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RESEARCH PAPER

Conversion of L-arabinose to L-ribose by genetically engineered *Candida tropicalis*

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Abstract

L-Ribose, a starting material for the synthesis of L-nucleoside, has attracted lots of attention since L-nucleoside is responsible for the antiviral activities of the racemic mixtures of nucleoside enantiomers. In this study, the L-ribulose-producing *Candida tropicalis* strain was engineered for the conversion of L-arabinose to L-ribose. For the construction of a uracil auxotroph, the *URA3* gene was excised by homologous recombination. The expression cassette of codon-optimized L-ribose isomerase gene from *Acinetobacter calcoaceticus* DL-28 under the control of the GAPDH promoter was integrated to the uracil auxotroph. The resulting strain, K1 CoSTP2 LsaAraA AcLRI, was cultivated with the glucose/L-arabinose mixture. At 45.5 h of fermentation, 6.0 g/L of L-ribose and 3.2 g/L of L-ribulose were produced from 30 g/L of L-arabinose. The proportion between L-ribose and L-ribulose was approximately 2:1 and the conversion yield of L-arabinose to L-ribose was about 20% (*w*/*w*). The L-ribose-producing yeast strain was successfully constructed for the first time and could convert L-arabinose to L-ribose in one-pot fermentation using the mixture of glucose and L-arabinose.

Keywords *Candida tropicalis* · L-Arabinose · L-Ribose · L-Ribose isomerase

Introduction

Infectious diseases including avian influenza, swine flu, and coronavirus disease 2019 (COVID-19) spread rapidly throughout the world, infect many people, and even lead to death [1]. Different kinds of viral diseases are threatening human beings every year. Moreover, it is difficult to prevent or treat them when various mutations appear. Hence, the need for effective antiviral drugs is getting bigger and bigger. Promising antivirals such as lamivudine, clevudine, and telbivudine are made from nucleoside and nucleotide analogs [2]. They are interrupting viral replication by the termination of viral DNA/RNA synthesis. As L-nucleosides, the enantiomers of D-nucleosides, are proven to be responsible for the antiviral activities of racemic mixtures of the D- and L-nucleosides in general, antivirals based on L-nucleosides have gathered much attention [3].

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The major starting material for the synthesis of L-nucleosides is L-ribose. Primarily, chemical reactions have been applied for the synthesis of L-ribose [4]. The synthesis of L-ribose is accomplished by multiple steps of complex reactions with various catalysts in harsh conditions (high temperature and high pressure). Also, extensive purifications are needed for the removal of unwanted by-products. Therefore, chemical production approaches are not suitable for the industrial scale. Biotechnological methods are promising alternatives as those processes are stereospecific and have simple reaction steps in moderate conditions without harmful catalysts.

The biological production of L-ribose is mainly achieved by enzymes or resting cells. Purified enzymes of mannose-6-phosphate isomerase (EC 5.3.1.8) from *Geobacillus thermodenitrificans* were used for the production of L-ribose from L-ribulose, and the conversion yield was 71% (*w*/*w*) [5]. The *Escherichia coli* cells expressing mannitol-1-dehydrogenase (EC 1.1.1.255) from *Apium graveolens* were investigated for the production of L-ribose from ribitol, and the yield was 55% (*w*/*w*) [6]. The *E. coli* cells expressing D-lyxose isomerase (EC 5.3.1.15) from *Thermoflavimicrobium dichotomicum*, in combination with L-arabinose isomerase (EC 5.3.1.4) from *Alicyclobacillus hesperidum*, converted

L-arabinose to L-ribose with the yield of 20.9% (*w*/*w*) [7]. The L-ribose isomerase (EC 5.3.1.B3) from *Acinetobacter calcoaceticus* DL-28 was expressed in L-ribulokinase-deficient *E. coli* for the conversion of L-arabinose to L-ribose and the yield was 20% (*w*/*w*) [8].

