https://doi.org/10.15407/microbiolj86.02.090

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# DISTRIBUTION, PROPERTIES, AND PRACTICAL SIGNIFICANCE OF α-GALACTOSIDASE

*The achievements of modern enzymology have greatly expanded the possibilities of practical use of enzymes, primarily in medicine and the food industry, which is due to their advantages over chemical catalysts: selectivity and stereospecificity of action, the possibility of achieving high rates of conversion of substrates under relatively mild technological conditions, harmlessness to the environment and humans. The microbial producers have proven themselves as a convenient and economical source of biotechnologically important enzyme preparations of hydrolytic action. One of such enzymes is α-galactosidase, which has the ability to cleave terminal α-linked galactosyl residues from a wide range of natural and synthetic compounds. The review presents data on the spread of the enzyme among different groups of microorganisms and provides a comparative description of their catalytic properties. A range of issues related to the physiological role of the enzyme in plant and animal organisms, localization and functional properties in bacteria and fungi are outlined. The place of α-galactosidase of microorganisms in the modern hierarchical classification of glycosidases is shown. The areas of possible use of the enzyme as an efficient tool for the modification of oligo- and polysaccharides in various industries are described.*

***Keywords:*** *α-galactosidase, microorganisms, distribution, properties, practical significance.*

Enzymatic modification is one of the priority directions of modern industrial biotechnology for obtaining food products of improved quality, food additives, and biologically active substances for humans and animal feed. In contrast to inorganic catalysts, enzymes have a number of valuable properties. This is, first of all, high specificity of action and selectivity to certain substrates, as well as extraordinary efficiency in conditions of moderate temperatures, normal pressure, and neutral pH values. In recent years, enzymes of microorganisms have become the main source of technological catalysts due to the rate of growth and controllable synthesis processes. In addition, the diversity of microorganisms and their adaptation systems, which ensure the existence of

Citation: Borzova N.V., Varbanets L.D. Distribution, Properties, and Practical Significance of α-Galactosidase. *Microbiological journal.* 2024 (2). P. 90—113. https://doi.org/10.15407/microbiolj86.02.090

microbes in the most extreme conditions, allow one to find among them producers of enzymes with any necessary properties. In addition, modern methods of genetic and molecular engineering make it possible to successfully modify them to improve technological properties.

O-glycosyl hydrolases, i.e., enzymes capable of cleaving a glycosidic bond, have been increasingly used in the current biotechnological processes in recent years, second only to proteases (Yi et al., 2021). Most of these enzymes are used in processing technologies of various vegetable raw materials.

**Classification, structure, and mechanism of action of α-galactosidases.** α-Galactosidases (melibiases) (EC 3.2.1.22) are glycosyl hydrolases capable of cleaving, as a rule, with preservation of their optical configuration, the terminal nonreducing residues of α-D-galactose from α-D-galactosides, including from galactooligosaccharides, galactomannans, and galactolipids. Their simplest natural substrates are melibiose disaccharide and raffinose trisaccharide. Transgalactosidase activity was detected in some of them. α-N-acetylgalactosaminidase (EC 3.2.1.49) is biochemically and evolutionarily close to α-galactosidase.

The traditional classification of glycosidases is based on the substrate specificity of the enzyme, and the modern hierarchical classification is based on the similarity of the amino acid sequences of the protein. Based on substrate specificity, Dey (Dey, 1984) divided α-galactosidase into two groups. One combined enzymes that hydrolyzed only small substrates (*p*-nitrophenyl-α-galactopyranoside (*p*-NPG), melibiose, raffinose, stachyose, galactooligosaccharides), and the other — enzymes that were also able to release galactose from highly polymerized substrates such as galactomannans. Based on the structure of the catalytic domain, all α-galactosidases were assigned to six families: GH4, GH27, GH36, GH57, GH97, and GH110 (according to the CAZy database (http://www.cazy.org/):

▪ GH4 — α-galactosidase from *Escherichia coli* and bifunctional enzymes with α-galactosidase and α-glucosidase (EC 3.2.1.20) activities from *Thermotoga maritime* and *Thermotoga neapolita*; + maltose-6-phosphate glucosidase (EC 3.2.1.122) from *Eubacteria*; + 6-phospho-α-glucosidases (EC 3.2.1.122) from *Eubacteria*; + 6-phospho-β-glucosidases (EC 3.2.1.86) from *Eubacteria*; + oligosaccharides raffinise family (ORFs) from *Archaea*.

▪ GH27 — mainly α-galactosidase of eukaryotes (plants, animals, fungi) and some *Eubacteria*; + α-N-acetylgalactosaminidase of eukaryotes (plants and animals); + isomalto-dextranase (EC 3.2.1.94) from *Arthrobacter globiformis*; + β-L-arabinopyranosidase (EC 3.2.1.88) from *Streptomyces avermitilis*.

▪ GH36 — mainly α-galactosidase from *Eubacteria* and *Eukaryota* (fungi and plants); + α-N-acetylgalactosaminidase from *Clostridium perfringens*; + α-galactosyltransferases (EC 2.4.1.67 and EC 2.4.1.82) from plants; + ORFs from *Archaea.*

▪ GH57 — α-galactosidase from *Pyrococcus furiosus* and *Thermococcus alcaliphilus* + α-amylase (EC 3.2.1.1), amylopululanase (EC 3.2.1.41) and 4-α-gluconotransferase (EC 2.4.1.25) from *Eubacteria* and *Archaea*.

▪ GH97 — α-galactosidase from *Bacteroides thetaiotaomicron*; + α-glucosidase (EC 3.2.1.20) from *B. thetaiotaomicron* and *Tannerella forsythia*; + ORF-protein from two species of the genus *Sulfolobus*.

▪ GH110 — α-galactosidase from *Bacteroides fragilis*, *B. thetaiotaomicron* and *Streptomyces avermitilis*; + α-1,3-galactosidase (EC 3.2.1.) from *B. fragilis*, *B. thetaiotaomicron*, *Pseudoalteromonas distincta*.

All multicellular organisms and most studied unicellular eukaryotes and bacteria have α-galactosidases, but among archaea they are found only in certain species. Most eukaryotic α-galactosidases belong to the GH27 family, and most bacterial α-galactosidases belong to the GH36 family. These two families contain almost exclusively α-galactosidases and α-N-acetylgalactosaminidases. The analysis of the phylogeny of the GH36 family showed its poly-

phyletic origin, which made it possible to split this family into four new ones. Families GH27 and GH36 at the highest hierarchical level form the GH-D clan and have, as believed (Naumoff, 2004), a common ancestral gene. The conserved catalytic domains of this clan have a three-dimensional structure in the form of a $(\beta/\alpha)_8$-barrel, also known as a triosephosphate isomerase barrel (TIM-barrel), and a C-terminal β-structural domain (Comfort et al., 2007). This three-dimensional structure is the most common among the catalytic domains of all glycosyl hydrolases and one of the most common among all proteins. To date, such crystal structures have been described for α-galactosidases of *Trichoderma reesei*, *Umbelopsis vinacea, Saccharomyces cerevisiae*, *Geobacillus stearothermophilus*, *Streptomyces* sp., and *Aspergillus nidulans* (Table 1) (Golubev et al., 2004; Fujimoto et al, 2009; Fernandes-Leiro et al., 2010; Foucault et al., 2006; Zhou et al., 2016; Nakai et al., 2010; Fushinobu & Hachem, 2021). α-Galactosidases of the GH36 family have an additional N-terminal domain, which is absent in the GH27 family enzymes. Also, α-galactosidases from the GH4, GH27, GH36, and GH57 families can use glutamic acid as a catalytic nucleophile (Comfort et al., 2007; Zechel & Withers, 2000).

