Yangzhou University
扬州大学

The Quorum Sensing Type I mediates pathogenic mechanism of Avian Pathogenic *E.coli*

Guoqiang Zhu (DVM,MSc,PhD)
COLLEGE OF VETERINARY MEDICINE
YANGZHOU UNIVERSITY

Email:yzgqzhu@yzu.edu.cn
Tel:0514-87972590(Office)
13773564198(Cell Phone)
Wechat:jonhzhu
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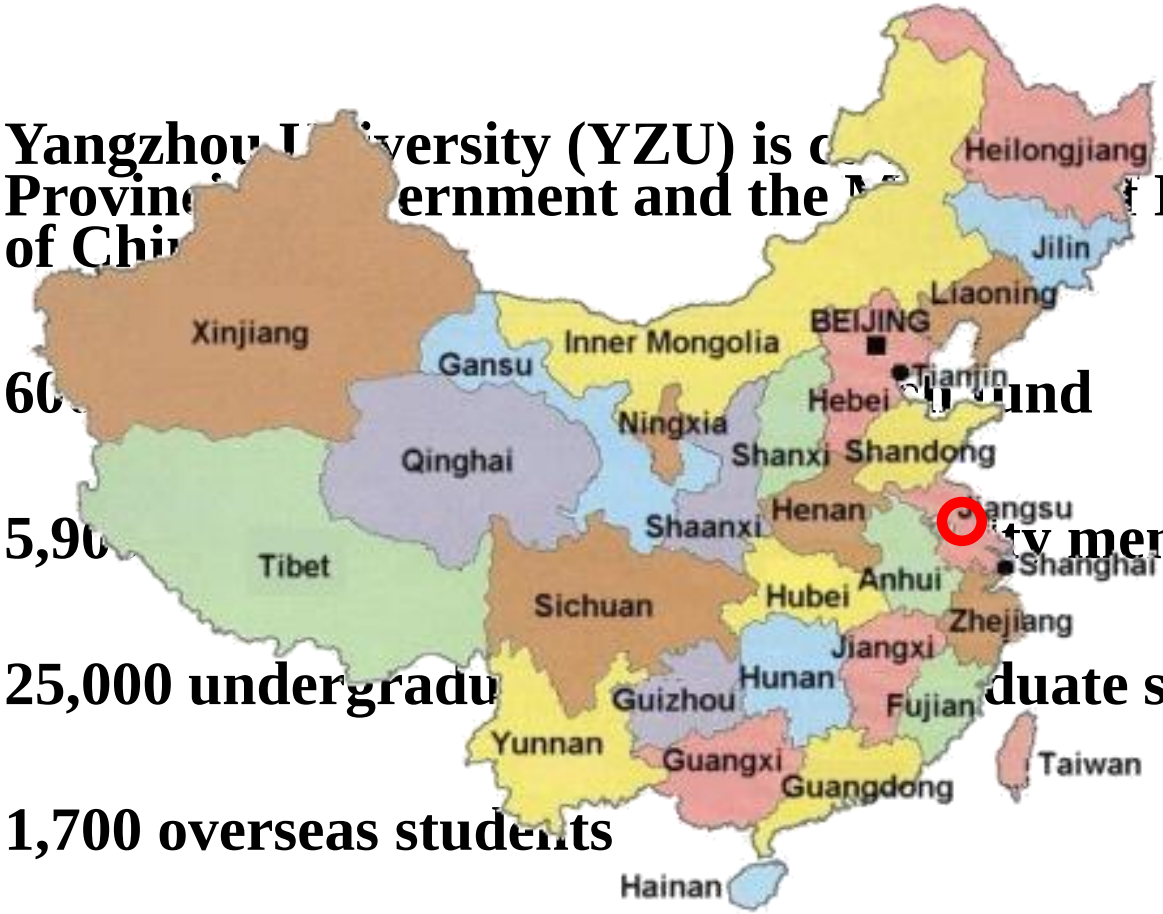
- Yangzhou U[illegible]versity (YZU) is c[illegible] Jiangsu Provinc[illegible]ernment and the M[illegible] Education of Chi[illegible]
- 60[illegible]und
- 5,90[illegible]ty members
- 25,000 undergradu[illegible]duate students
- 1,700 overseas students



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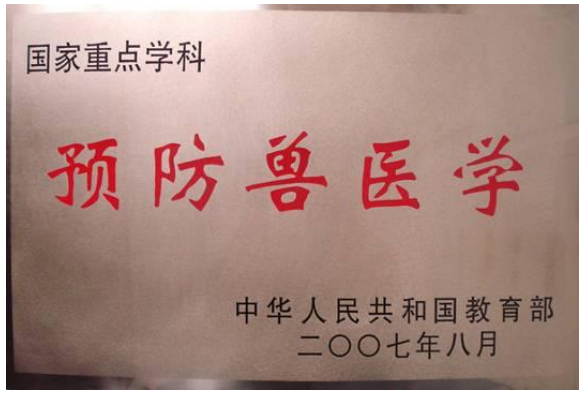
College of Veterinary Medicine



Ranking the third place by subject Veterinary Science in China in 2017-evaluation by the Ministry of Education,China

Veterinary Medicine: Seven-star specialty (top 2 position) according to Chinese alumni network

National Key Discipline: Preventive Veterinary Medicine

Academician of Chinese Academy of Engineering: 1 Professors: 44

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College of Veterinary Medicine

Faculty and Staff

- Staff: 45
- Faculty Members : 100
- Academician of Chinese Academy of Engineering: 1
- Professors: 44
- Associate Professors: 35

Departments

- Basic Veterinary Medicine
- Clinical Veterinary Medicine
- Preventive Veterinary Medicine
- Laboratory Animal Science

Enrolled Students

- Undergraduate students: 381
- Ms Students: 540
- PhD Students: 120

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Lab PI Guoqiang Zhu

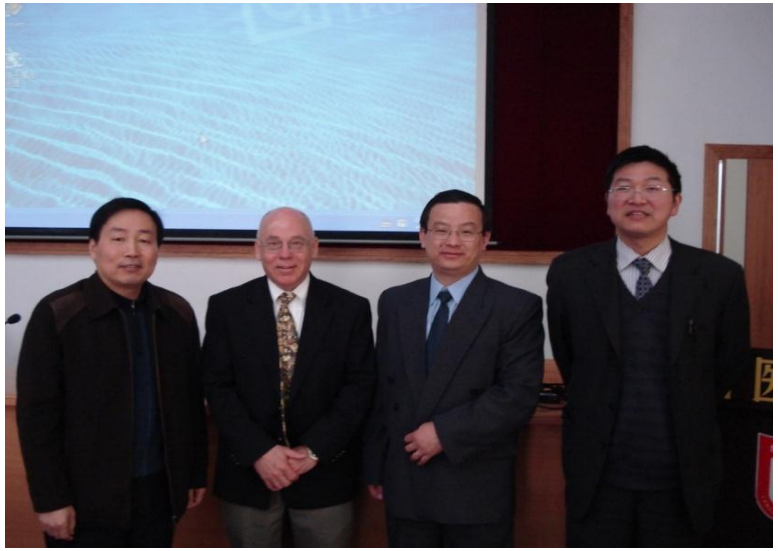
Guoqiang Zhu, Ph.D,

Professor and Chair of the Department of Preventive Veterinary Medicine

- Veterinary Microbiology and Microbial Disease
- Animal Pathogen Molecular Pathogenesis
- Interaction between Microorganisms and Host Cells, Particularly in Bacterial Adhesins-related Field
- *E.coli* and Adhesin Vaccine

