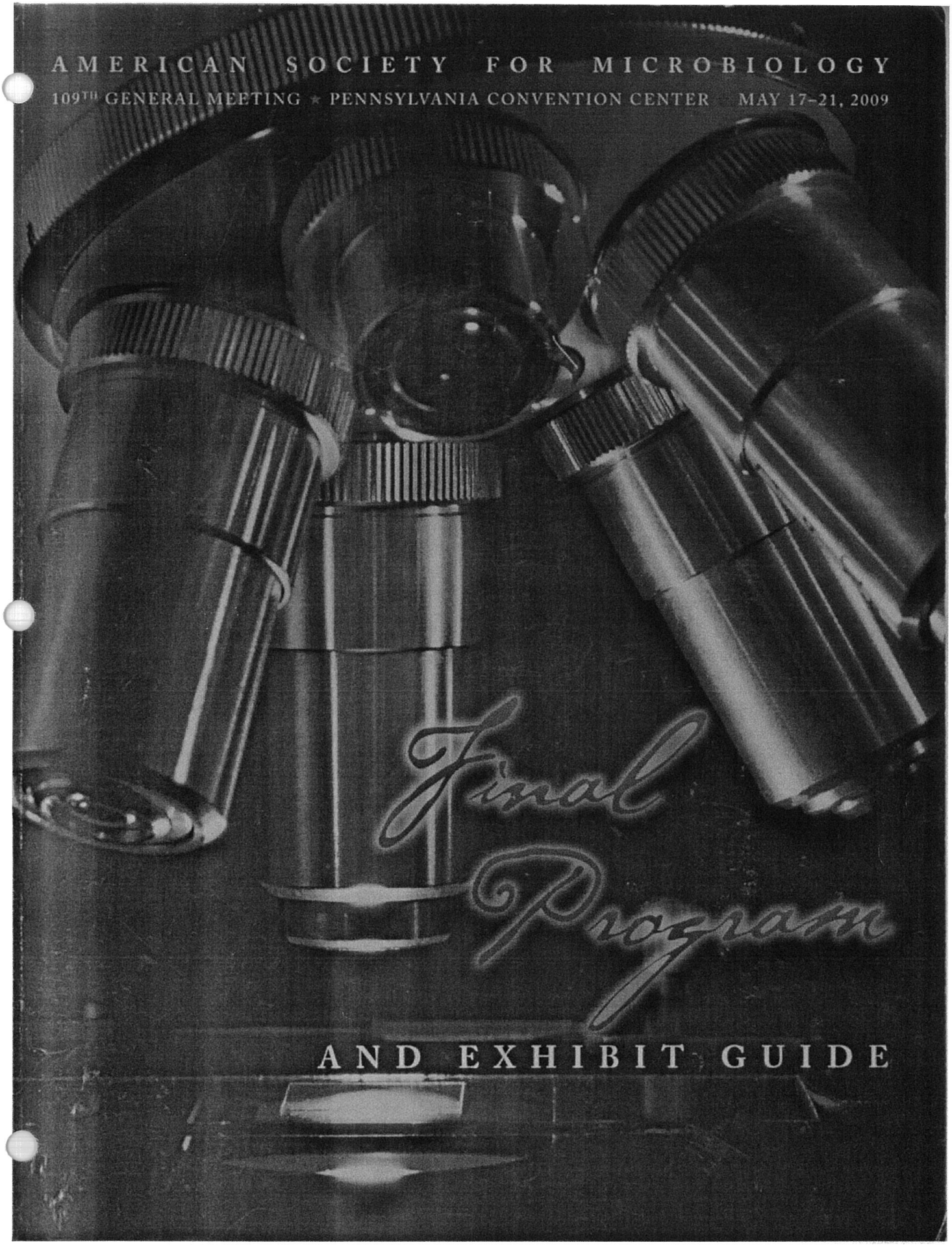
AMERICAN SOCIETY FOR MICROBIOLOGY
109TH GENERAL MEETING ★ PENNSYLVANIA CONVENTION CENTER MAY 17–21, 2009
Final Program
AND EXHIBIT GUIDE

