RUMEN MICROBIOME GENE CJD5-110 ENCODES A CELLULASE

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INTRODUCTION: Climate change, primarily driven by fossil fuel combustion and resulting greenhouse gas emissions, poses a threat to the environment. In response, bioethanol has emerged as a promising alternative to traditional fuels due to its renewable nature and reduced carbon footprint. Essential to bioethanol production is the enzymatic hydrolysis of cellulose, a component of plant cell walls. Cellulases play a crucial role in breaking down cellulose into glucose units, which can then be fermented to produce bioethanol. Wilson (2009) highlighted the significance of cellulases in this process, emphasizing their ability to hydrolyze cellulose into glucose. In the article Hess et al. (2011), the authors explored the enhancement of cellulase activity, illustrating the potential for optimizing enzymatic processes in bioethanol production. Building upon this foundation, the current research aims to explore novel approaches to further enhance cellulase efficiency. Our specific objective was to express the CJD5-110 gene, which had been identified by a previous cow rumen metagenomics study (Hess et al., 2011), and determine whether this gene encodes a functional cellulase enzyme. This research could contribute to the development of more efficient and sustainable bioethanol production methods, aligning with the broader objective of addressing climate change challenges through innovative biofuel solutions.

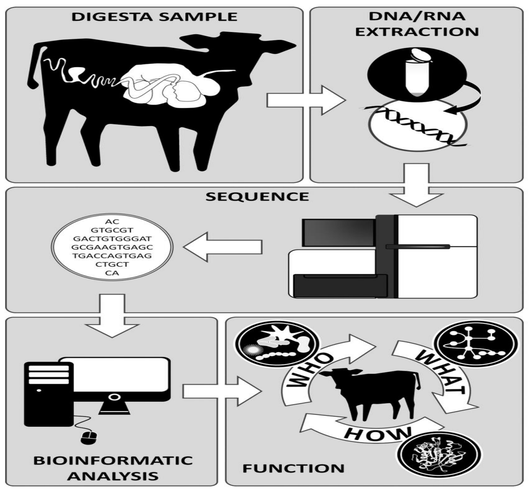

Figure 1: Rumen bioprospecting refers to the exploration and identification of industrially-useful microorganisms and enzymes present in the rumen of animals, or in this case cattle. The rumen is a stomach compartment where microbial fermentation breaks down complex plant materials, such as cellulose, into simpler compounds that can be absorbed by the host animal. This unique microbial ecosystem in the rumen has the potential to harbor a wealth of novel enzymes, especially in the case of cellulases, as degradation enzymes would help in strides to make affordable biofuels. Figure from Ribeiro et al. (2016).

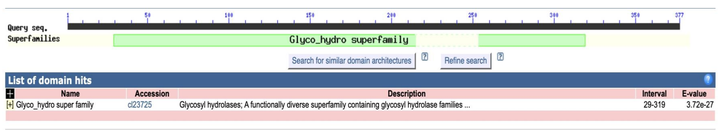

Figure 2: Domain structure of the protein encoded by the CJD5-110 gene, generated using *Conserved Domain (CD) Search*. It showcases the potential function of the protein which in this case is cellulase with the ability to break down cellulose. The figure contributes to overall objective of identifying novel cellulase genes from cellulolytic bacteria found in cow rumen, as cellulase was determined to be the protein isolated.

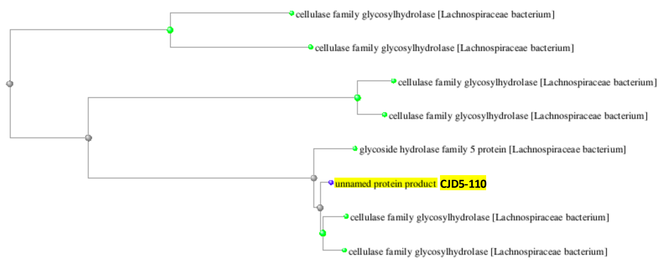

Figure 3: Phylogenetic tree generated from BLAST-P alignment data using the CJD5-110 protein sequence as a query. The figure shows the similarity between the CJD5-110 protein sequence and other sequences within the database that all originated from the Lachnospiraceae bacterium family. Lachnospiraceae represents a bacterial family commonly found in the intestinal microbiota of humans and mammals (Sagheddu et al, 2016). As for ability to break down cellulose, this type of bacteria seems to perform essential functions within the gut community by initiating the breakdown of complex substances, including plant cell walls, starch particles, and mucin (Flint et al, 2012). This is significant, as goal of gene isolation is to find a sequence that promotes cellulose degradation.

