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Review

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Metabolic Engineering for Improved Fermentation of L-Arabinose

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L-Arabinose, a five carbon sugar, has not been considered as an important bioresource because most studies have focused on D-xylose, another type of five-carbon sugar that is prevalent as a monomeric structure of hemicellulose. In fact, L-arabinose is also an important monomer of hemicellulose, but its content is much more significant in pectin (3–22%, g/g pectin), which is considered an alternative biomass due to its low lignin content and mass production as juice-processing waste. This review presents native and engineered microorganisms that can ferment L-arabinose. *Saccharomyces cerevisiae* is highlighted as the most preferred engineering host for expressing a heterologous arabinose pathway for producing ethanol. Because metabolic engineering efforts have been limited so far, with this review as momentum, more attention to research is needed on the fermentation of L-arabinose as well as the utilization of pectin-rich biomass.

Keywords: L-Arabinose, pectin, metabolic engineering, *Saccharomyces cerevisiae*, pentose

Significance of L-Arabinose as a Bioresource

Bioconversion of lignocellulosic biomass such as agricultural residues and wood waste materials into fuels and value-added chemicals is technically challenging due to a variety of factors [1–4]. Lignocellulosic biomass is composed of 40–50% cellulose, 25–30% hemicellulose, and 15–20% lignin, and the high lignin content requires strong physical and chemical pretreatment for its decomposition [5]. Also, hemicellulose is hydrolyzed into a mixture of glucose, xylose, and other minor sugars such as L-arabinose, and their fermentation is not efficiently done by any native industrial microorganisms [6]. For these reasons, industrial bioprocesses utilizing lignocellulosic biomass have not been realized so far, and the search for other alternative renewable biomass continues [7–10].

Meanwhile, fruit processing wastes such as orange peels are becoming abundant with the growth of the fresh juice industry, but are not being efficiently utilized [11, 12]. Fruit processing wastes are high in pectin (12–35%, g/g dry weight) with much less lignin content (approximately 2%, g/g dry weight) than lignocellulosic biomass [13]. Therefore, this pectin-rich biomass can be easily broken down into monomers; however, pectin monomers, like hemicellulose monomers, are not easily metabolized by common industrial hosts [14].

The primary chemical structure of pectin is methylated polygalacturonic acid in an alpha-(1-4) chain with branched oligosaccharides consisting of arabinose, galactose, xylose, and some minor sugars [15]. Among them, L-arabinose is one of the most abundant pentose sugars in pectin [16]. Arabinose content in various fruits and vegetables ranges from 3.3 to 21. 6 g/l (summarized in Table 1) [14, 17, 18]. It is contradictory to lignocellulosic biomass which has limited arabinose content (approximately 0.2%, g/g dry weight) [19].

L-Arabinose is a five-carbon sugar like xylose. Unlike other sugars that naturally occur in the D-form, such as D-xylose, L-arabinose is a component of pectin and hemicellulose, and it is more common than D-arabinose in nature. Although studies have been conducted extensively for xylose metabolism to realize lignocellulosic bioprocesses [20], L-arabinose metabolism has not received much attention. In the present review, microbial strains that can natively metabolize L-arabinose are summarized. In some studies, the strains were engineered to produce useful products such as ethanol. Moreover, metabolic engineering efforts to develop efficient L-arabinose-fermenting strains

Table 1. Representative pectin-rich biomass and their arabinose content.

Source	Arabinose, %[1)]	Other major sugars[2)]	Refs
Sugar beet pulp	21.6 (0.28)	Glu	[17, 18]
Lime peel	8.5	Glu	[14]
Pear peel	6.0	Glu, Xyl, Fru	[14]
Orange peel	5.6 (0.20)	Glu, Fru	[14, 17]
Apple pomace	5.5	Glu, Fru, Suc	[14]
Mandarin peel	3.3	Glu, Fru, Suc	[14]

[1)]% Dry matter (g/g pectin).

[2)]Higher content than arabinose. Glu, Glucose; Xyl, xylose; Fuc, Fucose; Suc, Sucrose.

using non-native but industrial hosts are discussed, focusing on *Saccharomyces cerevisiae*.

Native L-Arabinose-Fermenting Microorganisms

Arabinose catabolic pathways of native strains can be divided into the oxidoreductase (fungal) and isomerase (bacterial) pathways (Fig. 1). In both pathways, L-arabinose is converted into D-xylulose-5-phosphate, which is then canonically metabolized by the non-oxidative pentose phosphate pathway [21], or alternatively by the phosphoketolase pathway such as that in *Clostridium acetobutylicum* [22].