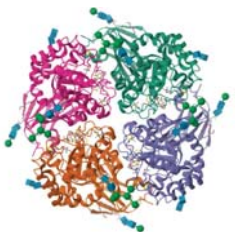
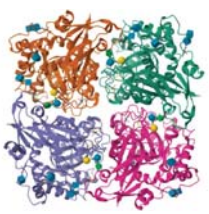
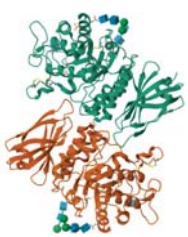
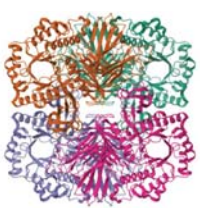
The catalytic domains of the GH57 family have a structure similar to the TIM-barrel, namely $(\beta/\alpha)_7$-barrel. The GH97 family contains both archaeal α-galactosidases known to date as well as a number of prokaryotic glycosyl hydrolases with other activities. The GH4 family includes bacterial glycosidases with broad substrate specificity, which use $NAD^+$ and $Mn^{2+}$ as cofactors (Rajan et al., 2004). The presence of α-galactosidase activity was shown for three of them. The GH110 family includes the only known α-galactosidase capable of removing α-D-galactose residues with inversion (rotation) of their optical configuration. This enzyme was discovered in the bacterium *B. fragilis* (Liu et al., 2008). According to modern ideas, the α-galactosidase genes of the GH27, GH36, and GH57 families have a common evolutionary origin.

Currently, there are experiment-based ideas that in the active centers of glycosidases, two areas can be conditionally distinguished: the sorption, the so-called carbohydrate-binding domain (CBD), and the catalytic domain (CD). The sorption site ensures the formation of a complex of the substrate with the enzyme; it is responsible for the selection of a substrate and the specificity of an enzyme. The CD is a set of functional groups that redistribute electron density and transfer groups directly in a chemical catalytic act.

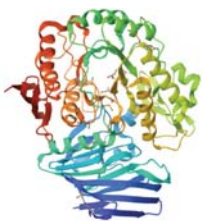
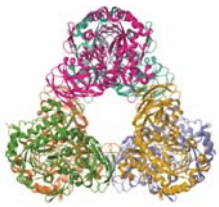
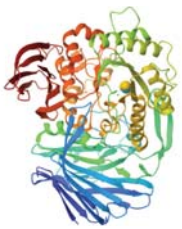
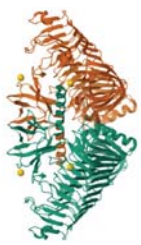
The study of the crystal structure of α-galactosidases with GH27 and 36 in a complex with their substrates allowed us to reveal some regularities of the mechanism of binding of the substrate to the active center of the enzyme. The monomeric enzymes of *T. reesei* and *T. maritima* form a shallow pocket of the active center, while the tetrameric α-galactosidases of *L. acidophilus* and hexameric enzyme from *Thermus thermophilus* are deep and narrow (Fredslund et al., 2011; Chen et al., 2020). The replacement of one amino acid residue involved in the formation of the active cavity of the enzyme can significantly affect both the substrate specificity and the enzyme stability. This was shown as a result of crystal structure and mutational studies of two α-galactosidases of *G. stearothermophilus* (Merceron et al., 2012). The crystal structure analysis of α-galactosidase from *B. thetaiotaomicron* of the GH97 family showed that the enzyme molecule consists of three domains, just like α-galactosidases of the GH36 family (Okuyama et al., 2009). In addition, the enzyme contains a calcium ion, which is important for the manifestation of catalytic activity. α-Galactosidases of the GH27 and 36 families are characterized by the mechanism of coordinated action of two carboxyl groups as a nucleophile (water activator) and an electrophile (substrate activator) (http://www.cazy.org/).

According to current data, α-galactosidases catalyze enzymatic reactions through the mechanism of preservation or inversion (rotation) of the anomeric configuration of the substrate

*Table 1.* **Electrophyle-nucleophyle systems of the active center of α-galactosidases**

| Source | GH | 3D Structure Status | Electrophyle | Nucleophyle | Structure | 3D View |
|---|---|---|---|---|---|---|
| Sweet almond (Dey & Pridham, 1977) | — | $(\beta/\alpha)_8$ barrel | $NH^+$ (His) | $COO^-$ (Asp) | — | — |
| *Umbelopsis vinacea* (Fujimoto et al., 2009) | 27 | $(\beta/\alpha)_8$ barrel | COOH (Asp) | $COO^-$ (Asp) | homo-tetramer |  |
| *Trichoderma reesei* (Golubev et al., 2004) | 27 | $(\beta/\alpha)_8$ barrel | COOH (Asp) | $COO^-$ (Asp) | monomer |  |
| *Saccharomyces cerevisiae* (Fernandes-Leiro et al., 2010) | 27 | $(\beta/\alpha)_8$ barrel | COOH (Asp) | $COO^-$ (Asp) | homo-tetramer |  |
| Lysosomal α-galactosidase (Guce et al., 2010) | 27 | $(\beta/\alpha)_8$ barrel | COOH (Glu) | $COO^-$ (Glu) | homo-dimer |  |
| *Geobacillus stearothermophilus* (Merceron et al., 2012) | 36 | $(\beta/\alpha)_8$ barrel | COOH (Asp) | $COO^-$ (Asp) | homo-tetramer |  |
| *Lactobacillus acidophilus* (Fredslund et al., 2011) | 36 | $(\beta/\alpha)_8$ barrel | COOH (Asp) | $COO^-$ (Asp) | homo-tetramer |  |

*Continuation of Table 1.*

| Source | GH | 3D Structure Status | Electrophyle | Nucleophyle | Structure | 3D View |
|---|---|---|---|---|---|---|
| *Thermotoga maritima* (Comfort et al., 2007) | 36 | $(\beta/\alpha)_8$ barrel | COOH (Asp) | $COO^-$ (Asp) | monomer |  |
| *Thermus thermophilus* (Chen et al., 2020) | 36 | $(\beta/\alpha)_8$ barrel | COOH (Asp) | $COO^-$ (Asp) | homo-hexamer |  |
| *Bacteroides thetaiotaomicron* (Okuyama et al., 2009) | 97 | $(\beta/\alpha)_8$ barrel | COOH (Glu) | $COO^-$ (Glu or Asp) | monomer |  |
| *Pseudoalteromonas distincta* (McGuire et al., 2020) | 110 | parallel β-helix | COOH (Asp) | Not known | homo-dimer |  |

«—« no data available

(Fig. 1). Most α-galactosidases carry out hydrolysis through a two-step mechanism, in which one of the catalytic groups acts as a nucleophile, forming an intermediate glycosyl enzyme, and the other as a general acid-base catalyst, which contributes to the formation and decomposition of the intermediate (Golubev et al., 2004). In a complete two-step mechanism, two Walden inversions are likely to occur, helping preserve the anomeric configuration of the resulting product. However, the formation of a carbonium ion in the intermediate step does not necessarily lead to racemization, and the configuration can be stabilized due to the specific binding of the intermediate to the enzyme. To date, only α-galactosidases from the new GH110 family have been shown to act by the inversion mechanism (Liu et al., 2008; McGuire et al., 2020).

Although more data on the structures of the active centers of α-galactosidases have appeared in recent years, many questions remain regarding the substrate specificity of these enzymes. All this indicates the need to accumulate and systematize facts in this area of research.

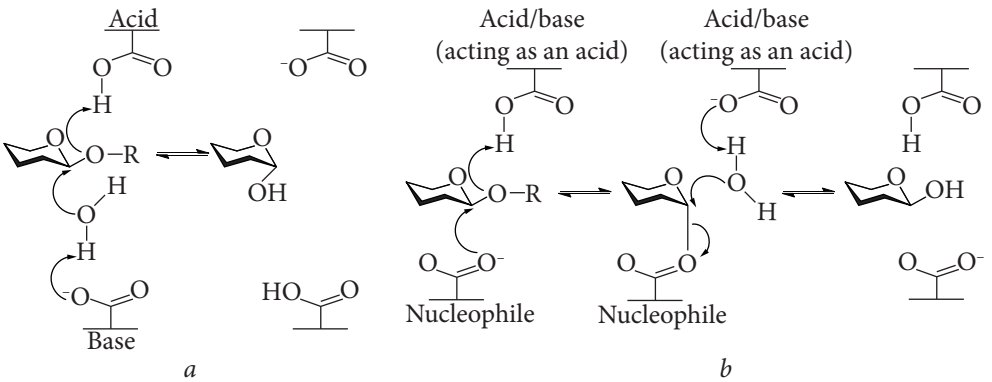



***Fig. 1.*** Mechanisms of hydrolysis carried out by glycosidases: *a* — one-step mechanism with inversion of stereochemistry; *b* — two-step mechanism with preservation of stereochemistry (Gloster & Davies, 2010)

