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

Jonathan K. Covington[1], Nicole Torosian[1], Allison M. Cook[1], Scott G. Bryan[1], Kasthuri Venkateswaran[2], Nitin K. Singh[2], Brian P. Hedlund[1,3]

[1] School of Life Sciences, University of Nevada, Las Vegas, Las Vegas, USA; [2] Biotechnology and Planetary Protection Group, Jet Propulsion Laboratory, California Institute of Technology, Pasadena, USA; [3] Nevada Institute of Personalized Medicine, University of Nevada, Las Vegas, Las Vegas, USA

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. Thus, 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.

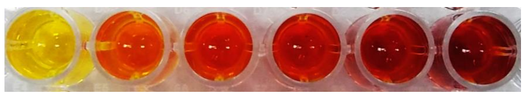

Figure 1: 3,5-Dinitrosalicylic acid (DNS) colorimetric assay. Reducing sugars cause an increased absorbance at 570 nm (2).

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 $^1/_5$ 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 5 imager.

Substrate, pH, and temperature assays

1. Enzyme was mixed 1:1 with 17 substrates (1% w/v) at pH 7 and incubated overnight at 80 °C.
   a) pH assay: 5.5 – 11.
   b) Temperature assay: 4 – 95 °C.
2. DNS solution was added and incubated 20 minutes **(Figure 1)**.
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.

RESULTS

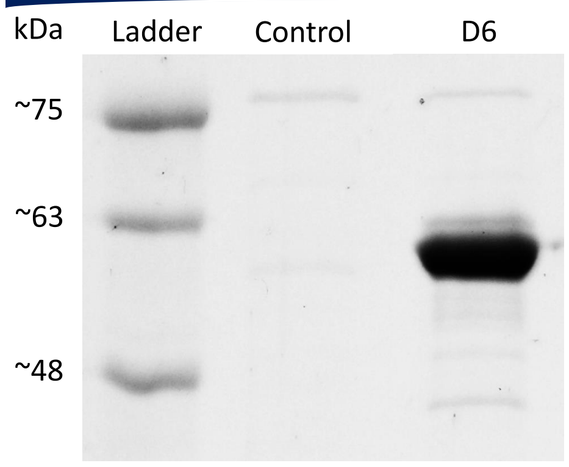

Figure 2: SDS-PAGE gel. D6 has a size of ~60 kDa with 92% homogeneity.

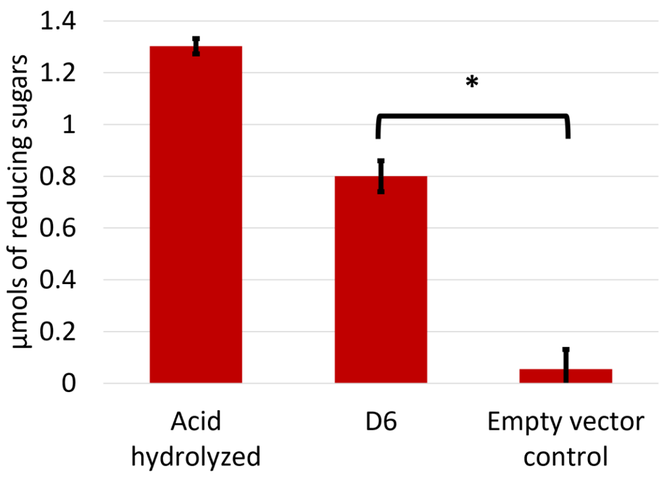

Figure 3: Substrate specificity. D6 degrades 61% of oat-derived beta-glucan after ~16 hours ($P < 0.05$).

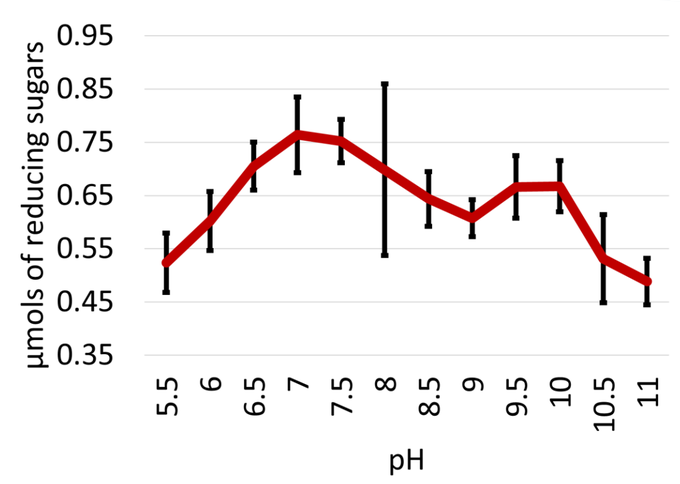

Figure 4: pH range and optimum. D6 is active at a broad range of pHs with an optimum of ~7.

