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Evaluation of a Novel Cellulase to Optimize Biofuel Production

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Evaluation of a Novel Cellulase to Optimize Biofuel Production

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Summary

Cellulose waste is utilized for biofuels, however cellulase enzymes are a limiting factor. We sought to evaluate a novel cellulase identified in cow rumen metagenomic analysis provided by the Joint Genome Institute. We performed DNA analysis, SDS PAGE, and a cellulase activity assay to theoretically calculate and compare to experimental molecular weight; and determine cellulase activity. The theoretical molar mass (64.9 kD) strongly correlates to the experimental molar mass (60.7 kDa). Purified cellulase has an activity, 6.27 cm^2/ug, 6x higher versus commercial cellulase enzyme. These results have positive implications for the creation of biofuels from agricultural waste products.

Introduction

- Cellulose is the most ample renewable biological resource and has a low-cost energy source based on energy content
- Cellulose is the primary component of the plant cell wall
- The enzyme cellulase breaks down the polysaccharide through hydrolysis at the β-1,4-glycosidic linkages

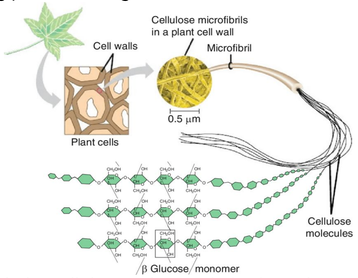


Figure 1. Cellulose the most abundant biomolecule on Earth is composed of glucose monomers. Image https://www.researchgate.net/publication/340136481_Nanocellulose_for_Sustainable_Future_Applications

- Cellulase enzymes break the cellulose polymer chains into glucose monomers which can be fermented to form bioethanol.
- Cellulosic Biofuel allows for renewable energy alternatives to traditional fossil fuel.
- Ruminant cows naturally contain systems to attempt to digest cellulose.
- Metagenomic discoveries attached to plant biomass in cow rumen identified potential biomass-degrading genes from the cow microbiome (Hess *et al*)
- **The objective of this study is to evaluate clones of putative cellulases for their activity compared to commercially available products and identify potential cellulase protein activity found in cow rumen in order to optimize biofuel production.**
- overexpression of cellulase in *E. coli* BL21*(DE3)* with a 6xHis tag
- **Strain CJD9-1 was evaluated and provided by the Joint genome Institute**

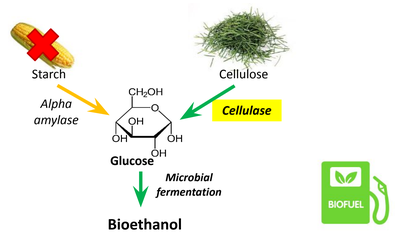
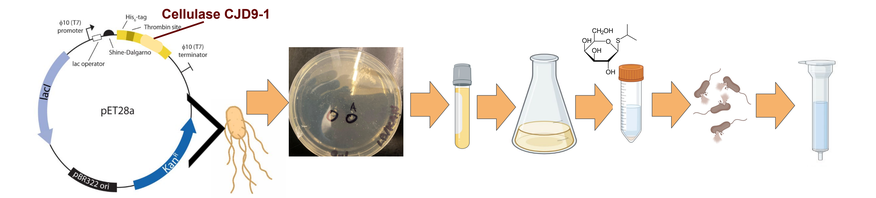
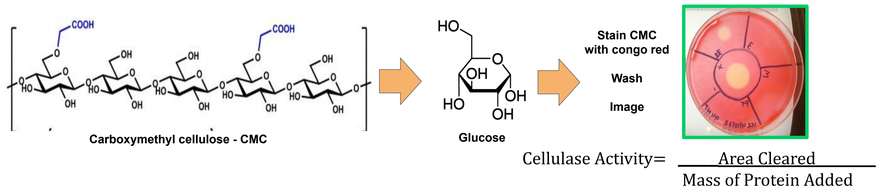


Figure 2. Cellulase enzyme optimization is key for converting bioethanol from feedstock to cellulose. Plant cells wall material is made up of cellulose microfibrils. Cellulose is composed of glucose monomers that with microbial fermentation, produce ethanol which can be used as a biofuel. Currently, food sources such as corn are used primarily for bioethanol. (Hess *et al*)

Methods





$$\text{Cellulase Activity} = \frac{\text{Area Cleared}}{\text{Mass of Protein Added}}$$

Figure 3: Semi-quantitative Carboxymethylcellulose plate assay using congo red detection. Clearing indicates enzyme activity. The control commercial cellulase is shown in the middle. Strains ME9-8 and CJD9-20 are depicted below with enzymatic activity shown in the buffer exchanged and wash samples.

