The Missing Link in the Fungal L-Arabinose Catabolic Pathway, Identification of the L-Xylulose Reductase Gene[†,‡]

Peter Richard,* Mikko Putkonen, Ritva Väänänen, John Londesborough, and Merja Penttilä

VTT Biotechnology, P.O. Box 1500, FIN-02044 VTT, Finland

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ABSTRACT: The fungal L-arabinose pathway consists of five enzymes, aldose reductase, L-arabinitol 4-dehydrogenase, L-xylulose reductase, xylitol dehydrogenase, and xylulokinase. All the genes encoding the enzymes of this pathway are known except for that of L-xylulose reductase (EC 1.1.1.10). We identified a gene encoding this enzyme from the filamentous fungus *Trichoderma reesei* (*Hypocrea jecorina*). The gene was named *lxr1*. It was overexpressed in the yeast *Saccharomyces cerevisiae*, and the enzyme activity was confirmed in a yeast cell extract. Overexpression of all enzymes of the L-arabinose pathway in *S. cerevisiae* led to growth of *S. cerevisiae* on L-arabinose; i.e., we could show that the pathway is active in a heterologous host. The *lxr1* gene encoded a protein with 266 amino acids and a calculated molecular mass of 28 428 Da. The LXRI protein is an NADPH-specific reductase. It has activity with L-xylulose, D-xylulose, D-fructose, and L-sorbose. The highest affinity is toward L-xylulose (K_m = 16 mM). In the reverse direction, we found activity with xylitol, D-arabinitol, D-mannitol, and D-sorbitol. It requires a bivalent cation for activity. It belongs to the protein family of short chain dehydrogenases. The enzyme is catalytically similar and homologous in sequence to a D-mannitol:NADP 2-dehydrogenase (EC 1.1.1.138).

L-Arabinose is a major constituent of plant material (*1*). An L-arabinose catabolic pathway is therefore a relevant pathway for microorganisms which live on decaying plant material and also for biotechnology when cheap raw materials are used. An example is to make fuel ethanol from agricultural waste residues such as corn fiber, which is particularly rich in L-arabinose. Approximately a third of the corn fiber xylan is L-arabinose (*2*). In this context, it is interesting to have an L-arabinose catabolic pathway in an ethanol- and inhibitor-tolerant organism like *Saccharomyces cerevisiae*.

Two distinct bacterial and fungal pathways are known for the catabolism of L-arabinose. The bacterial pathway consists of L-arabinose isomerase (EC 5.3.1.4), ribulokinase (EC 2.7.1.16), and L-ribulose phosphate 4-epimerase (EC 5.1.3.4). The pathway and the genes of this pathway are well-established (*3*). About the fungal pathway only little is known. The fungal pathway was first described by Chiang and Knight (*4*). It consists of aldose reductase (EC 1.1.1.21), L-arabinitol 4-dehydrogenase (EC 1.1.1.12), L-xylulose reductase (EC 1.1.1.10), D-xylulose reductase (EC 1.1.1.9), and xylulokinase (EC 2.7.1.17) which convert L-arabinose to L-arabinitol, L-xylulose, xylitol, D-xylulose, and D-xylulose 5-phosphate, respectively. The path from L-arabinose to D-xylulose is shown in Figure 1. The path consists of two reducing and two oxidizing steps with the reducing steps being NADPH-linked and the oxidizing steps being NAD-linked. Fungal aldose reductases which have activity with L-arabinose are known from *Pichia stipitis* (*5*) and *S. cerevisiae* (*6*), and their corresponding genes are known (*7*, *8*). The gene for an L-arabinitol 4-dehydrogenase is known from *Trichoderma reesei* (*9*). Genes for D-xylulose reductases are known from *P. stipitis* (*10*), *S. cerevisiae* (*8*), and *T. reesei* (*11*). A D-xylulokinase gene is known for *S. cerevisiae* (*12*). A fungal gene for L-xylulose reductase is not known. In this paper, we describe the identification of a gene from the filamentous fungus *T. reesei* (*Hypocrea jecorina*) encoding an L-xylulose reductase and the functional expression of this gene in *S. cerevisiae*. We also expressed the gene together with all the other genes of the L-arabinose pathway, which led to a functional pathway, i.e., growth of *S. cerevisiae* on L-arabinose.

