Hooked on α-D-galactosidases: from biomedicine to enzymatic synthesis

Irina Yu. Bakunina¹, Larissa A. Balabanova¹, Angela Pennacchio², and Antonio Trincone²

¹G.B. Elyakov Pacific Institute of Bioorganic Chemistry, Far Eastern Branch, Russian Academy of Sciences, Vladivostok, Russia and ²Istituto di Chimica Biomolecolare, Consiglio Nazionale delle Ricerche, Pozzuoli, Napoli, Italy

Abstract

α-D-Galactosidases (EC 3.2.1.22) are enzymes employed in a number of useful bio-based applications. We have depicted a comprehensive general survey of α-D-galactosidases from different origin with special emphasis on marine example(s). The structures of natural α-galactosyl containing compounds are described. In addition to 3D structures and mechanisms of action of α-D-galactosidases, different sources, natural function and genetic regulation are also covered. Finally, hydrolytic and synthetic exploitations as free or immobilized biocatalysts are reviewed. Interest in the synthetic aspects during the next years is anticipated for access to important small molecules by green technology with an emphasis on alternative selectivity of this class of enzymes from different sources.

Introduction

α-D-Galactosidases (EC 3.2.1.22) free galactose from different galactosides in nature; melibiose, raffinose and similar oligosaccharides or aryl and alkyl α-galactosides and polymers containing α-galactose (e.g. galactomannan), are all substrates. These enzymes are interesting activities from different points of view, from biomedicine to biocatalyzed synthesis.

Searching the database of the United States Patent and Trademark Office for patents containing the word α-D-galactosidase in the title, relatively few “hits” resulted starting from the early 1970s when interest in enzymatic manipulation of raffinose-containing food began (Kawamura et al., 1971) US Patent 1971, 3562113). Coeval is the interest for immobilization (Linden, 1982), however over time many α-D-galactosidases were identified from different sources starting with brewer’s yeast at the end of 1800s. In 1990, a dietary supplement called Beano was developed based on the research regarding gas-causing vegetables; the product containing the enzyme from Aspergillus niger (Di Stefano et al., 2007) was used to introduce students to enzymes (Hardee et al., 2000). It could also be appreciated as a ready-made biocatalyst for synthesis. Interest for these enzymes continues currently for the reduction of anti-nutritional factors in animal feeds (Opazo et al., 2012).

A search in Pubmed at the end of 2013 for the word α-D-galactosidase accounted for ca. 300 review articles. Most of them, if not all, are related to the importance of α-D-galactosidases in enzyme replacement therapy in Anderson–Fabry’s disease (deficiency of α-D-galactosidase A) (Pisani et al., 2012). In the biomedicine field, the use of α-D-galactosidases in the conversion of red blood cells of group B (removing of α-1,3-linked galactose), is known (Olsson et al., 2004) and for this application a marine enzyme has been interestingly proposed (Bakunina et al., 1998). α-D-Galactosidases also generated some interest in the selective hydrolysis of glycosphingolipids (Li & Li, 1999) in conjunction with NMR spectroscopy and mass spectrometry for the structural elucidation of complex lipid biomolecules.

α-D-Galactosyl linkage is present in so-called α-Gal epitope (Gal-α-1,3-Galβ1,3-GlcNAc-R), carbohydrate structures that are present on glycolipids and glycoproteins. The natural absence in humans of this epitope and the fact that a natural antibody to it, the anti-Gal antibody, is produced induced interest for the synthesis of these carbohydrate linkages present in drugs used in xenotransplantation and in immunotherapy (Galili, 2005).

Noteworthy is the recent involvement of α-D-galactosidases in analytical techniques for forensic and high-throughput applications like DBS (dried blood spots). The “lab-on-a-chip” approach, with very small volumes used (nano- to pico-liter samples), minimized cost and material consumption and holds promise as the next generation technology. Multiplexed enzymatic assays of acid α-glucosidase (GAA) and acid α-D-galactosidase, to screen for Pompe and Fabry disorders, have been recently developed (Demirev, 2013).
The biosynthetic aspect, genetic regulation, biological functions and structural classification of \( \alpha \)-D-galactosidases are all topics of current interest. Galactose containing oligo- and poly-saccharides are natural substrates and generally, \( \alpha \)-D-galactosidases are subject to induction by galactose and its derivatives (Pardee, 1957). Based on the understanding of the expression regulatory mechanisms and function of the \( \alpha \)-D-galactosidase, it may be possible to obtain more efficient and novel enzymes with new functionalities and applicability (Ademark et al., 2001). The usual transferring capability common to many glycosyl hydrolases (Trincone & Giordano, 2006) was evidenced for \( \alpha \)-D-galactosidases very early (Anagnostopoulos et al., 1954; Courtois & Petek, 1957).

We have depicted a general survey on \( \alpha \)-D-galactosidases from different origin with a special emphasis on marine example(s). Common structures and functions of natural \( \alpha \)-galactosyl containing compounds are reported; different sources of \( \alpha \)-D-galactosidases, genetic regulation and natural functions are covered first with subsequent emphasis on mechanisms of action, before discussing hydrolytic and synthetic exploitation as free or immobilized biocatalysts. In-depth aspects are covered in Supplementary Material.

**\( \alpha \)-Galactosides in nature**

A detailed analysis (Frias et al., 1996; Gebus et al., 2012; Huang et al., 2008; Keates et al., 1998; Kronzer & Schuerch, 1974; Lemieux & Driguez, 1975; Li et al., 2000; Martinez-Villaluenga et al., 2005, 2006, 2008; Saini, 1988; Sau et al., 2012; Shiozaki et al., 2010; Suzuki et al., 2003; Tester & Al-Ghazzewi, 2013; Weignerova et al., 1999; Yagi et al., 2010) of structure and biological functions of the most important \( \alpha \)-galactosides (Figure 1), found in nature, is reported in the Supplementary Material Part 1. \( \alpha \)-1,6-Galactosides are more common and easily used in the investigation of substrate specificity with respect to rarer \( \alpha \)-1,3- and \( \alpha \)-1,4-regioisomers that have been obtained by somewhat troublesome synthetic strategies. It is tempting to conclude that a direct influence of this distribution and substrate-availability, could have been reflected in the enzyme discovery; \( \alpha \)-1,3 and \( \alpha \)-1,4 specificity is still ascribed to few enzymes including marine \( \alpha \)-galactosidases.

The interest of synthetic chemists for the production of such structures was already active in 1970s and it has been traced in the literature as being generally prompted by discovery of biological functions of \( \alpha \)-galactosides. The production of these compounds using conventional techniques or innovative bio-based pathways is still currently supported.

**\( \alpha \)-D-Galactosidases**

\( \alpha \)-D-Galactosidases are widely distributed in nature. They have been purified from a number of sources including plants, where multiple forms of the enzyme exists (Brian & Webb, 1977), animals and microorganisms, such as fungi, bacteria and yeasts. \( \alpha \)-D-Galactosidases can be divided into acid and