As one of the xylose-assimilating yeasts, *Candida tropicalis* is well-known for its xylitol-producing ability [9–11], and the biological production of xylitol has been developed and showed a high conversion yield of over 90% [12]. *C. tropicalis* can also utilize even *n*-alkanes and fatty acids as carbon sources. Thus, it has been widely used to produce *ω*-hydroxy fatty acids and long-chain dicarboxylic acids [13–15]. However, *C. tropicalis* cannot assimilate L-arabinose, because the cells do not possess genes related to the L-arabinose metabolic pathway. Yoon et al. (2011) expressed the three genes related to the bacterial L-arabinose metabolic pathway, *araA* (L-arabinose isomerase), *araB* (L-ribulokinase), and *araD* (L-ribulose-5-phosphate 4-epimerase), in *C. tropicalis* and constructed an L-arabinose-assimilating strain to reduce L-arabitol accumulation as a by-product during xylitol production. As a result, the recombinant cells assimilated L-arabinose without the accumulation of L-arabitol in the medium [16].

Previously, the L-ribulose-producing strain, *C. tropicalis* K1 CoSTP2 LsaAraA, was constructed with constitutive expression of the transporter protein (AtStp2) and the L-arabinose isomerase (LsaAraA) [17]. In this study, to construct an L-ribose-producing yeast strain from L-arabinose as a substrate, the L-ribose isomerase gene from *A. calcoaceticus* DL-28 was integrated into the strain. The resulting strain, K1 CoSTP2 LsaAraA AcLRI, successfully converted L-arabinose to L-ribose from a glucose/L-arabinose mixture in one-pot fermentation with a conversion yield of about 20% (*w*/*w*).

Materials and methods

Strains and media

The L-ribulose-producing strain with constitutive expression of the transporter protein (AtStp2) and the L-arabinose isomerase (LsaAraA), *C. tropicalis* K1 CoSTP2 LsaAraA, was used in this study (Fig. 1). Genotypes of *C. tropicalis* strains are listed in Table 1. K1 CoSTP2 LsaAraAp, a uracil auxotroph, was used as a host strain for transformation. The *URA3* gene originated from *C. tropicalis* ATCC 20,336 (GenBank accession no. AB006207.1) was used as a selection marker. Partial sequences of *arg* gene obtained from *Bacillus subtilis* were used for pop-out the *URA3* marker. For growth and selection of *C. tropicalis*, YM medium (3 g/L of yeast extract, 3 g/L of malt extract, 5 g/L of peptone, 20 g/L of glucose), YNB medium (6.7 g/L of yeast nitrogen base without amino acids, 20 g/L of glucose), and YNB-5FOA medium (6.7 g/L of yeast nitrogen base without amino acids, 20 g/L of glucose, 0.1 g/L of uracil, 0.1 g/L of uridine, 0.8 g/L of 5′-fluoroorotic acid) were used. *E. coli* DH5*α* (Enzynomics, Daejeon, Korea) was used as a host for the transformation of plasmids. Luria–Bertani medium (5 g/L of yeast extract, 10 g/L of tryptone, 10 g/L of NaCl) containing 50 μg/mL of ampicillin was used to grow the transformed *E. coli* strains. The sequences of all primers used in this study are listed in Table 2.

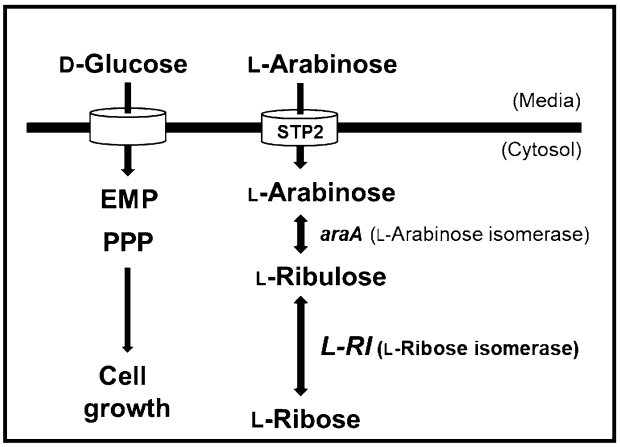

Fig. 1 Metabolic pathway for the production of L-ribose

Codon optimization and construction of expression cassette

The genomic sequences of L-ribose isomerase from *A. calcoaceticus* DL-28 (GenBank accession no. AB062121.1) was used as a source for codon optimization (Supplementary Materials Table S1). Because *C. tropicalis* belongs to the CTG clade species [18, 19], some codons should be changed for appropriate translation. Based on the codon usage database [20], the codon-optimized gene with *Xba*I or *Xho*I on each terminal was synthesized (Bioneer, Daejeon, Korea) and the resulting plasmid was designated as pBHA-AcLRI.

