The SOS response may promote the transfer of resistance genes and plasmids by indole in *Escherichia coli*

Shuoyan Lv
Jilin Agricultural University
Yue Guo
Changchun Institute of Biological Products Co Ltd
Hong Chen
Jilin Agricultural University
Ruisen Huang
Jilin Agricultural University
Songlei Guan (17736856@qq.com)
Jilin Agricultural University

Research

Keywords: resistance genes, SOS response, indole, horizontal transmission

Posted Date: October 20th, 2021

DOI: https://doi.org/10.21203/rs.3.rs-989737/v1

Abstract

Bacterial resistance is caused by a variety of causes. SOS response is the main stress mode for bacteria to adapt to environmental stress and repair damage, and the abuse of antibiotics is the main factor inducing SOS response.In order to clarify the role of SOS response in bacterial drug resistance.In this study, we induced SOS response by cefotaxime sodium,then exogenous indole, it was found that the conjugation efficiency increased significantly after SOS response and exogenous indole, at most increased 50 times,and drug-resistance genes (*bla*$_{CTX-M-9G}$, *fosA3*)and plasmids (IncI2,IncFIB) can be transferred between bacteria in the process of SOS.Moreover,the change trends of extracellular indole level, sensor pump (*acrEF* ,mtr mRNA) on cell membrane surface and *tnaA* mRNA were consistent with the *ibpA* mRNA downstream of SOS response.in which indole may play the role of "messenger".The above results show that SOS response can promote the horizontal transfer of drug-resistance genes, which is completed by regulating the level of indole, which plays a messenger role in the process of conjugation. Therefore, inhibiting SOS response by controlling the level of antibiotics in the environment, or directly interfering with the chemical synthesis of indole will help to reduce the transmission of drug resistance genes.

Background

Bacterial DNA damage repair procedure, also known as SOS response, is the stress response of bacteria to adapt to the environment and repair damage[1]. This phenomenon often appears in the during of bacteria survival. When bacterial DNA are severely damaged, the normal replication and repair system are useless, so the SOS response initiated. The classic SOS process mainly relies on the regulation of RecA and LexA molecules[2]. The process is as follow: once DNA is damaged, double strand breaks form single-stranded DNA (ssDNA). The ssDNA binds to the coenzyme RecA to form RecA filaments. The filaments interact with the repressor protein LexA cleavage of the repressor protein LexA, then more than 40 downstream genes involved in DNA repair are released. After the repair is over, ssDNA disappears. RecA loses its coenzyme activity, and LexA self-cleavage is reduced, reforms a dimer and binds to the promoter region, and continues to inhibit the expression of DNA repair genes[3]. Moreover, many exogenous factors can also initiate the response through non-dependent RecA pathway. For example, β-lactam antibiotics can activate the histidine kinase of the bacterial membrane, regulate the DpiA/B two-component system, regulate protein Hu directly promotes the transcription of RpoS or competitively replaces the LexA protein to initiate the SOS response[4].

Various pressure in the environment can induce SOS response, including physical (ionizing radiation, ultraviolet, hydrostatic pressure) and chemical factors (mitomycin C, oxidant, antibiotics, etc.), and antibiotics are the most common factors. The horizontal transmission of drug resistance genes is an important mechanism leading to the increasingly serious drug resistance of bacteria.Bianca Hochhut *et al* have confirmed that the SOS response can promote the transfer of some resistant plasmids and play an important role in the process of bacterial resistance[5]. However, the mechanism of SOS response

affecting resistance genes transfer has not yet been clarified. The small heat shock protein ibpA released by SOS reaction can indirectly regulate the level of indoles, and studies have shown that indoles also play a role in the transfer of drug resistance genes.In this study, β-lactam antibiotics were used to induce the SOS response in *E. coli*, the exogenous indole was added at the same time, and to observe their effect on resistance genes and plasmids conjugation transfer and mechanism.

1. Materials And Methods

1.1 Materials

1.1.1 Bacterial strain

E. coli BL21 (F-, pET28a-EGFP, Kana+) which was induced to establish SOS response by Cefotaxime sodium(CTX), was used as the recipient in process of conjugation(MIC_{BL21} is 2^{-4}μg/ mL). The two wild type chicken mutidrug resistant *E. coli* QALAK1-2 and FTK($MIC_{QALAK1-2\ and\ FTK}$ >2^{8}μg/ mL can be used as donors in the process of conjugation (the *trbC* [6] and *traF*[7] genes were used as genetic markers (see Additional file ,Tab.1) of the conjugative plasmid).