![Figure 1. Examples of natural \( \alpha \)-galactosides: compound 1, part of blood-group B antigenic determinant (Lemieux & Driguez, 1975); compound 2, receptor of pathogens and enterotoxins (Suzuki et al., 2003; Yagi et al., 2010); compounds 3, \( \alpha \)-galactosides of sucrose (Di Stefano et al., 2007; Frias et al., 1996; Martinez-Villaluenga et al., 2005, 2006, 2008; Saini, 1988); compound 4, antigenic determinants (Lemieux & Driguez, 1975); compound 5, 5-O-(\( \alpha \)-D-galactopyranosyl)-\( \delta \)-glycerol-2-enono-1,4-lactone (Keates et al., 1998); compounds 6, polygalatenosides (Huang et al., 2008); compounds 7, galactomannan type structure (Tester & Al-Ghazzewi, 2013); compounds 8, potential anticancer galactomannan derivatives (Li et al., 2000); compounds 9, \( \alpha \)-galactosylceramides ligand for the activation of CD1d-mediated invariant natural killer T cells (Shiozaki et al., 2010).](image-url)
alkaline enzymes, the former playing important roles in seed development and germination (Keller & Pharr, 1996). α-D-Galactosidases are ubiquitous in legume seeds and other vegetable sources (Balasubramanian & Mathew, 1986; Bayraktar & Onal, 2013; Bryant & Rao, 2000; Chinen et al., 1981; Feurtado et al., 2001; Kang & Lee, 2001; Kim et al., 2002, 2003; Malhotra & Dey, 1967; Marraccini et al., 2005; Zhifang & Schaffer, 1999), fungi (Galas & Miszkiewicz, 1996; Hashimoto et al., 1993; Krierger et al., 2004; Ohtakara et al., 1984; Puchart et al., 2000; Rezende et al., 2005; Wong et al., 1986) and other biological systems (Anisha et al., 2008; Cao et al., 2010; Shivam & Mishra, 2010) including many bacteria with marine examples (Blücher et al., 2000; Coombs & Brenchley, 2000; Gomes et al., 2000), in inexpensive agricultural residues (Gote et al., 2004), animals and human (Barnett, 1971; Bishop & Desnick, 1981; Dean & Sweeley, 1979) – detailed in the paragraph related to sources in Supplementary Material Part 2. Animal originating enzymes hydrolyse galactolipids. The reduced α-D-galactosidase activity results in Fabry’s disease in humans with progressive accumulation of globotriaosylceramide and related glycosphingolipids. Affected patients have microvascular disease of the kidneys, heart and brain (Feldt-Rasmussen et al., 2002). The enzyme is present in brain tissues with possible involvement in the hydrolysis of monogalactosyl diglycerides and digalactosyl diglycerides (Subba & Pieringer, 1970).

α-Galactosidases are classified according to substrate specificity in two groups (Dey et al., 1993). Group I includes enzymes catalyzing the hydrolysis of α-1,6 linkage in low molecular weight oligosaccharides and other galactosides. However, in this group, there are enzymes able to cleave α-1,2-, α-1,3- and α-1,4-glycosidic linkages (Varbanets et al., 2001; Zaprometova et al., 1990). Group II enzymes are active on branched macromolecular polysaccharides such as galactomannan and glucomannan. They originated mainly from plants (Kim et al., 2002) and fungi (Galas & Miszkiewicz, 1996). α-D-Galactosidases are included in the families GH-4, GH-27, GH-36, GH-57, GH-97 and GH-110 according to the classification of carbohydrate active enzymes (CAZy) (Cantarel et al., 2009; Henrissat et al., 1995). A detailed account (Anggreai et al., 2008; Brouns et al., 2006; Chakladar et al., 2011; Fernandez-Leiro et al., 2010; Fredslund et al., 2011; Gloster et al., 2008; Liljestrom & Liljestrom, 1987; Liu et al., 2007, 2008; Nagao et al., 1988; Naumoff, 2004, 2005; Okuyama et al., 2009; Rajan et al., 2004; Rigden, 2002; Shimamoto et al., 2001; Takami et al., 2000; Van Lieshout et al., 2003; van Schaik et al., 2010; Varrot et al., 2005; Yip et al., 2004) is reported in the Supplementary Material Part 3.

The crystal structures of free forms and their complexes with ligands (galactose and N-acetylglactosamine) of eight GH-27 members have been solved by the X-ray analysis (Clark & Garman, 2009; Garman et al., 2002) as detailed in Table 1 and Supplementary Material Part 4. All known subfamilies from I to V of GH-27 family are covered, improving knowledge on the activity of this important family of enzymes and giving deeper insight into the structural features that rule modularity and protein–carbohydrate interactions (Fernandez-Leiro et al., 2010). Less structural information is available for the GH-36 and GH-97 α-galactosidases. Crystal structures have been reported for six GH-36 members (Bruel et al., 2011; Comfort et al., 2007; Fredslund et al., 2011; Mercerón et al., 2012) without full information about the evolution of 3D structure within this family. Five from these are homotetrameric enzymes of symbiotic and probiotic bacteria from the human gut belonging to subfamily I. These are α-galactosidases AgaA and AgaB from *Geobacillus stearothermophilus* strain KVE39 (PDB 4fnp and 4nq, respectively) (Merceron et al., 2012), *Ruminococcus gnavus* E1 (PDB 2yfo) (Bruel et al., 2011), *Lactobacillus acidophilus* strain NCFM (PDB 2xn2) (Fredslund et al., 2011), *Lactobacillus brevis* strain ATCC 367 (PDB 3mif) (Fredslund et al., 2011) and one is from thermophile bacterium *Thermotoga maritima* of subfamily IV (PDB 1zy9) (Comfort et al., 2007) (Table 1, Supplementary Material Part 5). Only one 3D structure of GH-97 α-galactosidases of *Bacteroides thetaiotaomicron* BtGH97b (PDB 3a24) is known (Okuyama et al., 2009) (Table 1, Supplementary Material Part 6).

In general, tertiary structures of GH-27 enzymes consist of two domains whereas with GH-36 and GH-97 α-galactosidases, three domains are found. The large N-terminal β-supersandwich domain found in GH-36 and GH-97 proteins, is absent in the enzymes of the GH-27 family. It is formed by 16–20 antiparallel β-strands and some α-helices in the GH-36 member. However, β-supersandwich of GH-97 α-galactosidase is disordered and consists of the 21 antiparallel β-strands and 2 short 3 10-helices. The central classical (β/βα)-barrel domain in GH-36 α-galactosidases has high homology with GH-27 catalytic N-terminal domain. It was observed earlier in a triose phosphate isomerase (Banner et al., 1975) and now represents a common motif in many glycoside hydrolases of Clan-D, functioning as catalytic domain. It is deformed in GH-97 α-galactosidase containing several 3 10-helices in the loops connecting the parallel main β-strands and α-helices, moreover β-strand 7 is exchanged with a loop. Active cleft and the fundamental two catalytic residues Asp (nucleophile) and Asp (acid/base) are at the bottom of the (β/βα)-barrel in GH-27 and GH-36 enzymes, while Asp and Glu act as nucleophile and acid/base catalysts, respectively, in GH-97 α-galactosidases. The C-terminal domain is a β-sandwich resembling the structure of the Greek key motif taking place in the GH-27 GH-36 and GH-97 families. GH-27 and GH-36 enzymes do not require calcium ions for action unlike GH-97 enzymes in which a calcium-coordinated water molecule in the active site is involved. From the analysis of crystallographic results, excluding monomeric GH-36 α-D-galactosidase from *Thermotoga maritima*, the GH-36 enzymes exhibit a tetrameric organization and GH-97 enzyme functions in solution as a monomer (Supplementary Material Part 5 and 6, respectively). The structural comparison of GH-27, -36 and -97 families revealed not only significant similarity but also fine differences, despite the similarity of catalytic mechanisms and allowed the conclusion that they appear to have diverged from a common ancestor (Okuyama et al., 2009; Rigden, 2002).

