

Biofilm formation of a strain of bovine *Escherichia coli* and analysis of its influencing factors

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Abstract

This study investigated the biofilm (BF) formation of a bovine-derived *Escherichia coli* (*E. coli*) strain (designated DL1907) and analyzed the factors influencing its formation. The BF-forming capacity, optimal formation time and temperature, as well as the effects of different culture media, pH levels, NaCl concentrations, and glucose concentrations, were assessed using the crystal violet method. The results demonstrated that the strain had a strong BF-forming capacity, achieving the most intact BF structure after 36 h of culture at 37°C. Trypticase Soy Broth (TSB) medium promoted the strongest BF formation compared to Luria-Bertani (LB) and Müller-Hinton Broth (MH) media. The optimal BF growth was achieved at a NaCl concentration of 0.5%, while higher concentrations (> 0.5%) significantly inhibited BF formation. The strain demonstrated a strong capability for BF formation at pH 7, while acidic or alkaline conditions significantly impaired its BF-forming capacity. The minimum BF inhibitory concentrations (MBIC) of baicalin, berberine, andrographolide, and emodin against BF were 512 µg/mL, 64 µg/mL, 64 µg/mL, and 128 µg/mL, respectively. For the antibiotics, the MBIC values of ceftazidime, kanamycin, enrofloxacin, and florfenicol were 256 µg/mL, 128 µg/mL, 64 µg/mL, and 32 µg/mL, respectively. The above experimental results are intended to provide data support for the clearance of the isolated strain DL1907 BF.

Keywords: biofilm, bovine, *Escherichia coli*, influencing factors

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Introduction

E. coli is a facultative anaerobic bacteria that can grow in the range of 8-46°C, and the optimum growth temperature is 37°C (Jang *et al.*, 2017). *E. coli* is an important host organism in biotechnology and has a wide range of applications in industrial and medical fields because of its ease of manipulation, availability of whole genome sequence, and ability to grow under both aerobic and anaerobic conditions (Jiang and Sheng, 2014). Although *E. coli* usually appears to be a harmless commensal flora, some strains are still pathogenic and have been identified as the causative agents of food-borne enteritis, capable of causing intestinal or extra-intestinal disease in humans and many animals, as well as many disease syndromes, such as diarrhea, wound infection, meningitis, sepsis, atherosclerosis, hemolytic uremic syndrome, reactive and rheumatoid arthritis and other immune diseases, pose a serious threat to public health security (Leimbach *et al.*, 2013; Wang *et al.*, 2017; Cai *et al.*, 2020).

The term BF was coined by Costerton and his colleagues in 1978. It generally refers to the cell aggregation membrane-like substance formed by microorganisms by secreting a large amount of extracellular polysaccharide, protein, nucleic acid, and other extracellular polymeric substances (EPS) to wrap the cell itself in a three-dimensional structure to adapt to the environment (Schilcher and Horswill, 2020; Wu *et al.*, 2022). The mature BF structure is the main BF layer, connecting layer, condition layer, and matrix layer from outside to inside, which can form a physical barrier to protect living cells (Sharma *et al.*, 2016). In the process of BF formation, bacteria are regulated by the Quorum sensing system (QS), and a large amount of free DNA is released after bacterial lysis, which forms the skeleton of bacterial BF and maintains the spatial conformation of BF (Schilcher and Horswill, 2020). Many bacteria in BFs are in close contact with each other. Therefore, the emergence of BFs can increase the tolerance of bacteria to drugs by 600-1000 times and can resist oxidative stress, dehydration, starvation, and other adverse factors, thereby indirectly enhancing the virulence of bacteria, an important factor leading to the increase of bacterial resistance.

During BF formation, there are many influencing factors: incubation time, pH of the growing environment, availability of surface nutrients, nutrient concentration, etc. In this study, a pathogenic *E. coli* (DL1907) strain isolated from bovine was tested for BF formation time, some biological characteristics, and the minimum inhibitory concentration of BF by Chinese and Western medicine, to provide data for this strain's theoretical system of BF formation.

