Pakistan J. Zool., vol. 54(2), pp 701-708, 2022 DOI: https://dx.doi.org/10.17582/journal.pjz/20200617020636

Effects of Quorum Sensing AHL Signaling on the Biological Characteristics of Porcine Derived F4ac+ Enterotoxigenic *Escherichia coli*

Yang Yang[1,2], Ji Shao[1,2], Mingxu Zhou[3,4], Qiangde Duan[1,2], Xinyi Zhang[1,2] and Guoqiang Zhu[1,2]*

[1]*College of Veterinary Medicine, Yangzhou University, Yangzhou 225009, China*
[2]*Jiangsu Co-Innovation Center for Important Animal Infectious Diseases and Zoonoses, Yangzhou 225009, China*
[3]*Institute of Veterinary Immunology and Engineering, National Research Center of Engineering and Technology for Veterinary Biologicals, Jiangsu Academy of Agricultural Sciences, Nanjing 210014, China*
[4]*Jiangsu Key Laboratory for Food Quality and Safety-State Key Laboratory Cultivation Base, Ministry of Science and Technology, Nanjing 210014, China*

Yang Yang and Ji Shao contributed equally to this work.

Article Information
Received 17 June 2020
Revised 05 October 2021
Accepted 16 November 2021
Available online 07 January 2022 (early access)
Published 17 January 2022

Authors' Contribution
ZG and YY conceived and designed the experiments. SJ, ZX, ZM, DQ performed experiments. YY and SJ wrote this paper.

Key words
ETEC, Quorum sensing, Acyl-homoserine-lactone, Virulence factor, 3OC12-HSL and C6-HSL, AHL signals

ABSTRACT

Diarrhea of piglets is an important disease affecting the development of pig industry, while F4 enterotoxigenic *Escherichia coli* (ETEC) is an important pathogen causing diarrhea of newborn or weaned piglets, which seriously harms animal husbandry and causes huge economic losses. The pathogenicity of *E. coli* is closely related to its virulence factors, which are strictly regulated *in vivo*, and the quorum sensing system is involved in this process. To study the effect of quorum sensing signal molecule acyl homoserine lactone (AHL) on the biological characteristics of Enterotoxigenic *E. coli* (ETEC), gene *lasI* of *Pseudomonas aeruginosa* which is responsible for the synthesis of long side-chain AHL (3OC12-HSL), and gene *yenI* of *Yersinia enterocolitica* which is responsible for the synthesis of short side-chain AHL (C6-HSL), were selected and transformed into ETEC strain C83902, respectively, and endowed with the ability of endogenous synthesis of 3OC12-HSL and C6-HSL. At the same time, 200 µmol/L 3OC12-HSL and C6-HSL were added into the culture medium, respectively, and co-cultured with ETEC strain C83902. ETEC strains were stimulated by exogenous and endogenous long-chain and short-chain AHL molecules, respectively, and their biological characteristics, such as growth characteristics, biofilm formation ability, and adhesion ability to piglets intestinal epithelial cells IPEC-J2 were observed under the influence of the AHLs described above. Real-time PCR was used to detect the transcription levels of genes encoding major subunits of pathogenic factors such as fimbriae, flagella, enterotoxin, adhesin and hemolysin under the influence of the AHLs. The results showed that under the influence of quorum sensing AHLs, the biofilm formation ability and adherence ability of ETEC were significantly reduced, and the expression of *fliC* and other major virulence factors were regulated. AHL signaling molecules can regulate virulence related characteristics of F4ac+ ETEC.

INTRODUCTION

Porcine enterotoxigenic *Escherichia coli.* (ETEC) is an important pathogen that caused diarrhea in newborn and weaned piglets, which causes huge economic losses in pig industry worldwide (Ren *et al*., 2016). F4ac adhesin positive ETEC bacteria are relatively common in piglet diarrhea and can colonize the intestinal epithelium of piglets via F4ad adhesin, causing piglet diarrhea by releasing enterotoxins after extensive propagation (Johnson *et al*., 2009). However, its pathogenic mechanisms have not been fully elucidated.

