



Title	Arginine deiminase pathway is far more important than urease for acid resistance and intracellular survival in <i>Laribacter hongkongensis</i>: a possible result of arc gene cassette duplication
Advisor(s)	254499
Author(s)	Xiong, L; Teng, JLL; Watt, RM; Kan, B; Lau, SKP; Woo, PCY
Citation	The 23rd European Congress of Clinical Microbiology and Infectious Diseases (ECCMID 2013), Berlin, Germany, 27-30 April 2013.
Issued Date	2013
URL	http://hdl.handle.net/10722/204312
Rights	Creative Commons: Attribution 3.0 Hong Kong License

Arginine deiminase pathway is far more important than urease for acid resistance and intracellular survival in *Laribacter hongkongensis*: a possible result of *arc* gene cassette duplication



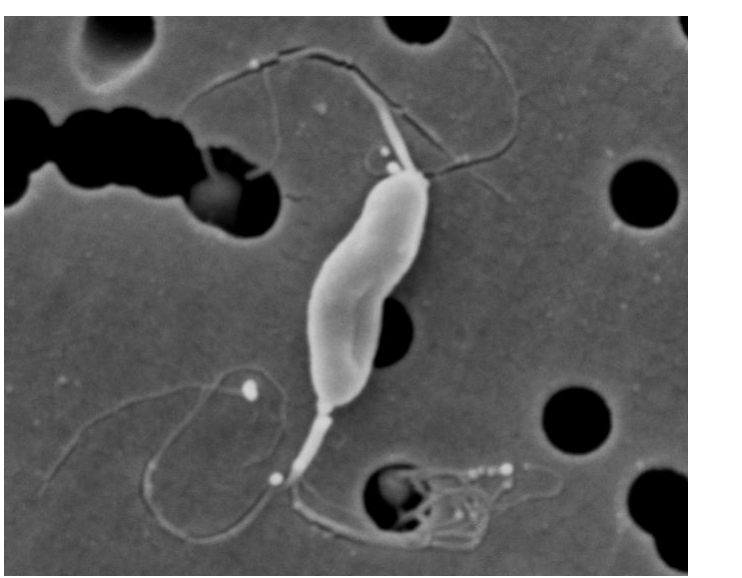
Lifeng Xiong,¹ Jade L. L. Teng,^{1,2} Rory M. Watt,³ Biao Kan,⁴ Kwok-Yung Yuen,^{1,2} Susanna K. P. Lau^{1,2} and Patrick C. Y. Woo^{1,2}

¹Department of Microbiology, The University of Hong Kong, Hong Kong

²Research Centre of Infection and Immunology, The University of Hong Kong, Hong Kong

³Oral Biosciences, The University of Hong Kong, Hong Kong

⁴State Key Laboratory for Infectious Disease Prevention and Control, National Institute for Communicable Disease Control and Prevention, Beijing, China



