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Research Article

Genetic analysis of D-xylose metabolism by endophytic yeast strains of *Rhodotorula graminis* and *Rhodotorula mucilaginosa*

Ping Xu, Renata Bura and Sharon L. Doty

School of Forest Resources, College of the Environment, University of Washington, Seattle, WA, USA.

Abstract

Two novel endophytic yeast strains, WP1 and PTD3, isolated from within the stems of poplar (*Populus*) trees, were genetically characterized with respect to their xylose metabolism genes. These two strains, belonging to the species *Rhodotorula graminis* and *R. mucilaginosa*, respectively, utilize both hexose and pentose sugars, including the common plant pentose sugar, D-xylose. The xylose reductase (XYL1) and xylitol dehydrogenase (XYL2) genes were cloned and characterized. The derived amino acid sequences of xylose reductase (XR) and xylose dehydrogenase (XDH) were 32%~41% homologous to those of *Pichia stipitis* and *Candida. spp.*, two species known to utilize xylose. The derived XR and XDH sequences of WP1 and PTD3 had higher homology (73% and 69% identity) with each other. WP1 and PTD3 were grown in single sugar and mixed sugar media to analyze the XYL1 and XYL2 gene regulation mechanisms. Our results revealed that for both strains, the gene expression is induced by D-xylose, and that in PTD3 the expression was not repressed by glucose in the presence of xylose.

Key words: Rhodotorula graminis, *Rhodotorula mucilaginosa*, D-xylose metabolism, xylitol, xylitol dehydrogenase, xylose reductase.

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Introduction

Lignocellulosic material containing cellulose, hemicellulose, and lignin is an abundant renewable organic resource that can be used for the production of energy and biochemicals (Weber *et al.*, 2010). The conversion of both the cellulose and hemicellulose fractions for producing biochemicals is being intensively studied. Between 23% to 40% of the lignocellulosic biomass consists of hemicellulose, the main component being xylose in most hardwoods and annual plants (Lee *et al.*, 1979). Whereas the fermentation of glucose can be carried out efficiently with the common brewer's yeast (*Saccharomyces cerevisiae*), the bioconversion of the pentose fraction (xylose and arabinose) presents a challenge since it is not metabolized by this species. Over the past decades, numerous studies have been carried out on various aspects of D-xylose bioconversion (Winkelhausen and Kuzmanova, 1998; Townsend and Howarth, 2010). D-xylose can be used by bacteria, yeasts and fungi through different pathways (Jeffries, 1983; Karhumaa *et al.*, 2007). In one of these, D-xylose can be directly converted to D-xylulose by xylose isomerase (Aristidou and Penttila 2000) without the participation of cofactors. In some yeasts and fungi, conversion of D-xylose to D-xylulose more often occurs by two enzymatic steps. First, D-xylose is reduced by a NADPH/NADH-linked xylose reductase (XR) to xylitol, whereupon the latter is oxidized to xylulose by an NAD-linked xylitol dehydrogenase (XDH) (Bruinenberg and van Dijken, 1983). D-xylulose is subsequently phosphorylated to D-xylulose-5-phosphate by D-xylulokinase before entering the pentose phosphate, Embden-Meyerhof, and phosphoketolase pathways (Skoog and Hahn-Hagerdal, 1988; Lin *et al.*, 2010).

The two major chemicals of interest that can be produced from D-xylose by yeasts are ethanol and xylitol. It is known that under normal growth conditions, certain pentose-fermenting yeasts (*e.g. Pichia stipitis*) produce mostly ethanol (Schneider 1989; Meyrial *et al.*, 1991; Frank and Guillermo, 2008); whereas others (*e.g. Candida guilliermondii* and *Candida tropicalis*) produce mainly xylitol as the end products (Gong *et al.*, 1981; Barbosa *et al.*, 1988; Ko *et al.*, 2006; Lima *et al.*, 2006). As an intermediate metabolite, xylitol is widely applied in the food and pharmaceutical industries due to its sweetness being equivalent to sucrose, the high negative heat of the solution (Borges, 1991; Passon, 1993), its anti-cariogenic and anti-infection effects (Sakai *et al.*, 1996; Pizzo *et al.*, 2000; Brown *et al.*, 2004), and the independent metabolism of insulin, which makes it useful for diabetic patients (Salminen *et al.*, 1989). Furthermore, the genus *Candida* is one of the most efficient xylitol producers (Mayer *et al.*, 2002).

