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The Quorum Sensing Type I enhances the acid resistance of EHEC O157:H7 by activating the *rpoS* and *gad* system

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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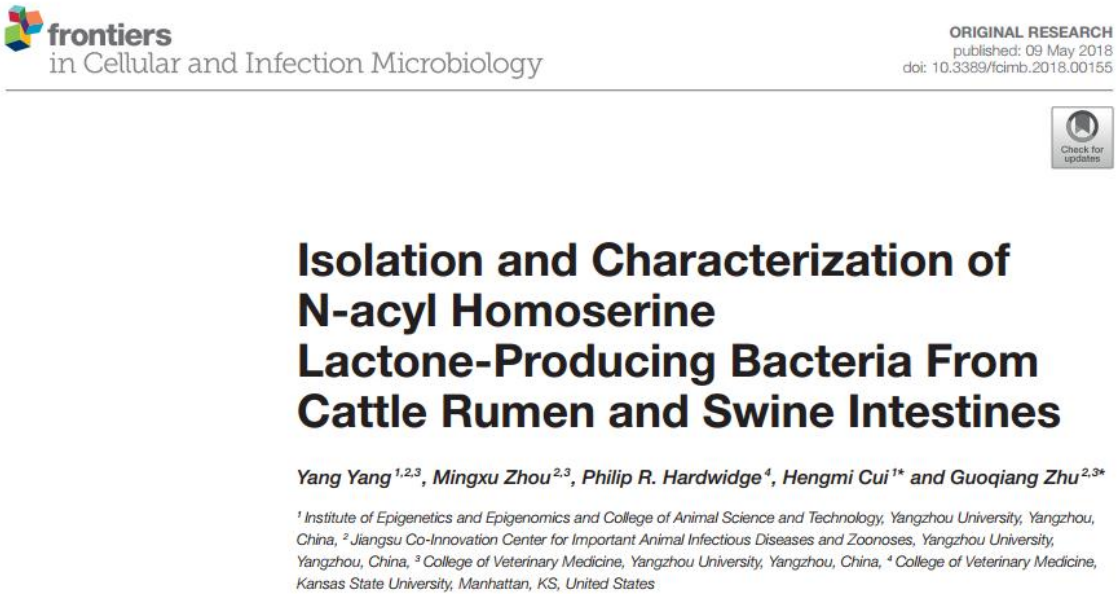
Background

The AHLs-produced strains in hosts（bovine, swine）

AHLs are prominent within the bovine rumen but undetectable in other areas of the GI tract such as rectum, colon and cecum.



frontiers in Cellular and Infection Microbiology

ORIGINAL RESEARCH
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Isolation and Characterization of N-acyl Homoserine Lactone-Producing Bacteria From Cattle Rumen and Swine Intestines

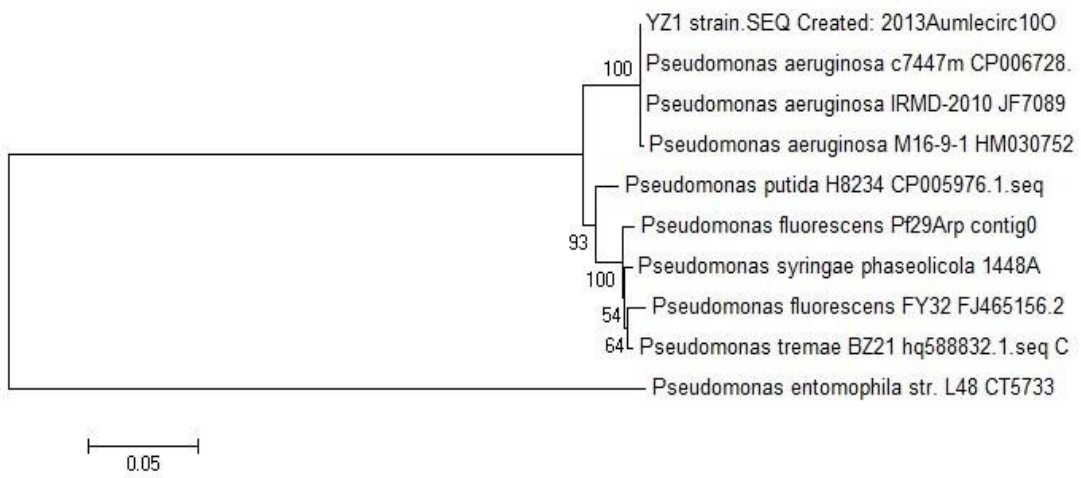
Yang Yang[1,2,3], Mingxu Zhou[2,3], Philip R. Hardwidge[4], Hengmi Cui[1] and Guoqiang Zhu[2,3]*

[1] Institute of Epigenetics and Epigenomics and College of Animal Science and Technology, Yangzhou University, Yangzhou, China, [2] Jiangsu Co-Innovation Center for Important Animal Infectious Diseases and Zoonoses, Yangzhou University, Yangzhou, China, [3] College of Veterinary Medicine, Yangzhou University, Yangzhou, China, [4] College of Veterinary Medicine, Kansas State University, Manhattan, KS, United States*

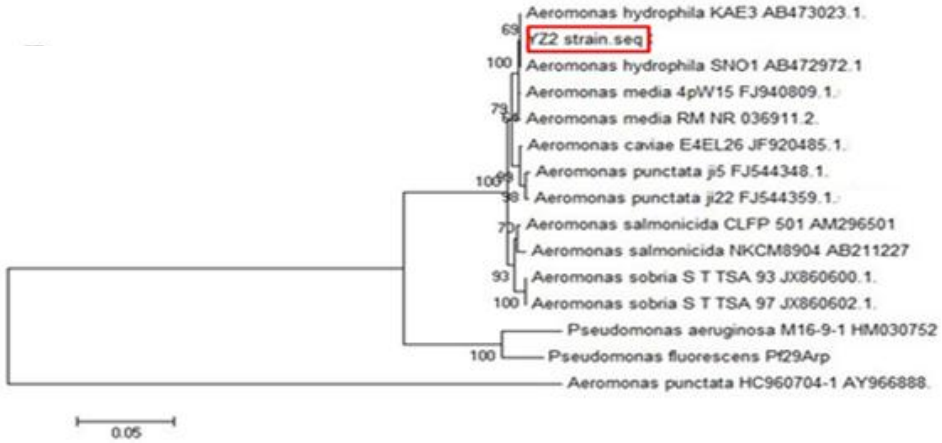
《Isolation and Characterization of N-acyl Homoserine Lactone-Producing Bacteria From Cattle Rumen and Swine Intestines》