In L-arabinose-fermenting native fungi, L-arabinose is converted into D-xylulose by two reduction and two oxidation reactions, which are composed of NAD(P)H-specific aldose reductase (AR or XR), NAD^+-specific L-arabitol-4-dehydrogenase (LAD), NAD(P)H-specific L-xylose reductase (LXR or ALX), and NAD^+-specific xylitol dehydrogenase (XDH) [21]. Then, D-xylulose is phosphorylated into D-xylulose-5-phosphate by D-xylulokinase (XK). As highlighted in Fig. 1, fungal pathways of L-arabinose and D-xylose share three enzymes: AR (XR), XDH and XK. Although the cofactor preferences of AR and LXR (ALX) vary among fungal species, the first reductase enzyme usually prefers NADPH, while the two dehydrogenases strictly use NAD^+. Therefore, redox balance of the pathway leading to efficient cell growth is achieved under aerobic conditions [23, 24]. Under oxygen-limited conditions, L-arabitol might be produced due to NAD^+ limitation, which is not found in the bacterial pathway of L-arabinose metabolism [25–27]. Because of the oxygen-dependent nature of the fungal pathway, ethanol production by these native strains is marginal [28, 29].

As early as 1990, a few native arabinose-fermenting fungi strains were identified, but the strains yielded a trace level of ethanol or even no ethanol production [26, 28]. In detail, 116 different yeast strains were screened for the ability to catabolize arabinose or xylose aerobically. As a result, four yeast strains (*Ambrosiozyma monospora*, and three *Candida spp.*) were found to ferment L-arabinose as a sole carbon source. Additionally, the ethanol yield was at most 0.18 (g/g

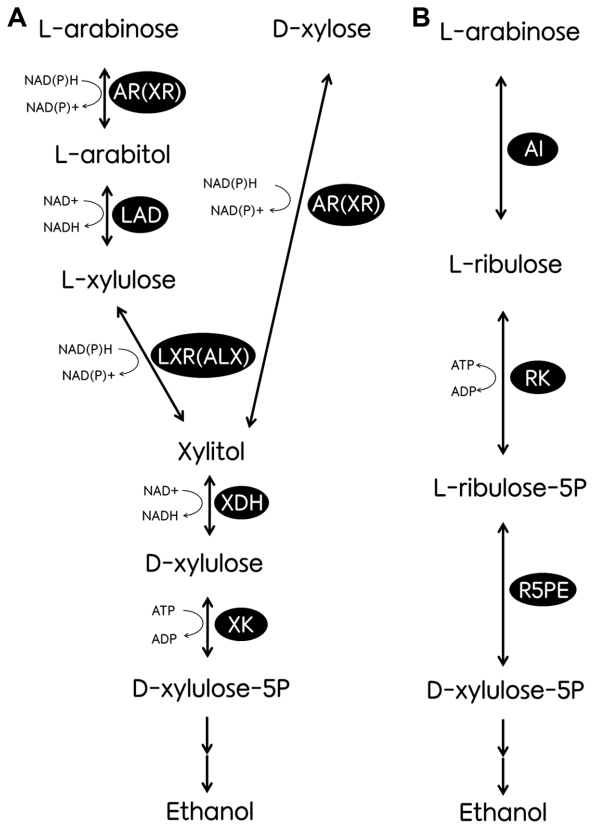

Fig. 1. Arabinose metabolic pathways in fungi (**A**) and bacteria (**B**).

AR(XR); Aldose reductase, LAD; L-arabitol-4-dehydrogenase, LXR(ALX); L-xylulose reductase, XDH; D-xylulose reductase, XK; Xylulokinase, AI; L-arabinose isomerase, RK; L-Ribulokinase, R5PE; L-Ribulose-5-P-4-epimerase.

Table 2. Native arabinose-assimilating microorganisms and their engineered strains producing ethanol.

Strain	Genotype[1)]	Fermentation conditions[2)]		Ethanol production		Refs
		Arabinose (g/l)	Aeration	Titer (g/l)	Yield (g/g)	
Yeast						
Ambrosiozyma monospora Y-1484	Wild type	80	OL	4.1	0.18	[28]
Candida succiphila Y-11998	Wild type	80	OL	3.9	0.05	[28]
Bacteria						
Sarcina ventriculi	Wild type	19	AN	4.7	0.31	[31]
Klebsiella oxytoca P2	*Zm_pdc*, *Zm_adhB*	80	OL	27.2	0.34	[32]
Escherichia coli FBR3	*Zm_pdc*, *Zm_adhB*, *ldh*Δ, *pfl*Δ	100	OL	44.4	0.46	[33]

[1)]*Zm*, *Zymomonas mobilis*; *pdc*, pyruvate decarboxylase gene; *adhB*, alcohol dehydrogenase gene; *ldh*, lactate dehydrogenase gene; *pfl*, pyruvate formate lyase gene.