The pink yeast strains WP1 (*Rhodotorula graminis*) and PTD3 (*Rhodotorula mucilaginosa*), identified in our laboratory (Xin *et al.*, 2009), are remarkable for their efficient performance in xylitol and ethanol production in the

Send correspondence to Sharon L. Doty. School of Forest Resources, College of the Environment, University of Washington, 98195-2100 Seattle, WA, USA. E-mail: sldoty@uw.edu.

presence of several common fermentation inhibitors (Vajzovic, A., manuscript in preparation). So far, investigation of xylitol production by yeasts has been limited to *Candida* and *Pichia* species, with few reports of studies of D-xylose metabolism in *Rhodotorula. spp*. Although XR and XDH activities were detected in *Rhodosporidium toruloides* (the teleomorph of *Rhodotorula glutinis*) (Freer *et al.*, 1997), none of the genes encoding XR and XDH were cloned from the *Rhodotorula* genus. This is the first report describing the cloning and characterization of the XR-encoding (XYL1) and XDH-encoding (XYL2) genes from both *Rhodotorula graminis* and *Rhodotorula mucilaginosa* yeast strains.

Materials and Methods

Yeast strains

The yeast strain WP1 isolated from within stems of wild cottonwood (*Populus trichocarpa*) was identified as *Rhodotorula graminis* whereas, another, PTD3, isolated from the stems of hybrid poplar plants *(Populus trichocarpa*X *P. deltoides*), was identified as *Rhodotorula mucilaginosa* (Xin *et al.*, 2009). A baker's yeast strain ATCC6037 was used as the control.

Growth requirements test

In order to study sugar utilization by the two endophytic yeast strains, WP1, PTD3, and the baker's yeast (BK), isolates were streaked from frozen glycerol stocks onto a yeast extract- peptone-dextrose (YPD) agar to obtain isolated colonies. A single colony was transferred to 10 mL of YPD broth and incubated overnight on a shaker at 30 °C. This overnight culture was harvested and washed twice with an MS medium (Caisson Labs MSP009). For growth curve assays, cells were grown in 25 mL of an MS medium containing either 3% glucose or 3% xylose at pH 5.8. Growth was monitored using a spectrophotometer measuring optical density at 600 nm (OD_{600}). Statistical analysis was with split plot ANOVA (Intercooled Stata 10.0, StataCorp LP, College Station, TX) in order to account for the multiple measures taken over time on each flask, as well as replicated flasks for each sample.

DNA and RNA isolation

WP1 and PTD3 genomic DNA was prepared according to a published protocol (Burke *et al.*, 2000) but with the following modifications: two extra phenol:chloroform/chloroform extractions as well as an isopropanol precipitation were carried out. For mRNA preparation, cells were grown in YPD, which was prepared as described (Kaiser *et al.* 1994), only that the sugars were autoclaved separately from the basal medium. YPX and YPGX were similar to YPD but dextrose was replaced with xylose or xylose plus glucose. mRNA isolation was according to the method described in (Laplaza *et al.*, 2006).

