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# Developing a Protocol for Purifying the malA Enzyme in Bdellovibrio bacteriovorus

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# Developing a Protocol for Purifying the malA Enzyme in *Bdellovibrio bacteriovorus*

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## BACKGROUND

The published genome of Bdellovibrio bacteriovorus, a Gram-negative bacterial predator, contains a number of maltose-associated genes, including malA[1]. The enzyme malA encodes for is similar to a an α-(1,4)-glucosidase, e.g. a maltase, and can also function as a glycosyltransferase. However, enzymological studies have shown that Bdellovibrio does not rely on carbohydrates for energy production, and its lack of glucose-transportation machinery suggests that it should be saccharolytically inactive[2]. The observed upregulation of a possible maltose porin during prey cell degradation indicates that maltose might be integral to predation in Bdellovibrio, though[3]. And, if it is, a thorough characterization of the enzyme – its kinetics, mode of action, substrate specificity, capacity for promotion and inhibition – would contribute much to a better understanding of Bdellovibrio physiology.

## GOALS

- To extract the *mal*A enzyme from Top10/p*mal*A E. coli.
- To test and quantify the enzyme's alpha-(1,4)-glucosidase activity using PNPG as a proxy substrate.
- To develop an effective and efficient protocol for the purification of malA.
- To assay the stability of the enzyme's activity at different temperature and pH conditions.

## MAKING *mal*A

### Cell Growth and Lysis

- Top10/pmalA *E. coli* were grown in 500 ml LB to saturation.
- Collected cells were resuspended to 50 ml at pH 6.8, sonically lysed, centrifuged; supernatant was collected and kept for experiments.

### Protein Identification by SDS-PAGE

- The molecular weight of malA is 64 kDa.

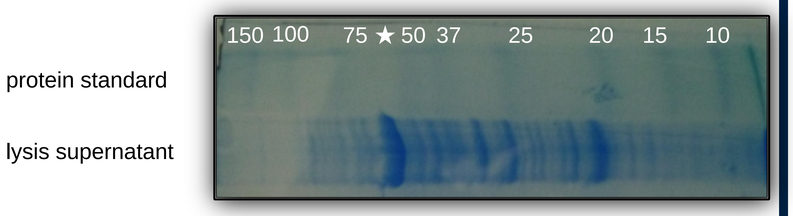
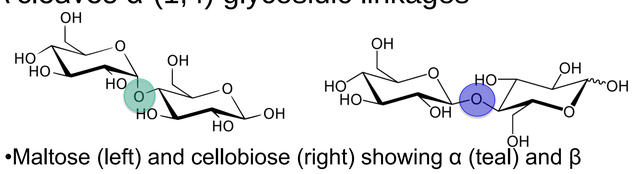



**Figure 1**. SDS-PAGE gel shows an intense band in supernatant sample near 64 kDa★ that is likely to be *mal*A.

## EXPERIMENTAL STRATEGY

### Activity Assay Using PNPG as Proxy Substrate

- malA cleaves α-(1,4) glycosidic linkages



•Maltose (left) and cellobiose (right) showing α (teal) and β (blue) 1,4-glycosidic linkages.

- Cleaving PNPG's α-(1,4) linkage forms yellow para-nitrophenol.

•PNPG (left) is clear; when mixed with sample containing malA (100 µl sample : 900 µl 1 mM PNPG), PNP (right) is formed.

- Quantify enzyme activity by recording absorbance changes at $\lambda_{max}$ = 403.5 nm (unit of activity = Unit = 1 AU / min / ml).

### Ion-Exchange Chromatography

- Theoretical pI of malA is 5.71; expect negative charge at pH 6.8.


•Columns with DEAE- and SP-impregnated agarose were selected. We expected our protein to stick to the "+" charged DEAE column and to recover it by eluting with NaCl after contaminants flowed through and column was washed; we expected the protein to flow through the "–" charged SP column, leaving contaminants stuck behind.