Materials and Methods

Raw materials for diseases: The *E. coli* strain (designated DL1907 in this study) was isolated from a clinical blood sample of diarrheic Simmental calves collected in 2019 from an intensive cattle farm in Tongliao, Inner Mongolia. Following routine blood culture, well-grown single colonies were selected for isolation and purification, with subsequent 16S rRNA

gene sequencing confirming the isolate is a strain of *E. coli*.

Main reagents and instruments: NaCl, glucose, and Nutrient agar were purchased from Zhejiang Changqing Chemical Co., LTD. Yeast extract and peptone were provided by the Aobox company. TSB and MH culture media were purchased from Shandong Topu Biological Engineering Co., LTD. 96 wells of cell culture plates and crystal violet dye solution were purchased from Shanghai Gefan Biology Science and Technology Co., LTD. Digital display gas bath constant temperature oscillator, electric constant temperature air blast drying box provided by Shanghai Countess Technology Co., LTD. The microplate reader was purchased from BioTeK (USA).

Determination of BF-forming ability of isolated strains: According to reference (Sharma *et al.*, 2016), the isolated strain was inoculated into LB broth and propagated for later use. The bacterial suspension was diluted 1:20 with fresh LB medium; 10 µL of the diluted culture and 190 µL of LB medium were dispensed into each well of a 96-well plate. Three replicates and one negative control (200 µL LB medium only) were prepared. The plate was incubated at 37°C for 36 h. After discarding the remaining culture medium and washing with PBS, the BF were fixed with 200 µL of 95% methanol for 20 minutes, washed once with PBS, and air dry. The samples were stained with 1% crystal violet solution for 15 minutes. PBS was rinsed 3 times and inverted to dry, 33% glacial acetic acid was added, and then shaken at low speed on a shaker (150 rpm, 15 min) to promote uniform dissolution of the dye, and the OD_{590 nm} value was detected by an enzyme marker. Three replicates and one control group (200 µL LB medium) were established.

The cutoff value (OD_c) was defined as twice the OD value of the control group. Using the mean normalized OD values (Normalized OD = Sample OD - Blank Control OD) from triplicate experimental groups, the BF formation capacity was classified as follows: OD ≤ OD_c, no BF formation (-); OD_c < OD ≤ 2OD_c, weak formation (+); 2OD_c < OD < 4OD_c, moderate formation (++); OD ≥ 4OD_c, strong formation (+++).

Effect of different media on BF formation ability: 10 µL of bacterial solution was added to 190 µL of LB, MH, and TSB liquid medium, respectively, and incubated at 37°C in a constant temperature box for 36 h. The BF was processed according to the method of 1.3, and the OD_{590 nm} value was detected by an enzyme labeling instrument, and the growth of BF was observed. Three replicates and one control group (200 µL LB medium) were established. The average OD value from the three replicate test groups was calculated (all OD values were normalized using the formula: Normalized OD = Sample OD - Blank Control OD). The BF formation capacity was evaluated according to the reference standard 1.3.

Effect of culture time on BF formation ability: The bacterial solution diluted with LB medium according to 1:20 (200 µL per well) was added to 96-well plates and incubated at 37°C in a constant temperature box for

6 h, 12 h, 24 h, 36 h, 48 h, and 72 h. At each period, BF was processed according to the method of 1.3 and detected by an enzyme labeling instrument with OD₅₉₀ nm values to observe BF formation at each stage. Three replicates and one control group (200 µL LB medium) were established. The average OD value from the three replicate test groups was calculated (all OD values were normalized using the formula: Normalized OD = Sample OD - Blank Control OD). The BF formation capacity was evaluated according to the reference standard 1.3.

Effect of incubation temperature on BF formation ability: The bacterial solution diluted with LB medium according to 1:20 (200 µL per well) was added to 96-well plates and incubated at 4°C, 25°C and 37°C, respectively, and the BF was processed according to the method of 1.3 at each stage and detected the OD₅₉₀ nm value by enzyme labeling instrument, and the formation of BF at each stage was observed stages of BF formation. Three replicates and one control group (200 µL LB medium) were established. The average OD value from the three replicate test groups was calculated (all OD values were normalized using the formula: Normalized OD = Sample OD - Blank Control OD). The BF formation capacity was evaluated according to the reference standard 1.3.