EXPERIMENTAL PROCEDURES

Yeast Strain for Screening. Although *S. cerevisiae* has the genes for aldose reductase, D-xylulose reductase, and xylulokinase in the genome, it cannot grow on xylose, because these genes are not adequately overexpressed. The yeast strain used for screening was therefore constructed from *S. cerevisiae* strain CEN.PK2 (VW1b) as follows. First, the *XYL1* and *XYL2* genes from *P. stipitis* encoding aldose reductase and D-xylulose reductase, respectively, and the *XKS1* gene from *S. cerevisiae* encoding xylulokinase were added. The *XYL1* and *XYL2* genes under constitutive promoters (*13*) and the *XKS1* gene under a consititutive promoter (*14*) were integrated into the chromosomes by targeted integration. The resulting strain (H2217) is auxotrophic for leucine and uracil. The L-arabinitol 4-dehydrogenase (*lad1*) was cloned as described previously (*9*). The *Eco*RI, *Bam*HI

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[‡] The nucleotide sequence reported in this paper has been submitted to GenBank (accession number AF375616).

* To whom correspondence should be addressed: VTT Biotechnology, Tietotie 2, Espoo, P.O. Box 1500, FIN-02044 VTT, Finland. Telephone: 358-9-456-7190. Fax: 358-9-455-2103. E-mail: Peter.Richard@vtt.fi.

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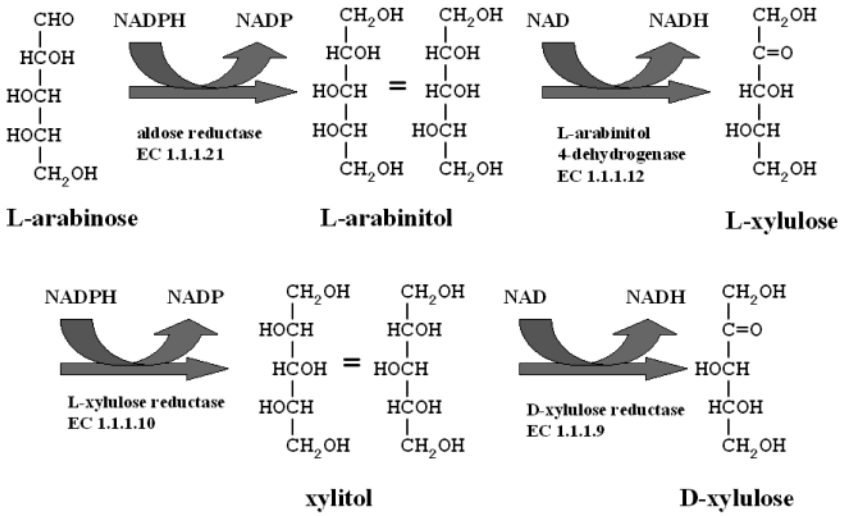

FIGURE 1: Fungal L-arabinose pathway. L-Arabinose is converted to D-xylulose in four steps (two reductions and two oxidations). The reductions are coupled to an oxidation of NADPH and the oxidations to a reduction of NAD. D-Xylulose is subsequently phosphorylated by xylulokinase to form D-xylulose 5-phosphate which is a metabolite of the pentose phosphate pathway. Carbon atom C-4 of L-arabinose becomes C-2 of L-xylulose and C-4 of D-xylulose. Both possible Fischer projections of L-arabinitol and xylitol are shown to clarify the stereochemistry.

fragment was ligated to the corresponding sites of pYX 242 (R&D Systems), which is a yeast expression vector with a constitutive promoter and with *LEU2* as a selection marker, and transformed to strain H2217, resulting in strain H2597. The activities of aldose reductase, D-xylulose reductase, xylulokinase, and L-arabinitol 4-dehydrogenase were confirmed in a yeast extract.

Screening. A cDNA library from *T. reesei* (*H. jecorina*) in the yeast expression vector pAJ401 which contained the URA3 gene as a selection marker (*15*) was transformed to strain H2597. Transformants were first grown on glucose plates with selective medium and then replica plated to plates containing 5% L-arabinose as the sole carbon source and selective medium.