The construction of the expression cassette was described previously [16]. To integrate the expression cassette into *XYL3* locus of *C. tropicalis* K1 CoSTP2 LsaAraAp, the pXK4 plasmid was synthesized containing the construct of a pair of 60-bp homologous fragments of *XYL3* (XK4F and XK4R) with the *Nru*I sites at both ends and the *Bam*HI site in the middle of XK4F and XK4R. The PTAUA fragment containing P_{GAPDH}-T_{GAPDH}-*arg*-*URA3*-*arg* with the *Bgl*II or *Bam*HI sites on each terminal was inserted into the *Bam*HI site of pXK4 and the resulting plasmid was designated as

Table 1 Genotypes of *C. tropicalis* strain used in this study

Strain	Genotypes[a]	References
K1 CoSTP2 LsaAraA	*ura3/ura3 xyl2Δ::hisG/xyl2Δ::hisG xyl1Δ::P_{GAPDH}-CoSTP2-arg/xyl1Δ::hph xyl1Δ::arg/xyl1Δ::trpR xyl3Δ::P_{GAPDH}-LsaAraA-AUA/XYL3*	Yeo et al. (2018)
K1 CoSTP2 LsaAraAp	*ura3/ura3 xyl2Δ::hisG/xyl2Δ::hisG xyl1Δ::P_{GAPDH}-CoSTP2-arg/xyl1Δ::hph xyl1Δ::arg/xyl1Δ::trpR xyl3Δ::P_{GAPDH}-LsaAraA-arg/XYL3*	This study
K1 CoSTP2 LsaAraA AcLRI	*ura3/ura3 xyl2Δ::hisG/xyl2Δ::hisG xyl1Δ::P_{GAPDH}-CoSTP2-arg/xyl1Δ::hph xyl1Δ::arg/xyl1Δ::trpR xyl3Δ::P_{GAPDH}-LsaAraA-arg/xyl3Δ::P_{GAPDH}-AcLRI-AUA*	This study

[a]P_{GAPDH} GAPDH promoter, *AUA arg-URA3-arg*

Table 2 Primers used in this study

Primer	Sequence[a]	Restriction site(s)
XK_F	**GTCTCTTCCATCTTAGCTAACATACC**	
XK_R	**GGAGAAGATGGTGAAGTTATAAGTCC**	
STP2_R	CTCGAGCTAATCCTTGAAGTACTTCTTCCATC	*Xho*I
LsaAraA_R	CTCGAGCTACTTGATGTTAACGTAG	*Xho*I
AcLRI_R	CTCGAGCTAGGAAATAGCGGTTTGC	*Xho*I
ARG_F	AGATCTCTTTCGTTATCGTTGACGCGAAGACC	
HRG_F	GGATCCGCATGTCTTTAGTTCTATGATG	*Bam*HI
Oligo dT	TTTTTTTTTTTTTTTT	
STP2_F	CGGTTACGATATTGGTATTTCCGGTGG	
LsaAraA_F	CCGGTTCCCAATCCTTGTACGGTGAAG	
AcLRI_F	GACCAGAACCTCCATCACTAGAAG	

[a]The restriction sites are underlined and the boldface types indicate homologous sequences to *C. tropicalis*

pXK4_PTAUA. The codon-optimized AcLRI was ligated into the *Xba*I and *Xho*I sites of pXK4_PTAUA and designated as pXK4_PTAUA-AcLRI. The constructed plasmid was linearized by digestion with *Nru*I before the transformation.

Transformation of yeasts

The lithium acetate/single-stranded carrier DNA/polyethylene glycol method with slight modifications [12, 21] was used to transform *C. tropicalis*. Cells were grown in 50 mL of YM media for at least 3 h, harvested by centrifugation at 2000×*g* for 5 min at 25 °C, and washed and resuspended in LiOAc solution (0.1 M LiOAc, 10 mM Tris–HCl, and 1 mM EDTA, pH 8.0). Next, 30 µL of cell suspension was mixed with 30 µL of DNA fragments (30 ng/µL) and 5 µL of salmon testis single-stranded DNA (Sigma, St. Louis, MO, USA). The mixture was added to 325 µL of polyethylene glycol solution (0.1 M LiOAc, 10 mM Tris–HCl, and 1 mM EDTA in 40% (*w*/*v*) polyethylene glycol 8000) and mixed thoroughly. After incubation at 30 °C for 45 min, the mixture was placed in a 42 °C heat block for 15 min. The cells were harvested by centrifugation at 3000×*g* for 3 min at 25 °C. The cell suspension in YNB media was spread onto YNB plates.