RT-PCR

Isolated RNA was quantified using a NanoDrop spectrophotometer (ND1000). Reverse transcription (RT) and subsequent PCR amplifications were performed using the OneStep RT-PCR Kit (QIAGEN). Entire WP1 XR and XDH-encoding genes were amplified by RT-PCR using two sets of primers (WP1-XR-F, WP1-XR-R and WP1-XDH-F, WP1-XDH-R), which were designed based on the XYL1 and XYL2 gene sequences in *Pichia stipitis* (GenBank accession numbers: CAA42072, AAD28251) (Jeffries *et al.*, 2007) as well as alignment results with WP1 whole genome sequences (sequencing by JGI available online) but with the following modifications: for cloning of the partial XR and XDH-encoding genes in PTD3 (genome sequences were not available), RT-PCR was with the degenerate primers PTD3-D-XR-F, PTD3-D-XR-R and PTD3-D-XDH-F, PTD3-D-XDH-R, which were designed based on multiple sequence alignment amongst PTD3, WP1 and other D-xylose-fermenting yeasts (CLUSTALW) (Thompson *et al.*, 1994). Following RT-PCR, samples underwent electrophoresis in a 1% agarose gel, using Sybersafe (Invitrogen) as a DNA intercalating and visualizing agent, at 100V for 1 h.

5' RACE and 3' RACE

Partial PTD3 XR and XDH-encoding genes were first amplified by RT-PCR and then sequenced, whereupon the complete nucleotide sequences were subsequently determined by 5' and 3' rapid amplification of cDNA ends (RACE) using a 5'/3' RACE kit (FirstChoice RLM-RACE Kit, Applied Biosystems). For 5'RACE, the gene-specific primers PTD3-XR-GSP1, PTD3-XR-GSP2, PTD3-XDH-GSP1 and PTD3-XDH-GSP2 were used. For 3' RACE, the gene-specific primer PTD3-XR-GSP3, PTD3-XR-GSP4, PTD3-XDH-GSP3 and PTD3-XDH-GSP4 were used. All sequences of primers used in this study are listed in Table 1.

18S rRNA semi-quantitative RT-PCR

In the gene repression studies, total RNA was isolated from cells grown in media containing 2%glucose, 2%xylose, 1%glucose+1%xylose or 2%glucose+2%xylose respectively. RT-PCR was applied to the same amounts of total RNAs from different media using primer sets (#XR-F, #XR-R and #XDH-F, #XDH-R) designed to work equally well for both WP1 and PTD3. To check whether the same amount of total RNA was used, 18S rRNA semi-quantitative RT-PCR was undertaken with WP1 and PTD3 under these different culture conditions using the primer set 515F and 1209R (downloaded from JGI for eukaryotic 18S rRNA gene amplification).

Molecular cloning and sequencing

The resulting PCR products, first purified using the QIAEXII gel extraction kit (Qiagen, Madison, Wisconsin), were then inserted into the pGEM-T Easy vector (Promega,

Table 1 - Primers used in the experiments.

Primer	Sequence
WP1-XR-F	ATGGTCCAGACTGTCCCC
WP1-XR-R	TCAGTGACGGTCGATAGAGATC
WP1-XDH-F	ATGAGCGCTCCCAGTCTCGC
WP1-XDH-R	TCACTCGAGCTTCTCGTCGAC
PTD3-D-XR-F [a]	GCYATCAAGKCGGGYTACCG
PTD3-D-XR-R [a]	GTGGWAGBTGTTCCASAGCTT
PTD3-D-XDH-F [a]	CCMATGGTCYTSGGNCACGA
PTD3-D-XDH-R [a]	CCGACVGGVCCDGCDCCAAAGAC
PTD3-XR-GSP1 (for 5' RACE)	GCCAGTGGATGAGGTAGAGG
PTD3-XR-GSP2(for 5' RACE)	GTGATGAAGATGTCCTTGCG
PTD3-XR-GSP3(for 3' RACE)	AGGTCTACGGCAACCAGAAG
PTD3-XR-GSP4(for 3' RACE)	ATCACCTCGAAGCTCTGGAAC
PTD3-XDH-GSP1 (for 5' RACE)	GATGAGCGATTTGAGGTTGAC
PTD3-XDH-GSP2 (for 5' RACE)	CCTTGGCAACTGCGTGGAC
PTD3-XDH-GSP3(for 3' RACE)	GCAAAGGTGGTCATTACGAAC
PTD3-XDH-GSP4(for 3' RACE)	CTCCTTGAGCCCATGTCGGT
#XR-F	ATCACCTCGAAGCTCTGGAAC
#XR-R	GCCAGTGGATGAGGTAGAGG
#XDH-F	CTCCTTGAGCCCATGTCGGT
#XDH-R	GATGAGCGATTTGAGGTTGAC
515F(18S rRNA)	GTGCCAAGGCAGCCGCGGTAA
1209R(18S rRNA)	GGGCATCACAGACCTG

a) K:G/T V:A/C/g M:A/C N:A/C/G/T R:A/g B:C/G/T S:C/g W:A/T Y:C/T D:A/T/g.

