GENETIC ENGINEERING OF *PICHIA STIPITIS* FOR FERMENTATION OF XYLOSE

T.W. Jeffries,[1,2] N.Q. Shi,[2] J.Y. Cho,[2] P. Lu,[2] K. Dahn,[3] J. Hendrick,[1] H.K. Sreenath[1] and B.P. Davis[2]

USDA, Forest Service, Forest Products Laboratory, Madison, WI; [2]Department of Bacteriology University of Wisconsin - Madison

ABSTRACT

A useful genetic system has been developed for the transformation of *Pichia stipitis.* This includes two selectable markers (*URA3* and *LEU2*), integrating and autonomous replication vectors, a pop-out cassette that enables multiple targeted disruptions, and a genomic X-library for rapid cloning. Using this system we have cloned two genes for alcohol dehydrogenase (*PsADH1* and *PsADH2*). two genes for pyruvate decarboxylase (*PsPDC1* and *PsPDC2*). and cytochrome c (*PsCYC1*). Disruption of *PsADH1,* but not PsADH2, resulted in increased xylitol production. Disruption of *PsCYC1* resulted in lower growth and higher ethanol yields. We have used it to heterologously express the gene for *S. cerevisiae* dihydroorotate dehydrogenase. This conferred the capacity for anaerobic growth.

INTRODUCTION

The yeast *Pichia stipitis* is one of the best naturally occurring xylose fermenting organisms known. It is capable of converting xylose and all of the other major wood sugars to ethanol. Some strains even ferment xylan and metabolize lignin moieties. Ethanol yields range between 0.3 and 0.44 g/g of substrate. Volumetric production rates are about 0.5 g/l-h [1]. Although this is suitable for some wood-derived waste streams, commercial fermentation of hydrolysates specifically for ethanol production requires higher performance. If a practicable solution to xylose fermentation can be found, more than 20 billion liters of ethanol could be produced annually from agricultural and silvicultural residues in the United States alone.

In an effort to better understand the mechanisms behind the fermentation of xylose yeasts, and to improve rates and yields, we have developed genetic tools to manipulate *P. stipitis*. Mutants deficient in uracil. and leucine metabolism (*ura3* and *leu2*) and selectable markers based on the corresponding native *P. stipitis* genes (*URA3* and *LEU2*) enable introduction of heterologous expression cassettes and identification of transformants. Extra chromosomal plasmids can be maintained on plasmids carrying a *P. stipitis* autonomous replication sequence (*ARS*), and random integration or targeted disruptions can be carried out using linearized fragments with appropriate flanking sequences. We have also developed a mating system that enables multiple transformations and crossings of various strains. We have Cloned and identified the primary fermentative genes for *P. stipitis* alcohol dehydrogenase (*PsADH1*), pyruvate decarboxylase (*PsPDC1*), cytochrome c (*PsCYC1*), plus several other genes of respirative and fermentative metabolism. We have demonstrated that (1) targeted disruption of *PsADH1* results in diminished fermentative growth and increased ethanol Production, (2) disruption of *PsCYC1* results in diminished aerobic growth and increased ethanol production, (3) incorporating multiple copies of xylose assimilative genes increases enzyme activity, and (4) heterologous expression of the *Saccharomyces cerevisiae* gene for dihydroorotate dehydrogenase (*ScURA1*) enables anaerobic growth on glucose. We are beginning to understand the basic mechanisms for oxygen regulation of fermentation, and we are combining the various traits that enable improved ethanol production into a single organism that should be useful for commercial ethanol production from hemicellulosic wood sugars. This paper provides a synopsis of the genetic system for *P. stipitis,* and it reviews our attempts at engineering the metabolism of this organism for improved ethanol production

MATERIALS AND METHODS

Strains. *Pichia stipitis* CBS 6054 (= NRRL Y-11545, ATCC 58785) was the parental strain for mutagenesis and the *source* of all cloned DNA. We obtained *P. stipitis ura3* auxotrophs by selecting for resistance to 5-fluoroorotic acid (FOA) in the presence of 100 mg/ml uridine after the method of Boeke et al. [3]. *Escherichia coli* DH5α™* (GibCo BRL, Gaithersburg, MD) (F *recA1 endA1 hsdRI7* (rk^-mk^+) *supE44-thi-1 gyrA relA1*) was used for all recombinant DNA experiments that required a bacterial host.