Quorum sensing (QS) is the way in which specific communication is made between bacteria (Fuqua *et al*., 1994; Nealson *et al*., 1970). Bacteria produce signal molecules and release them into the environment. when the bacteria reach a certain population number, and signal molecules in the environment also reach a certain threshold concentration, then the quorum sensing signal can bind to the receptor protein of the bacteria and activate downstream target genes expression to regulate bacterial quorum behaviors, such as bioluminescence, regulation

* Corresponding author: yzgqzhu@yzu.edu.cn
0030-9923/2022/0002-0701 $ 9.00/0

of virulence factor secretion, spore formation or biofilm formation, cell differentiation, motility, exopolysaccharide formation, etc. Since the discovery of bacterial quorum sensing in the 1990s, the study of quorum sensing in the regulation of bacterial traits on a population scale has continued to deepen, which is closely related to the synthesis process of a variety of pathogenic bacteria virulence factors. However, the regulatory mechanisms of acyl homoserine lactone (AHL) signaling on enterotoxigenic *E. coli* virulence factors for quorum sensing-1 system has not been fully clarified.

E. coli cannot synthesize AHL molecule on its own, but it has SdiA, the molecular receptor of AHL, which can monitor the AHL signal secreted by adjacent flora in the infection pathway through quorum sensing eavesdropping, so as to further regulate its own virulence (Culler *et al*., 2018; Styles and Blackwell, 2018; Dyszel *et al*., 2010; Yakhnin *et al*., 2011). In previous study of our laboratory, the AHL positive strains in the pig intestine has been screened, and its regulation on the virulence of Shiga toxin-producing *E. coli* (STEC), Enterohemorrhagic *E. coli* (EHEC) and avian pathogenic *E. coli* (APEC) has been verified (Smith *et al*., 2011; Yang *et al*., 2013). However, the effect of AHL on the biological characteristics of ETEC needs to be further explored. The previous results revealed that the analysis of the pathogenic mechanism of *E. coli* not only needs to proceed from the regulation mechanism of *E. coli* itself, but also needs to observe the impact of quorum sensing signals released by the adjacent intestinal flora in the path of intestinal infection (Yang *et al*., 2018b), which can further reveal the changes of the pathogenic mechanism and biological characteristics of ETEC strain in the complex flora environment.

In this study, *lasI* gene for synthesizing long side chain AHL (3OC12-HSL) in *Pseudomonas aeruginosa* and *yenI* gene for synthesizing short side chain AHL (C6-HSL) in *Yersinia enterocolitica* were transformed into ETEC strain C83902 respectively, to construct C83902/p*lasI* and C83902/p*yenI*, to construct the recombinant strains with the ability of endogenous synthesis of AHL signal molecule. Meanwhile, 200 μmol/L AHL was added to the culture medium. Effects of AHL from different species and sources upon ETEC were observed.

MATERIALS AND METHODS

Strains, cell lines and reagents

F4ac+ETEC strain C83902, the AHL signaling bioreporters (*Chromobacterium violaceum* CV026, *Agrobacterium tumefaciens* JZA1), and cell line IPEC-J2 of small intestinal epithelial cells from piglets were preserved and provided by our laboratory (Table I). Tryptone and Yeast extract were purchased from Oxoid Inc., DMSO, X-gal, chloramphenicol were purchased from Solarbio. AHLs standards (C6-HSL, 3OC12-HSL) were purchased from Sigma, USA. The Fastpure Plasmid Mini Kit Plasmid Extraction Kit was purchased from Nanjing Vazyme Inc., and 2% porcine RBCs were purchased from Nanjing Quanyi Biotechnology Co., Ltd.

