2010

BD2194 and BD2195 as Regulators of malA and Related Genes in the Predatory Bacterium, Bdellovibrio bacteriovorus

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BD2194 and BD2195 as Regulators of *mal*A and Related Genes in the Predatory Bacterium, *Bdellovibrio bacteriovorus*

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Background

Bdellovibrio is a Gram-negative δ-proteobacter that lives by preying on other Gram-negative bacteria. This organism undergoes two main phases of life: an attack phase (AP) and an intra-periplasmic growth, bdelloplast, phase (BD). During the AP phase, it swims rapidly in search of prey, meeting its target at random. Then during the BD phase, it invades its host's periplasm in order to take over cellular machinery and create progeny (Lambert, 2006).

Annotation of the published genome of *Bdellovibrio* HD100 revealed several maltose associated genes (MAGs), including *mal*A . *Bdellovibrio* cannot transport glucose (Ruby and McCabe, 1988), and thus its uses for genes related to maltose, an alpha 1,4-linked dimer of glucose, are unknown.

Using a host independent spontaneous mutant 109J-KAIRf (Ruby), transposon Tn*5*-17 mutagenesis has identified a possible regulator of *mal*A. This work aimed to identify this regulator as BD2194 or BD2195 as well as to determine whether or not this gene regulates MAGs other than *mal*A. Bioinformatic analysis yields no predicted function for either of these genes and therefore, they are both probable candidates as regulators.

Methods

RNA isolation

- Cultures were grown in PYE liquid to saturation.
- RNA was isolated with the MO BIO UltraClean Microbial RNA Isolation kit and residual genomic DNA was removed with the Baseline-Zero DNase Protocol.
- RNA was shown to be free of contaminating genomic DNA via control PCR reactions with 16s internal primers.

PCR and RT-PCR

- PCR was carried out using Invitrogen's Platinum PCR Supermix at 25 cycles of amplification.
- RT-PCR was completed using the Qiagen OneStep RT-PCR kit at 25 cycles of amplification for 16s and 35 cycles for all other genes.
- All primers were designed using the published genome for *Bdellovibrio* HD100. Amplicons were electrophoresed at 70V on gels of 1.5% agarose in 0.5X TBE buffer.

Complementation

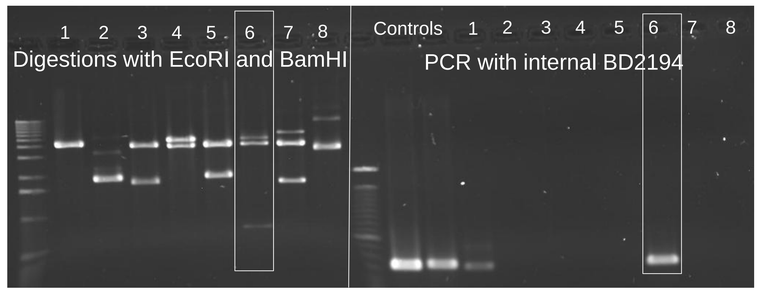
- 109J KAI Rf gDNA was amplified via PCR at 35 cycles with internal BD2194 and BD2195 primers with EcoRI and BamHI termini.
- Vector and inserts were purified using the Qiagen QIAquick PCR Purification kit.
- Ligations were done with the Epicentre QuickLink ligation kit.
- Plasmids with inserts were transformed into *E. coli* TOP10 and selected on LB+Cm and then screened on LB+Cm+X-gal.
- Plasmid DNA was extracted using the QIAprep Spin Miniprep Kit, digested with EcoRI and BamHI, and PCR-ed with internal primers.
- The plasmid was transformed into S17-1 using Studer's Quick and Dirty Electrotransformation technique.

Thank you to Dr. Mark Martin for mentoring me throughout my research and being a great advisor. Also, I'd like to express my gratitude to the Murdock Charitable Trust and ASM for funding my research. Finally, thank you to Greg Kirkpatrick, Kat Schmidt, and Chris Clark for helping to create a fun and productive lab environment.