Background

The AHLs-produced strains in hosts



Phylogenetic tree analysis determined that bovine derived YZ1 belongs to *Pseudomonas aeruginosa*



Phylogenetic tree analysis determined that swine derivedYZ2 belongs to *Aeromonas hydrophilia*

Background

Acid Resistance (AR) of *E. coli*

Most pathogenic bacteria require a large infectious dose (e.g., ***Vibrio cholerae*** requires~10^9organisms) in order to survive passage through the stomach. Remarkably, infectious **enterohemmorhagic *E.coli*** requires a substantially lower infectious dose (~10^2 organisms).

Type	Mechanism
AR1	**RpoS–dependent acid resistance system**
AR2	**Glutamic acid–dependent acid resistance system (GDAR)**
	Arginine-dependent acid resistance system (ADAR)
	Lysine-dependent acid resistance system (LDAR)





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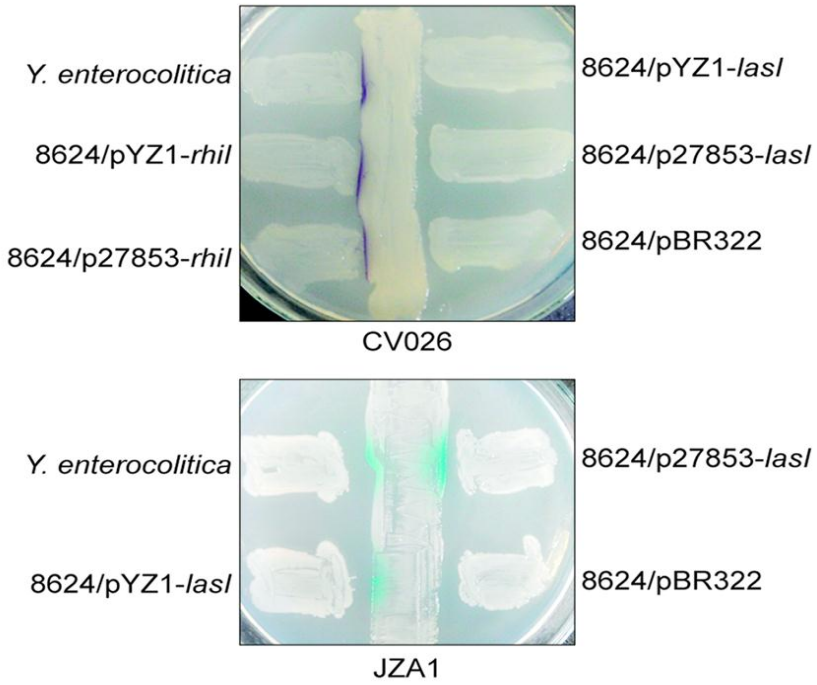
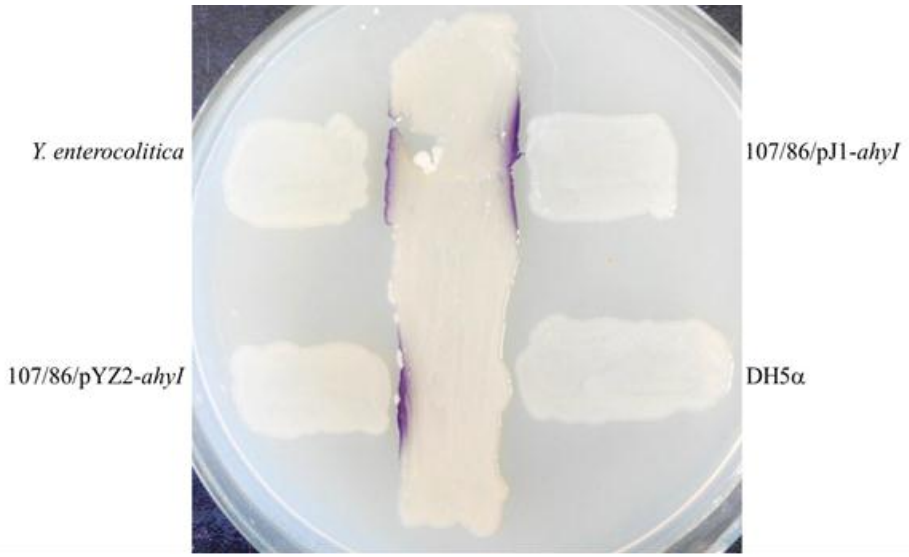
Results

To confirm E.coli recombinant expressing AHLs endogenously



O157:H7 strain 8624 recombinant
***rhlI* → short chain AHL**
***lasI* → long chain AHL**



