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The pentose catabolic pathway of the rice-blast fungus *Magnaporthe oryzae* involves a novel pentose reductase restricted to few fungal species

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ABSTRACT

A gene (*MoPRD1*), related to xylose reductases, was identified in *Magnaporthe oryzae*. Recombinant MoPRD1 displays its highest specific reductase activity toward L-arabinose and D-xylose. K_m and V_{max} values using L-arabinose and D-xylose are similar. *MoPRD1* was highly overexpressed 2–8 h after transfer of mycelium to D-xylose or L-arabinose, compared to D-glucose. Therefore, we conclude that MoPDR1 is a novel pentose reductase, which combines the activities and expression patterns of fungal L-arabinose and D-xylose reductases. Phylogenetic analysis shows that PRD1 defines a novel family of pentose reductases related to fungal D-xylose reductases, but distinct from fungal L-arabinose reductases. The presence of PRD1, L-arabinose and D-xylose reductases encoding genes in a given species is variable and likely related to their life style.

1. Introduction

Magnaporthe oryzae is a pathogen of a wide range of cereals [1] causing annual rice crop-loss of 10–30% worldwide. After its penetration into host plant leaves using an appressorium, *M. oryzae* invades rice leaf cells without causing major damage (biotrophic phase) during 4–5 days. After 6 days, the fungus switches to a necrotrophic phase and induces chlorotic and necrotic sporulating lesions associated. Rice cell walls are rich in arabinoxylan [2] that can be degraded by xylanases secreted by *M. oryzae* [3,4]. The resulting monosaccharides (L-arabinose and D-xylose) are likely taken up and metabolized by the fungus through the pentose catabolic pathway (Fig. 1). This pathway is still uncharacterized in *M. oryzae*, but has been extensively studied in saprobic filamentous fungi such as *Aspergillus niger* [5–10] and *Trichoderma reesei* [11–13]. In the pentose catabolic pathway, L-arabinose is converted to xylitol by the consecutive action of L-arabinose reductase, L-arabitol dehydrogenase and L-xylulose reductase (Fig. 1). D-Xylose is converted to xylitol in a single step by D-xylose reductase. Xylitol is then converted to D-xylulose-5-phosphate by xylitol dehydrogenase and D-xylulose kinase, which enters into the pentose phosphate pathway to be further metabolized. In this paper we present the initial characterization of a novel pentose reductase (PRD1) from *M. oryzae* that displays both L-arabinose and D-xylose reductase activities, and is only found in a small subset of fungal species. The absence of the recently described *A. niger* L-arabinose reductase [8] in other fungi, suggests a higher level of diversity for the first steps of the pentose catabolic pathway then for the later steps. PRD1 is here presented as an alternative for initial reduction of the pentoses.

2. Materials and methods

2.1. Strains, libraries and growth conditions

P1.2 (*MAT*1.2) and Guy11Δku80 are *M. oryzae* strains pathogenic on rice from CIRAD, Montpellier, France. The *M. oryzae* cDNA library was constructed with a lambda Zap Express kit using total

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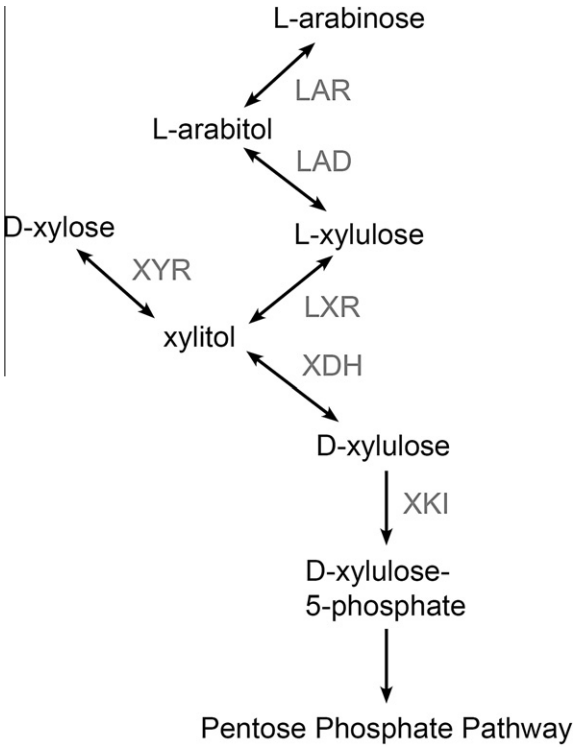

Fig. 1. Pentose catabolism in filamentous fungi LAR = L-arabinose reductase, LAD = L-arabitol dehydrogenase (EC 1.1.1.12), LXR = L-xylulose reductase (EC 1.1.1.10), XYR = D-xylose reductase (EC 1.1.1.307), XDH = xylitol dehydrogenase (EC 1.1.1.9), XKI = D-xylulose kinase (EC 2.7.1.17). LAR, LXR and XYR are NADPH/NADP+-dependent. LAD and XDH are NADH/NAD+ dependent.