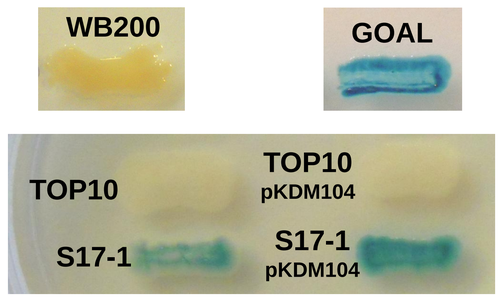
Abstract

Based on enzymology, *Bdellovibrio bacteriovorus* obtains energy via the tricarboxylic acid cycle and amino acid oxidation, as opposed to carbohydrate catabolism. In addition, *Bdellovibrio* cannot transport a number of sugars and lacks a PTS system, and thus appears to not possess a need for maltose metabolism. However, earlier work from this laboratory identified an alpha-glucosidase, *mal*A, and multiple other maltose associated genes (MAGs) within *Bdellovibrio's* genome. Since *Bdellovibrio* is not known to utilize some of the typical regulatory genes of maltose metabolism, such as *crp* or *mal*T, our objective was to identify regulation mechanisms of *mal*A. Through Tn*5*-17 transposon mutagenesis and phenotypic screening, we discovered two possible regulators of *mal*A, the putative uncharacterized genes BD2194 and BD2195. Here, work was begun to complement a *mal*A defective mutant, WB200, with intact copies of both BD2194 and BD2195. Also, semi-quantitative RT-PCR revealed that the regulation of MAGs *amy* and *amy*X was unaffected by WB200's mutation.

Complementation



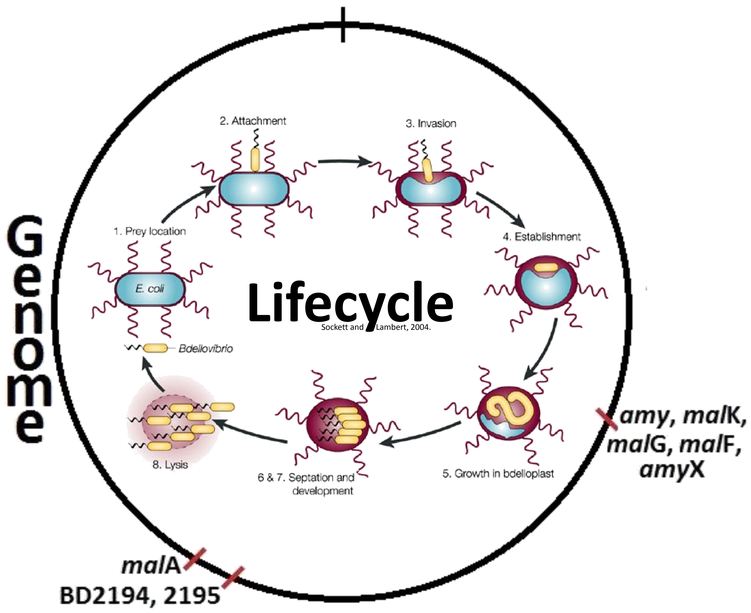
Samples with successful insertions of intact BD2194 should exhibit a ~1.3kb digestion fragment as well as a 500bp PCR amplicon as is shown in sample 6, pKDM104.



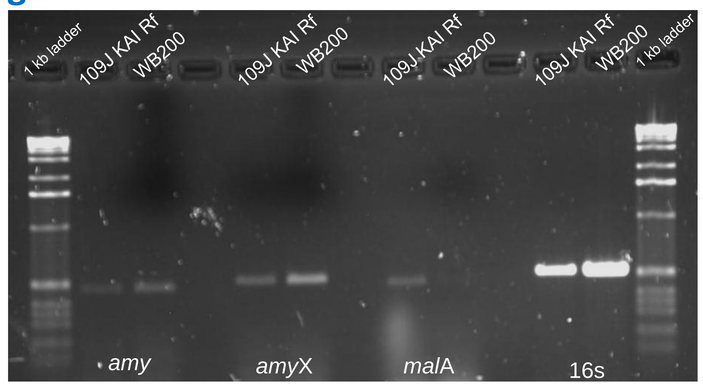
pKDM104 and pKDM105 have been successfully transformed into TOP10 and S17-1. Next, the S17-1 strains will be mated with WB200 to complement its mutation.

Conclusions

- BD2194/5 appears to be a regulator of *mal*A expression.
- As a regulator, BD2194/5 is specific to *mal*A and does not generally effect the other maltose-related genes *amy* and *amy*X.



Regulation



WB200's mutation results in decreased expression of the *mal*A gene. However, it appears to have no effect on such other maltose-related genes as *amy* and *amy*X.

In the future

- Mate S17-1 pKDM104 and pKDM105 with WB200.
- Test more maltose-related genes in WB200.
- Determine differential regulation of *mal*A and related genes throughout *Bdellovibrio*'s lifecycle.
- Search for more metabolic mutants by transposon mutagenesis and screening.

Citations

1. Lambert, C. C. 2006. *Bdellovibrio*: growth and development during the predatory cycle. Curr Opin Microbiol. 9(6):639-44.
2. Ruby, E. G. and J. B. McCabe. 1988. Metabolism of periplasmic membrane-derived oligosaccharides by predatory bacterium *Bdellovibrio bacteriovorus* 109J. J Bacteriol. 170(2):646-52.
3. Ruby, E. G. personal communication.
4. Image: Sockett, R. E. and Lambert, C. 2004. *Bdellovibrio* as therapeutic agents: a predatory renaissance? Nature Reviews Microbiol. 2: 669-675.