Poster Abstract P8

Thermo-activated xylanases from tropical strains of Aureobasidium pullulans

Pongtharin Lotrakul*[1], Saratsawadee Rotjanagusol[1], Sehanat Prasongsuk[1], Douglas E. Eveleigh[2] and Hunsa Punnapayak[1], (1)Chulalongkorn University, Bangkok 10330, Thailand, (2)Rutgers University, School of Environmental and Biological Sciences, New Brunswick, NJ

From various foliar samples collected in 4 provinces in Thailand, 20 strains of *Aureobasidium pullulans* were obtained. All strains were positive for xylanase production when grown on Yeast Malt Xylan Agar. In liquid culture, these *A. pullulans* strains produced xylanase ranging from 31 to 205 $U.l^{-1}$. The stability of these enzymes at high temperature varied widely. Incubation at 60°C for 1 hour showed activation of enzymes from 6 strains in comparison to their counterparts stored at 4°C for 1 hour. Xylanases from the other 14 strains were more sensitive to high temperature, losing most of their activity after 1 hour at 60°C. Thermo-activated xylanases were divided into 2 groups based on their responses to longer incubation times at 60°C. Xylanases in the first group showed a 2.5- to 4-fold increase in activity after 1 hour but completely lost their activity within 5 hours whereas those in the second group exhibited a 2- to 2.4-fold increase in activity after 1 hour and retained more than half of their activity after 5 hours. Clearly there is an interesting range of diversity in the thermostability of these Thai *A. pullulans* xylanases.

Poster Abstract P9

Characterization of the bacterial diversity in the goat rumen and identification of enzymes with potential use in the biofuels industry

Betania F. Quirino*[1], Isabel S. Cunha[2], Betulia M. Souto[1], Lucas S. Carvalho[1], Nidia S.P.L. Ramos[1], Ohana Y.A. Costa[2], Cristine C. Barreto[2] and Ricardo H. Kruger[3], (1)Embrapa-Agrienergy, Brasilia, Brazil, (2)Universidade Catolica de Brasilia, Brasilia, Brazil, (3)Universidade de Brasilia, Brasilia, Brazil

Ruminants have a powerful system for fermentation of fiber, mainly due to the presence of anaerobic microrganisms. To characterize the microbial communities present in the liquid and solid fractions of the goat rumen and identify enzymes that may have potential in the biofuels industry, a metagenomic approach was taken. Bacterial and archaeal 16S ribosomal gene libraries were constructed and clones of each library were sequenced and analyzed. The overall dominant genera in the rumen were *Clostridia* and *Bacteroides* known to play a role in plant fiber degradation in other ruminants. Unclassified bacteria accounted for 16% of the liquid fraction sequences and 23.8% of the solid fraction sequences, indicating that a majority of the rumen microbiota is not known. From the archaeal libraries only sequences from the phylum Euryarcheota, class Methanobacteria, were identified and a group of uncultured methanogenic *Archaea* not previously known to be associated with the rumen was identified. To explore the biotechnological potential of the goat rumen microbiota, a small insert metagenomic library was constructed in an expression vector and a number of functional screens for enzymatic activities were performed. Among the clones that showed different restriction digestion patterns and a stable phenotype upon retransformation were three clones with β-glycosidase activity and two clones with cellobiohydrolase activity. The characterization of these clones with potential use for the production of second generation ethanol is being pursued.

Poster Abstract P10

Lignocellulolytic enzyme production of tropical resupinate white rot fungi isolated in Thailand

Ponlada Permpornsakul*[1], Pongtharin Lotrakul[1], Sehanat Prasongsuk[1], Douglas E. Eveleigh[2], Donald Y. Kobayashi[2], Raymond Sullivan[2], Sheng-Hua Wu[3] and Hunsa Punnapayak[1], (1)Faculty of Science, Chulalongkorn University, Bangkok 10330, Thailand, (2)Rutgers University, School of Environmental and Biological Sciences, New Brunswick, NJ, (3)National Museum of Natural Science, Taichung, Taiwan

Resupinate white rot fungi (WRF) are abundant throughout tropical regions, but little is known about their potential as sources for novel lignolytic enzyme activity. In this study, tropical resupinate WRF were collected from dead-wood stumps in five different provinces of Thailand and screened for efficient lignin peroxidase (LiP) and lignocellulolytic enzyme production. Based on morphological characteristics, 25 of the resupinate WRF isolates were identified as members of the genera *Bjerkandera, Ceriporia, Fomes, Hyphodontia, Junghuhnia, Macrohyporia, Oxyporous, Pachykytospora, Peniophora, Perenniporia, Phanaerochaete,* and *Schizopora.* Among these isolates, lignin modifying enzymes (LMEs) were examined by direct visualization of specific color reactions using the compounds azure-B, phenol red or ABTS, which indicate production of lignin peroxidase (LiP), manganese peroxidase (MnP) and laccase, respectively. Nineteen of the isolates screened tested positive for LMEs activity. Among these 19 isolates, nine were positive for LiP activity. Most of LiP-positive isolates were found to be able to produce cellulases and xylanase on plate assays using 1% CMC or 2% xylan as a substrate, respectively. For further characterization, selected WRF isolates were screened for LiP activity using an azure-B (0.01%) decolorization liquid assay. Four isolates, identified as strains of *Peniophora sp., Phanerochaete sordida, Macrohyporia dictyopora* and *Schizopora apacheriensis*, significantly decolorized azure-B up to 50% within 4 days. These results suggest that tropical resupinate WRF are a taxonomically diverse group and may represent a potential untapped source of novel lignocellulolytic enzyme producers.