles to verify PCR product identity. Each sample was tested three times to obtain average data.

Data processing and analysis

The $2^{-\Delta\Delta Ct}$ method was used to determine relative quantification [14] (ΔCt = the mean expression level of *FUT1* - the mean expression level of *GAPDH*). The average expression level of *FUT1* in the muscle tissues was defined as 1.0 so that the expression levels of this gene in other tissues could be quantified ($\Delta\Delta Ct$ = ΔCt of different tissue - ΔCt of muscle). Statistical analyses were carried out using SPSS 11.0 software. T-test was carried out to analyze the differentiation significance of mRNA expression in 11 tissues among three populations.

Results

Results of real-time PCR

The total RNA met the required standards for purity and was reverse-transcribed successfully. The cDNA was used for Real-time PCR. After PCR reaction, the ABI System automatically generated amplification curves related to changes of fluorescence and melting point curves. Melting point curves for *FUT1* gene PCR primers have a single peak, which indicated that single PCR product was produced. Therefore, there was no indication of amplification of non-specific targets or primer dimers, which may complicate the quantification of target genes. The efficiency of the GAPDH PCR primers matched that of the *FUT1* gene, which indicated that relative differences in target genes can be calculated according to the $2^{-\Delta\Delta Ct}$ mathematical mode.

The results were shown in Table 1 and Figure 1. The expression of *FUT1* gene in 11 tissues in three different pig breeds shared almost the same characters. Relative to muscle (with a gene expression value of 1), *FUT1* gene was found to be expressed in all the tissues in three breeds with relatively high expression in lung, stomach, liver, duodenum and jejunum. We observed that piglet lymph node, heart, thymus and muscle all expressed the *FUT1* gene weakly.

On the whole, among Yorkshire, Meishan, and *E. coli* F18-resistant Sutai groups, the expression of *FUT1* gene was highest in Sutai pigs, followed by Yorkshire and Meishan pigs. There was a significant difference between the expression rates of Sutai and Meishan pigs together in stomach, duodenum and jejunum. Although most tissues of Yorkshire expressed *FUT1* gene higher than Meishan pigs', there was no Significant difference between two groups.

Table 1: Expression levels of *FUT1* gene in 11 tissues in three different pig populations.

Tissue	Meishan	Yorkshire	<i>E. coli</i> F18-resistant Sutai
Heart	2.018 ± 0.910	5.145 ± 2.300	5.260 ± 2.264
Liver	34.615 ± 13.719	53.512 ± 24.387	90.040 ± 49.205
Spleen	18.753 ± 5.301	41.890 ± 6.405	31.330 ± 15.125
Lung	66.820 ± 24.887	87.830 ± 38.216	133.300 ± 48.779
Kidney	18.102 ± 2.666	32.053 ± 11.455	25.620 ± 10.670
Stomach	30.487 ± 15.229 ^a	96.052 ± 35.522 ^{ab}	192.830 ± 50.262 ^b
Muscle	1.000 ± 0.000	1.000 ± 0.000	1.000 ± 0.000
Thymus	4.003 ± 1.657	4.436 ± 2.302	15.350 ± 10.288
Lymph node	12.389 ± 5.978	15.564 ± 4.082	18.350 ± 6.906
Duodenum	25.933 ± 6.510 ^a	57.693 ± 31.369 ^{ab}	61.370 ± 23.025 ^b
Jejunum	16.882 ± 9.928 ^a	20.404 ± 6.999 ^{ab}	61.460 ± 32.402 ^b

Note: Values in same organs with same letters indicate $P > 0.05$, and those with different letters indicate $P < 0.05$. Value = mean ± SD.