INTRODUCTION & PURPOSE

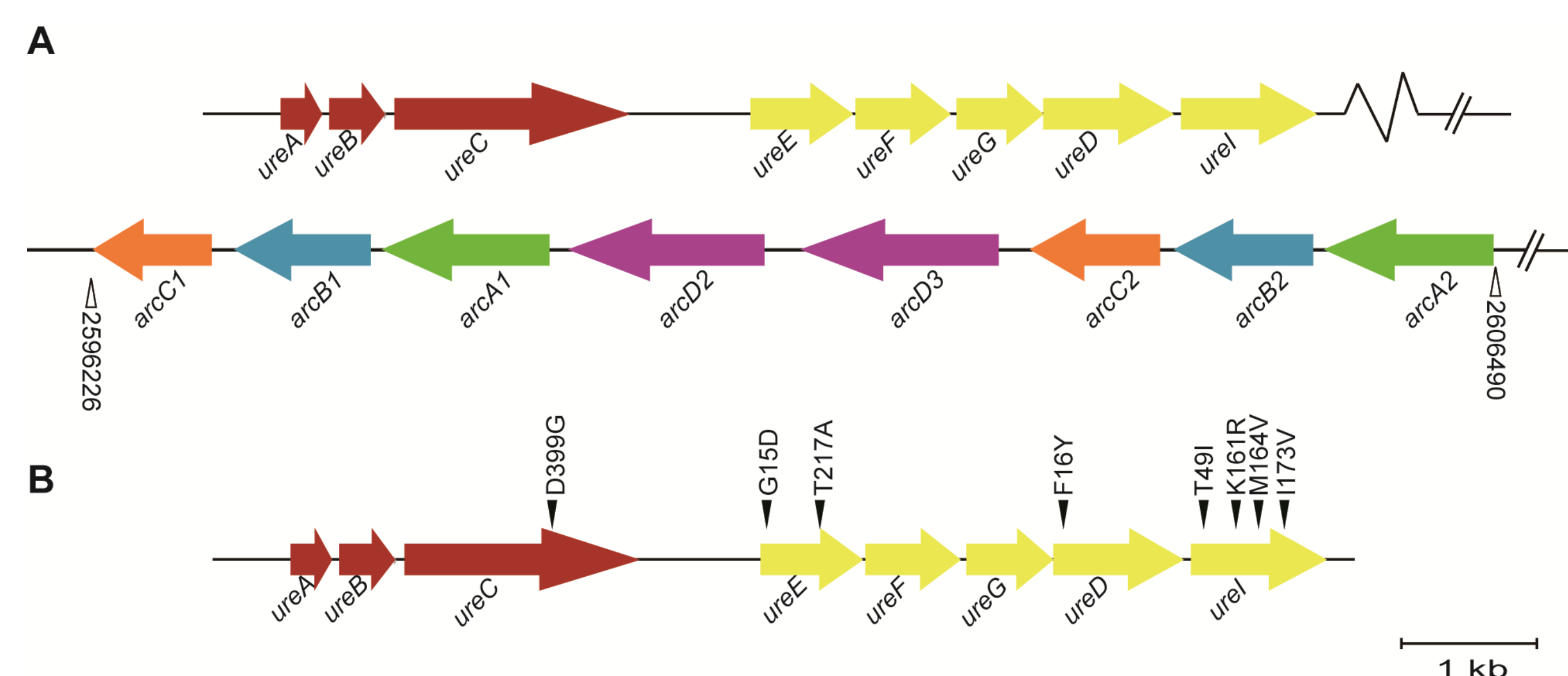


Fig. 1. Genetic organization of urease gene cassette and the two adjacent *arc* gene cassettes in the genome of *L. hongkongensis*.

Laribacter hongkongensis is a Gram-negative, urease-positive bacillus associated with invasive bacteremic infections in liver cirrhosis patients and fish-borne community-acquired gastroenteritis and traveler's diarrhea (1-2). Its mechanisms of acid resistance are unknown. A complete urease cassette and two adjacent *arc* gene cassettes (encoding enzymes of ADI pathway) were found in the genome (3). In this study, we investigated the mechanism for resisting acidic environment in vitro, in macrophages and in a mouse model.

METHODS

- Construction of non-polar deletion mutant strains (urease-negative and ADI-negative mutants);
- In vitro susceptibility of *L. hongkongensis* to acid pH (pH 2 to 6);
- Intracellular survival assays of wild type and mutants in J774 macrophages;
- Survival of *L. hongkongensis* in mouse model.

RESULTS

1. At pH 2 and 3, survival of HLHK9 Δ *arcA1/arcA2* and HLHK9 Δ *ureA/arcA1/arcA2* were markedly decreased ($P < 0.001$), while HLHK9 Δ *ureA* was slightly decreased ($P < 0.05$).