GH-4, GH-57, as well as GH-110, α-D-galactosidases crystal structures are unsolved at present. However, α-D-galactosidase from *Pyrococcus furiosus* is the first and
Table 1. Some characteristic of α-galactosidases of all CAZy families.

| CAZy Family | Subfamily | Evolution relations | Organism | cod PDB | 3D-structure | Mechanism | Substrate specificity |
|-------------|-----------|---------------------|----------|---------|--------------|-----------|----------------------|
| GH-4        |           |                     | Escherichia coli |         | Unsolved     | Retaining, elimination and hydration, Me^{2+} and NAD(H) | α-1,6 |
|             | GH-4      |                     | Bacillus subtilis |         |              |           | α-1,6                |
|             |           |                     | B. haloduranus (Takami et al., 2000) |         |              |           | α-1,6                |
|             |           |                     | Enterococcus faecium (van Schaik et al., 2010) |         |              |           | α-1,6                |
|             |           |                     | Citrobacter freundii (Shimamoto et al., 2001) |         |              |           | α-1,6                |
| GH-27       | II        | Common ancestor     | Oryza sativa | 1uas (Fujimoto et al., 2003) | N-terminal (β/α)-barel C-terminal β-sheet (Greek Kew motif) | Retaining, double displacement; nucleophyl, based/acids | α-1,3- α-1,6 |
| IIIa        |          |                     | Homo sapiens | 1r46 (Garman & Garboczi, 2004) |              |           | α-1,3- α-1,6         |
| V           |          |                     | Hypocrea jecorina (Trichoderma reesei) | 1szn (Golubev et al., 2004) |              |           | α-1,3- α-1,6         |
| I           |          |                     | Unbefulips vinacea | 3a5v (Fujimoto et al., 2009) |              |           | α-1,6                |
| I           |          | Saccharomices cerevisiae |            | 3lrk (Fernandez-Leiro et al., 2010) |              |           | α-1,6                |
| GH-36       | IV        | Thermotoga maritima | Geobacillus stearotherophilus strain | 1zy9 (Comforti et al., 2007) | N-terminal β-supersendvich Central (β/α)-barel C-terminal β-sheet (Greek Kew motif) | Retaining, double displacement; nucleophyl, based/acids | α-1,6 |
| I           |          | KVE39               |            |         |              |           | α-1,6                |
| I           |          | AgaA                |            | 4fnp (Merceron et al., 2012) |              |           | α-1,6                |
| I           |          | AgaB                |            | 4fnpq (Merceron et al., 2012) |              |           | α-1,6                |
| I           |          | Ruminococcus gaimus E1 |        | 2yfo (Bruel et al., 2011) |              |           | α-1,6                |
| I           |          | Lactobacillus acidophilus strain NCFM |      | 2xn2 (Fredslund et al., 2011) |              |           | α-1,6                |
| I           |          | Lactobacillus brevis strain ATCC 367 |      | 3mi6 (Fredslund et al., 2011) |              |           | α-1,6                |
| GH-97       | Retaining |                     | Bacteroides thetaiotaomicron BtGH97b | 3a24 (Okuyama et al., 2009) | N-terminal β-supersendvich Central deformed (β/α)-barel (contain 3_{10}-helix and loop instead of eighth β-stand) C-terminal β-sheet (Greek Kew motif) | Retaining, double displacement; nucleophyl, based/acids, Ca^{2+} | α-1,6 |
| GH-57       |          | Pyrococcus furiosus (Van Lieshout et al., 2003) |         |         | Unsolved     | Retaining, unknown | α-1,3 branch β-oligo |
| GH-110      | A        | Bacteroides fragilis | BiGal10A (Liu et al., 2007) |         | Unsolved     |               | α-1,3 terminal and branch β-oligo |
|             | B        |                     | BiGal10B (Liu et al., 2008) |         |               |               | α-1,3 terminal and branch β-oligo |
that the structural fold of the catalytic domain is (β/α)7-barrel found in some proteins (Dickmanns et al., 2006). Moreover, it was suggested that the structural fold of the catalytic domain is (β/α)7-barrel containing 310-helices far rare variation of the classical (β/α)n barrel found in some proteins (Dickmanns et al., 2006).

Examples of marine enzymes

Previous studies have shown that α-D-galactosidases are widespread in marine proteobacteria and Bacteroidetes (Bakunina et al., 2012; Ivanova et al., 1998). The α-D-galactosidase isolated from the marine bacterium Pseudoalteromonas sp. KMM 701 (PsGalA), attributed to the GH-36 family in accordance with its amino acid sequence, was the first to be fully characterized. Pseudoalteromonas sp. KMM 701 was isolated from the cold water of the Sea of Okhotsk. PsGalA is, indeed, an extremely cold active enzyme containing oligo- and poly-saccharides are the natural substrates of the enzyme, most of the microbial α-D-galactosidases are subject to induction by galactose and its derivatives (Cavazzoni et al., 1987; Delente et al., 1974; Pardee, 1957; Rosenow et al., 1999; Russell et al., 1992; Saier et al., 1996). As galactose containing oligo- and poly-saccharides are the natural substrates of the enzyme, most of the microbial α-D-galactosidases are subject to induction by galactose and its derivatives (Cavazzoni et al., 1987; Delente et al., 1974; Pardee, 1957; Rosenow et al., 1999; Russell et al., 1992; Saier et al., 1996).

Table 2. Results of Blast (Basic Local Alignment Search Tool) UniProt α-D-galactosidase of Pseudoalteromonas sp. KMM 701 and other marine predictive α-galactosidases.

| Entry | Organism | Length | Identity (%) |
|-------|----------|--------|--------------|
| Q19AX0 | Pseudoalteromonas sp. KMM 701 | 710 | 100.0 |
| F3BGP2 | Pseudoalteromonas haloplanktis ANT5/05 | 710 | 98.0 |
| G7EY75 | Pseudoalteromonas sp. BSI20429 | 710 | 96.0 |
| G7EE94 | Pseudoalteromonas sp. BSI20652 | 710 | 96.0 |
| M5GZD2 | Pseudoalteromonas sp. Bsw20308 | 710 | 95.0 |
| G7FWQ1 | Pseudoalteromonas sp. BSI20495 | 710 | 95.0 |
| N6W4T6 | Pseudoalteromonas agariarova S981 | 710 | 93.0 |
| G7FLJ4 | Pseudoalteromonas sp. BSI20480 | 710 | 93.0 |
| A0XX24 | Alteromonadales bacterium TW-7 | 710 | 93.0 |
| K7A5R1 | Glaciecola pallidula DSM 14239 = ACAM 615 | 709 | 64.0 |
| K0EAN5 | Alteromonas macrleodi (strain Balearic Sea AD45) | 709 | 59.0 |
| J9Y755 | Alteromonas macrleodi ATCC 27126 | 709 | 59.0 |
| K0CKM3 | Alteromonas macrleodi (strain English Channel 673) | 709 | 59.0 |
| F5ZF5 | Alteromonas sp. (strain SN2) | 214 | 59.0 |
| K6YXJ1 | Glaciecola mesophila KMM 241 | 718 | 51.0 |
| Q15P99 | Pseudoalteromonas atlantica (strain T6c/ATCC BAA-1087) | 718 | 52.0 |
| K6XR1 | Glaciecola chathamensis S18K6 | 718 | 51.0 |
| F4AU59 | Glaciecola sp. (strain 4H-3-7+YE-5) | 718 | 51.0 |
| K7A2L6 | Glaciecola polaris LMG 21857 | 718 | 51.0 |
| K6XF3M | Glaciecola agaritytica NO2 | 718 | 51.0 |
| K7AV2 | Glaciecola psychrophila 170 | 719 | 51.0 |
| Q1YSF9 | Gamma proteobacterium HTCC2207 | 736 | 49.0 |
| R0D1T0 | Caulobacter crescentor OR37 | 712 | 50.0 |
| Q2NBZ6 | Erythrobacter litoralis (strain HTCC2594) | 705 | 44.0 |
| A0Z897 | Marine gamma proteobacterium HTCC2080 | 710 | 41.0 |

α-D-Galactosidases: from biomedicine to enzymatic synthesis 237

Genetic regulation, biosynthesis and biological functions of α-D-galactosidases in microorganisms

Numerous studies have been published on the regulation of the biosynthesis of α-D-galactosidase in yeast and bacteria (Orskov & Orskov, 1973; Pardee, 1957; Rosenow et al., 1999; Russell et al., 1992; Saier et al., 1996). As galactose containing oligo- and poly-saccharides are the natural substrates of the enzyme, most of the microbial α-D-galactosidases are subject to induction by galactose and its derivatives (Cavazzoni et al., 1987; Delente et al., 1974; Pardee, 1957; Rosenow et al., 1999; Russell et al., 1992; Saier et al., 1996). The existence of the different regulatory mechanisms provides microorganisms to choose the preferred source of...
carbon and energy from the environment. However, some α-D-galactosidases were found to have other biological functions related to specific lifestyles of microorganisms in their ecological niche. It may be a result of mutations of α-D-galactosidase genes evolutionary adapted to more complex substrates and therefore modified by the expression patterns and the enzyme specificities. Detailed examples (Ademark et al., 1999; Bakunina et al., 2013; Boucher et al., 2003; Buchanan-Wollaston, 1997; Chrost et al., 2004; Civas et al., 1984; Crossman & Dow, 2004; Davey & O’Toole, 2000; de Vries et al., 1999; Ensor et al., 1999; Fong et al., 2010; Fridjonsson et al., 2000; Fujiki et al., 2000; Ishiguro et al., 2001; Kaplan et al., 2003; Luonteri et al., 1998; Mai-Prochnow, 2006; Margolles-Clark et al., 1996; Meier & Reid, 1982; Muiznieks et al., 1999; Pennycooke et al., 2004; Post-Beittenmiller et al., 1984; Prestidge & Pardee, 1965; Rice et al., 2005; Ryu et al., 2004; Schmid & Schmitt, 1976; Shnit-Orland et al., 2012; Silverstroni et al., 2002; Sou et al., 2006; Sumner-Smith et al., 1985; Taji et al., 2002) are reported on the dedicated Part 7 of the Supplementary Material.