F18 strain 107/86 recombinant
***ahyI* → short chain AHL**

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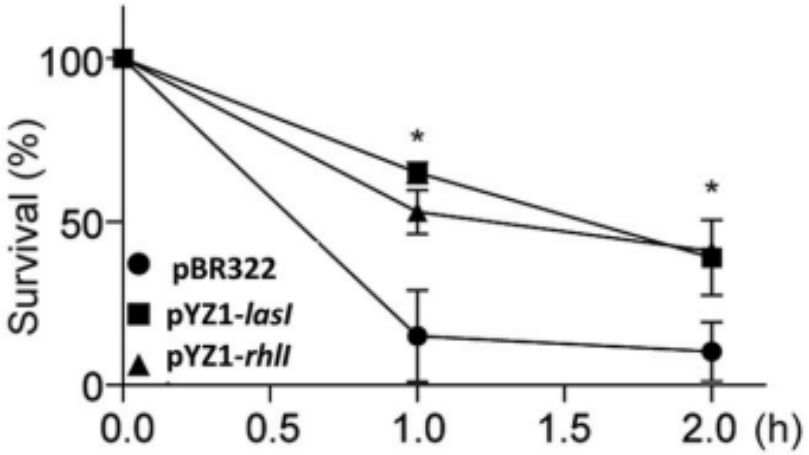
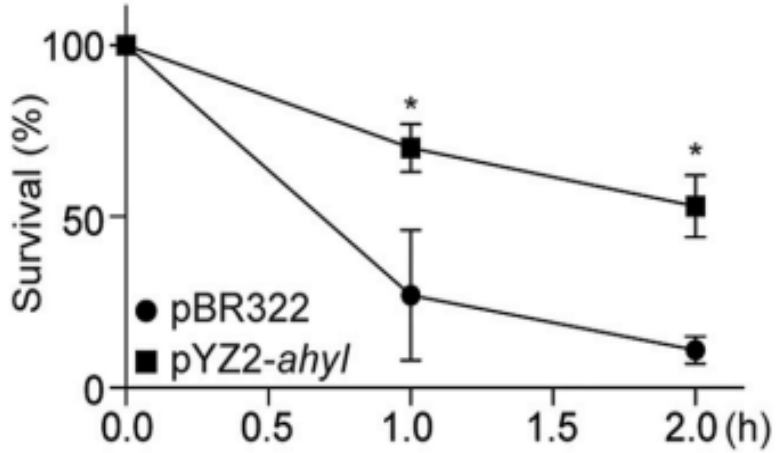
Results

Acid resistance (AR) assay

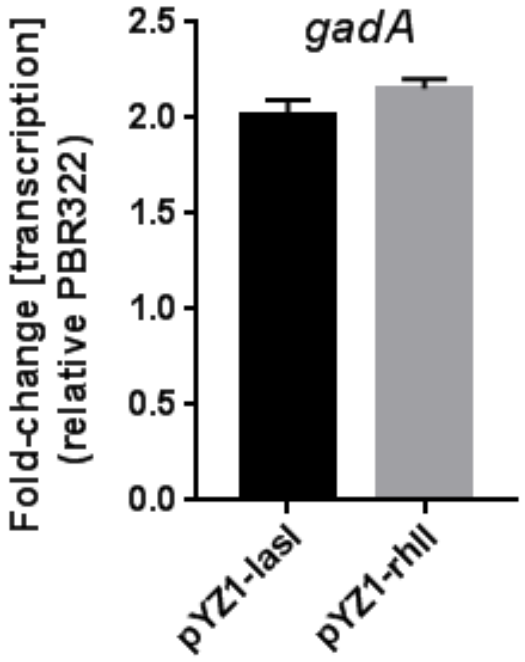




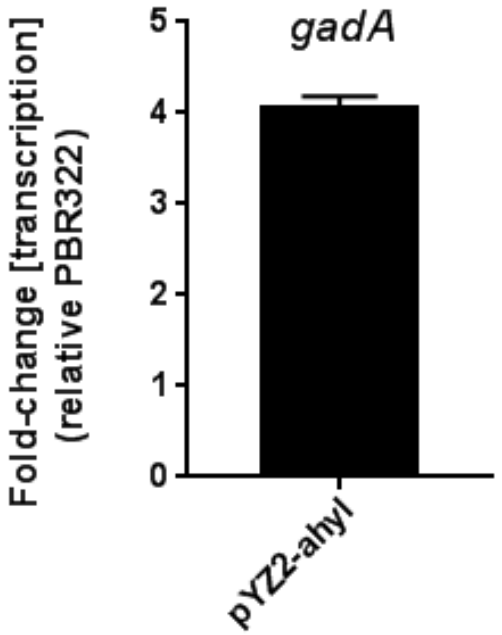
Compared with *E.coli* pBR322, the AR survival of *rhlI* and *lasI*, *ahyI*-expressing *E.coli* significantly increased **(4 folds)**

