

**Evaluation of the Role of Ammonia and Urea
Transporters on the Pathogenesis of
*Edwardsiella ictaluri***

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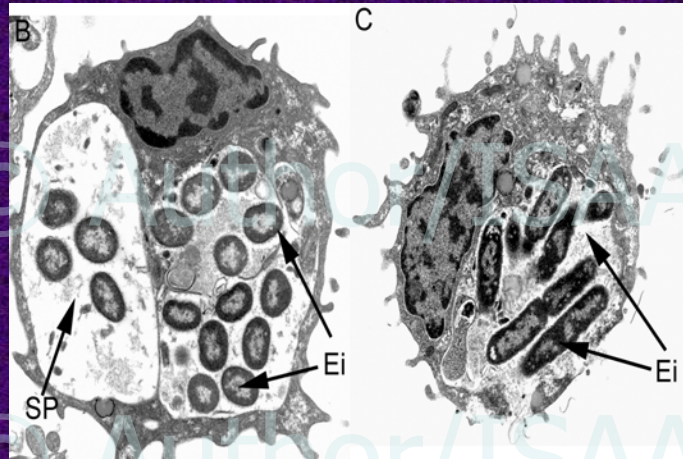


Edwardsiella ictaluri

**Enteric Septicemia
of Catfish**



***E. ictaluri* can survive and replicate in channel catfish macrophages.**



Booth et al. 2006. J. Aquatic Animal Health. 18:101.

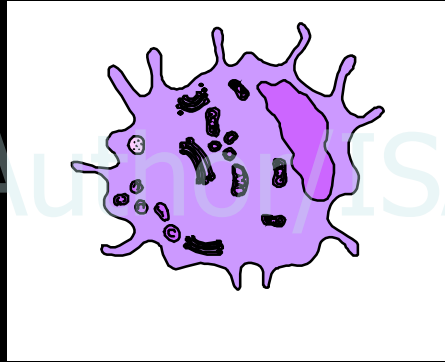
Signature tagged transposon mutagenesis

- **Survival and replication in channel catfish macrophages requires:**

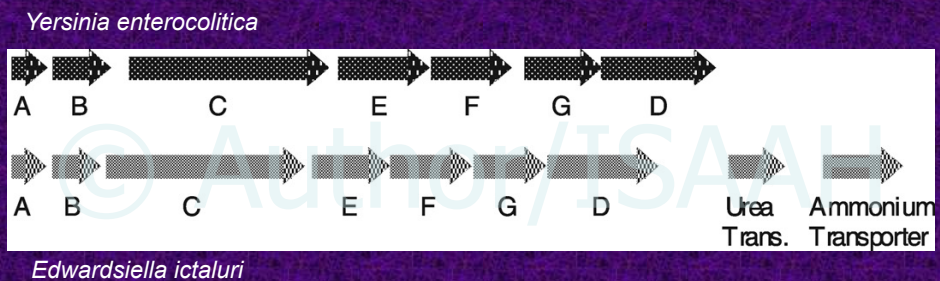
- **Type III secretion system** (Thune et al. 2007. Appl. Environ. Microbiol. 73:7934)

- **Acid activated urease enzyme** (Booth et al. 2009. Appl. Environ. Microbiol. 75:6712)

E. ictaluri and Macrophages



Urease genes of *E. ictaluri* and *Y. enterocolitica*



Escherichia coli and *Helicobacter pylori* also encode a similar gene cluster, without the transporters

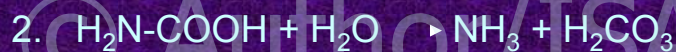
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- *Y. enterocolitica*, *E. coli* and *H. pylori* ureases are acid-inducible and are involved in survival in low pH conditions.
- *E. ictaluri* urease is not required for survival in low pH conditions.