Construction of recombinant strains

p*yenI*, p*lasI* plasmids and vector plasmid pBR322 were respectively electro-transformed into C83902 ETEC strain. Strains were centrifuged after culturing in LB medium for 1 h, and spread on LB plate containing optimal ampicillin. After 24 h of incubation, single colonies were selected and cultured in LB medium containing ampicillin. After 6 h, plasmids DNA were extracted using the Fast Pure Plasmid Mini Kit and identified by agarose gel electrophoresis.

Table I. Strains and plasmids used in this study.

Strains and plasmids	Details	Reference
Strains		
E. coli C83902	Wild-type (O8:H19:F4ac+ LT+ STa+ STb+)	Preserved in our laboratory
E. coli C83902/pyenI	C83902 carrying p*yenI*	Constructed in this study
E. coli C83902/p*lasI*	C83902 carrying p*lasI*	Constructed in this study
E. coli C83902/pBR	C83902 carrying pBR322	Constructed in this study
A. tumefaciens JZA1	AHL biosensor	Preserved in our laboratory
C. violaceum CV026	AHL biosensor	Preserved in our laboratory
Plasmids		
pBR322	Expression vector, Amp^r	Takara Ltd.
p*yenI*	pBR322 carrying LuxI ORF	Preserved in our laboratory
p*lasI*	pBR322 carrying LuxI ORF	Preserved in our laboratory

Verification of AHL producing ability

Plasmid p*yenI* can synthesize short side chain AHL signal C6-HSL, which can be detected by reporter CV026. Plasmid p*lasI* can synthesize long side chain AHL signal 3OC12-HSL, which can be detected by reporter JZA1.

According to previous method (Chu *et al*., 2011), strains C83902, C83902/p*yenI* and CV026 were streaked in parallel on LB plates and cultured at 28 °C for 24 h. The reporter strain was set as a negative control and C6-HSL as the positive control. The strains C83902, C83902/p*lasI* and JZA1 were cultured in LB medium with X-gal at 28°C for 24 h, to observe the color changes; 3OC12-HSL was chosen as the positive control.

The measurement of growth curve

Single colonies of wild strain C83902, recombinant strains C83902/pBR, C83902/pl*yenI* and C83902/p*lasI* were inoculated into LB liquid medium respectively, cultured overnight at 37 °C, then transferred to liquid LB broth at the ratio of 1:100 the next day. Moreover, the wild type C83902 was transferred to LB liquid medium containing 200 μmol/L C6-HSL and 3OC12-HSL at the ratio of 1:100. 100 μL solution samples were collected each hour to detect values of $OD_{600.}$The average value of three tests were took to draw the growth curve.

Detection of biofilm formation ability

According to previous method (Coffey and Anderson, 2014), in the endogenous AHL group, C83902, C83902/pBR, C83902/p*yenI*, C83902/p*lasI* with OD_{600} of 1.0 were inoculated in biofilm induction medium (tryptone 10.0 g/L, yeast extract 5.0 g/L, NaCl 2.5 g/L, KH_2PO_4 3.0 g/L, K_2HPO_4 7.0 g/L, $(NH4)_2SO_4$ 2.0 g/L, $FeSO_4$ 0.5 mg/L, $MgSO_4$ 1.0g/L, and thiamine hydrochloride 2.0 g/L). In the exogenous AHL group, the wild type C83902 was transferred to the 3 groups of biofilm induction mediums that without AHL, containing 200μmol/L C6-HSL, and 200μmol/L 3OC12-HSL. Medium were then added to 96 well plates, and 12 replicates were employed in each group. Strains were grown at 30 °C for 48-72h. The biofilm was detected by crystal violet method and quantified by OD_{600}.