Research experience

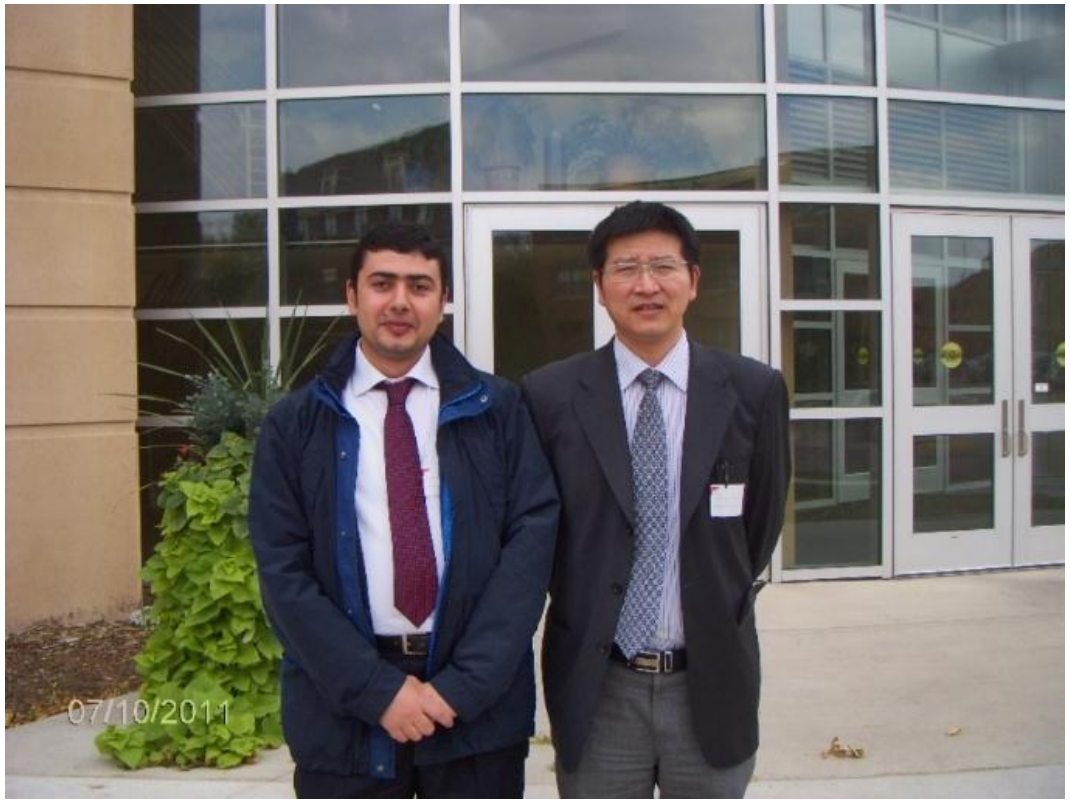
1994-1998 **China Academy of Military Medical Science**, Ph.D
2001-2004 **University of Pennsylvania**, Post doc.
2006-2008 McGill University, Visiting Scholar
2009-2010 University of Maryland, Visiting Scholar
2011 South Dakota State University, Visiting Scholar
2016 Kansas State University, Visiting Scholar



Drs.&Profs. David Francis and Weiping Zhang was visiting YZU in 2010

International collaboration

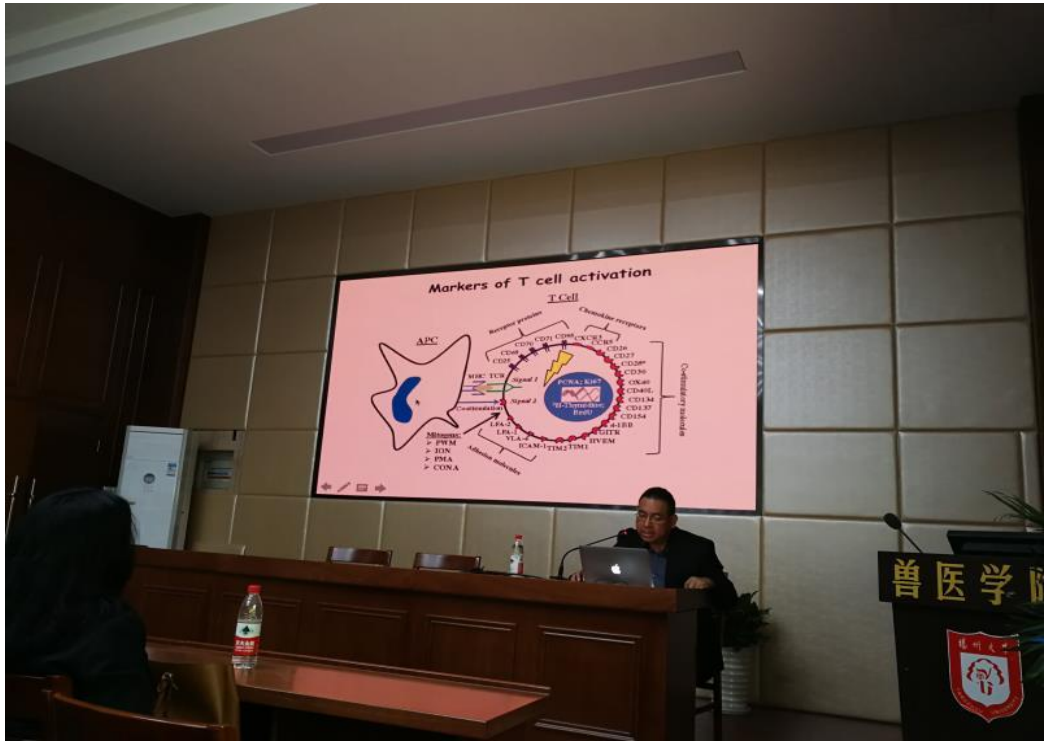
Philip R. Hardwidge, Professor
College of Veterinary Medicine Kansas state University,2013



Visiting Study in SDSU, 2011

International collaboration

Dieter M. Schifferli, Ph.D, Professor
University of Pennsylvania



Fernando Ruiz,Ph.D, assistant Professor
University of Virginia, 2018

International collaboration

Examples of Funding for International collaboration

- “Yangzhou University-Kansas State University of the United States” joint training project for students majored in Veterinary Medicine (Innovative Talents International Cooperation Programme , **2015-2021**, PI, Guoqiang Zhu)

- Cooperation agreement on Co-establishment of Joint Laboratory of Diagnosis of Zoonotic Pathogens between (College of veterinary medicine) Kansas State University and Yangzhou University, **2016-2020**, PI ,Guoqiang Zhu.

- Deep study of the interaction between bacteria and hosts (Bureau of National foreign experts, GDW20143200134, **2014-2019**, CoPI ,Guoqiang Zhu)

International collaboration

Scientific and Technical Friendship Award Yangzhou city

Scientific and Technical Friendship Award Jiangsu province

Background

- Quorum Sensing (QS) is a specific way of communication between bacteria
- Bacteria produce signal molecules and release signals into the environment; when environmental signals reach a certain threshold concentration, QS systems take effects
- QS induce bacteria to exhibit new behavioral characteristics on a population scale, such as bioluminescence, secretion regulation of virulence factors, formation of spores, or formation of biofilm

