MICROBIAL α-L-RHAMNOSIDASES: CLASSIFICATION, DISTRIBUTION, PROPERTIES AND PRACTICAL APPLICATION

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One of the important problems of current biotechnology is the usage of enzymes of microbial origin for destruction of poorly soluble compounds and synthesis of new drugs. In recent years a great deal of researchers' attention has been given to such technologically promising carbohydrases as O-glycosylhydrolases catalyzing the hydrolysis of O-glycoside links in glycosides, oligo- and polysaccharides, glycolipids, and other glycoconjugates.

Aim. The review provides data on the position of α-L-rhamnosidases in the modern hierarchical classification of glycosidases and presents data available in the literature on the features of the enzyme structure in various microorganisms.

Methods. The publications from the following databases were analyzed: PubMed (https://pubmed.nsbi.nlm.nih.gov/), the Carbohydrate-Active enZYmes (http://www.cazy.org/), the BRENDA Enzyme Database (https://www.brenda-enzymes.org/).

Results. Data on the physicochemical, catalytic, and kinetic properties of α-L-rhamnosidases in microorganisms of different taxonomic groups have been systematized. The peculiarities of the substrate specificity of the enzyme depending on the nature of the protein and the growing conditions of the producer are characterized.

Conclusions. Functional properties and specificity action of microbial α-L-rhamnosidases suggest their broad-range applicability for food and animal feed processing, as well as obtaining biologically active compounds for the pharmaceutical industry and medicine.

Key words: α-L-rhamnosidase; microorganisms; physicochemical properties; derhamnosylation; flavonoids.

Glycosidases (EC 3.2.1) are a group of enzymes responsible for the cleavage of O-glycosidic bonds in various substrates: oligo- and polysaccharides, glycoproteins, and glycolipids. Together with glycosyltransferases, they are the key enzymes of carbohydrate metabolism in almost all living organisms, so the study of their physicochemical, structural, functional and kinetic properties is of great theoretical and applied importance. Modern traditional nomenclature distinguishes more than 200 different glycosidases (EC 3.2.1.1 — 3.2.1.215), and on the basis of amino acid sequence homology, glycosidases are combined in the CAZy database into 164 families [1]. Enzymes with different glycosidase activities can be present within one family, while the same substrate specificity can be found in proteins from different families.

One of the representatives of the class of glycosidases is the enzyme α-L-rhamnosidase (α-L-rhamnoside-rhamnohydrolase — EC 3.2.1.40), which hydrolytically cleaves the terminal unreduced α-1,2, α-1,4 and α-1,6 bound L-rhamnose residues in α-L-rhamnosides. All reported α-L-rhamnosidases act by a configuration inversion mechanism, and based on amino acid sequence similarity, they are classified into three families of glycosidases GH28, GH78, and GH106 (http://www.cazy.org/) [1]: GH28 and GH106 families contain α-L-rhamnosidases involved in pectin metabolism, the GH78 family includes α-L-rhamnosidases that catalyze the cleavage of rhamnose, which is linked α(1→2), α(1→6) and, rarely , α(1→3) bonds with glucose of flavonoids [2−4]. For five enzymes of the GH78 and GH106 families, in addition to α-L-rhamnosidase, a very rare α-L-mannosidase activity was shown [5]. The use of enzymatic hydrolysis by α-L-rhamnosidase to obtain L-monosaccharides (in particular L-rhamnose and L-mannose), which are extremely rare in nature, is very attractive for the pharmaceutical and food industries, which require such monosaccharides for the synthesis of many active compounds [6].

It should be noted that there are very limited data on the structural and functional features of α-L-rhamnosidases. To date, sixteen genes encoding bacterial α-L-rhamnosidases of the GH78 family have been cloned and characterized, most of which are related to lactic acid bacteria such as *Bifidobacterium* [7, 8], *Lactobacillus* [9, 10], and *Pediococcus acidilactici* [11]. To date, only 4 structures of α-L-rhamnosidases GH78 and two GH106 are registered in the Protein Data Bank: enzymes isolated from *Bacillus* sp. GL1 [12], *Bacteroides thetaiotaomicron* [13], *Streptomyces avermitilis* [14], *Klebsiella oxytoca* [15], *Sphingomonas paucimobilis* [16] and *Novosphingobium* sp. [17]. The largest structure is the SaRha78a protein from *Streptomyces avermitilis*, which contains six domains, including the catalytic domain and the CBM67 carbohydrate-binding domain [14]. Enzyme from *Bacillus* sp. GL1 consists of five distinct domains [12], and *B. thetaiotaomicron* α-L-rhamnosidase contains four domains. In contrast, *K. oxytoca* α-L-rhamnosidase consists of only two domains and does not contain the CBM67 substrate-binding domain [15]. The catalytic domain of enzymes of the GH78 family has a typical (α/α)6-barrel structure. As shown by site-directed mutagenesis, the nucleophilic-electrophilic

pair for such enzymes consists of two carboxyl groups of glutamic acid residues [12, 14]. The involvement of glutamic and aspartic acid has also been established for *K. oxytoca* [15]. The GH106 family enzyme has been shown to have highly conserved residues in its structure that bind rhamnose and calcium in the shallow active site, and the catalytic domain has (β/α)8-barrel structure [17].