Results

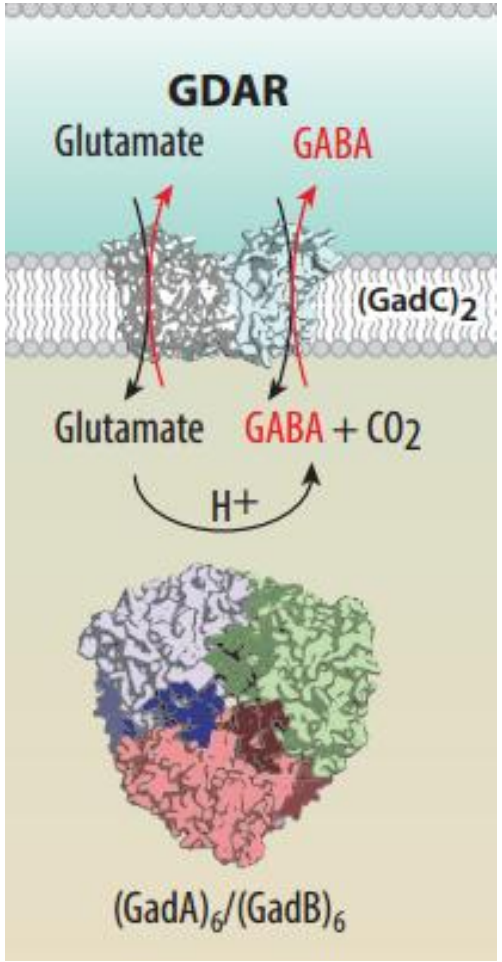
Changes in AR2 gene expression



gadA up-regulated **2** folds, compared with EHEC pBR322



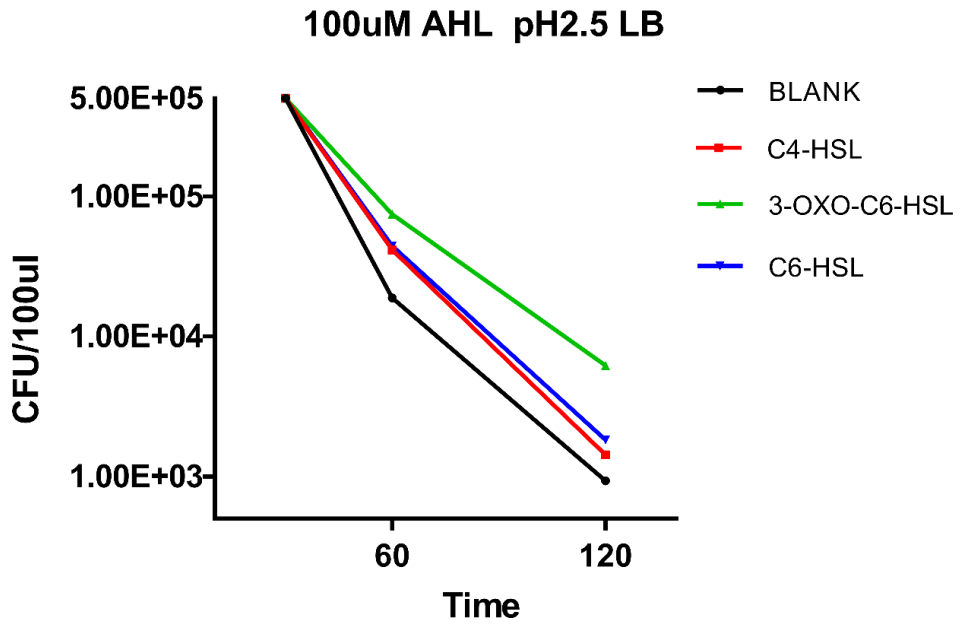
gadA up-regulated **4** folds , compared with EHEC pBR322



Results

AR assay of *E. coli* O157:H7 *under* different external AHLs

C4-HSL, **3-oxo-C6-HSL**, and C6-HSL as the external AHLs to test the AR



The increased folds with 100uM AHLs

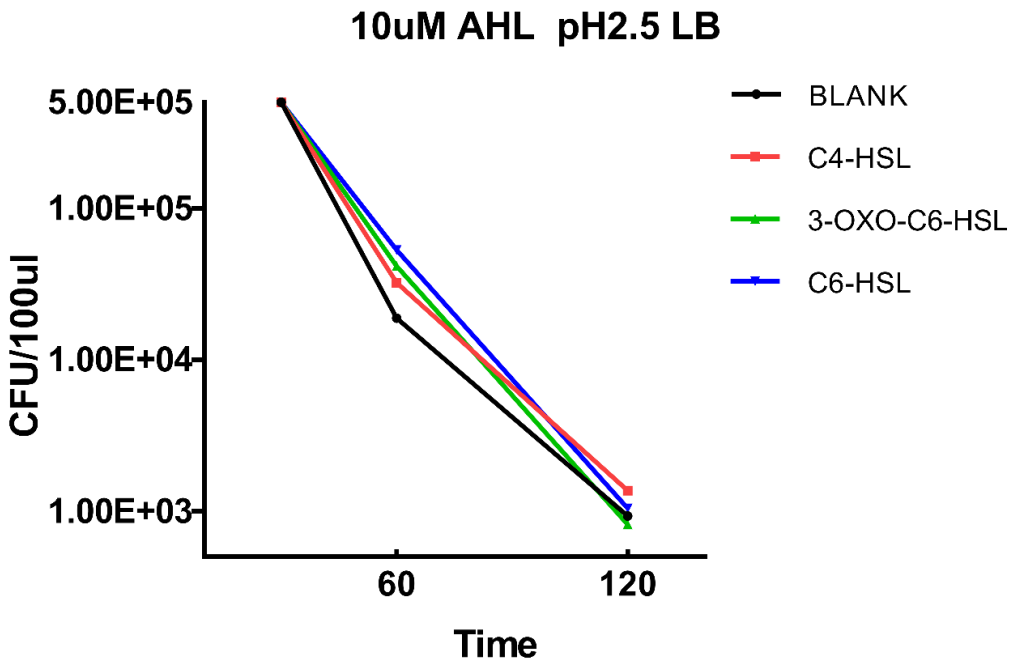
	C4-HSL	3-oxo-C6-HSL	C6-HSL
1h	~2.2	~4.0	~2.4
2h	~1.5	~6.6	~2.0

Folds= Survival with 100uM AHLs / Survival of blank

AR survival of O157:H7 was enhanced by different AHLs(100uM), especially the **3-oxo-C6-HSL** is significant

Results

AR assay of *E. coli* O157:H7 *under* different external AHLs



The increased folds with 10uM AHLs

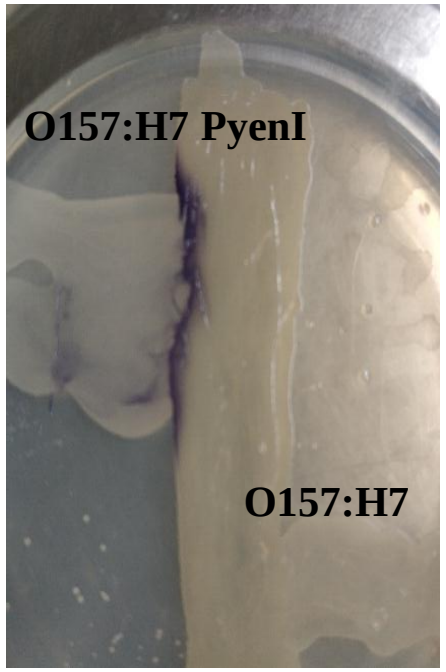
	C4-HSL	3oxo-C6-HSL	C6-HSL
1h	~1.7	~2.2	~2.8
2h	~1.5	~0.9	~1.1