Adherence assays

According to previous method (Zhou *et al*., 2013), C83902, C83902/pBR, C83902/p*yenI* and C83902/p*lasI* with OD_{600} of 1.0 were added to monolayer IPEC-J2 cells respectively with the multiplicity of infection (MOI) at 100:1, and incubated at 37 °C for 1 h. Then the cells were washed with PBS for 3 times to remove the non-adhered bacteria, then lysed with 0.5% tritonX-100 for 20 min. Medium were serial diluted at the ratio of 1:10, and finally spread on LB plate. After cultured in 37 °C overnight, CFU from each group were counted.

The wild strain C83902 was transferred to LB medium of 200 μmol/L C6-HSL or 3OC12-HSL. Strains were cultured at 37 °C for 6 h, and then were adjusted to OD600=1.0. Each group was centrifuged at 5000 r/min for 10 min, the supernatant was discarded, and bacterial precipitation was resuspended in DMEM at with 200 μmol/L C6-HSL or 3OC12-HSL. Then adherence assay was carry out.

Motility test

In the endogenous AHL group, C83902, C83902/pBR, C83902/p*yenI*, C83902/p*lasI* with OD_{600} at 1.0 were inoculated in LB medium at the ratio of 1:100, cultured at 37 °C until the logarithmic phase., then diluted to OD_{600} at 1.0. 1 μL solution of each strain was added in the middle of 0.3% semi-solid plates. After incubation at 37 °C for about 18 h, the diameter of bacterial motion halos was measured.

In the exogenous AHL group, wild type C83902 was transferred into LB medium, cultured overnight, took 1 μL bacterial solution and added to 0.3% semi-solid plate (one group without AHL, one group containing 200 μmol/L C6-HSL, one group with 3OC12-HSL), After incubation at 37 ˚C for about 18 h, the diameter of bacterial motion halos were measured.

Hemolytic activity test

The 2% porcine erythrocytes were purchased from Nanjing Quanyi Biotechnology Co., Ltd. The strains were cultured to logarithmic phase, then 1 mL bacterial solution was collected, centrifuged at 10000 rpm for 10min, and the supernatant was filtered use 0.22 μm filtration membrane. 100 μL filtered supernatant was collected in each group and mixed with 100 μL porcine erythrocytes. Solutions were incubated at 37 °C for 2 h and at 4 °C overnight. After the precipitation of insoluble erythrocyte, 100 μL supernatant from each group was took for OD_{400} value measurement.

Detection the expression level of virulence genes

According to previous method (Croxen and Finlay, 2010), the bacterial virulence genes (type I fimbria subunit *fimH* and *fimA*, flagellum subunit *fliC*, hemolysin *hlyA*, sfm fimbria subunit *sfmH*, and F4 fimbria subunit *faeG*) were selected and real-time PCR (rtPCR) was performed. Primers are shown in Table II.

The above strains were cultured to the logarithmic prophase (OD_{600} = 1.00), 1 mL of bacterial solution was taken to extract mRNA, and was transcripted into cDNA by TaKaRa Reverse Transcription Kit, and RT-PCR was carried out with corresponding cDNA as template. RT-PCR reaction system (20 μL): SYBR Green PCR Master Mix 10

μL, 1 μL of each upstream and downstream primers (0.5 μmol/L), 3 μL cDNA template (1.5 ng/μL), complement with ddH_2O up to 20 μL. PCR reaction conditions: 95 °C for 2 min; 95 °C for 5 s, 60 °C for 10 s, 40 cycles. The expression level of the target genes was analyzed and calculated by $2^{-\triangle\triangle CT}$ method.

Table II. Primers used in this study.

