Investigating the polysaccharide-degrading proteins of novel hyperthermophile *Ca.* Fervidibacter sacchari PD1

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Overview: Novel hyperthermophile *Candidatus* Fervidibacter sacchari encodes a plethora of putative carbohydrate-active enzymes (CAZymes) and glycoside hydrolases (GHs), enzymes involved in breaking down complex sugars. To investigate the functions of these predominantly uncharacterized proteins, we screened 30 substrates for use as a sole carbon and energy source, measured *Ca.* F. sacchari cell growth in each condition, and performed differential proteomics to link protein expression to substrate. These data will serve as beginning steps to elucidating protein function and will further our knowledge of heterotrophic metabolism in terrestrial geothermal systems, an environment proposed by some to be the origin of the first cells on Earth.

Introduction

Our lab has recently enriched and isolated *Ca.* Fervidibacter sacchari (Fig. 1) from ~85 °C sediments of Great Boiling Spring (GBS), a terrestrial geothermal system near Gerlach, Nevada. At present, *Ca.* F. sacchari remains the only axenic cultured member of *Ca.* Fervidibacteria, a strictly thermophilic proposed phylum within Domain Bacteria[1]. Beyond this phylum's placement as one of the deepest branching lineages within Bacteria[2], we have found that the isolate genome encodes 114 GHs and 190 CAZymes, believed to be the highest among known thermophiles (Table 1). However, most of these proteins have no annotated function. Here, we screen a mix of polysaccharides, monosaccharides, and lignocellulosic biomass as sole carbon and energy sources in *Ca.* F. sacchari growth experiments. Five of the most successful substrates were used for differential proteomics analysis. This work links bacterially expressed enzymes to individual carbohydrates, enabling preliminary function determination of these uncharacterized enzymes. More broadly, this work serves as a steppingstone to elucidating more novel activities and specificities among GHs, particularly those found in high-temperature environments.

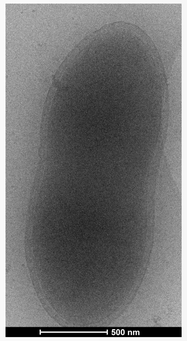
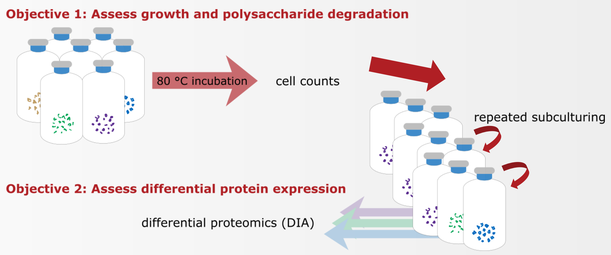


Figure 1. Cryo-EM of a *Ca.* F. sacchari PD1 cell.

	T_{opt} (°C)	Metabolism	No. of CAZymes	No. of GHs	Ref.
Caldicellulosiruptor kronotskyensis 2002	70	Fermentation	132	89	[5]
Dictyoglomus turgidum DSM6724	72	Fermentation	100	61	[6]
Thermotoga maritima MSB8	80	Fermentation	70	49	[7]
Ca. Fervidibacter sacchari PD1	**80-85**	**O_2 Respiration, Fermentation**	**190**	**114**	--

Table 1. Select features of some polysaccharide-degrading thermophilic bacteria. CAZyme counts according to dbCAN2 HMMdb v9.0[3]. Proteins for published organisms retrieved from RefSeq[4].

Methods



Results

	Galα1-6Man	Galβ1-2Xyl	Glcα1-6Glc	Glcβ1-3Glc	Glcβ1-4Glc	Glcβ1-4GlcA + GlcAβ1-4Glc	Manβ1-4Man	Rhaα1-3Glc	Xylα1-6Glc
β-glucan (oat)				✓	✓				
Locust bean gum (carob)	✓						✓		
Starch (potato)			✓		✓				
Xyloglucan (tamarind)		✓			✓				✓
Gellan gum (*Sphingomonas*)						✓		✓	