Folds= Survival with 10uM AHLs / Survival of blank

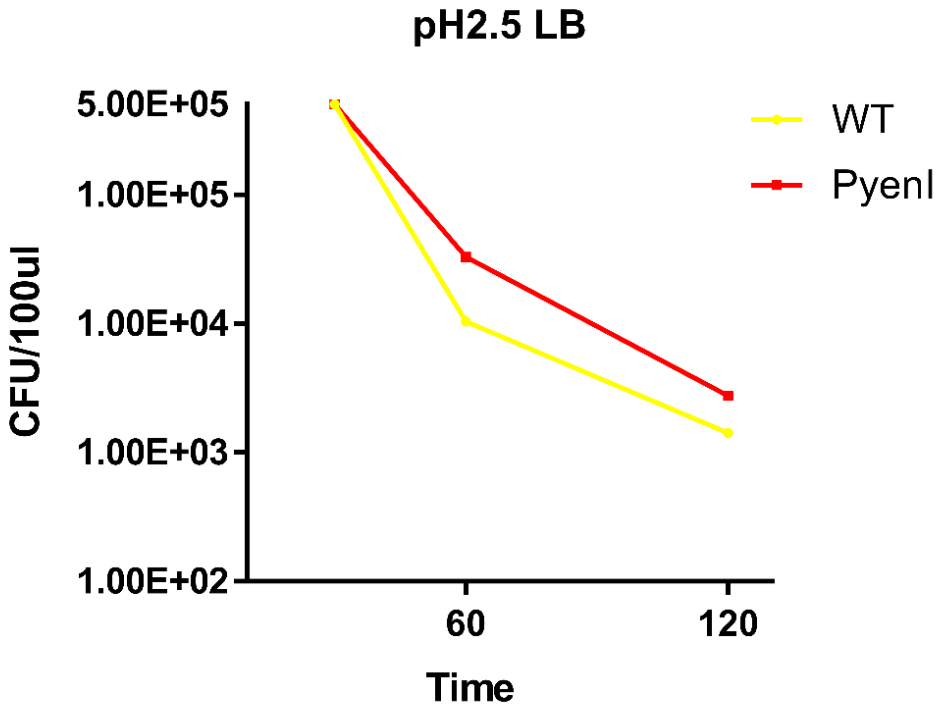
Compared with AHLs (100uM), effect of AHLs (10uM) on AR survival is not remarked

Results

AR assay of *E. coli* O157:H7 under endogenous AHLs



CV026 bioreporter



E. coli O157:H7 expressing *yenI* gene (luxI homologue), producing AHLs and getting the AR

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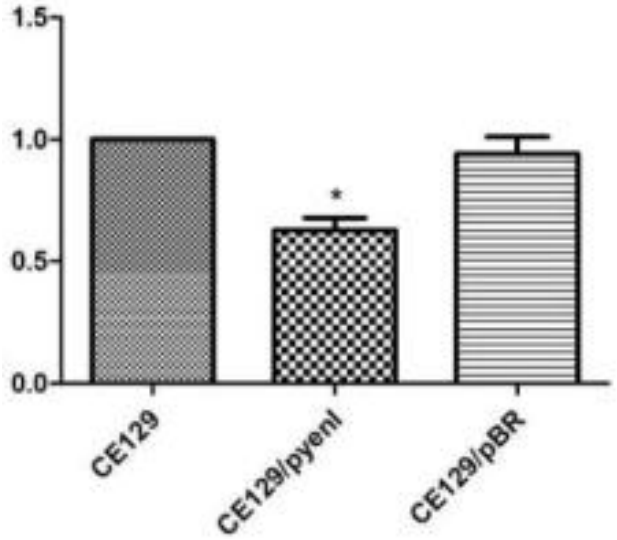
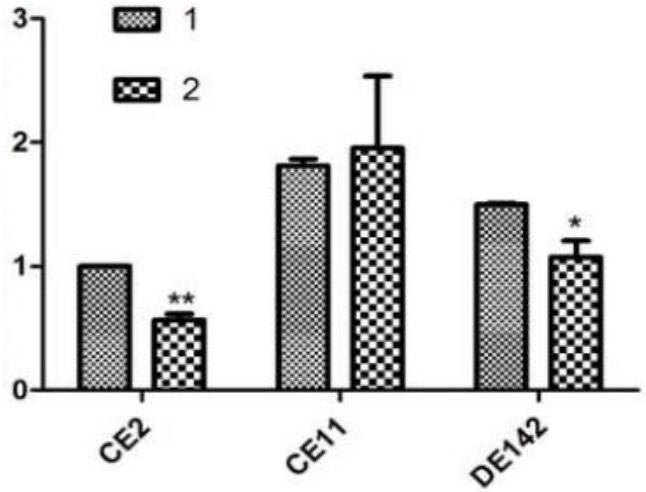
Results