A uracil prototroph, as a result of the transformation, was screened to allow the pop-out of the selection marker gene. The transformants were grown in 4 mL of YM media for at least 12 h, plated onto the YNB-5FOA plate for 2 days. Single colonies were picked and incubated onto YNB and YM plates for 2 days to confirm the deletion of the *URA3* gene. The specific removal of the *URA3* gene was examined by PCR.

Yeast total RNA isolation and RT-PCR analysis

A mini-prep version of yeast RNA isolation was performed with slight modifications [22]. Cells grown in YM medium were washed and resuspended in 400 µL of AE buffer (50 mM Na acetate pH 5.3, 10 mM EDTA). The resuspended cells were transferred to a microcentrifuge tube and 40 µL of 10% SDS, 440 µL of buffer-equilibrated phenol (Bioneer, Daejeon, Korea), and glass beads (Sigma) were added. The aqueous phase was extracted with 400 µL of phenol (pH 5.2):chloroform:isoamyl alcohol (25:24:1) (Bioneer) by centrifugation (13,000×*g*, 25 °C for 5 min). The aqueous phase

was added to 40 µL of 0.3 M Na acetate, pH 5.3, after which 2.5 volumes of 95% ice-cold ethanol were added. The tube was incubated for 30 min at -20 °C and centrifuged at 4 °C for 5 min. The pellet was dried at 60 °C for 5 min and resuspended in 55 µL of sterile water and stored at − 70 °C until use.

For cDNA synthesis from total RNAs, 400 pmol of oligo dT was incubated with extracted RNAs at 70 °C for 5 min. The mixture was then chilled rapidly on ice. Each cDNA was synthesized (AccuPower RT PreMix kit, Bioneer) and used in subsequent PCR with specific primers to amplify the protein-coding regions.

L-Arabinose isomerase and L-ribose isomerase activity assays

Cultured yeast cells were harvested by centrifugation and washed with 50 mM potassium phosphate buffer (pH 7.0). The cells were suspended in the same buffer and disrupted using glass beads. Cellular debris was separated by centrifugation (13,000×*g*, 4 °C for 10 min). Protein concentration was determined using a Quant-iT Assay Kit (Invitrogen, Carlsbad, CA, USA).

Under standard conditions, the reaction mixture contained 100 µg of crude extract, 100 mM of L-arabinose or L-ribulose (substrate), and 50 mM acetate buffer (pH 4) in a final volume of 500 µL. The mixture was incubated at 30 °C for 10 min, and the reaction was terminated by adding 50 µL of trichloroacetic acid (10%). The converted L-ribulose or L-ribose was detected by the cysteine carbazole sulfuric acid method [23], and the absorbance was measured at 540 nm. One unit of L-arabinose isomerase or L-ribose isomerase activity was defined as the formation of 1 µmol keto- or aldo-sugar per min under the above-specified conditions.

L-Ribose fermentation and analytical methods

L-Ribose fermentation with *C. tropicalis* K1 CoSTP2 LsaAraA AcLRI was performed in a 250-mL Erlenmeyer flask containing 50 mL of fermentation medium at 30 °C with 200 rpm. The fermentation medium was contained 30 g/L or 50 g/L of L-arabinose, 20 g/L of glucose, 10 g/L of yeast extract, 5 g/L of KH_2PO_4, and 0.2 g/L of $MgSO_4{\cdot}7H_2O$.

The concentrations of glucose, L-arabinose, L-ribulose, and L-ribose were analyzed by high-performance liquid chromatography equipped with a refractive index detector (Waters, Milford, MA, USA). The samples were separated by a Sugar-Pak I column (Waters) with degassed DDW at a flow rate of 0.3 mL/min. Cell growth was determined at 600 nm spectrophotometrically. One A_{600} was equivalent to 0.474 g (dry cell weight)/L.

Results

Homologous excision of the *URA3* gene

In the previous study, the L-ribulose-producing *C. tropicalis* strain was constructed by constitutive expression of proteins which were an L-arabinose isomerase (LsaAraA) and a pentose transporter (AtStp2). The resulting *C. tropicalis* K1 CoSTP2 LsaAraA strain is a uracil prototroph because the strain harbors a stably integrated *URA3* gene in its genome. For additional transformations to construct an engineered strain, however, the *URA3* gene should be removed from the strain. The excision of the selection marker gene was done by homologous recombination between the two *arg* genes on either side of the *URA3* gene (Fig. 2).

