INL/EXP-19-55116-Revision-0

Gene editing of Gluconobacter oxydans for improved xylose metabolism and bioleaching

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August 2019

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operated by Battelle Energy Alliance

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Prepared for the
U.S. Department of Energy
Office of Energy Efficiency and Renewable Energy
Under DOE Idaho Operations Office
Contract DE-AC07-05ID14517

Gene editing of *Gluconobacter oxydans* for improved xylose metabolism and bioleaching

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1. Introduction

Rare earth elements (REE) are critical materials utilized in many modern technologies[3]. The leaching and recycling of these materials from wastes or end-of-life products could be economically and ecologically advantageous.

Bioleaching is a safer and more environmentally conscious method than standard chemical leaching, and agricultural waste such as corn stover (Fig.1) can be used as a carbon source for production of organic acids for bioleaching elements from a solid matrix.

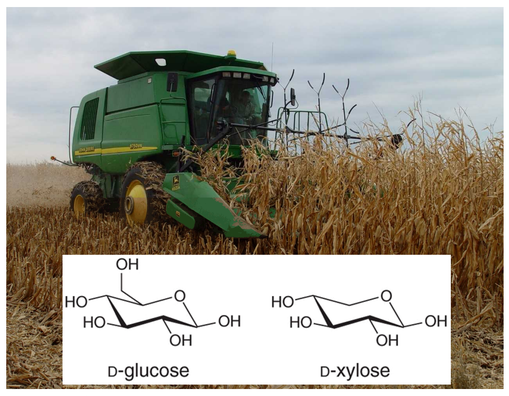

Figure 1 shows corn stover being harvested. The insert shows the molecular structures of glucose and xylose.

The bacteria chosen for bioleaching is *Gluconobacter oxydans* (*G.ox*), which is an acidophile and obligate aerobe[4]. However, this bacteria is much more adept at metabolizing glucose than xylose. Corn stover contains both glucose and xylose. The goal of this experiment was to introduce a plasmid into *G. ox* that would enable it to more efficiently utilize xylose and produce more xylonic acid. Xylonic acid has a lower pKa than gluconic acid, the product of glucose oxidation. Specifically, we want to enhance xylose metabolism. This was done by triparental mating, with a donor strain of *E.coli* containing a plasmid for xylose metabolism as well as resistance to the antibiotic kanamycin. Both kanamycin (kn) and cefoxitin (cf) were used as selective markers, as *G.ox* has a natural resistance to cefoxitin, while *E.coli* does not. The new *G.ox* transformant will be used in a new cycle of experimentation to produce bio-lixiviant from corn stover, in the hopes of producing a higher concentration of xylonic acid.

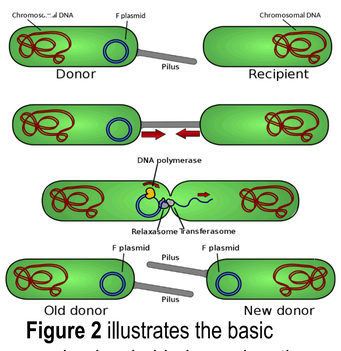

Figure 2 illustrates the basic mechanism behind a conjugation.

2. Methods

Conjugation: The donor, helper, and recipient strains were cultivated on agar plates specific to their strain. Both donor and helper had kn in their agar to ensure that they maintained the plasmid. Each strain was grown up in liquid media and then centrifuged to concentrate the cells[5]. Cells were then pipetted to yeast peptone growth media plates at a ratio of 3:1 of recipient to donor cells. Plates were incubated to allow copious growth and, ideally, sharing of plasmid[1,2].

Plasmid Prep: The new *G.ox* transformant was then grown for a plasmid purification. Some of this culture was frozen for later use, the remaining cells were lysed, and all cell proteins were precipitated and then removed from solution by centrifugation. The supernatant was treated to isolate the plasmid DNA and frozen.

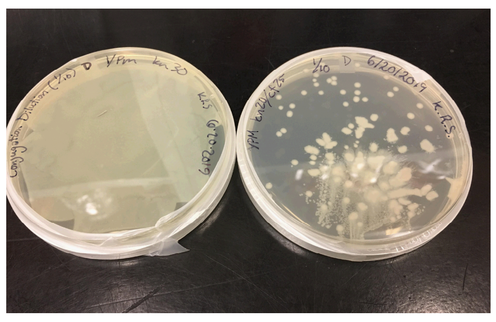

Figure 3 illustrates the difference in growth on two selective plates, based on the natural resistances of the strain being plated.