From transformants which exhibited growth on L-arabinose, the plasmids were rescued. Two types of plasmids were rescued from each colony, plasmids with the *lad1* and *LEU2* genes and plasmids from the cDNA library with the *URA3* marker. The plasmids with the *lad1* gene were identified by PCR, and plasmids without the *lad1* gene were sequenced.

Overexpression of the L-Xylulose Reductase. The open reading frame of the L-xylulose reductase was cloned by PCR from the cDNA library. The primer for the N-terminus (GCCGAATTCATCATGCCTCAGCCTGTCCCCACCGCC) contained an additional *Eco*RI restriction site (underlined), and the primer for the C-terminus (CGCCAAGCTTTTATCGTGTAGTGTAACCTCCGTCAATCAC) contained an additional *Hin*dIII restriction site (underlined). The resulting fragment was then cloned to a TOPO vector (Invitrogen). The resulting vector was digested with *Eco*RI and *Hin*dIII, and the resulting fragment of ~0.8 kb was ligated to the corresponding sites of the pYX242 vector (R&D Systems), which is a multicopy yeast expression vector with a triosephosphate isomerase (TPI) promotor, a strong constitutive promotor, and *LEU2* for selection. The plasmid was then transformed to *S. cerevisiae* strain CEN.PK2.

Histidine Tag. To make a histidine tag with six histidines at the C-terminal end of the protein, the *lxr1* gene was cloned by PCR with primers encoding the histidine tag. The following primers were used for the N-terminus (GCCGAATTCATCATGCCTCAGCCTGTCCCCACCGCC) and the C-terminus (GGATCCTTAATGATGATGATGATGATGTCGTGTAGTGTAACCTCCGTCAATCAC). The additional restriction sites *Eco*RI and *Bam*HI are underlined. The resulting PCR product was cloned to a TOPO vector, the resulting vector digested with *Eco*RI and *Bam*HI, and the resulting 0.8 kb fragment ligated to the corresponding sites of the pYX242 vector.

For the N-terminal histidine tag, primers were used for the N-terminus (CGAATTCATCATGCATCATCATCATCATCATGCCATCATGCCTCAGCCTGTCCCCACC) and the C-terminus (CGCCAAGCTTTTATCGTGTAGTGTAACCTCCGTCAATCAC). For the N-terminal histidine tag, six histidines were added after the start codon. Between the histidine tail and the original protein, an additional alanine and isoleucine were inserted. The cloning and ligation to an expression vector was carried out as with the C-terminal histidine tag except that the restriction enzymes *Eco*RI and *Hin*dIII were used. The plasmids were then transformed to *S. cerevisiae* strain CEN.PK2.

Purification of the Enzyme with the N-Terminal Histidine Tag. The *S. cerevisiae* cells overexpressing the L-xylulose reductase with the N-terminal histidine tag were grown on selective medium with glucose as a carbon source, harvested, and extracted by vortexing with glass beads. The enzyme was then purified from the crude extract with a Ni−NTA column (Quiagen) according to the manufacturer's description.

Enzyme Activity Measurements. For the standard procedure assay of L-xylulose reductase, the reverse reaction was assessed by adding the enzyme preparation to a reaction mixture containing 100 mM Tris-HCl (pH 9.0), 1.6 M xylitol, and 0.5 mM $MgCl_2$. The reaction was then started by adding NADP to a final concentration of 2 mM. The activity was then calculated from the rate of the increase in NADPH absorbance an 340 nm. Xylitol was replaced by other sugar alcohols when specified. The activity in the direction with

sugars as substrates was measured by adding the enzyme preparation to a reaction mixture containing 100 mM Hepes-NaOH (pH 7.0), 2 mM $MgCl_2$, and 0.2 mM NADPH. The sugar was then added to the desired final concentration. The activity was calculated from the rate of the decrease in NADPH absorbance at 340 nm. Magnesium ions were replaced with other ions if specified. All enzyme assays were carried out in a Cobas Mira automated analyzer (Roche) at 30 °C.