After incubation of the *C. tropicalis* K1 CoSTP2 LsaAraA strain for 12 h in YM medium, 200 µL of cell suspension was plated onto the YNB-5FOA plate for 2 days. The colonies on the plate were selected and the deletion of the *URA3* gene was confirmed by examining the growth of the cells on YM and YNB plates. Also, the specific removal of the *URA3* gene was examined by PCR. The resulting strain, a uracil auxotroph, was designated as K1 CoSTP2 LsaAraAp and used as a host strain for the construction of L-ribose-producing strain.

Chromosomal integration of L-ribose isomerase gene

Biotransformation of L-ribulose to L-ribose was done by the isomerization reaction of mannose-6-phosphate isomerase, D-lyxose isomerase, or L-ribose isomerase [5, 7, 8]. Here, L-ribose isomerase from *A. calcoaceticus* DL-28 was used to construct L-ribose-producing strain from L-arabinose since its optimal temperature was in the range of 30–40 °C [24], similar to the optimal temperature for the growth of *C. tropicalis*.

The genomic sequence of the L-ribose isomerase was codon-optimized for heterologous expression as *C. tropicais* utilizes alternative yeast nuclear code which decodes CUG codon as serine, not leucine [18, 19]. The optimized sequence designated as AcLRI was used for the construction of the expression cassette to chromosomally integrate into *XYL3* locus of the *C. tropicalis* K1 CoSTP2 LsaAraAp strain. The *XYL3* gene locus was chosen not to disturb the conversion of L-arabinose to L-ribose through minimizing any unwanted reactions by xylulokinase. The XK4F and XK4R fragments of the expression cassette, homologous to *XYL3*, was enabled the genetic integration of the cassette by homologous recombination. The GAPDH promoter fragments were enabled the constitutive expression of AcLRI.