[2)]All fermentations were performed with complex media with an initial arabinose concentration as shown above. Arabinose was the only carbon source available. OL, oxygen-limited conditions; AN, anaerobic conditions.

consumed arabinose) with *A. monospora* and *C. succiphila* (Table 2).

Meanwhile, 15 xylose-fermenting microorganisms were screened to evaluate the ability to ferment L-arabinose to ethanol [26]. As a result, one bacterium (*Erwinia chrysanthemi*), six yeast strains (*C. tropicalis, C. shehatae, Pachysolen tannophilusY-2460, P. tannophilus Y-12891, Scheffersomyces stipitis,* and *Torulopsis sonorensis*), and one mold strain (*Aspergillus oryzae*) were confirmed to assimilate arabinose with xylose and glucose as co-substrates. While *E. chrysanthemi* and *C. tropicalis* consumed xylose and arabinose simultaneously, all other fungal strains preferred xylose over arabinose. During arabinose metabolism, *S. stipitis* was the only fungal strain producing ethanol at a yield of 0.15 (g/g consumed sugar) and arabitol at a yield of 0.24 (g/g consumed sugar).

In comparison to the above-mentioned fungal pathways, bacterial pathways of L-arabinose metabolism are relatively simple; 1) only three enzymes are needed to convert to D-xylulose-5-phosphate, and 2) no cofactor is involved. The bacterial pathway consists of L-arabinose isomerase (AI), L-ribulose kinase (RK), and L-ribulose-5-phosphate-4-epimerase (R5PE) encoded by the *araA*, *araB*, and *araD* genes, respectively (Fig. 1B) [30]. In addition, native bacterial strains such as *Sarcina ventriculi* can ferment L-arabinose anaerobically and produce ethanol efficiently at a yield of 0.3 (g/g consumed arabinose) [31]. For some bacterial strains lacking the *pdc* and *adh* genes (encoding pyruvate decarboxylase and alcohol dehydrogenase, respectively) such as *Klebsiella oxytoca*, introducing the genes from *Zymomonas mobilis* enabled ethanol fermentation from L-arabinose [32]. For native L-arabinose-metabolizing *Escherichia coli*, in contrast, deletion of lactate dehydrogenase (*ldh*) and pyruvate formate lyase (*pfl*) genes was required to enable ethanol production from L-arabinose [33]. As summarized in Table 2, native bacterial strains assimilating L-arabinose can be promising hosts for ethanol fermentation.

Engineering *Saccharomyces cerevisiae* for L-Arabinose Fermentation

S. cerevisiae, the industrial host for bioethanol production, cannot utilize L-arabinose as efficiently as it can utilize xylose. For cellulosic ethanol production, a great amount of effort has been focused on the development of xylose-fermenting *S. cerevisiae* strains, while there have not been many examples for L-arabinose.

There are a limited number of studies on the development of *S. cerevisiae* expressing a heterologous fungal pathway of L-arabinose metabolism compared to that expressing a heterologous bacterial pathway [30, 34–38]. Usually, xylose-fermenting *S. cerevisiae* expressing heterologous AR, XDH, and XK is first engineered by expressing *Scheffersomyces stipitis XYL1*, *XYL2*, and *XYL3* genes, respectively, in most cases [20]. It has to be noted that an AR is NAD(P)H-specific aldose reductase with specificity for both xylose and L-arabinose [39] with a 50% higher rate for L-arabinose metabolism when using NADPH as a cofactor [40]. Next, the resulting strain is further engineered to express LAD and LXR, which are *T. reesei LAD1* and *A. monospora ALX1* genes, respectively [24, 34]. The *S. cerevisiae* 424A(LNH-ST)/pLXR$_{NAD}$_LAD strain, which was developed as above, produced 10 g/l ethanol from 45 g/l L-arabinose [35] as summarized in Table 3. In another study, *T. reesei LXR1* gene was expressed instead of *A. monospora ALX1* gene, but the resulting strain only produced 0.1 g/l ethanol from 50 g/l L-arabinose [35, 41]. The low ethanol production can be explained by the fact that *T. reesei LXR1* gene is now

Table 3. Ethanol fermentation by engineered *Saccharomyces cerevisiae* using arabinose as the sole carbon source.