***Distribution in nature and physiological significance of α-galactosidases.*** According to the data of the literature (Anisha, 2022), among microorganisms, the most active biosynthetics are fungi. The advantages of micromycetes as enzyme producers include high extracellular productivity and activity, as well as relative ease of enzyme isolation. The level of enzyme activity in the culture liquid of micromycetes is within 0.2—6.0 U/mL and exceeds the corresponding level of bacterial (0.02—0.569 U/mL) and yeast (0.6—0.8 U/mL) cultures. Sometimes micromycetes produce α-galactosidase isoenzymes, such as in *A. foetidus* (Liu & He, 2012). In the cultivation of fungal producers, both deep and solid-phase cultivations are equally successfully used (Elshafei et al., 2022; Borzova et al., 2020). Monosaccharides, galactooligosaccharides, galactomannan, and agricultural waste are usually used as carbon sources and inducers. Hyperproduction of α-galactosidase was successfully achieved using mutant strains of fungi, in particular *Thalaromyces emersonii* (Simila et al., 2010). Compared to micromycetes, α-galactosidases from yeasts have been less studied. Intra- and extracellular α-galactosidases have been described in *Candida guilliermondii* and *Debaryomyces hansenii* when grown on medium with melibiose and galactose (Viana et al., 2009). Highly active producers of α-galactosidase are also described among bacteria (Bhatia et al., 2020).

Despite all the advantages of microbial synthesis of enzymes, it should be remembered that the main condition for the use of such producers is their safety for humans. The search for producers of biotechnologically valuable metabolites must meet two main conditions: high productivity and harmlessness. Therefore, today lacto- and bifidobacteria, which are an integral part of the human gastrointestinal tract, are considered very promising producers of α-galactosidase (Fushinobu & Hachem, 2021). It was shown that some lactic acid bacteria (*L. acidophilus*, *L. reuteri*, and *L. fermentum*) produce α-galactosidase capable of hydrolyzing galactooligosaccharides (Ferdslund et al., 2011).

Since galactose-containing oligo-, polysaccharides, and lipids are widely distributed in plants, it is natural that the presence of a-galactosidase is usually observed in the tissues of seeds, rhizomes, roots, and tubers (Zhao et al., 2006). Thus, parallel synthesis of galactosyl carbohydrate derivatives takes place in maturing seeds against the background of a significant increase in a-galactosidase activity. During seed germination, the enzyme is directly involved in the hydrolysis of these oligosaccharides, which are a soluble energy reserve that can be easily metabolized.

a-galactosidase is also a key enzyme in the metabolism of plant galactolipids. It was shown (Dey, 1984) that bean leaves have all the necessary enzymes, not excluding a-galactosidase, for the complete decomposition of these substances into fatty acids, glycerol, and galactose. Galactolipids of chloroplast membranes are also substrates for α-galactosidases. Studying Hill's reaction in isolated spinach chloroplasts, the authors noted (Dey & Pridham, 1977) that the crude preparation, which contains galactolipases and α-galactosidases, is able to change the physiological activity of organelles. It is known that in higher plants, a-galactosidase is also involved in osmotic regulation (synthesis and cleavage of isofloridoside or a-galactosyl-1®1-glycerol).

According to the activity in the pH range, a-galactosidase of plants is divided into acidic and alkaline. Usually, both are represented in the genome of plants. According to the subcellular localization, acid a-galactosidases are divided into apoplast and vacuolar (Gu et al., 2018). The signal peptide of apoplastic α-galactosidases is involved in the secretory pathway that directs them to the extracellular space to hydrolyze cell wall-associated galactomannan during seed maturation or germination (Phoeurk et al., 2018). Apoplastic α-galactosidases also play an important role in leaf development, fruit ripening, or aerenchyma formation (Dey & Pridham, 1977). Vacuolar α-galactosidases are highly specific for oligosaccharides of the raffinose family, which are the source of reducing sugars in plants and can be rapidly converted to energy. Instead, alkaline α-galactosidases show the highest affinity for stachyose and are responsible for the rapid metabolism of galactooligosaccharides during seed germination (Arunraj et al., 2020). In addition, the accumulation of alkaline α-galactosidase is observed in plants during starvation or dehydration (Chuankhayan et al., 2023).

It should be noted that these enzymes are more common in the animal world. They are found in almost all human and animal tissues and organs; their greatest activity is noted in the intestinal mucosa, liver, and brain (Guce et al., 2010). The importance of the processes involving a-galactosidase for the body is evidenced at least by the fact that more than 30 human diseases are associated with the hereditary absence of lysosomal glycosidases, including Fabry disease and childhood neuroaxonal dystrophy (Schindler's disease) as hereditary disorders of sphingolipid metabolism caused by a lack of a-galactosidase and a-N-acetylgalactosaminidase (Azevedo et al., 2021). A change in the activity level of these enzymes in human blood can be a convenient indicator for the diagnosis of some liver disorders (Hilz et al., 2018). The physiological role of a-galactosidase in microorganisms is poorly studied and is related to the satisfaction of their trophic needs. It is believed that the main role of α-galactosidase in microorganisms is to provide the cell with sources of carbon and energy necessary for survival (Rattanaprasert et al., 2019).

The functions and properties of microbial α-galactosidases are closely related to the localization of the enzyme in the cell. Authors (de la Fuente & Sols, 1962) established that in *S. carlsbergensis,* the activity of a-galactosidase is localized in the outer part of the membrane, where its function, most likely, consists in the hydrolysis of substrates that would otherwise not be able to cross the cell membrane. For the yeast *Papiliotrema flavescens*, it was shown that long-chain α-galactosidase is present on the cell surface and in the cytosol (Stratilova et al., 2018). For the yeast species *D. hansenii*, bacteria of the genera *Bifidobacterium* and *Lactobacillus*, the intracellular localization of the enzyme was also shown (Viana et al., 2009; Xiao et al., 2000). In *P. atlantica*, the inactive a-galactosidase is localized in the periplasm, while the active form of the enzyme is formed in the cytoplasmic membrane. a-Galactosidase activity in *Micrococcus sp*. was determined in the cytoplasm. Extracellular α-galactosidases have been described in the vast majority of micromycetes (*A. niger*, *A. flavipes*, *A. nidulans*, *P. restrictum*, *Thermomyces lanuginosus*, *T. reesei*) (Elshafei et al., 2022; Borzova et al., 2020; Anisha, 2022).

***Physicochemical and catalytic properties and specificity of action of a-galactosidases of different microorganisms.*** α-Galactosidases isolated from different microorganisms can differ significantly in their biochemical properties and substrate specificity. The variability of the physicochemical properties of the enzyme is very important in view of its successful use in various technological processes. Unlike plant glycosidases, which are usually represented by two molecular forms: one high-molecular (167, 159, 121 kDa) and the other low-molecular (50, 23, 32 kDa) (Dey & Pridham, 1977), only one molecular form is characteristic of microbial glycosidases (Table. 1). Regarding bacterial a-galactosidases, it is known that their molecular weights are of the same order (Table 2), while fungal enzymes have a wider range: 210 kDa in *Rhizopus* sp. (Cao et al., 2009), 118 kDa in *A. awamori* (El-Gindy et al., 2008), 106 kDa in *A. foetidus* (Liu et al., 2012), 64, 99, and 430 kDa in *A. niger* (Awan et al., 2009; Othman et al., 2023; Borzova & Varbanets, 2007).

For many glycosidases described today, both of bacterial and fungal origins, the oligomeric structure of the enzyme molecule is characteristic. Monomeric forms (*T. maritima*, *Acinetobacter* sp., *T. reesei*), dimers (*Pseudoalteromonas* sp.), trimers (*Rhizopus* sp., *Sulfolobus solfataricus*), tetramers (*L. acidophilus*), pentamers (*A. terreus*), hexamers (*T. thermophilus*, *A. niger*, *P. canescens*) and octamers (*Thermus* sp.) have been described among α-galactosidases (www.cazy.org/GH36_structure.html, www.cazy.org/GH27_structure.html). Most of the studied extracellular enzymes are glycoproteins, but the percentage of the carbohydrate part varies in the range of 1.67—57%, and the main monosaccharides are mannose, galactose, and D-galactosamine. The glycoprotein nature of a-galactosidase of *Pycnoporus cinnabarinus* was proved by the method of disk electrophoresis and specific staining of gels for carbohydrates (Mitsutomi & Ochtakara, 1987). It was established that the carbohydrate component of a-galactosidase from *A. niger* includes mannose, galactose, and D-glucosamine (Borzova &Varbanets, 2007), and a-galactosidases of *A. tamarii* and *M. vinaceae* also contain glucose, in addition to those mentioned. The molecule of a-galactosidase of *S. carlsbergensis* consists of 43% of protein and 57% of carbohydrates, represented mainly by mannose (90—95%), and also contains about 7% of glucose and 1% of D-glucosamine. Intracellular and extracellular a-galactosidases of *D. hansenii* contain 34 and 40% of carbohydrates, respectively (Viana et al., 2009), and the glycoprotein of *Humicola* sp. contains 8.3% of carbohydrates (Kotwal et al., 1999). In the composition of the carbohydrate component of *P. canescens* a-galactosidase, in addition to mannose and glucosamine, arabinose and rhamnose were detected (Borzova et al., 2014), and the ratio of carbohydrates to protein was 1:6.