Media. Yeasts were routinely cultivated in YPD medium (1% yeast extract, 2% peptone, 2% glucose) or YMA (1% yeast extract, 2% malt extract). Yeast nitrogen base (YNB) without amino acids with 5 mg/ml ammonium sulfate (Difco, Detroit, MI) was used as the basal defined medium for all auxotrophic selections and transformations. Auxotrophs were grown in YNB (0.17%) with 2% glucose (YNBG). Uridine was supplied at 100 mg/ml in place of uracil, and leucine (100 µg/ml) was added as needed. *E. coli* was cultivated in Luria-Bertani (LB) media with 50 mg/ml ampicillin in liquid media or 100 mg/ml ampicillin in solid media.

Plasmids. Plasmid Bluescript $KSII^+$™ was obtained from Stratagene (La Jolla, CA). Plasmid pJM6 was constructed as described by Yang et al. [2].

Enzymes and chemicals. Restriction enzymes and other DNA modification enzymes were obtained from New England Biolabs (Beverly, MA), Stratagene (La Jolla, CA), or Promega (Madison, WI) and used as recommended by the suppliers. FOA was obtained from PCR, Inc. (Gainesville, FL). SeaKem GTG™ and SeaPlaque GTG™ agarose were obtained from FMC BioProducts (Rockville, ME).

TRANSFORMATION SYSTEM

Transformation of *P. stipitis* is required to advance genetic studies and development of xylose metabolism in this yeast. Our first step in the development of a useful transformation system was to clone the *P. stipitis URA3* gene through cross hybridization with its *S. cerevisiae* homolog [2]. This approach took advantage of the powerful positive selection system for *ura3* mutants afforded by 5'-fluoroorotic acid [3]. In order to increase the efficiency of transformation and the capacity to recover constructs, we also isolated a native *P. stipitis* autonomous replication sequence *(ARS).* The third part of the system is a *ura3* recipient host which we initially isolated as a spontaneous mutant (FPL-TJ26, *ura3-1*) from *P. stipitis* CBS 6054. With electroporation using circular *ARS*-bearing vectors, this system can produce 600 to 8,000 transformants per µg of DNA. Linearized vectors produced up to 12,000 transformants per µg of DNA. In order to introduce more than a few genes, it is necessary to have multiple selectable markers and a mating system. Also, the

*The use of trade or firm names in this publication is for reader information and does not imply endorsement by the U.S. Department of Agriculture of any product or service.

introduction of mutations often results in the degradation of complex physiological processes such as fermentation and growth. We therefore took special care to develop recipient hosts that showed high fermentative capacity.

SELECTION OF FERMENTATIVE STRAINS

A mutant strain of *Pichia stipitis*, FPL-061, was obtained by selecting for growth on L-xylose in the presence of respiratory inhibitors. The specific fermentation rate of FPL-061, was higher than that of the parent, *P. stipitis* CBS 6054, due to its lower cell yield and growth rate and higher specific substrate uptake rate. With a mixture of glucose and xylose, the mutant strain FPL-061 produced 30 g ethanol/L with a yield of 0.42 g ethanol/g sugar consumed. By comparison, CBS 6054 produced 26 g ethanol/L with a yield of 0.35 g/g under the same conditions. The fermentation was most efficient at an aeration rate of 9.2 mmoles O_2 L^{-1} h^{-1}. At high aeration rates (22 mmoles O_2 L^{-1} h^{-1}) the mutant cell yield was less than that of the parent. At low aeration rates, (1.1 to 2.5 O_2 L^{-1} h^{-1}), cell yields were similar, the ethanol formation rates were low, and xylitol accumulation was observed in both the strains. Both strains respired the ethanol once sugar was exhausted. We infer from the results that the mutant, *P. stipitis* FPL-061, diverts a larger fraction of its metabolic energy from cell growth into ethanol production than does the parent [4].

From FPL-061, we then selected mutants with the capacity to use xylose and glucose simultaneously. The glucose analog, 2-deoxyglucose (2-DOG) has been used to obtain mutants derepressed for pentose metabolism. Some researchers have used 2-DOG alone while others have used it in the presence of a glucose repressible carbon source. We examined both methods and screened mutant strains for improved use of xylose in the presence of glucose. *Pichia stipitis* mutants selected for growth on D-xylose in the presence of 2-DOG used xylose from a 1:1 glucose:xylose mixture more rapidly than did their parents. One of these, FPL-DX26 completely consumed xylose in the presence of glucose and produced 33 g/L ethanol in 45 h from 80 g/l of this sugar mixture. Mutants selected for growth on 2-DOG alone did not show significant improvement. Selection for growth on D-xylose in the presence of 2-DOG was useful in developing parental strains for further genetic manipulation [5].