Background

Quorum Sensing

QS: Not working at low bacterial population

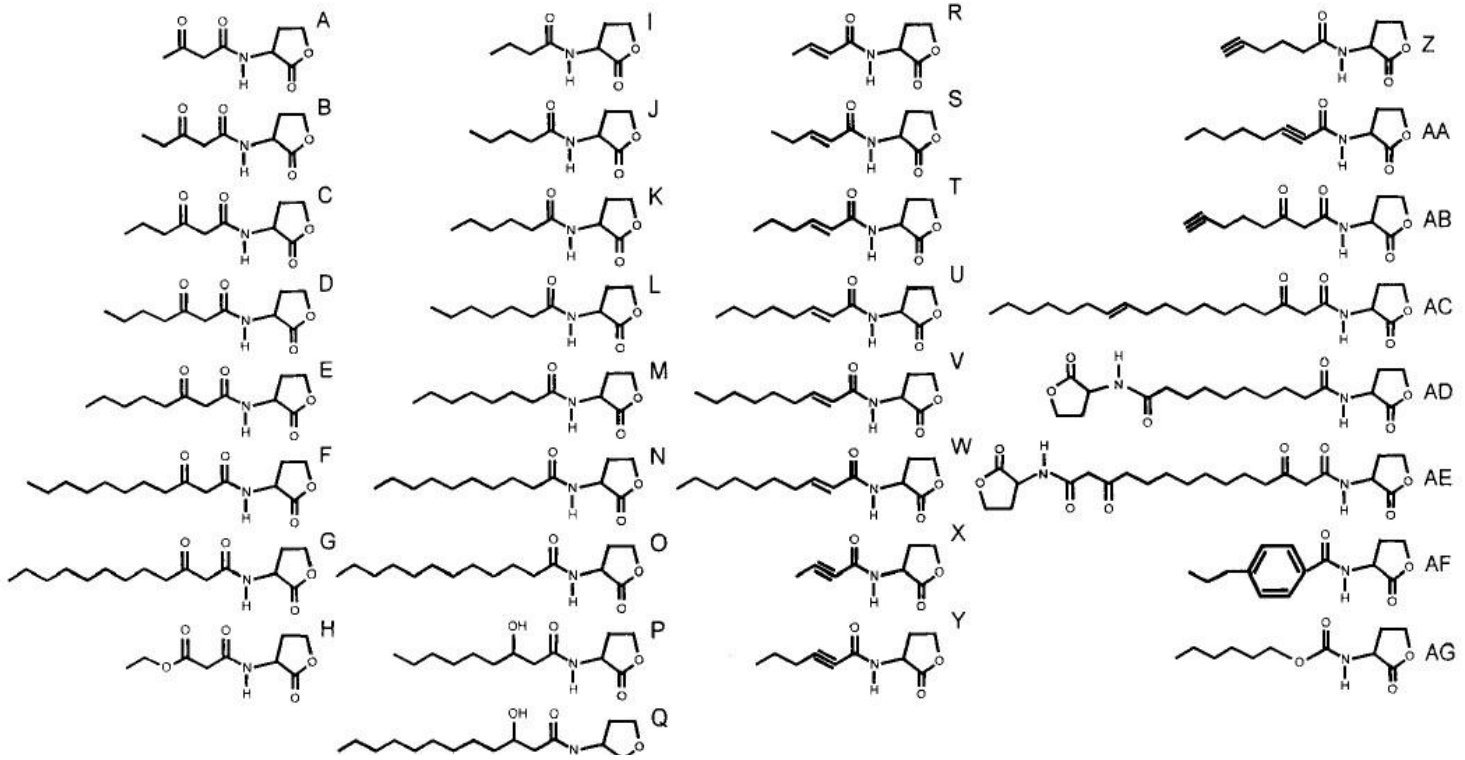
QS signals were released into the environment

Signal concentration reach a certain threshold then re-enter bacteria

QS signals induce virulence genes

Background

AHL species in nature

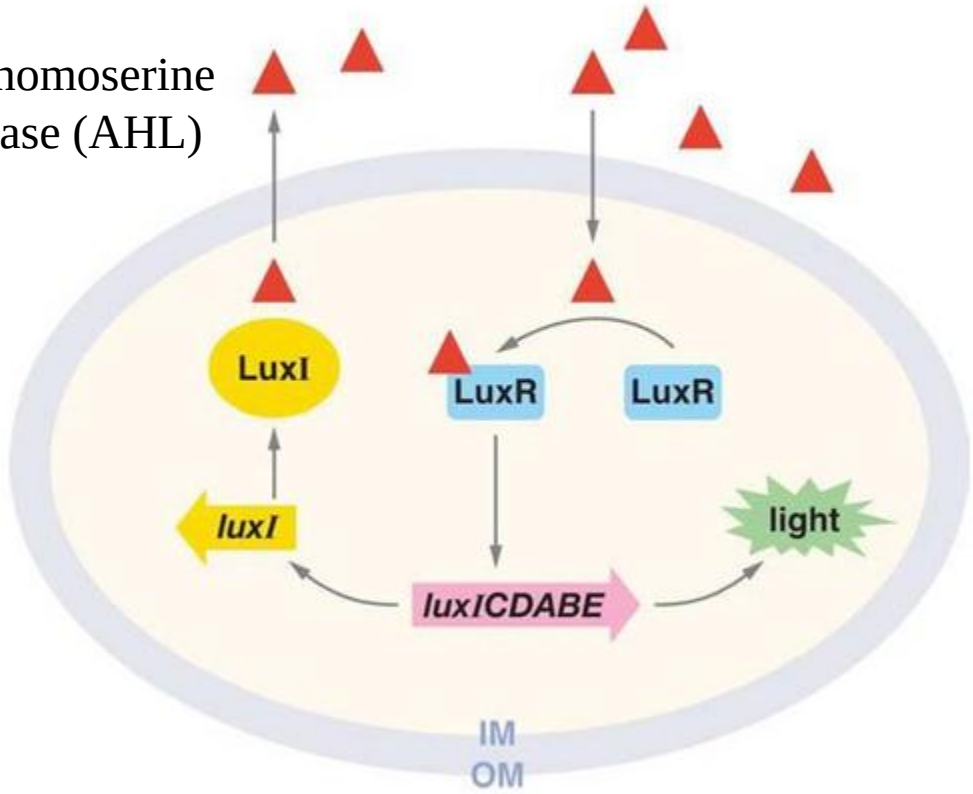


- **Each bacteria species produces one or several kinds of signals in this natural AHL pool.**
- **Quorum eavesdropping happens when different bacteria could use AHLs.**

Background

LuxR-LuxI system

Acyl-homoserine lactonase (AHL)



AI-1 pathway

1, *E. coli* is believed not to express the LuxI, which means *E.coli* can't produce AHLs, but only encodes a single LuxR homolog named SdiA

2, SdiA is expressed in insoluble inclusion bodies in the absence of AHLs, however, it is expressed in a folded, soluble form in the presence of AHLs.

Background

Avian Pathogenic *Escherichia coli* (APEC)

Avian colibacillosis is one of the most serious bacterial diseases caused by avian pathogenic *Escherichia coli* in poultry.

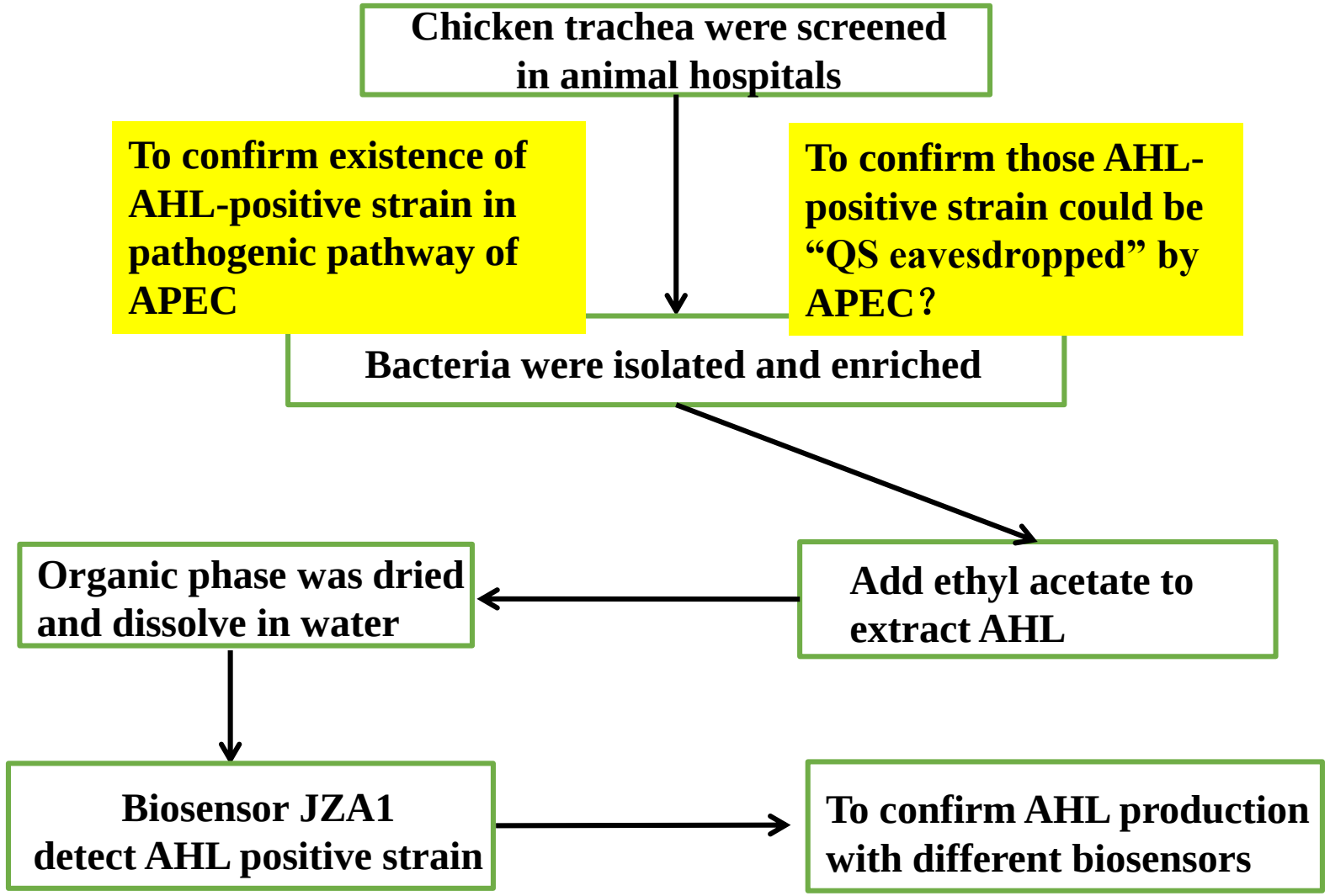
The virulence factors of APEC lead to the pathogenesis, and QS system is actively involved in the regulation process of virulence factors. However, QS-1 function of APEC have not been discussed.