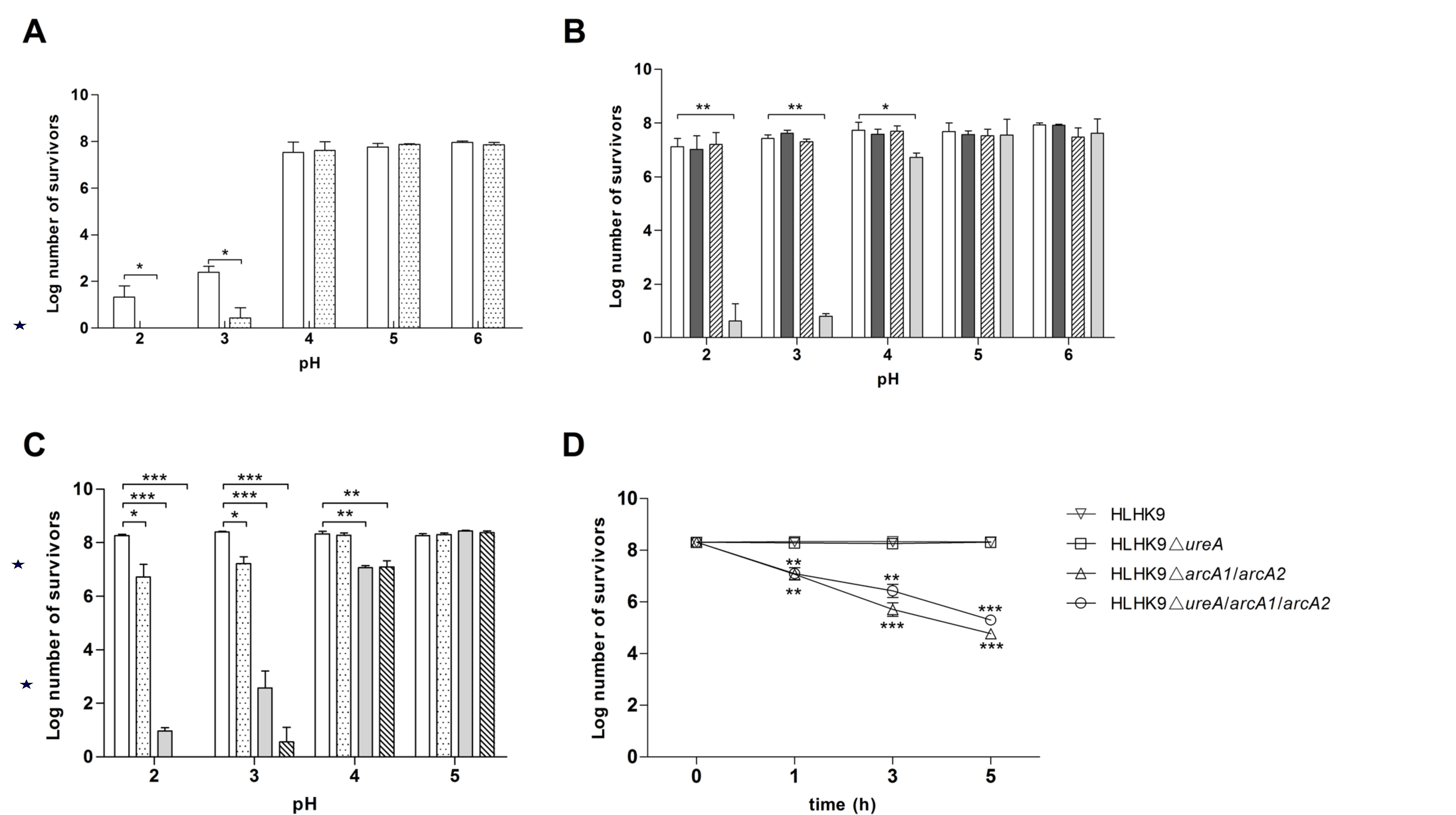


Fig. 2. Survival of wild type *L. hongkongensis* HLHK9 and derivative mutants under acidic conditions. An asterisk indicates a significant difference (*, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$).

2. HLHK9 Δ *ureA/arcA1/arcA2* and HLHK9 Δ *arcA1/arcA2* in macrophages were markedly decreased ($P < 0.001$ and $P < 0.01$ respectively) but that of HLHK9 Δ *ureA* was slightly decreased ($P < 0.05$), compared to wild type *L. hongkongensis* HLHK9. Although the mRNA levels of *arcA1*, *arcA2* and *ureA* genes were all significantly increased compared to those at 2 h post infection.