Primers	Sequencing (5'-3')
faeG-RT-F	ACTCAGAAAACCTGATGGTGAAACT
R	CCCCACCTCTCCCTAACACA
sfmH-RT-F	TGTGACATCAACGCCTTACGA
R	ATGCGCCGATATCACCAAAA
ipfA-RT-F	AACCGGCGTTGGTATTCGTA
R	TCATTTCAGCCTTGCCTTCA
fimH-RT-F	GGCTGCGATGTTTCTGCTC
R	CCCCAGGTTTTGGCTTTTC
hlyA-RT-F	TTGCACTCAGCAGGACAAAG
R	GGTGAGGCCAATGAGTTTCT
gapA-RT-F	CGTTAAAGGCGCTAACTTCG
R	ACGGTGGTCATCAGACCTTC
fliC-RT-F	TCGACAAATTCCGCTCCTC
R	GGTTGGTGGTGGTGTTGTTC

RESULTS

Verification of recombinant strains and the growth curves

The plasmids of C83902/pBR, C83902/pl*yenI* and C83902/p*lasI* were extracted from the recombinant strain, and verified by agarose gel electrophoresis. The results of AHL signal molecule detection showed that, the contact region between recombinant strain C83902/p*lasI* and CV026 turned purple, which was consistent with the positive control (Fig. 1A). The recombinant strain C83902/p*lasI* induce blue color in reporter strain JAZ1, which was consistent with the positive control, while the color with wild type C83902 did not change (Fig. 1B). It was proved that the recombinant strains with the ability to endogenously synthesize C6-HSL and 3OC12-HSL were successfully constructed. The control C83902/pBR that transformed with empty vector plasmid pBR322 could not induce the color change in CV026 or JZA1. It can be seen from the growth curves that both endogenous synthesis and exogenous addition of AHL had no significant effect on the growth of C83902 in aerobic environment (Fig. 2).

Results of biofilm formation

The results showed that under endogenous AHL, the biofilm forming ability of recombinant strains C83902/p*yenI* and C83902/p*lasI* decreased by 24.5% and 33.5% respectively, compared with the wild type C83902 (Fig. 3A). The biofilm forming ability of strain C83902 decreased by 47.6% and 25%% when exogenous C6-HSL and 3OC12-HSL was added, respectively (Fig. 3B).

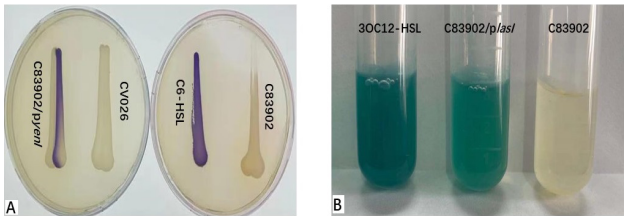

Fig. 1. The signal molecule detection of AHL. **A**, Detection the ability of C83902/p*yenI* to synthesize short side chain AHL by reporter strain CV026. **B**, Detection the ability of C83902/p*lasI* to synthesize long side chain AHL by reporter strain JZA1. C6-HSL and 3OC12-HSL were used as positive controls.

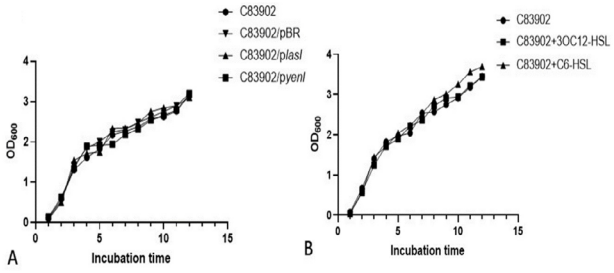

Fig. 2. The growth curves of C83902 and its recombinant strains under endogenous (A), and exogenous AHLs influence (B).

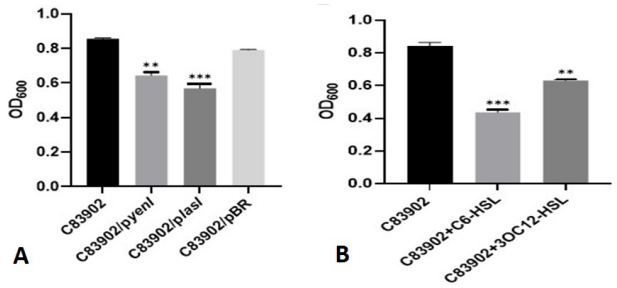

Fig. 3. Quantitative test of biofilm formation of C83902 and its recombinant strains under endogenous (A) and exogenous AHLs influence (B).