Identification of the Product by HPLC. The reaction products were identified by HPLC analysis. In the forward direction, 6 nkat/mL of purified protein was added to a reaction mixture containing 100 mM Hepes-NaOH (pH 7.0), 2 mM $MgCl_2$, 2 mM NADPH, and the specified sugar (20 mM). In the reverse direction, the same enzyme amount was added to a reaction mixture containing 100 mM Tris-HCl (pH 9.0), 2 mM $MgCl_2$, 10 mM NADP, and the specified sugar alcohol (20 mM). The reaction mixtures were incubated for several hours at room temperature before they were analyzed by HPLC. An Aminex Pb column (Bio-Rad) at a working temperature of 85 °C was used with water at a flow rate of 0.6 mL/min. The products were detected by a Waters 410 RI detector.

RESULTS

Screening for the L-Xylulose Reductase. A yeast strain, H2597 (see Experimental Procedures), which expressed all genes of the L-arabinose pathway, except for a gene for the L-xylulose reductase was used for the screening. A *T. reesei* cDNA library ligated into a yeast expression vector was transformed into this yeast strain, and 750 000 transformants were screened for growth on L-arabinose. After 3−4 weeks, approximately 16 colonies appeared. For yeast from one colony, the doubling time was estimated in liquid medium to be ~5 days. Eight of these colonies were analyzed further. They were not identical clones, but their plasmids contained identical open reading frames with different amounts of DNA between the expression vector and the start codon. We named the gene *lxr1* for L-xylulose reductase. The sequence was submitted to GenBank (accession number AF375616). The open reading frame encodes a protein with 266 amino acids and a calculated molecular mass of 28 428 Da. It belongs to the protein family of short chain dehydrogenases (*16*).

Overexpression of the L-Xylulose Reductase in S. cerevisiae. One of the plasmids with the *lxr1* gene obtained in the screening was retransformed to the CEN.PK strain. From the resulting strain, an extract was made by vortexing with glass beads and tested for L-xylulose reductase activity. We found an activity of 2−3 nkat of NADP reduced per milligram of extracted protein by using our standard procedure to assess the reverse reaction of L-xylulose reductase. The *lxr1* gene was also ligated into the pYX242 vector under the TPI promoter. Similar constructs using the same plasmid with the TPI promoter were made with an *lxr1* gene which was modified so that it would translate into a protein with either an N-terminal or a C-terminal histidine tag. These plasmids were also transformed to the CEN.PK strain. Activity was found with the construct with the *lxr1* gene without a histidine tag and the *lxr1* gene with an N-terminal histidine tag. These constructs had the same activity of 4 nkat per milligram of extracted protein. Using the construct with the C-terminal histidine tag, no L-xylulose reductase was detected in the yeast extract by the standard assay (<0.2 nkat/mg).

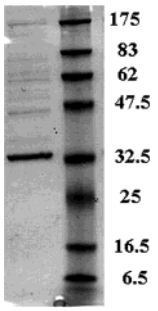

FIGURE 2: SDS−PAGE of the L-xylulose reductase with the N-terminal histidine tag. The left lane shows 1 μg of the enzyme after purification. The right lane shows standards. The molecular masses of the standards in kilodaltons are also indicated.

Catalytic Properties. The LXRI with the N-terminal histidine tag was purified and used for catalytic studies. An SDS−PAGE gel of the purified enzyme is shown in Figure 2. From this SDS−PAGE gel, one can estimate a molecular mass of 33 kDa which is in good agreement with the calculated molecular mass of the enzyme with the histidine tag (29 565 Da). The purified enzyme had an activity of ~150 nkat/mg in our standard procedure.

With the purified enzyme, we also tested the forward reaction with L-xylulose and NADPH as substrates. We found a V_{max} of 75 ± 10 nkat/mg and a K_m for L-xylulose of 16 ± 3 mM. With 100 mM phosphate instead of Hepes as a buffer, the V_{max} was lowered by a factor of 3. We tested if the $MgCl_2$ could be left out and found no activity in the absence of $MgCl_2$ under otherwise identical conditions. With $CaCl_2$ and Hepes as a buffer, we found an activity similar to that with $MgCl_2$; with $MnCl_2$, the activity was ~10% of the activity with $MgCl_2$. The enzyme had no activity with NADH as a cofactor.