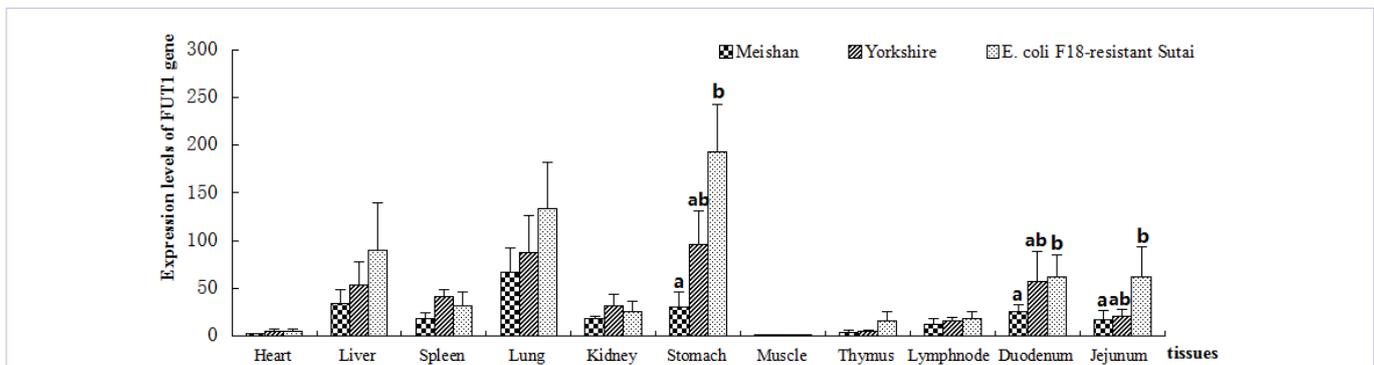


Figure 1: Expression levels of *FUT1* gene in 11 tissues in three different pig populations.

Note: Values in same organs with same letters indicate $P > 0.05$, and those with different letters indicate $P < 0.05$.

Meishan pig, one of the Chinese native pig breeds, is well known all over the world for the high prolificacy and high adversity resistance. Compared the expression of *FUT1* gene between Meishan and *E. coli* F18-resistant Sutai piglets, the result revealed that *FUT1* gene expressed higher in the latter in all tissues. And the expression of *FUT1* gene in stomach, duodenum and jejunum in *E. coli* F18-resistant Sutai piglets was significantly different from that in Meishan pigs.

Discussion

Newly weaned pigs suffer from post-weaning diarrhoea or oedema disease due to *E. coli* F18 infections [15]. The key point to the infection depends on the receptors expressed on the small intestinal epithelium. Recent findings demonstrate that the *E. coli* F18 receptor is known. The Fed F tip-adhesion of F18 fimbriae is bound to ABH type 1 glycosphingolipids, and the ABH type 1 H antigen is catalyzed by α 1-fucosyltransferase (*FUT1*) [2,16,17]. So the *FUT1* gene has been identified as a candidate gene for controlling the expression of the enterotoxigenic *E. coli* F18 receptor. Being a good genetic marker, the M307 mutation in *FUT1* gene was likely to control the resistance or susceptibility to *E. coli* F18 [7].

Here, we measured the expression of *FUT1* gene in different pig breeds (Yorkshire, Sutai, and Meishan piglets). The Sutai pigs were established as *E. coli* F18-resistant. The results showed that there was a similar trend in tissue-specific expression of *FUT1* gene. It expressed in all the tissues with relatively high expression in lungs, stomach, liver, duodenum and jejunum, which was in accordance with the result we've found before [12,18]). The previous researches in this field all proposed that *FUT1* gene on chromosome 6 was closely linked to the *E. coli* F18 receptor [4,7]). Therefore, the relatively high expression in small intestine such as duodenum and jejunum implied its function in influencing the expression or the structure of *E. coli* F18receptor. In addition, the lower expression in piglet lymph node and thymus indicated that *FUT1* gene might not participate in immune system directly.

What attracted our attention most was the different expression of *FUT1* gene in 11 tissues among three pig breeds. Due to the fact that Meishan pig is Chinese native pig breed and only possess GG genotype at the position of M307 in *FUT1* gene, all the individuals are sensitive to *E. coli* F18 in theory. Therefore, comparison of expression levels between the different breeds; Yorkshire, Meishan and Sutai. The Sutai pigs had the highest expression level of *FUT1* gene in all tissues, followed by

Yorkshire and Meishan pigs. The duodenum and jejunum, where the receptors of *E. coli* F18 are expressed, had higher expression levels of *FUT1* in *E. coli* F18-resistant Sutai and Yorkshire breeds. It was possible that the expression level in three breeds was due to the innate genetic background and environment.