Mechanisms of action

It is widely known that the stereochemical outcome of the enzymatic hydrolysis of O-glycosides can furnish products with inversion or retention of the anomeric configuration with respect to the starting substrates. Two mechanisms were considered initially. Retaining glycosidases maintain the anomeric configuration of the substrate in the products via a double displacement catalytic mechanism. Inverting glycosidases induced inversion in a one-step reaction. On this basis, a classification of glycosyl hydrolases has been adopted (Koshland, 1953), although other classifications are based on sequence similarities (Coutinho & Henrissat, 1999) and other mechanisms making use of different chemistry are today known (Jongkees & Withers, 2014). Of particular importance is the elimination–hydration mechanism found in the GH-4 family involving the presence of NAD⁰ and mercaptoethanol. Most, if not all, α-D-galactosidases belonging to GH-27 and GH-36 were ascribed to retaining enzymes but in a recent article, inverting examples were reported among α-D-galactosidases useful for removal of the immunodominant galactose (Liu et al., 2008).

The enzymes belonging to Bacillus fragilis and B. thetaiotaomicron were classified in two subfamilies of GH-110A and GH-110B and characterized by inverting mechanisms as determined by 1H NMR spectroscopy (Liu et al., 2008; Morley et al., 2009), a direct way for determining the stereochemical mechanism. Chemical shifts and coupling constants of the anomeric protons for substrates and products are distinct and readily observed. Retaining enzyme stereochemistry was assessed by this technique for GH-36 α-D-galactosidase from Thermotoga maritima (Comfort et al., 2007) and for three other GH-27 enzymes from Streptomyces griseolaulbus (Anisha et al., 2011).

It is known that many GH-27 and GH-36 retaining enzymes are capable of catalyzing transglycosylation. Transglycosylation activities remain unexplored for retaining GH-97 α-D-galactosidases. GH-4 belonging α-D-galactosidases from Bacillus halodurans and Citrobacter freundii catalyze galactoside hydrolysis via an NAD-dependent redox reaction that is coupled to an α,β-elimination process involving the formation of a glycal intermediate (Anggraeni et al., 2008; Chakladar et al., 2011). Addition of water to the anomeric center, reprotonation of C2–C3 double bond and reduction of 3-keto group by NADH previously formed, regenerate the pyranose ring with net retention of configuration. The overall result is the hydrolysis of the substrate. For optimal activity, the GH-4 α-D-galactosidase, found in the species Citrobacter freundii, requires two cofactors, NAD⁰ and Mn⁰²⁺, and the addition of a reducing agent, such as mercaptoethanol. It seems, not reported in literature, to be a possible transglycosylation event for this α-D-galactosidase. In addition, the proposed mechanism of the GH-4 galactosidases could be considered not prone to transglycosylation as for GH-109 α-N-acetylglactosaminidases (Liu et al., 2007). Indeed, the authors reported that when the enzyme from Citrobacter freundii catalyzed hydrolysis of the substrate in the presence of methanol (5 M), no trapping by methanol was observed. However, in the case of another enzyme (6-phospho-β-glucosidase) from Thermotoga maritima, it was proved that the biocatalyst had transglycosylation activity, as in the presence of methanol, methyl 6-phospho-β-glucoside was formed (Yip et al., 2004).

Practical applications of free and immobilized α-D-galactosidases

The α-D-galactosidase from coffee beans was one of the first to be purified and characterized and it is not a surprise that it is one of the most enzymes used in hydrolysis in the food industry and in biomedicine and for synthetic reactions described as follows. Hydrolytic properties of other enzymes from fungi or bacteria, especially regarding hydrolysis of antigens or highly improved performances by immobilization techniques, are detailed. Immobilization of enzymes has been one of the major activities in the field of biotechnology for any biocatalyst used in industry. The great draw that α-D-galactosidases have had in the food industry has made them biological materials for development of important immobilization techniques. A dedicated paragraph (Supplementary Material Part 8) is justified by the amount of studies starting with a didactic article (Mulimani & Dhananjay, 2007) and specialized ones (Bakunina et al., 2006; Corcherio et al., 2012; Filho et al., 2008; Hernaiz & Crout, 2000; Kuo & Goldstein, 1983; Ohtakara & Mitsutomi, 1987; Okutucu et al., 2010; Pessela et al., 2008; Prashanth & Mulimani, 2005; Shankar et al., 2011; Singh & Kayastha, 2012; Tippeswamy & Mulimani, 2003) as such as the study of human α-D-galactosidase A immobilization. The use of the synthetic ability of α-D-galactosidases by transglycosylation and reverse hydrolysis is described in Table 3.

Hydrolysis

Due to the presence of non-digestible raffinose-based sugars (Di Stefano et al., 2007) Figure 1) in soy products, the enzymatic hydrolysis by α-D-galactosidases has been of interest for years. The filamentous actinobacterium, Streptomyces griseolaulbus is a source of α-D-galactosidase that was used for the hydrolysis of soymilk and is also applied to reduce the
Table 3. Enzymatic synthesis of α-galactosides.