Although QS-1 effect in ETEC or EHEC have been discussed,

- Is there any QS-1 signals that can affect APEC pathogenic pathway and virulence?
- Does the QS-1 system play a role in the APEC pathogenesis?

Results

Isolation and Characterization of AHL positive strain



Results

Isolation and Characterization of AHL positive strain

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gtcttgacatccagagaatcctgcagagatgcgggagtgccttcgggaactctgagacag
gtgctgcatggctgtcgtcagctcgtgttgtgaaatgttgggttaagtcccgcaacgagc
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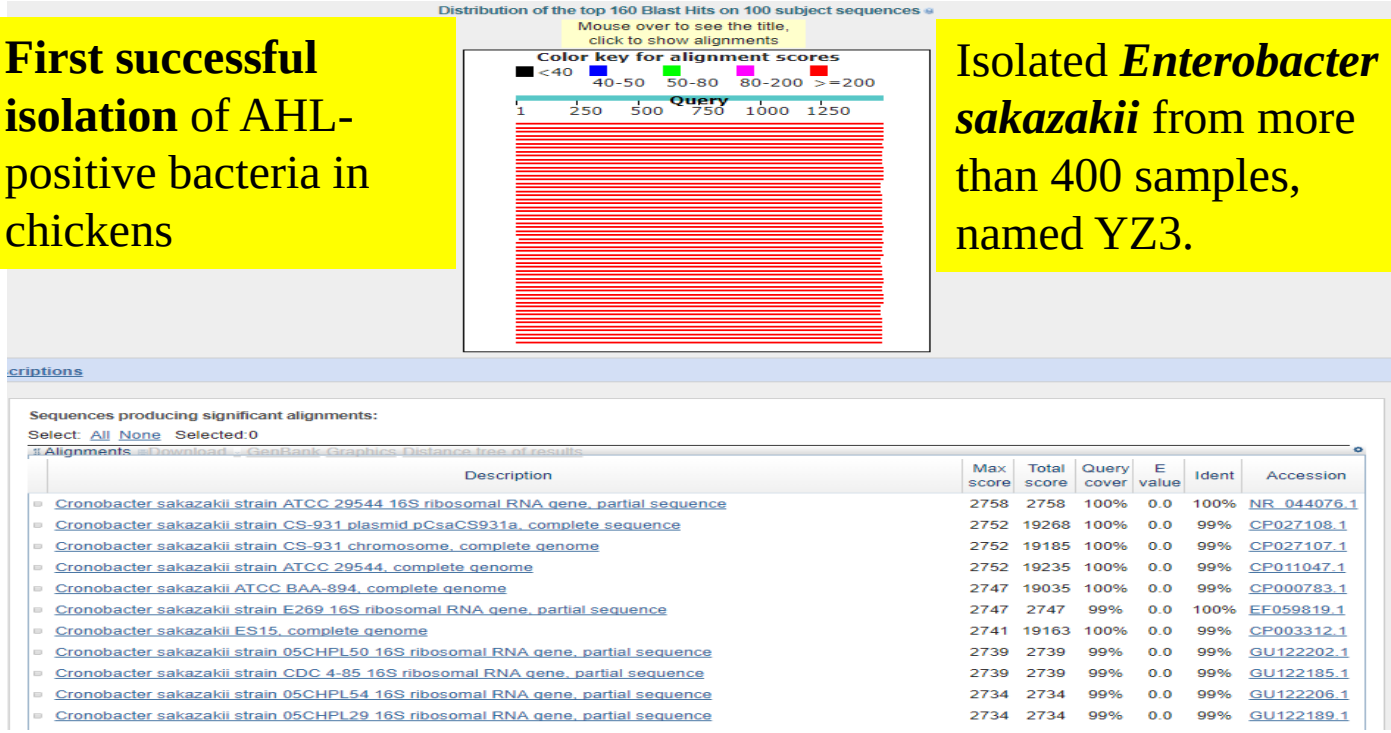


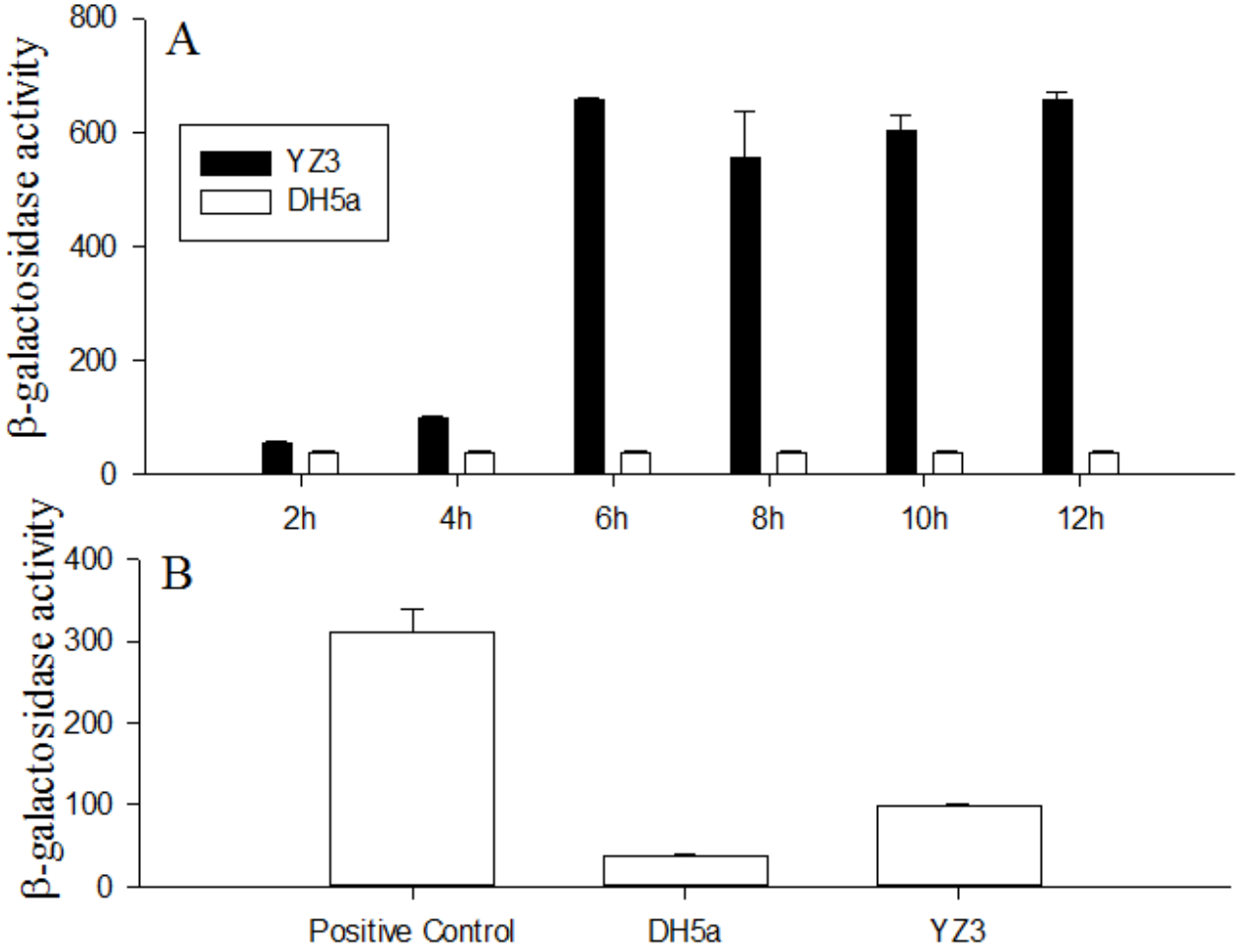
First successful isolation of AHL-positive bacteria in chickens