RNA from mycelium grown on complete medium [14]. cDNA-NW12C16 corresponds to *PRD1*. *Escherichia coli* M15[pREP4] (Qiagen) was used for production of PRD1. Subcloning was performed using pBluescript SK^+ [15], pGEM-Teasy (Promega) and pQE32 (Qiagen). *M. oryzae* was pre-grown in TNK medium (2 g/l $NaNO_3$, 2 g/l KH_2PO_4, 0.5 g/l $MgSO_4{\cdot}7H_2O$, 0.1 g/l $CaCl_2{\cdot}2H_2O$, 0.004 g/l $FeSO_4{\cdot}7H_2O$, 7.9 mg/l $ZnSO_4{\cdot}7H_2O$, 0.6 mg/l $CuSO_4{\cdot}5H_2O$, 0.1 mg/l H_3BO_3, 0.2 mg/l $MnSO_4{\cdot}H_2O$ and 0.14 mg/l $NaMoO_4{\cdot}2\ H_2O$, pH 5.5–5.8) with 2 g/l yeast extract and 1% D-fructose in 200 ml in a 1 L erlenmeyer flask. For flask inoculation, sporulating mycelium was harvested from 10 to 12 days old TNK-YE + 1% D-glucose agar plates by scraping off mycelial fragments and conidia in H_2O. Pre-cultures were grown at 120 rpm in a rotary shaker at 25 °C for 65 h and harvested over a piece of cheese-cloth, washed with TNK medium and 1.5 g mycelium (wet weight) was transferred to a 250 ml Erlenmeyer containing 50 ml TNK with 1 mg/l thiamine, 5 μg/l biotine [16] and either 25 mM D-glucose, L-arabinose or D-xylose. After 2 and 8 h of growth the mycelium was harvested with suction over a piece of cheese-cloth, dried between tissue paper and directly frozen in liquid nitrogen.

2.2. Molecular biology methods

Standard methods were used for DNA manipulations, such as subcloning, DNA digestions, and plasmid DNA isolations [17]. Chromosomal DNA was isolated as previously described [18]. Sequence analysis was performed using the Big Dye Terminator kit, Version 1.1 (Applied Biosystems, Foster City, CA) according to the supplier's instructions. The reactions were analysed with an ABI 310 (Applied Biosystems) or on an ABI 377 (Applied Biosystems) in which case Longranger Single Packs (Cambrex Bio Science, Rockland, Inc., Rockland, ME) were used. cDNA sequences for *PRD1* and *XYR1* were obtained from an EST library (see above) and are deposited at EMBL with accession numbers AJ890448 and AJ890447, respectively.

2.3. Sequence analysis

M. oryzae protein sequences were retrieved from *Magnaporthe* database at http://www.broadinstitute.org/annotation/genome/magnaporthe_grisea/MultiHome.html (v8). The amino acid sequences of XYR1 (*M. oryzae* MGG_03648), PRD1 (*M. oryzae* MGG_01404, check v8) and LarA (*A. niger* JGI 47818) were used as queries for BlastP analyses using a cut-off expected value of 1E-3 against fungal proteins from different databases such as NCBI, USA (http://www.ncbi.nlm.nih.gov/sutils/genom_table.cgi?organism=fungi) for *Botryotinia fuckeliana* T4, *Neurospora crassa* OR74A, *Podospora anserina* S mat+, *Penicillium chrysogenum* Wisconsin 54-1255, *Penicillium marneffei* ATCC 18224, *Verticillium albo-atrum* VaMs.102 and *Verticillium dahliae* VdLs.17, the Broad Institute, USA (http://www.broadinstitute.org/scientific-community/science/projects/fungal-genome-initiative/fungal-genome-initiative) for *Aspergillus* species, *Chaetomium globosum* CBS 148.51, *Fusarium* species, *Magnaporthe grisea* (*oryzae*) 70-15 and *Phaeosphaeria nodorum* SN15/*Stagonospora nodorum*, and the Joint Genome Institute of the Department of Energy, USA (http://genome.jgi.doe.gov/programs/fungi/index.jsf) for *Trichoderma reesei*, *Mycosphaerella graminicola* (anamorph Septoria tritici), *Nectria haematococca* Mating Population VI (MPVI)/*Fusarium solani*, *Trichoderma atroviride* IMI 206040 and *Trichoderma virens* Gv29-8/*Hypocrea virens*.

Candidate orthologs were verified by bi-directional BLAST P. Protein sequences were aligned using MAFFT (http://mafft.cbrc.jp/alignment/server/). Alignments were manually edited and trees were reconstructed using MEGA5. The evolutionary history was inferred by using the Maximum Likelihood method based on the Whelan and Goldman model [19]. The bootstrap consensus tree was inferred from 500 replicates [20]. A discrete Gamma distribution was used to model evolutionary rate differences among sites (5 categories, +*G*, parameter = 1.9082). The rate variation model allowed for some sites to be evolutionarily invariable ([+*I*], 7.0609% sites). The tree is drawn to scale, with branch lengths measured in the number of substitutions per site. The analysis involved 77 amino acid sequences. All positions with less than 80% site coverage were eliminated. There were a total of 301 positions in the final dataset. Evolutionary analyses were conducted in MEGA5 [21].