The main substrate for α-L-rhamnosidases is L-rhamnoside, which is part of plant glycosides, glycolipids, gums, pigments, resins, specific immunopolysaccharides, heteropolysaccharides of bacterial cell walls, cytotoxic saponins, antifungal glycoalkaloids, virulence factors of some bacteria [18, 19]. But most often, L-rhamnose is found in such plant life products as bioflavonoids, which are, in particular, part of the aromatic terpene glycosides of grapes and citrus fruits [20]. In addition, a high content of bioflavonoids is present in tealeaves, flowers and leaves of buckwheat, Japanese sophora, rose hips, black rowan, black currant, strawberry, raspberry, cherry, sea buckthorn, and some varieties of apples, plums, red pepper, which are raw materials for production medical drugs [21]. The biological effect of flavonoids is connected with their ability to inhibit the peroxidation of lipids, the oxidation of ascorbic acid catalyzed by heavy metal ions with which they form chelates, a high anti-inflammatory, capillary-strengthening, as well as antispasmodic effect on smooth muscles, an effect on the secretory activity of the stomach and liver [22].

Distribution of α-L-rhamnosidases among microorganisms

The glycolytic potential of microorganisms is undeniable, as their genomes encode a wide range of glycosidases [1], due to which they are an inexhaustible source of enzymes of various specificities. α-L-Rhamnosidase is found in various groups of organisms: in bacteria, fungi, higher plants and animals [23−30]. It is a secondary metabolite involved in the processes of synthesis and degradation of such components of cell membranes as glycolipids and glycoproteins. The localization of the enzyme in microbial cells, as well as the conditions of its biosynthesis, have not been studied much, but most of the works are devoted to extracellular α-L-rhamnosidases of micromycetes (*Aspergillus aculeatus*, *A. nidulans*, *Fusarium moniliforme*) and bacteria (*Bacillus* sp.) [29−32]. α-L-

Rhamnosidase activity was noted in most micromycetes with starch-degrading activity, especially in representatives of the genera *Acremonium*, *Alternaria*, *Aspergillus*, *Cladosporium*, *Eurotium*, *Fusarium*, *Mortierella*, *Penicillium*, *Phoma*, and *Talaromyces* [23, 29, 33]. Active producers are described among strains of *P. restrictum* and *P. roseopurpureum* [34]. Monti D. with coauthor. [35] created a database that summarizes information on fungal α-L-rhamnosidases, for which substrate specificity has been established, as well as stability in organic solvents has been studied. These data are used for the selective modification of deglucoruscin, a natural glycoside with pharmacological properties, which is used for the treatment of chronic venous insufficiency [36].

Secretory and intracellular α-L-rhamnosidases have been described in bacteria (*Sphingomonas* sp. [37], *Bacteroides* JY-6 [38], *Lactobacillius* and *Bifidobacteria* [8−10, 25]), *Clostridium stercorarium* [39], *Bacillus* sp. GL1 [40], Antarctic bacteria *Ralstonia pickettii*, *Pseudomonas mandelii* and *Pseudoalteromonas* sp. [41, 42]. Producers of α-L-rhamnosidases are also known among representatives of marine bacteria *Alteromonas macleodii*, *Halomonas variabilis*, *Marinomonas pontii*, *Oceanimonas smirnovii*, *Psychrobacter* sp., *P. citrea*, *P. undina*, *S. baltica*, *Bacillus* sp. [17, 43]. It is known that marine microorganisms are the main suppliers of organic compounds in the complex ecosystem of the World Ocean due to the presence of a pool of degradative enzymes, including those that modify glycopolymers [44, 45]. The special structure of the functionally significant parts of the molecule of such enzymes determines the mechanisms of adaptation of marine microorganisms to the conditions of existence, which is reflected in the reduction of intermolecular and intramolecular bonds and the increase in the efficiency of catalysis due to the increase in the number of turnovers of the active centers of enzymes [46]. All this stimulates scientific interest in the study of biodiversity and adaptation mechanisms of marine bacteria with the aim of studying the structural and functional features of their proteins, as well as for the involvement of such enzymes in various technological processes.

Mesophilic and psychrotolerant yeasts also exhibit α-L-rhamnosidase activity: producers have been described among Antarctic UV-resistant yeast species *Rodotorula mucilaginosa*, *Nadsoniella nigra* var. *hesuelica*, *Cryptococcus victoriae*, *C. terricola*, *Pichia angusta*, *Saccharomyces cerevisiae* and *Papiliotrema laurentii* [23, 47−50]. Although α-L-rhamnosidases are widely represented in various groups of microorganisms, α-L-rhamnosidases of fungal origin are the most studied. It is on the basis of fungal enzymes that the technologies for the use of α-L-rhamnosidases in the food industry have been developed to remove bitterness from citrus juices [11, 46, 51, 52], remove hesperidin crystals from orange juices, and enhance the aroma of wine by enzymatic hydrolysis of terpene glycosides [32, 53, 54]. However, despite significant industrial interest, only a few crude preparations of α-L-rhamnosidase from representatives of the genera *Aspergillus* and *Penicillium* (hesperidinase and naringinase) are currently being produced (Sigma, USA). However, such drugs, in addition to α-L-rhamnosidase, contain β-D-glucosidase as a concomitant component, which limits their use.

Since α-L-rhamnosidase, like most glycosidases, is an inducible enzyme, an increase in enzyme secretion is observed in the presence of various inducers. Because of the screening conducted by Monti D. with co-authors. [35], the different ability of micromycetes to form α-L-rhamnosidase in the presence of a number of rhamnosides: rutin, hesperidin and naringin is shown (Table 1). It is also known that naringin, rutin and rhamnose induce the synthesis of α-L-rhamnosidase in *A. terreus*, while arabinose, xylose, arabinogalactan, fructose, pectin, cellulose, xylan and arabans do not affect the synthesis of the enzyme [55]. However, the synthesis of α-L-rhamnosidase in some fungi, in particular *A. persicinium* and *M. alpina*, was not induced by L-rhamnose. Others could be identified as potential α-L-rhamnosidase producers only when using inducers such as hesperidin for *A. aculeatus* or rutin for *F. oxysporum*. Waste from juice production is also successfully used for growing producer crops (*A. terreus*) [56]. The possibility of formation of α-L-rhamnosidase using the mechanism of carbon catabolite repression was shown for *A. nidulans* [4]. Under conditions of catabolite repression (rhamnose + glucose), the wild strain of *A. nidulans* showed 2.96% of the specific activity obtained under conditions of induction (rhamnose).