Adherence assays

The results showed that, under endogenous AHL, the adhesion ability of recombinant strain C83902/p*yenI* and C83902/p*lasI* decreased by 20.9 and 42.9% respectively

compared with wild type C83902 (Fig. 4A). When exogenous C6-HSL and 3OC12-HSL were added, the adhesion ability of C83902 to IPEC-J2 cells decreased by 36.6 and 19.5%, respectively (Fig. 4B).

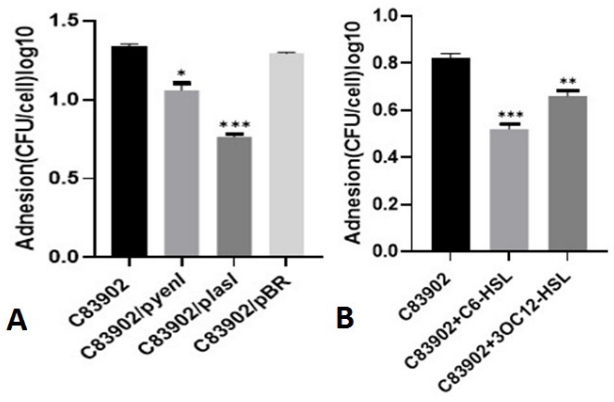

Fig. 4. The adherence ability of IPEC-J2 cells of C83902 and its recombinant strain under endogenous (A), and exogenous addition of AHL (B). **, $P<0.01$; ***, $P<0.001$.

The motility of strains

The results of motility test showed that under the effect of endogenous AHL, the diameter of motility circle of recombinant strains C83902/p*yenI* and C83902/p*lasI* decreased by 15.5% and 21.4%, respectively. When exogenous C6-HSL and 3OC12-HSL was added, the motility circle diameter of strain C83902 decreased by 12.3% and 16.1%, respectively.

Hemolytic activity

The results showed that under the endogenous AHL, the hemolytic activity of recombinant strains C83902/p*yenI* and C83902/p*lasI* increased by 12.5% and 11.4%, respectively (Fig. 5A); while the hemolytic activity of strain C83902 increased by 13.2% and 10.2%, respectively when exogenous C6-HSL and 3OC12-HSL was added.

Detection of virulence gene expression

The results showed that, compared with wild type C83902, the expression of *hlyA*, *sfmH*, *fimH* and *ipfA* in recombinant strain C83902/p*yenI* increased by 25, 27, 10 and 12%, respectively, while the expression of *fliC*, *fimA* and *faeG* decreased by 54, 21 and 24%. The expression of *hlyA*, *sfmH*, *fimH*, *ipfA* and *faeG* increased by 69, 79, 9, 25 and 9%, respectively after addition of 200 μmol/L exogenous C6-HSL, while the expression of *fliC* and *fimA* decreased by 16 and 15% (Fig. 5A).

Compared with wild type C83902, the expression of *hlyA*, *sfmH*, *stfG* and *ipfA* in recombinant strain C83902/p*lasI* increased by 85, 14, 7 and 8%, respectively, while the expression of *fimH*, *fliC*, *fimA* and *faeG* decreased by 19, 44, 29 and14%. And when addition of 200 μmol/L exogenous 3OC12-HSL, the expressions of *hlyA*, *sfmH*, *stfG* and *fimA* increased by 84, 23, 72 and 31%, while the expressions of *fimH*, *fliC*, *stfG* and *ipfA* decreased by 19, 27, 84 and 74%, respectively (Fig. 5B).