2.4. Quantitative real-time RT-PCR

Total RNA was extracted from mycelium ground in a microdismembrator (B Braun) using TRIzol reagent (Invitrogen) according to the instructions of the manufacturer. cDNA was prepared from total RNA (2.5 μg) using Thermoscript RT (Invitrogen) according to the instructions of the manufacturer. qPCR analysis was performed by using the ABI 7500 fast real-time PCR system (Applied Biosystems, Foster City, USA) and the ABI Fast SYBR Master Mix (Applied Biosystems, Foster City, USA) according to the instructions of the manufacturer. Primer sequences and optimal concentrations are listed in Table 1. The amplification reaction was as follow: 95 °C 20 s, 95 °C 3 s and 60 °C 30 s (40 cycles). A dissociation curve was generated to verify that a single product was amplified. Each cDNA was assayed in triplicate in a final volume of 20 μl containing 2 μl of cDNA (diluted 100-fold). Gene expression is relative to ILV5 (MGG_15774.7) expression [22] according to the formula $2^{-(Ct\ gene\ X\ -\ Ct\ ILV5)}$ [23]. Two biological replicates were analysed.

2.5. Production of recombinant PRD1

Based on the cDNA sequence of *PRD1*, oligonucleotides were designed for the cloning of the corresponding open reading frame in

Table 1
Primers pairs used for real-time RT-PCR assays.

Gene name	Function	Primer pair (5′–3′)	Optimized primer concentration (nM)
PRD1	Pentose	CACCCAGAGGGACATTGTTGT	900
MGG_01404.7	Reductase	TGACACAGTCGAGGTTCTGCTT	50
XYR1	D-Xylose	GCATCGTCGTCACTGCATACTC	900
MGG_03648.7	Reductase	GGCGTGCTCCATGTTGAACT	300
ILV5	Ketol-acid	CCAGCTCTACGACTCGGTCAA	50
MGG_15774.7	Reductoisomerase	AGTCGGGCTGGCTGTTGTAGT	50

The concentrations used for amplification were chosen after optimization tests.