Results

Cellulase CJD9-1 Bioinformatic Sequence Analysis

Translated Protein Sequence with Structure Prediction

A

```
1   ********************************** 60
    MKGKIIVITCALVMMACSYKNPDNGPIRLNQVGYSPMQEMTATIVMDEPVKEVFILNEKG
    CCCCCHHHHHHHHHHCCCCCCCCEEEECEECCCCCCEEEEEECCCCCEEEEEECCCCCC
61  ************************************************************ 120
    DTVWIGVPAVTLPNPISGKTCQIVDFSALDTPGTYTLYAKTQLSTVNCQFSIKEHPYREL
    EEEEEEEEEECCCCCEEEEEECCCCCCEEEEEEEEECCCCCECCCEEEECCCCHHHHH
121 ********** ********************************************** 180
    TRQALRAYYHQRASMATEEPFAEGYARPAGHPDDHVIVHASAATVERPEGTIISSPCGBY
    HHHHHHHHHHCCCCCCCCCCCCCEEEEEECCCCCCCCCCCCCCCCCCCCCCEEEEECCCCC
181 ************************************************************ 240
    DAGDYNKYIVNSGFTMGVMLMAYEMMKAYFDSLHLNIPESIVNRQSSNRQCPDMLAEALY
    CCCCCEEEHHHHHHHHHHHHHHHHHHHHHHHCCCCCHHHHHHHCCCCHHHHHHH
241 ********** ********************************************** 300
    NIRWMLTMQDTDGGVYHKLTEPDFEGFIRPDQCKKPRYVVMKTTAAALDFAATMALAARV
    CCHHHHHECCCCEEEEEEECCCCECCCCCCCCCCEEEEEEECCHHHHHHHHHHHHHH
301 ************************************************************ 360
    YAPFDADFCAQKAEAALRAYAWAEANPAVYYDQPKMNEEFKPAITTGAYDDFDVRDEFLW
    HHCCCHHHHHHHHHHHHHHHHHHCCCECCCCCCCCCCCCCCEEECCCCCCCCHHHHHH
361 ********** ********** ********** 420
    AATELYLLTGDEKYKSEITNYQSQITNYVPAVNGNVAELAYLELLMHGIENNLSPLTAHL
    HHHHHHHHCCHHHHHHHHHHHCCCHHHHCCCCCCHHHHHHHHHHHHHHHHHHHHHHH
421 ********** ********** ********** 480
    APFIDEVETSAFRSPYGNRESDFFWGCNSEGCCNRGIECLYAYRLTGDEQYRVNAERCLD
    HHHHHCCCCCCCCCCCCCCCCCEEECCHHHHHHHHHHHHHHHHHCCCHHHHHHHHH
481 ************************************************** 540
    YILGQNATGFCYVTGFGTHPTSHPHHRLSYSHPKGTIPGFLAGGPNPARQDAATDDYKYP
    HHHCCCCCCCEEEECCCCCCCCCCCEEEHHHHCCCCCCCCCEEEECCCCCCCCCEEEECCC
541 ********************************** 581
    KDVSADESYLDYQPSYASNEVTINWNVTLFALSAGLDALPQ
    CCCCCCCCCCCCCCCCCCECCCHHHHHHHHHHHHHCCC
```

Theoretical pI 4.96, Molar Mass 64913 g/mol

B Blastp Analysis
Sequence alignment
glycoside hydrolase family 9 protein [Paludibacteraceae bacterium]
Sequence ID: MBO4518690.1 Length: 605 Number of Matches: 1

Score	Expect	Method	Identities	Positives	Gaps
899 bits(2322)	0.0	Compositional matrix	438/591(**74%**)	487/591(82%)	21/591(3%)

C Protein Data Bank (PDB) Structural Homology Analysis

Organism	Bacteroides ovatus ATCC 8483
Macromolecule	Xyloglucan-specific endo-beta-1,4-glucanase BoGH9A
Sequence Match	Sequence Identity: **48%**, E-Value: 6.051e-154, Region: 15-567

D

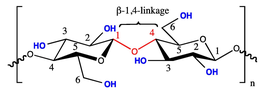


Figure 4. Genetic sequences for clone CJD9-1 were translated in programs Expasy. **A.** Proteus Structure Prediction where C, coil; H, helix; E, beta sheet. Top right. **B.** Translated sequences were compared for alignment in NCBI Blastp and **C.** the PDB. **D.** Cellulose structure with β-1,4 glycosidic linkage.