Effect of pH on BF formation ability: The pH of the LB liquid medium was adjusted to 4, 5, 6, 7, 8, 9, and 10, respectively, and incubated at 37°C for 36 h. Each group was treated with BF according to the method of 1.3 and detected the OD₅₉₀ nm value by an enzyme labeling instrument, to observe the effect of different pH on the formation ability of BF. Three replicates and one control group (200 µL LB medium) were established. The average OD value from the three replicate test groups was calculated (all OD values were normalized using the formula: Normalized OD = Sample OD - Blank Control OD). The BF formation capacity was evaluated according to the reference standard 1.3.

Effect of NaCl concentration on BF formation ability: Take 10 µL of bacterial solution and add it to 190 µL of LB liquid medium containing different concentrations of NaCl, set the concentration of NaCl to 0.5%, 1%, 1.5%, 2%, 2.5%, and the LB medium as the control group, and then treat the BF according to the method of 1.3 and detect the using an enzyme marker OD₅₉₀ nm value and observe the results. Three replicates and one control group (200 µL LB medium) were established. The average OD value from the three replicate test groups was calculated (all OD values were normalized using the formula: Normalized OD = Sample OD - Blank Control OD). The BF formation capacity was evaluated according to the reference standard 1.3.

Effect of glucose concentration on BF formation ability: Take 10 µL of bacterial solution and add it to 190 µL of LB liquid medium containing different concentrations of glucose, set the concentration of glucose to 1%, 2%, 3%, 4%, 5%, respectively, LB medium as the control group, and then treat the BF according to the method of 1.3 and detect using an enzyme marker, OD₅₉₀ nm values to compare BF

growth. Three replicates and one control group (200 µL LB medium) were established. The average OD value from the three replicate test groups was calculated (all OD values were normalized using the formula: Normalized OD = Sample OD - Blank Control OD). The BF formation capacity was evaluated according to the reference standard 1.3.

Determination of minimum BF formation inhibition concentration (MBIC) of Chinese and Western medicine: Four commonly used Chinese herbal extracts (Berberine, Emodin, Baicalin, and Andrographolide) and four commonly used antibiotics (Kanamycin, Enrofloxacin, Florfenicol, and Cefotaxime) were used to explore the inhibitory effects of Chinese and Western medicines on BFs. Under aseptic conditions, 100 µL bacterial solution was added to the 96-well plate in turn, The gradient dilution method was used to sequentially dilute to the 6th well, the concentration of the drug solution was 32 µg/mL, and 100 µL of bacterial solution and 100 µL of drug solution were added to the 7th and 8th wells as the control group, respectively. The BF was cultured at 37°C for 36 h, and the growth of the BF was observed.

Results

Determination of BF formation ability of isolated strains: As shown in the figure, there are obvious thin, uniform, and easily colored BFs visible on the sidewalls of the wells of the 96-well plate. This indicates that the BF formation ability of the test strains is strong (Fig. 1).

Effect of different media on BF formation ability: After 36 h of incubation in different media, it was found by determining the amount of BF formation (OD₅₉₀ nm) that *E.coli* BF grew best in TSB medium, with its OD₅₉₀ nm values were significantly higher than those of LB and MH media; in LB media, the amount of BF formation was intermediate between TSB and MH media; whereas, in MH media, the amount of BF formation was the lowest, and the OD₅₉₀ nm values were significantly lower than those of the other two media. The above results indicated that the type of medium had a significant effect on *E. coli* BF formation, with TSB medium being the most favorable for BF growth and accumulation (Fig. 2).

Effect of culture time on BF formation ability: Through the dynamic monitoring of the *E. coli* BF formation process (Fig. 3), the OD₅₉₀ nm values showed a trend of first increasing and then decreasing with incubation time. The OD value was the lowest at 12 h of incubation, and the BF formation ability was weak; it was at an intermediate level at 24 h, 48 h, and 72 h; and the OD value peaked at 36 h, indicating that the BF formation ability was the strongest at this time. Therefore, the optimum incubation time for BF formation was 36 h. The OD values of BF at 12 h were the lowest.

Effect of incubation temperature on BF formation ability : After 36 h of incubation at different temperatures, it was found by measuring the amount

of BF formation ($OD_{590\text{ nm}}$) that *E. coli* BF grew best at 37°C, and its $OD_{590\text{ nm}}$ values were significantly higher than those at 4°C and 25°C, and the OD values peaked at this temperature, indicating that BF formation was strongest at this temperature. Therefore, the optimal incubation temperature for BF formation was 37°C (Fig. 4).

