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REVIEW

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# α-L-Arabinofuranosidases: the potential applications in biotechnology

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**Abstract** Recently, α-L-arabinofuranosidases (EC3.2.1.55) have received increased attention primarily due to their role in the degradation of lignocelluloses as well as their positive effect on the activity of other enzymes acting on lignocelluloses. As a result, these enzymes are used in many biotechnological applications including wine industry, clarification of fruit juices, digestion enhancement of animal feedstuffs and as a natural improver for bread. Moreover, these enzymes could be used to improve existing technologies and to develop new technologies. The production, mechanisms of action, classification, synergistic role, biochemical properties, substrate specificities, molecular biology and biotechnological applications of these enzymes have been reviewed in this article.

**Keywords** α-L-arabinofuranosidases · Lignocelluloses · Synergistic role · Classification · Applications

## Introduction

Lignocelluloses of plant cell walls are composed of cellulose, hemicellulose, pectin and lignin. Hemicelluloses are one of the most abundant renewable polymers on the earth. Moreover, cellulose, hemicelluloses, lignin and pectins are the key components in the degradation of lignocelluloses. Many enzymes are involved in the degradation of these polymeric substrates [129]. L-arabinosyl residues are widely distributed in these polymers as side chains. The presence of these side chains restricts the enzymatic hydrolysis of hemicelluloses and pectins [93, 99, 101]. Further, it also represents a formidable technological barrier that retards the development of various industrial processes [99]. The use of a single accessory enzyme for partial or specific modification of lignocelluloses might offer new interesting options for the utilization of these low-cost raw materials [72, 110].

The α-L-arabinofuranosidases (α-L-AFases) are accessory enzymes that cleave α-L-arabinofuranosidic linkages and act synergistically with other hemicellulases and pectic enzymes for the complete hydrolysis of hemicelluloses and pectins [77, 113]. These enzymes warrant substantial research efforts because they represent potential rate-limiting enzymes in the degradation of lignocelluloses from agricultural residues [99]. The action of α-L-AFase alone or in combination with other lignocellulose-degrading enzymes represents a promising biotechnological tool as alternatives to some of the existing chemical technologies such as chlorination in pulp and paper industry [44, 46, 74], synthesis of oligosaccharides [94, 95] and pretreatment of lignocelluloses for bioethanol production [100, 101]. Considering the potential and future prospects of α-L-AFases, this paper reviews the various aspects of these enzymes with emphasis on their potential for biotechnology.

## Hemicelluloses and pectins

Hemicelluloses and pectins are the matrix polysaccharides of the plant cell wall. They account for 25–35% of lignocellulose biomass [99]. The hemicellulose xylans contain a β-1,4-linked D-xylose backbone [30]. In many plants, xylan backbone is substituted by different side chains with L-arabinose, D-galactose, acetyl, feruloyl, *p*-coumaroyl and glucuronic residues [1, 30]. Xylans from grasses, cereals, softwood and hardwood differ in their composition. This is due to the differences in the frequency and composition of the side chain substituents of xylans [30, 99, 100]. Similarly, arabinoxylans are found in the cell walls of the cereal plants and grasses belonging to the family Gramineae [1, 70]. They contain xylan backbone that is partially substituted at intervals with α-L-arabinofuranose residues [1]. Moreover, wheat arabinoxylan also contains other substituents as shown in Fig. 1 [1, 30].

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Pectins are a family of complex heteropolysaccharides that contain two well-defined regions called as smooth and hairy (Fig. 2) [17, 30]. The three pectic polysaccharides homogalacturonan, rhamnogalacturonan-I and substituted galacturonan have been isolated from plant cell walls [28, 30]. The dominant feature of the pectins is the presence of a linear backbone of galacturonic acid containing varying proportion of methyl ester groups. Pectin polymer backbone is interspersed at intervals with rhamnose residues carrying the neutral sugars side chains containing arabinose and galactose that form arabinans, arabinogalactans or galactans (Fig. 2) [17, 49]. Pectins are abundant in the soft tissues of citrus fruits [49, 131], sugar beet pulp and apple [20, 28].

## The α-L-AFases

The α-L-AFases (α-L-arabinofuranoside arabinofuranohydrolases, EC 3.2.1.55) are the enzymes involved in the hydrolysis of L-arabinose linkages. These enzymes have been purified from several bacteria, fungi and plants [51, 73, 93]. They form a part of the array of glycoside hydrolases required for the complete degradation of arabinose-containing polysaccharides [99, 115]. The action of these enzymes accelerates the hydrolysis of the glycosidic bonds by more than $10^{17}$ fold, making them one of the most efficient catalysts known [98, 107]. Such enzymatic hydrolysis releases soluble substrates, which are utilized by both prokaryotic and eukaryotic microorganisms [77]. The α-L-AFases specifically catalyze the hydrolysis of terminal nonreducing-α-L-1,2-, α-L-1,3- and α-L-1,5-arabinofuranosyl residues from different oligosaccharides and polysaccharides [99, 101, 112]. Whereas the nature of a glycone sugar can influence the catalytic activity of other arabinose-releasing enzymes, the α-L-AFases do not distinguish between the saccharide link to the arabinofuranosyl moiety and thus exhibit wide substrate specificity [93, 97]. Effective hydrolysis of α-L-arabinofuranosyl residues from various pectic, homo-hemicellulosic polysaccharides (branched arabinans, debranched arabinans), heteropolysaccharides (arabinogalactans, arabinoxylans, arabinoxyloglucans, glucuronoarabinoxylans, etc.) and different glycoconjugates is carried out by the α-L-AFases [8, 112]. Moreover, most microbial α-L-AFases are secreted into the culture media; thus, they are likely to attack polysaccharides [84].

## The synergistic role of α-L-AFases

The importance of α-L-AFases has come from the fact that arabinose side chains on hemicelluloses and pectins participate in cross-linking within the plant cell wall structure. The presence of these side chains also affects the form and functional properties of hemicelluloses and pectins [29]. They reduce the interaction between polymers chains due to their inherently more flexible water-hungry furanose conformations. Moreover, the L-arabinofuranoside substitutions on xylan strongly inhibit the action of xylan-degrading enzymes (Fig. 1), thus preventing the complete degradation of the polymer to its basic xylose units [99, 107]. Similarly, L-arabinofuranoside substitutions in pectin (Fig. 2) prevent the complete degradation of this polymer to its basic units. The α-L-AFases act synergistically with other hemicellulases and pectinases for the complete degradation of hemicelluloses and pectins, respectively [4, 29, 67, 102]. Moreover, in some cases, α-L-AFases possessing *β*-xylosidase activity or xylanases with α-L-arabinofuranosidase activity also have been described [73, 74, 83, 121]. Furthermore, some α-L-AFases with both exo- and endo-activity on arabinan, one of the major constituents of pectins, has been reported [11, 87].