1.1.2 Reagents and instruments: see Additional file, Tab.6 and Tab.7.

1.2 Methods

1.2.1 Detection mRNA levels of marker molecules of SOS response

SOS response was induced by CTX, and qPCR was used to detect the relative quantification of genes in RecA-dependent (*lexA & recA*) and non-RecA-dependent (*dpiA, dpiB, rpoS, hu*) pathways. Western-blot was used to detect the protein expression of RecA and LexA. The rabbit polyclonal antibodies of RecA and LexA were selected as the primary antibodies (1: 1000), and the goat anti-rabbit IgG was selected as the secondary antibody (1: 5000), with GAPDH as the internal reference (Schedule.2). The GraphPad Prism 6.01 and Image-J software was used for data analysis.

1.2.2 Conjugation

Next, the donor and recipient bacteria were subjected to bacterial conjugation in the process of the SOS response. Experiments used the filter membrane method. The recipient bacteria were cultured in a shaker at 37°C and 180rpm for 14~16h. 1ml of recipient and donor bacteria were respectively inoculated into LB medium (recipient bacteria 50μg/mL Kan+), cultured at 37°C and 180rpm with shaking for 4~6h until OD_{600}=0.8. The culture of 1ml donor bacteria, 7000rpm, 2min, discarded supernatant precipitation. The

donor and recipient bacteria were mixed with 1: 1 and 1: 2 to 200 μL. The mixed bacterial solution was placed on the filter membrane and cultured at 37°C for 10~12h or 15~18h. Screening of conjugant based on CTX resistance (8μg/ml) and green fluorescence colony. The number of conjugant were calculated in each group, and the average value was taken three times. The conjugation freqency = the average colony number of conjugant T / the average colony number of recipient bacteria R. The validation of conjugant colonies was based on the same resistant genes and plasmids as the donor bacteria (see Additional file, Tab.3). In addition, 2 mM, 1 .5mM, 1mM and 0.5 mM exogenous indole were added respectively during the conjugation, and concertration of the donor and recipient bacteria were mixed at 1:1, 1:2 and 2:1. The mixed bacterial solution was grown on the filter membrane. After culture, the bacteria liquid on the filter membrane was diluted in 10 ml LB liquid medium by shock, and then diluted and coated in solid medium successively.Finally, CTX resistance and green fluorescent colonies were used as the standard for conjugonss.(Fig. 2A, Fig. 2B) In addition, the conjugons were verified by electrophoresis to see whether there were drug-resistant genes and plasmid transfer in the conjugons (see Additional file, Fig. 6). The resistant genes of *E. coli* QALAK1-2 included *mcr-1,tetA,qnrA,fosA3,bla*$_{CTX-M-9G}$, and the plasmids included IncFIB and IncI2. The resistant genes of *E. coli* FTK include *mcr-1,tetA,oqx A,oqx B,qnr B,fosA3 and bla*$_{CTX-M-9G}$, and its plasmids contain IncY, IncHI2 and IncI2(see Additional file ,Tab.4,Tab.5).

1.2.3 Determination Of The Extracellular Indole Concentration

The concentration of indole in the culture broth of recipient strain *E. coli* BL21 was determined by HPLC-MS. Mass spectrometry detection conditions (see Additional file, Tab.8). Liquid phase analysis conditions: Waters BEH C18 column, 2.1×50mm, 1.7 μm, column temperature 25°C. The injection volume was 10 μL, samples were determined twice. The liquid phase gradient elution conditions (see Additional file, Tab.9).

(1) Preparation of standard products:

Configure 1mg / mL ethanol indole standard solution, according to the proportion of 1: 9 to add 5 % acetic acid and ethanol (indole acidification easy ionization), make up 0.25μg / ml, 0.50μg / mL, 0.75μg / mL, 1.00μg / mL, 2.50μg / mL, 3.75μg / mL.Y = 1184.73x-60.48, R^2 = 0.9965(see Additional file,Fig. 1).

(2) Preparation of samples:

50μL bacterial liquid was inoculated in 50ml LB liquid medium (Kan+), 200rpm, 37 ℃ shaking culture about 2~3h to OD600 was 0.6, 0.01μg / mL CTX was added, induced 5h or 15h to collect bacterial liquid, each set of 4 parallel samples, set without adding bacterial liquid as the control group. 4000 rpm for 10 min, discard the precipitation, the supernatant was filtered with a filter membrane and dried at 60°C. The dry powder was added with 5 % acetic acid and ethanol according to 1: 9, 4000 rpm for 5 min, discard the precipitation and leave the supernatant.