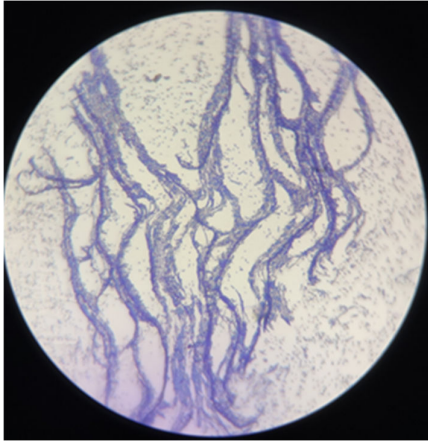


Figure 1 BF morphology of *E. coli* at 36 h of incubation (100×).

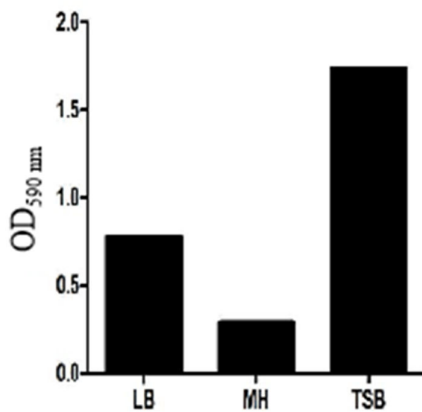


Figure 2 $OD_{590\text{ nm}}$ value of *E. coli* BF in different media.

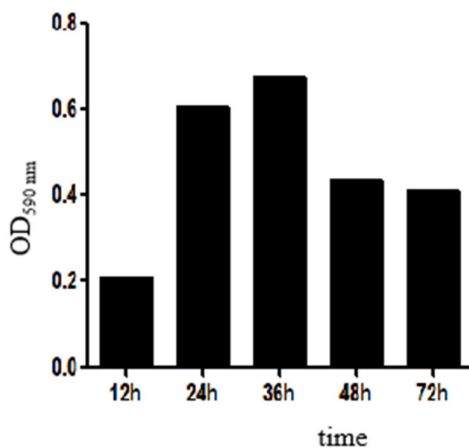


Figure 3 $OD_{590\text{ nm}}$ value of *E. coli* BF at different culture times.

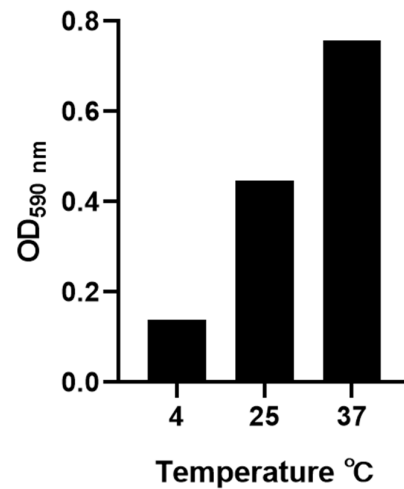


Figure 4 $OD_{590\text{ nm}}$ values of *E. coli* BF at different incubation temperatures.

Effect of pH on BF formation ability: There were significant differences in the total biomass of BF at maturity under different pH conditions. Total BF biomass ($OD_{590\text{ nm}}$) decreased significantly when the environmental pH was 4, 5, 9, and 10, indicating that environmental conditions that were too acidic or too alkaline had a significant inhibitory effect on the growth of *E. coli* BF, which was unfavorable to the formation and maturation of BF. In contrast, at pH 6 and 8, the growth process of BF showed typical stages of adhesion, proliferation, and maturation, with a faster rate of BF formation and a significant increase in biomass. The fastest rate of BF formation and the highest level of biomass were observed when the environmental pH was 7. Therefore, the environmental condition of pH 7 was most favorable for the formation and accumulation of *E. coli* BF (Fig. 5).