| Entry | Year [Reference] | Enzyme/donor | Product(s) | Conditions (biocatalysis, yields, immobilization, etc.) | Notes |
|-------|------------------|--------------|------------|-------------------------------------------------------|-------|
| 1     | 1992;1995 (Kitahata et al., 1992; Koizumi et al., 1995) | Coffee bean α-galactosidase/ melibiose | 6-O-α-D-galactosyl α-cyclodextrin and minor 2-O-α-D-galactosyl α-cyclodextrin | 2:1 w/w ratio donor/acceptor, 28% molar yield, 600 mg/ml donor initial concentration | Transgalactosylated derivative of the donor was also present |
| 2     | 1996 (Vic et al., 1996) | Aspergillus oryzae α-galactosidase/melibiose | α-D-Gal-O-(CH₂)₆-OH, α-D-Gal-O-(CH₂)₃-CH=CH₂ | Reverse hydrolysis, 80-90% acceptor solution in H₂O. Reaction time: 24 h to 6 days | |
| 3     | 1997 (Matsuo et al., 1997) | Coffee bean α-galactosidase/ PNP-α-D-Gal | Gal-α-1,3-Gal-PNP | PNP-α-D-Gal 250 mg/ml phosphate buffer/DMF solution (30%) + 26.7 U enzyme | |
| 4     | 2001 (Weignerova et al., 2001) | Extracellular α-galactosidases from fungi/PNP-α-D-Gal | Gal-α-1,2-Gal-PNP, Gal-α-1,6-Gal-PNP | Efficient 6-O-α-D-galactopyranosylation of substrate (15-22% conversion). | |
| 5     | 2001 (Biely et al., 2001) | Coffee bean α-galactosidase/ melibiose bean α-galactosidase | 6-O-α-D-galactopyranosyl derivative of cyclo{3(α-D-Glc)-6(α-D-Glc)-1(α-D-Glc)} from alternan | Molar excess acceptor = 8, temperature of reaction = −15°C; 15 days incubation | Studied with ¹⁹F NMR spectroscopy |
| 6     | 2001 (André et al., 2001) | Coffee bean α-galactosidase/ α-D-GalF; AgaB Bacillus stearothermophilus α-galactosidase/ α-D-GalF | Regioisomers of disaccharidic fluorides | Transglycosylation reactions or reverse hydrolysis | |
| 7     | 2002 (Casali et al., 2002) | Aspergillus terreus, A. commune, Penicillium viraceum, P. brasiliensis α-galactosidase/melibiose | Allyl, 2-nitroethyl, 2-(2'2'-tri-fluoracetamido)-ethyl α-D-galactosides | Transglycosylation yield of 51% | |
| 8     | 2002 (Spangenberg et al., 2002) | Coffee bean α-galactosidase/ α-D-GalF | α-D-galactosides of methyl α-D-galactosides | Molar excess acceptor = 8, temperature of reaction = −15°C; 15 days incubation | |
| 9     | 2003 (Nieder et al., 2003) | Bifidobacterium adolescentis α-galactosidase/melibiose | α-1,3 linked galactosides of UDP-Glc and UDP-Gal | Melibiose donor: 100-500 mM; 0.5 U/ml α-galactosidase at −5°C. Reaction time: 21 days | |
| 10    | 2003 (Simerska et al., 2003) | Talaromyces flavus α-galactosidase/PNP-α-D-Gal | Galactoside of 4-nitrophenyl 6-O-acetyl α-D-galactopyranoside | Biocatalysis was tested in organic solvent to assess activity and stability of the α-galactosidase. Solvents used: acetone, acetonitrile, DMF, DMSO, 1,4-dioxane, 2-methoxyethanol, pyridine, 2-methylpropan-2-ol, THF, propargyl alcohol. | |
| 11    | 2006 (Simerska et al., 2006) | Extracellular α-galactosidase of T. flavus CCF 2686/PNP-α-D-Gal | Sterically hindered alcohols (primary, secondary and tertiary) as galactosyl acceptors | Donor 0.03 M/acceptor 2.6 M | Yields from 7 to 35% |
content of raffinose oligosaccharides in horse gram and green gram flours. In comparison to traditional techniques, such as soaking and cooking, the enzymatic treatment was most effective and the raffinose content was reduced by 97.5% while stachyose was lowered by 93.2% (Anisha & Prema, 2008). The same reaction using Phaseolus vulgaris (family GH-27) and different crude enzymes from fungi was studied with essentially the same positive results (Song & Chang, 2006).

An interesting application of α-D-galactosidase in the biomedicine domain for treatment of red blood cells is known from the early 1980s when the idea of converting blood group A and B antigens to H using specific exo-glycosidases capable of removing the immune-determinant sugar residues, appeared. The monosaccharide determining type A specificity is the terminal α-1,3 linked N-acetylgalactosamine, while the corresponding monosaccharide for B type specificity is an α-1,3 linked galactose. In group O, cells lack either of these terminal monosaccharides with presence of fucose α-1,2 linked. Very expensive acid enzyme extracted from coffee beans (GH-27) was initially used for conversion of B red blood cells but lack of appropriate biocatalyst for A antigens has been the reason of a lag in the progress of this specific aspect. Therefore, novel glycosidases with improved kinetic properties and specificities for this kind of reaction are always of interest (Olsson et al., 2004). Recently, one of the novel isoforms of α-D-galactosidases derived from B. fragilis was applied for the enzymatic removal of the major α-3-Gal xenotransplantation antigen using porcine and rabbit red blood cells (Liu et al., 2008). However, α-D-galactosidases inactivating serological activity of human B red blood cells are relatively rare enzymes. Such an effect on the B red blood cells was shown for the CAzy unclassified enzymes from the eukaryotes Colocacia esculenta (Chien & Lin-Chu, 1991), Trichomonas foetus (Yates et al., 1975) and Streptomyces sp. (9917S2) (Oishi & Aida, 1972) as well as Cephalosporium acremonium (Zaprometova et al., 1990) and Penicillium sp. 23 α-galactosidase (Varbanets et al., 2001). Complete conversion of B red blood cells to O red blood cells, was achieved with wild and recombinant GH-27 α-D-galactosidases from green coffee beans Coffea canephora (Harpaz et al., 1974; Harpaz et al., 1975; Goldstein, 1989) and the action of recombinant GH-27 α-D-galactosidase of soybean Glycine max (Davis et al., 1996; Hobbs et al., 1996). It was reported that recombinant GH-27 α-D-galactosidase from the cell culture of rice Oryza sativa, expressed by cells Pichia pastoris, has acquired the ability to completely transform B red blood cells to O red blood cells (Chien et al., 2008). Clinical tests on volunteers showed that modified red blood cells are viable and can function practically as native ones (Goldstein, 1984; Gong et al., 2005; Kruskall et al., 2000; Zhang et al., 2007; Zhu et al., 1996). Data on clinical trials of red blood cells transformed by GH-110 enzymes have not been found in the literature.

There is another interesting application of hydrolytic capabilities of α-D-galactosidases applied in chemical synthesis. Developing a ‘natural strategy’ of these biocatalysts Fessner and collaborators used these enzymes to resolve anomeric product mixtures obtained from a simple acid-catalyzed Fischer galactosylation, providing single diastereomers easy to isolate (Ruiz et al., 2001).

### Synthesis

Examples (André et al., 2001; Biely et al., 2001; Bonnet et al., 2003; Casali et al., 2002; Kitahata et al., 1992; Koizumi et al., 1995; Matsuo et al., 1997; Nieder et al., 2003; Okada et al., 2011; Scigelova & Crout, 2000; Simerska et al., 2003, 2006; Spangenberg et al., 1999, 2002; Vic et al., 1996; Weignerova et al., 2001) of reports about enzymatic synthesis of α-galactosides are listed in Table 3. The coffee bean enzyme was one of the first biocatalyst used for the synthesis of α-galactosyl derivatives of cycloexetins. Reaction details and enzymatic performances are reported in the Supplementary Material Part 9.

Among important members of the human gastrointestinal microflora Bifidobacteria possess high α-D-galactosidase activity. Only in rare cases, enzymes have been shown to be able to synthesize galactooligosaccharides with melibiose, stachyose and raffinose as starting substrates. The products have the potential to boost the growth of bifidobacteria in the human gastrointestinal tract. Under optimum pH conditions for activity (pH 6.0) and high melibiose concentration (40% w/v), the enzyme from Bifidobacterium bifidum was able to form oligosaccharides with degree of polymerization (DP) ≥3 with a total yield of 20.5% (w/w) (Goulas et al., 2009). The enzyme from Bifidobacterium breve 203 (Aga2) synthesized a trisaccharide (Gal-α-1,4-Gal-α-1,6-Glc) using melibiose as a substrate. It was a new oligosaccharide containing Gal-α-1,4 linkage, a novel galactosidic link formed by microbial α-D-galactosidase. In a reaction using 100 mM melibiose, ca. 11% of the trisaccharide was formed which was isolated by a Biogel P2 column and characterized by 2D NMR spectroscopy (Zhao et al., 2008). Although the yield was modest, the authors conducted an analysis on acceptor specificity using different structures. In the presence of the aryl galactoside donor, Aga2 was able to catalyze glycosyl transfer to various acceptors including monosaccharides, disaccharides and sugar alcohols. Among them lactose was of interest being the disaccharidic fragment of globotriose. α-D-Galactosidases Aga1 and Aga2 from Ruminococcus graminus E1 were both able to perform transglycosylation reactions with α-(1,6) regioselectivity, leading to the formation of product structures up to [Hex]H2 and [Hex]H4, respectively, in the presence of melibiose. Aga1 and Aga2 also catalyzed transglycosylation reactions with PNP-Gal as the donor and various sugar acceptors such as Man, Gal, Glc, Mel, Suc, Lac and Raf. Aga1 could transglycosylate all hexoses tested and α-(1,6) linked oligosaccharides (Raf and Mel) but could not transglycosylate Xyl or the β-linked sugars. It was suggested that Aga1 and Aga2 play essential roles in the metabolism of dietary oligosaccharides and could be used for the design of galacto-oligosaccharide (GOS) prebiotics (Cervera-Tison et al., 2012).