1.2.4 The relative quantityof ibpA, tnaA, acrEF, mtr **genes**

The mRNA levels of tryptophanase tnaA[8,9], *ibpA* (small heat shock protein) gene released by SOS response, indole sensor pump *acrEF, mtr*[10] in recipient strain *E.coli* BL21 before and after conjugation were detected by qPCR(see Additional file, Tab.5).

2. Results

2.1 Establishment Of Sos Response

After the induction of CTX at three concentrations of 0.01 μg/mL, 0.03 μg/mL and 0.05 μg/mL, finally the optimal concentration for the SOS response in both RecA-dependent and independent pathways was 0.01 μg/mL CTX, and the time was>4h (Additional file,Fig. 2).The results showed that, compared with the untreated group (control group), the expression of LexA and RecA protein both increased significantly at 3h, and decreased at 4h, and SOS response was triggered after 4h. It was consistent with the trend of mRNA transcription(see Additional file,Fig. 3).

2.2 Conjugation

2.2.1 The Selection Of Donor And Recipient Bacteria

The *trbC* gene was detected at 255bp and the *traF* gene was detected at 143bp. It proves that QALAK1-2 carry conjunctive plasmids(see Additional file ,Fig. 4A,B), which belongs to F (+).*E. coli* QALAK1-2 carried *mcr-1*, $bla_{CTX-M-9G}$, and *fosA3* resistance genes, and the plasmid types were Inc I2 and Inc FIB (see Additional file,Fig. 4C). Therefore, *E. coli* QALAK1-2 with multidrug resistance genes and F (+) plasmids can be used as a donor for subsequent conjugation procedure. Electrophoretic verification of another drug-resistant *E. coli* FTK showed that it contained the genetic markers trbC and traF genes of conjugation plasmids (see Additional file,Fig. 9). FTK can also be used as donor bacteria in conjugation transfer experiments.

2.2.2 Screening Conjugonss

The donor strain *E. coli* QALAK1-2 and recipient strain *E. coli* BL21 were used for conjugation. The colonies showing resistance to CTX and green fluorescence were conjugants, in which the concentration ratio of recipient to donor was 1: 2, and the conjugation frequency was the highest at 15~18 h(see Additional file,Fig. 5). Selected colonies carried drug resistance genes and plasmids such as $bla_{CTX-M-9G}$ *or foxA3 or mcr-1 or* IncI2 *or* IncFIB ,indicating that the conjugants was successful(see Additional file,Fig. 6).

After sorting out the data,the conjugation freqency of the control group was approximately 5.12×10^{-1}, 1.86×10^{-1}, 2.56×10^{-1}, and 5.12×10^{-1}, respectively. Under the 0.01μg/mL CTX, the conjugation freqency (6.28×10^{-1}, 6.05×10^{-1}, 5.58×10^{-1}, 1.00×10^{0}) was significantly higher than that of the control group (P<0.05 or P<0.01). (Fig. 2C). The maximum frequency increase can be 50 times.

2.2.3 The concentration of indole in the extracellular medium of the recipient strain BL21 increase after the SOS response

The results show that with the addition of 0.01μg/ml CTX, the concentration of indole in *E. coli* BL21 culture broth at 5h and 15h was significantly higher than the control group (P<0.05), and there was no significant difference between 5h and 15h (P>0.05). The transcription level of tryptophanase (*tnaA*), which regulates indole production, was significantly increased at 4h and 15h after adding the CTX (P<0.05), which was consistent with the changing rend and time of extracellular indole concentration (see Additional file,Fig. 8). The highest indole concentration in E.coli BL21 was 0.358μg/ml after CTX was added for 5h. After 15h, the highest indole concentration was 0.51μg/ml.It shows that the amount of indole production increases at 5h (SOS response starts) and accumulates at 15h (conjugation transfer stage). Tab.1 and Additional file,Fig. 7-8.

Tab.1 Comparison of indole concentration in *E. coli* BL21 culture medium

Group	Retention time /min	Concentration(±s, n=4)	Area Y	*t value*	*P value*
Control	12.57	0.09±0.31	49.116	–	–
CTX 5h	12.62	0.27±0.088	256.433	3.707	0.010
CTX 15h	12.60	0.18±0.33	126.424	3.687	0.010

Note: *vs* control; *co*ntrol; *vs* 5h group, t=0.073,P=0.118.