Effect of NaCl concentration on BF formation ability: Detection of the effects of different NaCl concentrations on *E. coli* BF formation revealed that the highest $OD_{590\text{ nm}}$ value and the largest amount of BF formation were observed under the condition of 0.5% NaCl; after the NaCl concentration exceeded 0.5%, the $OD_{590\text{ nm}}$ value gradually decreased, and the BF structure tended to be loose. Therefore, 0.5% NaCl was most favorable for *E. coli* BF formation (Fig. 6).

Effect of glucose Concentration on BF formation ability: The effects of different concentrations of glucose on the formation ability of *E. coli* BF were examined, and the results showed that the $OD_{590\text{ nm}}$ value reached the maximum when the concentration of glucose in the medium was 3%, which indicated that the highest amount of BF was formed and the best growth of BF was achieved at this concentration, while the amount of BF formed decreased when the concentration of glucose was lower or higher than 3%, the amount of BF formation decreased, indicating that

either too high or too low glucose concentration had a certain inhibitory effect on the formation of *E. coli* BF. Therefore, 3% glucose concentration is the optimal condition for *E.coli* BF formation (Fig. 7).

Inhibitory effect of Chinese and Western medicines on BF: Table 1 shows that among the herbal extracts, berberine and andrographolide had the best inhibitory effect on bovine-derived *E. coli* BF, both with an MBIC of 64 µg/mL; followed by rhodopsin (128 µg/mL) and baicalin (512 µg/mL). Among the antibiotics, florfenicol had the strongest inhibitory effect, with an MBIC of 32 µg/mL; followed by enrofloxacin (64 µg/mL), kanamycin (128 µg/mL), and ceftazidime (256 µg/mL). Intact BF was formed in the negative control group, and no bacterial growth was observed in the drug control group.

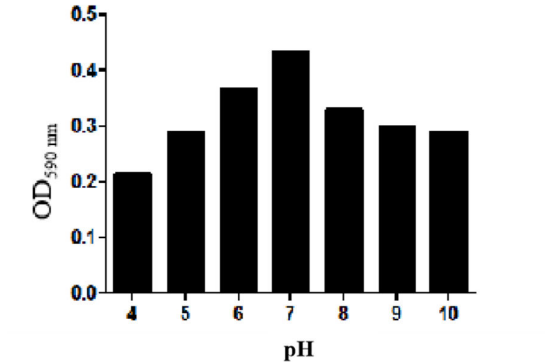


Figure 5 OD_{590 nm} value of *E. coli* BF at different pH values.

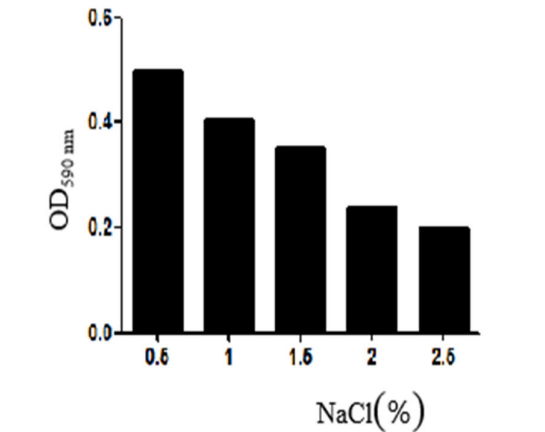


Figure 6 OD_{590 nm} value of *E. coli* BF in different concentrations of NaCl.

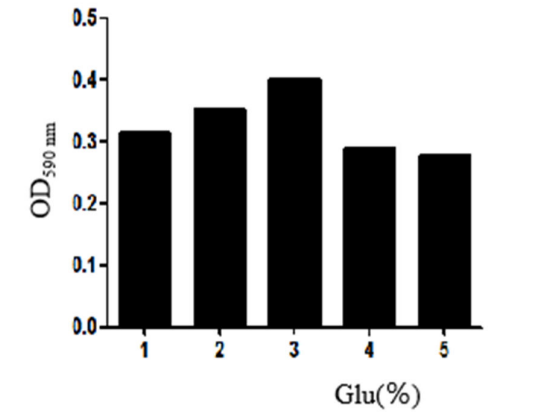


Figure 7 OD_{590 nm} value of *E. coli* BF in different concentrations of glucose.

Table 1 Results of drug inhibition on BF.

