Novel thermophilic cellulolytic isolates belonging to the phylum Chloroflexi

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Repository Citation

Palisoc, Maryknoll; Guy, Jessica K.; Peacock, Joseph P.; Trinh, Duy C.; Dodsworth, Jeremy A.; and Hedlund, Brian P., "Novel thermophilic cellulolytic isolates belonging to the phylum Chloroflexi" (2011). Undergraduate Research Opportunities Program (UROP). 11.  
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Novel Thermophilic Cellulolytic Isolates Belonging to the Phylum Chloroflexi

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Introduction
Current biofuel technologies utilize valuable foodstuffs, such as corn kernels and cane sugar, as sources of easily metabolized sugars. Microbes are used to ferment these sugars into bioethanol, a first-generation biofuel. However, in order to avoid diverting foodstuffs from the food supply, the development of second-generation biofuels technology is necessary. Second-generation biofuels are produced by converting structurally complex lignocellulosic biomass, such as agricultural and municipal wastes, to fermentable sugars or directly to biofuels (1).

The major technological hurdle limiting the mass production of second-generation biofuels is the difficulty in efficiently converting structurally complex lignocellulosic materials to fermentable sugars or directly to biofuels. The discovery of novel thermophilic microorganisms and enzymes that have high activities or broad substrate ranges on plant polymers addresses this challenge.

Research Objectives

Objective I: To obtain pure cultures of thermophilic microbes capable of cellulose degradation

Objective II: To characterize the morphology and physiology of the isolates

Objective III: To identify isolates through DNA sequencing of the 16S rRNA gene and phylogenetic analysis

Experimental Design

Objective I: Corn stover and aspen shavings were incubated in Great Boiling Spring in Gerlach, Nevada (Fig. 2) and used as inocula for 72 enrichments designed to support the growth of strongly cellulolytic microorganisms. The enrichments were tubes containing Filter Paper Medium I (Castenholz Medium D with filter paper as the sole organic carbon source and additional vitamins and nitrogen).

One enrichment generated a biofilm capable of degrading insoluble cellulose, and this consortium was found to contain a mixture of rods and filaments. Rods belonging to the genera Genobacillus and Rhodothermus were isolated by plating and found to have weak cellulolytic activity on insoluble cellulose. It was hypothesized that the filaments were responsible for the strong cellulolytic activity displayed by the community, but the filaments were not observed to grow on plates. Hence, optical tweezers and a microfluidic cell sorting device (Fig. 3) were employed to physically isolate the filaments from the community. 33 single filaments, six small groups of filaments, and one negative control were sorted into Filter Paper Medium I and incubated at 55°C.

Objective II: Six cultures inoculated with single filaments grew to high cell density and efficiently degraded the filter paper at temperatures ranging from 45-65°C (Fig. 4).

Of the 28 different conditions tested, the best growth was observed in R2A Medium while shaking at 65 RPM. Filaments sometimes aggregated into spherical (Fig. 6A) or thread-like (Fig. 6B) structures and were observed to cling to glass surfaces. The isolates stained Gram-negative. In addition, some dense cultures were visibly pink or red and all cell pellets produced through centrifugation were pink or red.

Objective III: The DNA of two pure cultures, BG1 and JGK2, was isolated, the 16S rRNA genes were amplified and the PCR products were sent for DNA sequencing. The resulting near-full length 16S rRNA gene sequences were submitted to NCBI BLAST to find and compare regions of local similarity to other microorganisms. These sequences were aligned in mothur, and then imported into ARB, where neighbor-joining and maximum likelihood trees were produced.

Results and Discussions

Fig. 2. Photograph of Great Boiling Spring.

Fig. 3. Photograph of a microfluidic cell sorting device.

Fig. 4. Photograph depicting the extent of filter paper degradation performed by isolates after 16 days of incubation in Filter Paper Medium I at 55°C.

Fig. 5. Phase-contrast micrographs displaying cultures aggregating into spherical (A) and thread-like (B) structures in R2A medium.

Fig. 6. Phase-contrast micrographs displaying filaments aggregating into spherical (A) and thread-like (B) structures in R2A medium.

Future Directions
Detailed phylogenetic and phenotypic characterization is underway to determine whether the organisms represent a novel order or family within the Chloroflexi, further resolve the branching pattern of the phylogenetic tree, and to delineate isolate activities against specific biopolymers. Future enzymatic and genomic characterization will determine whether these organisms or their enzymes may be useful for increasing the efficiency of second-generation biofuels technology.

Acknowledgements
This work was possible due to the generous support of the NSF Career Grant MCB-0546665, Nevada Renewable Energy Consortium (DOE), Urban21 (DOE) and NSF REU (DBI 1005223).

Literature Cited