Enzyme engineering has been used to enhance the transglycosylation activity of glycosidases and to modify other features of interest in biocatalysis (Trincone, 2013). It is important in this paragraph to discuss α-galactosynthase using β-galactosyl-azide as a possible donor. The α-D-galactosidase (TM1192) from the hyperthermophilic bacterium Thermotoga maritima (TmGalA), belonging to family GH-36, was chosen as a model system. The mutant Asp327Gly is an efficient
α-galactosynthase producing different galactosylated disaccharides from a β-galactosyl-azide donor and 4-nitrophenyl-α- and β-glycosides as acceptors. This is the first α-galactosynthase produced so far and the authors concluded that the instability of fluoride derivatives as substrates could have hampered the development of α-glycosynthases, thus β-azide derivatives can serve as an attractive alternative for the future production of novel α-glycosynthases (Cobucci-Ponzano et al., 2011).

Conclusion

From the general analysis of the literature reported in this review, the result is clear that α-D-galactosidases are known enzymes that have found a number of useful applications. The deficiency of α-D-galactosidase A in the Anderson–Fabry’s disease has made the topic to be almost exclusively covered in biomedicine. Further interest in the same domain has been added when the possible conversion of group B of red blood cells, removing enzymatically α-1,3-linked galactose, was reported. However, applications of α-D-galactosidases in food technology have not been neglected since concern in food science is still active in particular for new immobilizations and analytical techniques or in applications for design of galacto-oligosaccharide prebiotics using the synthetic capabilities of these enzymes. Improvements in enzyme production and properties, with regard to industrial applications, are being attempted by genetic approaches. Genomic data from marine microorganisms that play a crucial role in the global carbon cycle, suggest that the importance of the variable genome in tailoring individual strains to their specific lifestyles and functional repertoire. In addition, in this context, these studies are of great interest to turn wild types in optimal synthetic catalysts by site directed mutagenesis.

It has to be said, that the synthetic abilities appear to be the less exploited aspect for α-D-galactosidases and in future years interest for this specific aspect could increase for access to important small molecules by green technology. The emphasis on alternative sources of this class of enzymes, reported in this review, focuses on this need, as new enzymes could possess characteristics suitable for synthesis (selectivity, resistance to reaction conditions, etc.) more pronounced than the ones found in known examples.

Acknowledgements

Special thanks are due to the industrial research projects: PON01_02512 BIA-EPI Ricerca e Sviluppo di bioregolatori attivi sui meccanismi epigenetici dei processi infiammatori nelle malattie croniche e degenerative and PON ‘‘EnerbioChem Filiere agro-industriali integrate ad elevata efficienza energetica per la messa a punto di processi produzione eco-compatibili di energia e bio-chemicals da fonte rinnovabile e per la valorizzazione del territorio’’.

Declaration of interest

This work was supported by the Short Term Mobility fund in favour of A.T. by Consiglio Nazionale delle Ricerche and by grants from RFBR 13-04-00806-a, FEB RAS 12-III-A-05-019 and Programm FEB RAS 12-I-116-10.

References

Ademark P, de Vries RP, Hagglund P, et al. (2001). Cloning and characterization of Aspergillus niger genes encoding an α-galactosidase and a β-mannosidase involved in galactomannan degradation. Eur J Biochem, 268, 2982–90.

Ademark P, Lundqvist J, Hagglund P, et al. (1999). Hydrolytic properties of a β-mannosidase purified from Aspergillus niger. J Biotechnol, 75, 281–9.

Anagnostopoulos C, Courtois JE, Petek F (1954). The transferring action of α-galactosidase from coffee. Bull Soc Chim Biol (Paris), 36, 1115–23.

André C, Spangenberg P, Gentil E, Rabiller C. (2001). In situ 19F NMR spectroscopy study of enzymatic transglycosylation reactions using α-D-aldohexopyranosyl fluorides as donors and acceptors. Tetrahedron Asymmetry, 12, 779–83.

Angraeeni AA, Sakka M, Kimura T, et al. (2008). Characterization of Bacillus halodurans α-galactosidase Mel4A encoded by mel4A gene (BH2228). Biosci Biotechnol Biochem, 72, 2459–62.

Anisha GS, John RP, Prema P. (2011). Substrate specificities and mechanism of action of multiple α-galactosidases from Streptomyces griseololbus. Food Chem, 124, 349–53.

Anisha GS, Prema P. (2008). Reduction of non-digestible oligosaccharides in horse gram and green gram flours using crude α-galactosidase from Streptomyces griseololbus. Food Chem, 106, 1175–9.

Anisha GS, Rojan PJ, Nicemol J, et al. (2008). Production and characterization of partially purified thermostable α-galactosidases from Streptomyces griseololbus for food industrial applications. Food Chem, 111, 631–5.

Bakunina IYu, Nedashkovskaya OI, Balabanova LA, et al. (2013). Comparative analysis of glycoside hydrolases activities from phylogenetically diverse marine bacteria of the genus Arenibacter. Mar Drugs, 11, 1977–98.

Bakunina IYu, Nedashkovskaya OI, Kim SB, et al. (2012). Diversity of glycosidase activities in the bacteria of the phylum Bacteroidetes isolated from marine algae. Microbiology, 81, 688–95.

Bakunina IYu, Nedashkovskaya OI, Zvyagintseva TN, Shchupinov YA. (2008). Immobilization of α-Galactosidase inside hybrid silica nanocomposites containing polysaccharides. Russ J Appl Chem, 79, 827–32.

Bakunina IYu, Sova VV, Nedashkovskaya OI, et al. (1998). α-Galactosidase of the marine bacterium Pseudalteromonas sp. KMM 701. Biochemistry (Moscow), 63, 1209–15.

Balabanova LA, Bakunina IYu, Nedashkovskaya OI, et al. (2010). Molecular characterization and therapeutic potential of a marine bacterium Pseudalteromonas sp. KMM 701 α-galactosidase. Mar Biotechnol, 12, 111–20.

Balasubramaniam K, Mathew D. (1986). Purification of α-galactosidase from coconut. Phytochemistry, 25, 1819–21.

Banner DW, Bloomer AC, Petsko GA, et al. (1975). Structure of chicken β-D-galactosidase and a β-glycosidase producing different galactosylated disaccharides from a β-galactosyl-azide donor and 4-nitrophenyl-α- and β-glycosides as acceptors. This is the first α-galactosynthase produced so far and the authors concluded that the instability of fluoride derivatives as substrates could have hampered the development of α-glycosynthases, thus β-azide derivatives can serve as an attractive alternative for the future production of novel α-glycosynthases (Cobucci-Ponzano et al., 2011).
Comfort DA, Brouns SJ, Smiths N, Wu H, et al. (2006). Production of a novel α-D-galactosidase from soybean (Glycine max). Biochem Biol Intern, 39, 471–85.

de Vries RP, van den Broeck HC, Deckers E, et al. (1999). Differential expression of three α-D-galactosidase genes and a single β-D-galactosidase gene from Aspergillus niger. Appl Environ Microbiol, 65, 2453–60.

Dean KJ, Sweetee CC. (1979). Studies on human liver α-galactosidases. III. Partial characterization of carbohydrate-binding specificities. J Biol Chem, 254, 10006–10.

Delboni K, Johnson HJ, Kuo MJ, et al. (1974). Production of a new thermolabile α-d-galactosidase from a strain of Bacillus steatherophilus. Biotechnol Bioeng, 16, 1227–43.

Demirev PA. (2013). Dried blood spots: analysis and applications. Anal Chem, 85, 779–89.

Dey PM, Patel S, Brownleander MD. (1993). Induction of α-D-galactosidase in Penicillium ochrochloron by guar (Cyanoapis tetragonobola) gum. Biotechnol Appl Biochem, 17, 361–71.

Di Stefano M, Micelli E, Gotti S, et al. (2007). The effect of oral α-D-galactosidase on intestinal gas production and gas related symptoms. Dig Dis Sci, 52, 78–83.