Isolated ***Enterobacter sakazakii*** from more than 400 samples, named YZ3.

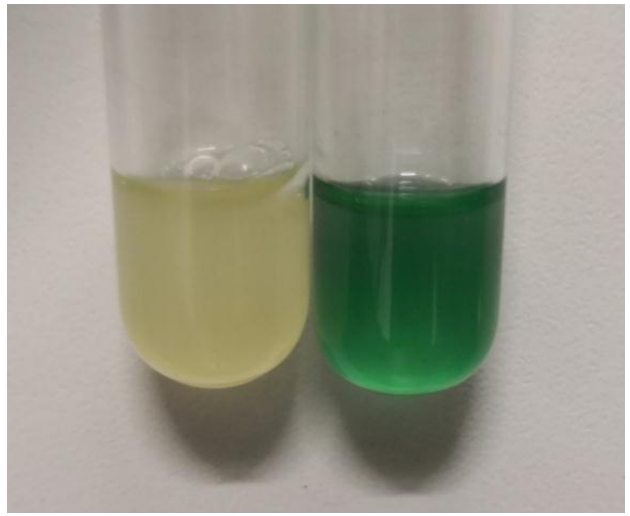
Results

Isolation and Characterization of AHL positive strain

Biosensor JZA1 detection



JZA1, sensitive to all AHLs and stimulate galactosidase activity under AHL induction.
Currently the most sensitive reporter for AHLs.



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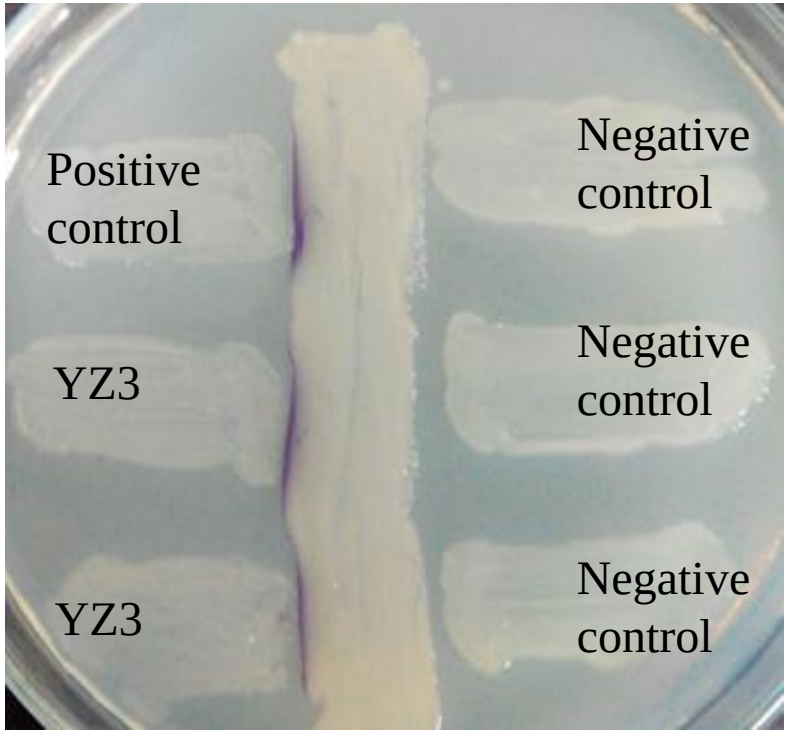
Results

Isolation and Characterization of AHL positive strain

Biosensor CV026



CV026 produces purple pigment under short side-chain with high sensitivity

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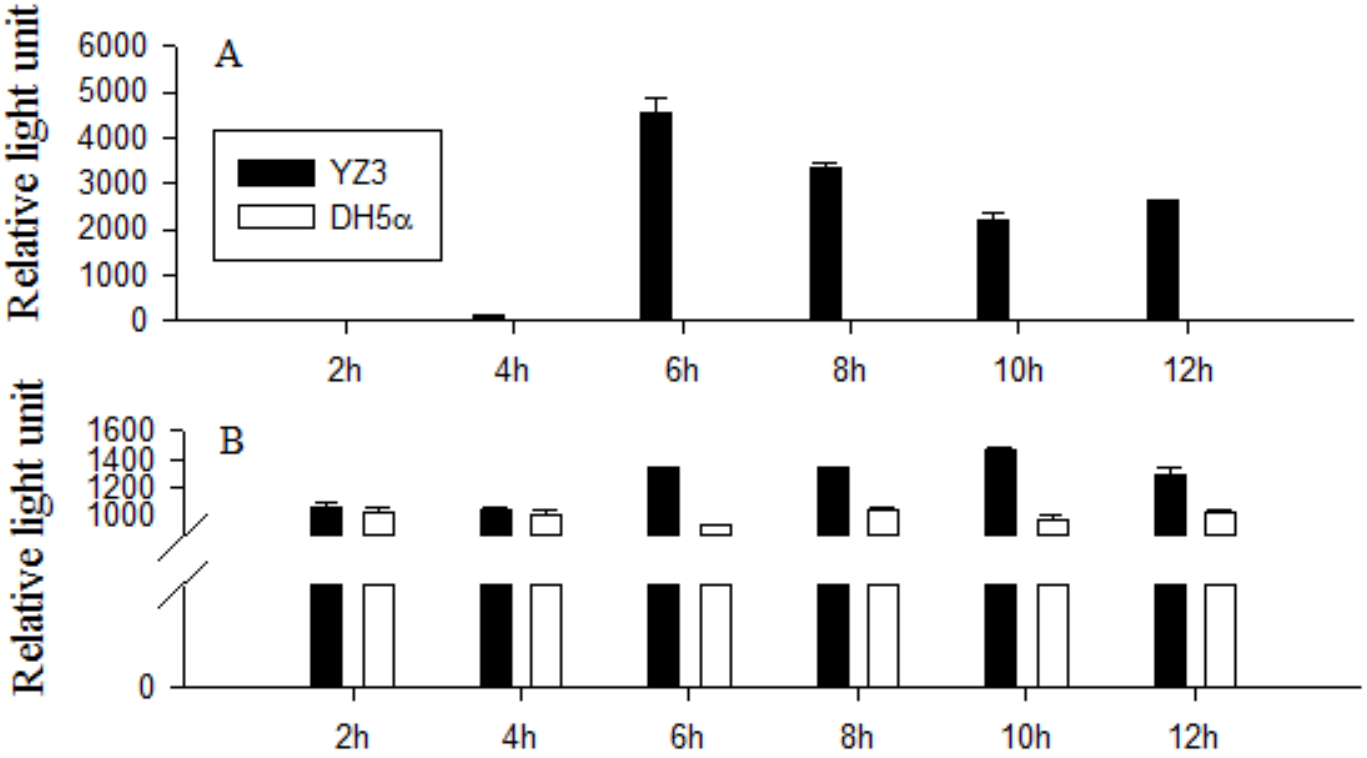
Results

Isolation and Characterization of AHL positive strain

Biosensor PSB401/1142



pSB401/1142 strain emits a firefly-like bioluminescence under exogenous AHL.
pSB401 for short side-chain AHL detection,
pSB1142 for long side-chain AHL detection.

