FEBS Letters 587 (2013) 1346–1352

FEBS Letters

journal homepage: www.FEBSLetters.org

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

Sylvia Klaubauf [a,c], Cecile Ribot [b,c,1], Delphine Melayah [b,c], Arnaud Lagorce [b,c,2], Marc-Henri Lebrun [c,d], Ronald P. de Vries [a,c,e,*]

[a] CBS-KNAW Fungal Biodiversity Centre, Utrecht, The Netherlands
[b] Bayer Cropscience, Lyon, France
[c] MPA, UMR 2847 CNRS, Bayer Crop Science, Lyon, France
[d] BIOGER, UR 1290 INRA, Thiverval-Grignon, France
[e] Microbiology & Kluyver Centre for Genomics of Industrial Fermentation, Utrecht, The Netherlands

ARTICLE INFO

Article history:
Received 18 February 2013
Accepted 2 March 2013
Available online 13 March 2013

Edited by Judit Ovadi

Keywords:
Pentose catabolism
Pentose reductase
D-Xylose
L-Arabinose
Magnaporthe oryzae

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

* Corresponding author at: CBS-KNAW Fungal Biodiversity Centre, Uppsalalaan 8, 3584 CT Utrecht, The Netherlands. Fax: +31 302512097.
E-mail address: r.devries@cbs.knaw.nl (R.P. de Vries).
[1] Current address: mRNA Regulation and Development, Institute of Human Genetics, CNRS UPR1142, 141 Rue de la Cardonille, 34396 Montpellier Cedex 5, France.
[2] Current address: Institut de Génétique et Microbiologie, UMR 8621, CNRS, Université Paris-Sud, France.

0014-5793/$36.00
http://dx.doi.org/10.1016/j.febslet.2013.03.003

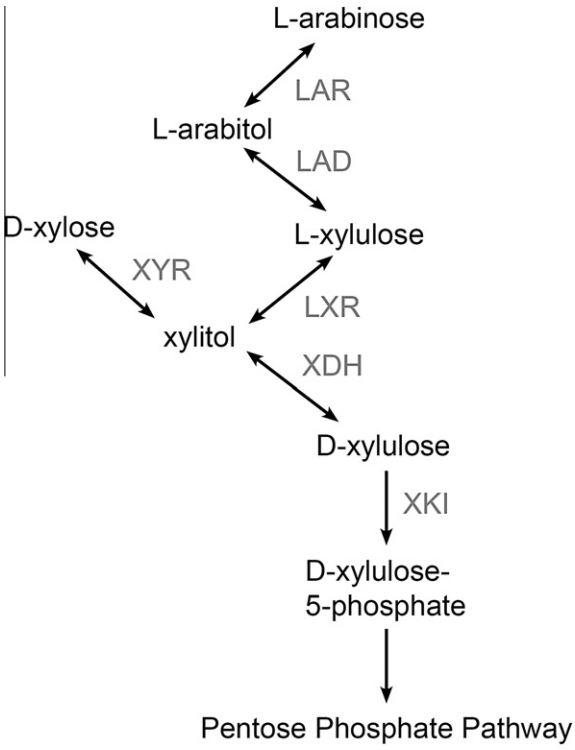

Fig. 1. Pentose catabolism in filamentous fungi LAR = L-arabinose reductase, LAD = L-arabitol dehydrogenase (EC 1.1.1.12), LXR = L-xylulose reductase (EC 1.1.1.10), XYR = D-xylose reductase (EC 1.1.1.307), XDH = xylitol dehydrogenase (EC 1.1.1.9), XKI = D-xylulose kinase (EC 2.7.1.17). LAR, LXR and XYR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