It has been clear that glycoproteins or glycolipids, expressed on the surface of the cells are the main receptors targeted by Gram-negative bacteria. The highly specific binding sites on these receptors are usually part of the sugar chains. The function of *FUT1* gene implied more detailed relations between fucose transferred by *FUT1* and the structure of *E. coli* F18 receptors. Bao [19]; Meijerink, et al. [6] reported that *FUT1* enzyme was likely important in the synthesis of a structure which enables adhesion of *E. coli* F18 bacteria to small intestinal mucosa. Considering that this complement of proteins does not necessarily match to the complement of mRNA transcripts within the cells, we could speculate that the higher level of *FUT1* gene was able to increase the proportions of the receptors' expression in F18-sensitive Sutai and Yorkshire, and result in their individual predisposition to disease. On the contrary, for Meishan pigs with relatively lower expression of *FUT1* gene, there would be less chance of forms of receptors or being affected by toxins produced by *E. coli* F18.

In addition, it is well known that Meishan pigs have a character for higher stress resistance [20,21], such as the invasion of *E. coli* F18, even though they only harbour GG genotype, which was contradictory to the findings reported by Meijerink, et al [7]. Therefore, we made the comparison between Meishan and *E. coli* F18-resistant Sutai piglets. The expression of *FUT1* gene in stomach, duodenum and jejunum in *E. coli* F18-resistant Sutai piglets was significantly higher than that in Meishan. We also know that the 307G >A mutation had no effect on the expression of *FUT1* gene [12]. Those indicated that the function of *FUT1* gene in influencing the *E. coli* F18 adhesion might be regulated by two ways, differential expression and amino acid change. This also suggested that there was a genetic difference in *E. coli* F18 resistance between Sutai and Meishan. Using genetic and enzymatic technology, Meijerink, et al. [7]; Meijerink [6] investigated that the levels of $\alpha(1,2)$ -fucosyltransferase activity were significantly lower in intestinal mucosa of *E. coli* F18-resistant pigs than in susceptible pigs. Moreover, lysates of CHO cells transfected with *FUT1* constructs encoding threonine at amino acid position 103 also showed significantly reduced enzyme activity compared with constructs encoding alanine at this position. So it was possible that for foreign piglets, a favorable mutation occurred and reserved in the course of evolution in order to protect organism from the infection of *E. coli* F18 and meet with the hostile environment. Under natural condition, the frequency of the gene mutation in foreign pigs, such as Duroc and Landrace, was around 0.20, which was quite low [10,22].

Meanwhile, for Meishan pigs, it also possesses the strong immune system that decreases the risk of *E. coli* F18 infection. As Coddens, et al. [23] reported, colonization begins after adhesion of the bacteria with their fimbriae to the small intestine and stops when anti-fimbrial antibodies can be detected in the intestinal lumen of the infected pig. So we could presume that there was no

need for Meishan pigs to have the mutation by virtue of its original higher resistance and lower expression of *FUT1* gene. This may be the reason why there were no polymorphisms of *FUT1* gene in Chinese domestic pigs, which suggested a complicated immune-regulating mechanism and genetic heterogeneity.

In this study, we conducted the expression of *FUT1* gene in Yorkshire, Meishan and Sutai piglets, and Sutai piglets were established as *E. coli* F18-resistant. It prompted the speculation that the high expression of *FUT1* in jejunum and duodenum was probably responsible for the structure of *E. coli* F18 receptors, and despite that the 307G >A mutation in *FUT1* gene had no correlation with higher expression of the gene itself, it may inhibit the *FUT1* activity and exerted a negative impact on the formation of the receptors. Apart from the reason for different genetic background, the variation in promoter of *FUT1* gene, the methylation or the regulation by adjacent genes in the same pathway may lead to the lowest expression of *FUT1* gene in Meishan pigs. And further investigations involving determination of *E. coli* F18 receptors and the mechanism of resistance to *E. coli* F18 in Chinese native breeds must be of great importance.

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