It was established that the a-galactosidase of *A. awamori* contains about 30 water-labile O-hydrocarbon chains, and about half of them are linked to serine residues (Neustroev et al., 1993). In addition, N-glycosylation is also shown for this glycosidase, and the number of carbohydrates connected to the protein by N-glycosidic bonds is 3 per enzyme molecule. Glycosylation according to the mixed type was also established in α-galactosidase of *T. reesei* (Savel'ev et al., 1997). There are 6 O-glycans per protein molecule, which are attached to serine and threonine and can be released by β-elimination.

Among them, there are monomers: glucose, mannose and galactose; dimers: α-1,6-mannopyranosyl-α-D-glycopyranoside and α-1,6-glucopyranosyl-α-galactopyranoside, as well as one trimer: α-glucopyranosyl-α-1,2-mannopyranosyl-α-1,6 -galactopyranoside. N-linked glycans are of the mannose-rich type and are released when the protein is treated with endo-β-N-acetylglucosaminidase F or by hydrazinolysis. A mixed type of glycosylation was established for a-galactosidases of *P. canescens*, *S. cladosporioides*, and *A. niger* (Borzova et al., 2014).

*Table 2.* **Biochemical properties of α-galactosidase from different producers**

| Microorganism | Glycosidase family | Media, inductor | Mm, kDa |
|---|---|---|---|
| | | | Fungi |
| *A. awamori* (El-Gindi et al., 2008) | — | Corn bran | — |
| *A. foetidus* (Liu et al., 2012) | GH27 | Soy and wheat flour | 106.3; 49.7; 109.9 |
| *A. niger* (Othman et al., 2023) | — | Mannose | 64 |
| *A. niger* (Borzova & Varbanets, 2007) | — | Soy flour | 430 hexamer |
| *Aspergillus* sp. (Chen et al., 2023) | — | Wheat bran | 125 monomer |
| *A. oryzae* (Wang et al., 2020) | GH36 | — | 100 |
| *D. hansei* (Zou et al., 2023; Viana et al., 2009) | GH27 | Galactose | 54.5 (60) |
| *Hericium erinaceus* (Ye et al., 2018) | GH27 | — | 57 |
| *Humicola* sp. (Kotwa et al., 1999) | | Wheat bran extract | 371 tetramer |
| *P. chrysogenum* (Yakimova et al., 2017) | — | Soy flour | — |
| *P. flavescens* (Stratiliva et al., 2018) | GH36 | Lactose | 110 |
| *Penicillium* sp. (Varbanets et al., 2001) | — | Galactose | 400 hexamer |
| *Pleurotus djamor* (Hu et al., 2017) | — | — | 60 monomer |
| *Pseudobalsamia microspora* (Yang et al., 2015) | — | — | 62 monomer |
| *Rasamsonia emersonii* (An et al., 2019) | GH27 | Potato dextrose medium | 362 hexamer |
| *Rhizopus sp.* (Cao et al., 2009) | GH36 | Potato dextrose broth | 210 trimer |
| *Talaromyces flavus* (Simerska et al., 2007) | GH27 | 6-Deoxy-D-glucose | 63 monomer |
| *T. lanuginosus* (Rezessy-Szabo et al., 2007) | GH36 | Sucrose | 93 monomer |
| *Thielavia terrestris* (Saad & Fanzi, 2012) | — | Chickpea seeds | 82 |
| | | | Bacteria |
| *Acinetobacter* sp. (E et al., 2015) | — | Raffinose | 65 dimer |
| *Anoxybacillus vitaminiphilus* (Wang et al., 2021) | GH36 | Raffinose | 320 tetramer |
| *Bacillus flexus* (Bhatia et al., 2023) | — | Guar gum soy meal | 86 dimer |
| *Bacillus megaterium* (Huang et al., 2018) | GH36 | Raffinose | 271.3 trimer |
| *B. stearothermophilus* (Gote et al., 2004) | GH36 | Galactose | 165 dimer |
| *Bifidobacterium longum* subsp. *longum* (Sasaki et al., 2021) | GH36 | Glucose | 80 |
| *Thermotoga neapolitana* (Duffaund et al., 1997) | GH36 | Guar gum | 61 |
| *S. griseoloalbus* (Anisha et al., 2009) | GH27<br>GH36<br>GH36 | Soy flour | 72<br>57<br>35 |
| *Sulfolobus solfataricus* (Brouns et al., 2006) | GH36 | Raffinose | 225 trimer |

«—« no data available, «$K_i$» inhibition constant

| Thermal optimum, °C | pHoptimum | Activity, U/mg (*p*-NPG) | Inhibitor |
|---|---|---|---|
| 55 | 4.8 | 0.35 | $Na^+$, $Li^+$, $Ag^+$, $Hg^{2+}$, $Zn^{2+}$, $Mo^{2+}$, $Cd^{2+}$, $Pb^{2+}$ |
| 60 | 5.0<br>4.0 | 101 | $Ag^+$, $Cu^{2+}$, SDS |
| 60 | 3.5 | 5.41 | $Hg^{2+}$, $Cu^{2+}$, $Mn^{2+}$ |
| 60 | 4.0 | 25.3 | $Ag^+$, $Hg^{2+}$, galactose ($K_i$ 6.2 mM), *p*-CMB, urea |
| 65 | 5.0 | 0.27 | $Cu^{2+}$, $Mn^{2+}$ |
| 50 | 4.66 | 66.7 U/mL | $Cu^{2+}$ |
| 60 | 5.0,<br>pI 4.1 | 11.9 | $Ag^+$, $Cu^{2+}$, galactose ($K_i$ 0.7 mM) |
| 60 | 6 | 46 | $Ag^+$, $Fe^{3+}$, $Zn^{2+}$, SDS, N-bromosuccinimide, galactose, melibiose |
| 65 | 5.0 | 1.33 | $Ag^+$, $Hg^{2+}$, galactose ($K_i$ 11) |
| 50 | 4.5 | 0.46 | — |
| 60 | 4.8 | 8.8 pkat/mg | — |
| 50 | 5.0 | | $Ag^+$, $Hg^{2+}$, *p*-CMB, galactose ($K_i$ 6.7 mM) |
| 53.5 | 5.0 | 52.18 | $K^+$, $Cd^{2+}$, $Cu^{2+}$, $Hg^{2+}$, $Al^{3+}$, $Fe^{3+}$, $Ag^+$, N-bromosuccinimide, *p*-CMB |
| 55 | 5 | 11.275 | $Hg^{2+}$, $Cd^{2+}$, $Cu^{2+}$, $Fe^{3+}$, N-bromosuccinimide, dithiothreitol |
| 80 | 4.0 | 38 | $Ag^+$, galactose ($K_i$ 2.77 mM), guanidine hydrochloride |
| 50 | 4.8 | 74.6 | $Ag^+$, $Hg^{2+}$, $Mn^{2+}$, SDS |
| 50 | 3.5—4.5 | 5.7 | Galactose (0.38 mM), xylose, melibiose, metyl-α- and β-galactose, lactose |
| 65 | 5-5.5<br>pI 3.9 | 15.9 U/mL | $Ca^{2+}$, $Zn^{2+}$, $Hg^{2+}$, $Ag^+$ |
| 70 | 6.5 | 85.8 | $Ag^+$, $Hg^{2+}$, $Zn^{2+}$, $Ba^{2+}$, $Mg^{2+}$, $Mn^{2+}$, $Fe^{2+}$, EDTA, sodium arsenate, L-cysteine, iodoacetate |
| 50—60 | 7.0 | 8—12 | $Ag^+$, $Hg^{2+}$, *p*-CMB, SDS, glucose, galactose, lactose, stachyose |
| 60 | 7.5 | 70 | $Ni^{2+}$, $Ca^{2+}$, $Cu^{2+}$, $Zn^{2+}$, $Fe^{2+}$, $Mn^{2+}$, SDS, CTAB, acetonitrile |
| 55 | 7.0 | — | $Ag^+$, $Hg^{2+}$, $Cu^{2+}$ |
| 37 | 7.4 | 1.22 | $Ag^+$, $Cu^{2+}$, $Co^{2+}$, $Hg^{2+}$, SDS, β-mercaptoethanol, DMSO |
| 65 | 6.5<br>pI 4.9 | 1 | $Ag^+$, $Cu^{2+}$, $Hg^{2+}$, *p*-CMB, galactose |
| 50 | 6.0 | 337 | Tris |
| 105 | 7.3<br>pI 4.9 | 0.5 | — |
| 65 | 5.0 | 176.9 | $Ag^+$, $Hg^{2+}$, $Cu^{2+}$, melibiose |
| 50 | 6.5 | 168 | |
| 55 | 5.5 | 61.8 | |
| 90 | 5.0 | 4.25 | ($Ag^+$, $Ca^{2+}$, $Cd^{2+}$, $Co^{2+}$, $Cu^{2+}$, $Hg^{2+}$, $Mn^{2+}$, $Ni^{2+}$, $Zn^{2+}$ |