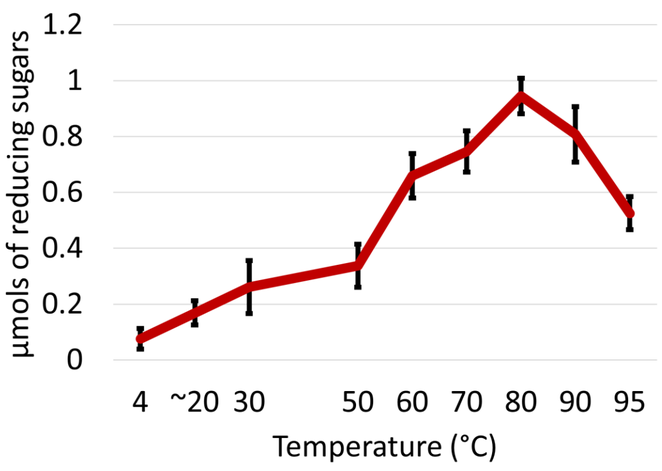

Figure 5: Temperature range and optimum. D6 is highly active between 60 and 95 °C with an optimum of ~80 °C.

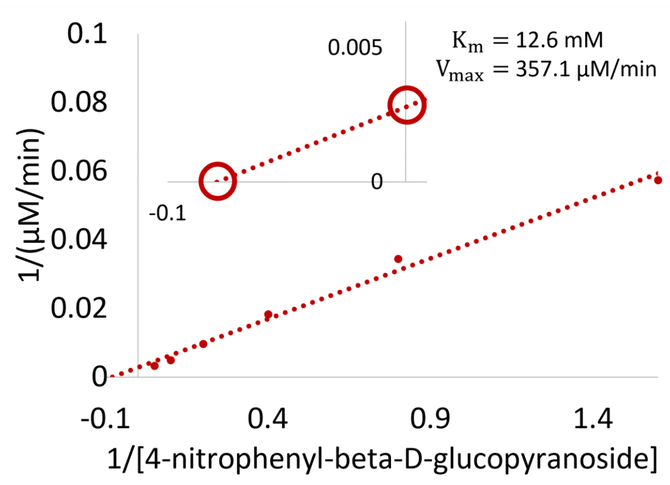

Figure 6: Lineweaver-Burk plot. V_{max} and K_m is calculated from the reciprocals of the y and x-intercepts, respectively. Substrate use confirms beta-glucanase activity of D6.

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.

ACKNOWLEDGEMENTS

We thank the DOE Joint Genome Institute Functional Genomics Group for transforming *F. sacchari* GHs into *E. coli*. We also thank Casey Hall-Wheeler and the UNLV Genomics Core facility for training to use their SpectraMax Plus plate reader and Typhoon 5 imager. This material is based upon work supported by the National Aeronautics and Space Administration under Grants No. 80NSSC20M0043, 80NSSC17K0548, 80NSSC21M0157, and the Office of Science of the U.S. Department of Energy under Contract No. DE-AC02-05CH11231.

REFERENCES

(1) Rinke, C., Schwientek, P., Sczyrba, A., Ivanova, N., Anderson, I., Cheng, J., et al. (2013). Insights Into the Phylogeny and Coding Potential of Microbial Dark Matter. *Nature*, 499(7459), 431-437.

(2) Kim, J., Kwon, Y., Kim, J., Heo, S., Lee, Y., Lee, S., et al. (2014). Effective Microwell Plate-Based Screening Method for Microbes Producing Cellulase and Xylanase and Its Application. *Journal of Microbiology and Biotechnology*, 24(11), 1559-1565.

(3) Ball, A., Chambers, K., Hewinson, M., Navaratnarajah, S., Samrin, L., Thomas, N., et al. (2008). A Microtitre Plate Assay for Measuring Glycosidase Activity. *Journal of Enzyme Inhibition and Medicinal Chemistry*, 23(1), 131-135.

(4) Drula, E., Garron, M., Dogan, S., Lombard, V., Henrissat, B., & Terrapon, N. (2022). The carbohydrate-active enzyme database: Functions and literature. *Nucleic Acids Research*, 50(D1), D571-D577.