ADDITIONAL SELECTABLE MARKERS

Fermentation studies with the initial transformable host, FPL-TJ26 (*ura3-1*), showed that it has relatively little capacity for ethanol production, so in subsequent mutagenesis studies, we isolated additional *ura3* mutants by from CBS 6054 (FPL-PSU1, *ura3-2*) and from the highly fermentative mutant FPL-DX26 (FPL-UC7, *ura3-3*). All three of these recipient hosts can be transformed with *PsURA3* or with the *URA3.* gene from *S. cerevisiae.* Multiple hosts give a wider variety of genetic backgrounds for expression, but it is necessary to have multiple selectable markers if one wishes to introduce more *than* a few genes. To this end, we used *P. stipitis URA3* (*PsURA3*) to disrupt *P. stipitis LEU2* in *P. stipitis* FPL-UC7. A *URA3*:lacZ "pop-out" cassette was constructed containing *PsURA3* flanked by direct repeats from segments of the lacZ reading frame. The *P. stipitis LEU2* gene (*PsLEU2*) *was* cloned from a *P. stipitis* CBS 6054 genomic library through homology to *S. cerevisiae LEU2,* and a disruption cassette was constructed by replacing the *PsLEU2* reading sequence with the *PsURA3*:lacZ cassette. FPL-UC7 (*ura3-3*) was transformed with the disruption cassette, and a site-specific integrant was identified by selecting for the Leu^- Ura^+ phenotype. The *ura3* marker was recovered from this strain by plating cells onto 5'-FOA and screening for spontaneous *URA3* deletion mutants. Excision of the flanked *PsURA3* gene resulted in the Leu^- Ura^- phenotype. The double auxotrophs are stable and can be transformed at a high frequency by *PsLEU2* or *PsURA3* carried on ARS-based plasmids [6].

FERMENTATIVE GENES

We were interested in knowing how the expression various fermentative genes would affect ethanol production. Two genes coding for isoxymes of alcohol dehydrogenase. designated *PsADH1* and *PsADH2*, were identified and isolated from *P. stipitis* CBS 6054 genomic DNA by Southern hybridization to *S. cerevisiae ADH* genes, and their physiological roles were characterized through disruption. The amino acid sequences of *PsADH1* and *PsADH2* show high identity (80.5%) to one another. and are about 72% to 75% identical to *S. cerevisiae ADH1.* They also show high identity with other group I ADH proteins. The PsADH isozymes are presumably localized in the cytoplasm as they do not possess the ammo terminal extension characteristic of mitochondrion targeted ADHs. Genetic studies showed that PsADH1 plays a major role in xylose fermentation because its disruption result. in a lower growth rate and a profoundly greater accumulation of xylitol. Disruption of *PsADH2* does not significantly affect ethanol production or aerobic growth on ethanol as long as *PsADH1* is present. We did not observe expression on *PsADH2* to any significant extent under aerobic on fermentative conditions unless *PsURA1* was disrupted. The expression of is PsADH1 and PsADH2 isozymes appear to be equivalent in their ability to convert ethanol to acetaldehyde and either is sufficient to allow cell growth on ethanol However, disruption of both genes blocks growth on ethanol *P. stipitis* strains disrupted in either *PsADH1* or *PsADH2* accumulate ethanol, although in different amounts, when grown on xylose under oxygen-limited conditions. The *PsADH* double disruptant, which is unable to grow on ethanol, still produces ethanol from xylose at about 13% of the rate seen in the parental strain. Thus, deletion of both *PsADH1* and *PsADH2* blocks ethanol respiration but not its production, implying separate paths for ethanol fermentation and respiration [7].

In *P. stipitis*, fermentative activities and enzymes increase in response to the availability of oxygen rather than to the availability of fermentable sugars. This makes its behavior considerably different from the more studied system in *S. cerevisiae*. To better characterize *PsPDC* expression and regulation, two genes for PDC (*PsPDC1* and *PsPDC2*) we cloned and sequenced from *P. stipitis* CBS 6054, and the structures were studied. Aside from *S. cerevisiae*, from which three PDC genes have been characterized, *P. stipitis* is the only other yeast from which multiple genes for PDC have been identified and characterized. *PsPDC1* and *PsPDC2* have diverged almost as far from one another as they have from the next most closely related known yeast gene. *PsPDC1* contains an open reading frame of 1791 nucleotides encoding 597 amino acids. *PsPDC2* contains a reading frame of 1791 nucleotides encoding 570 amino acids. An 81-nucleotide segment in the middle of the beta domain of *PsPDC1* codes for a unique segment of 27 amino acids, which may play a role allosteric regulation. The 5' regions of both *P. stipitis* genes include two putative TATA elements that make them similar

to the PDC genes from *S. cerevisiae, Kluyveromyces marxianus,* and *Hanseniaspora uvarum* [8].