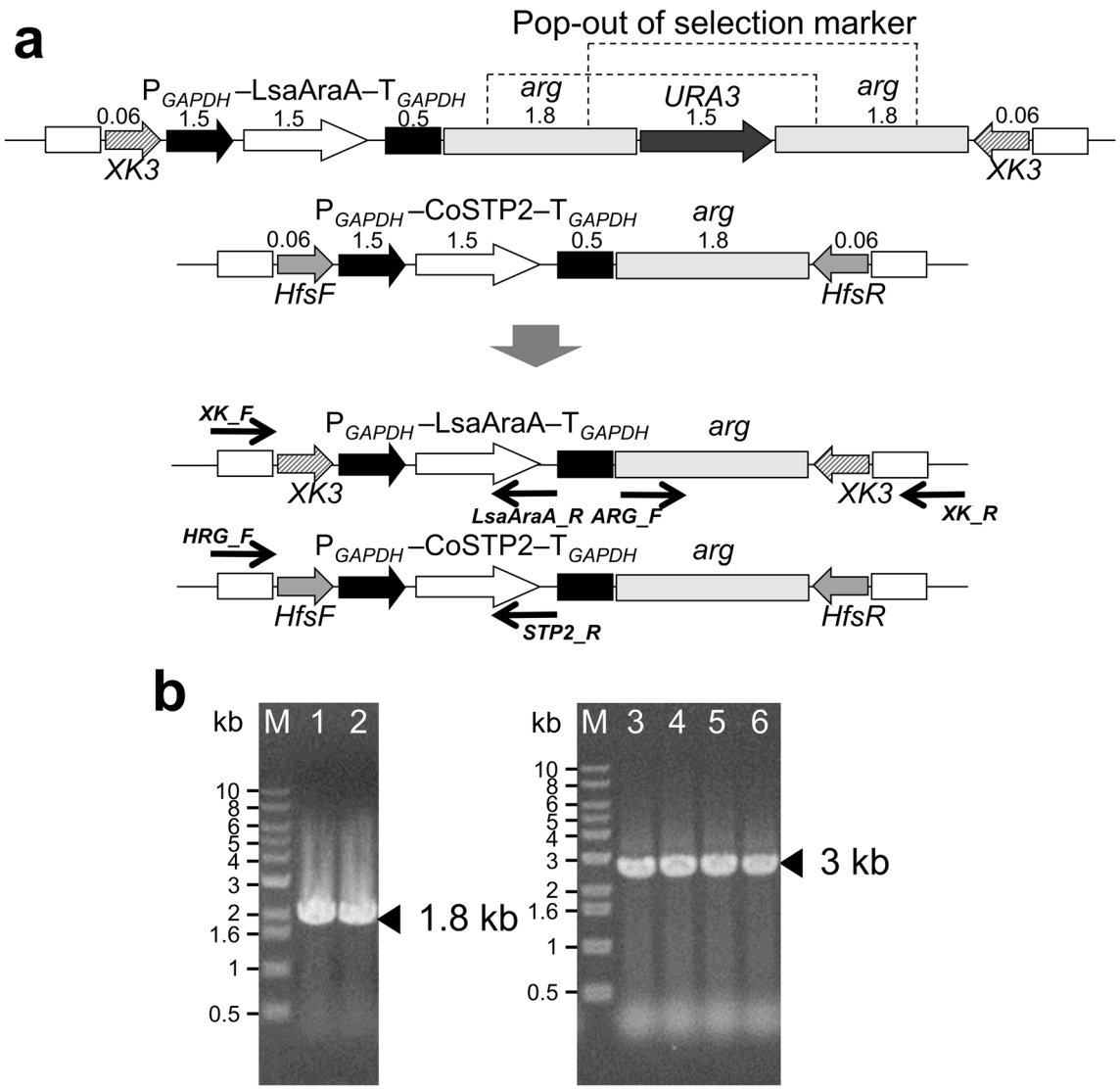

Fig. 2 Homologous excision of the selection marker; **a** Schematic diagram of pop-out of the selection marker; **b** PCR confirmation of pop-out of the K1 CoSTP2 LsaAraA strain. Lane 1 and 2 represent PCR products with ARG_F and XK_R. Lane 3 and 4 represent PCR products with XK_F and LsaAraA_R. Lane 5 and 6 represent PCR products with HRG_F and STP2_R. Lane M contains a DNA size marker. XK3, 60-base pair homologous fragments of the *XYL3*; P_{GAPDH}, the promoter of the *GAPDH*; T_{GAPDH}, the terminator of the *GAPDH*; *arg*, repeating fragment used for pop-out; *URA3*, selection marker; *HfsF* and *HfsR*, homologous fragments of *hph*; LsaAraA and CoSTP2, codon-optimized *araA* from *Lactobacillus sakei* 23 K and *STP2* from *Arabidopsis thaliana*, respectively; XK_F and XK_R, primers that bind to *XYL3*; LsaAraA_R and CoSTP2_R, primers that bind to LsaAraA and CoSTP2, respectively

Uracil prototrophs, as a result of the transformation, formed colonies on YNB plates. Single colonies were selected and the specific integration of the expression cassette was confirmed by PCR with the appropriate primer sets (Fig. 3). The transcription of the AcLRI, LsaAraA, and AtStp2 was confirmed by reverse transcription PCR (Supplementary Materials Fig. S1). The stably integrated strain was designated as K1 CoSTP2 LsaAraA AcLRI and the specific activity of L-arabinose isomerase and L-ribose isomerase was determined as 81.3 ± 13.4 and 98.8 ± 16.7 U/mg, respectively.

Fermentation for the conversion of L-arabinose to L-ribose

It was expected that the newly constructed *C. tropicalis* K1 CoSTP2 LsaAraA AcLRI strain could convert L-arabinose to L-ribose. For investigating the conversion of L-arabinose to L-ribose, fermentation was performed using a medium containing L-arabinose as a substrate. And glucose was used for supporting the growth of cells.

Flask fermentation was performed with 50 mL of fermentation medium containing 20 g/L of glucose and