Similar results regarding the inhibition of α-L-rhamnosidase synthesis by glucose and xylose and the absence of such an effect in the presence of arabinose and lactose were also obtained by other researchers [23].

In addition, it is known that the synthesis of various extracellular enzymes is subject to the regulatory effect of pH. In acidic growing conditions (pH 5.5), the specific activity of α-L-rhamnosidase of *Aspergillus* strains is 2 times higher than in alkaline conditions (pH 7.6). Such data are explained by the fact that the synthesis of extracellular protein is much more intense in alkaline conditions than in acidic conditions. It is interesting to note that the *A. terreus* strain was completely unable to produce α-L-rhamnosidase at pH values lower than 5.8, which is uncharacteristic for micromycetes [57].

Isolation and properties of microbial α-L-rhamnosidases

Today, a fairly wide range of enzymes with α-L-rhamnosidases activity has been obtained from the culture fluid of micromycetes, bacteria, and yeast. In order to purify intracellular enzymes in the initial stages, various methods of cell destruction are used, more often — ultrasonic devices. So α-L-rhamnosidase from *Bacteroides* sp. JY-6 was isolated after ultrasonic destruction of cells followed by salting out with ammonium sulfate. Sequential chromatography on DEAE-cellulose, Silica-PAE, Sephacryl S-300

Table 1. **Extracellular α-L-rhamnosidase activity of micromycetes using various inducers of biosynthesis [35]**

Strain	Activity, U/ml			
	Inducer			
	L−Rhamnose	Rutin	Hesperidin	Naringin
A. persicinum CCF 1850	–	2.3	–	–
A. aculeatus CCF 108	19.0	4.5	60.0	14.0
A. aculeatus CCF 3134	12.0	5.5	46.0	–
A. aculeatus CCF 3138	4.1	–	3.1	–
A. niger CCIM K2	12.0	5.6		6.2
A. terreus CCF 3059	230.0	170.0	64.0	110.0
Circinella muscae CCF 2417	–	5.7	–	–
Emericella nidulans CCF 2912	46.0	–	130.0	76.0
Eurotium amstelodami CCF 2723	–	2.5	–	–
F. oxysporum CCF 906	–	49.0		3.7
M. alpina CCF 2514	–	5.1	–	–
Mucor circinelloides griseo−cyanus CCIM	–	8.5	–	–
P. oxalicum CCF 2430	8.3	–	–	–
R. arrhizus CCF 100	–	3.2	–	–
T. flavus CCF 2686	1.6	–	–	24.0
T. harzianum CCF 2687	–	1.8	–	–

Note: "–" — not studied, the activity in the enzyme medium was determined using *p*-nitrophenyl-α-L-rhamnopyranoside (*p*-NPR) as a substrate.

and hydroxyapatite was used to purify the enzyme [38]. To purify α-L-rhamnosidase from the bacterium *Fusobacterium* sp. K-60 was used column chromatography on Butyl-Toyopearl, hydroxyapatite, Sephacryl S-300 and Q-Sepharose [58]. A similar isolation and purification scheme was used to purify α-L-rhamnosidases of *Bacillus* sp. and *L. plantarum* [10, 40], which made it possible to obtain homogeneous enzyme preparations and characterize them. Highly purified intracellular α-L-rhamnosidase was also obtained from the yeast culture *P. angusta*. For this purpose, gel filtration, ion exchange and affinity chromatography on concavalin A-Sepharose, DEAE Bio Gel A agarose, Rhamnose-Sepharose 6B and hydroxyapatite were used [48]. Using affinity chromatography, the highest level of purification was also obtained when separating the components of the *P. paucimobilis* enzyme complex [59].

The physicochemical properties of α-L-rhamnosidases isolated from different sources vary significantly. Thus, according to literature data (Table 2), the molecular mass (Mm) of the enzyme ranges from 36 to 135 kDa for fungal producers and from 12 to 500 kDa for bacterial producers. Thermooptimums for mesophilic bacteria and fungi were mostly noted at 50−60 °C, while for thermophiles — at 70−95 °C. The majority of enzymes were stable during storage at 0−4 °C. It is also known that during freezing to −20 °C α-L-rhamnosidase from *P. paucimobilis* remained stable for several months at optimal pH values [59].

There are also data that α-L-rhamnosidases obtained on different carbon sources (rhamnose or naringin) can exhibit different stability under thermal denaturation conditions. Thus, more stable forms of α-L-rhamnosidases of *E. erubescens* and *C. albidus* were obtained using naringin [73, 74]. The importance of the carbon source for the formation of protein glycosylation sites and their number was also shown by other researchers for *A. fumigatus* α-galactosidase and *A. terreus* α-L-rhamnosidases [57, 75].

α-L-Rhamnosidases of different origins also differ in their sensitivity to metal cations and chemical compounds of various nature. It was shown that in the presence of Hg^{2+}, Mn^{2+}, Zn^{2+}, Ba^{2+}, Cu^{2+}, Co^{2+}, Pb^{2+}, Fe^{2+}, Fe^{3+} and Al^{3+} cations, the activity of *P. tardum* α-L-rhamnosidase decreases by 20−90% [69]. Inactivation by Hg^{2+} ions was noted in enzymes from *B. thetaiotaomicron* [13], *P. angusta* [48], *Bacillus* sp [31], *C. stercorarium* [39].