Structure of Homologous Cellulase

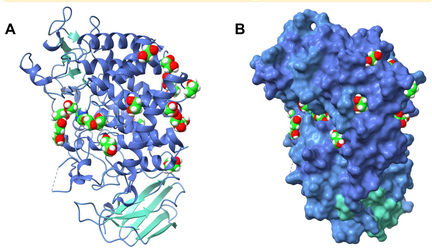


Figure 4. Crystal Structure of PDB Entry 6DHT the highest homology to Cellulase CJD 9-1 putative cellulase. Made in Chimera$X^{1.9}$. The ligand in 6DHT is di(hydroxyethyl)ether (A) The alpha helices and random coils of the protein are colored in a cornflower blue the beta sheets are colored in cyan. The ligand is colored lime heterochrome. (B) Surface of PDB entry 6DHT. The same color scheme is adopted from image A.

Cellulase CJD9-1 Concentration

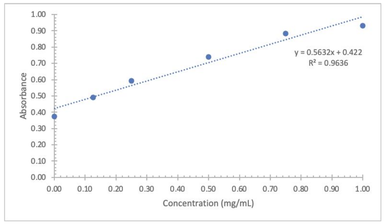


Figure 5. Standard Curve for Bradford Assay. Absorbance was measured at 540 nm. The equation of the line and the R^2 value are at the top right of the graph. The standards were measured at 0, 125, 250, 500, 750, and 1000 g/ml of BSA.

Results

Cellulase CJD9-1 is Expressed

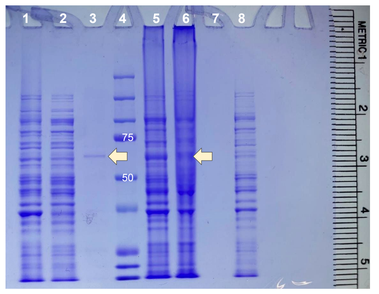
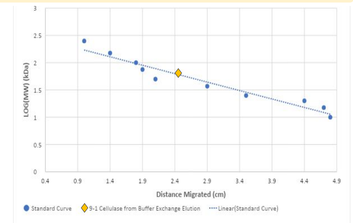


Figure 6. SDS PAGE for CJD9-1 Cellulase. From left to right the lanes are 1. flow through, 2. wash, **3. buffer exchange elution**, 4. kaleidoscope molecular weight ladder, 5 soluble lysate, 6. induced pellet and 8. uninduced pellet.

Molar Mass of CJD-1



Molar Mass Determination	MW (kD)
Theoretical from sequence	64.9
Experimental	60.7

Figure 7 Theoretical and Experimentally Determined Molecular Weight of Cellulase CJD9-1. (Top) Standard Curves for Kaleidoscope Migration in SDS PAGE with 9-1 Cellulase from Buffer Exchange as the Unknown. The equation of the line and R^2 value are at the top right of the graph. The standard curve is in blue. The unknown is gold. (Bottom) determined molar mass of Cellulase 9-1.

Cellulase CJD9-1 Activity is 6x Better

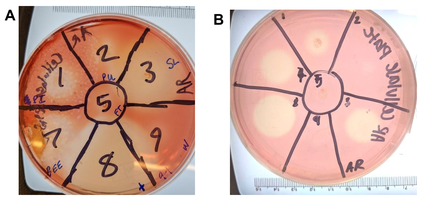


Figure 8 Cellulase Activity Plates. The numbering scheme 1. induced pellet, 2. uninduced cell pellet, 3. soluble lysate, 5. flow through, 7. buffer exchanged elution, 8. positive control, and 9. wash. **Plate A.** This plate was left to incubate too long and the cellulases began attacking overlapping areas. However, an interesting finding from this plate is that the cellulase also began attacking the agar. **Plate B.** This is the cellulase plate used for the calculations for the cellulase activity.

$$\text{Cellulase Activity} = \frac{\text{Area Cleared}}{\text{Mass of Protein Added}}$$

Table 1. Cellulase Activity from Figure 6 B
Cellulase Activity Plate Assay

SAMPLE	Activity (cm/ug)
1. Induced Pellet	0.676
2. Uninduced Pellet	0
3. Soluble Lysate	0.651
5. Flowthrough	0
7. Buffer Exchange Elution	6.27
8. Positive Control (Aspergillus Niger Cellulase)	1
9. Wash	0

Conclusions

- **Cellulase CJD9-1 a novel cellulases displayed 6x the activity of a commercial cellulase making it a target for enhance biofuel feedstock production**
- The majority of metagenomic cellulases analyzed by did not exceed the activity of the control cellulase (data not shown)
- Larger cultures must be prepared in order to evaluate the kinetic activity with enough protein.
- Sequence analysis indicated homology with the β-1,4 hydrolase family and Enzyme Class EC: 3.2.1.4
- The molar mass of the isolated protein is consistent with the predicted mass with signal sequence, amino acids 1-15, removed.

Acknowledgments

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CIRM also granted use of the bead beater for this research

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