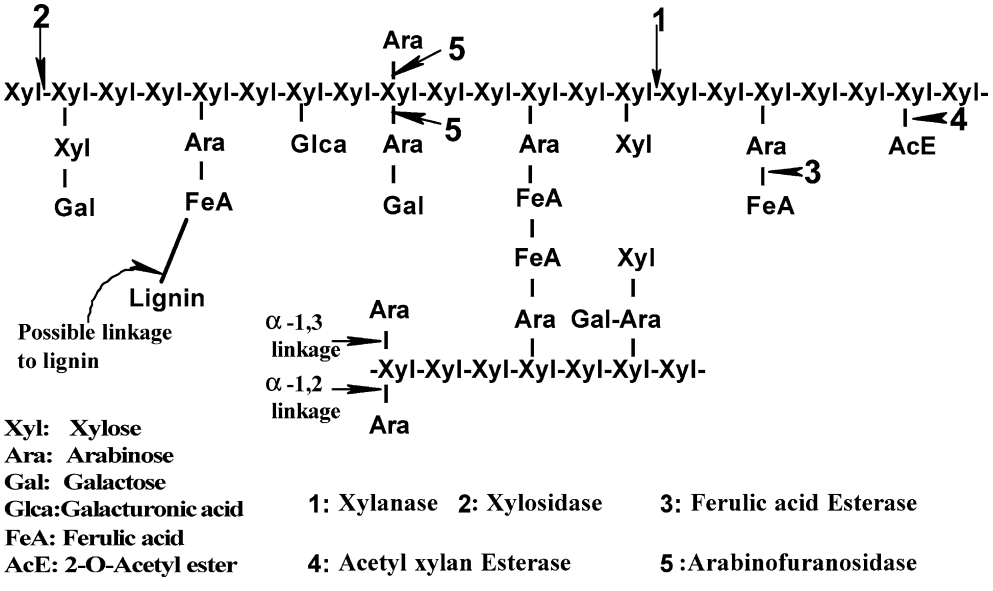



**Fig. 1** The main structural features considered present within water-soluble wheat arabinoxylans. The diagram also indicates the variety of enzymes that are active against arabinoxylans. Modified from Ref. [1]

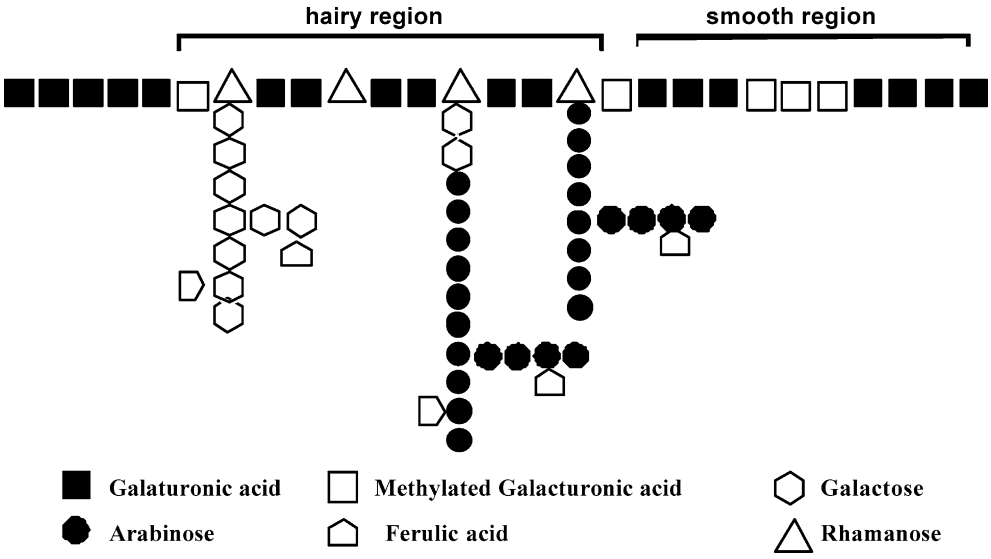



**Fig. 2** Schematic drawing of pectin (rhamnogalcturonan I) showing the smooth and hairy regions. Modified from Ref. [30]

The role of α-L-AFases in the degradation of arabinose-containing polymers is well known. They have a cooperative role facilitating the action of other lignocellulose-degrading enzymes [118, 120]. This has been confirmed for α-L-AFase from *Thermomonospora fusca* that worked in truly synergistic relationship with endoxylanase from the same bacterium releasing 0.6 and 0.3 mg of reducing sugars from oat spelt xylan and ball-milled wheat straw, respectively [4]. α-L-AFase played an important role to increase the release of reducing sugars from these lignocelluloses. However, other authors report the synergistic action of these enzymes with other pectinases and hemicellulases on lignocelluloses. For instance, the two enzymes α-L-AFases (kabfA and kabjB) from *Aspergillus kawachii* acted synergistically with xylanase in the degradation of arabinoxylan, releasing higher amounts of ferulic acid in the presence of feruloyl esterase [68]. Furthermore, Hashimoto and Nakata [51] showed that hemicellulose from soy sauce materials was decomposed synergistically by xylanase, β-xylosidase and α-L-AFase produced by *Aspergillus oryzae* HL15 during moromi[1] fermentation. They also suggested that α-L-AFase of *A. oryzae* HL15 was very closely involved in releasing not only arabinose but also xylose into moromi mash. The same effect has been shown when these enzymes act synergistically on arabinoxylan. Moreover, an exo-arabinanase, Abnx from *Penicillium chrysogenum*, released very little arabinobiose from arabinan, as the action of Abnx was inhibited by the arabinofuranose unit linked as a side chain [102]. When Abnx acted in combination with either α-L-AFases (AFQ1 or AFS1), from the same fungus, the arabinose contents in the reaction mixtures were higher than the sum of those by the two enzymes acting separately [102].

[1]Moromi is a fermenting mixture or mash of rice, water, koji (malted soybeans) and *A. oryzae*, which is produced during the traditional fermentation of soy sauce and in the production of sake, the traditional alcoholic beverage in Japan.

Furthermore, Morales et al. [88] reported that the two α-L-Afases, i.e., AF64 and AF53 from *Bacillus polymyxa*, facilitate the action of the endoxylanase on oat spelt xylan and wheat bran arabinoxylan. An increase in the production of smaller xylooligosaccharides has occurred because of the cooperative action of α-L-AFases used in these experiments. α-L-AFases also act synergistically with endo-arabinanase and cinnamoyl esterase (CinnAE) from *Aspergillus niger*. When sugar-beet pulp (SBP) was incubated with the mixture of the former enzymes, the esterase was able to release 14 times more of the alkali-extractable ferulic acid present in the whole pulp as free acid than CinnAE alone [70].