*Thermo- and pH-optimum action, stability of enzymes of various origins*. The optimum pH of bacterial a-galactosidases is usually in the neutral pH range, and in fungal ones — 3.5—5.0 (Table 2), although some microbial enzymes are active in a wide range pH (3—12), which is a valuable property from the viewpoint of technological use. A wide pH-optimum, as in *A. niger* a-galactosidase (Awan et al., 2009), is a characteristic feature of extracellular fungal glycosidases and favorably distinguishes them from intracellular analogues.

The temperature optimum for the action of most a-galactosidases is noted at 37—60 ºC (Table 1). But the greatest interest is attracted by microbial enzymes (*R. emersonii*, *S. solfataricus*, *T. lanuginosus*, *T. terrestris*, *T. neapolitana*) in which the thermooptimum is in the range of 70—100 ºC,and high stability is noted at temperatures close to the thermooptimum. α-Galactosidases of the yeasts *Humicola* sp., *D. hansenii,* and *S. cerevisiae* also demonstrate quite high thermal stability in the range of 55—60 ºC (Alvarez-Cao et al., 2019a; Viana et al., 2009; Kotwal et al., 1999). Such enzymes can be used in the sugar and cellulose industry, where production needs a long-term use of high temperatures. There are also described α-galactosidases of *Pseudoalteromonas* marine bacteria, which are highly active at low temperatures (20—30 ºC) (Golotin et al., 2016).

Microbial a-galactosidases are sensitive to the presence of carbohydrates: galactose and a-galactosides are strong and competitive inhibitors of many a-galactosidases (Table 2). Thus, it was shown that galactose and *p*-NPG inhibited the activity of a-galactosidases of *P. purpurogenum* and *Penicillium* sp. (Varbanets et al., 2001; Morales-Quintana et al., 2017). a-Galactosidase from *A. ficuum* NRRL is competitively inhibited by galactose ($K_i$ = 64 mM) and fructose ($K_i$ = 60.3 mM), and non-competitively by glucose ($K_i$ = 83.2 mM) and mannose ($K_i$ = 64 mM) (Zapater et al., 1990). $K_i$ values of galactosides closely correlate with Michaelis constants when these compounds are used as substrates. At the same time, α-galactosidases of *A. niger*, *R. miehei*, and *S. griseoloalbus* showed high tolerance to galactose concentrations up to 100 mM (Anisha et al., 2009; Katrolia et al., 2014). Structural analogues of D-galactose such as L-arabinose and D-fucose also inhibit enzymes, while their enantiomers are ineffective. To date, most studies have shown that 2-deoxy-D-galactose, D-glucose, D-mannose, D-fructose, D-xylose, and D-ribose do not inhibit a-galactosidase (Bhatia et al., 2020).

Most of the investigated α-galactosidases strongly inhibit metal ions, especially $K^+$, $Cd^{2+}$, $Cu^{2+}$, $Hg^{2+}$, $Al^{3+}$, $Fe^{3+}$, and $Ag^+$ (Tables 2, 3). Inhibition by silver and mercury ions may be related to the effect on cysteine and dicarboxylic acid residues in the active center of the enzyme. The importance of cysteine for the manifestation of catalytic activity is also confirmed by inhibition of the enzyme in the presence of *p*-chloromercurybenzoate (*p*-CMB) (Table 2). For some α-galactosidases, inhibition by N-bromosuccinimide has been shown, which indicates the important role of the tryptophan residue. Oligomeric α-galactosidases are characterized by inhibition by sodium dodecyl sulfate (SDS) and urea (Table 2). High resistance to the action of such proteases as trypsin, pepsin, subtilisin A, proteinase K, alkaline protease, collagenase, and α-chymotrypsin has been shown for a significant number of α-galactosidases of microorganisms (Cao et al., 2009; Wang et al., 2020). Noteworthy is the insufficiency of data on the mechanisms of inactivation-stabilization of α-galactosidase. At best, the authors indicate the fact of enzyme inactivation under certain conditions, for example, unsuitable temperature, or the nature of inhibition by a certain substance (competitive/non-competitive), but most often there are data only on the degree of inhibition. However, the data on the mechanisms of inactivation and denaturation of enzymes make it possible to regulate hydrolysis in various technological processes.

***Substrate specificity of a-galactosidase.*** As mentioned above, a-galactosidase cleaves terminal

a-galactosidic bonds, splitting the corresponding residues from the non-reducing end of glycosides such as oligosaccharides, polysaccharides, glycolipids, glycoproteins, and mucopolysaccharides (Fig. 2). At the same time, the bond between the C-1 atom of the monosaccharide residue and the glycosidic oxygen atom is split, as was shown using $^{18}O$. a-Galactosidases hydrolyze the following natural and synthetic a-D-galactosides:

▪ **galactosides**: methyl-, ethyl-, *p*-propyl-, phenyl-, o-nitrophenyl-, *p*-nitrophenyl-, *m*-nitrophenyl-, *o*-cresyl- , *p*-cresyl-, *m*-chlorophenyl-, 1-naphthyl-, 2-naphthyl- and 6-bromo-2-naphthyl a-D-galactosides, 1-*o*- and 2-*o*-a-galactosyl