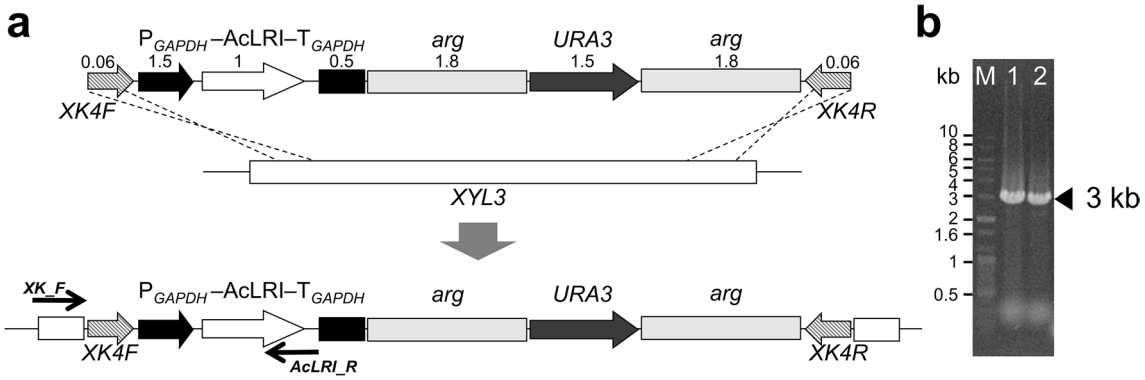

Fig. 3 Construction of the L-ribose-producing strain; **a** Schematic diagram of genomic integration; **b** PCR confirmation of transformants. Lane 1 and 2 represent PCR products with XK_F and AcLRI_R. Lane M contains a DNA size marker. XK4F and XK4R, 60-base pair homologous fragments of the *XYL3*; P_{GAPDH}, the promoter of the *GAPDH*; T_{GAPDH}, the terminator of the *GAPDH*; *arg*, repeating fragment used for pop-out; *URA3*, selection marker; *XYL3*, the gene encoding xylulokinase; AcLRI, codon-optimized *L-RI* from *A. calcoaceticus* DL-28; XK_F, primers that bind to the *XYL3*; AcLRI_R, primers that bind to AcLRI

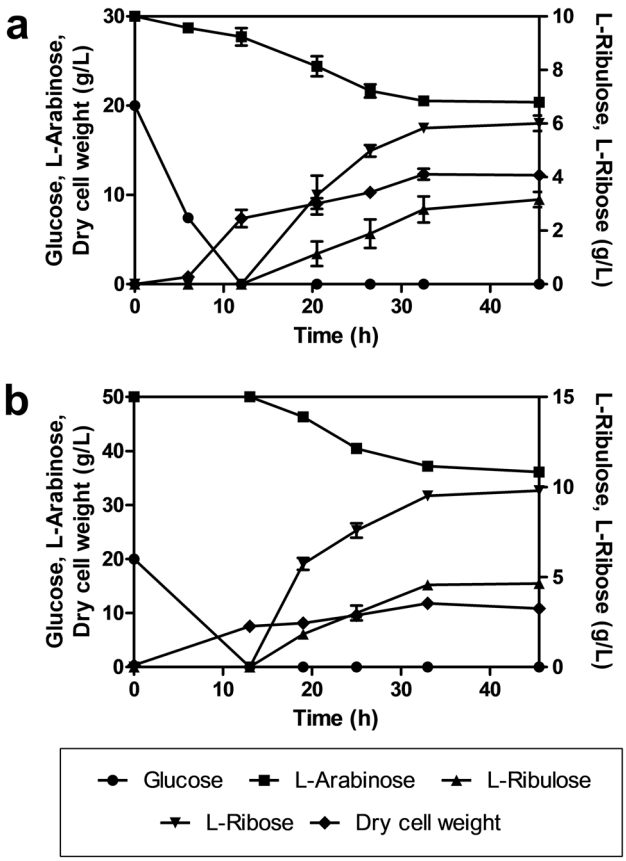

Fig. 4 Flask fermentation for the production of L-ribose; **a** flask fermentation with 30 g/L of L-arabinose as a substrate; **b** Flask fermentation with 50 g/L of L-arabinose as a substrate. All fermentation medium contained 20 g/L of glucose as a carbon source. The error bars indicate the standard deviation

Table 3 The production rates of L-ribulose and L-ribose with 30 g/L or 50 g/L of L-arabinose

Substrate concentration	Production rate of L-ribulose (g/L h)	Production rate of L-ribose (g/L h)
30 g/L of L-arabinose	0.069	0.132
50 g/L of L-arabinose	0.102	0.215

30 g/L of L-arabinose (Fig. 4a). L-Arabinose was gradually converted to L-ribose with the growth of the cell. Upon the exhaustion of glucose, the consumption rate of L-arabinose was increased and L-arabinose was converted into L-ribulose and L-ribose. After 45.5 h of fermentation, 6.0 g/L of L-ribose and 3.2 g/L of L-ribulose were produced from the consumption of 9.2 g/L of L-arabinose. Further increase of L-ribose or L-ribulose was not observed after 32.5 h. The proportion between L-ribose and L-ribulose was approximately 2:1 and the conversion yield of L-arabinose to L-ribose was 20% (*w*/*w*).