## Classification of arabinose-releasing enzymes

Kaji [60] classified α-L-AFases based on their sources and substrate specificity. Beldman et al. [8] classified arabinose-releasing enzymes depending on the mode of action and their substrate specificity. However, both classifications were not effective as they were too broad to define the substrate specificities of these enzymes. Moreover, newly isolated enzymes have shown different modes of action than those enzymes classified before. Because of this, further subclasses and a new class need to be added to the existing system of classification proposed by Beldman et al. [8]. In view of this, three subclasses of the existing arabinoxylan-α-L-arabinofuranohydrolases class could be introduced [38, 122, 123] and designated Subclass (1) AXHB-md 2, 3, Subclass (2) AXHB-m 2,3 and Subclass (3) AXHd3.

Subclass (1) AXHB-md 2,3 includes enzymes that release arabinose from both singly and doubly substituted xylose, and able to hydrolyze *p*-nitrophenyl α-L-arabinofuranoside at a rate similar to that for oligosaccharide substrates. This subclass was exemplified by the enzyme arabinoxylan arabinofuranohydrolase isolated from germinated barley [38].

Subclass (2) AXHB-m 2,3 includes enzymes that hydrolyze arabinose residues from C2 or C3 linked to a single-substituted xylose residue and do not hydrolyze *p*-nitrophenyl α-L-arabinofuranoside. The enzyme isolated from *Bifidobacterium adolescentis* [122] represents this subclass.

Subclass (3) AXHd3 includes enzymes that are able to release only C3-linked arabinose residues from double-substituted xylose residues but do not hydrolyze *p*-nitrophenyl α-L-arabinofuranoside. This subclass was represented by the enzyme isolated from *B. adolescentis* [123].

Recently, new types of α-L-AFases have been isolated with properties that have not been reported earlier. Such enzymes could not be assigned to any of the arabinose-releasing enzyme classes. These enzymes have the ability to act on both interior α-1,5 backbone and α-1,3–side chains of arabinan and debranched arabinans; in addition, they are able to act on *p*-nitrophenyl α-L-arabinofuranoside. In view of this, these enzymes should be assigned into a new class represented by α-L-AFase isolated from the thermophilic bacterium PRI-1686 [11] and Tm-AFase from the hyperthermophilic bacterium *Thermotoga maritima* MSB8 [87].

The most recent classification scheme based on amino acid sequences, primary structure similarities and hydrophobic cluster analysis has classified α-L-AFases into five glycosyl hydrolases families (GHs), i.e., GH3, GH43, GH51, GH54, and GH62 [23, 52]. This classification is useful to study evolutionary relationship, mechanistic information and structural features of these enzymes [25].

## Mechanisms of action of α-L-AFases

Like other glycoside hydrolases, α-L-AFases mediate glycosidic bond cleavage via acid/base-assisted catalysis employing two major mechanisms, giving rise to either an overall retention or an inversion of the anomeric configuration [26, 136]. In both mechanisms, as shown in Fig. 3, the hydrolysis usually requires two carboxylic acids, which are conserved within each glycoside hydrolases family [98] and proceed through an exocarbonium ion-like transition state [92, 98, 107].

Retaining α-L-AFases are members of GH3, GH51 and GH54 families that cleave the glycosidic bond using a two-step double-displacement mechanism, as shown in Fig. 3a. This was also confirmed by the crystal structure studies and snapshots along the reaction pathway of GH51 described by Hövel et al. [56]. In the first step of the reaction (glycosylation), the acid–base residue acts as a general acid, protonating the glycosidic oxygen and stabilizing the leaving group. The nucleophilic residue attacks the anomeric carbon of the scissile bond, forming a covalent glycosyl-enzyme intermediate with the opposite anomeric configuration of the substrate. In the second step (deglycosylation), the acid–base residue, acting this time as a general base, activates a water molecule that attacks the anomeric center of the glycosyl-enzyme intermediate from the same direction of the original bond, liberating the free sugar with an overall retention of the anomeric configuration [36, 56].

Inverting α-L-AFases that represent GH43 family use a single displacement mechanism, in which one carboxylate acts as a general base catalyst, deprotonating the nucleophilic water molecule that attacks the bond, while the other carboxylic acid acts as a general acid catalyst by protonating the leaving aglycone (Fig. 3b) [107, 136].

## Production of α-L-AFases

The α-L-AFases production is influenced by the carbon source and composition of the growth medium. Various carbon sources including monomeric sugars and complex polysaccharides have been used to assess their effect on the production, induction and substrate specificity of α-L-AFases (Table 1). For example, pentoses D-arabinose, L-arabinose, D-xylose and hexoses D-galactose, D-glucose, D-mannose, L-sorbose have been commonly used. Other sugars cellobiose, lactose, lactulose, maltose, mellibose, sucrose, trisaccharide, raffinose, D-arabitol, L-arabitol, D-mannitol, D-sorbitol and xylitol also have been used. Sugar beet pulp (starch-free), wheat bran (starch-free), wheat straw, oatmeal, rice straw and corn cob are some of the lignocelluloses that have been used for the production of α-L-AFases. Polysaccharides such as oat spelt xylan, birchwood xylan, beechwood xylan, wheat arabinoxylan, arabinogalactan, larch wood arabinogalactan, sugar beet arabinan, galactan CMC, guar gum, gum Arabic and locust bean gum have also been used. Pectins, schizophyllan, starch, xanthan, carboxymethyl cellulose, potato *β*-1,4-galactan, carob galactomannan, Me-*β*-xyloside and lactobionic acid are some other carbon sources utilized for α-L-AFases production.

Generally, arabinose-containing substrates are essential for the efficient production of α-L-AFases [9, 68]. Monomeric compounds L-arabitol and L-arabinose induce the genes involved in the production of these enzymes in some microorganisms [27]. Conversely, other monosaccharides such as glucose and galactose may inhibit the production of α-L-AFases [9, 68]. Arabinogalactans and oatmeal were found to be the best inducers for α-L-AFase isolated from *Bacillus pumilus* PS213 [32]. α-L-AFase was produced by *Rhodothermus marinus* when the culture was grown on birchwood xylan [46]. L-Arabitol was the inducer for the production of α-L-AFases enzymes araA and araB by the *A. niger* mutants [26]; ABF1 by the *Penicillium purpurogenum* [15, 27] and kabfA and kabjB by the *A. kawachii* [68]. α-L-AFase production by *Pseudomona cellulosa* was repressed when glucose was used in the production medium [9].

