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Thomas W. Jeffries received his PhD in Microbiology from Rutgers University in 1975 then joined the staff at Lawrence Livermore National Laboratory where he engaged in on strategic planning for the development of renewable fuels from biomass. Following two years in the Department of Chemical Engineering at Columbia University he joined the staff at the USDA Forest Products Laboratory in Madison in 1979. At FPL, he served as the Director of the Institute of Microbial and Biochemical Technology from 1998 to 2004 before promotion to his current role as Microbiologist ST. Complementing his USDA position, Dr. Jeffries joined the University of Wisconsin Dept. of Bacteriology as an Associate Professor in 1990, and was promoted to Professor in 1998 where he teaches Biotechnology and Industrial Microbiology and conducts research into the pathway engineering of xylose metabolism in *Scheffersomyces stipitis*, *Spathaspora passalidarum* and *Saccharomyces cerevisiae*.

Dr. Jeffries has authored approximately 150 peer-reviewed publications and 11 patents and has presented numerous lectures to national and international audiences. He serves on the editorial board for various journals including *Applied and Environmental Microbiology*, *Metabolic Engineering*, and *Applied Microbiology and Biotechnology*. He is a Senior Editor for the *Journal of Industrial Microbiology and Biotechnology*. He is a fellow of the Society for Industrial Microbiology, the American Academy of Microbiology and the International Academy of Wood Science. In 2003 Dr. Jeffries received the Charles D. Scott award for the development of technology for ethanol from cellulose. He has served as Chairman of the ASM Fermentation Division, and the Society for Industrial Microbiology Board of Directors and is President Elect for the SIM.

Title: Recent advances in pentose utilization by native and engineered yeasts

Abstract: Fermentation of hemicellulosic sugars remains an issue in the development of commercial bioconversion processes, even after 30 years of research. Today, however, the emphasis is shifting toward the creation of strains that can handle industrial feedstocks under commercial conditions and on the co-fermentation of xylose, cellobiose and glucose. Yeasts must ferment xylose in highly challenging hydrolysates with only minimal supplemental nutrients. Co-fermentation would enable lower capital costs but also runs counter to strong regulatory mechanisms. Both of these traits are being addressed through metabolic engineering, and evolutionary adaptation. Metabolic engineering of *Saccharomyces cerevisiae* for xylose utilization started shortly after the discovery that yeasts could ferment xylulose directly to ethanol. This was about the same time that the first native xylose fermenting yeasts, *Pachysolen tannophilus*, *Candida shehatae*, and *Scheffersomyces (Pichia) stipitis* were described. The vast literature and genetic tools available with *S. cerevisiae* has set the pace for metabolic engineering – but studies of native xylose and cellobiose fermenting organisms have established the context within which such engineering occurs. In recent years, our laboratory has developed a highly functional system for the transformation and genetic manipulation of *S.stipitis* based on a LoxP-flanked synthetic selectable marker (NAT1), zeocin and bleomycin (Shble), an engineered version of Cre and a synthetic version of hph. We have constructed and mated multiple transformants of *S. stipitis* that carry various

combinations of genes for sugar transport, xylose metabolism and fermentation, most of which exhibit significantly improved fermentation kinetics. Genes borrowed from *S. stipitis*, such as XYL1, XYL2 and XYL3 along with sugar transporters from *S. stipitis*, and *Candida tenuis* and xylose isomerase from various sources have all been engineered into *S. cerevisiae* to improve its performance. The heterologous expression of a cellulose-oligosaccharide transporter from *Neurospora crassa* in *S. cerevisiae* and its subsequent co-utilization of cellobiose and xylose was a notable advance. Advances are continuing with native xylose utilizing yeasts as well. Recently we found that a highly unusual beetle-gut associated yeast, *Spathaspora passalidarum*, will ferment xylose to ethanol significantly faster than it ferments glucose and it will co-ferment glucose and xylose when the glucose level is below 30 g/l. Utilization of real-life hydrolysates remains a challenge in many cases. Agricultural residues such as corn stover can be enzymatically hydrolyzed following treatment with ammonia, which converts acetic to acetamide, and the resulting mixture of sugars ($\approx 2/3$ glucose and $1/3$ xylose) can be fermented. With this and many other pretreatments, however, the presence of degradation products in the pretreated substrate inhibits cellulase activity and the initial fermentation of glucose impedes subsequent xylose utilization. Metabolic engineering – guided in some cases by transcriptomics and metabolomics– along with evolutionary adaptation continues to push the field closer to commercialization.