We also found activity with NADPH and the sugars D-xylulose, D-fructose, and L-sorbose and no activity with D-tagatose. We also found no activity with the aldose sugars D-glucose, D-mannose, and D-xylose. For D-fructose, we found a K_m of 165 ± 20 mM and a V_{max} of 420 ± 30 nkat/mg. With D-xylulose, the activity increased in a linear manner with the substrate concentration up to a concentration of 63 mM; i.e., a Michaelis−Menten value was not determined. At a substrate concentration of 63 mM, the activities were 80 nkat/mg for L-xylulose, 13 nkat/mg for D-xylulose, 100 nkat/mg for D-fructose, and 3.5 nkat/mg for L-sorbose.

In the reverse direction, we found activity with xylitol, D-arabitol, D-mannitol, and D-sorbitol. At a sugar alcohol concentration of 800 mM, we found activities of 20 nkat/mg with xylitol, 200 nkat/mg with D-mannitol, 15 nkat/mg with D-sorbitol, and 20 nkat/mg with D-arabinitol. We found no activity with L-arabinitol.

With HPLC analysis, we identified the reaction products. From L-xylulose, xylitol was formed, and from D-xylulose, arabinitol was formed. In the reverse direction, we saw that xylulose was the product of xylitol and xylulose was also the product of D-arabinitol. The HPLC method that was used does not distinguish between L- and D-xylulose. The products of D-mannitol and D-sorbitol were fructose and sorbose, respectively.

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                  10        20        30        40        50        60
                   |         |         |         |         |         |
LXR1 T.r.    MPQPVPTANRLLDLFSLKGKVVVVTGASGPRGMGIEAARGCAEMGADLAITYSSRKEGAE
MtDH A.b.    MAPGFTIS--------FVNKTIIVTGGN--RGIGLAFTRAVAAAGANVAVIYRSAKDAVE
DR hamster   ----MDLG--------LAGRRALVTGAG--KGIGRSTVLALQAAGAHVVAVSRTQADLDS
              .  .  .         : .:  :***..  :*:*   . .    **.:.    :  :  .

                  70        80        90       100       110       120
                   |         |         |         |         |         |
LXR1 T.r.    KNAEELTKEYGVKVKVYKVNQSDYNDVERFVNQVVSDFGKIDAFIANAGATANSGVVDGS
MtDH A.b.    V-TEKVGKEFGVKTKAYQCDVSNTDIVTKTIQQIDADLGAISGLIANAGVSVVKPATELT
DR hamster   LVSECPGVET------VCVDLADWEATEQALS----SVGPVDLLVNNAAVALLQPFLEVT
               :*    * . . .    : :: : . : :..  :..* :. :: **..:  .   : :

                 130       140       150       160       170       180
                   |         |         |         |         |         |
LXR1 T.r.    ASDWDHVIQVDLSGTAYCAKAVG-AHFKKQGHGSLVITASMSGHVANYP-----QEQTSY
MtDH A.b.    HEDFKFVYDVNVFGVFNTCRAVAKLWLQKQQKGSIVVTSSMSSQIINQSSLNGSLTQVFY
DR hamster   KEAFDMSFNVNLRAVIQVSQIVARGNIARGAPGAIVNVSSQASQRALAN-------HSVY
              . :.   :*:: ..   .: *.   : :   *::* .:* :.:            :  *

                 190       200       210       220       230       240
                   |         |         |         |         |         |
LXR1 T.r.    NVAKAGCIHLARSLANEWR-DFARVNSISPGYIDT--GLSDFIDEKTQELWRSMIPMGRN
MtDH A.b.    NSSKAACSNLVKGLAAEWASAGIRVNALSPGYVNT--DQTAHMDKKIRDHQASNIPLNRF
DR hamster   CSTKGALDMLTK----------IRVNAVNPTVVMTSMGRTNWSDPHKAKVMLDRIPLGKF
               :*..   *.:. : .      ***::.*  : *  . :   * :  .   . **:.:

                 250       260       270
                   |         |         |
LXR1 T.r.    GDAKELKGAYVYLVSDASSYTTGADIVIDGGYTTR
MtDH A.b.    AQPEEMTGQAILLLSDHATYMTGGEYFIDGGQLIW
DR hamster   AEVENVVDAILFLLSHRSNMTTGSTLPVDGGFLVT
             .: ::: .  : *:*. :.  **.   :***
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FIGURE 3: Amino acid sequence alignment of the L-xylulose reductase from *T. reesei* (LXR1 T.r.), the D-mannitol dehydrogenase from the button mushroom *Ag. bisporus* (GenBank accession number AF053764) (MtDH A.b.), and the hamster liver diacetyl reductase which has also L-xylulose reductase activity (DR hamster). Asterisks indicate identity. Colons indicate a high degree of homology. Periods indicate a low degree of homology.