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Urease reaction

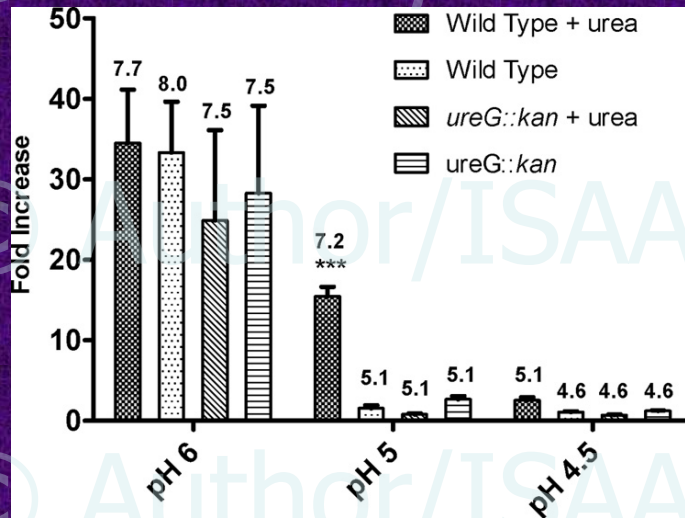
Urease



Results in net increase in the pH of the environment

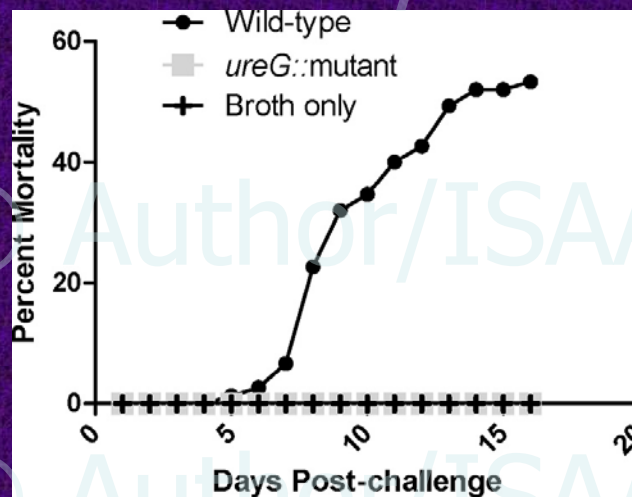
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Growth of WT and *ureG* mutant in BHI



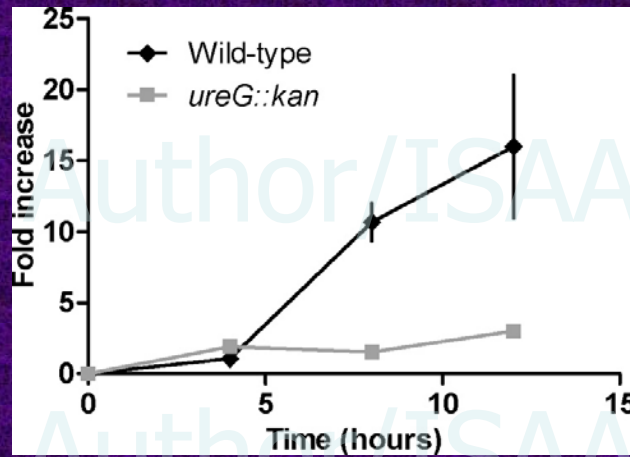
Booth et al. 2009. Appl. Environ. Microbiol. 75:6712

Percent mortality for wild-type and urease mutant *E. ictaluri* following immersion challenge



Booth et al. 2009. Appl. Environ. Microbiol. 75:6712

% Increase of wild-type and urease mutant in HKDM in a gentamicin survival assay



Booth et al. 2009. Appl. Environ. Microbiol. 75:6712

Conclusion

- *E. ictaluri* encodes an acid inducible urease operon that can modulate environmental pH and is involved in intracellular replication.
- *E. ictaluri* is the first bacterial pathogen known to involve the urease enzyme in intra-cellular pathogenesis

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E. ictaluri is the only bacterium that encodes urea and ammonia transporters in a urease gene cluster

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- **Hypothesis:** The *E. ictaluri* urea and ammonia transporters are actively expressed

- **Aim 1:** Demonstrate transcription of the *E. ictaluri* urea and ammonia transporters
- **Aim 2:** Demonstrate translation of the *E. ictaluri* urea and ammonia transporters

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RT-PCR to Demonstrate Transcription of the Ammonia and Urea Transporter Genes in WT, Δ AT, and Δ UT *E. ictaluri*



Western Blots with plasmid encoded Flag-tagged AT and UT in Δ AT and Δ UT *E. ictaluri* strains using anti-Flag antibody failed to demonstrate translation

