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

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Background

*Bdellovibrio* is a Gram-negative β-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 *malA*. *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 Tn5-17 mutagenesis has identified a possible regulator of *malA*. 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 *malA*. 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.
- Ligation reactions 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 QIAGEN Prep 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.

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.

Conclusion

BD2194/5 appears to be a regulator of *malA* expression.

As a regulator, BD2194/5 is specific to *malA* and does not generally effect the other maltose-related genes *amy* and *amyX*.

In the future

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