RESULTS:

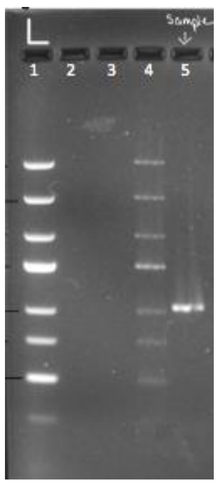

Figure 4: Analysis of PCR-amplified CJD5-110 gene by agarose gel electrophoresis. Lane 1 contains the GeneRuler Express DNA ladder (ThermoFisher Scientific). Lane 5 contains the polymerase chain reaction product for the CJD5-110 cellulase gene, which was amplified from cow rumen DNA. The calculated size of the band in lane 5 was 1069 base pairs. The expected size of the CJD5-110 gene is 1036 base pairs. The calculated value is within ± 5% of the expected value.

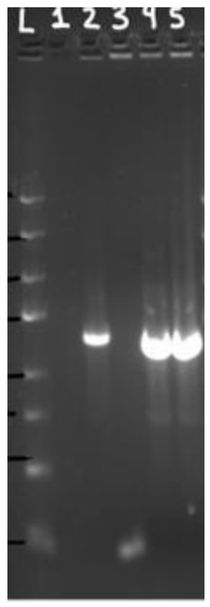

Figure 5: Agarose gel electrophoresis of colony PCR reactions amplifying the pET101 vector in transformed *E. coli* cells to identify recombinant vectors of pET101-CDJ5-110. Lane L contains the Gene Ruler Express DNA ladder (ThermoFisher Scientific). Lane 1 did not have a band to analyze. The band for lane 3 is at 260 base pairs, the expected size for vector self-ligation. The calculated base pair size for lane 2 is 1247. The calculated base pair size for lanes 4 and 5 is 1180. The expected base pair size of the recombinant pET vector DNA and CJD5-110 gene is 1296 base pairs. The calculated values are within ± 10% of the expec ted value. Lanes 2, 4 and 5 contain recombinant products.

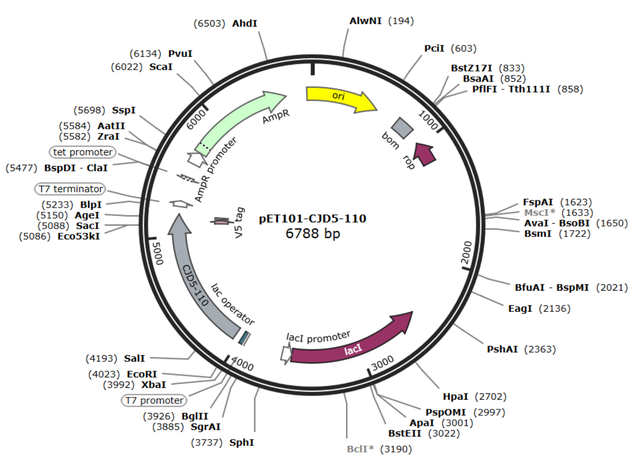

Figure 6: pET101-CJD5-110 plasmid map, which was generated from the DNA sequence of the recombinant plasmid identified in Figure 5, lane 2. The image was generated using the SnapGene Viewer software package. It showcases locations of major identifiable landmarks on DNA like restriction enzyme sites, genes of interest, plasmid name and length. Another important aspect of the figure is 6788 bp which was the actual size of the vector with an expected value of 6789, which are very close in value. The figure is significant as it verifies the insertion of target gene CJD5-110 in the pET 101 vector, as indicated with the grey arrow.

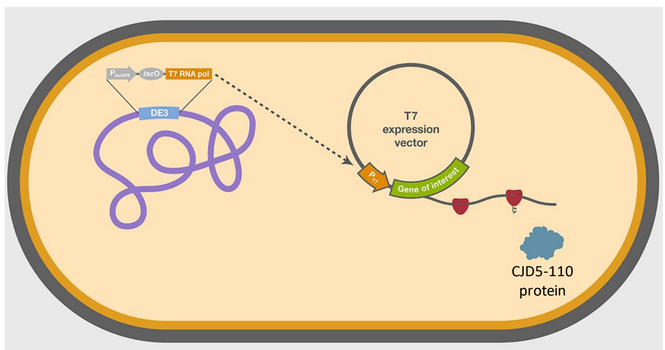

Figure 7: Visual concept of expression of CJD5-110 gene in *E. coli* BL21. BL21 cells were transformed with the pET101-CJD5-110 plasmid. IPTG was added to the transformed cells to activate transcription of the CJD5-110 gene. High levels of CJD5-110 proteins were produced (Figure adapted from Thermofisher.com, Figure 1A).