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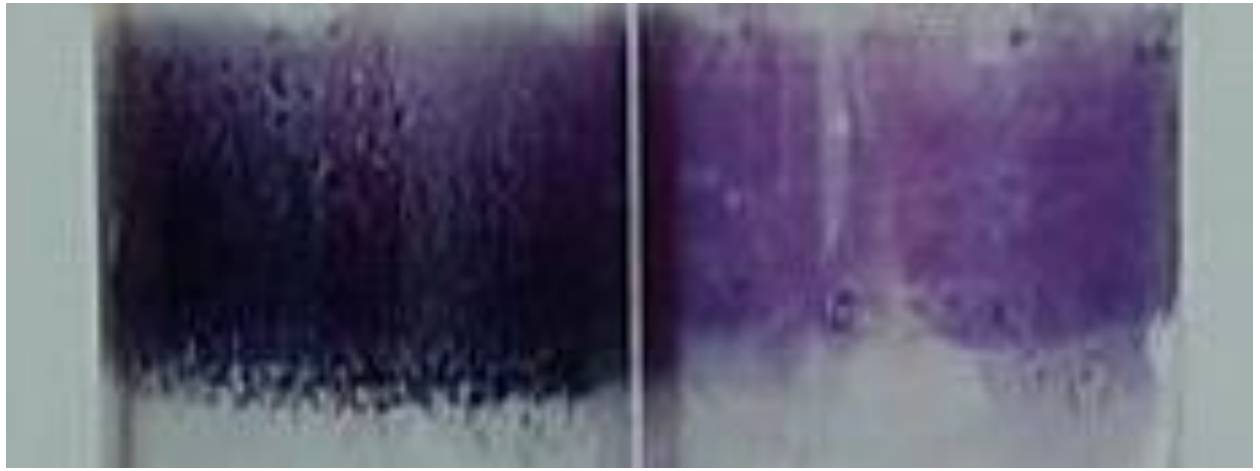
Results

Isolation and Characterization of AHL positive strain

AHLs extracted from YZ3 reduce biofilm formation of APEC CE129

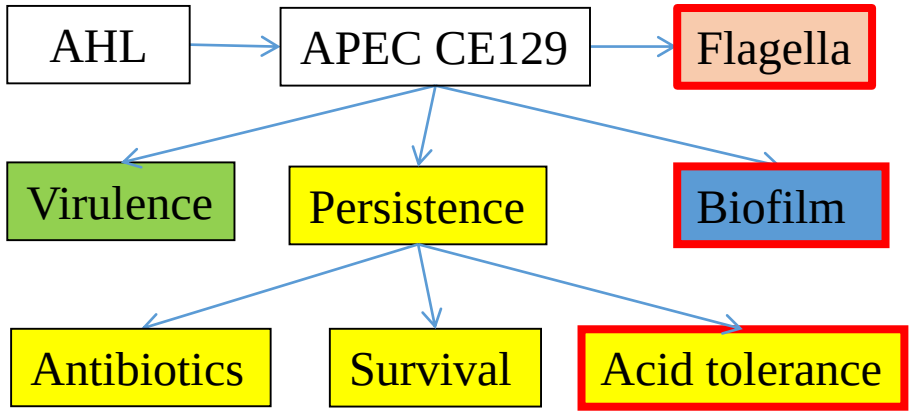


APEC CE129　　APEC CE129+AHLs

This result showed that AHLs- produced YZ3 can be “QS eavesdropped” by APEC

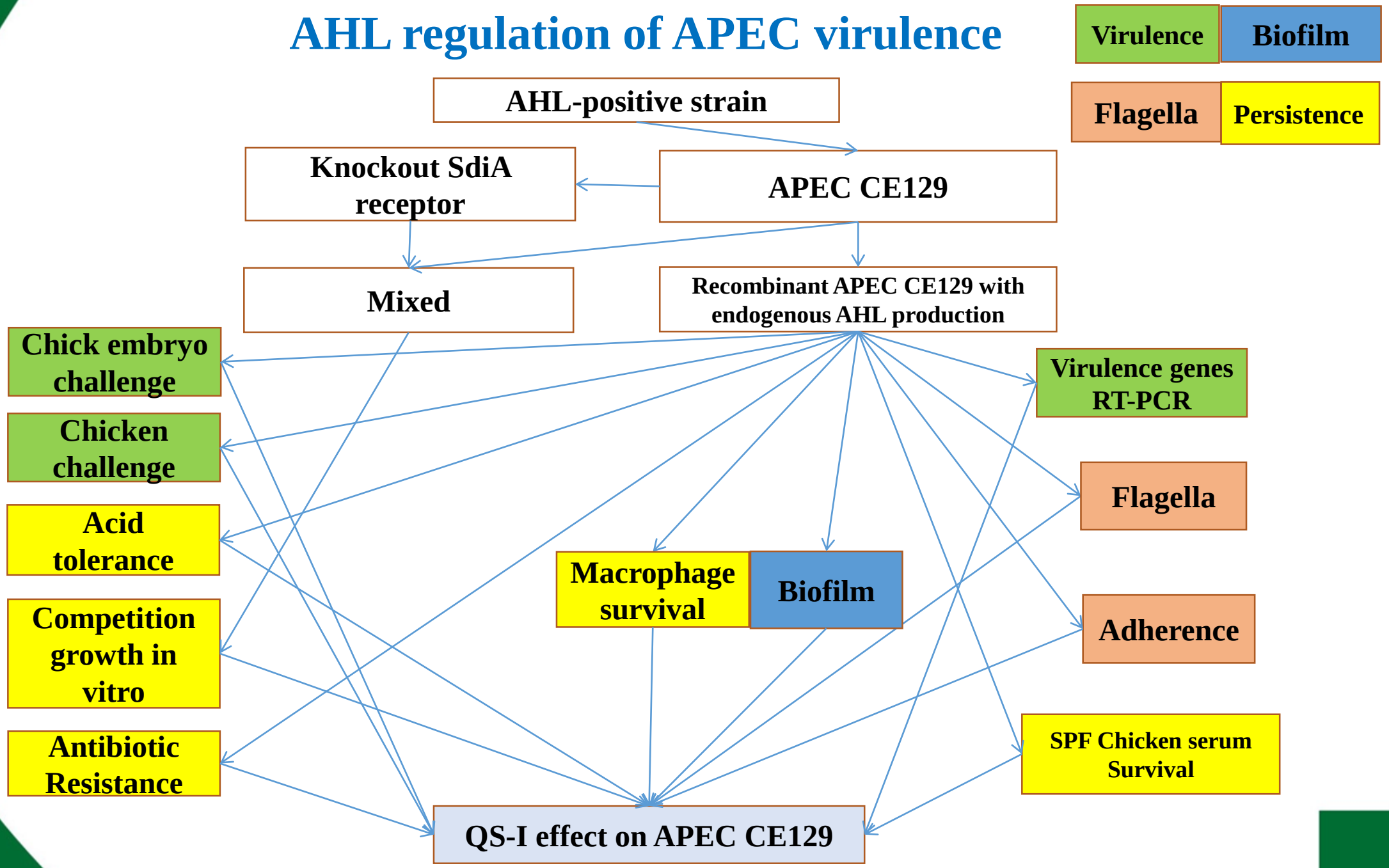
Results

AHL regulation of APEC virulence



Focus Points: Virulence, Flagella, Biofilm, Persistence

AHL regulation of APEC virulence



Results

AHL regulation of APEC virulence

Constructs for CE129△sidA and CE129 p*yenI*.