O-015. Mining *Clostridium thermocellum* for Enzymatically Active Carbohydrases

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The efficient hydrolysis of biomass to 5 carbon and 6 carbon sugars is limited by the lack of affordable, high specific activity enzymes. Screening of genomic and metagenomic libraries for new biomass-degrading enzymes has had only limited success. We examined a number of screening strategies using *Clostridium thermocellum* (*Cth*) as a target-rich model organism to validate the efficiency of capturing carbohydrases that may prove useful for biomass degradation. The *Cth* genome has been sequenced and is predicted to have genes for over 60 potential biomass-degrading enzymes associated with the cellulosome, and another 18 enzymes that are noncellulosomal. Two different cloning systems were used for gene expression and two different screening methods were utilized for identification of positive clones. A comparison of the methods showed large differences both in the total number of positive clones identified as well as large differences in the total number of different enzymes captured. This poster also describes the gene products that were captured by the individual screens, including a new cellulase that was not previously annotated.

O-016. Benzylic and aryl Hydroxylations of *m*-xylene by *o*-xylene Dioxygenase from *Rhodococcus* sp. strain DK17

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The *o*-xylene dioxygenase (XDO) from *Rhodococcus* sp. strain DK17 possesses the ability to perform distinct regioselective hydroxylations with the size and position of the substituent group determining the number and position of the dihydroxylation on the aromatic ring. *Escherichia coli* cells harboring a recombinant plasmid with the DK17 XDO genes were cotransformed with the pKJE7 chaperon plasmid for higher expression and stable maintenance of XDO, and then used for bioconversion of *m*-xylene. Gas chromatography-mass spectrometry analysis of the oxidation products detected 3-methylbenzylalcohol and 2,4-dimethylphenol as a major and a minor metabolite in a ratio of 8:2, respectively. A molecular modeling study suggests that the DK17 XDO holds xylene isomers at a kink region between α6 and α7 helices of the active site in a position, so that one side of the aromatic ring is blocked by helix α6. Although the substrate-binding pocket of XDO is modeled to be spacious when a xylene isomer binds as a substrate, *m*-xylene is unlikely to locate at the active site with a methyl group facing the kink region because such a configuration would not fit within the substrate-binding pocket. *m*-Xylene molecule can flip horizontally to expose the *meta*-position methyl group to the catalytic motif. In this configuration, 3-methylbenzylalcohol could be formed as a product presumably due to the *meta* effect, which states that the *meta* methyl group of *m*-xylene would not allow binding and catalysis of aryl hydroxylation of XDO. Alternatively, *m*-xylene molecule can rotate counterclockwise from the previously mentioned position, which would allow the catalytic motif to hydroxylate at C-4 yielding 2,4-dimethylphenol as a product. To our best knowledge, 2,4-dimethylphenol have not been seen before in the oxidation of *m*-xylene. The present work demonstrates that the DK17 *o*-xylene dioxygenase has an ability to oxidize *m*-xylene through both benzylic and aryl hydroxylations although the former reaction is preferred.
Acknowledgments/References: This work was supported by a grant from MEST through the 21C Frontier Microbial Genomics and Applications Center Program and also by a grant from KOSEF through AEBRC at POSTECH.

O-017. Biochemical Characterization of a Novel Laccase from *Trametes versicolor*

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Background: Laccases are versatile biocatalysts present in diverse groups such as plants, fungi, bacteria and plants. In fungi, laccases have been widely described and characterized as biotechnological tools in diverse fields including bioremediation. However, the finding of novel laccases with improved bioconversion abilities is required in order to be used in process like bioconversion of xenobiotics. **Methods:** We have identified previously novel induced laccases produced by *Trametes versicolor*, a basidiomycete, induced by different sawdust substrates used to grow this fungi. In this work, we report the purification and characterization of a novel laccase (named lacc CI produced by *T. versicolor*. Kinetic parameters such as optimal pH, Km, substrate specificity. The CI enzyme was produced in liquid medium in presence of cedar sawdust and branflakes extract in order to encrease the amount of enzyme for biochemical studies. **Results:** Our results indicate that CI laccase from *T. versicolor* present different kinetic properties compared with the constitutive laccase produced by this same fungi. **Conclusion:** Induced laccase CI show distinct kinetical properties compared with the constitutive laccase. These differences could account for a particular function induced by the chemical nature of the substrate used to grow this fungi.
Acknowledgments/References: Financial support was provided by PROMEP/ 103.5/07/2674.
Baldrian, P. (2006). "Fungal laccases - occurrence and properties." FEMS Microbiology Reviews **30**(2): 215-242.