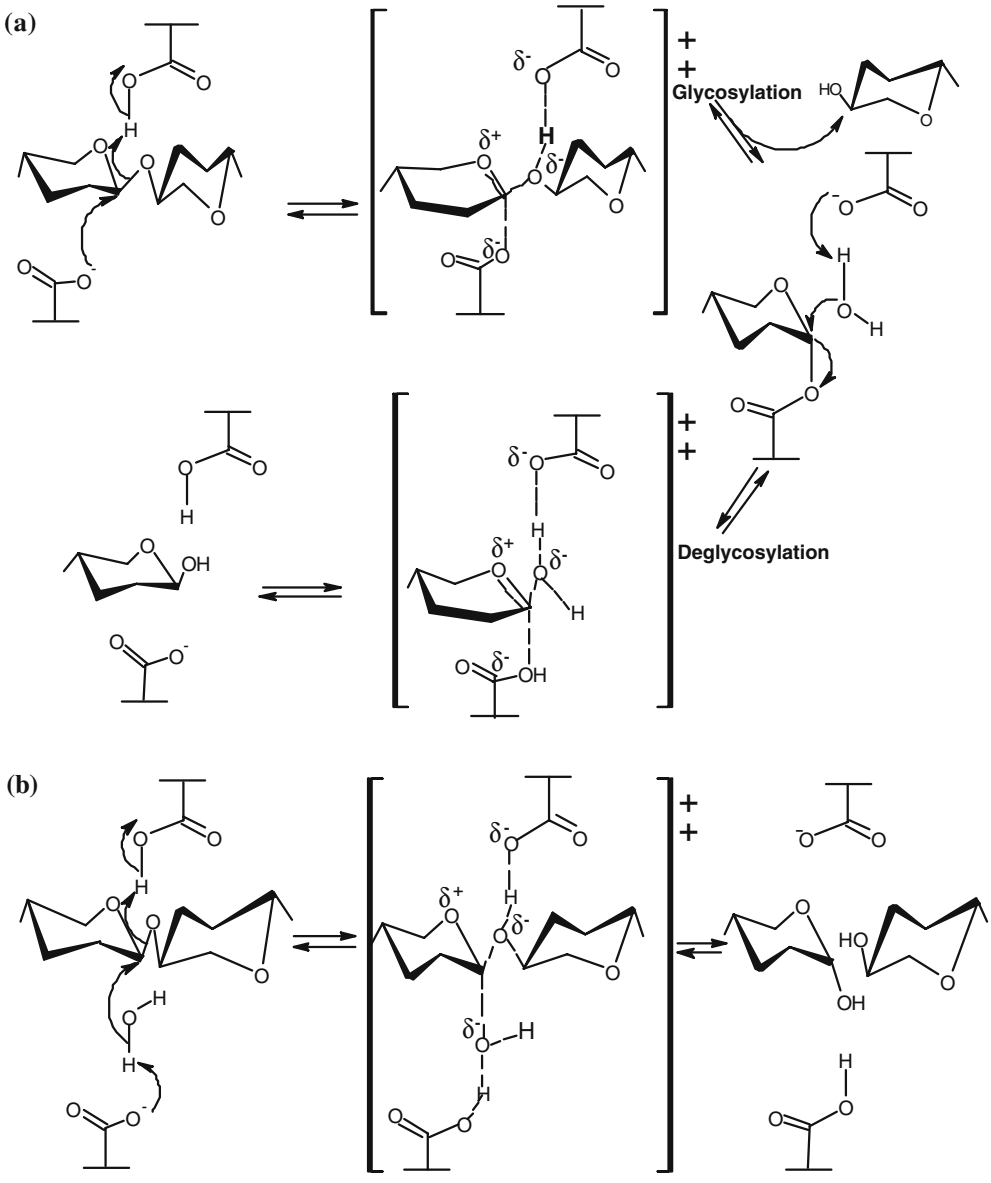



**Fig. 3** General mechanisms for **a** retaining and **b** inverting glycosidases. Adapted from Ref. [98]

The experiments carried out by Gomes et al. [46] indicated that carbon and nitrogen sources influence the production of α-L-AFase by *R. marinus*. In these experiments, different concentrations of xylan (2–6 g/l) and yeast extract (4–12 g/l) were used to increase the enzyme production. The highest enzyme activity (108 nkat/ml) was obtained with medium containing 3 and 9 g/l of Birchwood xylan and yeast extracts, respectively. The lowest enzyme activity (86 nkat/ml) was obtained with medium containing 5 and 7 g/l of Birchwood xylan and yeast extracts, respectively [46]. *A. niger* showed highest α-L-AFase activity (243 U/ml) when grown on a solid-state medium with C: N ratio of 15:9. The carbon and nitrogen sources used were dried skins of grape pomace and casein peptone, respectively [57]. *T. fusca* BD25 showed highest α-L-AFase activity (0.136 U/mg protein) when grown in a medium containing 0.6% (w/v) oat spelt xylan and 0.6% (w/v) yeast extract corresponding to C:N ratio of 4:1 [117–119].

Production of α-L-AFase by *Aspergillus nidulans* was high when ammonium sulfate or ammonium chloride was used as nitrogen source [37]. *A. kuwachii* IF04308 produced highest amounts of α-L-AFase when grown on a medium supplemented with a mixture of bactotryptone, yeast extract and $NaNO_3$ as a nitrogen source [68]. Similarly, when a mixture of urea, ammonium sulfate and neopeptone was used as a source of nitrogen, the production of α-L-AFase by *P. purpurogenum* was enhanced [27].

Both temperature and pH of the growth medium are known to influence growth and enzyme production by microorganisms. The thermophilic bacterium *R. marinus* produced 5.32 U/mg of α-L-AFase when grown in a shake flask for 96 h at 61°C and pH 8. Similarly, the

**Table 1** Properties of some microbial α-L-arabinofuranosidases

| Microorganism | Enzyme | Molecular mass (kDa) | Optimum pH | Optimum temperature (°C) | Polymers attacked | GHs family | References |
|---|---|---|---|---|---|---|---|
| *A. oryzae* HL15 | Afase | 60[a] 110[b] | 5.5 | 60 | AX, AG, OSX | – | [51] |
| *A. oryzae* | abfA | 228 | 5 | 50 | – | 51 | [82] |
| *A. Kawachii* | AkAbfA AkAbfB | 80 62 | 4 | 55 | AX | 51 | [68] |
| *Fusarium oxysporum f. sp. dianthi* (Fod) | abfB | – | 4.0 | 50 | OSX | – | [18] |
| *P. purpurogenum* | ABF1 | 49.6 58 | 5 | 50 | AX, BX, OSX, IAG, WS, WB | 54 | [15, 27] |
| *P. chrysogenum* | AFQ1 AFS1 | 79 52 | 4 3.3–5.0 | 50 50 | BA, DA, AX, SAG, A2, A3 | 51 | [102] |
| *Rhizomucor pusillus* HHT1 | AFase | – | – | – | BA, A2, A3, A4, A5 | – | [93] |
| *B. pumilus* PS213 | AF | 22056[c] | 7 | 55 | OSX, AG | 51 | [32] |
| *B. breve* K-110 | AFase | 60 | – | – | Ginsenoside Rb2 and Rc | 51 | [108] |
| *Clostridium cellulovorans* | rArfA | 138 | 6 | 40–50 | BA, AX | 51 | [69] |
| *Bifidobacrerium longum* B667 | abfB | 61[c]260[b] | 6.0 | 45 | BA, AX, A2, A3, A4, A5 | 51 | [76] |
| *P. cellulosa* | abf51A | 57 | 5.5 | < 55 | WA, BA, A2, A3, A4, A5 | 51 | [9] |
| | abf62A | – | – | – | WA, A2, A3, A4, A5 | 62 | |
| *S. chartreusis* GS901 | AFase I | 80 | 5.5 | 55 | BA, AX, AG, A2, A3 | 51 | [84] |
| | AFase*II* | 37 | 7 | 50 | BA, AX, AG, A2 | 43 | |
| *Streptomyces thermaviolaces* OPC-520 | STX-IV | 37 | 5 | 60 | AX, OSX | 62 | [116] |
| *Thermoanaerobactere ethanolicus* JW200 | xarB | 85 | – | 65 | – | 3 | [74] |
| *Thermobacillus xylaniliticus* D3 | AbfD3 | 56.071 | 6.2 | 75 | WA, IX, OSX | 51 | [31] |
| *T. fusca* | | ~92 | 9 | 65 | – | – | [118, 119] |
| Bacterium PRI-1686 | AraF | 350 | 6 | 70 | BA, DA, OSX | 51 | [11] |
| *T. maritima* MSB8 | AFase | 332 | 7 | 90 | BA, DA | 51 | [87] |