Antimicrobial agents	MBIC µg/mL
Baicalin	512
Berberine	64
Andrographolide	64
Emodin	128
Ceftazidime	256
Kanamycin	128
Enrofloxacin	64
Florfenicol	32

Discussion

In recent years, the detection rate of pathogenic *E. coli* has increased annually, and *E. coli* varies with host and geographic region. According to relevant reports, the detection rate of pathogenic *E. coli* from mink is about 51.3%, that of pathogenic *E. coli* from sheep is as high as 48% (Yang *et al.*, 2015; Zhu *et al.*, 2018), and that of pathogenic *E. coli* from cattle is 28.4%-50% (Xia *et al.*, 2017). *E. coli* can also cause urinary, respiratory, and digestive system infections in humans and animals, posing a serious threat to the medical and livestock industry.

BF has multiple formation pathways, and its spatial structure depends on species and environmental conditions (Tolker-Nielsen, 2015). The formation process of BF is dynamic, which can be divided into four stages: initial adhesion stage, agglomeration and proliferation stage, maturity stage, shedding, and spreading stage. At maturity, the BF is divided into four layers: the main BF layer, the connective layer, the conditioned layer, and the matrix layer, from the outside to the inside. Under different environmental conditions and nutrients, BFs are formed in different states and with different degrees of integrity. Relevant studies have shown that changes in nutrient supply can induce BFs to spread to different degrees. Under nutrient-poor conditions, bacteria tend to detach from the BF to escape from unfavorable environments, resulting in a loose and incomplete structure, while under nutrient-rich conditions, bacteria prioritize the allocation of metabolic resources to proliferation and colonization, which promotes the formation of dense and structurally complete BF. In addition, BF growth was also regulated by incubation time and environmental pH, suggesting that BF formation is a

highly dynamic and multifactorial biological process (Tolker-Nielsen, 2015; Roy *et al.*, 2018; Liu *et al.*, 2020).

Nutrient content has a certain effect on BF formation, the results of this experiment showed that LB medium containing 3% glucose favored the formation of *E. coli* BF, while increasing or decreasing the concentration of sugar was detrimental to the production of BF. Tao Jian (Tao *et al.*, 2015) showed that the highest BF formation was achieved when *E. coli* was cultured with M63 medium containing 3% glucose for 72 h, which is consistent with the results of this experiment. In the study of Pillai *et al.* (2004), the concentration of NaCl <1% had an enhancing effect on BF, and 0.5% was the optimum concentration. The appropriate concentration of NaCl can promote the formation of BF, but with the increase of the content shows the phenomenon is observed that the formation of BF is inhibited, which is also confirmed by the results of the present test. Gu Xiaoxiao (Gu *et al.*, 2020) used experiments to prove that *E. coli* grew best in LB medium under the same conditions. In contrast, among the three media used in this experiment, TSB, MH, and LB, the best growth was observed in TSB medium, which indicates that there are differences in the formation of BF in different strains.

According to the in vitro antibacterial effect of traditional Chinese medicine on *E. coli* studied by Zhang *et al.* (2022), the MIC of *Coptis coptitis* was 6.250 mg /mL, and the antibacterial effect was the strongest, followed by Cozi, Phellodendri, and Cen. Berberine and andrographolide had the best inhibitory effect on the growth of *E. coli* BF, and the minimum inhibitory concentration was 64 µg/mL, next is emodin (128 µg/mL). The three herbal active ingredients, berberine, andrographolide, and emodin, may inhibit the formation of *E. coli* BF through their different mechanisms. Among them, berberine mainly blocks inter-bacterial communication and adhesion by interfering with the bacterial group sensing (QS) system (Engelbertsen *et al.*, 2019), inhibiting extracellular polysaccharide (EPS) synthesis and flagellar motility (Yao *et al.*, 2020); Andrographolide inhibits bacterial growth and BF formation by inhibiting the expression of bacterial virulence factors and inducing intracellular oxidative stress (Lee *et al.*, 2017; Katara *et al.*, 2020), while emodin further hinders BF stabilization and maturation by disrupting bacterial cell membrane structure, inhibiting the synthesis of EPS, and enhancing the sensitivity of bacteria to antibiotics (Walter and Bex, 2021). The above mechanisms may be the important pathways through which the three agents exert their anti-BF effects. The active ingredients of the above three Chinese medicines can synergistically inhibit bacterial BF formation through different mechanisms. Together, the three act on key aspects of the QS signaling pathway, EPS synthesis, and membrane structural integrity, which have the potential advantages of multi-targeting and low risk of drug resistance, and provide a new strategy for anti-BF therapy.