## STABILITY OF ENZYME ACTIVITY

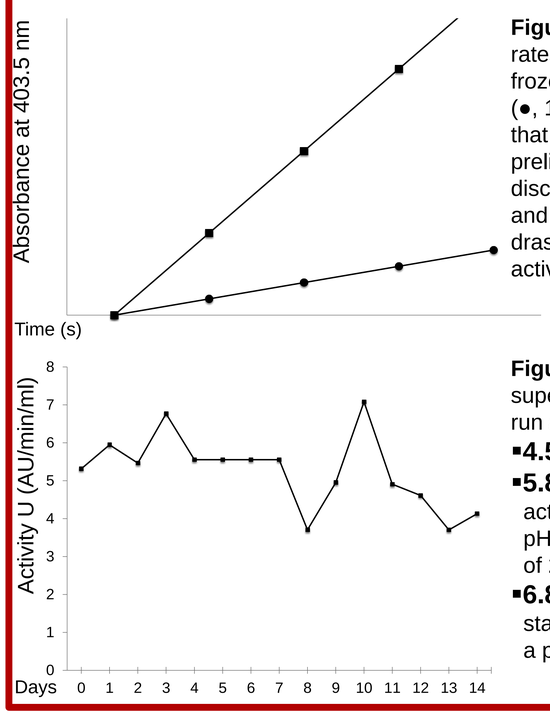



**Figure 2**. PNPG → PNP reaction rates for samples that had been frozen and thawed prior to testing (●, 1.09 Activity Units) and those that had not (■,5.53 U.). During preliminary activity testing we discovered that repeated freezing and thawing of the supernatant drastically reduced its catalytic activity.

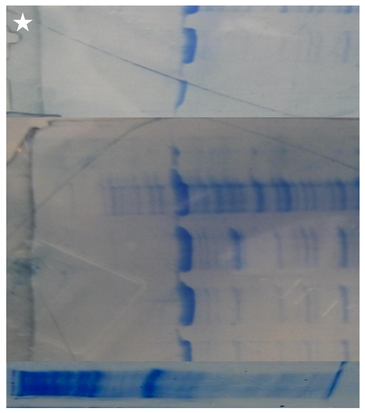
**Figure 3.** Stability assay for supernatant at pH 6.8. Assays were run at four different pH values:

- **4.5, 8.8**: Enzyme was inactive.
- **5.8**: Lost over half of total enzyme activity after buffer exchange from pH 6.8 with subsequent daily loss of 25% activity.
- **6.8** (pictured): Enzyme was very stable, losing only 23% activity over a period of two weeks.

## PURIFICATION

### DEAE Column

- Pushed lysis supernatant through column.
- Our protein did not stick: flowthrough and early wash fractions had the most 64 kDa protein, most activity.
- Purer protein came out: elution contains contaminants

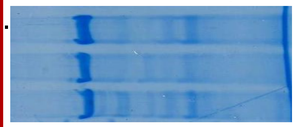


**1**: protein MW standard
**2**: supernatant load (3.70 U)
**3**: first 2 ml flow (0.64 U)
**4**: second 2 ml flow (2.41 U)
**5**: third 2 ml flow (3.22 U)
**6**: first 2 ml wash (2.23 U)
**7**: second 2 ml wash (0.94 U)
**8**: 5 ml 500 mM NaCl elution

**Figure 4**. SDS-PAGE analysis of fractions from DEAE purification. Desired protein appears near 64 kDa.★ The loss of extraneous bands in lanes 3 through 7 and the mess that was eluted indicate purification.

### SP Column and Affinity Chromatography.

- The SP column provided no additional purification



**1**: post-DEAE protein load (7.02 U)
**2**: flowthrough (1.18 U)
**3**: first 2 ml wash (2.15 U)

**Figure 5**. Gel from a second attempt at SP purification after buffer exchange to pH 5.8. Neither our protein nor any contaminants stuck to the column.

- We attempted affinity chromatography with heparin and hydroxyapatite columns; no purification.

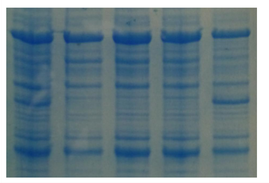


**Figure 6**. Gel from our attempt at affinity chromatography: neither a HiTrap heparin column (pictured) nor our self-packed hydroxyapatite column provided any purification. Left to right: MW standard (1), flowthroughs (2, 3, 4) and washes (5).

## FUTURE

### Purification Continued

- Develop method for gel filtration chromatography.

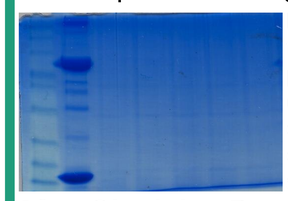


**Figure 7.** We assembled a column for gel filtration, but preparatory issues precluded purification: the protein standards we ran, BSA (66 kDa) and myoglobin (17 kDa), were too dilute to be located after running through the column. Left to right: MW standard (1), protein standard load (500 µl, 1 mg/ml)(2), flow (3-7).

### After Obtaining Pure 64 kDa Protein

- Sequencing the protein to confirm that it is *mal*A.
- Quantify amount of protein present in our productions.
- Determine specific activity (µmol/min/mg) of *mal*A.
- Continue studies to determine the enzyme.'s native substrate, mode of action, and regulatory properties.

**LITERATURE CITED**

- 1: Rendulic, S. et al. (2004) A Predator Unmasked: Life Cycle of *Bdellovibrio bacteriovorus* from a Genomic Perspective. *Science 303*, 689-692.
- 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: Sockett, R.E. (2009) Predatory Lifestyle of Bdellovibrio bacteriovorus. *Annu. Rev. Microbiol 63, 523–39.*

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