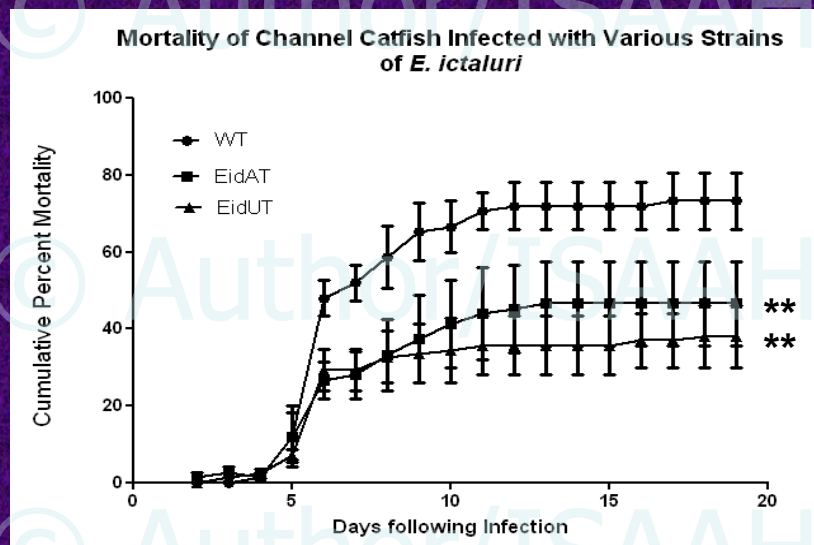
Conclusions

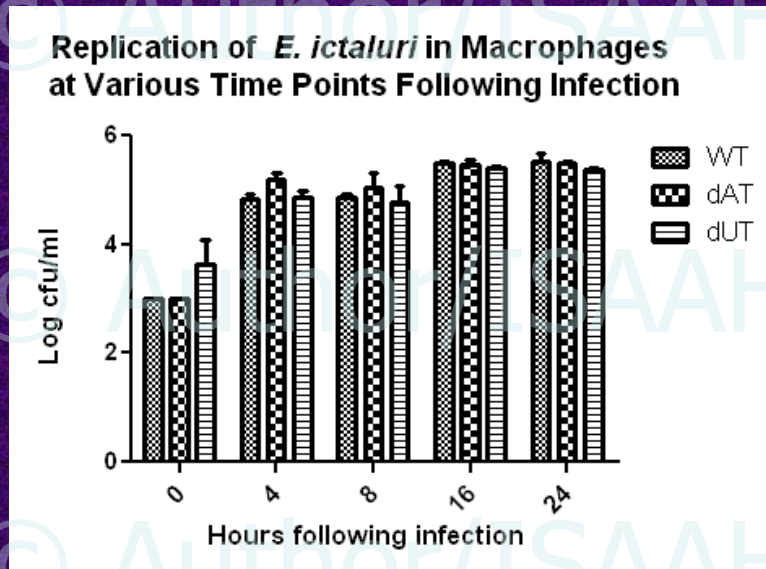
- The *E. ictaluri* urea and ammonia transporters are actively transcribed
- Additional work is required to demonstrate translation.

- **Hypothesis** *E. ictaluri* urea and ammonia transporters are important to *E. ictaluri* pathogenesis
 - **Aim 1.** Construct isogenic mutants of the *E. ictaluri* urea and ammonia transporters
 - **Aim 2.** Evaluate the pathogenesis of the *E. ictaluri* urea and ammonia transporters *in vivo*
 - **Aim 2.** Evaluate the role of the *E. ictaluri* urea and ammonia transporters in intracellular replication

Mutant Construction

- Ammonia and urea transporter genes were amplified from WT *E. ictaluri* genomic DNA and inserted into pBluescript.
- Deletion/insertion mutants with a kanamycin marker were constructed by inverse PCR
- The mutated genes were transferred to the suicide vector pRE107 and transferred to wild-type *E. ictaluri* by conjugation and allelic exchange
- Mutations in the genome were verified by PCR and DNA sequencing.





Conclusions

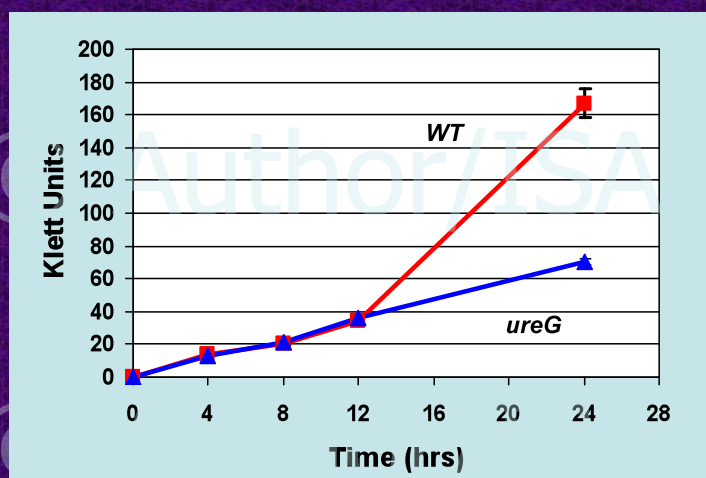
- The *E. ictaluri* urea and ammonia transporters are involved in pathogenesis
- The gentamycin exclusion assay failed to demonstrate a role for the *E. ictaluri* urea and ammonia transporters in intracellular survival and replication

Acknowledgements



United States Department of Agriculture
National Research Initiative
Competitive Grant Program

Growth of WT and *ureG* mutant in BHI at pH 5.0 with 6mM Urea



**Western Blots with plasmid encoded Flag-tagged
AT and UT in Δ AT and Δ UT *E. ictaluri* Strains**