O-018. Putative Unique Gene Approach for the Design of Species Specific Probes as Modeled by *Lactobacillus plantarum*

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The concept of utilizing putative unique genes for the design of species specific probes was tested. The abundance profile of assigned functions within the *Lactobacilus plantarum* genome was used for the identification of a putative unique gene (*csh*) used as the template for PCR amplification and construction of a non-radioactive DIG labeled probe, which aided in the identification of this lactic acid bacterium from mixed cultures using Southern hybridization. This approach to species identification could be applied to any microorganism for which there are complete genomes sequences for both the target organism and some closely related organisms.
Acknowledgments/References: The author thanks Ms. Ashlee Webber at the USDA-ARS, SAA Food Science Research Unit located in Raleigh, NC for technical assistance.

O-019. Biological Catalysts for Conversion of Gas to Ethanol and Methane

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There is a need to find new and improved ways to obtain alternative fuels that do not compete with food sources. Any carbonaceous material can be converted to syngas (a mixture of CO, CO_2, N_2, and H_2) by means of gasification. Some acetogenic bacteria are able to ferment syngas resulting in alcohol, organic acids and hydrogen as fermentation byproducts. Conversion of syngas to ethanol using microbial strains achieves high yields and is more environmentally friendly than traditional chemical catalysis. *Clostridium ljungdahlii* ferments syngas to ethanol resulting in the conversion of the CO_2 and CO present in the syngas to ethanol and acetic acid. ATP synthesis resulting from acetate production skews the final liquid products to higher acetate production compared to ethanol. Therefore, development of a microbial fermentation system that can support the conversion of acetic acid to other fuel components, such as methane, would increase the effective fuel yield of the gasification-fermentation process. *Methanosarcina* species can convert acetate to methane. These aceticlastic methanogens could be used in the production of biofuels from the acetate generated by the fermentation of syngas by *C. ljungdahlii*. In addition, it is desirable to find appropriate fermentation conditions that will shift product ratios towards ethanol. There have been previous studies reporting that certain acetogens can produce higher amounts of ethanol compared to acetate when oxygen was added to the cultures. For the two-step fuel production, *C. ljungdahlii* was grown anaerobically on liquid media and syngas was used in the headspace. Spent media was collected and use to grow *Methanosarcina barkeri* and ethanol, acetate and methane concentrations were quantified. For the oxygen exposure experiments, different concentrations of oxygen were added to the culture headspace and ethanol and acetate production was quantified. In the present study we demonstrate the use of microbial catalysts for the orchestrated production of ethanol and methane and show that the addition of oxygen to the acetogen culture improves ethanol production as well as ethanol to acetate ratios.

180/O. Antibiotics, Antimicrobials and Mycotoxins

Tuesday, 1:00 pm | Poster Hall

O-020. The Antimicrobial Compound Reuterin Mediates Bacterial Growth Inhibition by Causing Oxidative Stress

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Reuterin (3-hydroxyropionaldehyde) is a broad-spectrum antimicrobial compound produced by many strains of probiotic *Lactobacillus reuteri*. Reuterin has been proposed to mediate, in part, the beneficial health effects exhibited by probiotic *L. reuteri*. The goals of this study were to determine how reuterin is produced and to elucidate the mechanism of action of reuterin in mediating bacterial growth inhibition. Reuterin is produced from glycerol by the action of glycerol dehydratase and is converted to 1,3-propanediol by propanediol oxidoreductase. We have created disruptions in the *dhaT* and *pduC* genes encoding propanediol oxidoreductase and the large subunit of glycerol