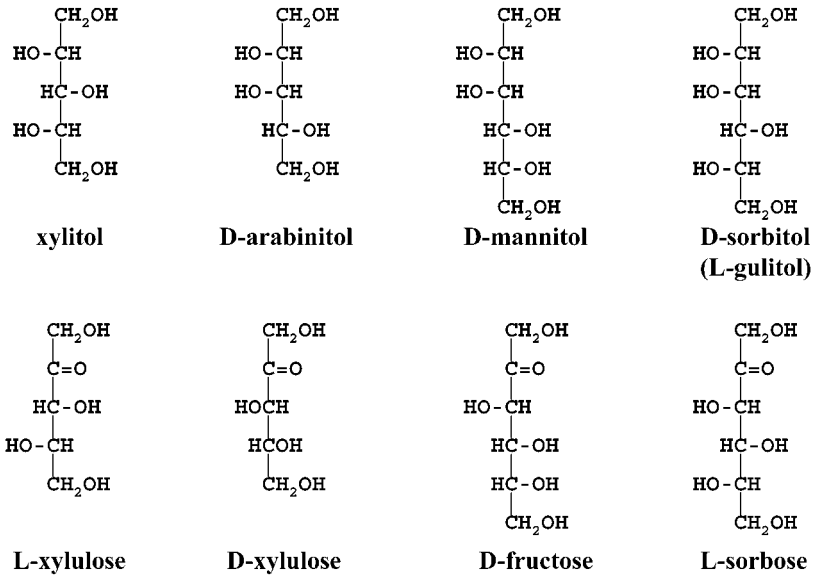

FIGURE 4: Sugar alcohols which are substrates for the L-xylulose reductase (top row) and the corresponding sugars (bottom row).

DISCUSSION

The fungal pathway for L-arabinose catabolism as it was first described by Chiang and Knight (*4*) consists of five enzymes. For four of these enzymes, the corresponding genes are known. We expressed these four known genes in a strain of *S. cerevisiae* and screened a cDNA library from a mold which can use L-arabinose as a carbon source for growth on L-arabinose. In this way, we found a gene encoding an L-xylulose reductase.

L-Xylulose reductases from molds and higher animals have been reported. For molds, L-xylulose reductase was described for the filamentous fungi *Penicilium chrysogenum* (*4*) and *Aspergillus niger* (*17*). For higher animals, L-xylulose reductase activity was found in guinea pig liver (*18*−*21*). A partly purified L-xylulose reductase from pigeon liver is commercially available (Sigma Aldrich). L-Xylulose reductase was also reported in humans where the absence of this activity caused a disease called essential pentosuria (*22*, *23*). From hamster liver, a gene was cloned which encoded a diacetyl reductase that also exhibited activity with L-xylulose (*24*). The liver enzymes and the fungal enzymes share the fact that they are all NADPH-linked but have otherwise

different enzymatic properties. The *A. niger* enzyme has a K_m for L-xylulose of 17 mM (925 mM for xylitol, 0.03 mM for NADPH, and 0.13 mM for NADP) (*17*). Liver enzymes have K_m values for L-xylulose of 0.29 mM (guinea pig) (*18*) and 0.26 mM (hamster liver) (*24*).