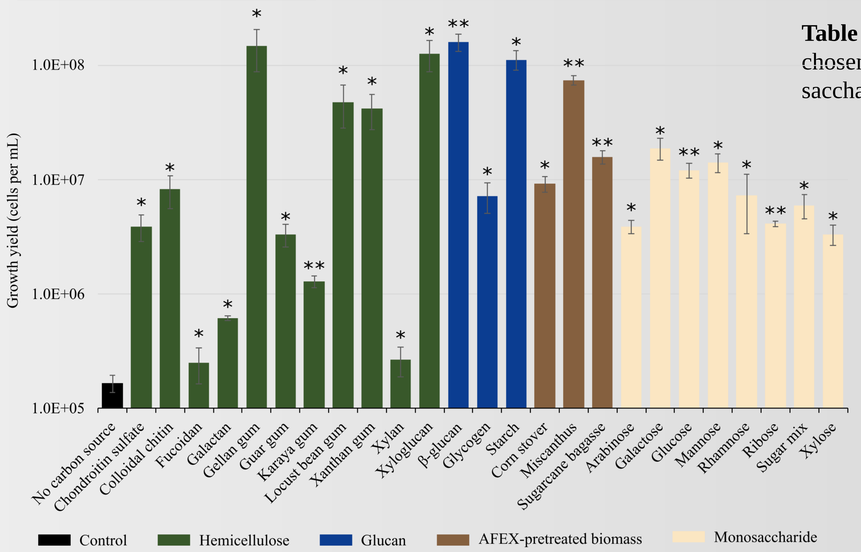


Figure 2. Substrates that supported *Ca.* F. sacchari growth. Triplicate cultures used each substrate as sole carbon source and electron donor. Cells were counted at five and nine days with highest yield reported. Error bars, SD; *, $p < 0.05$; **, $p < 0.001$, Welch's t-test. Y-axis is log scale.

Table 2. Linkages present in select substrates used for carbon source testing. Substrates were chosen based on characterized enzymatic activities in GH families predicted to be present in *Ca.* F. sacchari by dbCAN2. Shown here are the five polysaccharides used in the DIA proteomics analysis.

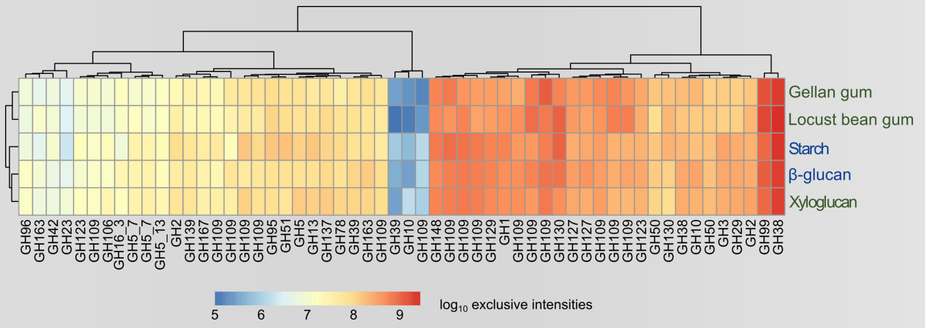


Figure 3. Preliminary proteomic analysis of *Ca.* F. sacchari growing on five different polysaccharides in biological triplicate. Differentially abundant GHs (ANOVA $p < 0.05$ after Benjamini-Hochberg correction, $q = 0.05$; FDR 1%; ≥ 2 unique peptides/protein) are shown. Expression patterns appear largely similar for individual GHs across all conditions, but whole proteomes were distinct between conditions, suggesting a regulatory network beyond GHs.

Conclusions & Future Directions

***Ca.* Fervidibacter sacchari grows on a wide range of organic carbon sources but preferentially grows on polysaccharides** (Figure 2). This lends credence to *Ca.* F. sacchari being a polysaccharide specialist but raises the question of polysaccharide availability in hot spring sediments. Future work will probe the ability of *Ca.* F. sacchari to use native sugars, e.g., from biofilms and cyanobacterial mats.

***Ca.* F. sacchari proteomes differed between growth conditions.** Preliminary proteomics analysis showed 100/114 GHs expressed in all conditions, with 56 GHs differentially expressed in at least one condition. GH expression patterns did not strongly correlate with glycoside bond stereochemistry present in individual substrates besides grouping of substrates containing β1,4-linked glucose (Table 2, Figure 3). Whole-proteome analysis, paired with future complementary RNA-Seq, will reveal if genetic regulatory networks are responsible for GH expression. Biochemical characterization of GHs heterologously expressed in *E. coli* will unveil the specific, possibly multiple, activities of these enzymes.

References

1. Rinke et al., *Nature* 2013. doi.org/10.1038/nature12352
2. Blank et al., *Appl. Environ. Microbiol.* 2002. doi.org/10.1128/AEM.68.10.5123–5135.2002
3. Zhang et al., *Nucleic Acids Res.* 2018. doi.org/10.1093/nar/gky418
4. O'Leary et al., *Nucleic Acids Res.* 2016. doi.org/10.1093/nar/gkv1189
5. Miroshnichenko et al., *IJSEM* 2008. doi.org/10.1099/ijs.0.65236-0
6. Brumm et al., *Front. Microbiol.* 2016. doi.org/10.3389/fmicb.2016.01979
7. Huber et al., *Arch. Microbiol.* 1986. doi.org/10.1007/BF00409880
8. Dodsworth et al., *IJSEM* 2014 doi.org/10.1099/ijs.0.055855-0

Acknowledgements

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