Madison, Wisconsin), according to the manufacturer's instructions. Bidirectional sequencing of the inserts was carried out by the UW Biochemistry Department Sequencing Facility using the BigDye Terminator v3.1 Cycle sequencing kit (Applied Biosystems).

Analysis of gene sequences

The genome sequence of WP1 was provided through DOE Joint Genome Institute sequencing efforts. Putative XYL1 and XYL2 genes were first found in the JGI sequence by using BLAST. The resultant sequences were employed in the design of primers for cloning the mRNA sequences of the two genes from WP1. Sequence comparison of the cloned genes with public databases was through Internet at the National Center for Biotechnology Information site, by applying the tblast algorithm (Altschul *et al.*, 1997). GenomeScan was used to predict gene exon/intron structures and putative XR and XDH mRNA sequences in WP1. All the resulting sequences in WP1 and PTD3 were aligned with homologous protein sequences of other D-xylose-fermenting yeasts (*e.g. Pichia stipitis*, *Candida. spp*) by means of the local BLAST program (Bl2seq).

The GenBank accession numbers for the four newly-determined nucleotide sequences in this study are: HM038238 (WP1 XYL1 gene), HM038239 (WP1 XYL2 gene), HM038240 (PTD3 XYL1 gene) and HM038241 (PTD3 XYL2 gene) (data was released on April 10, 2010).

Results

Growth of WP1 and PTD3 on different sugars

In order to study the sugar utilization of the two endophytic yeast strains, WP1 and PTD3, growth rate was monitored in media with different sugars. Growth curve experiments showed that both WP1 and PTD3 grew well in glucose (Figure 1a) and xylose (Figure 1b) sugars. As reported previously, Baker's yeast did not utilize xylose. It is also noteworthy that PTD3 grew better than WP1 under the two conditions and PTD3 was a better xylose utilizer (Figure 1b). There was an approximate 24-hour-delay before WP1 and PTD3 started growing in glucose and xylose. The delay was most likely through the shift from a rich medium (YPD) (Ito *et al.*, 1983) to a minimal one (Murashige and Skoog medium) (Murashige and Skoog, 1962).

Sequences of WP1 XR and XDH-encoding genes

XR and XDH-encoding genes were cloned and sequenced from WP1 by using primers based on the genomic sequence of WP1 provided by the DOE JGI sequencing project. Subsequent analysis of the two genes was with the