Dickmanns A, Ballschmiter M, Liebl W, Ficner R. (2006). Structure of the novel α-amylase AmyC from Thermotoga maritima. Acta Cryst, D62, 262–70.

Ensor L, Storz S, Weiner R. (1999). Expression of multiple complex polysaccharide-degrading enzyme systems by marine bacterium strain 2-40. J Ind Microbiol Biotechnol, 23, 123–6.

Feld-Rasmussen U, Rasmussen AK, Mersebach H, et al. (2002). Fabry disease a metabolic disorder with a challenge for endocrinologists. Mt. Sinai J Med, 58, 259–65.

Fernandez-Leiro R, Pereira-Rodrı´guez A, Cerdan ME, et al. (2010). Structural analysis of Saccharomyces cerevisiae α-galactosidase and its complexes with natural substrates reveals new insights into substrate specificity of GH-27 glycosidases. J Biol Chem, 285, 28020–33.

Ferrutão JA, Banik M, Bewley JD. (2001). The cloning and characterization of α-D-galactosidase present during and following germination of tomato (Lycopersicon esculentum) seed. J Exp Bot, 52, 1239–49.

Filo M, Pessela BC, Mateo C, et al. (2008). Immobilization-stabilization of an α-D-galactosidase from Thermus sp. strain T2 by covalent immobilization on highly activated supports: selection of the optimal immobilization strategy. Enzyme Microb Technol, 42, 265–71.

Fong JCN, Syed KA, Klose KE, Yildiz FH. (2010). Role of Vibrio polysaccharide (vps) genes in VPS production, biofilm formation and Vibrio cholerae pathogenesis. Microbiology, 156, 2757–69.

Fredslund F, Hachem M, Larsen RJ, et al. (2011). Crystal structure of α-galactosidase from Lactobacillus acidophilus NCFM: insight into tetramer formation and substrate binding. J Mol Biol, 412, 466–80.

Frias J, Price KR, Fenwick CL, et al. (1996). Improved method for the analysis of α-galactosides in pea seeds by capillary zone electrophoresis. Comparison with high-performance liquid chromatography triple-pulsed amperometric detection. J Chromatogr A, 719, 213–19.

Fridjonsson O, Watzlawick H, Mattes R. (2000). The structure of the α-D-galactosidase gene loci in Thermus brockianus ITI 360 and Thermus thermophilus TH125. Extremophiles, 4, 23–33.

Fujiki Y, Ito M, Nishida I, Watanabe A. (2000). Multiple signalling pathways in gene expression during sugar starvation: pharmacological analysis of din gene expression in suspension-cultured cells of Arabidopsis. Plant Physiol, 124, 1139–47.

Fujimoto Z, Kaneko S, Kim WD, et al. (2009). The tetramer structure of the glycoside hydrolase family 27 α-galactosidase I from Umbelopsis vinacea. Biosci Biotechnol Biochem, 73, 2360–40.

Fujimoto Z, Kaneko S, Momma M, et al. (2003). Crystal structure of rice α-galactosidase complexed with D-galactose. J Biol Chem, 278, 20313–18.

Galas E, Miszkiewicz H. (1996). Purification and some properties of α-D-galactosidase from Mortierella vinacea IBT-3. Acta Microbiol Pol, 45, 143–54.

Galli U. (2005). The α-Gal epitope and the anti-Gal antibody in xenotransplantation and in cancer immunotherapy. Immunol Cell Biol, 83, 674–86.

Galperin MY, Fernandez-Suarez XM. (2012). The 2012 Nucleic acids research database issue and the online molecular biology database collection. Nucleic Acids Res, 40, D1–8.
α-D-Galactosidases: from biomedicine to enzymatic synthesis

Kaplan JB, Meyenhofer MF, Fine DH. (2003). Biofilm growth and detachment of Actinobacillus actinomycetemcomitans. J Bacteriol, 185, 1399–404.

Kawamura S, Kagawa-ken T, Kasi T, et al. (1971). Rapid microbiological production of alpha-galactosidase. US Patent 3,562,113.

Keates SE, Loewus F, Helms GL, Zink D. (1998). 5-O-(α-D-galactopyranosyl)-D-glycerol-pent-2-enone-1,4-lactone: characterization in the oxalate-producing fungus, Sclerotinia sclerotiorum. Phytochemistry, 49, 2397–401.

Keller F, Pfarr DM. (1996). Metabolism of carbohydrates in sink and source: galactosyl-oligosaccharides. In: Zamski E, Schaffer AA, eds. Photoassimilate distribution in plants and crops. New York: Marcel Dekker, 157–83.

Kim WD, Kaneko S, Park GG, et al. (2003). Purification and characterization of α-galactosidase from sunflower seeds. Biotechnol Lett, 25, 353–8.

Kim WD, Kobayashi O, Kaneko S, et al. (2002). α-Galactosidase from cultured rice (Oryza sativa L. var. Nipponbare) cells. Phytochemistry, 61, 621–30.

Kitahata S, Hara K, Fujitaya K, et al. (1992). Synthesis of O-α-galactosyl-β-cyclodextrin by coffee bean α-galactosidase. Biosci Biotechnol Biochem, 56, 1518–19.

Koizumi K, Tanimoto T, Okada Y, et al. (1995). Isolation and characterization of novel heterogeneous branched cyclomalto-oligosaccharides (cyclodextrins) produced by transgalactosylation with α-galactosidase from coffee bean. Carbohydr Res, 278, 129–42.

Koshland DEJ. (1953). Stereospecificity and the mechanism of enzymatic reactions. Biol Rev Camb Phil Soc, 28, 416–36.

Krieger N, Steiner W, Mitchell D. (2004). Foreword, Frontiers in biocatalysis. Food Technol Biotechnol, 42, 19–21.

Kronzer F, Schuerch C. (1974). Use of positively charged leaving groups in the synthesis of O-α-D-linked galactosides. Attempted synthesis of 3-O-(α-D-galactopyranosyl)-D-galactose. Carbohydr Res, 33, 273–80.

Kruskall MS, AuBuchon JP, Anthony KY, et al. (2000). Transfusion to blood group A and O patients of group B RBCs that have been enzymatically converted to group O. Transfusion, 40, 1290–8.

Kuo JY, Goldstein J. (1983). α-Galactosidase immobilized on a soluble polymer. Enzyme Microf Technol, 5, 285–90.

Lemieux RU, Driguez H. (1975). The chemical synthesis of 2-O-(α-L-fucopyranosyl)-3-O-(α-D-galactopyranosyl)-D-galactose. The terminal structure of the blood-group B antigenic determinant. J Am Chem Soc, 97, 4069–75.

Li H, Li Q, Cai MS, Li ZJ. (2000). Synthesis of galactosyl and lactosyl derivatives as potential anti metastasis compounds. Carbohydr Res, 328, 611–15.

Li YT, Li Sc. (1999). Enzymatic hydrolysis of glycosphingolipids. Anal Biochem, 273, 1–11.

Liljestrom PL, Liljestrom P. (1987). Nucleotide sequence of the melA gene, coding for α-galactosidase in Escherichia coli K-12. Nucleic Acids Res, 15, 2215–20.

Linden JC. (1982). Immobilized α-D-galactosidase in the sugar beet industry. Enzyme Microb Technol, 4, 130–6.

Liu QP, Sulzenbacher G, Yuan H, et al. (2007). Bacterial glycosidases for the production of universal red blood cells. Nat Biotechnol, 25, 454–64.

Liu QP, Yuan H, Bennett EP, et al. (2008). Identification of a GH-110 subfamily of α-L-1,3-galactosidases, novel enzymes for removal of the α3Gal xenotransplantation antigen. J Biol Chem, 283, 8545–54.

Luong NT, Alatalo E, Sinko-Abi M, et al. (1998). α-galactosidases of Penicillium simplicissimum: production, purification and characterization of the gene encoding AGL1. Biotechnol Appl Biochem, 28, 179–88.