OXYGEN REGULATED EXPRESSION

We have studied the expression of *PsADH1* and *PsADH2* in *P. stipitis* CBS 6054. Cells expressed *PsADH1* approximately ten times higher under oxygen-limited conditions than under fully aerobic conditions when cultivated on xylose. However, transcripts of *PsADH2* were not detectable under either aeration condition. Beta galactosidase production driven by a *PsADH1::lacZ* fusion construct showed that expression of *PsADH1* increased as aeration decreased. These results suggested that PsADH1 expression is affected by oxygen. Oxygen regulation may in turn be mediated by heme: The level of *PsADH1* transcript in cells grown on xylose under oxygen-limited conditions in the presence of heme was about one-tenth the level of its transcript in cells grown in its absence. Concomitantly with the induction of *PsADH1, PsCYC1* expression was repressed. The disruption of *PsADH1* caused a dramatic increase in *PsADH2* expression on non-fermentable carbon source under fully aerobic conditions, providing a provisional indication that the expression of *PsADH2* is. subject to feedback regulation under these conditions [9].

GENES FOR XYLOSE ASSIMILATION

The major enzymes involved in xylose assimilation ate xylose reductase (XOR or *XYL1*), xylitol dehydrogenase (XDH or *XYL2*), and xylulose kinase (XUK or *XYL3*). The *P. stipitis* XYL1 gene was inserted into an autonomous plasmid (pJM6) that *P. stipitis* maintains in multi-copy. The resulting plasmid, pXOR, or a control plasmid pJM6 without *XYL1* were introduced separately into *P. stipitis* FPL-TJ26. When the transformed cells were grown on xylose under aerobic conditions, the strain with pXOR had up to 1.8 fold higher XOR activity than the control strain. Oxygen limitation led to higher XOR activity in both experimental and control strains grown on xylose. However, the XOR activities of the two strains grown on xylose were similar under oxygen-limitation. When grown on glucose under aerobic or oxygen-limited conditions, the experimental strain had XOR activity up to ten times higher than that of the control strain. Ethanol production was not improved but rather it decreased with the introduction of pXOR compared to the control, and this was attributed to non-specific effects of the plasmid [10].

LIMITED RESPIRATION

A gene encoding the cytochrome c protein (*PsCYC1*) was cloned from *P. stipitis* CBS 6054, by cross-hybridization to a *CYC1* gene from *S. cerevisiae*. The 333 bp open-reading frame showed extensive sequence similarity with other cloned fungal cytochrome c genes. Putative binding sites for the transcriptional activator complex Hap2/3/4/5 and for Hap1 were found adjacent to each other in one upstream activating sequence region. Disruption of *PsCYC1* was successfully carried out in a *P.* stipitis FPL-UC7 which is sensitive to 2-deoxy-glucose. The disruptant Strain was able to support growth by using the alternative electron transport chain. This Confirmed the existence of the alternative respiratory pathway in *P. stipitis*. Furthermore, the disruptant strain could not grow when the respiratory inhibitor, salicylhydroxamate (SHAM), was present alone implying there are no more than two electron transport chains in *P. stipitis*. The resultant strain grew very slowly. The disruptant strain also was unable to grow on the non-fermentable carbon source, glycerol. Cytochrome spectra indicated that there is only a single functional cytochrome c gene in *P. stipitis* [11].