2.2.4 The mRNA levels of ibpA and indole sensing pumps acrEF, mtr were increased

After the SOS response started, the untreated group served as the control group, and the transcription levels of the recipient bacteria's *ipbA* and the efflux pump *acrE* and *acrF* genes were significantly increased (P<0.01 or P<0.05). After bacterial conjugation, the transcription levels of the *ibpA* and the efflux pumps *acrE* and *acrF* increased significantly (P<0.01). The transcription levels of *acrEF* and *mtr* genes were significantly increased when CTX was added for 5h (P<0.01 or P<0.05), and the transcription levels of *acrE* and *mtr* were significantly increased when CTX was added for 5h (P<0.05). (Fig. 1)

2.2.5 Changes Of Conjugation Efficiency After Exogenous Indole

In the process of conjugation between another multidrug resistant *E. coli* FTK and BL21, indole with concentration of 2,1.5,1,0.5 mM was added externally. The result showed that conjugons frequency was improved significantly at 1,1.5,2 mM (Fig. 2A). After adding exogenous indole at a concentration of 2,1.5 and 1mM, the colonies developed after dilution and coating contained more zygotes, that is to say, conjugation frequency was higher (Fig. 2B).Moreover, in the SOS reaction group, that is, the solid medium with CTX and exogenous indole added, the number of conjugates produced by coating was higher, and the conjugation frequency increased more obviously. In addition of 2mM exogenous indole and CTX induced medium, the growth of green fluorescent colonies increased significantly.The results indicate that both indoles and SOS response can promote the transfer of drug-resistant genes.

3. Discussion

The presence of some drug-resistant bacteria in the environment makes it difficult to treat the disease, while the horizontal spread of resistant genes is one of the main reasons for the increasing severity of bacterial resistance. Resistant genes are mainly transmitted between bacteria with movable elements (including plasmids, Integrons, transposons, etc.). In 2004, Bianca Hochhut et al. found that the SOS response can promote the horizontal transfer of resistance plasmid[5], but so far the mechanism has not been clarified. Antibiotic pressure is one of the most common reasons that induce bacterial SOS response. Some scholars believe that long-term abuse of antibiotics and the subsequent SOS response are important factors for bacteria to acquire drug resistance [11–12].

3.1 SOS responses promote the horizontal transmission of resistance genes and plasmids

In this study, CTX-induced SOS response in *E. coli* BL21, and the conclusions of Bianca Hochhut et al. were verified[10]. After *E. coli* BL21 was induced with 0.01 μg/mL CTX, the levels of *recA* and *lexA* mRNA were the highest at 3h, decreased after 4h, and stabilized at 5h (see Additional file,Fig. 2). The protein expression trend is consistent with the gene transcription (see Additional file,Fig. 3), indicating that the SOS response was triggered after 4h. In the independent pathway, the trends of *dpiA/B*, *hu*, and *rpoS* mRNA also indicated the SOS response has been triggered(see Additional file,Fig. 1). From the results of the conjugation experiment, the SOS response promotes the transfer of resistance genes $bla_{CTX-M-9G}$ and *fosA3* which may be transferred with the plasmid IncI2 or IncFIB from donor to recipient. By inducing SOS response with CTX, a significant increase in the number and frequency of conjugated colonies was observed, in particular the conjugated frequency was about 50 times that of the control group.It indicated that SOS response could promote the horizontal transmission of resistance genes.

As early as 1986, it was reported that β-lactam antibiotics can increase the horizontal transfer freqency of tetracycline-resistant plasmids by 100 to 1000 times. β-lactam antibiotics promote the transfer of SCCmec carrying the methicillin-resistant Staphylococcus drug resistance genes *mecA* and *mecC* transfer elements by inducing the SOS response [13]. SXT is a junction transfer element carrying multiple

resistance genes of chloramphenicol, sulfamethoxazole, trimethoprim and streptomycin. Researchers tried to use SetR to inhibit the transfer of SXT, but the SOS response could promote the self-lysis of SetR. After the inactivation of SetR, the inhibition of SetC and SetD was relieved. The latter two were the activators of SXT conjugation transfer and integrase, but increased the frequency of SXT conjugation transfer and promoted the horizontal transfer of drug resistance genes. Horizontal transfer of genes promotes the spread of resistance genes not only restricts resistance to a single cell, but promotes the resistance and adaptation of the entire flora to drugs, which reflects the "smartness" of bacteria in evolutionary stfreqencygies and functional mechanisms [5].