[a]Molecular mass determined by SDS-PAGE gel
[b]Molecular mass determined by gel filtration
[c]Molecular mass determined by mass spectrometry
*OSX* oat spelt xylan, *AG* arabinogalactan, *BA* arabinan, *BiWX* birchwoodxylan, *BeWX* beechwood xylan, *WA* wheat arabinoxylan, *BX* sugar beet pulp xylan, *WBX* wheat bran, *IX* larch xylan, *SAG* soybean arabinogalactans, *IAG* larchwood arabinogalactan, *WS* wheat straw, *WB* wheat bran, *DA* Debranched arabinans, *A2* arabinobiose, *A3* arabinotriose, *A4* arabinotetraose, *A5* arabinopentaose

fungus *P. chrysogenum* 31B produced higher amounts of two α-L-AFases when grown under static conditions for 12 days at pH 5.0 and 30°C [102, 103].

Batch cultivation system in shake flasks has been used for α-L-AFases production by bacteria [32, 46] and fungi [51, 68, 102]. Yields of α-L-AFase were relatively better (88.7 nkat/mg protein) when *R. marinus* was grown in shake flasks as compared to that obtained when grown in a bioreactor (54.5 nkat/mg protein) [46]. Solid-state fermentation (SSF) has been used successfully for α-L-AFases production from different fungi [40, 96, 57]. SSF system resembles the natural habitat of microbes and, therefore, may prove efficient in producing certain enzymes and metabolites. However, not much is known about α-L-AFases production by bacteria using SSF.

## Biochemical properties of α-L-AFases

The available information on the biochemical properties of α-L-AFases is mostly derived from the studies carried out on the enzymes isolated from bacteria, fungi and plants. Microbial α-L-AFases vary in their molecular masses, which can be as high as 495 kDa for α-L-AFase from *Streptomyces purpurascens* IFO 3389 [66] (Table 1).

The effect of temperature and pH on the α-L-AFase depends on the source from which the enzyme is isolated (Table 1). The highest temperature stability has been obtained for α-L-AFase from *T. maritima* MSB8. This cloned enzyme has an optimal temperature of 90°C at pH 7. Moreover, at this temperature (90°C) and pH (7), the enzyme was stable for 24 h. It also retains 50% of its activity at 100°C over a period of 20 min [87]. The other example is α-L-AFase from *R. marinus*, which is stable at 85°C for 8.3 h in a pH range of 5.0–9.0 [46, 76].

The activities of α-L-AFases are affected by metal ions, ionic and nonionic detergents, and chelating and reducing agents depending on the enzyme and concentration of the agent used [54, 76]. For instance, the activities of α-L-AFase (abfB) from *Bifidobacterium longum* B667 [76] and α-L-AFase (AbfD3) from *Thermobacillus xylanilyticus* D3 were not affected by EDTA, DTT, but were affected by $Cu^{2+}$ ions [31, 76]. Metal ions such as $Ag^{+}$, $Hg^{2+}$, $Zn^{2+}$, $Cd^{+2}$, $Co^{+2}$ and Ni had an inhibitory effect on some of these enzymes [76, 102, 116].

## Molecular biology of α-L-AFases

Some α-L-AFases have been studied up to molecular level. The genes coding for these enzymes have been identified, cloned and expressed in different bacterial and fungal systems. In addition, the protein products of these genes have been sequenced and the evolutionary relationship among some of the sequenced proteins has been reported using the phylogenetic tree analysis [32]. For example, some of the cloned genes, i.e., STX-IV from *Streptomyces thermoviolaceus* OPC-520 chromosome [116], AkabfA and AkabfB from *A. kawachii* and AwabfA and AwabfB from *Aspergillus awamori* [68], *xarB* from the thermophilic anaerobe *Thermoanaerobacter ethanolicus* JW200 [74], α-L-AFase gene from *B. longum* B667 [76], *Bifidobacterium breve* K-110 [108] and from the *Clostridium cellulovorans* genomic library [69] have been characterized. Similarly, genes such as Abf51A from the genomic library of *Psuedomonas cellulosa* [9], abf1 from *P. purpurogenum* [15], abfA from *T. maritima* TM0281 [87] and abfB from *Fusarium oxysporum f. sp. dianthi* (Fod) [18] also have been well characterized.

Amino acid sequencing as well as crystal structure studies indicate the presence of substrate-binding domain (SBD) in some of the reported enzymes. The SBD may take part in the efficiency of the enzyme function [71]. However, the possible role of α-L-AFases in the release of arabinofuranosyl residues is not yet clear [65]. Some α-L-AFases with SBD have been reported, i.e., α-L-AFases from *Pseudomonas fluorescens* and *Streptomyces lividans* have a cellulose-binding domain (CBD) [63] and a xylan-binding domain (XBD) [125], respectively. Other α-L-AFases such as those produced by *Streptomyces chartreusis* GS901 possess additional functional domains at both the N-terminal and the C-terminal regions. However, these domains did not show any similarities to the known SBD observed in many other types of glycanases. It might represent a novel kind of SBD [84]. *A. kawachii* IFO4308 α-L-AFase (AkAbfB) was found to have an arabinose-binding domain (ABD) that showed a number of distinct characteristics that are different from those of carbohydrate-binding module (CBM) [86]. Recently, Bolam et al. [13] showed that the X4 modules from a *Cellvibrio japonicus* α-L-AFase (Abf62A) binds to polysaccharides. This protein comprises a new family of CBMs, designated as Abf62A-CBM35. There are more than 13 α-L-AFases that have been grouped in family 42 of CBM [23].