Mai-Prochnow A. (2006). Autolysis in the development and dispersal of biofilms formed by the marine bacterium Pseudolalteromonas tunicate. PhD thesis, School of Biotechnology and Biomolecular Sciences, Faculty of Science, The University of New South Wales, Sydney, Australia.

Malhotra OP, Dey PM. (1967). Purification and physical properties of sweet almond α-D-galactosidase. Biochem J, 103, 508–13.

Margaroles-Clark E, Tenkanen M, Luonten E, Penttilä M. (1996). Three α-galactosidase genes of Trichoderma reesei cloned by expression in yeast. Eur J Biochem, 240, 104–11.

Marraccini P, Rogers WJ, Caillet V, et al. (2005). Biochemical and molecular characterization of α-D-galactosidase from coffee beans. Plant Physiol Biochem, 43, 909–20.
Silvestrini A, Connes C, Sesma F, et al. (2002). Characterization of the melA locus for α-galactosidase in Lactobacillus plantarum. Appl Environ Microbiol, 68, 5464–71.

Simerska P, Kuzma M, Monti D, et al. (2006). Unique transglycosylation potential of extracellular α-D-galactosidase from Talaromyces flavus. J Mol Cat B Enzym, 39, 128–34.

Simerska P, Kuzma M, Pivsvecova A, et al. (2003). Application of selectively acylated glycosides for the α-galactosidase-catalyzed synthesis of disaccharides. Folia Microbiol, 48, 329–37.

Singh N, Kayastha AM. (2012). Cicer α-galactosidase immobilization onto chitosan and Amberlite MB-150: optimization, characterization, and its applications. Carbohydr Res, 358, 61–6.

Song D, Chang SKC. (2006). Enzymatic degradation of oligosaccharides in pinto bean flour. J Agric Food Chem, 54, 1296–301.

Sou C-P, Ali ZM, Lazan H. (2006). Characterisation of an α-galactosidase with potential relevance to ripening related carbohydrate changes. Phytochemistry, 67, 242–54.

Spangenberg P, André C, Langlois V, et al. (2002). α-Galactosyl fluoride in transfer reactions mediated by the green coffee beans α-galactosidase in ice. Carbohydr Res, 337, 221–8.

Spangenberg P, Chiffoleau-Giraud V, André C, et al. (1999). Probing the transferase activity of glycosidases by means of in situ NMR spectroscopy. Tetrahedron Asymmetry, 10, 2905–12.

Subba RK, Pieringer RA. (1970). Metabolism of glyceride glycolipids-IV: enzymatic hydrolysis of monogalactosyl diglycerides in rat brain. J Neurochem, 245, 2520–4.

Sumner-Smith M, Bozzato RP, Skipper N, et al. (1985). Analysis of the inducible MEL1 gene of Saccharomyces carlsbergensis and its secreted product, α-galactosidase (melibiose). Gene, 36, 333–40.

Suzuki N, Khoo KH, Chen CM, et al. (2003). N-glycan structure of pigeon IgG. A major serum glycoprotein containing Galα1,3Galβ1,4GlcNAc. J Biol Chem, 278, 46293–306.

Taji T, Ohsumi K, Iuchi S, et al. (2000). Complete genome sequence of the alkaliphilic bacterium Bacillus halodurans and molecular characterization of a novel type of α-galactosidase from Melibooza ostreae. J Mol Cat B Enzym, 39, 128–34.

Takami H, Nakasone K, Takaki Y, et al. (2000). Complete genome sequence of the alkaliphilic bacterium Bacillus halodurans and genomic sequence comparison with Bacillus subtilis. Nucleic Acids Res, 28, 4317–31.

Testor RF, Al-Ghazzewi FH. (2013). Mannans and health, with a special focus on glucosamannans. Food Res Int, 50, 384–91.

Tippeswamy S, Mulimani VH. (2003). Enzymic degradation of raffinose family oligosaccharides in soymilk by immobilized α-galactosidase from Gibberella fujikuroi. Process Biochem, 38, 635–40.

Triconce A, Giordano A. (2006). Glycosyl hydrolases and glycosyltransferases in the synthesis of oligosaccharides. Curr Org Chem, 10, 1163–93.

Triconce A. (2013). Angling for uniqueness in enzymic preparation of glycosides. Biomolecules, 3, 334–50.

Van Lieshout JFT, Verhees CH, Ettema TJG, et al. (2003). Identification and molecular characterization of a novel type of α-galactosidase from Pyrococcus furiosus. Biocatal Biotransform, 21, 243–52.

van Schaik W, Top J, Riley DR, et al. (2010). Pyrosequencing-based comparative genome analysis of the nosocomial pathogen Enterococcus faecium and identification of a large transferable pathogenicity island. BMC Genomics, 11, 239.

Varbanets LD, Malanchuka VM, Buglova TT, Kuhlmann RA. (2001). Penicillium sp. 23 α-galactosidase: purification and substrate specificity. Carbohydr Polym, 44, 357–63.

Varrot A, Yip VLY, Li Y, et al. (2005). NAD+ and metal-ion dependent hydrolysis by family 4 glycosidases: structural insight into specificity for phospho-β-D-glucoylsides. J Mol Biol, 356, 423–35.

Vic G, Hastings JJ, Crout DHG. (1996). Glycosidase-catalyzed synthesis of glycosides by an improved procedure for reverse hydrolysis: application to the chemoenzymatic synthesis of galactopyranosyl-(1-4)-O-α-galactopyranoside derivatives. Tetrahedron Asymmetry, 7, 1973–84.

Weignerova L, Hunkova Z, Kuzma M, Kren V. (2001). Enzymatic synthesis of three pNP-α-galactobiopyranosides: application of the library of fungal α-galactosidases. J Mol Cat B Enzym, 11, 219–24.

Weignerova L, Sedmera P, Hunkova Z, et al. (1999). Enzymatic synthesis of iso-globotriose from partially protected lactose. Tetrahedron Lett, 40, 9297–9.

Wong H, Hu C, Yeh H, et al. (1986). Production, purification and characterization of α-galactosidase from Monascus pilosus. Appl Environ Microbiol, 52, 1147–52.

Yagi H, Yamamoto M, Yu SY, et al. (2010). N-glycosylation profiling of turtle egg yolk: expression of galabiose structure. Carbohydr Res, 345, 442–8.

Yates AD, Morgan WTJ, Watkins WM. (1975). Linkage-specific α-D-galactosidases from Trichomonas foetus: characterization of the blood-group B-destroying enzyme as a 1,3,α-galactosidase and the blood-group PI-destroying enzyme as a 1,4,α-galactosidase. FEBS Lett, 60, 281–5.

Yip VLY, Varrot A, Davies GJ, et al. (2004). An unusual mechanism of glycoside hydrolysis involving redox and elimination steps by a family 4 β-galactosidase from Thermotoga maritima. J Am Chem Soc, 126, 8354–5.

Zaprometova OM, Ulejivo IV, Lakhin VM. (1990). Structure and properties of Cephalosporium acremonium α-galactosidase. Glycoconj J, 7, 287–300.

Zaprometova OM, Ulejivo IV. (1988). Isolation and purification of a mold α-galactosidase. Biotechnol Appl Biochem, 10, 233–46.

Zhang YP, Gong F, Bao GQ, et al. (2007). B to O erythrocyte conversion by the recombinant α-galactosidase. Chin Med J, 120, 1145–50.

Zhang H, Lu L, Xiao M, et al. (2008). Cloning and characterization of a novel α-galactosidase from Bifidobacterium breve 203 capable of synthesizing Galα1,4 linkage. FEMS Microbiol Lett, 285, 278–83.

Zhifang G, Schaffer A. (1999). A novel alkaline α-galactosidase immobilization for phospho-α-D-glucosides. J Mol Cat B Enzym, 1163–93.

Zhu A, Leng L, Monahan C, et al. (1996). Characterization of recombinant α-galactosidase for use in sereocconversion from blood group B to O of human erythrocytes. Arch Biochem Biophys, 327, 324–9.

Supplementary Material available online

Supplemental Parts 1–9.