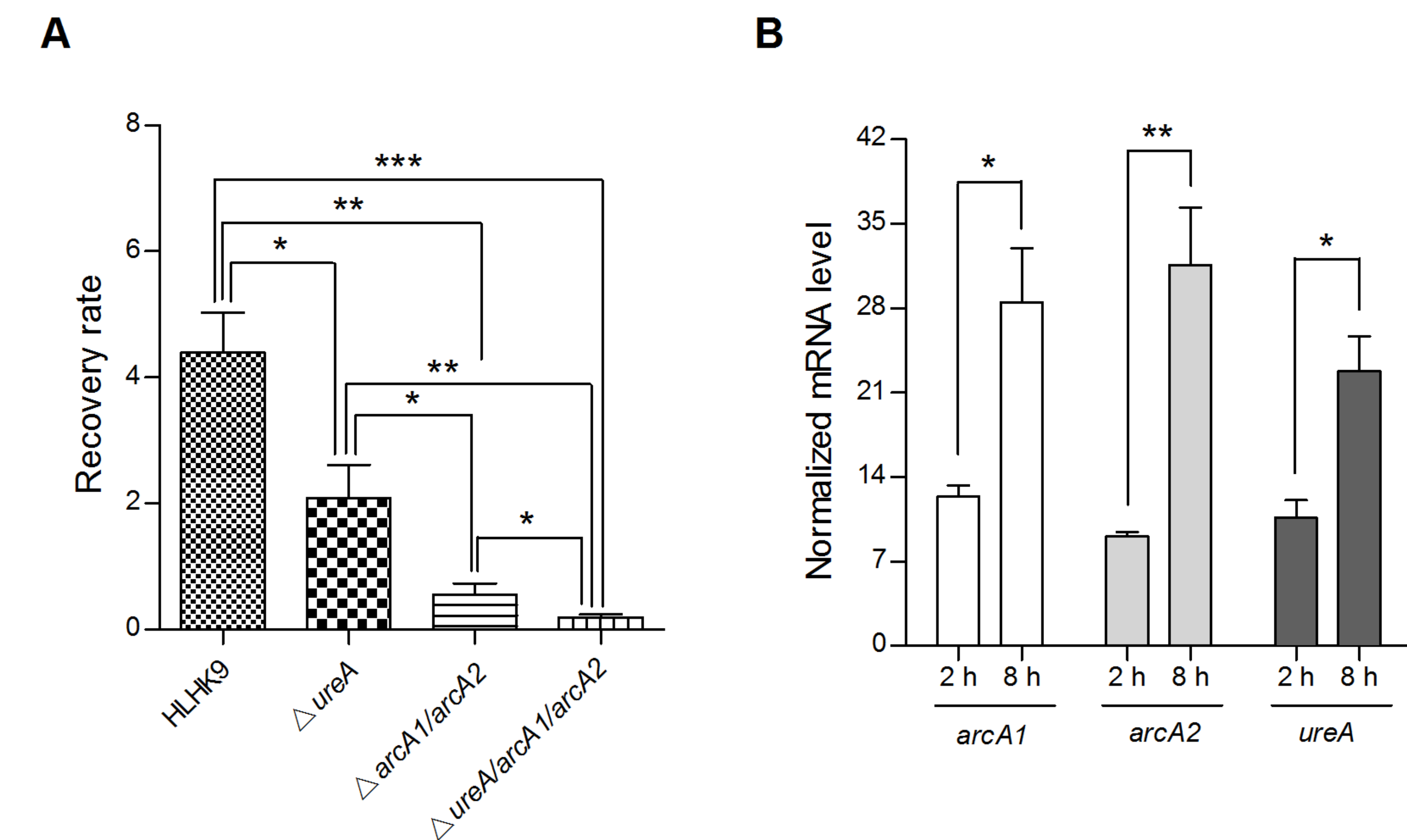


Fig. 3. (A) Recovery rates of wild type *L. hongkongensis* HLHK9, HLHK9 Δ *ureA*, HLHK9 Δ *arcA1/arcA2* and HLHK9 Δ *ureA/arcA1/arcA2* in J774 macrophages. (B) Expression level of ADI genes (*arcA1* and *arcA2*) and *ureA* gene of HLHK9 in macrophages.

3. HLHK9 Δ *ureA* exhibited similar survival compared to HLHK9, but survival of HLHK9 Δ *arcA1/arcA2* and HLHK9 Δ *ureA/arcA1/arcA2* were markedly reduced ($P < 0.01$).