So far, only three α-L-AFases have been studied for their three-dimensional structure. There appears considerable diversity in the three-dimensional structure of these enzymes. These enzymes are α-L-AFase B (AkabfB) (EC 3.2.1.55) from *A. kawachii* IFO 4308 located within GH 54 family [86], α-L-AFase (AbfA) (EC 3.2.1.55) from *Geobacillus stearothermophillus* T-6 located within GH 51 family [56, 107] and the bifunctional xylanase D/ α-L-arabinofuranosidase (XynD)/(Xyn43A) (EC 3.2.1.8 and EC 3.2.1.55, respectively), of *Paenibacillus polymyxa* located within GH 43 family [56].

## Biotechnological applications of α-L-AFases

The importance of lignocellulose-degrading enzymes is well defined because of their role in many industrial and biotechnological processes. This resulted in re-establishment of a new era for the efficient utilization of the cheap agricultural waste materials. α-L-AFases, with their synergistic action with other lignocellulose-degrading enzymes, are the promising tools in various agro-industrial processes [3, 99]. These include production of important medicinal compounds, improvement of the wine flavors, bread quality, pulp treatment, juice clarification, quality of animal feedstock, production of bioethanol and the synthesis of oligosaccharides.

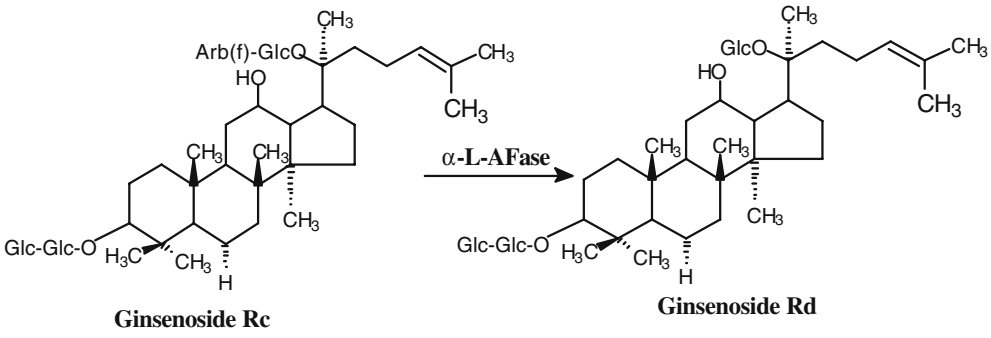
## Production of arabinose as antiglycemic agent

Recently, there is a growing interest for L-arabinose as a possible food additive because of its sweet taste, and its low uptake due to its poor absorption by the human body [84]. Moreover, it has been proved that L-arabinose selectively inhibits intestinal sucrase in a competitive manner and thus reduces the glycemic response after sucrose ingestion in animals [106]. Studies carried out on mice suggest that L-arabinose dose-dependently suppressed the increase of blood glucose level after the ingestion of sucrose [108]. Furthermore, L-arabinose delays and reduces the digestion, absorption and the net energy derived from sucrose when both are ingested simultaneously. Based on these findings, L-arabinose can be used as a physiologically functional sugar that inhibits sucrose digestion. In this way, L-arabinose is useful in preventing postprandial hyperglycemia in diabetic patients [104[2]]. Therefore, effective L-arabinose production is a vital perquisite for its use in this respect as well as for its importance in food industry. To achieve this goal, it is necessary to use arabinose-releasing enzymes α-L-AFases, and defined polysaccharides and oligosaccharides from different agricultural raw materials [84, 93, 115].

## Production of antimetastatic and anticarcinogenic compounds

Ginsenosides Rb2 and Rc are the main components of ginseng (the root of *Panax ginseng* C.A. Meyer, Araliaceae). These roots are frequently used as a traditional medicine in China, Korea, Japan and other Asian countries. Ginsenosides Rb2 and Rc are L-arabinofuranoside- and L-arabinopyranoside-bound glycosides,

[2] as cited by [108].




**Ginsenoside Rc** **Ginsenoside Rd**

**Fig. 4** Proposed metabolic conversions for the ginsenoside Rb2 by α-L-arabinofuranosidase from *B. breve* K-110. Modified from Ref. [108]

respectively, in ginsenoside Rd [108]. These ginsenosides are transformed to compound K, via ginsenoside Rd, by intestinal bacteria in human intestine by the action of α-L-AFase [7] (Fig. 4). The pharmacological actions of these ginsenosides have been explained based on the biotransformation of ginsenosides by glycosidases of human intestinal bacteria [2, 6, 7, 50, 128]. The latter bacteria utilize α-L-AFase to transform the protopanaxadiol ginsenosides to compound K that exhibits antimetastatic and/or anticarcinogenic effects. Moreover, compound K can be produced effectively by different arabinosidases including α-L-AFases and α-L-arabinopyranosidase [61].

## α-L-AFases and wine industry

One of the most important characteristics of wine quality is its aromatic fragrance. It is now well established that certain monoterpenes contribute significantly to the flavor of wine [80]. Terpenols are strongly aromatic molecules that represent an important part of aromas [42]. They are not volatile and are directly accessible to the olfactory mucosa [12, 127, 132]. A major portion of these monoterpenols in grapes musts, wines, other alcoholic beverages (brandy, bitters, etc.) and fruit juices (apple, apricot, peach, papaya, passion fruit etc.) [12, 105] are linked to disaccharide moieties, in which the major terminal non-reducing sugar is α-L-arabinofuranose which can be released by the action of α-L-AFases [12]. It is now clear that the glycosidically bound volatiles can be released by sequential enzymatic hydrolysis in two stages. In the first step, and depending on the precursor, the glycosidic linkage is cleaved by α-L-AFases, followed by the action of the other glycosidase, which then liberates the monoterpenols (Fig. 5) [42, 43, 78, 113, 114, 133]. Thus, α-L-AFases treatment followed by the addition of other glycosidases can be used for the enhancement of wine flavor by the release of free terpenols. Moreover, Yannai and Sato [134] have reported that α-L-AFase from *Pichia capsulata* X91 is active at ethanol concentrations found in wine and able to release considerable amount of monoterpenols, especially linalool, citronellol and geraniol, thereby increasing the aromatic flavors of different wines. Furthermore, the immobilized α-L-AFase, β-D-glucopyranosidase and α-L-rhamnopyranosidase from *A. niger* increased the aroma of a model wine solution to more than 600 mg/l of total free terpenols [113, 114].