Strain names	Strain backgrounds	Arabinose pathways	Optimization strategies	Media	Arabinose (g/l)	Aeration	Titer (g/l)	Yield (g/g consumed)	Refs
Heterologous fungal pathways									
H2561	CEN.PK2	Ss*XYL1*, Ss*XYL2*, Sc*XKS1*, Tr*LAD1*, Tr*LXR1*	-	Minimal	50	AN	0.1	-	[34]
424A(LNH-ST)/ pLXR$_{NAD}$-LAD	424A	Ss*XYL1*, Ss*XYL2*, Ss*XYL3*, Tr*LAD1*, Am*ALX1*	-	Complex	45	OL	9.4	0.22	[35]
Heterologous bacterial pathways									
JBY25-4M	CEN.PK2-1C	Bs*araA*, Ec*araB*, Ec*araD*, Sc*GAL2*	-	Minimal	20	OL	6	0.3	[36]
BWY1-4S	CEN.PK2-1C	Bs*araA*, Ec*araB*, Ec*araD*, Sc*GAL2*	Codon optimization Adaptive evolution	Minimal	30	AN	9	0.39	[43]
IMS0002	CEN.PK2-1C	Lp*araA*, Lp*araB*, Lp*araD*	PPP overexpression Adaptive evolution	Complex	20	AN	8.92	0.45	[30]
BSW3AP	CEN.PK102-3A	Lp*araA*, Lp*araB*, Lp*araD*	PPP overexpression Adaptive evolution	Minimal	20	OL AN	6.9 -	0.43 0.42	[37]
BSW3AG	CEN.PK102-3A	Lp*araA*, Lp*araB*, Lp*araD*, ScGAL2	PPP overexpression Adaptive evolution	Minimal	20	AN	-	0.43	[37]

OL, oxygen-limited conditions; AN, anaerobic conditions; Bs, *Bacillus subtilis*; Ec, *Escherichia coli*; Sc, *Saccharomyces cerevisiae*; Tr, *Trichoderma reesei*; Am, *Ambrosiozyma monospora*; Pi, *Piromyces sp.*; Lp, *Lactobacillus plantarum*; Nc, *Neurospora crassa*; Mt, *Myceliophthora thermophile*; Ss, *Scheffersomyces stipitis*; PPP, pentose phosphate pathway.

functionally identified as mannitol dehydrogenase [42]. Another factor determining L-arabinose fermentation efficiency is aeration. The fungal L-arabinose pathway is not redox-neutral because of the dual cofactor preference of AR and LXR (NADPH and NADH) while XDH and LAD are NAD^+-specific. Therefore, the cofactor imbalance issue could be more severe than xylose fermentation requiring just AR and XDH.

Meanwhile, the bacterial L-arabinose pathway is redox-neutral; thus, more studies have been performed for heterologous expression in *S. cerevisiae* from genes of *Bacillus subtilis*, *Escherichia coli* and *Lactobacillus plantarum*. The bacterial L-arabinose pathway consisting of *araA, araB* and *araD* genes was tested with various combinations from different origins (Table 3). The *araA* gene from *Bacillus subtilis* [36], *Bacillus licheniformis* [43, 44] and *Lactobacillus plantarum* [30, 37] and the *araB* and *araD* genes from *Escherichia coli* [43, 44] or *L. plantarum* [30, 37] were tested. Ethanol production from the engineered strains varied between 6–9 g/l from 20 g/l L-arabinose. Regardless of the origin of the heterologous genes, various approaches to improve L-arabinose fermentation have been performed. In general, the overexpression of the non-oxidative pentose phosphate pathway genes (*TAL1, TKL1, RPE1* and *RKI1*) and adaptive evolution were required [30, 36, 43]. However, despite all optimizations, the fermentation productivity is limited by a bacterial pathway possibly because of the unfavorable thermodynamic properties of L-arabinose isomerase under ambient conditions [45].

It should be noted that adaptive evolution is proven to be an effective metabolic engineering strategy to improve xylose fermentation by engineered *S. cerevisiae* strains for both fungal and bacterial pathways [20, 46]. However, for L-arabinose fermentation, only the engineered strains with a bacterial pathway have been subjected to adaptive evolution (Table 2). It can be explained by the fact that L-arabinose fermentation has not been performed systematically and extensively compared to xylose fermentation. It is also possible that the heterologous expression of a fungal pathway in *S. cerevisiae* requires multiple strategies to be optimized to overcome the severe redox imbalance issue.

Arabinose fermentation can be improved by expressing L-arabinose-specific sugar transporters (Table 4). *S. cerevisiae*

Table 4. Characterization of putative arabinose transporters over-expressed in a hexose transporter null mutant of *Saccharomyces cerevisiae*.