These data indicate the importance of sulfhydryl groups for maintaining the active conformation of α-L-rhamnosidases and the manifestation of its activity. Inhibition of activity (by 50−99%) in the presence of Cu^{2+}, Fe^{2+}, Fe^{3+}, Hg^{2+}, Ni^{2+} was established for α-L-rhamnosidases of *B. thetaiotaomicron* [13], Cd^{2+} and Pb^{2+-} in *P. paucimobilis* and *Bacteroides* JY-6 [38, 59], Ba^{2+}, Zn^{2+}, Mn^{2+} — in *P. laurentii* and *T. bacterium* [47, 63], Mg^{2+} — in *A. nidulans* [30], cations Co^{2+}, Cu^{2+}, and Ni^{2+} — in *A. alternata* [65], 0.2 mM Zn^{2+} completely inactivated the α-L-rhamnosidase of *A. luteo albus* [66]. α-L-Rhamnosidase of *P. angusta* was also inhibited by Cu^{2+} and Hg^{2+} at a concentration of 10^{-3} M by 89 and 94% [48]. α-L-Rhamnosidase of *B. thetaiotaomicron* was also activated by Ca^{2+} and Mg^{2+} by 30 and 26%, respectively [13]. For the α-L-rhamnosidase of *A. alternata*, 1.35- and 1.2-fold activation by Ba^{2+} and Al^{3+} cations, respectively, was noted [65]. Ca^{2+}, Co^{2+}, Cu^{2+} at a concentration of 100 mM activated α-L-rhamnosidase of *A. niger* by 1.4−1.6 times, but did not affect the activity of α-L-rhamnosidase of *A. luteo albus* [66]. The stimulating effect of Ca^{2+}, Zn^{2+}, Mg^{2+}, Co^{2+} cations on α-L-rhamnosidase activity of micromycetes was established [55, 69, 76]. It is known that Ca^{2+} cations participate in the stabilization of the tertiary structure of some proteins through the formation of ionic bonds with two different amino acid residues, that is, they perform the function of a stabilizing bridge similar to disulfide bonds. Most of the currently known α-L-rhamnosidases are metal-independent enzymes, however, the metal dependence of the *E. erubescens* enzyme has been shown [70]. Also, calcium chelators ethylenediaminetetraacetic acid (EDTA) and ethylene glycol-bis(β-aminoethyl ether)-N,N,N′,N′-tetraacetic acid (EGTA) at a concentration of 1 mM reduced the activity of the *P. paucimobilis* enzyme to 0.7 and 10%, respectively [59].

Dithiothreitol did not affect the activity of α-L-rhamnosidases in *A. terreus*, which made it possible to conclude that SH-groups play a minor role in its activity [55], at the same time, *p*-chlormercuribenzoate is an inhibitor of many α-L-rhamnosidases [37, 70]. The inactivating effect of SO_2 was noted on the enzyme of *A. nidulans* [30]. α-L-Rhamnosidase of *D. thermophilum* was highly tolerant to the presence of various solvents (acetonitrile, dimethylsulfoxide and dimethylformamide) and 30% methanol [61], ethanol (42%) reduced the activity of the *P. decumbens*

Table 2. **Biochemical properties of microbial α-L-rhamnosidases**

Microorganism	Structure	Mm, kDa	pH-Optimum	Thermal optimum, °C	Activity, U/mg	K_m, mM (p−NPR)	Inductor
Bacteria							
Bacillus sp. [31]	Pentamer	500	7.0	50	54	0.119	gellan
B. thetaiotaomicron [13]	–	86	6.5	60	48.7	0.53	–
Bacteroides sp. [38]	Dimer	240	7.0	–	89.9	0.29	–
D. thermophilum [61]	Monomer	106	5.0	95	–	0.054	–
Fusobacterium sp. [58]	Tetramer	170	5.0−6.5	40	3.4	0.057	Soy flour
Novosphingobium sp. [62]	Monomer	12	6−7.5	35−45	–	0.157	–
Thermomicrobia bacterium [63]	Dimer	210	5.0	70	109	0.46	–
Micromycetes							
Acrostalagmus luteo albus [66]	–	109	8	55	526	3.38	Rhamnose
A. alternata [65]	Dimer	135	5.5	60	21,7	4.16	–
A. aculeatus [3]	Monomer Glycoprotein (15−24 %)	85 90	4.5	–	13	0.3 2.8	Hesperidin
A. kawachii [60]	– Glycoprotein (22%)	68 90	4.0	50	–	17.9	–
A. oryzae [64]	–	75	4.5	65	224	5.2	–
A. tubingensis [52]	Monomer Glycoprotein (12%)	110	4.0	50	–	–	–
Eupenicillium erubescens [70]	Monomer Glycoprotein (1%)	40	5.0	60	121	1.09	Rhamnose, naringin
Fusarium moniliforme [32]	Monomer	36	9.5	50	–	0.5	–
P. decumbens [67]	–	–	4.0	50	13.3	6.1	Naringin
Penicillium sp. [68]	–	90	3.5	57	–	1.52	Naringin
P. tardum [69]	Monomer Glycoprotein (12%)	95	5.0	60	33.1	0.7	Rhamnose, naringin
T. longibrachiatum [71]	–	–	4.5	60	3.9	–	Rhamnose
Yeast							
Cryptococus albidus [72]	Monomer Glycoprotein (5%)	50	5.0	60	12.5	4.48	Rhamnose, naringin
P. angusta [48]	Monomer	88	6.0	40	33.9	–	Rhamnose

Note: " – " — no data available.

enzyme by 20% [77]. Rhamnose and glucose are competitive inhibitors of many microbial α-L-rhamnosidases [23, 37, 38, 48, 55, 69, 70, 72].