Restriction Digest: To determine how successful the plasmid purification was, a restriction digest was performed. The full DNA sequence for the plasmid and the inserted gene were analyzed to determine which enzymes would work best for the digest. PstI and KpnI were the chosen enzymes, and the digest was performed. The reaction was allowed to run for one hour. The digested plasmid was then run through a gel using electrophoresis to determine the presence and quality of the plasmid.

3. Results & Discussion

Conjugation: The conjugation of *G.ox* was successful, as can be seen in Figure 4. The transformant and control strains were plated on a plate containing both antibiotics, and another plate with Pkm media. The Pkm would be cleared if the cells produced an organic acid (a verification of the acidophlic nature of *G.ox*). The antibiotic plate required the cell to have resistance to both kn and cf.

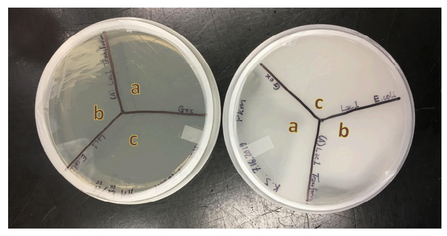

Figure 4 shows the verification of a successful conjugation. The left has both kn and cf in the media, and the right is a Pkm plate. Both the *G.ox* control (a) and transformant (b) cleared the Pkm. The *E.coli* (c) did not grow on the antibiotic plate, and although it did grow on the Pkm, it did not clear the media.

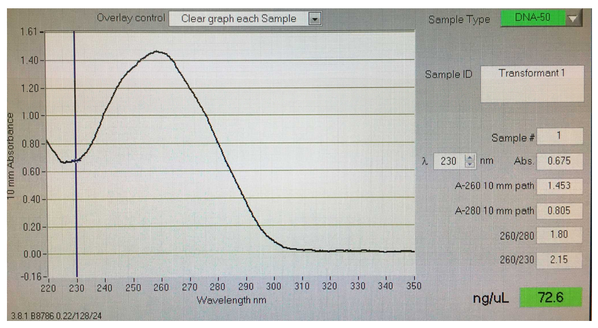

Figure 5 shows a nanodrop graph, which indicates a high-purity plasmid in solution.

Plasmid and Nanodrop: A Nanodrop spectrophotometer was used to gauge the success of the plasmid prep. The absorbance readings in Figure 5 indicate a high concentration of nucleic acids in solution. Since all the genomic DNA was removed, these values point to a pure plasmid sample.

Electrophoresis/Digest: The plasmid digests were run in the gel shown in Fig.6 below. Lanes 3-6 were the DNA from the restriction digest, with 3 being the original plasmid, and 4-6 were from the transformants. As shown, the transformant DNA appears highly degraded. This could be caused by several factors, the most probable of which is that the purified plasmid was highly degraded, and the enzymes were therefore entirely ineffective.

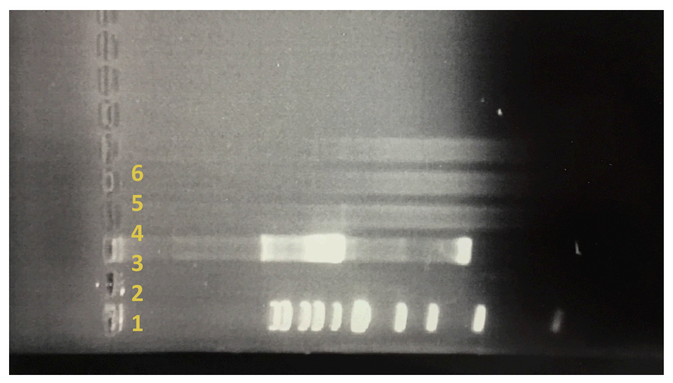

Figure 6 shows the second gel run with the transformant *G.ox* strain. Lane 1 is the 1kb ladder, Lane 3 is the original plasmid, and Lanes 4-6 are the transformant plasmid from the previously mentioned plasmid prep.

4. Conclusions / Moving Forward

- *Gluconobacter oxydans* can be genetically altered by triparental mating.
- Due to some apparent plasmid damage, another plasmid prep must be completed.
- A successful *G. ox* transformant will be tested on corn stover to verify improved xylonic acid production-bioleaching.

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Acknowledgements

I would like to acknowledge mentors Dr. David Reed, Dr. Dayna Daubaras, Dr. Vicki Thompson, and Dr. Yoshiko Fujita , from the Idaho National Laboratory, for their guidance and support in this internship. Thanks is due also to the CMI organization for funding this project through DOE Idaho Operations Office Contract DE-AC07-05ID14517, and to Idaho National Laboratory for awarding me this position. Thanks as well to ATUM for providing their plasmid engineering services.