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Characterization of the thermophilic xlanase Fsa272 from Candidatus Fervidibacter sacchari belonging to glycoside hydrolase family GH10

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Characterization of the thermophilic xylanase Fsa272 from *Candidatus* Fervidibacter sacchari belonging to glycoside hydrolase family GH10

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ABSTRACT

Candidatus Fervidibacter sacchari is a novel, facultatively anaerobic, hyperthermophilic bacterium found in terrestrial geothermal springs globally. Its genome encodes 115 putative glycoside hydrolase enzymes that are predicted to hydrolyze glycosidic bonds between carbohydrates. Fsa272, a member of the glycoside hydrolase family 10, was synthesized and cloned into *Escherichia coli* strain T7 Express. The transformed *E. coli* was grown with LB broth and ampicillin at 37°C. Fsa272 expression was induced with isopropylthio-beta-galactoside (IPTG), and the lysate was heat purified for 15 minutes at 80° C. The 3,5-dinitrosalicylic acid assay identified xylanase activity with a pH range of 4.5 to 10.5 (pH_{opt} 5.5) and a temperature range of 60 to 90°C (T_{opt} 80-90°C). The *para*-nitrophenol assay was used to determine the Michaelis-Menten kinetic parameters of Fsa272, resulting in K_M of 1.8 mM and V_{max} of 232.6 µM/min. The characterization of Fsa272 provides critical information on *Ca.* F. sacchari and its potential application in converting polysaccharide waste to biofuels.

INTRODUCTION

Candidatus Fervidibacter sacchari is the first isolated member of the bacterial class *Fervidibacteria* and is the only known hyperthermophile specializing in aerobic polysaccharide catabolism. This bacterium was isolated from Great Boiling Spring, Nevada. *Ca.* F. sacchari grows optimally at 85°C on various hemicelluloses, glucans, and ammonia fiber expansion-pretreated biomass substrates.

The *Ca.* F. sacchari genome encodes 115 annotated thermostable glycoside hydrolases (GHs), enzymes that hydrolyze the glycosidic bonds between complex carbohydrates. GHs can be used to produce monosaccharides that can then be fermented into biofuels such as bioethanol. GHs are thus potentially useful for industrial-level biowaste degradation and biofuel production.

Figure 1: Great Boiling Spring
Ca. Fervidibacter sacchari was isolated from Great Boiling Spring, NV.

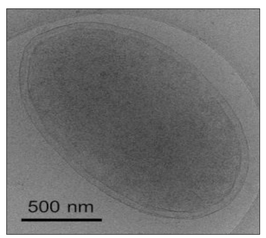

Figure 2: Cell structure
Ca. F. sacchari shown by a cryo-electron micrograph.

ACKNOWLEDGEMENTS

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RESULTS

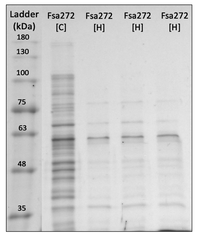

Figure 3: SDS-PAGE
Heat-purified protein (H) is ~15% pure from the crude lysate (C). Fsa272 has a size of ~60 kDa on this gel.

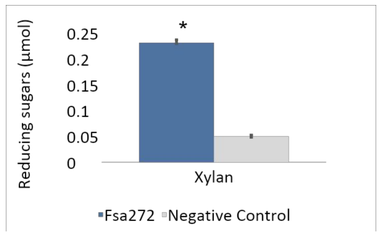

Figure 4: Activity Screen
Fsa272 was screened with 14 complex polysaccharides and was active on xylan using an unpaired t-test ($p < 0.005$).

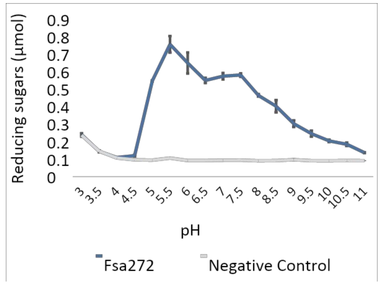

Figure 5: Activity at various pH values
Fsa272 had an optimum of pH 5.5, when tested with xylan dissolved in different pH-adjusted buffers from pH 3.0 to 11.0.