3.2 Indole may be used as a signal molecule to help the horizontal transfer of resistance genes during the SOS

Indole is a hydrophobic Nitrogen-containing heterocyclic aromatic compound synthesized by bacteria using tryptophan under the catalysis of tryptophanase. So far, studies have found that about 150 bacteria, including *Escherichia coli*, can synthesize indole with tryptophan as a raw material under the action of tryptophanase [14]. Since the discovery of indole, it has been considered only a physiological and biochemical identification indicator of bacteria. Until recently, it has not been confirmed that indole is a new interspecies and transboundary signaling molecule [15–19]. The results of this study suggest that indole may play a role as a signal molecule promoting resistance genes transmission in SOS response: The signal molecule must be produced and accumulated in a specific period or environment[16]: This study found that the concentration of extracellular indole increased after the SOS response was initiated and accumulated during the conjugation phase(Tab.1 and Additional file,Fig. 7). The mRNA of *tnaA* gene was consistent with the trend and time of extracellular indole level (see Additional file ,Fig. 8), suggesting that indole has the characteristics of production and accumulation of signal molecules. The accumulation of signal molecules outside the cell can be recognized by specific sensors[16]:In this study,the mRNA of *acrEF,mtr* (sensing pump)in the recipient strain and the conjugants were significantly increased (Fig. 1), consistent with the changing trend of indole concentration measured in extracellular, suggesting that MTR and AcrEF-TolC pump sense and recognize accumulation of indole inside and outside the cell. The cumulative effect of the signal molecule is consistent with the response it regulates[16]: In this study,the trend of indole concentration in extracellular is consistent with the drug induction time established by the SOS response. Signal molecules must cause physiological changes caused by excess metabolism and decomposition[16]: In this study, indole also promoted the transfer of resistance genes except normal metabolism and decomposition. Exogenous indole content can significantly increase the efficiency of drug resistance gene transfer(Fig. 2A). In addition, the efficiency of the transfer of drug-resistant genes was also different with different indole concentrations, and the efficiency of drug-resistant genes and plasmids was most obviously improved when the indole concentration was 2mM, 1.5Mm and 1mM. Combined with SOS response, the efficiency of drug resistant gene transfer was more significant.The above results suggest that indole is likely to play the role of “messenger” to help resistance genes dissemination.

3.3 SOS responses regulate indole level by activating ibpA

The *ibpA* that released after LexA self-cleavage can regulate the level of indole [20]. DorotaKuczyńska-Wiśnik's[21] study confirmed that *ibpA* can regulate the level of extracellular indole and affect the formation of biofilm, thus affecting drug resistance.In this study,the *ibpA* mRNA levels of the recipient and the conjugants were significantly increased (Fig. 1), Indole's regulatory enzyme *tnaA* mRNA also increased significantly at 4h and 15h (vs 0h, $P<0.05$) (see Additional file,Fig. 7). In addition, the concentration of indole in the extracellular also increased significantly during these two processes (Tab.1 and Additional file,Fig. 7). It shows that SOS response promotes indole production by activating *ibpA*. The process may be as follows: The SOS response caused the self-cleavage of LexA, resulting in the release of DNA repair genes downstream of LexA, including *ibpA*, which regulates the synthesis of indole by tryptophan,then the increased indole stimulates the AcrEF/Mtr sensor pump to efflux indole, resulting in the increase of extracellular indole, and then transmits the "signal for help from antibiotic stress" to the donor bacteria through a certain way,so the resistance genes were transferred to the recipient srain to help resist the pressure of antibiotics. However, the specific pathway of indole information transmission is not clarified [22]. Therefore, it is urgent to verify the pathways and targets of indole, which will help to comprehensively reveal the regulatory mechanism of resistance genes dissemination.

Abbreviations

SOS：Bacteria adapt to environmental stress and repair damage

CTX: Cefotaxime sodium

HPLC-MS: High performance liquid chromatography-mass spectrometry technology

Waters BEH c18: Column name

RT-PCR: Reverse Transcription-Polymerase Chain Reaction

Declarations

Funding

No

Authors’contributions

GSL conceived the project. GY and LSY completed the experment and analyzed the data togetherly. GY and LSY wrote the manuscript. CH and HRS participated the experamental operation. All the authors read and discussed the manuscript. All authors read and approved the final manuscript.

Ethics approval and consent to participate

Not Applicable.

Consent for publication

No

Competing interests

The authors declare that they have no competing interests.

Availability of data and materials

The data and materials are all available from this research.