Today, a lot of interest has been generated in the involvement of α-L-AFases in enhancing the aroma. This is mainly achieved by using the recombinant yeast strain (YCA1) [*Saccharomyces cerevisiae* strain $T_{73}$ (CECT1894) transformed with YCAbfB from *A. niger* N400 (CPS 120.49)] that was capable of efficiently secreting α-L-AFase directly in vinification process or by directly adding the purified enzyme obtained from it [110]. Preliminary experiments carried out with this recombinant yeast strain (YCA1) have shown increased levels of some volatile compounds involved in wine aroma [110]. Furthermore, during wine aging, a number of the fragrant precursors (such as linalol, nerol and geraniol) turn into less-fragrant compounds (α-terpineol, diols, and triols, oxides, etc.) so that after 6–7 months of aging for an aromatic wine (Muscato wines), the final result is often a reduction in the more fragrant-free terpenes. The addition of glycosidases to the wine increases its aroma without this disadvantage [12]. For

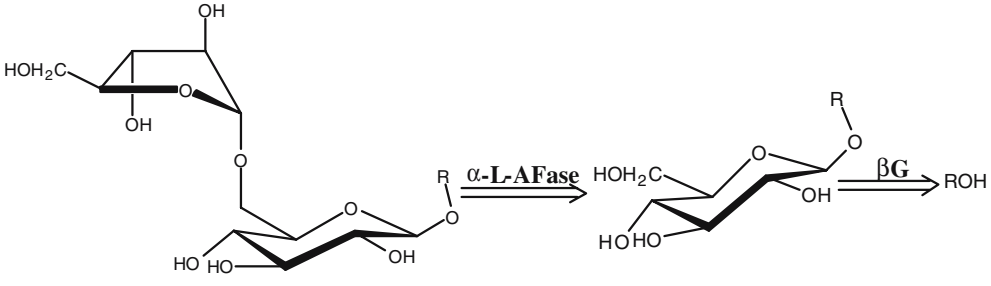



**Fig. 5** Mechanism of action of the glycosidase α-L-arabinofuranosidase and β-D-glucopyranosidase (βG) on diglycosidic precursors. ROH is a volatile aglycone such as monoterpenols and other alcohols. Modified from Ref. [113]

instance, α-L-arabinofuranosidase (EC 3.2.1.55) and β-D-glucopyranosidase (βG, EC 3.2.1.21) are currently produced on an industrial scale from *A. niger* [3], and are used in the aromatization of musts, wines and other alcoholic beverages [12, 105].

## α-L-AFases, acetic acid production and quality of the bread

Staling is probably the main problem that occurs during bread storage. This results in a decreased bread shelf life and causes serious economic losses to the bread industry [45]. Pentosans are important functional ingredients in bread and their positive role in bread texture and staling is well known [16, 58, 64]. Pentosans added to the dough may be moderately hydrolysed by wheat flour enzymes and especially by exogenous enzymes such as xylan-degrading system including α-L-AFases [39, 59]. These enzymes produce free pentoses (mainly arabinose and xylose) thereby increasing the availability of soluble carbohydrates in the dough [44, 45, 59, 79]. This positively interferes with the metabolism of sourdough lactic acid bacterium *Lactobacillus hilgardii* [44]. This bacterium increases the acidification rates and the production of acetic acid. For example, Gobbetti et al. [45] showed that by using pentosans, α-L-AFase from *A. niger* and *Lactobacillus plantarum* 20B, soluble carbohydrate availability, acidification rate and production of acetic acid increased during sourdough fermentation. (α-L-AFase mainly hydrolyse the exterior arabinofuranosyl linkages of pentosans in the dough thereby making pentoses available for fermentation by *L. plantarum* [45]. Recently, α-L-AFases along with pentosanse and other enzymes have been considered as natural improvers that greatly enhance the overall quality of bread [59, 79]. The enzyme treatment delayed the bread staling and increased the shelf life of the bread thereby giving economic benefits to the bread industry [45, 59, 79].

## α-L-AFases in pulp and paper industry

Several commercial xylanase preparations are available for the treatment of pulp [124]. Application of α-L-AFase would further enhance the delignification of pulp as the enzyme acts to release the arabinose side chain that retards the action of other bleaching enzymes [10, 48]. The removal of lignin from semi-bleached kraft pulp was improved when the pulp was treated with α-L-AFase from *B. stearothermophilus* L1 together with xylanase [10]. The enzyme acted synergistically with a thermophilic xylanase in the delignification process, releasing 19.2% of lignin. Delignification obtained using the combined enzyme treatment exceeded the sum of the amounts obtained using the enzymes individually [10]. According to Margolles-Clark et al. [77], *Trichoderma reesei* α-L-AFase ( could also liberate >60% of the arabinose from arabinoglucuronoxylan isolated from pine kraft pulp. The treatment of softwood kraft pulp with the crude α-L-AFase-rich xylanase and mannanase from *R. marinus* increased the bleachability of the pulp when used in a X–Q–D–Q–P bleaching sequence, where X was enzyme treatment, Q was chelation, D was chlorine dioxide treatment with $NaClO_2$ in acidic solution and P was the peroxide bleaching [46]. The highest increase in brightness (1.8% ISO) was achieved when the mixture of α-L-AFase-rich xylanase and mannanase was used for the pulp treatment. The observed increase in the brightness (1.9–2.1%) was similar to the value obtained using commercial enzyme preparation [46]. The high thermal and pH stability, broad pH optima and lack of cellulose activity of the α-L-AFase, xylanases [48, 75] and mannanase produced by *R. marinus* are most useful for biobleaching of pulp and paper [24, 46].

## α-L-AFases and animal feedstock

The digestion of feedstuffs by ruminal microorganisms results in the production of acids and microbial cells, which provide the host animal with its main sources of energy and protein [35]. Although hemicelluloses (mainly xylans) represent 30–40% of the total forage carbohydrate, their contribution to dietary energy available to the animal is often decreased because of low overall (40–60%) digestion [21, 34, 130]. The increase in digestibility of feedstuffs is well correlated with the decrease in the degree of substitution of the hemicellulose polymers with arabinosyl residues [47, 89]. L-arabinose residues prevent the total hydrolysis of xylans. Therefore, any mechanism able to remove the arabinosyl side chains from hemicellulose should increase its digestibility [21, 22, 33, 34, 53]. The utilization of cell wall polysaccharides by poultry and pigs was improved by the addition of cellulases, pectinases and xylanases [19]. Moreover, the addition of α-L-AFases removes arabinose side groups that restrict the action of glycanases and could further promote the hydrolysis of solubilized cell wall polysaccharides [47, 54, 67]. It has been shown that the use of commercial enzymes preparation containing α-L-AFases enhanced the activity of xylanase because the latter prefers unsubstituted regions of xylan as a substrate, thereby reducing the viscosity of the feedstuffs used [81]. Cotta [22] reported that α-L-AFase isolated from *Ruminococcus albus* 8 removed arabinosyl residues from alfalfa cell wall (ACW), pectic and hemicellulosic polysaccharides, thereby making these substrates more susceptible to attack by other glycanases. For a given species, such as *R. albus*, digestion can vary from a low of 5 to a high of 88% for corn [53]. α-L-AFases helps endo-xylanases in the hydrolysis of arabinoxylan, thereby improving the feed digestibility [14, 96]. The addition of mixture of xylanases and α-L-AFases as a strategy to increase digestion is currently being used in some countries [96]. This approach has been

considered in the European Community (AIR contract number AIR1 CT92) [96]. Genetic manipulation of anaerobic bacteria and ruminal organisms is yet another strategy to increase the production of xylan-degrading enzymes, which can be used to improve the digestion of plant materials [62, 90, 121]. This has to involve cloning of α-L-AFase genes into the manipulated ruminal bacteria to increase the efficiency of xylan-degrading enzymes. This approach has been proved good when the cloned α-L-AFase from *Prevotella ruminicola* was used [41].