Substrate specificity of α-L-rhamnosidases

Natural substrates for α-L-rhamnosidases from various sources are α-L-rhamnoside-containing flavonoids and saponins of plants, glycoproteins, gums, resins, pigments in which the L-rhamnose residue is connected α-1,2; α-1,4- and α-1,6-bonds with β-D-glucoside or attached directly to the aglycone in the C1 position (Fig.).

The most common are naringin, poncirin, neohesperidin (α-1,2-bond), rutin, hesperidin (α-1,6-bond), saikosaponin, asiaticoside (α-1,4-bond), quercitrin and proscyllaridin A, in which the L-rhamnose residue is connected to an aglycone. Table 3 presents data on the ability to hydrolyze some natural rhamnoglycosyl substrates by enzymes isolated from various microorganisms. Thus, α-L-rhamnosidase from *A. niger* [78] hydrolyzes naringin, rutin,

but not hesperidin and quercitrin; while an enzyme from another strain of *A. niger* [79] is able to hydrolyze hesperidin as well. α-L-Rhamnosidase from *Bacteroides* JY-6 [38] acts on neohesperidin, naringin, poncirin, hesperidin, rutin, saikosaponin C. α-L-Rhamnosidase from *P. paucimobilis* FP 2000 hydrolyzed hesperidin best, followed by proscyllaridin A, rutin, naringin, quercitrin, saikosaponin C [59].

The affinity of α-L-rhamnosidases of different producers to various substrates can vary greatly depending on their specificity. The value of K_m for *p*-NPR varies in the range of 0.057−2.8 mM, for naringin 0.021−1.9 mM, for hesperidin 0.02−1.33 mM, for rutin 0.028−1.44 mM, for quercitrin 0.077−0.89 mM and for ponzirin 0.02−0.93 mM. These data indicate that the affinity of the enzyme for natural flavonoid rhamnoglycosides is higher than for synthetic substrates. Most of α-L-rhamnosidases attack the α-1,2-glycosidic bond, some also hydrolyze substrates with α-1,6 linked rhamnose. α-L-Rhamnosidases, which hydrolyze α-1,4- and other bonds, are extremely rare. Despite steric hindrance due to the direct linkage of
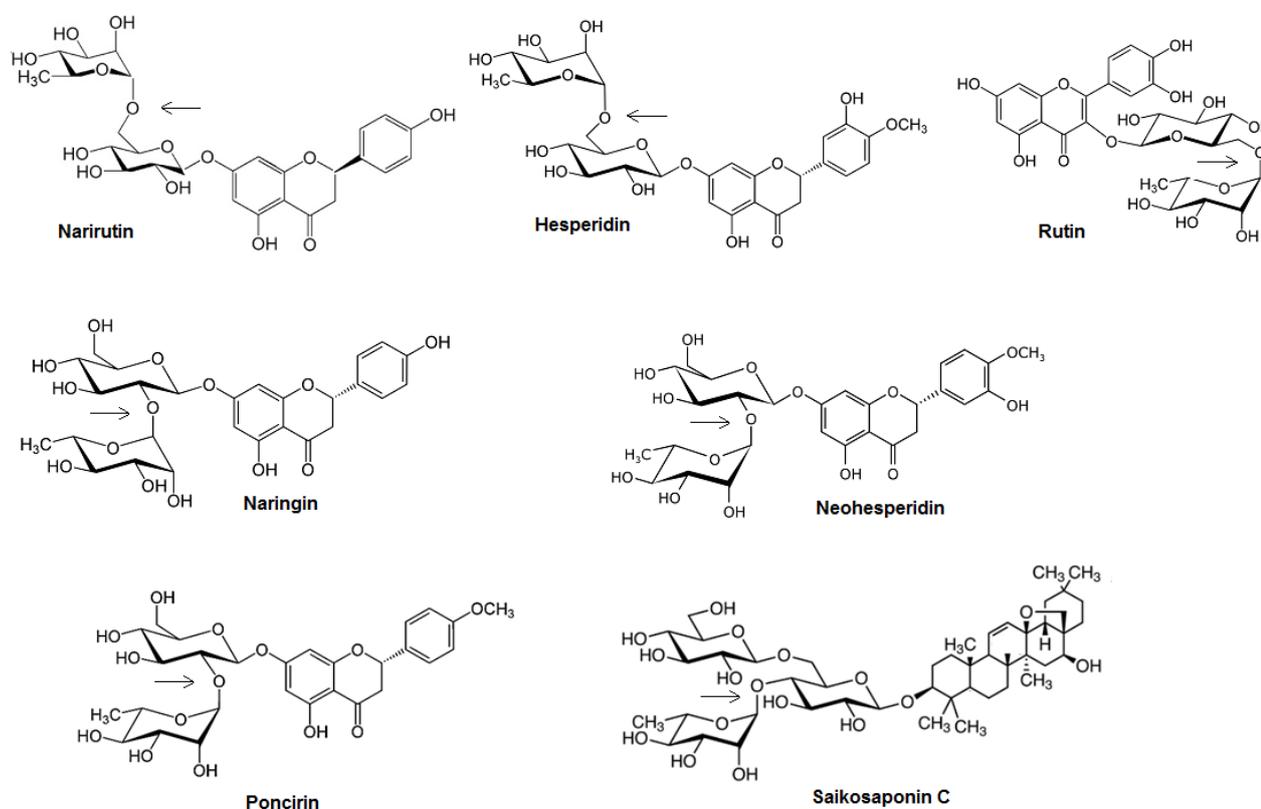
Structural formulas of the most common substrates for α-L-rhamnosidases. The arrows highlight to the sessile bond

