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Greg Kirkpatrick
University of Puget Sound

His-Tag Protein Purification of the MalA Enzyme in *Bdellovibrio*

Greg Kirkpatrick, Dr. Mark O. Martin
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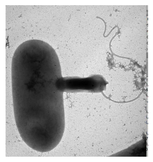
Abstract



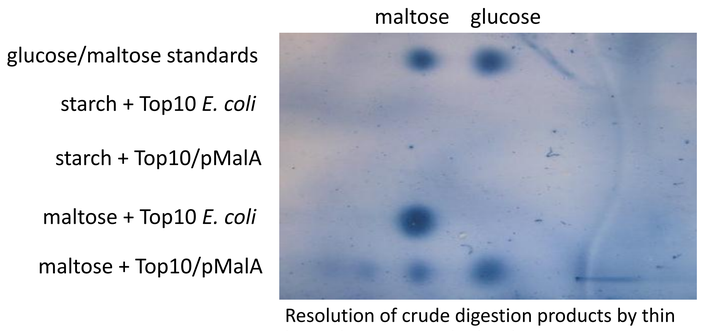
Bdellovibrio invading a prey cell

To obtain a purified preparation of the MalA enzyme from the bacterial predator *Bdellovibrio bacteriovorus,* the gene of interest was cloned into a Novagen pET-30 series vector. This approach adds a histidine tag to the protein, allowing for single-step purification on a nickel column. Cloning and protein overproduction in *E. coli* strain BL21(DE3) were completed, but nickel column purification was unsuccessful. Improved primers should afford a pure enzyme preparation in the near future.

Background and Previous Work

The published genome of the predatory bacterium *Bdellovibrio bacteriovorus* contains the *mal*A gene, coding for an α (1->4) glucosidase-like enzyme. Enzymologically, *Bdellovibrio* appears non-saccharolytic; it posseses low glycolytic and high TCA enzyme levels and is known to not transport glucose. However, recent research showed upregulation of a probable maltose porin at a prey cell degradation stage of predation, suggesting maltose may be included in *Bdellovibrio's* "diet." A glucosidase could potentially be relevant in both predation and metabolism, meaning detailed characterization of MalA may contribute to poorly understood dimensions of this bacterial predator.

In summer 2009, preliminary biochemical assays of MalA using crude cell extract indicated that the enzyme acts on small sugars such as maltose, but not larger polysaccharides. However, a pure enzyme preparation was necessary for confirmation of early results and more sophisticated assay by liquid chromatography coupled with mass spectrometry (LC-MS).

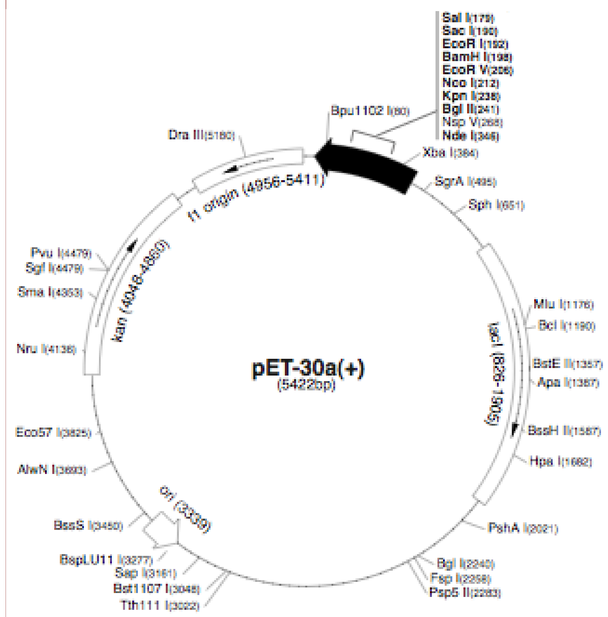


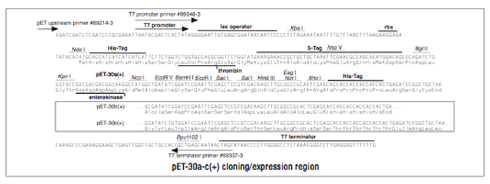
Resolution of crude digestion products by thin layer chromatography (TLC)

Steps Toward Purification

1. Vector and Restriction Enzyme Selection

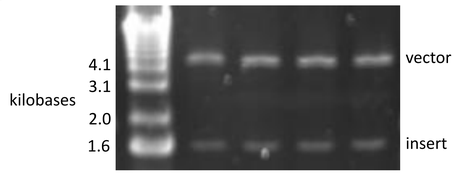
The pET-30 series vectors can add six histidine residues to either the N or C terminus of the desired protein. We selected two different restriction enzymes, EcoRI and SalI, to prevent recircularization of the vector. Use of these enzymes dictated that the histidine tag would be applied to the C terminus of MalA.





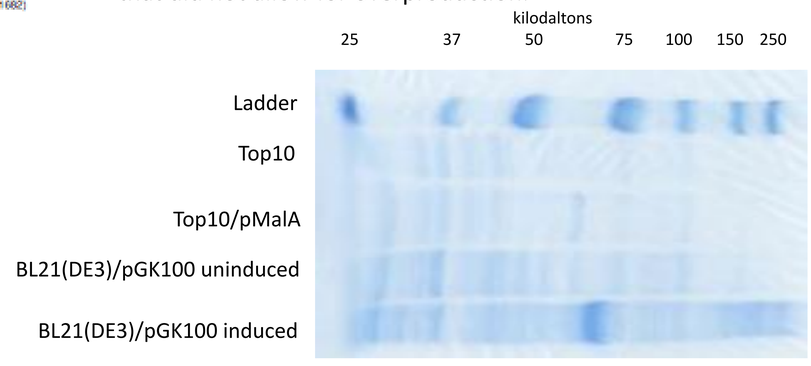
2. Cloning confirmation by gel electrophoresis

Top10 *E. coli* cells transformed with the probable plasmid (pGK100) were grown in liquid culture, then plasmid DNA was extracted and digested with EcoRI and SalI and run on a gel. The appearance of two bands at sizes corresponding to the vector and insert confirmed successful cloning of the *mal*A gene.



3. Transformation and overproduction confirmation by polyacrylamide gel electrophoresis

After successful transformation into Top10 *E. coli,* pGK100 was transformed into BL21(DE3) *E. coli* for overproduction of MalA, induced by addition of IPTG to 1 mM concentration. The results were compared by SDS-PAGE to a previous transformation (pMalA) that did not allow for overproduction.



Future Work

Once the enzyme is purified to an acceptable level, the initial TLC results can be confirmed and the LC-MS method determined by Jordan Carelli can be employed for precise and quantitative identification of sugars present in MalA-catalyzed reactions. This will allow for kinetic characterization of the enzyme, as well as investigation of the active site by incubation with maltose derivatives synthesized by the Hanson group.

Acknowledgements

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