Characterization of a Novel beta-Glucanase From the Hyperthermophile
Fervidibacter sacchari, the Sole Isolate of an Ancient Bacterial Class

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INTRODUCTION
Recycling plant waste into usable products is a requirement for long-term space exploration. However, the lignocellulosic backbone of these foods prevents easy degradation. NASA has sought a solution in the form of lignocellulose-degrading enzymes like glycoside hydrolases (GHs). Of particular interest are GHs active at high temperatures, which can be heat-purified without the use of costly chromatography-based approaches. The sole isolate of a novel class, Fervidibacter sacchari, is a hyperthermophilic bacterium that has 114 genes with GH domains and has been found to grow on 17 diverse polysaccharides (1). However, the biochemical characteristics of these GHs remain unknown, leaving a knowledge gap that prevents us from fully utilizing their potential. Here, we focus on one F. sacchari GH, “D6,” and identify its size, substrate specificity, pH/temperature optima, and Michaelis Menten kinetic parameters $V_{max}$ and $K_{m}$.

METHODOLOGY
Expression and purification
1. D6-producing E. coli was grown to OD$_{600}$ = 0.6 – 0.8, then induced overnight with 0.5 mM IPTG.
2. Cultures were resuspended in 1/3 volume lysis buffer (pH 5.5 – 11).
3. Cells were lysed and the enzyme was purified by heating at 80 °C then removing the pellet.

Protein quantification, size, and purity
1. Protein content was assessed with a Pierce™ BCA Protein Assay Kit.
2. Enzymes were electrophoresed through an SDS-PAGE gel.
3. Gels were stained with Coomassie Brilliant Blue staining solution.
4. Gels were destained and viewed with a Typhoon S imager.

RESULTS
Figure 1: 3,5-Dinitrosalicylic acid (DNS) colorimetric assay. Reducing sugars cause an increased absorbance at 570 nm (2).

Expression
Expression and purification
1. Enzyme was mixed 1:1 with 4-nitrophenyl-beta-D-glucopyranoside (0.625 – 20 mM) and incubated at optimal pH and temperature (3).
2. 300 µL of disodium phosphate was added to each.
3. Absorbance was read at 570 nm.

Michaelis Menten kinetic parameters
1. Enzyme was mixed 1:1 with 4-nitrophenyl-beta-D-glucopyranoside (0.625 – 20 mM) and incubated at optimal pH and temperature (3).
2. 300 µL of disodium phosphate was added to each.
3. Absorbance was read at 400 nm.

Figure 2: SDS-PAGE gel. D6 has a size of ~60 kDa with 92% homogeneity.

Figure 3: Substrate specificity. D6 degrades 61% of oat-derived beta-glucan after ~16 hours (P < 0.05).

Figure 4: pH range and optimum. D6 is active at a broad range of pHs with an optimum of ~7.

Figure 5: Temperature range and optimum. D6 is highly active between 60 and 95 °C with an optimum of ~80 °C.

Figure 6: Lineweaver-Burk plot. $V_{max}$ and $K_{m}$ is calculated from the reciprocals of the y and x-intercepts, respectively. Substrate use confirms beta-glucanase activity of D6.

CONCLUSIONS
1. D6 possesses novel beta-glucanase activity in GH family GH50, a family of agarases (4).
2. D6 is the first characterized hyperthermophilic GH50, with high activity between 60 and 95 °C, and an optimum of ~80 °C. High-temperature activity allows easy purification by heat.
3. D6 is active at a broad range of pHs, from 5.5 to 11 and beyond, with an optimum of ~7. A wide pH range allows for freedom of solvent pH.
4. D6 has a low affinity of $K_{m}$ = 12.6 mM but high reaction velocity of $V_{max}$ = 357.1 for the given substrate. This may allow rapid reaction when processing large volumes of plant waste.

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