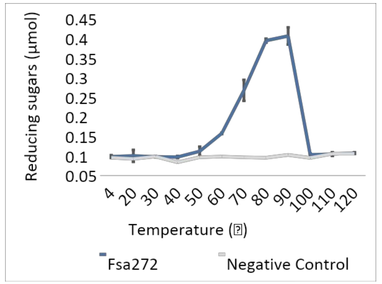

Figure 6: Activity at various temperatures
Fsa272 had a temperature optimum of 80-90℃, when tested with xylan from 4℃ to 120℃.

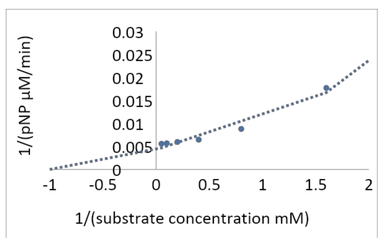

Figure 7: Lineweaver-Burk Plot
This plot was used to calculate a V_{max} of 232.5 µM/min and a K_M of 1.814 mM at a final Fsa272 concentration of 4.96 µM.

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METHODOLOGY

Gene Expression and Purification of Fsa272

1. The transformed *Escherichia coli* with Fsa272 and an empty vector control were grown at 37°C in LB broth with 100 µg/mL of ampicillin.
2. Fsa272 expression was induced with 0.5 mM of IPTG overnight at 37°C.
3. The cells were sonicated then centrifuged to isolate the soluble fraction.
4. The soluble lysates were heated to 80°C for 15 minutes to purify by heat treatment.
5. The purified lysates were centrifuged, and the supernatant was stored at -20°C.

Analysis of protein size, purity, and concentration (Figure 3)

1. Crude and purified proteins were run on an SDS-PAGE gel (1).
2. Gel was stained with Coomassie Brilliant Blue-R-250 staining solution for 30 minutes, then destained for 30 minutes.
3. Amersham Typhoon 5 Biomolecule Imager was used to image and assess the purity.

Screening for Fsa272 activity on polysaccharides (Figure 4)

1. The activity assay solution contained 4.56 µg protein and 0.5% substrate and incubated overnight at 80°C .
2. The mixture was incubated with 160 µL of DNS solution at 100°C.
3. A spectrophotometer (570 nm) measured the µmoles of reducing sugars.

Determining optimal pH and temperature on xylan (Figures 5 & 6)

1. To test pH, the DNS assay was repeated using substrates dissolved in pH-adjusted buffers from 3.0 to 11.0.
2. To test temperature, the DNS assay was repeated at varying temperatures from 4°C to 120°C.

Kinetic analysis of Fsa272 on *para*-nitrophenyl-beta-xylobioside (Figure 7)

1. Fsa272 (4.96 µM) was mixed with a range of *para*-nitrophenyl-beta-xylobioside dilutions (final concentration 0.625 to 20 mM) at pH 5.5 in triplicate and incubated for 60 minutes at 80°C.
2. The reaction was terminated with disodium phosphate (final concentration 0.6%) and the absorbance (400 nm) was measured using an absorbance plate reader.

CONCLUSIONS

Ca. Fervidibacter sacchari encodes many glycoside hydrolase enzymes that specialize in polysaccharide degradation, including Fsa272, a xylan-degrading member of the glycoside hydrolase family 10 (GH10). Fsa272 works optimally at a pH of 5.5 between 80 to 90°C. The Michaelis-Menten kinetic parameters showed that Fsa272 has a relatively high velocity and specificity for glycosidic linkages found in xylan, although this experiment must be repeated with a higher protein purification and shorter incubation time.

Fsa272 is effective at degrading xylan, one component of lignocellulosic biomass waste that is expelled in abundance each year from agriculture. The ability to decompose this biomass using an inexpensively purifiable thermophilic enzyme will aid the push for renewable energy and biofuels. *Ca.* F. sacchari GHs have the potential to be used to degrade diverse polysaccharide wastes, encouraging the study of this broader GH cache to overcome this step in producing biofuels.

FUTURE DIRECTIONS

- Improve protein lysate purity to at least 95%.
- Repeat Michaelis-Menten kinetics to improve linearity of the Lineweaver-Burk plot.
- Study the remaining highly expressed glycoside hydrolases from *Ca.* F. sacchari.