The L-xylulose reductase from *T. reesei* has properties similar to those of the corresponding enzyme that was purified from *A. niger*. Both enzymes use NADPH as a cofactor, and the affinities for L-xylulose are similar, 16 and 17 mM (*17*) for the enzymes from *T. reesei* and *A. niger*, respectively. The molecular masses of the two enzymes are also similar. The purified enzyme from *A. niger* has a molecular mass of 32 kDa as estimated by SDS−PAGE (*17*). The *T. reesei* enzyme has a calculated molecular mass of 28 428 Da. One apparent difference between the two enzymes is that the *A. niger* enzyme has no activity with D-fructose. One could now suggest that the enzyme we identified belongs to a different class of enzymes than the *A. niger* enzyme. Because it has even higher activity with D-fructose and D-mannitol than with xylitol, it could be called a mannitol dehydrogenase (EC 1.1.1.138) which also exhibits L-xylulose reductase activity. We think, however, that the two enzymes are very similar and the difference is only apparent. The activity of the *A. niger* enzyme was measured in phosphate buffer (*17*). When we used phosphate buffer for the *T. reesei* enzyme, we also could not detect any activity with D-fructose. We found some, but significantly decreased, activity with L-xylulose when using phosphate buffer. This indicates that the two enzymes from *T. reesei* and *A. niger* might be very similar, but the D-mannitol dehydrogenase activity of the *A. niger* enzyme might have been overlooked.

The enzyme described in this publication has an amino acid sequence similar to that of the D-mannitol 2-dehydrogenase from *Agaricus bisporus* (*25*) (Figure 3). The level of sequence similarity with the mannitol dehydrogenase is even higher than with an enzyme with reported L-xylulose reductase activity, i.e., the hamster liver diacetyl reductase (*24*) (Figure 3). The *T. reesei* L-xylulose reductase has also D-mannitol 2-dehydrogenase activity. It is, however, different from the D-mannitol 2-dehydrogenase that was purified from *Aspergillus parasiticus*. The *A. parasiticus* enzyme had no activity with xylitol; i.e., it was not an L-xylulose reductase (*26*).

Whether the diacetyl reductase/L-xylulose reductase from hamster liver described by Ishikura et al. (*24*) has mannitol dehydrogenase activity is not clear, since it was not tested.

The L-xylulose reductase from *T. reesei* has a broad substrate specificity. All sugar alcohols identified as substrates have a hydroxyl group on the second atom of the carbon chain in the L-configuration, which is oxidized to a ketone group. From the activity measurements and the HPLC analysis of the products, we conclude that the following sugar−sugar alcohol couples are interconverted: L-xylulose and xylitol, D-xylulose and D-arabinitol, D-fructose and D-mannitol, and L-sorbose and D-sorbitol (Figure 4).

We found no activity when the histidine tag was expressed at the C-terminus. This can mean that the enzyme with the C-terminal histidine tag was not expressed or that the enzyme is inactive. There is structural information about an enzyme of the same protein family, i.e., a short chain dehydrogenase (*27*), which suggests that the catalytic site and the C-terminal end of the protein are close together. It might be that adding a histidine tag to the C-terminal end of the protein interferes with the binding to the catalytic site and this leads to an inactive enzyme. The N-terminus, however, is distant from the catalytic domain in this structure, and also, the LXRI is active with an N-terminal histidine tag; i.e., it has the same standard activity as the nontagged enzyme.

In this paper, we describe for the first time the functional overexpression of the complete fungal L-arabinose pathway in a heterologous host. Sedlak and Ho previously tried to express the bacterial pathway in *S. cerevisiae*, but even though the individual enzymes were reported to be functionally expressed, this did not result in either growth on L-arabinose or ethanol production from L-arabinose (*28*).

The expression of the fungal pathway led to growth of *S. cerevisiae* on L-arabinose, which is not a natural substrate for this yeast. However, the growth rate of the recombinant *S. cerevisiae* strain on L-arabinose is very slow. One possible reason for this is that the L-arabinose uptake into the cell is very slow. We are not aware of any information in the literature dealing with L-arabinose uptake into cells of *S. cerevisiae*. Another possible reason for the slow growth is the cofactor imbalance. The conversion of L-arabinose to D-xylulose is redox neutral. However, the two reductions are coupled to NADPH utilization, whereas the oxidations are coupled to NAD utilization. Since yeasts are believed to have no transhydrogenase activity (*29*), the cofactors are not efficiently regenerated.

