Phenotype, Virulence and Immunogenicity of *Aeromonas salmonicida* Cyclic Adenosine 3',5'-Monophosphate Receptor Protein (Crp) Mutants in Fish Host Toxicity

Amanda Loh, Roy Curtiss III and Javier Santander
Biodesign Institute, Arizona State University, Tempe, 85287, AZ.
aloh1@asu.edu

ABSTRACT

Aeromonas salmonicida is a Vibrionaceae family member that causes a lethal disease called furunculosis in marine and freshwater fish. Being a mucosal facultative intracellular pathogen, this bacterium is an excellent candidate for developing immersion-oral live attenuated vaccines for the salmon-trout aquaculture industry. Deletion of the cyclic 3',5'-adenosine monophosphate (cAMP) receptor protein (*crp*) gene has been utilized in live attenuated vaccines for mammals and birds. Here we characterize the *crp* gene and report the effect of a *crp* deletion in *A. salmonicida*. The *A. salmonicida crp* gene and encoded protein are similar to other Enterobacteriaceae and Vibrionaceae family members, complementing *Salmonella enterica* and *Edwardsiella* Δ*crp* mutants in a cAMP-dependent fashion. The *A. salmonicida* Δ*crp-12* in frame deletion mutant demonstrated slight growth defects, loss of maltose utilization among other sugars, and lack of brown pigment synthesis. We found that the *A. salmonicida* Δ*crp-12* mutant was attenuated and conferred immune protection against *A. salmonicida* infection to the fish. We propose that deletion of the *crp* gene in *A. salmonicida* is an effective strategy for developing immersion live attenuated antibiotic-sensitive vaccines for the catfish aquaculture industry.

BACKGROUND

Bacteria typically utilize glucose as their primary source of carbon, using ATP to transport glucose into the cell. However, when glucose is not available, adenylate cyclase is activated, converting ATP into cAMP. cAMP binds to Crp, initiating the transcription of certain genes related to carbohydrate utilization and in pathogenic bacteria, related to virulence.

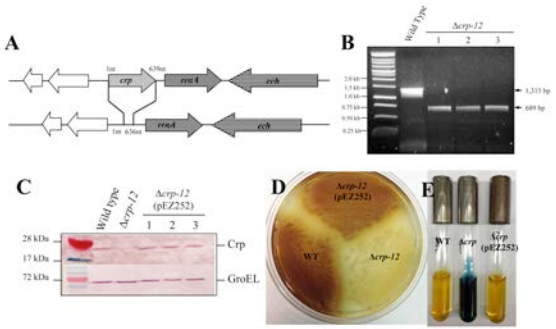

Figure 1. Deletion of *crp* gene, phenotype and complementation of *A. salmonicida crp* mutant. A. Gene deletion map; **B.** PCR verification of *crp* deletion; **C.** Western blot of *A. salmonicida* Δ*crp-12* mutant and *A. salmonicida* Δ*crp-12* complemented with pEZ252 (P_{crp}-*crp*); **D.** Synthesis of brown pigmentation on Tripticase-Soy Agar; **E.** Maltose utilization in OF-media. Yellow indicates utilization of the maltose to acid products. Green-Blue indicates no utilization of maltose.

HYPOTHESIS

We hypothesized that *crp* is well conserved across species and that the complementation of the *A. salmonicida crp* gene into *S.* Typhimurium and *E. ictaluri* would provide the same function. We also thought measuring cAMP dependence would demonstrate that *Crp* is linked to carboyhydrate metabolism.

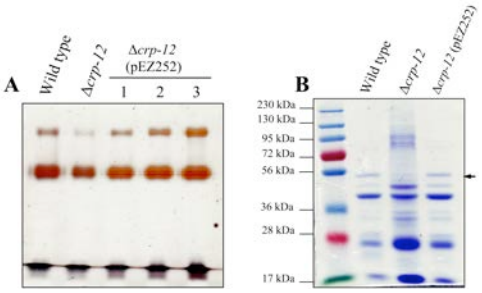

Figure 2. Effect of *crp* deletion on *A. salmonicida* outer membrane. A. Lipopolysaccharide profile; **B.** Outer membrane protein profile. The arrow indicates the A-layer protein.

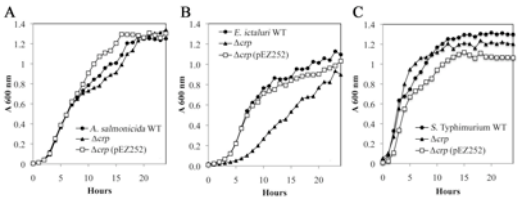

Figure 3. Growth curve. A. Growth of *A. salmonicida* in LB broth at 37°C*;* **B.** Growth of *E. ictaluri* in BHI broth at 28°C*;* **C.** Growth of *S.* Typhimurium in LB broth at 37°C.

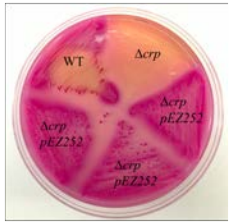

Figure 4. *crp* complementation of *A. salmonicida crp* in *S.* Typhimurium. *S.* Typhimurium wild type, Δ*crp-12* mutant and pEZ252 complements on MacConkey maltose plate at 37°C.

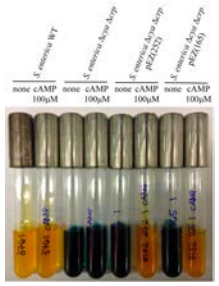

Figure 5. *crp*-cAMP dependence test. Strains grown in OF media with 1% maltose.

Figure 6. *crp* complementation of *A. salmonicida crp* in *E. ictaluri*. *E. Ictaluri* wild type, Δ*crp* mutant and pEZ252 complements in OF media with maltose at 37°C.

Strain	Dose	Survival/total
Wild type	3.75×10^8	0/5
	3.75×10^7	0/5
	3.75×10^6	4/5
	3.75×10^5	5/5
Δ*crp-12*	1.67×10^8	2/5
	1.67×10^7	5/5
	1.67×10^6	5/5
	1.67×10^5	5/5

Table 1. Virulence of *A. salmonicida* strains in adult zebrafish (*D. rerio*) intra muscular infected.

CONCLUSIONS

1. Crp is well conserved through evolution outside of the enterobacteriaceae family.
2. Crp regulates the transcription of genes related to carbohydrate utilization in a cAMP-dependent fashion as well as those related to pathogenesis.

FUTURE WORK

1. Identify genes that are regulated by *crp* including the ones that synthesize the brown pigment and allow for the utilization of maltose.
2. Locate the promoter regions and *crp* consensus binding sites to find other unknown genes controlled by *crp*.
3. Perform animal experiments on zebrafish to test the immunogenic properties expected of a *crp* gene deletion.

REFERENCES

1. Santander J, Mitra A, Curtiss III R. Phenotype, virulence and immunogenicity of *Edwardsiella ictaluri* cyclic adenosine 3',5'-monophosphate receptor protein (Crp) mutants in catfish host. Fish & Shellfish Immunology 2011; 31:1142-1153.
2. Bullock GL, Cipriano RC, Snieszko, SF. Furunculosis and other diseas caused by Aeromonas salmonicida. 1983.

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ASU BARRETT THE HONORS COLLEGE ARIZONA STATE UNIVERSITY
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