To verify the conversion of L-arabinose to L-ribose, the culture was conducted again with the medium where the concentration of L-arabinose was elevated to 50 g/L (Fig. 4b). Similar to the previous experiment, L-arabinose was consumed after the depletion of glucose. By utilizing 14.4 g/L of L-arabinose, 9.8 g/L of L-ribose and 4.6 g/L of L-ribulose were produced after 45.5 h of fermentation. About 1.5-fold increase in the production rates of L-ribulose and L-ribose were observed (Table 3). But, the ratio of L-ribose to L-ribulose and the conversion yield of L-arabinose to L-ribose remained 2:1 and 20% (*w*/*w*), respectively.

Discussion

Biological production of L-ribose from L-arabinose is possible by enzymatic reactions of L-arabinose isomerase and L-ribose isomerase. In the previous study, the efficient L-ribulose-producing strain was constructed by constitutive expression of an L-arabinose isomerase (LsaAraA) and a pentose transporter (AtStp2) [17]. For establishing L-ribose-producing strain, the *C. tropicalis* K1 CoSTP2 LsaAraA strain was engineered to stably integrate the codon-optimized L-ribose isomerase gene from *A. calcoaceticus* DL-28 into its chromosome. The newly transformed strain, designated as K1 CoSTP2 LsaAraA AcLRI, were then fermented to determine the conversion of L-arabinose to L-ribose and 6.0 g/L of L-ribose and 3.2 g/L of L-ribulose were produced from the medium containing 30 g/L of L-arabinose and 20 g/L of glucose.

Purified enzymes or resting cells have been investigated for the production of L-ribose from L-arabinose, L-ribulose, or ribitol [5–8]. For the utilization of enzymes or resting cells, however, the pre-cultivation and harvest steps are necessary. This makes the production process more complicated and expensive. Besides, bacterial endotoxins and the use of antibiotics can cause health problems [25–27]. In this study, to overcome these limitations, the L-ribose-producing yeast strain was constructed with expressing L-arabinose isomerase and L-ribose isomerase under the control of the GAPDH promoter. This strain successfully converted L-arabinose into L-ribose in one-pot fermentation.

For the conversion of L-arabinose to L-ribose, L-ribose isomerization proceeds in concert with L-arabinose isomerization [28]. The equilibrium ratio among L-arabinose/L-ribulose/L-ribose was reported as 71:6:23 in the free enzymes, and 63:16:21 in resting cells [5, 7]. In this study, the ratio was calculated as 7:1:2. Although the exact values are somewhat different, it is consistent that most of the L-arabinose remains after the reaction because of the inefficient conversion from L-arabinose to L-ribulose [29].

In general, the isomerization of aldopentose (L-arabinose) to ketopentose (L-ribulose) is thermodynamically unfavorable [30, 31]. The addition of borate resulting in stable borate-ketose complexes could improve the yield of L-ribulose [32], but extra purification for borate removal is required, which makes the production process complicated. Another promising candidate for the enhanced L-ribulose production to overcome the limited conversion of isomerization is the dehydrogenation of L-arabitol by L-arabitol 2-dehydrogenase. This energetically favored reaction, however, has limitations because the enzyme is catalytically efficient in reduction reaction rather than oxidation under physiological conditions [33]. Further studies for the discovery of novel enzymes or protein engineering are needed for the oxidation of L-arabitol. When efficient L-arabitol 2-dehydrogenase is expressed in combination with the expression of L-arabinose reductase, it is expected that increased production of L-ribulose could be possible by the recombinant strain with L-arabinose as a substrate.

To the best of our knowledge, it is the first recombinant yeast strain to convert L-arabinose to L-ribose in a one-step fermentation. As a substrate for L-ribose production, L-arabinose can be obtained from agricultural wastes such as birch bark, sugarcane bagasse, corncob, and coconut shell. By applying this developed fermentation process industrially, the biological production of L-ribose will be economically feasible.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s00449-020-02506-2.

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Availability of data and materials All data generated or analyzed during this study are included in this published article and its supplementary information files.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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