## α-L-AFases in fruits juice industry

α-L-AFases are receiving attention for their applications in fruit juice clarification [97]. The preparations of pectinolytic enzymes utilized so far contain significant amounts of α-L-AFases [91, 130, 132]. These enzymes specifically remove the 1,3-side chains present on the main 1,5-linked arabinan chains. This results in a precipitate (haze) consisting of 1,5 arabinans. The α-1,5 arabinanase acts on 1,5 arabinans that help to increase the solubility of the precipitate [20, 28, 126]. As industrial enzymes often do not require extensive purification, the juice industry can use α-L-AFases and arabinanase-containing plant extracts [55, 109]. For example, in apple and pear juice production, haze formation is a problem due to the presence of solubilized arabinans [20]. The precipitates can most probably be avoided by adding sufficient amounts of α-L-AFase and endo-arabinanase [85, 126, 131]. Birgisson et al. [11] reported an α-L-AFase from the thermophilic bacterium PRI-1686 belonging to the recently described phylum of Thermomicrobia. This enzyme has the ability to degrade the interior α-1,5 backbone as and α-1,3-side chains of arabinan. Moreover, Miyazaki [87] described a thermophilic α-L-AFase from the hyperthermophilic bacterium *T. maritima* MSB8 that had the ability to degrade arabinan and debranched arabinan. Such properties are useful to avoid haze formation in fruits juice industry.

## Production of fermentable sugars for bioethanol industry

Enzyme-catalyzed conversion of sugarcane, sugar beet, corn or wheat to ethanol by distillers yeast *S. cerevisiae* is the current process for the industrial production of bioethanol [111]. These substrates contain non-fermentable hemicelluloses. These hemicelluloses remain unutilized and accumulate as by-product residues (˜70 % by weight of the total residue) during the process of ethanol production [1, 5, 111]. The utilization of these residual hemicelluloses is essential for the efficient conversion of these compounds to ethanol, value-added products and industrial chemicals [99, 100, 135]. Nevertheless, these substrates require a suitable pretreatment before they can be used for the production of ethanol [100]. For instance, acid hydrolysis can be used for the hydrolysis of arabinoxylans in hemicelluloses to monosaccharides. However, enzymatic hydrolysis is preferred due to reduced formation of byproducts that may inhibit the subsequent microbial fermentation [99]. The complexity and heterogeneity of the arabinoxylans in hemicelluloses demand enzyme systems that convert these substrates into fermentable sugars [40, 72, 100, 111]. Such an enzyme system needs to include de-polymerizing and the side-group cleaving enzymes to degrade hemicelluloses into pentoses monosaccharides [100]. Moreover, such a system will also need a microorganism not only capable of utilizing pentoses, but also able to withstand high concentrations of ethanol produced during the process [72, 100, 101, 135]. Therefore, tailored enzymes are required to hydrolyse lignocellulosic substrates to fermentable sugars [100, 111]. The synergistic action of α-L-AFases with lignocellulose-degrading enzymes makes them potential agents for saccharifying various pretreated agricultural and forestry residues to monomeric sugars for the production of fuel and chemicals [99]. Designed hemicellulosic enzymes consisting of Celluclast 1.5 l from *T. reesei* and Ultraflo L from *Humicola insolens* exhibited a strong synergistic interaction in catalyzing the release of xylose and arabinose from wheat arabinoxylans, which otherwise will be accumulated as by-products during the production of ethanol. This was mainly due to the cooperative action of α-L-AFases, endo-1,4-xylanases and xylosidase present in the two enzyme preparations. Moreover, this study suggested that such synergistic interaction might be useful for the production of efficient enzyme cocktails to improve the utilization of wheat hemicellulose by-products produced during the production of ethanol [111]. Furthermore, Saha and Bothast [101] suggested that the high activity of the α-L-AFase from *Aureobasidium pullulans* on both arabinan and debranched arabinan, its ability to release L-arabinose from arabinoxylans, and its high thermostability make this enzyme a promising candidate for the production of fermentable sugars from hemicellulosic biomass for ethanol production [101].

## Synthesis of pentose-containing compounds

Increasingly, enzymes are being adopted for the synthesis of oligosaccharides and glycoconjugates via enzymatic or mixed chemo-enzymatic routes. The glycoside hydrolases (EC 3.2.1) and glycosyltransferases (EC 3.2.4) are promising enzymes as they play an important role in the synthesis strategies by performing glycosylation in one stereoselective step. Glycoside hydrolases (mainly exo-acting hydrolases) often display more relaxed regioselectivity, and unlike glycosyltransferases, an extensive palette of glycoside hydrolases, displaying a wide range of sugar specificities, are available [36]. Some α-L-AFases are robust and ther-

mostable and do not require the use of costly sugar donors. For example, thermostable α-L-AFase (AbfD3) from *T. xylanilyticus* [31] has the ability to catalyze transglycosylation in the presence of *p*-nitrophenyl α-L-arabinofuranoside (*p*NPAraf) and various alcohols. Moreover, Rémond et al. [95] reported the synthesis of several pentose-containing oligosaccharides using this enzyme. The enzyme AbfD3 possessed the ability to synthesize oligosaccharides in kinetically controlled transglycosylation reactions. The products of these reactions could be useful analytic tools as reference compounds for the analysis of hemicellulase action, and for raising antibodies to well-defined motifs for immunochemical-based analysis of plant cell walls [94, 95]. Moreover, α-L-AFases that display transglycosylation ability constitute potentially interesting tools for chemoenzymatic synthesis of arabinose-containing compounds that are difficult to access via organic synthetic methods [95].

## Future prospects

Achieving efficient breakdown of the plant cell wall polysaccharide hemicelluloses and pectins represents an important and lucrative goal for biotechnologists. For that, further research has to be carried out to explore many aspects of α-L-arabinofuranosidases, in much detail. Further, studies on the synergistic effects of the robust enzyme on the action of other hemicellulases and pectinases that already exist as commercial enzymes may lead to improvement of many existing industrial products. Understanding how these enzymes interact and act on lignocelluloses and the relationship between their structure and function at molecular level are other aspects that need to be studied. Moreover, isolation and characterization of robust α-L-AFases and genes encoded for these enzymes will likely have significant implications in the design of industrial processes that can be accomplished within a wide range of conditions and in commercial production of biomass-degrading enzymes. Manipulation and genetics engineering of bacteria for obtaining complete and a more efficient lignocellulose-degrading enzymes system including α-L-AFases genes will be a novel path into complete saccharification system, which is required for many technologies including ethanol production. Furthermore, chemical treatments in some industries such as paper and pulp bleaching and ethanol production which cause environmental problems could be reduced or replaced by using an efficient hemicellulose-degrading enzymes system.

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