No Effect of AHLs on AR of APEC rather than enteropathogenic *E. coli*





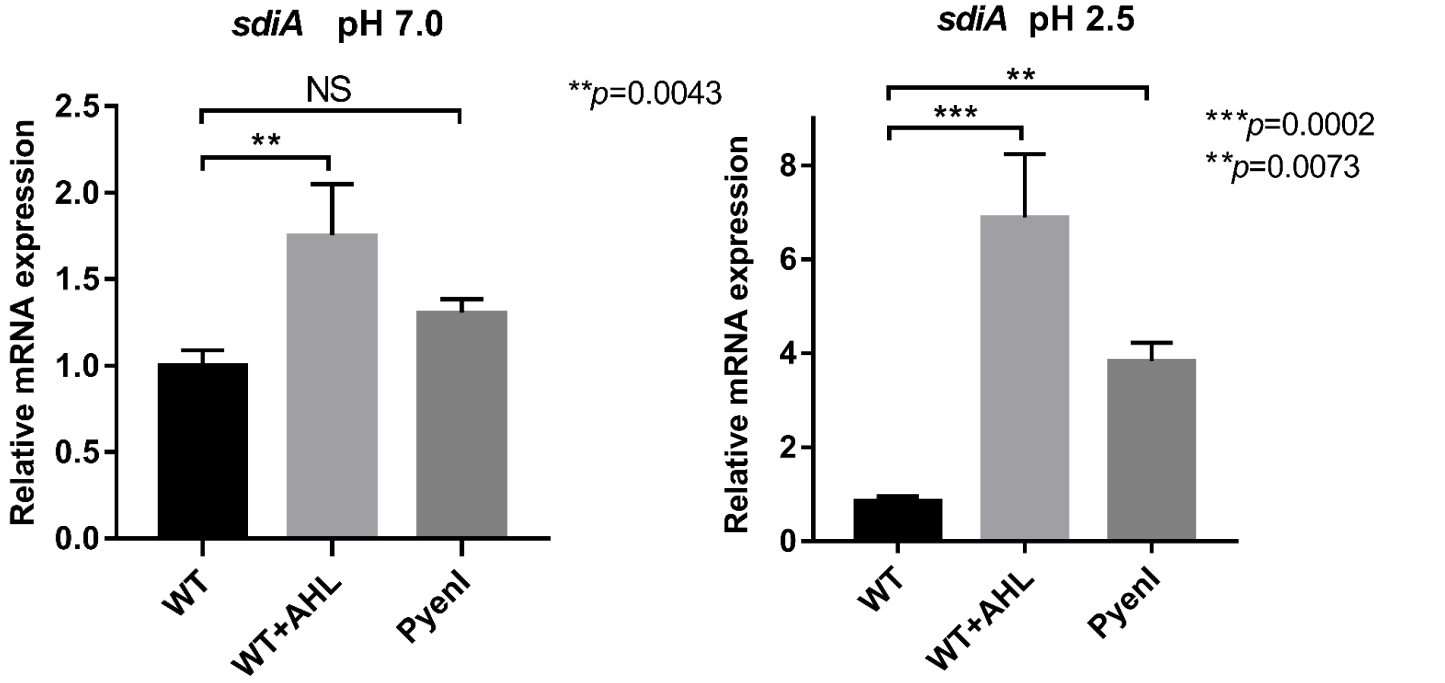
1, Blank group 2, externally added AHLs

Relative mRNA expression of *gadA*

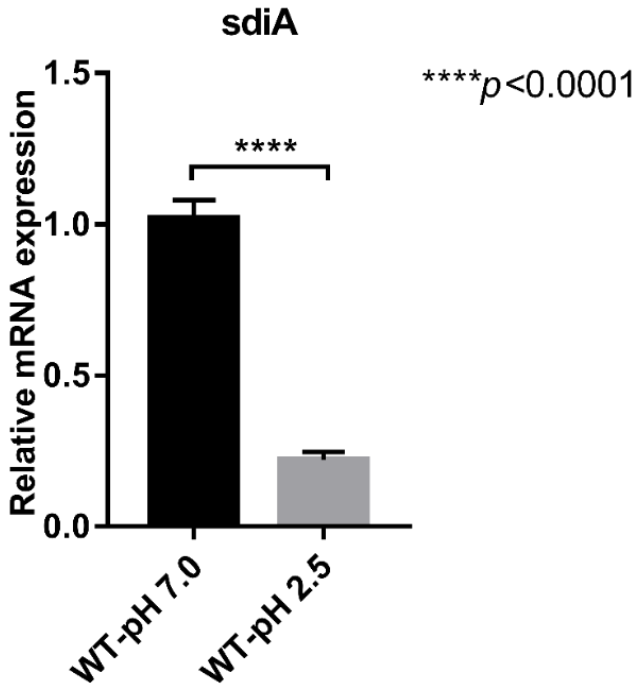
- APEC CE129 strain expressing yenI gene have no effect on AR survival
- APEC (CE2, CE11, DE142) strains with external AHLs also have no effect on AR survival