Table 3. **Specificity of various α-L-rhamnosidases for rhamnoglycoside substrates**

Enzyme source	p-NPR	Naringin	Saikosaponin C	Rutin	Hesperidin	Quercitrin	Proscilaridine A	Poncirin	Neohesperidin
	α-1	(α-1,2)	(α-1,4)	(α-1,6)	(α-1,6)	(C$_1$)	(C$_1$)	(α-1,2)	(α-1,2)
A. aculeatus [3]	+	+	*	+	+	−	*	*	*
A. nidulans [30]	+	+	*	+	+	*	*	*	*
A. niger [78]	+	+	*	+	−	−	*	*	*
A. niger [79]	+	+	*	+	+	−	*	*	*
A. terreus [57]	+	+	*	+	−	*	*	*	*
Penicillium sp. [68]	+	+	*	*	*	*	*	*	*
Bacteroides JY-6 [38]	+	+	+	+	+	*	*	+	+
C. stercorarium [39]	+	+	*	*	+	*	*	*	*
P. paucimobilis [59]	+	+	+	+	+	+	+	*	*
P. angusta [48]	−	+	*	+	+	+	*	*	*
Sphingomonas sp. R [37]	+	+	*	+	+	+	*	*	*

Note: "+" — hydrolysed; "−" — not hydrolysed; "*" — not determined.

the α-L-rhamnosyl residue to the aglycone, the enzymes obtained from *M. alpina*, *P. oxalicum*, and *T. flavus* were able to utilize quercitrin as a substrate, whereas its hydrolysis had previously only been noted in bacterial [37] and animal [79] glycosidases. The complex glycoside ginsenoside Re effectively derhamnosylated preparations from *A. aculeatus*, *E. nidulans*, and *P. oxalicum*. It is interesting that previously only α-L-rhamnosidase obtained from the strain of *Absidia* sp. 39, which was cultivated in the presence of an aqueous extract from ginseng roots [80], was able to hydrolyze ginsenoside, however, the preparations from the three foregoing strains were obtained with an inducer of a different nature. The terpene glycoside asiaticoside was derhamnosylated by α-L-rhamnosidases from *A. terreus*, *E. nidulans*, *F. oxysporum*, *T. flavus*, *A. aculeatus*, and *A. niger* [23, 78]. In some cases, enzymes obtained from the same strains in the presence of different inducers showed different substrate specificity. For example,

α-L-rhamnosidase induced by naringin from *T. flavus* CCF 2686 had broad substrate specificity, while the enzyme obtained on medium with L-rhamnose hydrolyzed only rutin and quercitrin [35]. α-L-Rhamnosidases obtained from *E. nidulans* CCF 2912, when induced by L-rhamnose, hesperidin, and naringin, also revealed different substrate specificity [35]. The enzyme obtained with hesperidin as an inducer of synthesis was able to deglycosylate a wider range of substrates. α-L-Rhamnosidases from *A. niger* [79] and *P. decumbens* [77] are also able to hydrolyze monoterpenyl disaccharides from grapes: geraniol-β-D-rutinoside and 2-phenylethyl-β-D-rutinoside.

Substrates for α-L-rhamnosidase can also be α-L-rhamnoside-containing disaccharides containing α-D-xylose, β-D-fucose, α-D-galactose and α-N-acetyl-D-glucosamine in reduced terminals: O-α-rhamnopyranosyl-α-1,3-α-D-xylose; O-α-L-rhamnopyranosyl-α-1,2-β-D-fucose, O-α-L-rhamnopyranosyl-α-1,2-α-D-galactose,

O-α-L-rhamnopyranosyl-α-1,4-α-D-galactose, O-α-L-rhamnopyranosyl-α-1,3-N-acetyl-D-glucosamine, O-α-L-rhamnopyranosyl-α-1,6-N-acetyl- D-glucosamine, O-α-L-rhamnopyranosyl-α-1,4-N-acetyl-D-glucosamine. For some α-L-rhamnosidase was shown endo-glycosidase activity and ability to hydrolysed of rhamnogalacturonan regions of pectin occurs [19].

It was also established that α-L-rhamnosidases of micromycetes, in particular *A. oryzae* and *A. niger*, exhibit transglycosylation properties [64, 81] and the ability to synthesize rhamnosyl mannitol and disaccharides containing α- or β-L-rhamnopyranosidic group through the reactions of transglycosylation with inexpensive rhamnose as the glycosyl donor.

Specific data on the mechanism of action of α-L-rhamnosidases are very scarce, since the mechanism and kinetics of enzyme reactions have rarely been studied. Although, by analogy with other glycosidases, it is likely that the bonds between the C-atom of rhamnose and the oxygen of the substrates are splitted. Thus, it was established [39] that the bond break occurs from the direction of the oxygen atom to which the non-reducing residue of the oligo- and polysaccharide is adjacent and to which the glycosidase shows the greatest specificity. It is known that α-L-rhamnosidases act on C1−O or O−Cn in substrates (for α-L-rhamnosidases $s = 2, 4, 6$). During the study of the type of enzymatic mechanism of the rhamnosidase reaction, the hydrolysis products of *p*-NPR were analyzed using ^{1}H-NMR spectrum. Anomeric protons that were cleaved from rhamnose appeared as peaks at 5.06 parts per million (α) and 4.83 parts per million (β) [39]. In studies after the interaction of the substrate and the enzyme, the peak, which was determined as the β-isomer of rhamnose, appears slightly earlier than the α-peak. However, the peak area between α- and β-protons increased from 0.36 to 0.88, and they were balanced between both isomeric forms of α-rhamnopyranose. This type of kinetic behavior determines the mechanism of hydrolysis, in which β-rhamnose acquires the form of α-rhamnoside through a single displacement mechanism, spontaneously mutating into the α-form. A similar mechanism was observed in rhamnogalacturonan hydrolase from *A. aculeatum* [19].