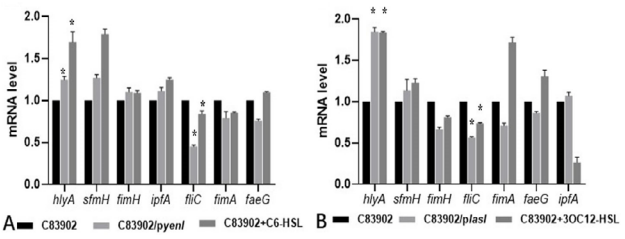

Fig. 5. Transcriptional levels of virulence related genes of C83902 and its recombinant strains endogenous and exogenous C6-HSL (A) and OC12-HSL (B). * $P<0.05$

DISCUSSION

F4ac ETEC is a widely prevalent pathogen causing diarrhea in piglets, which causes huge economic losses in pig industry all over the world (Sugiharto *et al*., 2012; Van den Broeck *et al*., 2002). The pathogenicity and virulence regulation mechanism of ETEC have been well deeply studied (Luppi, 2017). However, these studies usually focus on ETEC, observing that ETEC regulates virulence factors through its own pathway and mediates the pathogenic effect on the host, while ignoring the impact on ETEC from the perspective of adjacent and intestinal flora (Smith *et al*., 2008). Since the discovery of bacterial quorum sensing in the 1990s, the study of quorum sensing in population scale regulation of bacterial traits has continued to be deepen, and it is closely related to the expression process of a variety of pathogenic bacteria virulence factors. It is usually considered that *E. coli* only has quorum sensing-II system, but does not have a complete quorum sensing-1 system, so it cannot synthesize AHLs, the signal molecular of quorum sensing-1. *E. coli* encodes AHL receptor protein SdiA, which can sense and respond to AHL signals produced by other bacteria, so as to regulate *E. coli* biological functions (Smith *et al*., 2011; Yakhnin *et al*., 2011). The research of our laboratory has confirmed the virulence regulation function of AHL in Shiga toxin producing *Escherichia coli* STEC, EHEC (enterohemorrhagic *E. coli)* and APEC (avian pathogenic *Escherichia coli*) (Yang *et al*., 2013, 2014, 2018a, b). Mechanism of AHLs upon ETEC is still unknown, through which ETEC sense and respond to AHL synthesized by adjacent flora in intestinal environment, so as to start quorum sensing eavesdropping to change its

own characteristics.

In this study, *lasI* and *yenI*, the homologous genes of quorum sensing *luxI*, were transformed into F4ac + ETEC C83902 to confer it with the ability of endogenous synthesis of C6-HSL and 3OC12-HSL. These two AHL signals are more important in more than 30 kinds of AHL in nature, and they have been studied in depth. These two types of AHL can represent short-chain side-chain AHL and long-chain side-chain AHL molecules respectively through the difference in the number of side chain carbon atoms. Using these two different AHL molecules with different traits can reveal the AHL function more comprehensively. In this study, CV026 which can detect short side chain of AHL, and JZA1 which can detect long side chain of AHL, were used to prove the successful construction of the recombinant strains. Moreover, by adding C6-HSL and 3OC12-HSL to the culture medium, 4 different culture conditions (endogenous, exogenous of short side chain or long side chain of AHL) were formed, so as to more comprehensively demonstrate the virulence regulation effect of AHL on F4ac ETEC strain.

The results showed that, different sources and types of AHL had no significant effect on the growth curve of F4ac+ETEC. Biofilm (BF) is a microbial population formed by the adhesion of bacterial surface polysaccharides, proteins and nucleic acids to abiotic or bioactive surfaces (Rabin *et al*., 2015). It can improve the tolerance of bacteria to antibiotics, environmental pressure and host immune attack (Płusa, 2019; Sharma *et al*., 2019). It is an important factor that cannot be ignored in the process of ETEC infection. The biofilm forming ability of ETEC strain decreased significantly under the effect of different sources and different kinds of AHL. In most strains of *Pseudomonas aeruginosa*, the expression of QS factor stimulates biofilm formation, while in *E. coli*, it shows a unique biofilm inhibition phenomenon under AHL (Li *et al*., 2015; Yang *et al*., 2013). There are many factors affecting biofilm, researchers generally believe that biofilm synthesis in *E. coli* is related to the expression of flagella. During biofilm formation, flagella can help bacteria adhere to the surface of solid medium and help bacteria gather and form biofilm through movement chemotaxis.