Acknowledgments

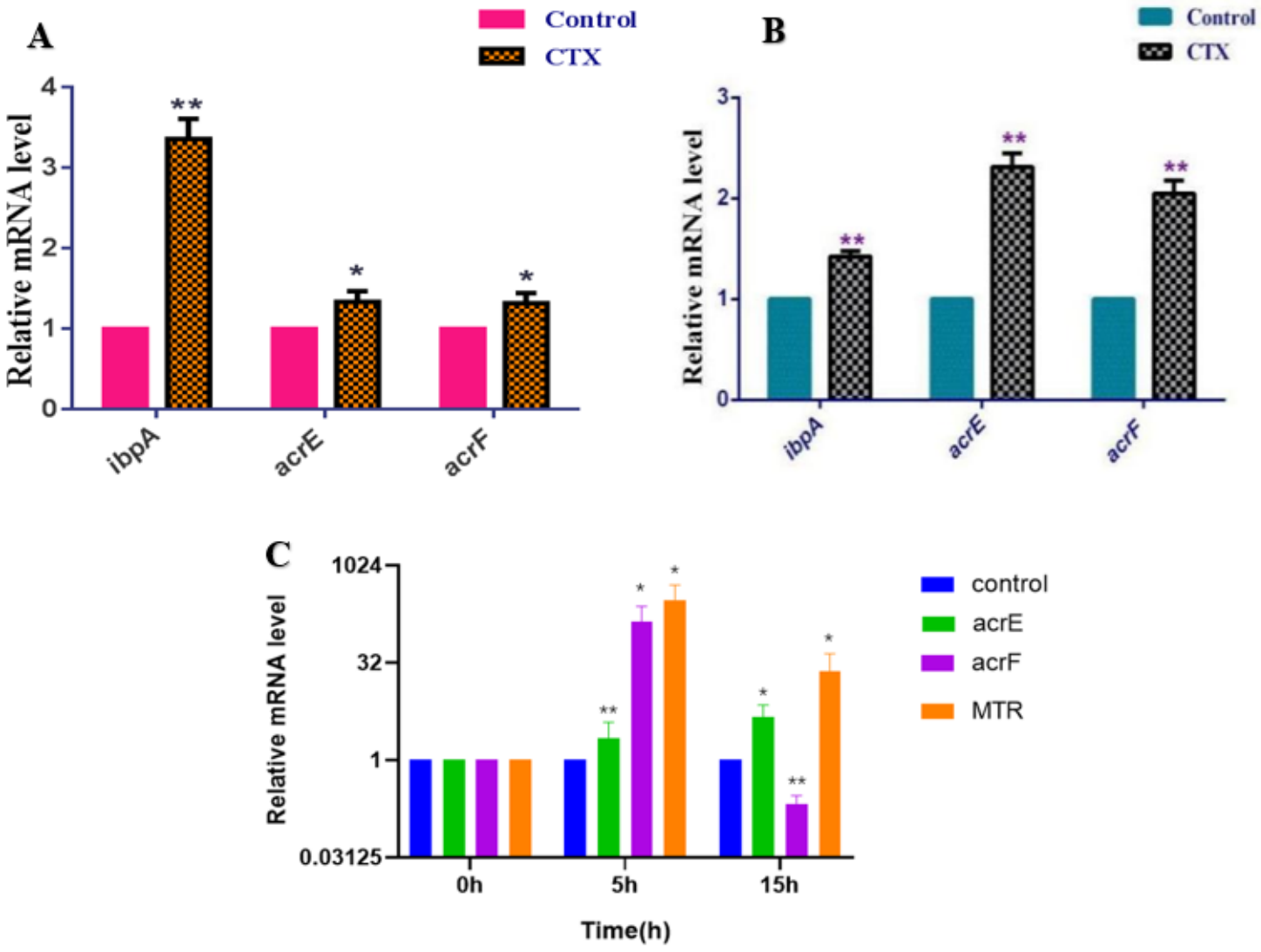
We thank all the teachers and students in Science Building 511 of Jilin Agricultural University for their help