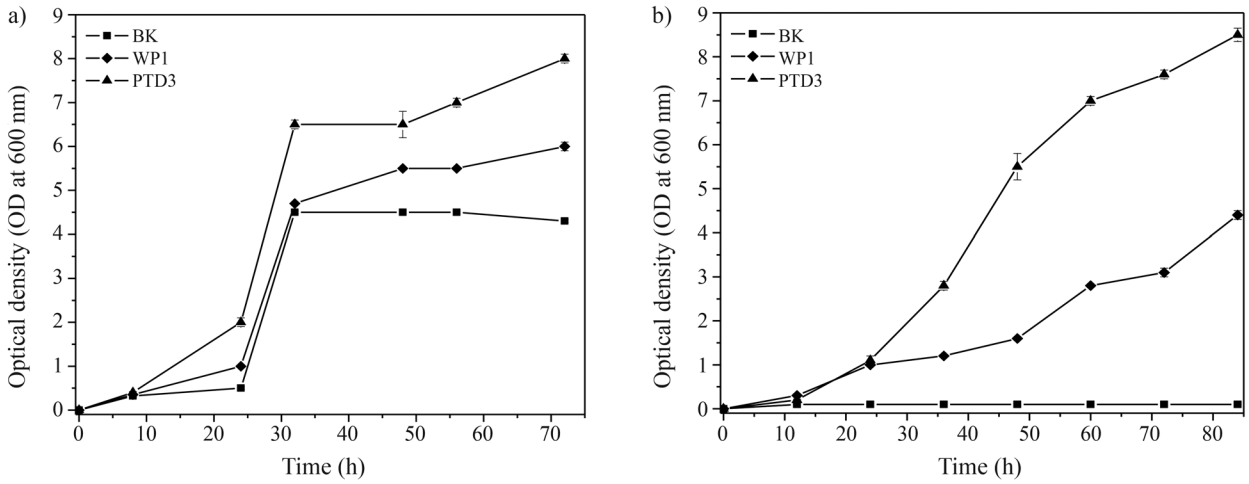

Figure 1 - Growth of WP1 and PTD3 (a) in an MS medium, with 3% glucose as the carbon source, (b) in an MS medium with 3% xylose as the carbon source. Baker's yeast (BK; ATCC6037) was used as positive control. The experiments were carried out in triplicate, the error bars indicating standard deviation.

cloned sequences (not directly from the JGI sequences provided). The 1259 bp nucleotide sequence of WP1-XR contains an open reading frame of 987 bp nucleotides encoding a polypeptide of 322 amino acids, while the 1216 bp nucleotide sequence of WP1-XDH contains an open reading frame of 1191 bp nucleotides encoding a polypeptide of 396 amino acids. At the amino acid level, the WP1-XR gene is 37% and 36% identical to the XYL1 genes of *Pichia stipitis* and *Candida guilliermondii*, respectively; whereas the WP1-XDH gene is slightly more conserved with 41% identity to the XYL2 genes of both *Pichia stipitis* and *Candida tropicalis*. On using Vector NTI 10, the visualized annotation pictures showed that both XR and XDH genes are more complex than those of *Pichia stipitis* which lacks introns (Figures 2 and 3) (Amore *et al.*, 1991).

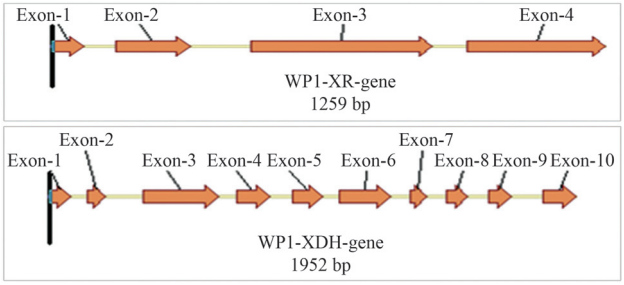

Figure 2 - Exon/intron structures visualization of XR and XDH-encoding-genes of the *Rhodotorula graminis* WP1 strain.

WP1 XR and XDH gene expression levels in glucose and xylose

To investigate XR and XDH gene expression in WP1, cells were grown in media containing either glucose or xylose. RNA was subsequently purified from the cultures. mRNA segments were amplified using RT-PCR with primers specific for each of the two genes. As shown in Figure 4 (left), the XR and XDH genes were expressed in WP1 cells grown in xylose, indicating not only that they were certainly transcribed but also that XR and XDH gene expression was upregulated by xylose. The XR gene expression was not detectable when the cells were grown in glucose; however, there was some low-level constitutive expression of the XDH gene in glucose.