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REFERENCES

1. Hayn, A., Steiner, W., Klinger, R., Steinmüller, H., Sinner, M., and Esterbauer, H. (1993) in *Bioconversion of forest and agricultural plant residues* (Saddler, J. N., Ed.) pp 33−72, CAB International, Wallingford, U.K.
2. Hespell, R. B. (1998) *J. Agric. Food Chem. 46*, 2615−2619.
3. Lee, N., Gielow, W., Martin, R., Hamilton, E., and Fowler, A. (1986) *Gene 47*, 231−244.
4. Chiang, C., and Knight, S. G. (1960) *Biochem. Biophys. Res. Commun. 3*, 554−559.
5. Verduyn, C., van Kleef, R., Frank, J., Schreuder, J. H., van Dijken, J. P., and Scheffers, W. A. (1985) *Biochem. J. 226*, 668−677.
6. Kuhn, A., van Zyl, C., van Tonder, A., and Prior, B. A. (1995) *Appl. Environ. Microbiol. 61*, 1580−1585.
7. Amore, R., Kötter, P., Kuster, C., Ciriacy, M., and Hollenberg, C. P. (1991) *Gene 109*, 89−97.
8. Richard, P., Toivari, M. H., and Penttilä, M. (1999) *FEBS Lett. 457*, 135−138.
9. Richard, P., Londesborough, J., Putkonen, M., Kalkkinen, N., and Penttilä, M. (2001) *J. Biol. Chem. 276*, 40631−40637.
10. Kötter, P., Amore, R., Hollenberg, C. P., and Ciriacy, M. (1990) *Curr. Genet. 18*, 493−500.
11. Wang, T., Penttilä, M., Gao, P., Wang, C., and Zhong, L. (1998) *Chin. J. Biotechnol. 14*, 179−185.
12. Ho, N. W. Y., and Chang, S.-F. (1989) *Enzyme Microb. Technol. 11*, 417−421.
13. Toivari, M. H., Aristidou, A., Ruohonen, L., and Penttilä, M. (2001) *Metab. Eng. 3*, 236−249.
14. Richard, P., Toivari, M. H., and Penttilä, M. (2000) *FEMS Microbiol. Lett. 190*, 39−43.
15. Margolles-Clark, E., Tenkanen, M., Nakari-Setälä, T., and Penttilä, M. (1996) *Appl. Environ. Microbiol. 62*, 3840−3846.

16. Bateman, A., Birney, E., Durbin, R., Eddy, S. R., Howe, K. L., and Sonnhammer, E. L. L. (2000) *Nucleic Acids Res. 28*, 263−266.
17. Witteveen, C. F. B., Weber, F., Busink, R., and Visser, J. (1994) *Microbiology 140*, 1679−1685.
18. Touster, O., Reynolds, V. H., and Hutcheson, R. M. (1954) *J. Biol. Chem. 221*, 697−709.
19. Hickman, J., and Ashwell, G. (1958) *J. Biol. Chem. 234*, 758−761.
20. Hollman, S. (1959) *Hoppe-Seyler's Z. Physiol. Chem. 317*, 193−216.
21. Arsenis, C., and Touster, O. (1969) *J. Biol. Chem. 244*, 3895−3899.
22. Lane, A. B. (1985) *Biochem. Genet. 23*, 61−72.
23. Lane, A. B., and Jenkins, T. (1985) *Ann. Hum. Genet. 49*, 227−235.
24. Ishikura, S., Isaji, T., Usami, M., Kitahara, K., Nakagawa, J., and Hara, A. (2001) *Chem.-Biol. Interact. 130−132*, 879−889.
25. Stoop, J. M., and Mooibroek, H. (1998) *Appl. Environ. Microbiol. 64* (12), 4689−4696.
26. Niehaus, W. G., and Dilts, R. P. (1982) *J. Bacteriol. 151*, 243−250.
27. Benach, J., Atrian, S., Gonzales-Duarte, R., and Ladenstein, R. (1998) *J. Mol. Biol. 282*, 383−399.
28. Sedlak, M., and Ho, N. W. Y. (2001) *Enzyme Microb. Technol. 28*, 16−24.
29. Bruinenberg, P. M., Jonker, R., van Dijken, J. P., and Scheffers, W. A. (1985) *Arch. Microbiol. 142*, 302−306.

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