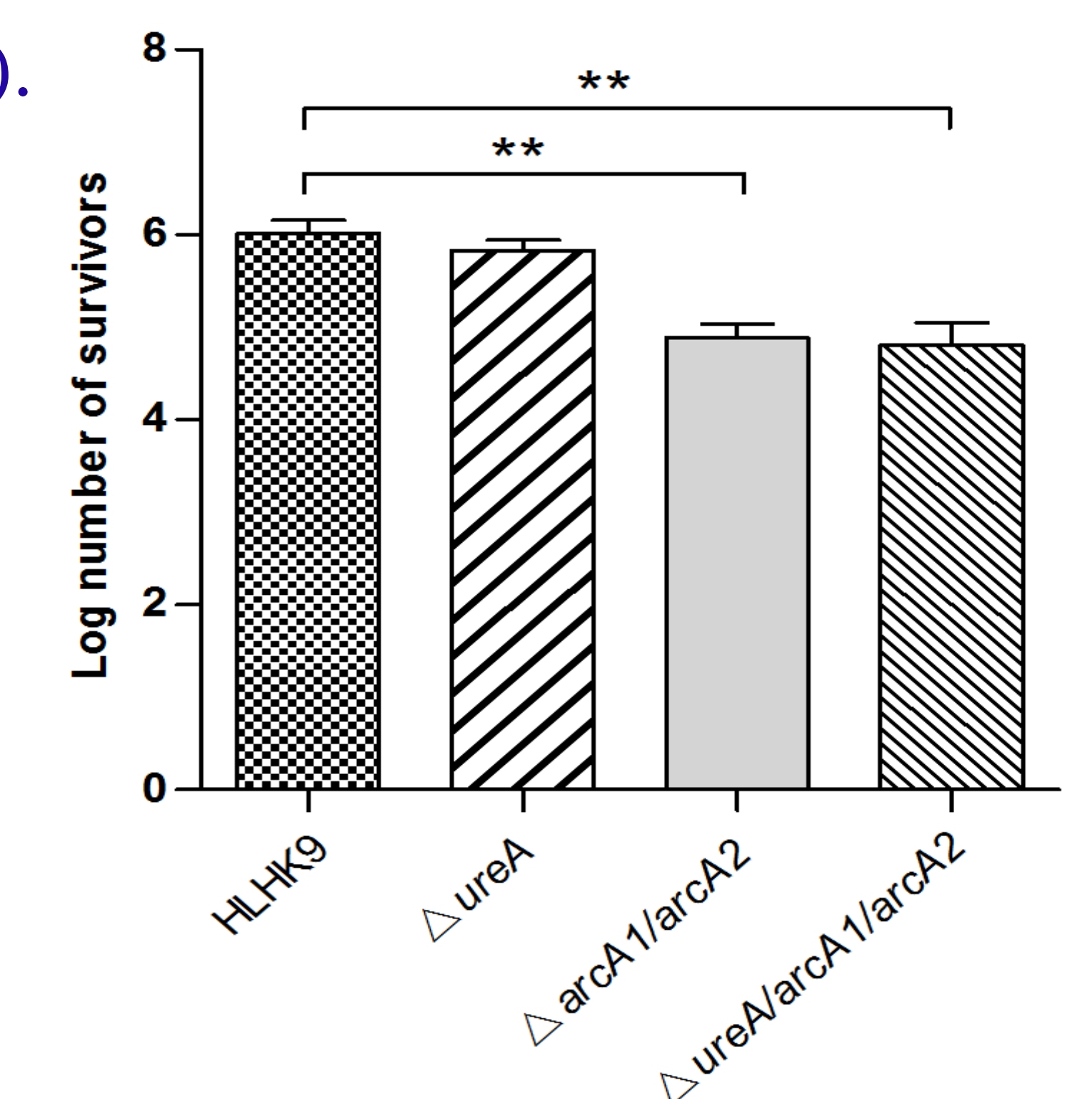


Fig. 4. Survival of wild type *L. hongkongensis* HLHK9 and derivative mutants in a mouse gastric passage model. Comparison of the survival of wild type *L. hongkongensis* HLHK9, HLHK9 Δ *ureA*, HLHK9 Δ *arcA1/arcA2* and HLHK9 Δ *ureA/arcA1/arcA2* after passing through the stomach of mice. Error bars represent means \pm SEM of three independent experiments. An asterisk indicates a significant difference.

CONCLUSIONS

- ❖ The ADI pathway of *L. hongkongensis* plays a much more important role than the urease in resisting acidic environments;
- ❖ Both of ADI pathway and urease system have significant contribution to the replication of *L. hongkongensis* in macrophages; however, the ADI pathway plays a more important role.
- ❖ The ADI pathway plays a more significant role than urease in the survival of *L. hongkongensis* under the acidic conditions encountered during passage through the mouse gastric environment.

REFERENCES

1. Yuen, K. Y., et al (2001) *Laribacter hongkongensis* gen. nov., sp. nov., a novel gram-negative bacterium isolated from a cirrhotic patient with bacteremia and empyema. J Clin Microbiol 39: 4227-4232.
2. Woo, P. C., et al. (2004) Association of *Laribacter hongkongensis* in community-acquired gastroenteritis with travel and eating fish: a multicentre case-control study. Lancet 363: 1941-1947.
3. Woo, P. C., et al. (2009) The complete genome and proteome of *Laribacter hongkongensis* reveal potential mechanisms for adaptations to different temperatures and habitats. PLoS Genet 5: e1000416.