Flagella is a very important virulence factor of *E. coli*. It not only provides motility, but also participates in the adhesion, colonization and invasion of bacteria to host cells (Zhou *et al*., 2015). In this study, the changes of adhesion ability of ETEC to porcine small intestinal epithelial cells IPEC-J2 under AHL were detected by adherence assay. The adhesion ability of ETEC strains decreased significantly under different sources or types of AHL, and the decrease of adhesion ability may come from the inhibition of the expression level of adhesion factors such as flagella by AHL. The results of flagella motility test showed that ETEC motility was inhibited, which was consistent with the results of adherence test.

Flagella usually interacts with the expression of other virulence factors. Virulence factors of ETEC mainly include fimbria, flagella, enterotoxin, adhesin, hemolysin, etc. (Nagy *et al*., 1996). In this study, the expression level of virulence factor related genes such as flagella was tested by qPCR. After endogenous synthesis and exogenous addition of C6-HSL, the gene expression levels of *hlyA, sfmH, fimH, stfG* and *ipfA* increased, while the expression levels of *fliC* and *fimA* decreased. Endogenous C6-HSL inhibited *faeG* expression, while exogenous C6-HSL activated *faeG* expression. After endogenous synthesis and exogenous addition of 3OC12-HSL, the expression of *hlyA* and *sfmH* increased, while the expression of *fimH* and *fliC* decreased. Endogenous of 3OC12-HSL up-regulated the expression of *stfG* and *ipfA*, but inhibited the expression of *fimA* and *faeG*. Its regulatory effect was opposite to that of exogenous 3OC12-HSL. Among these virulence factors, under different sources or different kinds of AHL, the expression level of flagella gene *fliC* and hemolysin gene *hlyA* maintained the same trend and the difference is significant, suggesting that AHL may inhibit flagella expression and activate the synthesis of α-hemolysin. The inhibition effect on the synthesis of flagella by AHL may be closely related to the changes of virulence related biological characters such as biofilm formation and decreased adhesion of F4ac ETEC. α-hemolysin is a Repeats in toxin (RTX) protein. It has cytolytic activity and cytotoxicity to most mammalian cells such as erythrocytes, granulocytes, monocytes and endothelial cells (Askari *et al*., 2016). It can integrate into the host cell membrane, affect the stability of cell membrane and induce lysis, thus it is an important virulence factor for ETEC extraintestinal infection and penetrating the intestinal vascular barrier (Burgos and Beutin, 2010). Hemolytic activity test showed that α-hemolysin activity was up-regulated by AHL. This study found that AHL is related to hemolysin for the first time, and its regulation mechanism urgently needs further analysis.

CONCLUSION

In conclusion, for quorum sensing-1 system, this study selected two different kinds of important AHL signals, verified the regulation of AHL on virulence related biological characteristics of F4ac ETEC by endogenous synthesis and exogenous addition of AHL, and revealed AHL effects on flagella expression, biofilm formation, adhesion ability and hemolysin synthesis. It is suggested that the adjacent flora in the ETEC infection pathway

can interfere with ETEC biological characteristics from the population scale, which lays a foundation for the comprehensive disclosure of the pathogenic pathway of ETEC.

ACKNOWLEDGEMENT

This study was supported by grants from the Chinese National Science Foundation Grants (Nos. 31972708, 31502075, 31873010, 31672579), "13th Five-Year" National Key Development Program (2016YFD0501000), the Priority Academic Program Development of Jiangsu Higher Education Institutions.

Ethical compliance

There are no researches conducted on animals or humans.

Statement of conflict of interest

The authors have declared no conflict of interest.

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