*Table 3.* **Inhibition of α-galactosidase by some metal ions**

| Ion of metal | Source | Metal concentration (mM) | Inhibition (%) | Character of inhibition | $K_i$ (M) |
|---|---|---|---|---|---|
| $Ag^+$ | *A. ficuum* (Zapater et al., 1990) | $21.5 \times 10^{-3}$ | 90 | competitive | — |
| | *S. olivaceus* (Dey & Pridham, 1977) | $2.0 \times 10^{-3}$ | 50 | competitive | — |
| | *P. cinnabarinus* (Mitsutomi & Ochtakara, 1978) | 1.0 | 95 | — | — |
| | *C. guillermondii* (Dey & Pridham, 1977) | 1.0 | 90 | — | — |
| | *Acinetobacter* sp. (E et al., 2015) | 5.0 | 100 | competitive | — |
| | *A. niger* (Borzova & Varbanets, 2007) | 1.0 | 95 | noncompetitive | $2.5 \times 10^{-4}$ |
| | *T. lanuginosus* (Rezessy-Szabo et al., 2007) | 10.0 | 100 | — | — |
| | S. *solfataricus* (Brouns et al., 2006) | 10.0 | 100 | — | — |
| $K^+$ | *Acinetobacter* sp. (E et al., 2015) | 5.0 | 43 | — | — |
| $Hg^{2+}$ | *S. olivaceus* (Dey & Pridham, 1977) | 0.2 | 63 | — | — |
| | *D. pneumonia* (Dey & Pridham, 1977) | 0.2 | 100 | — | — |
| | *M. vinaceae* (Dey & Pridham, 1977) | $1.0 \times 10^{-4}$ | 23 | noncompetitive | — |
| | *T. reesei* (Golubev et al., 2004) | $2.0 \times 10^{-2}$ | 100 | competitive | $2 \times 10^{-5}$ |
| | *Acinetobacter* sp. (E et al., 2015) | 5.0 | 100 | competitive | — |
| | *A. niger* (Borzova & Varbanets, 2007) | 1.0 | 95 | noncompetitive | $4.5 \times 10^{-6}$ |
| $Ca^{2+}$ | S. *solfataricus* (Brouns et al., 2006) | 10 | 100 | — | — |
| $Cu^{2+}$ | *D. pneumonia* (Dey & Pridham, 1977) | 0.2 | 100 | — | — |
| | *Acinetobacter* sp. (E et al., 2015) | 5.0 | 44 | — | — |
| $Zn^{2+}$ | *A. ficuum* (Zapater et al., 1990) | 0.11 | 85 | competitive | — |
| | *Acinetobacter* sp. (E et al., 2015) | 5.0 | 25 | — | — |
| | S. *solfataricus* (Brouns et al., 2006) | 10.0 | 100 | — | — |
| | *T. lanuginosus* (Rezessy-Szabo et al., 2007) | 10.0 | 100 | — | — |
| $Mg^{2+}$ | *Rhizopus* sp. (Cao et al., 2009) | 1.0 | 2 | — | — |
| | *P. microspora* (Yang et al., 2015) | 1.25 | 37 | — | — |
| $Mn^{2+}$ | *P. microspora* (Yang et al., 2015) | 5.0 | 33 | — | — |
| | *Rhizopus* sp. (Cao et al., 2009) | 1.0 | 50 | — | — |
| | S. *solfataricus* (Brouns et al., 2006) | 10.0 | 100 | — | — |
| $Cr^{3+}$ | *Rhizopus* sp. (Cao et al., 2009) | 1.0 | 46 | — | — |
| $Al^{3+}$ | *Rhizopus* sp. (Cao et al., 2009) | 1.0 | 44 | — | — |
| | *P. microspora* (Yang et al., 2015) | 2.5 | 46 | — | — |

«—» no data available

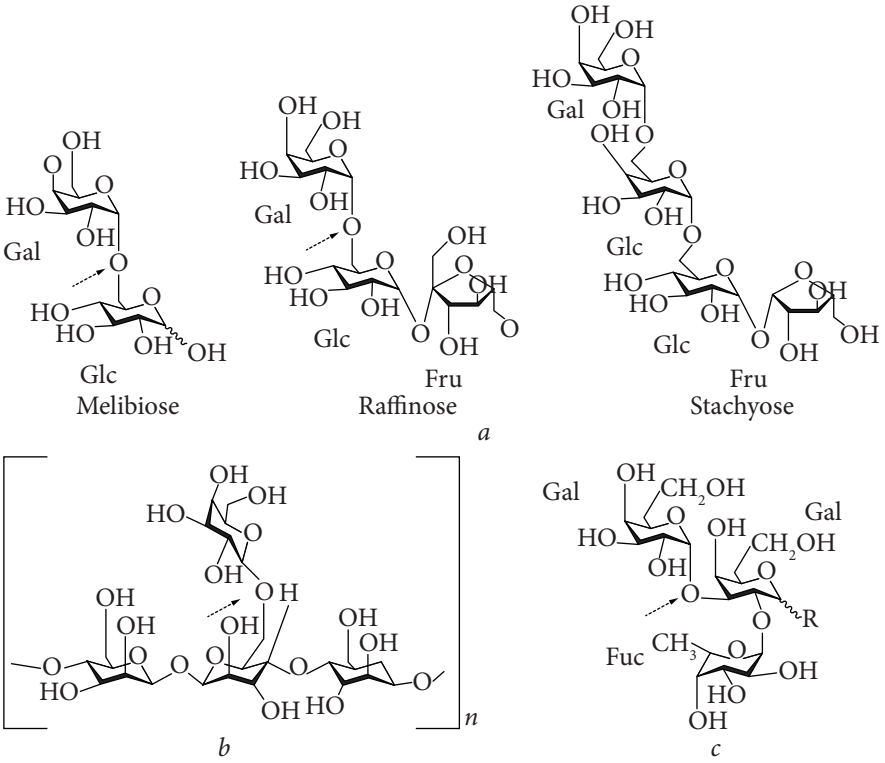



***Fig. 2.*** Natural substrates of α-galactosidase: *a* — galactooligosaccharides; *b* — galactomannan polysaccharides; *c* — blood group B antigen trisaccharide with α-1,3-linked D-galactose. Gal — galactose; Glc — glucose; Fru — fructose; Man — mannose, and Fuc — fucose. Arrows indicate the sites of cleavage of the bond by α-galactosidase

glycerol, galactinol, digalactosyl glycerol, and a-D-galactosyl fluoride;

▪ **oligosaccharides**: melibiose, epimelibiose, *o*-a-D-gal(1,4)-D-gal, melibiitol, melibionic acid, raffinose, umbelliferose, planteose, mannotriose, mannotreitol, mannotreonic acid, stachyose, verbascose and higher homologues, lychnose, isolichnosis, and higher homologues;

▪ **polysaccharides**: galactomannans and pectins;

▪ **a-D-galactosides of a specific substance of blood group B (II):** Galα1-3(Fucα1-2)Gal and tetrasaccharide, Galα1-3(Fucα1-2)Galβ1-4GlcNAc.

Some authors carried out a quantitative assessment of glycon specificity (Dey, 1984) and showed that the affinity of plant α-galactosidase to the substrate depends on the glycon fragment and is located in the series: a-D-galactoside > a-D-fucoside > b-L-arabinoside. This suggests that one of the specific areas of attachment of the substrate to the enzyme is the primary alcohol group in the galactose structure. A study of the specificity of a-galactosidase of *Penicillium* sp. showed that the enzyme, like most fungal a-galactosidases, exhibits a narrow specificity regarding the structure of the glycon hydrolyzing only a-D-galactosides (Varbanets et al., 2001). The configuration near the C-1 and C-4 atoms of the substrate and the presence of substituents in the C-2 and C-6 positions are essential for interaction with the enzyme. In contrast to this enzyme, the specificity of a-galactosidase from *C. cladosporioides* with respect to glycon is much wider: in addition to a-D-galactosides, it also hydrolyzes the nitrophenyl derivatives of aminosaccharides in the b-configuration and b-D-glucosides.

It is shown that in general aryl-a-D-galactosides are more suitable substrates than alkyl derivatives or disaccharides. The relationship be-

tween $K_m$ and $V_{max}$ can vary significantly for different aglycones, and a high affinity of an enzyme to any substrate does not necessarily coincide with a high $V_{max}$ value and vice versa. It has been proven that the factors affecting the affinity are complex and include the position and size of the substituent groups, their electronic effect, and the degree of hydration (Dey, 1984). In the homologous series of a-D-galactosides, the rate of hydrolysis decreases with increasing chain length, but there are some exceptions among microbial enzymes. According to the affinity to various substrates, most a-galactosidases can be placed in the following sequence: *o*-NPG > *p*-NPG >> raffinose > methyl-a-D-galactopyranoside.

There are reports that a-galactosidases of *A. niger* hydrolyze only synthetic substrates and do not hydrolyze terminal a-1,6-linked galactose in linear structures such as melibiose, stachyose, raffinose, and galactomannans (Borzova & Varbanets, 2007; Kaneko et al., 1991). Some α-galactosidases show synergism with β-mannanases and β-mannosidases in the hydrolysis of galactomannans, which makes it possible to obtain larger amounts of monosaccharides with their participation (Phoeurk et al., 2018; Song et al., 2018). α-Galactosidases isolated from strains of the same species often have different enzymatic characteristics, substrate specificity and belong to different families — GH27 or GH36. α-Galactosidases of the GH27 family are known as key enzymes for the hydrolysis of galactomannans. Moreover, yeast α-galactosidases cleave only the terminal galactose of the main chain, while micromycete enzymes are able to attack the galactose of the side chains of galactomannan (Fernandez-Leiro et al., 2010; Malgas et al., 2015). Affinity to the same substrates for enzymes from different producers varies in a wide range, as evidenced by the absolute values of $K_m$ (Table 4), however, for natural substrates, this indicator is usually lower than for *p*-NPG.