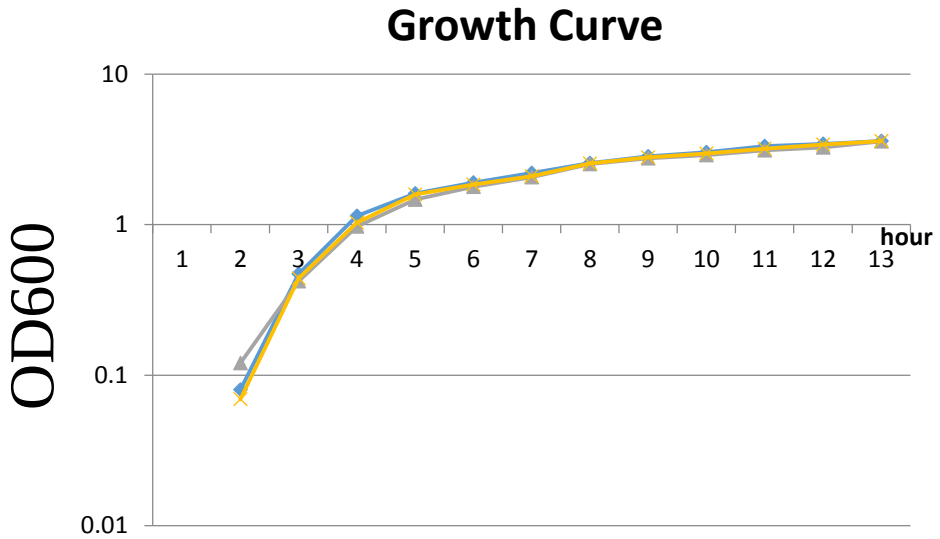
CE129/△sidA, lacking AHL eavesdrop ability.

CE129 p*yenI* , producing endogenous AHL signals.

Chick developing embryo challenge

Chicken challenge

No significant difference



Growth curve of CE129, CE129/p*yenI*, CE129/pBR

No significant difference

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Results

AHLs regulation of APEC virulence

MIC Experimental data

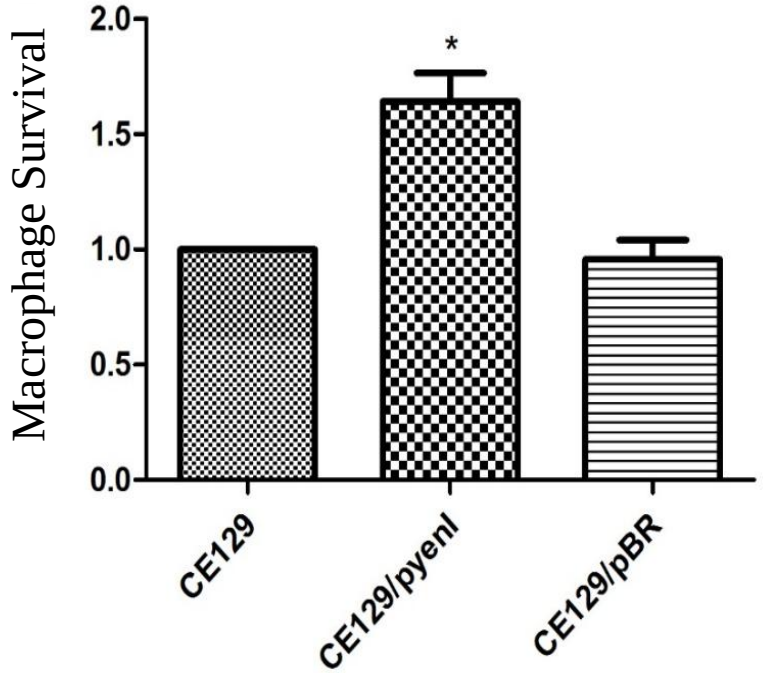
	CHL	Tet	OFX	NOR
CE129	8μg/mL	R	16μg/mL	16μg/mL
CE129/pyenI	8μg/mL	R	16μg/mL	16μg/mL
CE129/pBR	8μg/mL	R	16μg/mL	16μg/mL