Presently, there has been extensive research and an in-depth understanding of the formation mechanism of *E. coli* BF. BF has an important role in enhancing bacterial drug resistance, warding off the host immune system, and contributing to recurrent infections, and is

one of the major causes of the persistence of many chronic infectious diseases. Clinically, when antimicrobial therapy is ineffective, in addition to considering drug resistance, attention should be paid to BF formation in pathogenic bacteria. *E. coli* often attaches to medical devices such as catheters and tracheal tubes, or forms BF in bladder epithelial cells, triggering chronic or persistent infections. Because BF can induce adaptive resistance, it is often difficult to completely clear the infection with antibiotics alone. Therefore, it is necessary to combine physical methods, phages, metal ions, and other means to destroy the structure of BF, and then combine with antimicrobial drugs to treat free-living bacteria, in order to improve the therapeutic efficacy (Chen *et al.*, 2015; Lee *et al.*, 2019). BF is highly adaptable to survive in unfavorable environments, making it difficult to be cleared by antibiotics and recognized by the immune system, and is a significant cause of hospital-acquired and chronic infections, placing a heavy burden on the healthcare system.

Therefore, inhibition of BF formation is important for controlling pathogenic *E. coli* infections. In this study, we analyzed the BF formation ability and influencing factors of a bovine-derived pathogenic *E. coli* in Tongliao area and found that changes in environmental and nutritional conditions significantly affected the growth of BF. This study provides theoretical support for an in-depth exploration of the relationship between BF and *E. coli* pathogenicity.

Acknowledgments

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References

- Cai Y, Zhou XK, Jiang CY, Zeng YB, Xu ZW and Zhu L 2020. Isolation and identification of pathogenic *Escherichia coli* and *Cryptobacterium septicum* mixed infections of porcine lung origin and detection of major virulence factors. *Jiangsu J Agric Sci.* 36(01): 254-256.
- Chen S, He N, Yu J, Li L, Sun F, Hu Y, Deng R, Zhong S and Shen L 2015. Inhibitory effect of 2-mercaptoethane sulfonate on the formation of *Escherichia coli* biofilms in vitro. *Mol Med Rep.* 12(4): 5223-5230.
- Engelbertsen D, Autio A, Verwilligen RAF, Depuydt MAC, Newton G, Rattik S, Levinsohn E, Saggu G, Jarolim P, Wang H, Velazquez F and Lichtman AH, Luscinskas FW 2019. Increased lymphocyte activation and atherosclerosis in CD47-deficient mice. *Sci Rep.* 9(1): 10608.
- Gu XX, Wu Q, Tao QXC, Ma X, Li J, Han ML, Zhou X, Huang X, Wu TZ, Zhang XX and Zhong FG 2020. Analysis of biofilm formation of *E. coli* from calf encephalitis and its influencing factors. *J Anim Sci Vet Med.* 52(07): 92-96.