Results

Transcription study of *sdiA*, *rpoS* and *gadA* under AHLs



Expression of *sdiA* *under AHLs at pH=2.5 or 7.0*

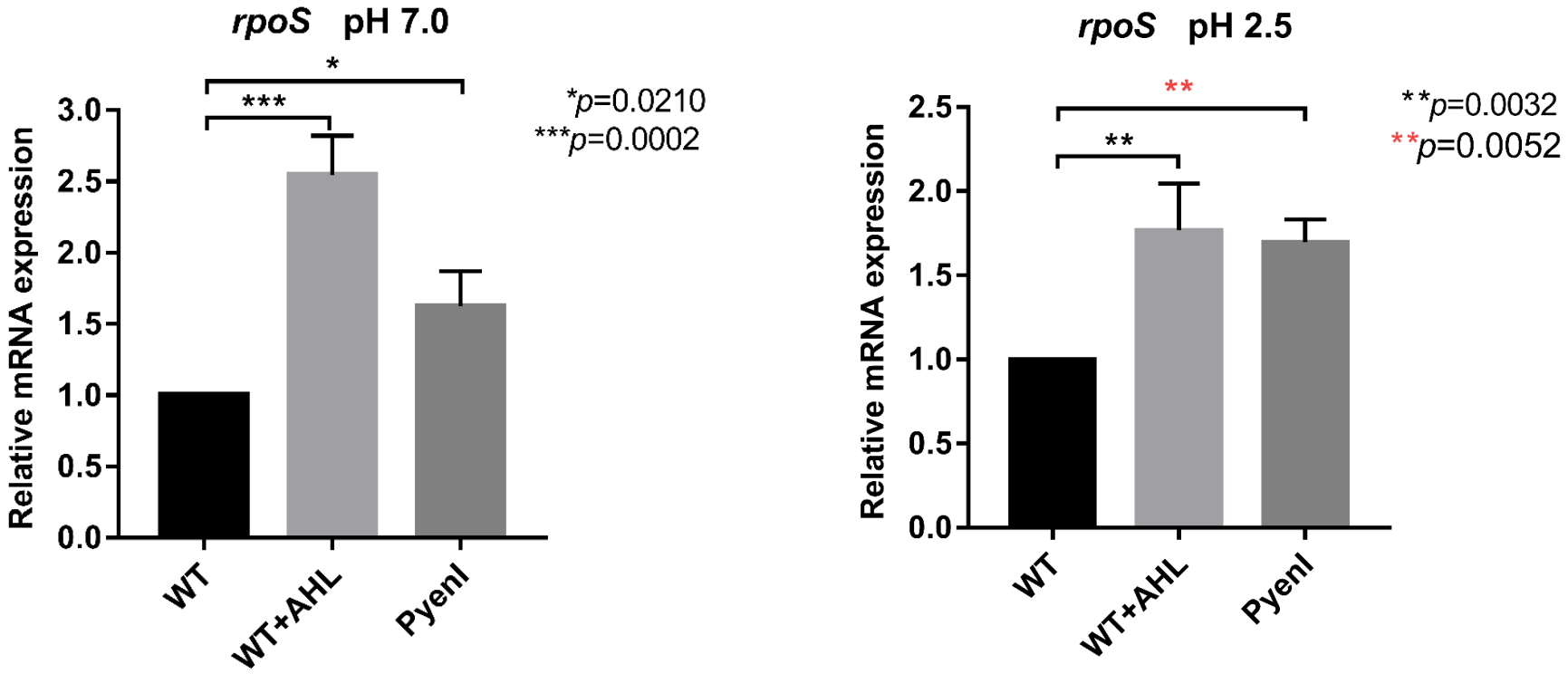


Expression of *sdiA* at pH=2.5 or 7.0

- The *sdiA* transcription is different in normal and acid environment under AHLs
- The functional activation of *sdiA* needs AHLs and acidic condition together