No significant difference

Results

AHL regulation of APEC virulence

Experimental data for Macrophage HD11 Survival

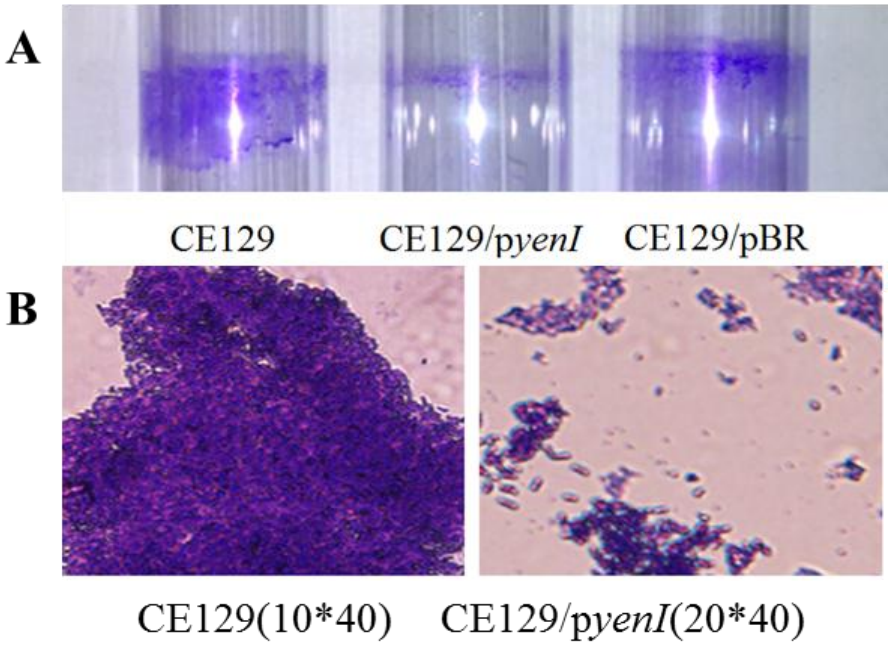


Under AHL, intracellular survival ability of APEC **increased**

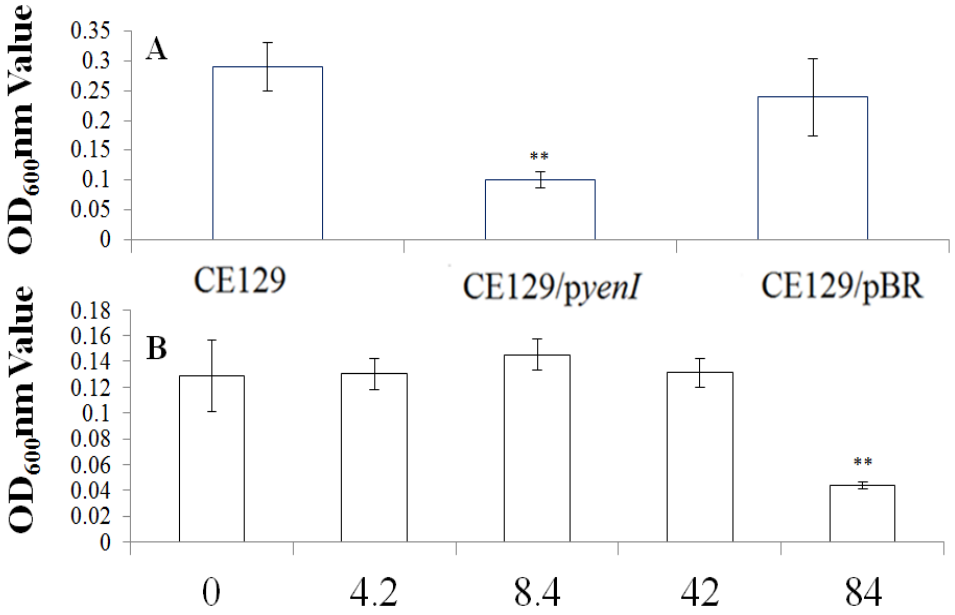
Results

AHLs regulation of APEC virulence

Qualitative test of biofilm formation



Quantitative test biofilm formation

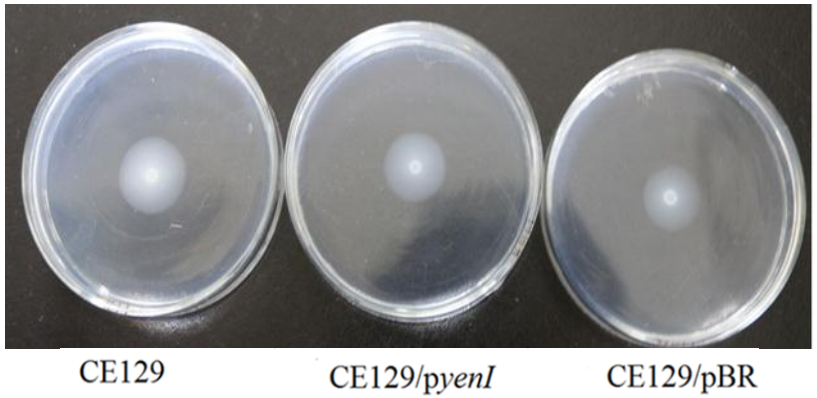


Under AHLs, biofilm formation of APEC **decreased**

Results

AHL regulation of APEC virulence

Mobility test



Expression level of flagella was not reduced under AHL induction

This phenotype is different from intestinal pathogenic *E. coli*

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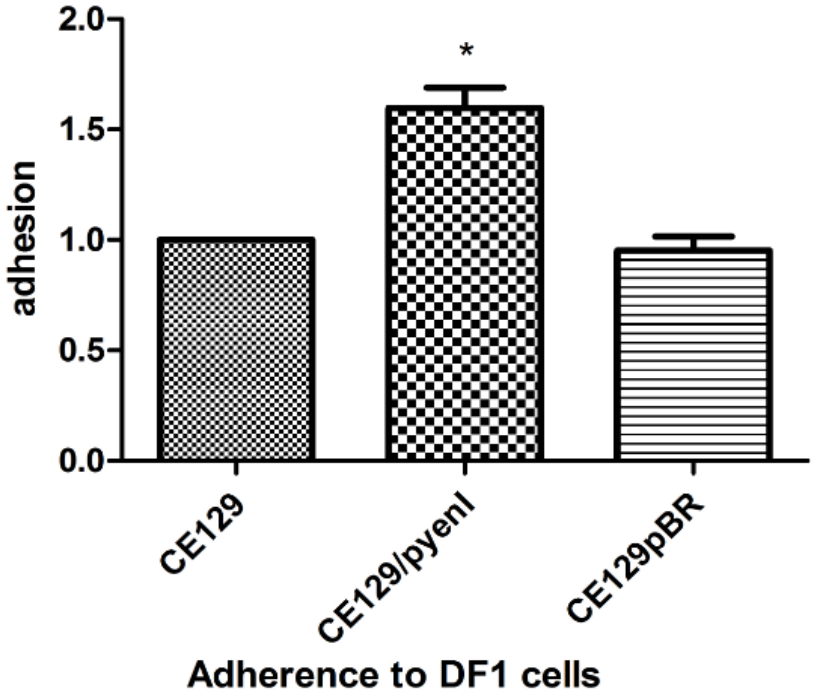
Results

AHL regulation of APEC virulence

Adherence test



Gene	CE129 WT	CE129/ p*yenI*	CE129/ pBR
fimA	100	70	106
fliC	100	103	111

Real-Time PCR of *fimA* gene

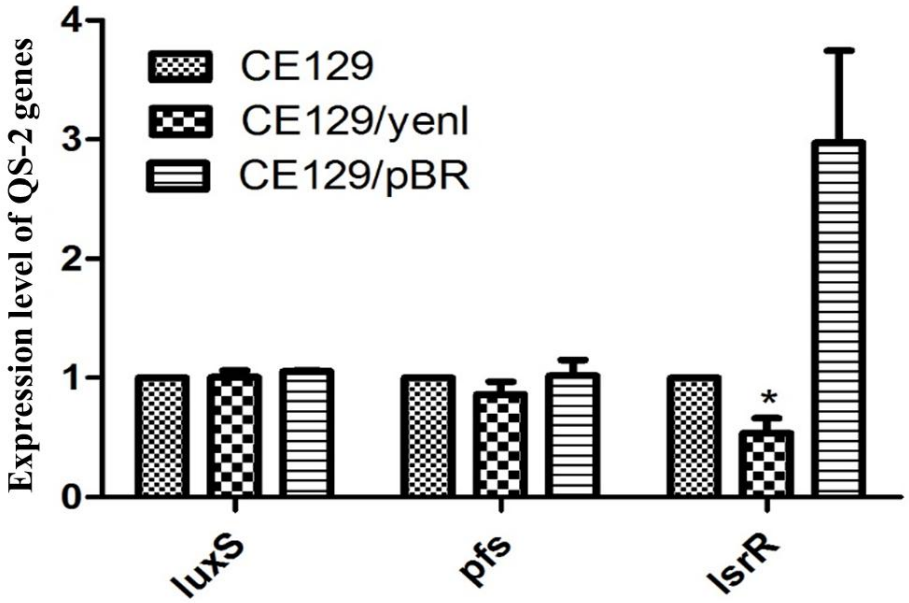
AHLs **increase** APEC adherence to DF1 cell

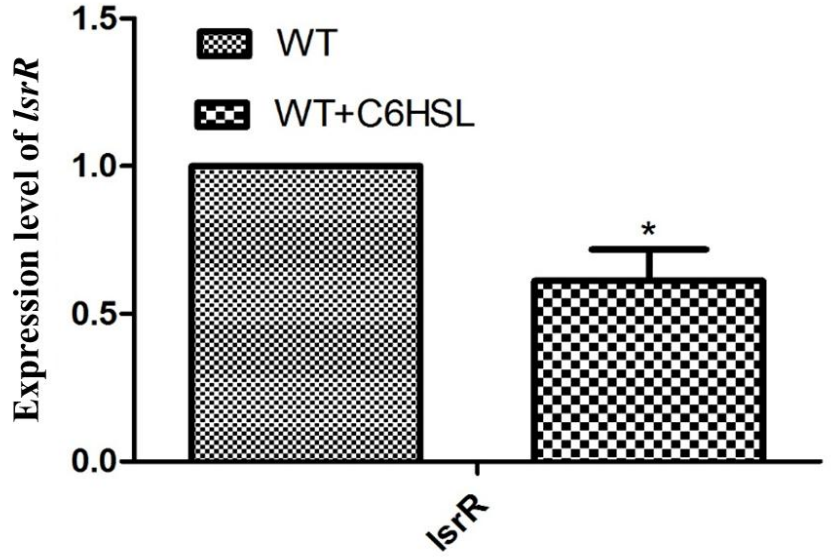
Adherence changes not from type 1 fimbriae or flagella

Results

AHL regulation of APEC virulence

QS-1 regulation of QS-2





QS-2 function of APEC has been fully discussed in previous study, QS-2 regulate flagella expression, pathogenicity and other characteristics in APEC. We found QS-1 **influenced** QS-2 through ***lsrR*** gene.

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Summary

- Screening out AHL-positive bacteria *Enterobacter sakazakii* YZ3, and confirming its QS-1 signals can be QS- Eavesdropped by APEC strain.
- Constructs of APEC recombinant strains CE129△sidA and CE129 p*yenI*. CE129△sidA lacks AHL receptor and QS-eavesdrop ability. CE129 p*yenI* could produce endogenous AHL signals. On this basis, QS-1 function upon APEC virulence have been discussed in this study.
- QS-1 influence QS-II activity in APEC, as well as adherence and invasion ability. Biofilm formation of APEC was also suppressed under AHL signals. lsrR, the important gene in QS-Ⅱ, decreased by 46.6%.
- **Interestingly, APEC strain showed different phenotypes of acid tolerance and flagella expression, compared with Enterotoxigenic *E. coli*.**

Acknowledgements

Collaborators

- Dr. Philip R. Hardwidge Kansas State University
- Dr. David H. Francis South Dakoda State University
- Dr. Weiping Zhang Illinois University

Lab experiment members (Ph.D and Ms.)

- **Yang Yang, Ph.D student**
- **Zhendong Li, Ms. student**
- Yun Liu
- Binbin Zhang

Major Founding resource: NSFC National Natural Science Foundation of China