Regarding the specificity for the type of connection, the most common are a-galactosidases, which are able to hydrolyze a-1,2-, a-1,4-, and a-1,6-linked galactose (Katrolia et al., 2014). More unique are a-galactosidases, which attack the a-1,3-bond (Liu et al., 2008; McGuire et al., 2020; Varbanets et al., 2001). Enzymes of such specificity are necessary for the transformation of erythrocytes of blood group B (III).

As mentioned above, some α-galactosidases can catalyze the reverse reaction, or the transglycosylation reaction. For the first time, such activity was described in plant a-galactosidase (Dey & Pridham, 1977). To date, transferase activity has been shown for some bacterial (*B. bifidum*, *B. stearothermophilus*, *L. reuteri*) and fungal (*A. parasiticitic*, *A. fumigatus*, *A. nidulans*, *A. niger*) a-galactosidases. With the help of p-NPG, the formation of p-NP-α(1,2)-galactobiose, p-NP-α(1,3)-galactobiose, and p-NP-α(1,6)-galactobiose by fungal α-galactosidases was shown (Weignerova et al., 2001).

It was established that the α-galactosidase of *L. plantarum* has high regioselectivity, efficiency, and diversity concerning involved donors and acceptors of substrates for the synthesis of α-galactooligosaccharides through transglycosylation reactions (Delgado-Fernandez et al., 2020), with the advantage of the transfer of galactosyl residues to the C6-hydroxyl group of galactose, which lengthens a galactooligosaccharide chain with terminal sucrose (raffinose, stachyose) or terminal glucose (melibiose, mannotriose, verbascotetraose). High transglycosylation activity and synthesis of new glycosides was shown for the enzyme of a mutant strain of *B. thetaiotaomicron* (Okuyama et al., 2017). Addition of glycerol to the mixture of glycosides formed as a result of the hydrolysis of guar gum by α-galactosidase of *A. niger* made it possible to obtain a high yield of galactosyl-glycerol (Kurakake et al., 2015).

***Practical importance of α-galactosidase.*** Enzymes of microorganisms have enormous potential in various industrial fields, which has led to the growth of their commercial use in recent

years. Optimizing the conditions for growing microbial producers and using effective cleaning methods, one can achieve a significant increase in the yield of enzymes, which makes their large-scale production cheaper. Cloning of new α-galactosidase genes and their heterologous expression in a suitable host is also gaining popularity. In addition, the enzyme immobilization makes it possible to obtain long-acting catalysts with exceptional thermal stability and activity as well as tolerance to pH and salt concentration (Celem & Onal, 2022). All this makes the use of microbial enzymes commercially attractive. Since a-galactosidase is a highly specific enzyme, this property can be used in the structural analysis of complex natural and synthetic glycoconjugates containing D-galactosyl residues (Anisha, 2022; Bhatia et al., 2020). Exoglycosidases allow us to learn more about the structure of glycoproteins in human blood plasma. The use of transglycosylation properties of endoglycosidases makes it possible to synthesize new oligosaccharides (Oh et al., 2018; Meszaros et al., 2021). In addition, glycoproteins consisting of serine and threonine linked by O-glycosidic bonds, as in mucin, are known to have a significant effect on cell-cell interactions, many of which are mucus and serum glycoproteins involved in formation of the body's immune response (Martinez-Ocana et al., 2020). Therefore, it is not surprising that the role of a-galactosidase in metabolism in the human and animal body, as well as the possibility of its practical application in the field of medicine, has been the main topic of a significant number of works in recent decades

*Table 4.* **Substrate affinity and effective hydrolysis of substrates by a-galactosidases from different sources**

| Enzyme producer | $K_m$, mM | | | | $k_{cat}/K_m$, $mMs^{-1}$ |
|---|---|---|---|---|---|
| | *p*-NPG | Melibiose | Raffinose | Stachyose | *p*-NPG |
| *T. maritima* (Comfort et al., 2007) | 0.08 | — | — | — | 11.57 |
| *T. neapolitana* (Duffaund et al., 1997) | 0.5 | 15.11 | 5.32 | — | 305 |
| *Acinetobacter sp.* (E et al., 2015) | 0.33 | nd | 3.26 | nd | 22.46 |
| *A. vitaminiphilus* (Wang et al., 2021) | 0.12 | nd | nd | nd | 763.92 |
| *A. niger* (Borzova & Varbanets, 2007) | 1.19 | — | — | — | 152.1 |
| *A. terreus* (Ferreira et al., 2011) | 0.72 | 1.92 | 27.93 | 54.74 | nd |
| *B. thetaiotaomicron* (Okuyama et al., 2017) | 0.31 | — | — | — | 778 |
| *D. hansenii* (Viana et al., 2009) | 0.32 | 2.12 | 32.8 | 10.8 | nd |
| *S. cerevisiae* (Fernandez-Leiro et al., 2010) | 4.5 | 11.2 | 54.1 | — | 63.5 |
| *B. breve* (Xiao et al., 2000) | 0.27 | 4.1 | — | — | nd |
| *Rhizomucor miehei* (Katrolia et al., 2012) | 0.36 | 16.9 | 47.9 | 27.6 | 17.5 |
| *B. longum* subsp. *longum* (Sasaki et al., 2021) | 0.774 | — | 29.5 | — | 138 |
| *Rhizopus* sp. (Cao et al., 2009) | 2.9 | 33.0 | — | — | nd |
| *T. lanuginosus* (Rezessy-Szabo et al., 2007) | 1.13 | nd | 1.61 | 1.17 | 3455 |
| *T. reesei* (Golubev et al., 2004) | 1.2 | 1.3 | 3.8 | 1.8 | nd |
| *P. cinnabarinus* (Mitsutomi & Ochtakara, 1987) | 0.31 | 0.8 | 2.16 | — | nd |
| *Penicillium* sp. (Varbanets et al., 2001) | 1.0 | 4.0 | 5.7 | — | nd |
| *S. solfataricus* (Brouns et al., 2006) | 0.08 | — | — | — | nd |
| *Hericium erinaceus* (Ye et al., 2018) | 0.36 | nd | 40.07 | 54.71 | nd |
| *Humicola* sp. (Kotwa et al., 1999) | 0.279 | nd | 1.45 | 1.42 | nd |

«—» does not hydrolyze substrate, «nd» no data available

(Nam et al., 2012; Anisha, 2023). Carbohydrates, due to their ability to form a significant variety of structures from a small number of monomers, are used by nature as substances that can be recognized by the complementary structures of other cells or macromolecules. Thus, the carbohydrate determinant of blood substances of groups B (III) contains a-1,3-linked D-galactose (Gao et al., 2016). The ability of α-galactosidase to cleave the galactosyl residue from blood erythrocytes can be used in hematological practice to develop a technology for creating universal donor blood (Rahfeld & Withers, 2020).

This question is of great practical importance, as it becomes possible to avoid some complications arising during blood transfusion, especially in extreme situations. To date, the possibility of bioconversion of erythrocytes of group III into donor erythrocytes of group I has been shown for enzymes obtained from plants (Chern et al., 2012) and some micromycetes and bacteria (Katrolia et al., 2014). However, only one a-galactosidase preparation suitable for use in hematological practice has been studied in detail — this is a preparation from green coffee beans (Sigma-Aldrich, USA). A number of successful tests of enzymatically transformed erythrocytes have already been conducted on a group of volunteers (Lenny et al., 1991). In addition, a recombinant form of a-galactosidase has already been developed and successfully tested on erythrocytes in the USA. But the search for more promising and cheap enzymes suitable for carrying out large-scale transformation of blood is actively continuing.

New α-galactosidases of the GH110 family are very promising due to their catalytic properties (activity at neutral pH, high specific activity, substrate specificity) (Liu et al., 2008). Such enzymes will be useful in hemotherapy and xenotransplantation. Today, it is believed that the cause of all or most reactions of rejection of foreign organs is the presence of a large number of antibodies to the Gala-1,3-Gal carbohydrate epitope in humans (Zeyland et al., 2013). In humans and great apes, the α-1,3-galactosyltransferase gene is inactivated, although their immune system produces IgM/IgG antibodies targeting this epitope. It was shown (Osman et al., 1997) that a-galactosidase GH110 and a-1,2-fucosyltransferase can effectively reduce the manifestation of these glycotopes on the cell surface and therefore can be used to obtain transgenic animals with a negligible content of bound galactose on the cell surface of their organs**,** not interacting with human serum, which will significantly increase their engraftment.