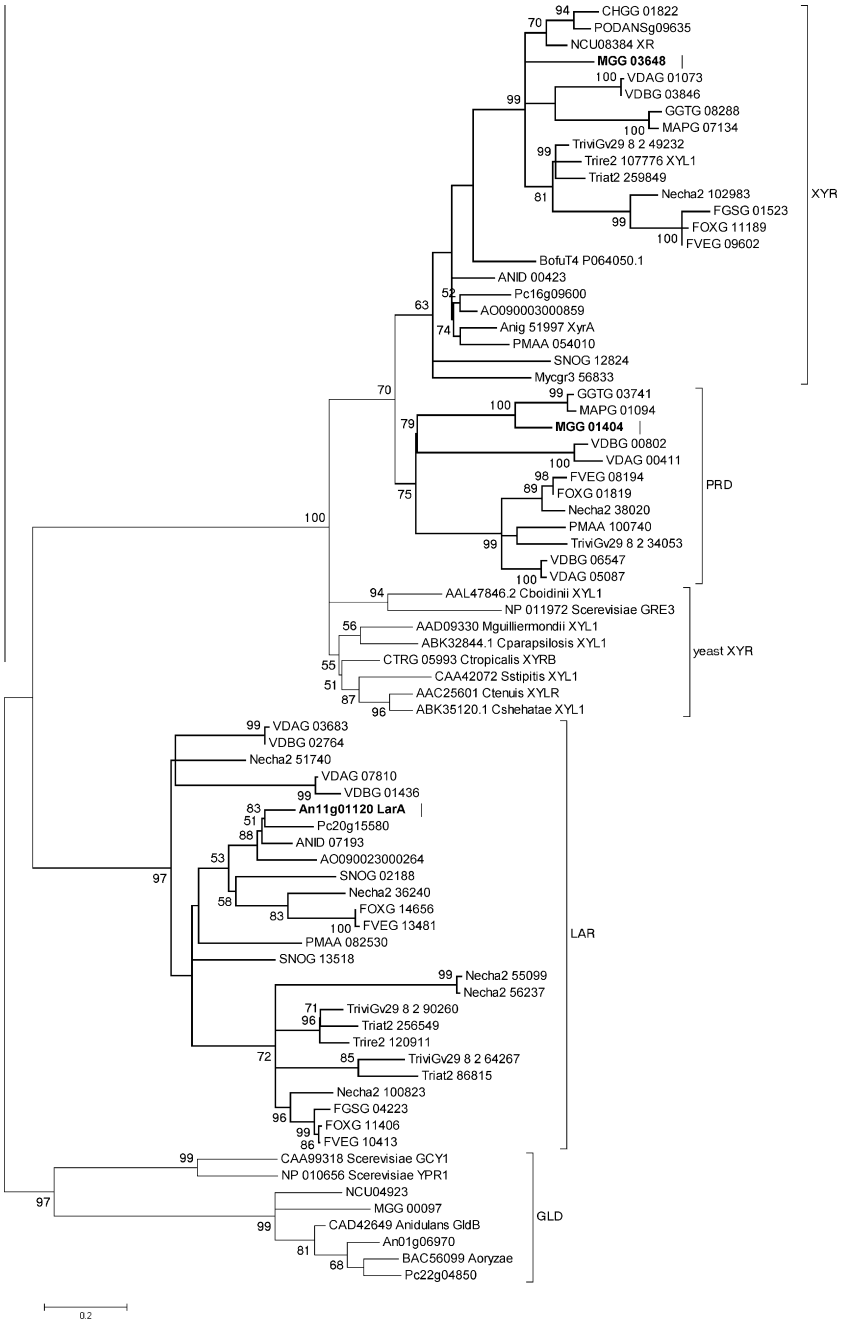

Fig. 2. Phylogenetic tree of fungal pentose reductases. A Maximum Likelihood tree (500 bootstraps) was constructed as described in Section 1 to demonstrate the two groups within the XYR/PRD clade that separate clearly from the LAR clade. GLD = Glycerol dehydrogenase, ANID = *Aspergillus nidulans*, Anig = *Aspergillus niger*, AO = *Aspergillus oryzae*, Bofu = *Botryotinia fuckeliana*, CHGG = *Chaetomium globosum*, FGSG = *Fusarium graminearum*, FOXG = *Fusarium oxysporum* f. sp. *lycopersici*, FVEG = *Fusarium verticillioides*, GGTG = *Gaeumannomyces graminis*, MGG = *Magnaporthe oryzae*, MAPG = *Magnaporthe poae*, Mycgr = *Mycosphaerella graminicola*, Necha = *Nectria haematococca*, NCU = *Neurospora crassa*, Pc = *Penicillium chrysogenum*, PMAA = *Penicillium marneffei*, PODANS = *Podospora anserina*, Triat = *Trichoderma atroviride*, Trivi = *Trichoderma virens*, Trire = *Trichoderma reesei*, SNOG = *Phaeosphaeria nodorum*, VDBG = *Verticillium albo-atrum*, VDAG = *Verticillium dahlia*.

E. coli expression vector pQE32 (Qiagen) using *PRD1* cDNA as template. One oligonucleotide was designed just after the ATG starting codon of these genes and contained a *Bam*HI site (5′-CAACAACAGCAGTCAGGATCCCTAAGACATCTATC-3′). The other oligonucleotide was designed after the STOP codon and contained a *Hind*III (5′-GAGACCAGGACATCCCAAGCTTCGGTTTCCTCGC-3′). The PCR fragment was cloned in pGEM-T easy (Promega) and confirmed by sequence analysis. A fragment was isolated from this constructs using the restriction enzymes mentioned above and cloned into pQE32. The resulting construct was transformed to *E. coli* M13 cells as indicated by the supplier (Qiagen). Purification of the recombinant protein was performed according to the supplier's recommendations (Qiagen).

2.6. Enzyme assays

All enzyme assays were performed at 2 °C. Dehydrogenase activities were determined in a 100 mM glycine buffer at pH 9.6, with 0.4 mM NAD^+ and 100 mM of substrate. Reductase activities were determined in a 50 mM sodium phosphate pH 7.6 buffer, with 0.2 mM NADPH and 100 mM substrate. Changes in absorbance at 340 nm ($\varepsilon = 6.22\ mM^{-1}\ cm^{-1}$) were recorded on a Unicam UV-1 spectrophotometer (Spectronic Unicam, Rochester, NY). Kinetic values were determined using a concentration range of 5–200 mM for L-arabinose and D-xylose. K_m and Vmax values were calculated using the solver tool of Microsoft Excel.

3. Results

3.1. Identification and phylogenetic analysis of M. oryzae PRD1

The *M. oryzae* protein database [24] was searched using BlastP and *A. niger* XyrA [6] as a query, for possible D-xylose reductase encoding genes. Two hits were identified, one (*MGG_03648*, EHA49921) with 66% amino acid sequence identity to *A. niger* XyrA, and the other (*MGG_01404*, EHA54542) with 53% identity. A comparison of cDNA clones and ESTs from public *M. oryzae* databases to its genome sequence revealed the presence of 1 intron in *MGG_03648* and 3 introns in *MGG_01404*, that confirmed the gene models and the deduced protein sequences.

