2010

# Genetic regulation of adenylate cyclase genes in Bdellovibrio bacteriovorus

Kat Schmidt
*University of Puget Sound*

# Genetic regulation of *cya* genes in *Bdellovibrio bacteriovorus*

Kat Schmidt, Dr. Mark O. Martin

Biology Department, University of Puget Sound, Tacoma, Washington

University of Puget Sound
1500 N. Warner Street
Tacoma, Washington
98416

## Abstract

*Bdellovibrio bacteriovorus* is a deltaproteobacterium with an obligatory predatory lifestyle, consuming a wide variety of other Gram-negative bacteria. Its unusual life cycle and genome make it a particularly interesting organism to study. In this experiment, two of *Bdellovibrio's* five adenylate cyclase genes, *cya*A and *cya*K, and their roles in the different stages of *Bdellovibrio's* life cycle were investigated. *Cya* genes encode for adenylate cyclase, an enzyme that synthesizes cyclic-AMP (cAMP), which serves as a global secondary messenger for gene regulation. The two *cya* genes, upon which research was focused, appear to be differentially regulated during predation, which makes this subject of specific interest. Plasmids that are either *cya*A$^-$ or *cya*K$^-$ were created and introduced into *Escherichia coli*, creating new mutant strains. From there a *cya*K$^-$ strain of *Bdellovibrio* was created. Future work will involve creating a *Bdellovibrio* strain that lacks a functional *cya*A gene, and comparing the predation efficiency of the two mutated *Bdellovibrio* strains to normal *Bdellovibrio*.

## Introduction

*Bdellovibrio bacteriovorus* is a relatively small, host-dependent (HD) Gram-negative delta-proteobacterium that preys upon larger Gram-negative bacteria, and was discovered in the 1960s by Hans Stolp and Mortimer Starr (Stolp, 1963). Inspection of *Bdellovibrio's* single, circular chromosome shows it to contain very few repeated DNA elements. Two notable exceptions to *Bdellovibrio's* generally non-repeating genome are the multiple *pil* and *cya* genes, which code for pilin components and adenylate cyclase, respectively. *Bdellovibrio's* unusual genome emphasizes its uniqueness among bacteria (Evans, 2007; Sockett, 2009).

Of particular interest regarding *Bdellovibrio's* life cycle is the role and regulation of adenylate cyclase (AC). Generally, adenylate cyclase in organisms is encoded by the *cya* gene (Baker, 2004; Botsford, 1992). While most bacteria, such as *E. coli,* have only one *cya* gene, *Bdellovibrio* has five *cya*-like genes (see Figure 1). Four of *Bdellovibrio's* five *cya* genes have been successfully cloned by Jillian Waters in Dr. Martin's laboratory. It was observed from semi-quantitative RT-PCR analysis that two of these genes, *cya*A and *cya*K, appear to be differentially regulated, depending upon the life-stage of the *Bdellovibrio* (Waters, 2008). *Cya*A appears to be upregulated in growth phase *Bdellovibrio*, while *cya*K is upregulated in attack phase *Bdellovibrio*.

Although *cya*A and *cya*K both synthesize cAMP, bioinformatic analysis of both genes shows significant differences in their domain structure and character (Waters, 2008). *Cya*A has a HAMP domain, which is generally located in adenylate cyclases, and histidine kinases. Since the HAMP domain is usually a part of integral membrane proteins, it is likely that this AC is an integral membrane protein (Aravind, 1999). *Cya*K has a CHASE2 domain, which is an extracellular sensory domain. CHASE2 is generally located in histidine kinases, diguanylate cyclases, and phosphodiesterases, and recognizes short peptide series (Mougel, 2001). It may be that the extracellular portion of *cya*K "senses" some signal regarding life in attack phase for *Bdellovibrio* while out "hunting" for prey cells, while *cya*A is involved in growth related maintenance. Mutational analysis may provide an answer to these questions.