Another problem is associated with the presence of Gala-1,3-Gal oligosaccharide in the tissues of non-primates, namely human allergies. Consumption of pork and beef meat can cause food allergy in some people due to the presence of specific IgE against this glycoside (Roman-Carrasco et al., 2021). a-Galactosidase is also important as a means of enzyme replacement therapy. The basis of many human genetic diseases are molecular changes that are associated with an impaired carbohydrate metabolism, most often — complex glycoconjugates. The cause of sphingolipid metabolism disorder is a hereditary deficiency of lysosomal a-galactosidase, this is the so-called Fabry disease, and some other sphingolipid metabolism disorders (Azevedo et al., 2021; Anisha, 2023). With these diseases, there is an accumulation of excess triglycosylceramide in the blood, so one of the possible approaches to the treatment of Fabry disease may be the administration of a-galactosidase preparations. There are already reports of some success in the treatment of Fabry disease using microencapsulated preparations of recombinant a-galactosidase of microbial origin (Fabrazyme™ and Replagal™) (Tsuboi & Yamamoto, 2012).

a-Galactosidase preparations have an important applied value in the food industry, in particular in sugar production, where with their help, an increase in the yield of sugar from molasses is achieved and its quality is improved (Katrolia et al., 2014). In the process of sugar production from sugar beets, an increase in the concentra-

tion of raffinose, stachyose, and verbascose is observed. These oligosaccharides complicate the crystallization of sucrose. Hydrolysis of these galactooligosaccharides by a-galactosidase leads to the formation of galactose and sucrose, the latter is extracted from molasses in the traditional way in the form of marketable sugar (Alvarez-Cao et al., 2019b). Thus, the use of the so-called melibiase process not only eliminates obstacles during crystallization, which are caused by a high concentration of galactooligosaccharides in molasses, but also promotes the formation of sugar crystals. In many countries, in particular the USA and Japan, enzyme preparations from *Absidia* sp., *Circenella* sp., and *M. vinaceae* are used to increase sugar yield (manufacturers of the product are Beano, GHC ND Licensing, Ltd., LeaderPharma, AkPharma, Inc., GlaxoSmith-Kline, and Deerland enzymes).

The use of industrial preparations of a-galactosidase makes it possible to more widely use various soy products in human and animal nutrition (Li et al., 2020; Bayraktar & Onal, 2019). Soy contains up to 55% of complete protein, the amino acid composition of which is similar to animal protein and is 90% digestible, however, soy products contain compounds that cause negative reactions in the human body and make soy products unsuitable for use. Such compounds include proteins — trypsin inhibitors and low molecular weight oligosaccharides of stachyose and raffinose. The presence of such oligosaccharides, which are difficult for the body to absorb, significantly complicates the digestion process, since raffinose and stachyose in their native state are not digested in the intestines of mammals, which leads to excessive gas formation. Treatment of soy flour and milk with a-galactosidase promotes the breakdown of raffinose and stachyose into glucose, galactose, and fructose and eliminates this deficiency of soy products. Today, there are α-galactosidase preparations from *A. niger* and *S. cerevisiae* «VeganZyme», «Nutriteck», «Vitacost», «Beano», and «Capsozyme SB Plus» available on the market, which improve the process of digestion of leguminous products in humans and domestic animals.

Until recently, glycosidases were not very actively involved in the industry of synthesis of compounds, they were mainly used to break down carbohydrates. With the creation of glycosynthase and transglycosidase technology through genetic engineering, the view of glycosidases as tools of industrial biotechnology began to change. Their ease of preparation, availability, reliability, and specificity, combined with the ability to control unwanted side hydrolysis by enzyme engineering, have made glycosidases competitive synthetic tools. Promising applications of glycosidases include the production of well-defined chitooligomers, valuable galactooligosaccharides, or special chemicals such as glycosylated flavonoids, breast milk oligosaccharides, or engineered antibodies (Oh et al., 2018; Delgado-Fernandez et al., 2020).

α-Galactosidases are very widely used in modern technologies for obtaining functional food additives based on galactooligosaccharides (GOS). As a result of transglycosylation activity of α-galactosidases of probiotic bacteria *B. thetaiotaomicron, B. adolescentis,* and *L. plantarum*, new prebiotic trisaccharides α-D-Galp-(1→6)-α-D-Galp-(1→6)-D-Glcp are formed from melibiose and raffinose, which are metabolized by intestinal microbiota (Delgado-Fernandez et al., 2020, Okuyama et al., 2017; Liu et al., 2021). The ability to form GOS was also shown for the enzymes of *A. nidulans* and *A. niger* (Nakai et al., 2010). The high yield of α-GOS, consisting of α-galactobiose, α-galactotriose, stachybiose, and larger oligosaccharides, allows us to hope for the prospects of commercial production of new functional food products based on them (Anisha, 2022, Katrolia et al., 2014). Thermostable a-galactosidases are also widely used in the paper industry along with hemicellulases (Clarke et al., 2000). By hydrolyzing side galactosyl residues, α-galactosidase facilitates the hydrolysis of the hemicellulose main chain by xylanases and mannanases. It was shown

that the use of α-galactosidase of *P. simplicissimum* in combination with β-mannanase leads to an increase in the yield of galactose during the processing of coniferous cellulose (Anisha, 2022), α-galactosidase from *P. fluorescens* enhances the combined effect of xylanase and β-mannanase (Song et al., 2018), and the synergistic action of *T. reesei* β-mannanase, *B. subtilis* α-galactosidase, and β-glucosidase accelerates the hydrolysis of galacto- and glucomannans of pine wood and pulp (Katrolia et al., 2014; Clarke et al., 2000).

With the help of α-galactosidase, it is possible to improve the gelling ability of guar galactomannan. This will make it possible to replace more expensive raw materials in the food industry, such as locust bean gum, with more affordable guar gum (Phoeurk et al., 2018; Song et al., 2018). In addition, enzymatic hydrolysis of galactomannans, which are present in large quantities in plant biomass, can provide biorefineries with a sufficient amount of raw materials for ethanol production. Today, there is a certain number of α-galactosidase preparations for various purposes on the market, but they cannot satisfy all needs. Therefore, broad prospects for the use of microbial glycosidases require the development of both technologies for obtaining active producers (through selection and genetic engineering methods) and strategies for stabilizing enzyme preparations for multiple use. Effective approaches are various forms of enzyme immobilization, encapsulation, and obtaining cross-linked aggregates, which allow obtaining stable and active forms of enzymes for practical use in medicine and the food industry (Bayraktar et al., 2019; Liu et al., 2021; Cabrera et al., 2016).

**Conflict of interest.** The authors declare that there are no conflicts of interest.

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Received 5.09.2023

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РОЗПОВСЮДЖЕНІСТЬ, ВЛАСТИВОСТІ ТА ПРАКТИЧНЕ ЗНАЧЕННЯ α-ГАЛАКТОЗИДАЗИ

Досягнення сучасної ензимології значно розширили можливості практичного використання ензимів насамперед в медицині та харчовій промисловості, що обумовлено їхніми перевагами над хімічними каталізаторами: вибірковістю та стереоспецифічністю дії, можливістю досягнення високих швидкостей перетворення субстратів за відносно м'яких технологічних умов, нешкідливістю для навколишнього середовища і людини. Мікробні продуценти зарекомендували себе як зручне та економічне джерело біотехнологічно важливих ферментних препаратів гідролітичної дії. Одним з таких ензимів є α-галактозидаза, яка має здатність відщеплювати термінальні α-зв'язані галактозильні залишки від широкого кола природних та синтетичних сполук. В огляді наведені сучасні дані про розповсюдження ензиму в різних групах мікроорганізмів, а також порівняльна характеристика їхніх каталітичних властивостей. Окреслено коло питань, які стосуються фізіологічної ролі ензиму у рослинних і тваринних організмах та локалізації і функціональних властивостей у бактерій і грибів. Показано місце α-галактозидази мікроорганізмів у сучасній ієрархічній класифікації глікозидаз. Описано можливості використання ензиму як тонкого інструменту модифікації оліго- та полісахаридів у різних галузях промисловості.

***Ключові слова:*** *α-галактозидаза, мікроорганізми, розповсюдження, властивості, використання.*