Amino acid sequences of D-xylose reductases from 23 fungal genomes as well as characterized D-xylose reductases from Saccharomycotina, and the recently identified *A. niger* L-arabinose reductase (LarA) [8], were included in the analysis. Glycerol dehydrogenase and related sequences were added for phylogenetic rooting. A representative tree of these protein sequences (Fig. 2) revealed two distinct clusters, one gathering all the known fungal D-xylose reductases and one containing all the known fungal L-arabinose reductases including *A. niger* LarA (LAR clade, Fig. 2). No LarA ortholog was found in *M. oryzae* genome, although numerous genes related to *larA* were found in the genomes of other Ascomycota, frequently as paralogs. Within the D-xylose reductase cluster, the Saccharomycotina proteins were grouped in a basal clade (yeast XYR clade, Fig. 2) rooting the two Pezizomycotina D-xylose reductase clades. One of these two clades, gathers all the biochemically characterized D-xylose reductases from Pezizomycotina, such as *A. niger* XyrA [6,8], *Neurospora crassa* XR [25] and *Hypocrea jecorina* XYL1 [26], and their orthologs (clade XYR, Fig. 2). This clade includes *M. oryzae* MGG_03648 that was therefore denominated as XYR1. The other clade contains *M. oryzae* MGG_01404 that was denominated PRD1 (for Pentose ReDuctase1) and related proteins from a limited number of species (clade PRD, Fig. 2; Suppl. Table 1). These PRD encoding genes were identified in the genomes of both Sordariomycetes species highly related to *M. oryzae* (*Magnaporthe poae* and *Gaeumannomyces graminis*), as well as in most (5 out of 11 species, 45%) of the other Sordariomycetes species analyzed (*Verticillium, Fusarium, Nectria, Trichoderma*), but barely in other Pezizomycotina species (1 out of 8 species, 12%). This discontinuous distribution of PDR encoding genes among fungal genomes differs strongly from the distribution of XYR encoding genes that were detected as a single copy gene in all the genomes analyzed.

The aligned amino acid sequences of *M. oryzae* PRD1 and XYR1, *A. niger* LarA and XyrA as well as *Candida tenuis* XYLR were aligned to evaluate the conservation of active site residues (Fig. 3). For *C. tenuis* XYLR the crystal structure is available [27] as well as information about the catalytic mechanism and coenzyme specificity

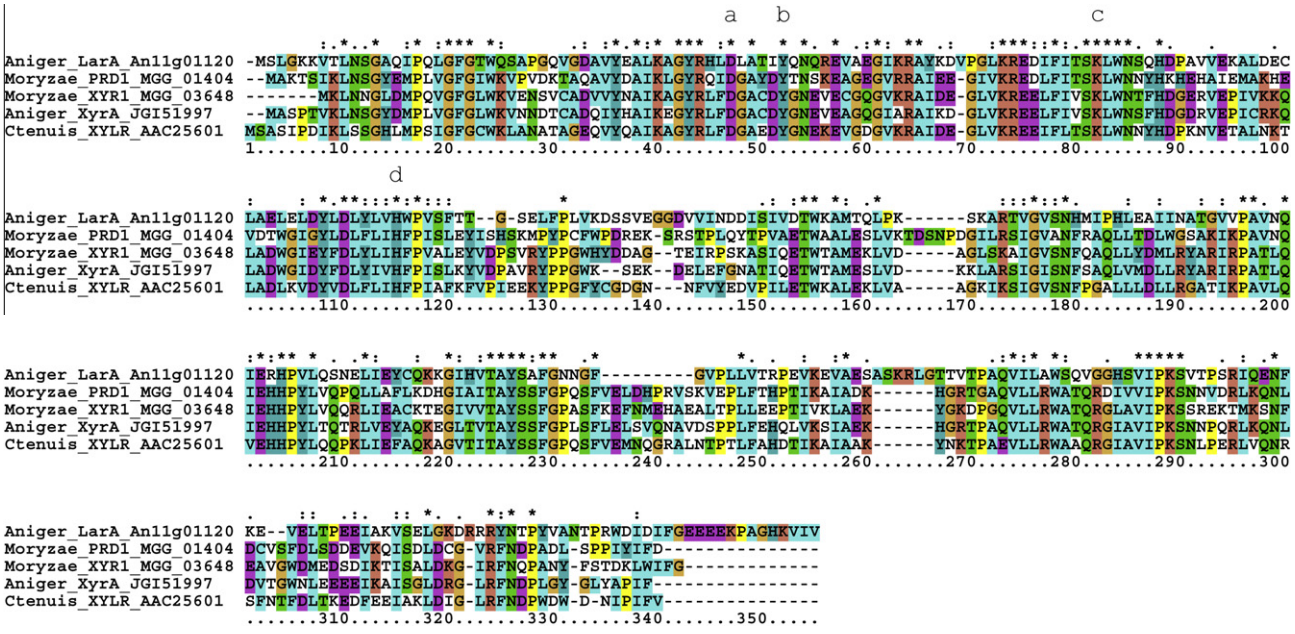

Fig. 3. Amino acid sequence alignment of five fungal pentose reductases. Amino acid sequence alignment of *A. niger* XyrA (D-xylose reductase) and LarA (L-arabinose reductase), *M. oryzae* XYR1 (D-xylose reductase) and PRD1 (pentose reductase) and *C. tenuis* XYLR (D-xylose reductase). The catalytic residues of *C. tenuis* XYLR are indicated with a, b, c and d and are conserved in all sequences.