Results

Transcription study of *sdiA*, *rpoS* and *gadA* under AHLs

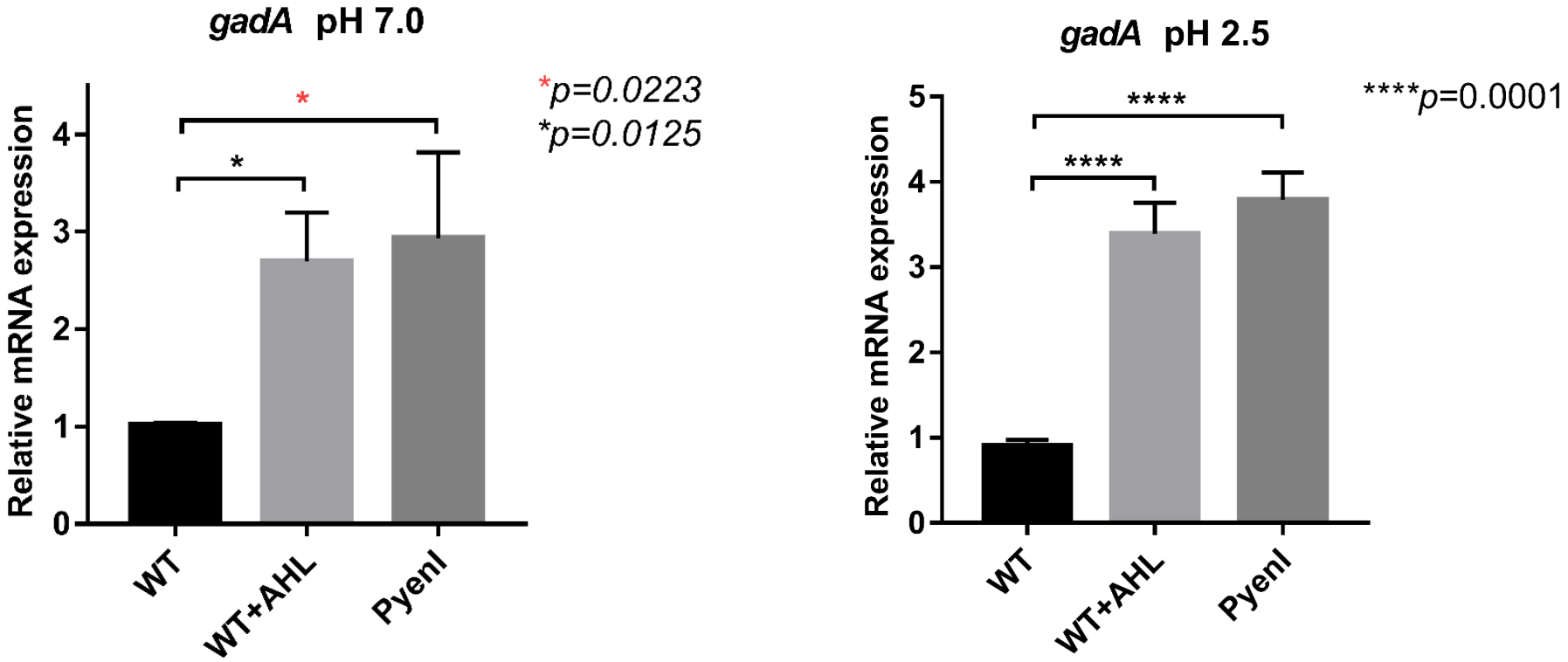


Expression of *rpoS under AHLs at pH=2.5 or 7.0*

The *rpoS* can be activated by AHLs in normal and acid enviroment

Results

Transcription study of *sdiA*, *rpoS* and *gadA* under AHLs

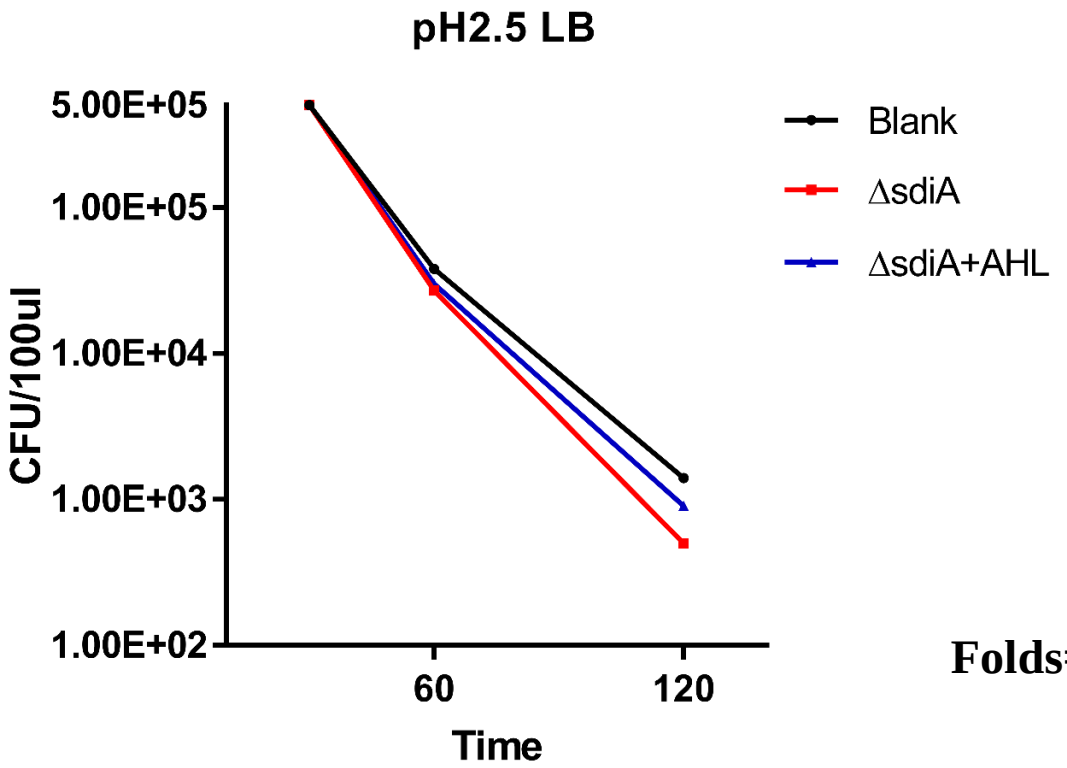


Expression of *gadA* under AHLs at pH=2.5 or 7.0

The *gadA* can be activated by AHLs in normal and acid enviroment

Results

AR assay of O157:H7, O157:H7 ΔsdiA, O157:H7 Δ*sdiA*+AHL



The decreased folds of Δ*sdiA* and Δ*sdiA*+AHL

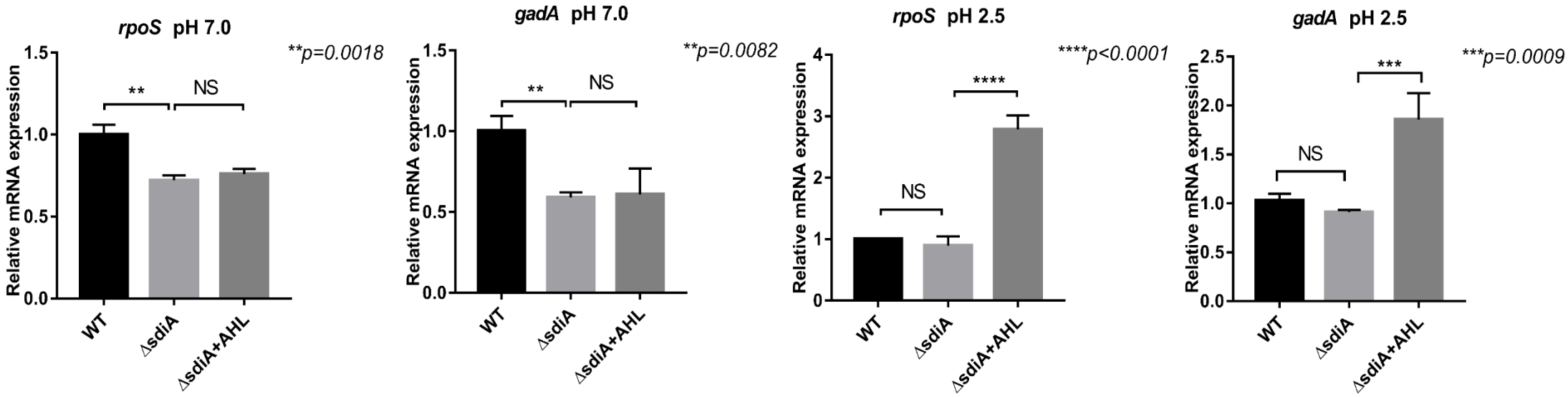
	ΔsdiA	ΔsdiA+AHL
1h	~0.71	~0.78
2h	~0.36	~0.64

Folds= Survival of Δ*sdiA* and Δ*sdiA*+AHL/ Survival of blank

The *sdiA* deletion strain reduces the AR survival of O157:H7, and AHLs can enhance the AR survival of O157:H7 Δ*sdiA* strain

Results

Transcription study of *rpoS* and *gadA in O157:H7 ΔsdiA strain*



Transcription of *rpoS and gadA of* Δ*sdiA* at pH=7.0 *and* pH=2.5

In normal condition, Δ*sdiA* and Δ*sdiA*+AHL reduce the expression of *rpoS* and *gadA*
In acidic condition, Δ*sdiA* can't affect *rpoS* and *gadA*, and Δ*sdiA*+AHL can upregulate *rpoS* and *gadA*

This result indicates that AHL with some unknown factors work synergeticly to enhance AR

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Summary

1. AHLs can enhance the AR of enteropathogenic *E. coli* to a certain extent, especially the AHL molecules containing carbonyl, but does not increase the AR for non-enteric pathogenic *E. coli*.

2. The AHL-SdiA system can enhance AR by activating *rpoS* and *gad* AR system, and this regulatory mechanism mainly function in acid stress enviroments.

3. AHLs with some unknown factors were involved in the gene regulatory mechanism of AR for *E. coli*.

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