ANAEROBIC GROWTH

Respirative and fermentative pathways co-exist to support growth and product formation in *P. stipitis. This* yeast grows rapidly without ethanol production under fully aerobic conditions, and it ferments glucose or xylose under oxygen-limited conditions, but it stops growing within one generation under anaerobic conditions. Expression of *Saccharomyces cerevisiae URA1* (*ScURA1*) in *P. stipitis* enabled rapid anaerobic growth in minimal defined medium containing glucose when essential lipids were present. *ScURA1* encodes a dihydroorotate dehydrogenase that uses fumarate as an alternative electron acceptor to confer anaerobic growth. Initial *P. stipitis* transformants grew and produced 32 g/l ethanol from 78 g/l glucose. Cells produced ethanol even higher and faster following two anaerobic serial subcultures. Control strains without *ScURA1* were incapable of growing anaerobically and showed only limited fermentation. *P. stipitis* cells bearing *ScURA1* were viable in anaerobic xylose medium for long periods, and supplemental glucose allowed cell growth, but xylose alone could not support anaerobic growth even after serial anaerobic subculture on glucose. These data imply that *P. stipitis* can grow anaerobically using metabolic energy generated through fermentation but that it exhibits fundamental differences in cofactor selection and electron transport with glucose and xylose metabolism [12].

DISCUSSION

Genetic engineering for improved fermentation or product formation has been termed metabolic engineering [13,14]. It has been used successfully to impart the capacity for ethanol production and xylose fermentation in *E. coli* [15], *Zymomonas mobilis* [16] and *S. cerevisiae* [17, 18, 19, 20]. In general, attempts at metabolic engineering have been more successful in bacteria than in yeasts. Although the reasons ate not entirely clear, the smaller genome and fewer feedback regulatory factors found in bacteria make these organisms much easier to work with.

The metabolic engineering of xylose fermentation in *S. cerevisiae* has been progressively more successful. The introduction of *XYL1* from *P. stipitis* did not by itself enable ethanol production or growth. To the contrary, *XYL1* alone enables *S. cerevisiae* to make xylitol from xylose, as long as glucose is provided as a supplemental carbon source [17]. The presence of both *XYL1* and *XYL2* enables *S. cerevisiae* to grow on xylose [18,19], but it is necessary to overexpress the gene for xylulokinase, *XYL3* in order to obtain significant growth or ethanol production on xylose [20].

Our studies have shown that metabolic engineering succeeds in *P. stipitis* either when genes for new activities are introduced or when existing activities are eliminated. The overexpression of particular genes thought to be rate limiting does not necessarily result in improved metabolic flux. This is probably due to feedback effects and other factors such as internal redox level, ATP supply or changes in the copy number of the ARS-based vectors. With experience, the development of stable, integrated transformants, and the use of better promoters, this approach should prove valuable.

ACKNOWLEDGMENT

This research was supported by in part by the National Renewable Energy Laboratory under subcontract no. AUX-4-11193-02, and by USDA NRICGP grant No. 96-35500-3172.