Table 2
Specific enzymatic activity of the recombinant pentose reductase 1 (PRD1) from *M. oryzae*.

Reductase substrate	Specific activity (mU/mg)	Dehydrogenase substrate	Specific activity (mU/mg)
L-Arabinose	**101.6** ± 5.0	L-Arabitol	**11.0** ± 0.7
D-Arabinose	2.3 ± 0.4	D-Arabitol	0.3 ± 0.0
D-Xylose	**93.0** ± 2.3	Xylitol	**7.0** ± 0.3
L-Xylose	2.0 ± 0.3	D-Sorbitol	5.5 ± 0.6
D-Glucose	18.5 ± 0.1	D-Ribitol	ND
D-Ribose	**54.5** ± 1.0	D-Mannitol	0.3 ± 0.1
D-Mannose	3.0 ± 0.5		
D-Galactose	24.0 ± 1.2		
L-Rhamnose	1.3 ± 0.2		
D-Fructose	1.0 ± 0.3		

ND = not detected. Standard deviations are given behind the values.

(reviewed in [28]). The catalytic tetrad of the active site is conserved in all reductases (XYR, PRD1 and LarA) used for the alignment and phylogenetic tree, confirming former results about the conservation of these catalytic residues in both sequence and structure in most aldo–keto reductases [28].

3.2. Characterization of recombinant M. oryzae PRD1

Prd1 encodes a protein of 328 amino acids with a calculated molecular mass of 37.1 and a pI of 6.3. PRD1 was expressed in *E. coli* and purified as described in Section 1. Recombinant PRD1 had its highest reductase activities with L-arabinose (100%) and D-xylose (90%) as substrates, and it displayed much lower activities with D-ribose (54%), D-galactose (24%) and D-glucose (18%) (Table 2). The dehydrogenase activity of PRD1 was highest with L-arabitol as substrate (100%), followed by xylitol (65%) and D-sorbitol (50%) (Table 2). No or very low enzymatic activities were detected with other pentose and hexose substrates. The K_m values were 19.7 and 19.9 mM for L-arabinose and D-xylose respectively, while the V_{max} values were 0.360 and 0.244 U/mg, for L-arabinose and D-xylose respectively. These values are similar to those obtained for known fungal L-arabinose and D-xylose reductase [8,25,26]. The K_m and V_{max} values of *A. niger* L-arabinose reductase LarA for L-arabinose are 54 mM and 2.3 U/mg, respectively, while these values are 155 mM and 1.65 U/mg for D-xylose [8]. The K_m value of *A. niger* D-xylose reductase XyrA for L-arabinose is 21 mM, while this value is 6 mM for D-xylose [8]. Therefore, PRD1 has a wider enzymatic activity toward L-arabinose and D-xylose than classical fungal L-arabinose or D-xylose reductases (Table 3).

3.3. Expression of pentose catabolic pathway genes in M. oryzae

mRNA levels of *MoPRD1* and *MoXYR1* were analysed using qRT-PCR. *M. oryzae* mycelium was pre-grown in minimal medium with 1% D-fructose and transferred to 25 mM D-glucose, 25 mM D-xylose or 25 mM L-arabinose for 2 and 8 h. Both *MoPRD1* and *MoXYR1* were highly expressed after 2 and 8 h of growth on either D-xylose or L-arabinose (Table 4), while only a low level of expression of *MoPRD1* and *MoXYR1* was observed on glucose at 2 and 8 h. *MoPDR1* was clearly over-expressed at 2 h on D-xylose (sevenfold) and L-arabinose (30-fold) compared to glucose. At 8 h, the induction of *MoPRD1* slightly decreased on both D-xylose and L-arabinose (D-xylose fourfold and L-arabinose 20-fold compared to glucose). *MoXYR1* was also clearly over-expressed at 2 h on D-xylose (24-fold) and L-arabinose (78-fold) compared to glucose. At 8 h, the induction of *MoXYR1* compared to glucose increased on D-xylose (63-fold), while it slightly decreased on L-arabinose (62-fold). The induction of *MoPRD1* expression by both D-xylose and L-arabinose is characteristic of the expression patterns of fungal D-xylose and L-arabinose reductase encoding genes [5,6,8,26,29,30].

Expression of *MoPRD* and *MoXYR1* was also monitored by qRT-PCR at different stages of an infection of barley leaves (0–4 days) by *M. oryzae* isolate Guy11. Barley leaves were infected with drops of

Table 3
Comparison of kinetic properties of fungal pentose reductases.

	K_m L-Arabinose	K_m D-xylose	V_{max} L-Arabinose	V_{max} D-Xylose	Reference
A. niger LarA	54	155	2.3	1.6	[8]
A. niger XyrA	21	6	Nd	Nd	[8]
M. oryzae PRD1	20	20	0.36	0.24	This study
N. crassa XR	40	34			[25]

Values for PRD1 are averages of three individual experiments. Variations between the experiments were below 1%.