Sequences of PTD3 XR and XDH-encoding genes

Since strain PTD3 was a more effective utilizer of xylose, compared to WP1, xylose metabolism genes from the former were cloned. However, as the PTD3 genome has not yet been sequenced, a different approach was used for cloning the two genes than that for WP1. The partial PTD3 XR and XDH-encoding genes were cloned using degenerate primer sets that were designed based on multiple sequence alignment amongst PTD3, WP1 and other D-xylose-fermenting yeasts (Table 1). The complete nucleotide sequences were subsequently determined by 5' and 3' rapid amplification of cDNA ends (RACE) by using gene specific primers based on cDNA fragment sequences. The 1087 bp nucleotide sequence of cloned PTD3-XR contained an open reading frame of 975 bp nucleotides encoding a polypeptide of 324 amino acids. Alignment revealed that the PTD3-XR protein is 67% identical to the WP1 XR. The 1409 bp nucleotide sequence of PTD3-XDH contains an open reading frame of 1185 bp nucleotides encoding a polypeptide of 394 amino acids. Alignment showed that the PTD3- XDH protein is 69% identical to the WP1 XDH. Alignments with other yeasts were also carried out, in order to study homology with the two genes in PTD3 (Table 2). The XR and XDH proteins of WP1 and PTD3 were 69%-73% identical, whereas they were only 37%-41% identical to these same proteins from other known xylose-utilizing species.

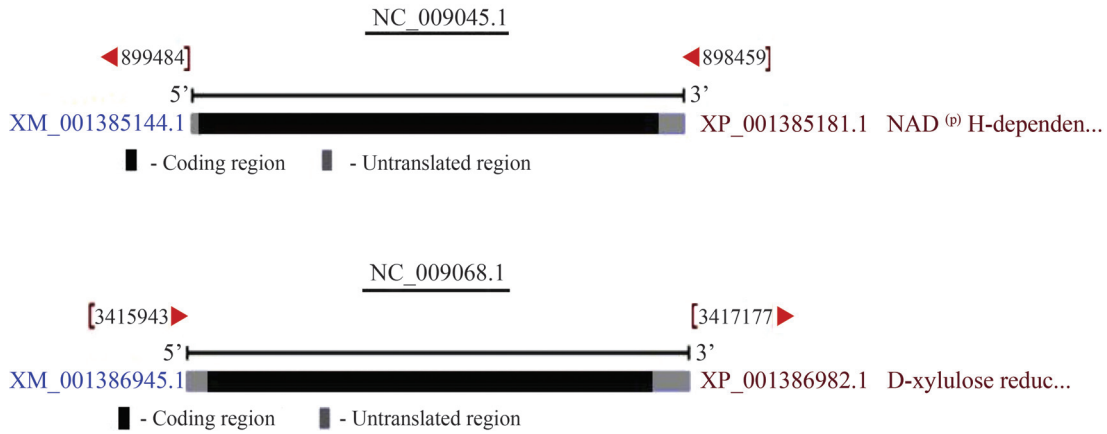

Figure 3 - Exon/intron structures visualization of XR and XDH-encoding-genes of *Pichia stipitis*.

Table 2 - XR and XDH identities between homologous proteins in several yeast strains[a].

Identity	WP1	*Pichia stipitis*	*Candida guilliermondii*	*Candida tropilis*
PTD3 XR	73%	38%	37%	39%
PTD3 XDH	69%	37%	Null	41%

[a]GenBank Accession No.: XR: CAA42072 (*P. stipitis*); ABX60132 (*C. tropicalis*); AAD09330 (*C. guilliermondii*). XDH: AAD28251 (*P. stipitis*); ABB01368 (*C. tropicalis*).The XDH protein sequence of *Candida guilliermondii* was unavailable.

PTD3 gene expression levels in glucose and xylose

To investigate the expression of the two genes in PTD3, cells were grown in glucose and xylose media as with the WP1 study. PTD3 gene specific primers were used to amplify the segments from mRNA by using RT-PCR. We observed different bands corresponding to XR and XDH in mRNA from cells grown on either glucose or xylose (Figure 4, right). These results indicate that the genes are indeed transcribed within mRNA, and that XR and XDH gene expression was induced by xylose. As in WP1, the genes were barely expressed in medium containing only glucose as the carbon source.