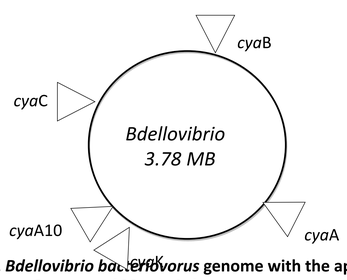


**Figure 1. *Bdellovibrio bacteriovorus* genome with the approximate positions of the five adenylate cyclase genes. – Courtesy of Martin Laboratories.**

## Life Cycle

Like other predatory prokaryotes, *Bdellovibrio* has an unusual life cycle (see Figure 2) as well as an unusual genome (Martin, 2002; Rendulic, 2004). Host-dependent (Wild type) *Bdellovibrio's* life cycle is characterized by two stages: attack phase (AP) and growth phase (BD). When in attack phase, *Bdellovibrio* is free-swimming, searching for and finding prey (Koval, 1997). Once it has invaded the prey cell, *Bdellovibrio* inhabits the periplasmic region between membranes, and enters growth phase.

During growth phase, *Bdellovibrio* digests its host from within using an array of hydrolytic enzymes, and the original invading cell undergoes replication and division, via elongation and septation of a growing filament. The progeny become flagellated cells, the host cell is lysed, and the new *Bdellovibrio* cells are released to seek out new prey (Rendulic, 2004; Sockett, 2009).

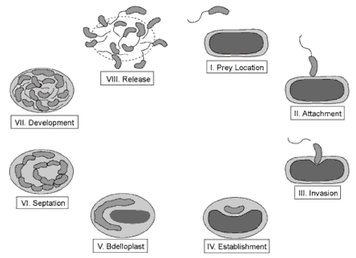


**Figure 2. Life cycle of *Bdellovibrio bacteriovorus*. – Courtesy of Max Planck Institute for Developmental Biology .**

## Materials and Methods

1. *Escherichia coli* strain PD200 was tested for *cya*$^-$ genotype by phenotypic assays: Colonies were grown overnight at 37.0 degrees Celsius on MacConkey plus 1% maltose plates, and plates that yielded only small, white colonies were selected.
2. Plasmids bearing either *cya*A or *cya*K were extracted from frozen stock of *E. coli* strain TOP10 using QIAprep Spin Miniprep Kit.
3. These plasmids were tested by transforming them via electroporation (using BIO-RAD MicroPulser$^{TM}$) into electrocompetent PD200 and plating onto MacConkey plus 1% maltose. Colonies were grown overnight at 37.0 degrees Celsius. A phenotypical assay was performed, where pink colonies confirmed successful transformation.
4. Plasmids confirmed to be carrying either *cya*A or *cya*K were selected for transposon mutagenesis. Following the HyperMu$^{TM}$ <CHL-1> Insertion Kit protocol, a transposon carrying chloramphenicol resistance (Cm$^R$) was inserted into each *cya* gene, effectively disabling it.
5. These new plasmids were transformed via electroporation into *E. coli* strain PD200, and spread onto LB plates containing chloramphenicol (15ug/ml). Colonies were grown overnight at 37.0 degrees Celsius. Any resulting colonies were Cm$^R$ and therefore had successfully been both mutated and transformed. To confirm presence of *cya* genes, PCR was performed using BIO-RAD MyCycler thermal cycler.
6. Colonies were then selected and patched onto both MacConkey plus 1% maltose and LB plus chloramphenicol (15ug/ml) plates, and grown overnight at 37.0 degrees Celsius. Colonies that were both white on MacConkey maltose and grew well on plates with chloramphenicol were then selected and stored.
7. The plasmid bearing a non-functional copy of *cya*K was then transformed via electroporation into electrocompetent host-independent *Bdellovibrio* strain 109J KAI Rf. Colonies were grown over a week at 30.0 degrees Celsius on plates containing PYE media and chloramphenicol (15ug/ml). Any resulting colonies were Cm$^R$ and therefore had successfully been both mutated and transformed.