Table 4
Expression of *M. oryzae* pentose reductase genes.

Gene	D-glucose	D-xylose	L-arabinose	Ratio*
2 h				
PRD1 fold change	0.36	2.48 × 7	10.97 × 30	4.4
XYR1 fold change	0.10	2.39 × 24	7.88 × 78	3.3
8 h				
PRD1 fold change	0.39	1.45 × 4	7.64 × 20	5.3
XYR1 fold change	0.07	4.45 × 63	4.39 × 62	1.0

Expression levels are relative to the level of constitutively expressed gene *ILV5* using data from technical triplicates. *Ratio of expression levels on L-arabinose compared to D-xylose.

Table 5
Expression of *M. oryzae* pentose reductase genes during barley leaf infection.

Gene	Barley leaves infection (hai, hours after inoculation)		
	24 hai	48 hai	72 hai
MoXYR1	0.02	0.60	1.00
MoARD1	0.10	1.00	1.75

Expression of *M. grisea* genes was monitored by qRT-PCR. Average expression level is calculated relative to constitutively expressed gene *ILV5* using data from 3–5 technical replicates (standard deviation less than 10 %) and 2–3 biological replicates. Barley leaves were infected by drop spores inoculation. At 24 hai (hours after inoculation) the fungus has successfully penetrated into host leaf. At 48 hai and 72 hai, the fungus is growing inside barley leaves (no visible lesion).

Guy11 spore suspensions and collected at 24, 48 and 72 h after infection (hai). Infected areas were removed from inoculated leaves for RNA extraction. At 24 hai, the fungus is penetrating into host plant leaves. Between 48 and 72 hai, the fungus is growing inside barley leaves without causing apparent damages (no visible lesions). Both *MoPRD1* and *MoXYR1* are significantly expressed during infection of barley leaves as their levels of expression are similar to those of the constitutively expressed gene ILV5 (Table 5). Both genes have the same pattern of expression in that were only expressed at later stages of infection (48 and 72 hai) when *M. oryzae* is growing inside infected tissues (Table 5).

4. Discussion

D-Xylose and L-arabinose reductases are required for the conversion of pentose sugars through the pentose catabolic pathway and have received much attention due to the applications of these sugars in food and feed and biofuel industries. In the industrial workhorse *A. niger*, the *xyrA* gene encoding a D-xylose reductase [6] and more recently the *larA* gene encoding a L-arabinose reductase [8] have been identified. In our study, we have searched the genome of the rice-pathogen *M. oryzae* for genes encoding such enzymes. Two genes were detected using XyrA as a query, both of which gave a best reciprocal Blast hit with *A. niger* XyrA, while we did not find any homologs for LarA. Based on phylogenetic analysis, the gene encoding the protein MG_03648 displaying the highest protein sequence similarity to XyrA, is clearly an ortholog of *A. niger xyrA*, (Fig. 2) and was denominated as *XYR1*. The other gene encoding a protein (MG_01404) with similarities to XyrA, is a member of a clade (PRD) distinct, but related to the XYR clade, and was denominated as *PRD1*. Phylogenetic analysis clearly showed that the XYR and PRD clades are related (Fig. 2) and rooted by the clade gathering all the D-xylose reductases from Saccharomycotina. Therefore, these three clades define a superfamily of proteins encoding fungal D-xylose reductases and pentose reductases distinct from the fungal L-arabinose reductase clade (LAR, Fig. 2). The topology of the phylogenetic tree suggests that PDR and XYR encoding genes result from the duplication of an ancestral D-xylose reductase encoding gene early after the divergence of Pezizomycotina from Saccharomycotina. According to this hypothesis, most of the PRD encoding genes have been lost in Dothideomycetes (0 out of 2 species contain a PRD homolog) and Eurotiomycetes (1 out of 5 species contained a homolog), while they are frequently conserved in Sordariomycetes (8 out of 15 species contained a homolog). The discontinuous distribution of PRD encoding genes among fungal species could be partly correlated with a plant pathogenic life style. Indeed, among Sordariomycetes, nearly all the saprobes lack PRD encoding genes, while plant pathogens have one or two copies. This suggests that PDR encoding genes might have been selected in relation to the growth/survival of a given fungal species as a plant pathogen. This correlates with the expression of the reductase encoding genes during later stages of infection of barley by *M. oryzae* as demonstrated in this study.

Enzymatic analysis of PRD1 demonstrated that it was able to convert L-arabinose and D-xylose with a similar affinity, but with a slightly higher specific activity for L-arabinose. In contrast, XyrA and LarA from *A. niger* had a much higher affinity for D-xylose or L-arabinose, respectively, but similar activity on both substrates [6,8]. A higher affinity for D-xylose has also been reported for the D-xylose reductase from *Hypoctea jecorina* [26] and *N. crassa* [25]. Based on these results, PRD1 appears to be a novel pentose reductase with a wider substrate affinity than L-arabinose reductases from the LAR clade and D-xylose reductases from the XYR clade, since it is able to bind both L-arabinose and D-xylose with a similar affinity.