Figure 8: Carboxymethylcellulose (CMC) plate cellulase activity assay. The plate on the left contains the whole cell extract from *E.coli* BL21 containing the empty pET101 vector (EV WC) with no clear zone, the purified CJD5-110 recombinant protein (Pure YC) with a clear zone area of 283.5 mm^2, and *Aspergillus niger* cellulase (ANC) with a clear zone area of 660.5 mm^2. The plate on the right contains the total soluble protein isolated from *E. coli* BL21 harboring the CJD5-110 gene (SP) with a clear zone area of 572.6 mm^2, the whole cell extract of the *E. coli* BL21 harboring the CJD5-110 gene (WC) with a clear zone area of 594 mm^2, and the total soluble protein from *E.coli* BL21 containing the empty pET101 vector (EV) with no clear zone. The positive control is *Aspergillus niger* cellulase (ANC), which has the expected large clear zone. The negative control is the empty pET101 vector, (EV WC, EV), which has the expected missing clear zone. All protein extracts of the *E. coli* BL21 harboring the CJD5-110 gene have a clear zone, indicating cellulase activity in the recombinant protein sample. The purified sample has a smaller zone area, indicating less activity than the soluble and whole cell protein extracts.

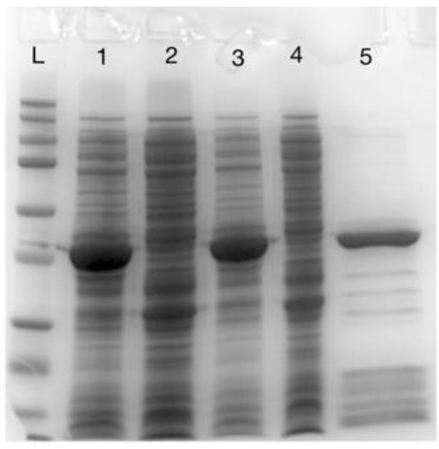

Figure 9: Stained SDS-PAGE gel of five protein extracts from *E. coli* BL21 harboring the pET101-CJD5-110 vector or a pET101 empty vector. Lane L contains the Precision Plus Protein Standard (ThermoFisher Scientific). Lane 1 contains total soluble protein from *E. coli* pET101-CJD5-110. Lane 2 contains total soluble protein from *E. coli* pET101-empty vector. Lane 3 contains whole cell extract from *E. coli* pET101-CJD5-110. Lane 4 contains the whole cell extract from *E. coli* pET101-empty vector. Lane 5 contains the purified recombinant protein from *E. coli* pET101-CJD5-110. There are thick recombinant protein bands for the CJD5-110 extracts compared to the thin bands of the empty pET101 vector protein extracts, indicating recombinant protein production. The calculated size of the recombinant protein bands is 44 kD, which matches the expected mass of 43.5 kD for the CJD5-110 recombinant protein. Purification of the His-tagged CJD5-110 protein via nickel ion chromatography was successful given the strong recombinant protein band at 44 kD in Lane 5.

CONCLUSIONS: The objective of the study was to identify novel cellulase genes from cellulolytic bacteria found in cow rumen. We were able to identify the gene CJD5-110 to be a cellulase gene. The CJD5-110 gene was inserted into a pET101 vector and transformed into *E. coli* BL21 cells that produced the recombinant protein. The protein extract was able to degrade CMC, proving the protein's active cellulase activity. The His-tagged CJD5-110 was able to be purified by nickel ion chromatography. The purified protein extract did not have an enhanced or improved cellulase activity, as compared to the whole cell and soluble cell protein extracts. Other researchers have also used metagenomic screening to identify, isolate, and characterize novel cellulase genes. These researchers were able to identify a novel cellulase protein and purify it for further characterization. They found the purified protein extract's specific pH range and temperature for optimal activity (Gong et al, 2012). Further studies can be done to determine optimal pH and temperature of CJD5-110 that could improve the purified CJD5-110 protein's cellulase activity.

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