Fields of α-L-rhamnosidases practical use

Modern enzyme technologies are an alternative to chemical processes of destruction of poorly soluble compounds and synthesis of new biologically active drugs. In recent years, the nomenclature of biotechnologically important enzymes, including glycosidases, has expanded significantly due to the improvement of methods of obtaining biocatalysts from microbial sources. α-L-Rhamnosidase is used in the structural analysis of complex glycoconjugates of oligo- and polysaccharides, glycosides, glycolipids, and can also be involved in the production of L-rhamnose and L-mannose from natural glycosides by hydrolysis of prunin from naringin, [5, 6, 64, 81]. The enzyme can also be used to cleave rhamnosyl residues present in flavonoid glycosides to improve the quality of food products [20, 21]. For example, the ability of α-L-rhamnosidase to modify naringin is used to remove the bitterness of some citrus juices, and enzymatic derhamnosylation of hesperidin glucoside makes it possible to avoid its crystallization during the production of orange and tangerine juice [51, 52, 53, 57, 69, 82]. The use of α-L-rhamnosidases for the processing of fruit and berry and vegetable purees and juices, as well as green tea, makes it possible to obtain functional beverages with a high content of bioavailable flavonoid glucosides [51, 57, 72, 76]. Also, α-L-rhamnosidases can be involved in the processing of vegetable and fruit pectins. As a result of processing, the content of prebiotic oligosaccharides increases, which increases the functional value of such products [19, 20].

α-L-Rhamnosidase is also widely used in the winemaking process to enhance the aroma of the final product [54]. One of the important characteristics of wine quality is the aromatic bouquet, the monoterpenes present in grapes make a significant contribution to its creation. These components are partly contained in the form of free volatile forms and partly as glycoside-linked non-volatile precursors. The latter are represented by β-D-glucopyranosides and diglycosides, namely: 6-O-α-L-arabinofuranosyl-β-D-glucopyranoside and 6-O-α-L-apiofuranosyl-β-D-glucopyranoside, which are a potential source of bouquet in the traditional winemaking process. Glycoside-bound volatile substances can be released by two-stage enzymatic hydrolysis [54]. At the 1st stage, the glycosidic bond can be cleaved by α-L-rhamnosidase, α-L-arabinofuranosidase or β-D-apiosidase, releasing the corresponding

monoterpenyl-β-D-glucoside, and at the 2nd stage, β-D-glucosidase releases monoterpenol. Orejas and others. [83] showed that α-L-rhamnosidase activity of *A. nidulans* was very weakly inhibited by glucose and SO_2 and partially inhibited by ethanol, indicating the potential of using the enzyme to enhance wine aroma. Also, high tolerance to significant concentrations of glucose and ethanol was shown for α-L-rhamnosidase from *P. angusta* [48].

α-L-Rhamnosidase can be involved in the production of many drugs and their precursors [29]. To date, it has been shown that the course of inflammatory processes can be influenced by the presence of biologically active substances, in particular, glycosylated polyphenolic compounds, which have antioxidant, antitumor, anti-inflammatory and antidiabetic properties. The bioavailability and activity of such compounds largely depends on the degree of their glycosylation. In order to increase the biological activity of flavonoids, several approaches are used, among which is derhamnosylation of natural glycosides with the help of α-L-rhamnosidase.

Saikosaponins and their metabolites have been shown to increase the level of corticosteroids in the blood [84]. By hydrolyzing saponin, α-L-rhamnosidase releases rhamnose and diosgenin, which are used in the synthesis of clinically useful steroid drugs such as progesterone [85]. Other deglycosylated steroids, such as desglucoruscin, ginsenoside-Rg2, etc., are of clinical importance [35, 86]. The effect of ginsenoside Rg 1, which was obtained from ginsenoside Re under the action of α-L-rhamnosidase, on the number of insulin receptors in the membranes of the liver and brain, as well as on the content of extracellular cyclic adenosine monophosphate (cAMP) was established [87].

Detailed histopathological and immunochemical analyzes showed that lifetime administration of enzymatically modified quercetin improved LPS-induced proinflammatory and oxidative responses in mice that lead to brain damage [88]. Deglycosylated flavonoids also have anti-inflammatory, cytoprotective and antiaging biological properties [89].

The enzyme's ability to modify such bioflavonoids as rutin and quercitrin can be used to prevent and treat hemorrhagic diatheses, capillary toxicosis, hemorrhages in hypertensive conditions, atherosclerosis, and radiation sickness [88]. Derhamnosylated flavonoids quercitin and hesperitin exhibit antioxidant, anticarcinogenic, anti-inflammatory, antiaggregation and vasodilator effects [90].