REFERENCES

1. DU PREEZ, J.C., VAN DRIESSEL, B., AND PRIOR, B.A. "Effect of Aerobiosis on Fermentation and Key Enzyme Levels During Growth of *Pichia stipitis, Candida shehatae* and *Candida tenuis* on D-xylose", *Arch. Microbiol.* 152:143–147 (1989)
2. YANG, V.W., MARKS, J.A., DAVIS B.P., JEFFRIES T.W. "High-Efficiency Transformation of *Pichia stipitis* Based on its *URA3* Gene and a Homologous Autonomous Replication Sequence, *ARS2*", *Appl. Environ. Microb.* 60(12):4245–4254 (1994).
3. BOEKE, J.D., LACROUTE, F., AND FINK, G.R. A Positive Selection for Mutants Lacking Orotidine-5'-Phosphate Decarboxylase Activity in Yeast: 5-Fluoroorotic Acid Resistance", *Mol. Gen. Genet.* 197: 345–346 (1984)
4. SREENATH, H.K. AND JEFFRIES, T.W. "Diminished Respirative Growth and Enhanced Assimilative Sugar Uptake Result in Higher Specific Fermentation Rates by the Mutant *Pichia stipitis* FPL-061", *Appl. Biochem. Biotechnol.* 63/65:109-116 (1997).
5. SREENATH, H.K. AND JEFFRIES, T.W. "2-Deoxyglucose as a selective agent for derepressed mutants of *Pichia stipitis", Appl. Biochem. Biotechnol* (submitted).
6. LU, P., HENDRICK, J., DAVIS, B.P. AND JEFFRIES, T.W. "Disruption of the β-Isopropylmalate Dehydrogenase Gene (*LEU2*) of *Pichia stipitis* with *URA3* and Recovery of the Double Auxotroph", *Appl. Microbiol. Biotechnol.* 49(4):141-146 (1998)
7. CHO, J.-Y. AND JEFFRIES, T.W. "*Pichia stipitis* Genes for Alcohol Dehydrogenase With Fermentative and Respirative Functions", *Appl. Environ. Microbiol.* 64(4):1350-1358 (1998)
8. LU, P., DAVIS, B.P. AND JEFFRIES, T.W. "Cloning and Characterization of two Pyruvate Decarboxylase Genes from *Pichia stipitis*", *Appl. Environ. Microbiol.* 64:94-97 (1998)
9. CHO, J.-Y. AND JEFFRIES, T.W. " Transcriptional Control of *PsADH* genes in the Xylose Fermenting Yeast, *Pichia stipitis", Mol. Gen. Genet.* (submitted)
10. SHI, N.Q. AND JEFFRIES, T.W. "Anaerobic Growth and Improved Fermentation of *Pichia stipitis* Bearing a *URA1* Gene from *Saccharomyces cerevisiae*", *Appl. Microbiol. Biotechnol.* (in press)
11. DAHN, K.M., DAVIS, B.P., PITTMAN, P.E., KENEALY, W.R., AND JEFFRIES, T.W. "Increased xylose reductase activity in the xylose-fermenting yeast *Pichia stipitis* by overexpression of *XYL1", Appl. Biochem Biotechnol.* 57/58:267-276. (1996)
12. SHI, N.-Q., DAVIS, B.P., SHERMAN, F., AND JEFFRIES, T.W. "Disruption of The Cytochrome C Gene in Xylose-fermenting Yeast *Pichia stipitis"*, (submitted)
13. STEPHANOPOULOS, G. AND, SINSKEY A.J. "Metabolic Engineering–Methodologies and Future Prospects", *Trends Biotechnol* 11(9):392-396 (1993)
14. CAMERON, D.C., AND CHAPLEN, F.W. "Developments in Metabolic Engineering", *Curr. Opin. Biotechnol.* 8(2):175-180 (1997)
15. INGRAM, L.O. AND CONWAY, T. "Expression of Different Levels of Ethanologenic Enzymes from *Zymomonas mobilis* in Recombinant Strains of Escherichia coli", *Appl. Environ. Microbiol.* 54(2):397-404 (1988)
16. ZHANG, M., EDDY, C., DEANDA, K. FINKELSTEIN, M. AND PICATAGGIO, S. "Metabolic Engineering of a Pentose Metabolism Pathway in Ethanologenic *Zymomonas mobilis*", *Science* 267:240-243.
17. KOTTER, P., AND CIRIACY, M. Xylose fermentation by *Saccharomyces cerevisiae*", *Appl. Microbiol. Biotechnol.* 38:776-783 (1993)
18. TANTIRUNGKIJ, M., IZUISHI, T., SEKI, T., AND YOSHIDA, T. "Fed-batch fermentation of xylose by a fast-growing mutant of xylose assimilating recombinant *Saccharomyces cerevisiae*", *Appl. Microbiol. Biotechnol.* 41:8-12 (1994)
19. MEINANDER, N., ZACCHI, G., AND HAHN-HÄGERDAL, B. "A heterologous reductase affects the redox balance of recombinant *Saccharomyces cerevisiae*", *Microbiology* 142:165-172 (1996)
20. TOON, S.T., PHILIPPIDIS, G.P., HO, N.W.Y., CHEN, Z.D., BRAINARD, A., LUMPKIN, R.E., AND RIELY, C.Y. "Enhanced cofermentation of glucose and xylose by recombinant *Saccharomyces* yeast strains in batch and continuous operating modes. *Appl. Microbiol. Biotechnol"*, 63-65: 243-255 (1997)

Hyatt Regency Hotel
Vancouver, BC Canada
June 16 – 19, 1998

7th International Conference on

Biotechnology

in the

Pulp and Paper Industry

Poster Presentations

Vol. C

Institute for Microbial and Biochemical Technology FPL-4712

Problem 4 Bioconversion of Low-grade Lignocellulosic Materials into Fuels and Chemicals

FY1999 Research Attainments

Publications

Research Unit

Jeffries, T. W.; Shi, N. Q.; Cho, J. Y.; Lu, P.; Dahn, K.; Hendrick, J.; Sreenath, H. K.; Davis, B. P. 1998. Genetic engineering of Pichia stipitis for fermentation of xylose. In: Seventh international conference on biotechnology in the pulp and paper industry: Poster presentations vol. C; 1998 JUNE 16-19; Vancouver, BC, Canada Montreal, Quebec, Canada: Canadian Pulp and Paper Association: C215-C218.