Comparison of XR and XDH gene expression levels between WP1 and PTD3

The expression levels of the XR and XDH genes were compared in order to better understand the differences in utilization of xylose between the two endophytic yeast strains. By means of aligned WP1 and PTD3 sequences, primers were designed to the gene regions of sequence identity so that the expression of XR and XDH-encoding genes as determined by RT-PCR could be directly comparable. RT-PCR was applied for amplifying mRNA segments from WP1 and PTD3 cells grown in a YP medium containing different sugars (2% glucose, 2% xylose, 1% glucose + 1% xylose, 2% glucose + 2% xylose). As shown

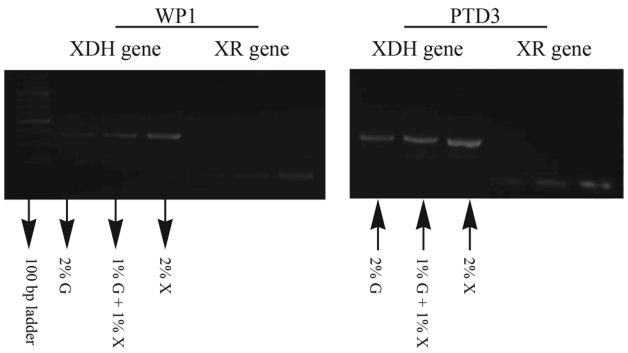

Figure 4 - WP1 and PTD3 XR/XDH gene-expression in cells grown in 2% glucose (lanes 1,4,7,10), 1% glucose+1% xylose (lane 2, 5, 8,11) and 2% xylose (lanes 3,6,9,12). Lanes 1-6: WP1gene expression; Lanes 7-12: PTD3 gene expression.

in Figure 4, when the yeasts were grown in a xylose medium, both the XDH and XR genes were expressed at higher levels in PTD3 than in WP1. Expression of the two appeared slightly suppressed in 1% xylose + 1% glucose medium compared to 2% xylose medium. In order to investigate whether the expression differences resulted from the lower xylose concentration in the mixed sugar medium or from repression by glucose, an RT-PCR experiment was also conducted under 2% glucose + 2% xylose culture conditions. As shown (Figure 5), in WP1, gene expression of the two was still slightly suppressed in 2% xylose+2% glucose medium compared to 2% xylose medium. Whereas in PTD3, there was no suppression by glucose. In the latter strain, the level of XR and XDH gene expression was about equal in both the mixed sugar medium and the xylose medium. 18S rRNA RT-PCR functioned as an internal control to show that equal amounts of total RNA were used under the various culture conditions (data not shown).

Discussion

In this study, XR and XDH encoding genes were cloned from *Rhodotorula graminis* WP1 strain, expression

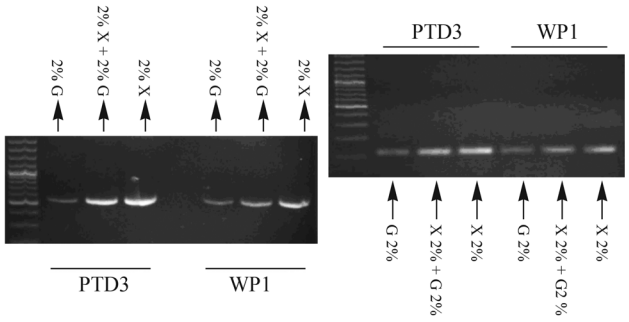

Figure 5 - WP1 and PTD3 XR/XDH gene-expression in cells grown in 2% glucose, 2% xylose and 2% glucose+2% xylose. RNA templates used under different conditions are labeled in the figure.

of the two genes being verified by RT-PCR. It was shown that D-xylose is an efficient inducer of XR and XDH in both strains, thus similar to the trend found with *Candida guilliermondii* (Sugai and Delgenes, 1995) and *Pichia stipitis* (Bichio *et al.*, 1988). Furthermore, the novel characteristic of no inhibition by glucose for these genes was reported.