## Conclusions

- By evidence of our phenotypic assays, and PCR, using transposon mutagenesis to "knock-out" *cya*A and *cya*K genes was successful, as was transforming plasmids carrying one of the disabled genes into *E. coli* and *Bdellovibrio*.
- We now have plasmids carrying non-functional *cya*A and *cya*K genes, respectively, and *E. coli* strain PD200 containing these plasmids. In addition, we have *Bdellovibrio* host-independent strain 109J KAI Rf bearing a plasmid with a non-functional *cya*K gene.

## In the Future

The next step will be to use transformation via electroporation to create a *cya*A$^-$ strain of host-independent *Bdellovibrio*, and then host-dependent strains of *Bdellovibrio* bearing one of the plasmids containing a knock-out *cya* gene. Once that is accomplished, the *cya*A$^-$ and *cya*K$^-$ strains of *Bdellovibrio* can be compared to normal *Bdellovibrio* using predation assays, the goal of which will be to see if one or both of the mutations makes *Bdellovibrio* a less efficient predator.

## Literature

1. Aravind, L., and C.P. Ponting. (1999). "The cytoplasmic helical linker domain of receptor histidine kinase and methyl-accepting proteins is common to many prokaryotic signaling proteins." *FEMS Microbiol. Lett.* 176: 111-6.
2. Baker, D.A., and J. M. Kelly. (2004). "Structure, function and evolution of microbial adenylyl and guanylyl cyclases." *Molecular Microbiology* 52(5): 1229-1242.
3. Botsford, James L., and James G. Harman. (1992). "Cyclic AMP in Prokaryotes." *Microbiol. Reviews* 56(1): 100-22.
4. Evans, Katy J., Lambert, Carey, and R. Elizabeth Sockett. (2007) "Predation of *Bdellovibrio bacteriovorus* HD100 Requires Type IV Pili." *J Bacteriol.* 189: 4850-9.
5. Koval, S.F., and Bayer, M.E. (1997). "Bacterial Capsules: no barrier against *Bdellovibrio*. *Microbiology* 143: 749-753.
6. Martin, M.O. (2002). "Predatory Prokaryotes: An Emerging Research Opportunity." *J. Mol. Microbiol. Biotechnol.* 4(5): 467-477.
7. Mougel, Christophe, and Igor B. Zhulin. (2001). "CHASE: an extracellular sensing domain common to transmembrane receptors from prokaryotes, lower eukaryotes and plants." *Trends in Biochemical Sciences* 26(10): 582-4.
8. Rendulic, Snjezana, et al. (2004). "A Predator Unmasked: Life Cycle of *Bdellovibrio bacteriovorus* from a Genomic Perspective." *Science* 303(5658): 689-692.
9. Sockett, Renee Elizabeth. (2009). "Predatory Lifestyle of *Bdellovibrio bacteriovorus*." *Annual Review of Microbiology* 63: 523-539.
10. Stolp, H., and Starr, M.P. (1963). "*Bdellovibrio bacteriovorus* gen. et sp. N., a predatory, ectoparasitic, and bacteriolytic microorganism." *Antonie van Leewenhoek* 29: 217-248.
11. Waters, Jillian. (2008). "Preliminary Characterization of Multiple *Cya*-like Genes of the Predatory Bacterium *Bdellovibrio bacteriovorus*." Senior thesis. University of Puget Sound. May 16.

*I would like to express my gratitude and appreciation to my mentor, Dr. Mark Martin, for his patience and guidance; to Kim Dill-McFarland, Greg Kirkpatrick, and Chris Clark for being inspiring labmates; to the American Society for Microbiology for funding my research, and to the Murdock Foundation for financially supporting Dr. Martin's and our work with Bdellovibrio bacteriovorus. Finally, I would like to say thank you to the University of Puget Sound, for their support of student-driven research.*