References

1. Shinagawa H. SOS response as an adaptive response to DNA damage in prokaryotes. Exs. 1996;77:221–35.
2. Selwood T, Larsen BJ, Mo CY, Culyba MJ, Hostetler ZM, Kohli RM, Reitz AB, Baugh SDP. Advancement of the 5-Amino-1-(Carbamoylmethyl) -1H -1,2,3 -Triazole -4-Carboxamide Scaffold to Disarm the Bacterial SOS Response. Front Microbiol. 2018 Dec 18; 9:2961. doi: 10.3389/fmicb.2018.02961.
3. Qin TT, Kang HQ, Ping M, et al. SOS response and its regulation on the fluoroquinolone resistance. Annals of Translational Medicine. 2015;3(22):358.doi. 10.3978/j.issn.2305-5839.2015.12.09.
4. Brown ED, Wright GD. Antibacterial drug discovery in the resistance era. Nature. 2016;529(7586):336–43.
5. Piñero-Fernandez S, Chimerel, et al. Indole Transport across Escherichia coli Membranes. J Bacteriol. 2011. doi:10.1128/JB.01477-10. Epub 2011 Feb 4.
6. Gutierrez A, Laureti L, Crussard S, et al. β-lactam antibiotics promote bacterial mutagenesis via an RpoS-mediated reduction in replication fidelity. Nat Commun. 2013;4:1610. doi:10.1038/ncomms2607.
7. Maneewannakul S, Maneewannakul K, Ippen-Ihler K. Characterization of trbC, a new F plasmid tra operon gene that is essential to bacterial conjugation. J Bacteriol. 1991 Jun;173(12):3872–8. doi:10.1128/jb.173.12.3872-3878.1991.
8. [8]Tanimoto K, Ike Y. Analysis of the conjugal transfer system of the pheromone-independent highly transferable Enterococcus plasmid pMG1: identification of a tra gene (traA) up-regulated during conjugation. J Bacteriol. 2002,184(20):5800–4. doi:10.1128/jb.184.20.5800-5804.2002.

9. Kuczyńska-Wiśnik D, Matuszewska E, Laskowska E. Escherichia coli heat-shock proteins IbpA and IbpB affect biofilm formation by influencing the level of extracellular indole. Microbiology. 2010 Jan;156(Pt 1):148–57. doi:10.1099/mic.0.032334-0. Epub 2009 Oct 1.
10. Sasaki-Imamura T, Yano A, Yoshida Y. Production of indole from L-tryptophan and effects of these compounds on biofilm formation by Fusobacterium nucleatum ATCC 25586. Appl Environ Microbiol. 2010 Jul;76(13):4260–8. doi:10.1128/AEM.00166-10. Epub 2010 May 14.
11. Beaber JW, Hochhut B, Waldor MK. SOS response promotes horizontal dissemination of antibiotic resistance genes. Nature. 2004 Jan 1;427(6969):72-4. doi: 10.1038/nature02241. Epub 2003 Dec 21.
12. Garrec H, Drieux-Rouzet L, Golmqrd JL, et al. Comparison of nine phenotypic methods for detection of extended-spectrum β-Lactamase production by. EnterobacteriaceaeJ Clin Microbiol. 2011;5:1048–57. doi:10.1128/JCM.02130-10.
13. Da Re S, Garnier F, Guérin E, et al.The SOS response promotes qnrB quinolone- resistance determinant expression.EMBO Rep,2009,10(8):929-933. doi:10.1038/embor.2009.99.
14. Han Y, Sun MM, Wang JP, et al. Progress on indole: an intercellular signal molecule in microbial communities. Microbiol China. 2015;42(4):736–48. (in Chinese).
15. Wang DD, Ding XD, Rather PN. Indole can act as an extracellular signal in Escherichia coli. J Bacteriol. 2001;183(14):4210–6. doi:10.1128/JB.183.14.4210-4216.2001.
16. Winzer K, Hardie KR, Williams P. Bacterial cell-to-cell communication: sorry, can't talk now gone to lunch! Curr Opin Microbiol. 2002;5(2):216–22. doi:10.1016/s1369-5274(02)00304-1.
17. Lee JH, Lee J. Indole as an intercellular signal in microbial communities. FEMS Microbiol Rev. 2010;34(4):426–44. doi:10.1111/j.1574-6976.2009.00204.x.
18. Lee JH, Kim YG, Baek KH, et al. The multifaceted roles of the interspecies signalling molecule indole in Agrobacterium tumefaciens. Environ Microbiol. 2015;17(4):1234–44. doi:10.1111/1462-2920.12560.
19. Kim J, Park W. Indole: a signaling molecule or a mere metabolic byproduct that alters bacterial physiology at a high concentration? J Microbiol. 2015 Jul;53(7):421–8. doi:10.1007/s12275-015-5273-3. Epub 2015 Jun 27.
20. Pavlov MY,Ehrenberg M.Optimal control of gene expression for fast proteome adaptation to environmental change.PANS,2013,110(51):20527–20532.
21. DorotaKuczyńska-Wiśnik, Matuszewska E, Laskowska E.Escherichia coli proteins IbpA and IbpB affect biofilm formation by influencing the level of extracellular indole.Microbiology, 2010,156(Pt1):148-157. doi: 10.1099/mic.0.032334-0. Epub 2009 Oct 1.
22. Zarkan Ashraf et al. Local and universal action: The paradoxes of indole signaling in bacteria. Trends in microbiology, 2020, 28(7): 566-577. doi: 10.1016/j.tim.2020.02.007. Epub 2020 Mar 20.