The XI (xylose isomerase)-encoding gene was not encountered in the WP1 genome sequence provided by JGI. Thus it can be concluded that WP1 utilizes the two-step redox pathway in D-xylose metabolism as occurs with other yeasts (Gong *et al.*, 1981). However, alignments showed that homology between the XR and XDH sequences and other XRs and XDHs from *Candida spp*. and *Pichia stipitis* yeasts was low (32%-41%). Furthermore, the WP1 XR and XDH-encoding genes have multiple introns and the exon/ intron structures are more complicated and advanced than in the homologous genes in *Pichia stipitis* and *Candida spp* (Handumrongkul *et al.*, 1998; Lin *et al.*, 2010)**.** These differences, besides possibly introducing greater variability of protein sequences translated from a single gene, might also enhance XR and XDH gene expression (Mayer *et al.*, 2002; Smith and Lee, 2008). From a macro perspective, the gene differences imply the possibility of long evolution distances between WP1 and *Pichia stipitis* and *Candida. spp* which could lead to further mutual differences in the xylose metabolism pathway.

Research on WP1 XR and XDH-encoding-gene expression levels in xylose and glucose media showed that the expression was induced by xylose. The two genes in WP1 were expressed to low levels while grown in glucose medium. Additionally, the expression level of the XDH-encoding gene (XYL1) was higher than that of the XR-encoding gene (XYL2).

Similarly, full-length XR and XDH encoding genes were cloned from the *Rhodotorula mucilaginosa* PTD3 strain, respective expression being verified by RT-PCR. By alignment it was shown that homology between XR and XDH sequences and those of other XRs and XDHs from *Candida spp.* and *Pichia stipitis* yeasts was also low (37% ~ 41% identities). Since the genome sequence of PTD3 was unavailable, it was impossible to investigate their respective exon/ intron structures. However, based on high homology (73% and 69% identity for XR, XDH) with WP1, PTD3 gene structures may be somewhat more like that of WP1.

As with WP1, the expression of PTD3 XR and XDH-encoding genes was also induced by xylose. The two genes in PTD3 were expressed to low levels while grown in glucose medium. As in WP1, the expression level of the XDH-encoding gene (XYL1) in PTD3 was higher than that of the XR-encoding gene (XYL2).

Since PTD3 grew better in D-xylose medium compared to WP1, a possible explanation could be the higher production of those enzymes involved in xylose metabolism by the former (Yablochkova *et al.*, 2004). Hereby it was noted that both XR and XDH-encoding gene expression levels were much higher in PTD3 than in WP1, thus supporting this hypothesis. Studies of both resultant protein levels and xylose uptake mechanisms in these yeast strains are required.

Single and mixed sugars were investigated, in order to analyze their potential for inducing XR and XDH-encoding gene expression in both WP1 and PTD3. As to pentose and hexose co-fermentation, the yeast strains under study are not unique, for certain other strains as *Candida guilliermondii* were found capable of fermenting glucose and xylose simultaneously (Lee *et al.*, 1996). Nevertheless, for many yeasts as *Saccharomyces cerevisiae*, *Pichia stipitis* and *Candida. spp*, D-glucose is the preferred substrate for growth and fermentation, when both D-glucose and D-xylose are present in the medium (Bichio *et al.*, 1988; Townsend and Howarth, 2010). Although the xylose assimilation genes (XYL1, XYL2) were not expressed in *Pichia stipitis* in the presence of glucose in the medium (Bichio *et al.*, 1988), herein it was shown that in the case of both WP1 and PTD3, and under the same circumstances, the two continued to be so in response to xylose. Furthermore, the band quantities of RT-PCR in single sugar (xylose) and mixed sugar (glucose+xylose) revealed that both genes were not suppressed by glucose in PTD3, whereas they were slightly so in WP1. This is significant since xylose reductase and xylitol dehydrogenase are pivotal for growth and xylitol formation during xylose metabolism (Jeffries, 2006). Furthermore, the high-level expression of both genes in the mixed sugars of xylose and glucose will not only greatly increase xylitol yield in mixed sugars from real hydrolytes, but also contribute to optimizing fermentation conditions of lignocellulosic biomass. In addition, a better understanding of gene-regulation mechanisms, as well as identification of XR and XDH-encoding-genes and xylose uptake genes, will help in defining strategies for the genetic engineering of industrial strains, such as *S. cerevisiae*, for further improvement in productivity.

Acknowledgments

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