Transporter genes	Arabinose uptake rate (mmol/h/g DCW)	Arabinose affinity (mM)	References
Neurospora crassa LAT-1	116.7	58.12	[50]
Myceliophthora thermophile LAT-1	10.29	29.39	[50]
Penicillium chrysogenum AraT	5.30	0.13	[51]
Saccharomyces cerevisiae GAL2	0.13	57.00	[48]
Arabidopsis thaliana Stp2	0.04	4.50	[48]
Scheffersomyces stipites AraT	0.02	3.80	[48]

could uptake arabinose through some hexose transporters such as Hxt5 and Hxt7 with low affinity [47]. *S. cerevisiae* Gal2 had the highest affinity to L-arabinose (57 mM) among other native hexose transporters [48, 49]. It is also reported that *S. cerevisiae* Gal2 contributed to anaerobic arabinose fermentation when arabinose is the sole carbon source [37]. Several heterologous arabinose transporters have been identified from *Neurospora crassa* [50], *Myceliophthora thermophile* [50], *Penicillium chrysogenum* [51], *Arabidopsis thaliana* [48], and *Scheffersomyces stipitis* [48], and their ability to uptake L-arabinose varies significantly (0.02–116.7 mmol/h/g DCW). *N. crassa* LAT-1 was the most efficient L-arabinose transporter reported with a rate of 116.7 (mmol/h/g DCW), which is 2 orders of magnitude higher than that of *S. cerevisiae* GAL2 0.13 (mmol/h/g DCW) [50]. *P. chrysogenum* AraT was a high-affinity arabinose transporter with no activity with glucose and xylose, although it was still inhibited by the presence of glucose and xylose [51, 52]. At the present stage, no heterologous sugar transporter was reported to either improve L-arabinose fermentation or allow simultaneous uptake of arabinose and glucose. In arabinose metabolism, as in xylose's case [53, 54], it can be assumed that arabinose catabolism is currently more limiting than non-specific arabinose uptake in engineered *S. cerevisiae*.

Engineering of Other Microorganisms for Arabinose Fermentation

Some non-native arabinose fermenting microorganisms have also been engineered to assimilate L-arabinose and produce ethanol or other products. *Z. mobilis* (pZB206), which natively carries *pdc* and *adhB* but lacked arabinose-assimilating enzymes, was constructed by introducing *E. coli araABD*, *talB*, *tktA* to metabolize arabinose to ethanol [55]. The resulting strain showed an ethanol yield of 0.49 by consuming 25 g/l L-arabinose. Unlike *S. cerevisiae*, *Corynebacterium glutamicum* expressing *E. coli araABD* was easily engineered to produce amino acids such as L-glutamate, L-lysine, L-ornithine and L-arginine with arabinose as the sole carbon source [56]. For example, one of the engineered *C. glutamicum* strains produced L-glutamate at a yield of 0.07 from 75 g/l L-arabinose. *Rhodococcus opacus* expressing *Streptomyces cattleya araABD* fermented 16 g/l L-arabinose as the sole carbon source and produced fatty acids at a yield of 0.13 (g/g consumed arabinose). The fatty acids were mostly palmitic acid with some cis-10-heptadecenoic acid, oleic acid, myristic acid, pentadecanoic acid, palmitoleic acid, heptadecanoic acid, and stearic acid. Although the examples are limited, non-*Saccharomyces cerevisiae* strains can also be engineered to ferment L-arabinose and produce various value-added products other than ethanol.

Future Outlook

Both crop biomass and cellulosic biomass do not support sustainable bioprocesses due to their low contribution to greenhouse gas reduction [57] and limited technologies to overcome the recalcitrance [58], respectively. Alternatively, pectin-rich biomass such as fruit-processing wastes can be an attractive choice due to low lignin content and the growing demands for fresh juice. L-Arabinose is a primary sugar of pectin structure and its content is minimal in other biomass. As discussed in this review, research for L-arabinose fermentation is in an early stage. Thus, all options are open to either optimizing native arabinose-assimilating strains or engineering non-native strains such as *S. cerevisiae*, *Z. mobilis*, *C. glutamicum*, and *R. opacus* depending on the desired products. Engineered *S. cerevisiae* strains fermenting L-arabinose are still limited to ethanol production, however, various chemicals and value-added

products are expected to be studied as well. Additionally, the development of strains fermenting other pectin-derived monomers such as galacturonic acid and L-rhamnose needs to be considered.

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Conflict of Interest

The authors have no financial conflicts of interest to declare.

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