Aug 9th, 10:15 AM - 12:00 PM

Novel thermophilic cellulolytic isolates belonging to the phylum Chloroflexi

Maryknoll Palisoc  
University of Nevada, Las Vegas

Jessica K. Guy  
University of Nevada, Las Vegas

Joseph P. Peacock  
University of Nevada, Las Vegas

Duy C. Trinh  
University of Nevada, Las Vegas

Jeremy A. Dodsworth  
University of Nevada, Las Vegas

See next page for additional authors

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.  
https://digitalscholarship.unlv.edu/cs_urop/2011/aug9/11

This Event is brought to you for free and open access by the Undergraduate Research at Digital Scholarship@UNLV. It has been accepted for inclusion in Undergraduate Research Opportunities Program (UROP) by an authorized administrator of Digital Scholarship@UNLV. For more information, please contact digitalscholarship@unlv.edu.
Presenters
Maryknoll Palisoc, Jessica K. Guy, Joseph P. Peacock, Duy C. Trinh, Jeremy A. Dodsworth, and Brian P. Hedlund

This event is available at Digital Scholarship@UNLV: https://digitalscholarship.unlv.edu/cs_urop/2011/aug9/11
Novel Thermophilic Cellulolytic Isolates Belonging to the Phylum Chloroflexi

Maryknoll M. Palisoc, Jessica K. Guy, Joseph P. Peacock, Duy C. Trinh, Jeremy A. Dodsworth, and Brian P. Hedlund
School of Life Sciences, University of Nevada Las Vegas, Las Vegas, Nevada

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).

Fig. 2. Photograph of Great Boiling Spring.

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 plate 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.

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

Results and Discussions

Objective I:
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).

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.

Objective II:
All six isolates were unbranched multicellular filaments ranging from 20 to >600 μm in length and 0.7-0.8 μm in width (Fig. 5A,B). All isolates were capable of gliding motility but were not motile in liquid medium.

Fig. 5. Phase-contrast micrographs of the isolates in Filter Paper Medium I (A) and in R2A medium (B).

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.

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

BLAST results showed that the isolates are members of the Phylum Chloroflexi, with 84% sequence identity similar to their nearest cultured relative, Roseiflexus castenholzii, indicating that the isolates are only distantly related to cultivated microorganisms. The evolutionary distance tree (Fig. 7) places the isolates within the Class Chloroflexi, branching between Herpetosiphon aurantius (non-photosynthetic) and Roseiflexus castenholzii (photosynthetic).

Fig. 7. Neighbor-joining tree of the Phylum Chloroflexi. 16S rRNA sequence alignments were performed and tree support of at least 70% is indicated. Outgroup sequences are Escherichia coli (X84248), Bacillus subtilis (D26185), and Corynebacterium diphtheriae (D66224, after Hugenholtz et al. 2004).

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