A sharp increase in the biosphere of the number of new chemical compounds — products of industrial and laboratory synthesis, which are essentially xenobiotics and have potential allergenic properties, leads to an increase in the prevalence of occupational and other allergies. Derhamnosylated flavonoids baicalein and quercitin, which are powerful inhibitors of leukotriene synthesis, histamine release from antigen-stimulated basophils and mast cells of humans and animals, and secretion of lysosomal enzymes and active oxygen radicals by neutrophils, can contribute to solving this problem [87]. Plant flavonoids affect lipoxygenase and cyclooxygenase cascade oxidation of arachidonic acid [91].

The pharmaceutical industry of Ukraine today produces quercitin in the form of granules under the commercial name "Corvitin". It not only inhibits the synthesis and secretion of mediators of anaphylaxis, but also has a direct relaxing effect on anaphylactic smooth muscle contractions induced by histamine, acetylcholine and prostaglandin E2. It was the search for substances with antibacterial activity among natural compounds that drew the attention of scientists to hesperidin and its aglycone hesperitin, which were obtained from citrus processing waste. It was established that hesperidin in the studied concentrations has a slight antibacterial effect, while its aglycone form — hesperitin, exhibits a pronounced antibacterial effect and inhibits the growth of all tested pathogenic staphylococci and bacilli. The well-known glycopeptide antibiotic chlorosporin C is obtained by derhamnosylation of chlorosporin B [92]. Prunin, a product of enzymatic hydrolysis of naringin, also exhibits anti-inflammatory and antiviral activity [93].

It should be noted a wide range of techniques that are used today to obtain stable and active forms of α-L-rhamnosidases for biotechnological use. In addition to the above-mentioned hesperidinase and naringinase from "Sigma-Aldrich", recombinant prokaryotic α-L-rhamnosidase ("Megazyme") and α-L-rhamnosidase *Streptomyces coelicolor* ("Protzomyx") are also available for analytical purposes in the form of ammonium sulfate efflorescence. However, technological processes require,

first of all, stable reusable preparations. To this end, new methods for the purification and binding of proteins on various carriers are being actively developed. Thus, the production of a thermostable *Clostridium stercorarium* α-L-rhamnosidase by immobilization of a His-tagged protein on calcium alginate for the hydrolysis of naringin in quinoa juice was reported [94]. The effectiveness of the use of related drugs has also been demonstrated for other carriers: celite, polyethylene terephthalate (Dacron-hydrazide), polysiloxane/polyvinyl alcohol (POS/PVA), poly(vinyl alcohol) cryogelsand chitosan [95−97]. Enzyme aggregates crosslinked with glutaraldehyde [98], including α-L-rhamnosidases from *Brevundimonas* sp. and *P. tardum* [69, 76], show high activity and stability. The promise of using cell surface engineering for naringin hydrolysis is shown by the example of the *Alternaria* sp. enzyme attached to yeast cells [99].

Therefore, microorganisms as producers of industrially important enzymes certainly have many advantages. These include extracellular secretion, multiple forms of protein, and a high level of expression. In addition, the initial levels of crop productivity can be significantly improved by optimizing growing conditions as well as by molecular genetic methods. One of the main problems, the solution of which requires the use of enzyme technologies, is to reduce the cost of obtaining an enzyme and increase its efficiency due to high activity and stability under the necessary technological conditions. And for this, it is necessary to understand the mechanisms of enzyme inactivation in order to develop effective methods of their stabilization on this basis. Although α-L-rhamnosidases have attracted the attention of researchers for more than 7 decades, some issues still remain poorly understood compared to other enzymes. This concerns, first of all, studies of the kinetics and mechanism of catalytic action, the relationship between their structure and function, and substrate specificity. The molecular mechanism of catalysis has hardly been studied. All this indicate the need for further research to understand the specifics of the functioning of these biotechnologically important enzymes.

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α-L-РАМНОЗИДАЗИ МІКРООРГАНІЗМІВ: КЛАСИФІКАЦІЯ, ПОШИРЕННЯ, ВЛАСТИВОСТІ ТА ПРАКТИЧНЕ ВИКОРИСТАННЯ

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Однією з важливих проблем сучасної біотехнології є використання ензимів мікробного походження для деструкції важкорозчинних сполук та синтезу нових лікарських препаратів. За останні роки значну увагу дослідників привертають такі технологічно перспективні карбогідрази, як О-глікозилгідролази, які каталізують гідроліз О-глікозидних зв'язків у глікозидах, оліго- та полісахаридах, гліколіпідах та інших глікокон'югатах.

Метою роботи було проаналізувати дані про положення α-L-рамнозидаз у сучасній ієрархічній класифікації глікозидаз та представити наявні у літературі дані щодо особливостей будови ензиму у різних мікроорганізмів.

Методи. Проаналізовано публікації з таких баз даних: PubMed (https://pubmed.nsbi.nlm. nih.gov/), Carbohydrate-Active enZYmes (http://www.cazy.org/), BRENDA Enzyme Database (https://www.brenda-enzymes.org/).

Результати. Систематизовано дані щодо фізико-хімічних, каталітичних та деяких кінетичних властивостей α-L-рамнозидаз у мікроорганізмів різних таксономічних груп. Охарактеризовано особливості субстратної специфічності ензиму залежно від природи протеїну та умов вирощування продуцента. Окреслено перспективи та шляхи використання α-L-рамнозидаз у біотехнологічних процесах різних галузей: хімічній, харчовій, фармацевтичній промисловості та медицині.

Висновки. Функціональні властивості та специфічність дії мікробних α-L-рамнозидаз свідчать про можливість їхнього широкого застосування для перероблення продуктів харчування та кормів для тварин, а також для отримання біологічно активних сполук для фармацевтичної промисловості та медицини.

Ключові слова: α-L-рамнозидаза; мікроорганізми; фізико-хімічні властивості; дерамнозилювання; флавоноїди.