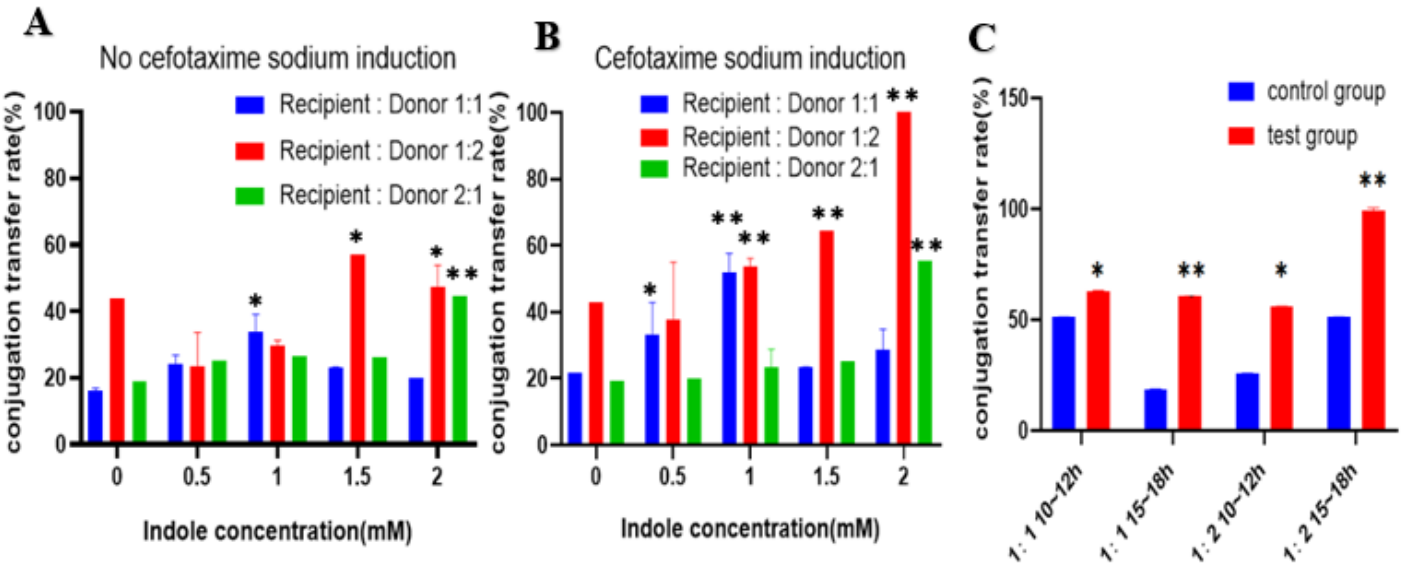
Figures



Note: A,-relative quantification of ibpA, acrE and acrF mRNA in recipient;vs control group,*P<0.05,**P<0.01.B- relative quantification of ibpA, acrE and acrF mRNA in conjugant;vs control group,*P<0.05,**P<0.01.C- relative quantification of acrEF and mtr mRNA of 0.01μg/ml CTX on E. coli BL21 in different time periods; vs 0h, *P<0.05, **P<0.01.

Figure 1

Relative quantification of ibpA, acrE ,acrF and mtr mRNA in recipient and conjugant



Note: A- transconjugative frequency without 0.01μg/mL CTX induction(no SOS response was induced). B-transconjugative frequency induced by 0.01μg/mL CTX(SOS response was induced).*vs* 0mmol/L indole,**P*<0.05,***P*<0.01.C- transconjugative frequency induced by 0.01μg/mL CTX(SOS response was induced). induced by 0.01μg/mL CTX(SOS response was induced) *vs* Endogenous indole.

Figure 2

Effect of exogenous and endogenous indole on conjugation frequency

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- Additionalfile.docx