- Jang J, Hur HG, Sadowsky MJ, Byappanahalli MN, Yan T and Ishii S 2017. Environmental *Escherichia coli*: ecology and public health implications-a review. J Appl Microbiol. 123(3): 570-581.
- Jiang Y and Sheng PF 2014. Detection of HPI and LEE virulence island related genes in chicken *Escherichia coli*. China Poultry. 36(21): 54-56.
- Katara JL, Verma RL, Parida M, Ngangkham U, Molla KA, Barbadikar KM, Mukherjee M, C P, Samantaray S, Ravi NR, Singh ON and Mohapatra T 2020. Differential expression of genes at panicle initiation and grain filling stages implied in heterosis of rice hybrids. Int J Mol Sci. 21(3): 1080.
- Lee G, Chung HS, Lee K, Lee H, Kim M and Bae H 2017. Curcumin attenuates the scurfy-induced immune disorder, a model of IPEX syndrome, with inhibiting Th1/Th2/Th17 responses in mice. Phytomedicine 33: 1-6.
- Lee JH, Kim YG, Raorane CJ, Ryu SY, Shim JJ and Lee J 2019. The anti-biofilm and anti-virulence activities of trans-resveratrol and oxyresveratrol against uropathogenic *Escherichia coli*. Biofouling 35(7): 758-767.
- Leimbach A, Hacker J and Dobrindt U 2013. *E. coli* as an all-rounder: the thin line between commensalism and pathogenicity. Curr Top Microbiol Immunol. 358(1): 3-32.
- Liu Y, Pan C, Ye L, Si Y, Bi C, Hua X, Yu Y, Zhu L and Wang H 2020. Nonclassical Biofilms Induced by DNA Breaks in *Klebsiella pneumoniae*. mSphere 5(3): e00336-20.
- Pillai SK, Sakoulas G, Eliopoulos GM, Moellering RC Jr, Murray BE and Inouye RT 2004. Effects of glucose on *fsr*-mediated biofilm formation in *Enterococcus faecalis*. J Infect Dis. 190(5): 967-970.
- Roy R, Tiwari M, Donelli G, Tiwari V 2018. Strategies for combating bacterial biofilms: A focus on anti-biofilm agents and their mechanisms of action. Virulence 9(1): 522-554.
- Schilcher K and Horswill AR 2020. Staphylococcal biofilm development: Structure, regulation, and treatment strategies. Microbiol Mol Biol Rev. 84(3): e00026-19.
- Sharma G, Sharma S, Sharma P, Chandola D, Dang S, Gupta S and Gabrani R 2016. *Escherichia coli* biofilm: development and therapeutic strategies. J Appl Microbiol. 121(2): 309-19.
- Tao J, Cao BB, Wei FS, Zhou QQ, Han L, Cheng HF, Wang YB and Hu H 2015. A study of the factors influencing the formation of the biofilm of pathogenic *Escherichia coli* O157:H7. China J Prev Vet Med. 37(08): 600-603.
- Tolker-Nielsen T 2015. Biofilm development. Microbiol Spectr. 3(2): MB-0001-2014.
- Walter K and Bex P 2021. Cognitive load influences oculomotor behavior in natural scenes. Sci Rep. 11(1): 12405.
- Wang XY, Song YH, Jing W, Zhu XQ, Xu Q and Du XD 2017. Localisation and genetic environment of the ESBL gene of chicken origin in *Escherichia coli* BlaCTX-M-125. Jiangsu J Agric Sci. 33(01): 141-145.
- Wu LN, Liu YG, Zhang YM, Mao YW, Liang RR, Yang XY, Luo X, Dong PC and Zhu LX 2022. Inhibitory effect of lactic acid and peracetic acid on *Escherichia coli* O157:H7 biofilm and its mechanism. Food Sci. 43(09): 10-18.
- Xia QQ, Wen SS, Ma SQ, Wu Q, Zhao LL, Wei CW, Ge JW and Chen HY 2017. Detection and analysis of biofilm formation ability, drug resistance and virulence island genes of pathogenic *Escherichia coli* from mink. China J Prev Vet Med. 39(05): 361-365.
- Yang SQ, Ao RGL, Wang CJ and Zhou T 2015. Serotype, virulence gene detection and drug resistance analysis of pathogenic *Escherichia coli* from cattle in Hohhot, Inner Mongolia. China J Prev Vet Med. 37(10): 761-764.
- Yao X, Lv Y, Yu H, Cao H, Wang L, Wen B, Gu T, Wang F, Sun L and Xin F 2020. Site-directed mutagenesis of coenzyme-independent carotenoid oxygenase CSO2 to enhance the enzymatic synthesis of vanillin. Appl Microbiol Biotechnol. 104(9): 3897-3907.
- Zhang TY, Huang SH, Dai Y, Tao SR, Ma Y, Peng JJ 2022. In vitro bacteriostatic effect of Baigongxuan in combination with other traditional Chinese medicines on *Escherichia coli* in vitro, China J Vet Med. 41(02): 78-81.
- Zhu CT, Mei YY, Zhu LL, Xu Y, Sheng S and Wang J 2018. Recombinant *Escherichia coli* BL21-pET28a-egfp cultivated with nanomaterials in a modified microchannel for biofilm formation. Int J Mol Sci. 19(9): 2590.