The ability of an enzyme to convert a compound in vitro is not sufficient evidence for its biological function. To be able to perform this function in vivo, the corresponding gene needs to at least be expressed under relevant culture conditions. Recently it was shown, that a gene previously characterized to encode an L-xylulose reductase [31], encodes in fact a mannitol dehydrogenase based its expression patterns on different sugars in *A. niger* and *H. jecorina* and detailed enzymatic analysis [32]. The expression patterns of *xyrA* and *larA* in *Aspergillus nidulans* are indeed well correlated with the biological relevance of their enzymatic activities, since *xyrA* is almost only overexpressed in the presence of D-xylose while *larA* is almost only overexpressed in the presence of L-arabinose [29]. Our study showed that *M. oryzae MoPRD1* is overexpressed during fungal growth on both L-arabinose and D-xylose compared to D-glucose. This overexpression on both sugars suggests that PDR1 is involved in the conversion of both pentoses, although more efficiently for L-arabinose since it is more highly expressed on this sugar than on D-xylose. *MoXYR1* is also overexpressed during fungal growth on both L-arabinose and D-xylose compared to D-glucose. However, the expression level of *MoPRD1* on L-arabinose is higher than *MoXYR1* (1.8-fold at 8 h). This high expression on L-arabinose combined with its higher activity on this substrate, suggests that PRD1 is more efficient for L-arabinose conversion than XYR1. In view of the absence of a LAR encoding gene in *M. oryzae*, we hypothesize that PDR1 is the major enzyme for the first step of the L-arabinose catabolic pathway in this fungus, while XYR1 is the major enzyme for the first step of the xylose catabolic pathway.

The expression patterns of *MoXYR1* and *MoPDR1* strongly differ from those of *xyrA* and *larA* from *A. nidulans*. These differences could be due to the occurrence of different pentose regulatory networks. Indeed, although the D-xylose responsive regulator XlnR controlling the expression of the XYR encoding genes is present in all filamentous ascomycetes analyzed, the L-arabinose responsive regulator AraR controlling the expression of LAR encoding genes, was only identified in Aspergilli and related fungi [30]. In *A. niger* XlnR and AraR regulators have an antagonistic relationships, resulting in activation of L-arabinose related genes on D-xylose in an *xlnR* disruptant and of D-xylose related genes on L-arabinose in an *araR* disruptant [33]. Currently no L-arabinose responsive regulator has been identified in *M. oryzae*, but expression studies demonstrated that the XlnR homolog of *M. oryzae* (Xlr1) is not responsible for L-arabinose-related gene expression (Klaubauf & de Vries, unpublished results).

Our combined phylogenetic, biochemical and expression analyses of fungal D-xylose and L-arabinose reductases have revealed a novel family of pentose reductases (PRD) and differences in fungal pentose catabolic pathways among fungi. First, the distribution of LAR, XYR and PRD encoding genes among fungal species strongly differs, with XYR encoding genes being present in all species, while the distribution of LAR or PRD encoding genes among species is discontinuous. Second, the expression of these genes in response to D-xylose and L-arabinose also differs among fungal species. These different evolutionary patterns may to have led to four types

of pentose catabolic pathways in Sordariomycetes. Some species have only a single XYR encoding gene suggesting that the corresponding enzyme is involved in both L-arabinose and D-xylose conversion (*N. crassa*). In contrast, all the Magnaporthacae species have a XYR and a PRD encoding gene suggesting that each corresponding enzyme is involved in either L-arabinose or D-xylose conversion. *Fusarium* and *Trichoderma* species contain a single XYR and 1–2 LAR encoding genes, but only some of them contain a PRD encoding gene, suggesting some enzymatic redundancies for L-arabinose conversion. In other Pezizomycotina these gene combinations are also found such as a single XYR, 0–1 PRD and 1–2 LAR in *Aspergillus* species and some Dothidiomycetes, while the presence of only a single XYR was found in other Dothidiomycetes and the Leotiales. This demonstrates a high variation in the content and combination of genes involved pentose catabolism in fungi suggesting that these enzymes have play a role in the adaptation of these fungal species to different ecological niches.

The presence of PRD1 is unlikely to be essential for pathogenicity as XYR1 can compensate for its function. Also, other research in our lab showed that deletion of XKI1 encoding *d*-xylulose kinase, the last step of pentose catabolism, will abolish growth of *M. oryzae* on D-xylose and L-arabinose, but does not affect pathogenicity on barley or rice (unpublished data). Therefore, and also due to the absence of a deletion strain in the ATMT library (http://atmt.snu.ac.kr/), we have not further pursued this. Interestingly, while the pentose catabolic pathway does not appear to be essential for pathogenicity of *M. oryzae*, a recent study has shown that the oxidative part of the pentose phosphate pathway is involved in pathogenicity [34]. While these two pathways are linked, the pentose phosphate pathway has a more general role in carbon metabolism, which might explain the difference.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.febslet.2013.03.003.

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