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MINI-REVIEW

β-Diglycosidases from microorganisms as industrial biocatalysts: biochemical characteristics and potential applications

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Abstract
Flavonoid glycoside degradation can proceed through two alternative enzymatic pathways: one that is mediated by monoglycosidases, and the other catalyzed by a diglycosidase. β-Diglycosidase performs the flavonoid deglycosylation in a single reaction. The characterized β-diglycosidase activities recognize the following disaccharidic sugar moieties: β-primeverose, acuminose, vicianose, and β-rutinose. The present paper reviews the biochemical characteristics and potential industrial applications of microbial β-diglycosidases that break down plant diglycoconjugated flavonoids.

Keywords β-Diglycosidase · Enzyme catalysis · Fungi · Bacteria · Archaea

Introduction

The major flavonoid glycosides found in plants or fruits are quercetin 3-*O*-rutinoside (rutin), hesperetin 7-*O*-rutinoside (hesperidin), kaempferol-3-*O*-rutinoside, and naringenin 7-*O*-neohesperidoside (naringin) (Mazzaferro and Breccia 2011a). These compounds are involved in the bitter taste or clouding in plant-based foods or beverages, respectively.

Flavonoid glycoside degradation can proceed through two alternative enzymatic pathways: one that is mediated by monoglycosidases, and the other catalyzed by a diglycosidase. Monoglycosidases (e.g., EC 3.2.1.40: α-L-rhamnosidase), the main catalysts for deglycosylation, firstly cleave the glycosidic bond between the monosaccharide moiety and glucose. Subsequently, a β-glucosidase hydrolyzes the link between glucose and the aglycone. In contrast to this, β-diglycosidase performs the flavonoid deglycosylation in a single reaction. Many β-diglycosidases have been identified and characterized from several plants (Imaseki and Yamamoto 1961; Yasuda and Nakagawa 1994; Ogawa et al. 1997; Wirth et al. 2001; Lizotte and Poulton 1988; Mizutani et al. 2002; Suzuki et al. 2002; Baumgertel et al. 2003; Ahn et al. 2004, 2007; Nakanishi et al. 2005; Chuankhayan et al. 2005). To our knowledge, the crystal structure of β-primeverosidase (EC 3.2.1.149) from plant *Camellia sinensis* has been the only reported β-diglycosidase crystal structure (Saino et al. 2014). There is only one previous review about these enzymes that summarize the functional and biotechnological insights into diglycosidases (Mazzaferro and Breccia 2011a). The present paper reviews the biochemical characteristics and potential industrial applications of β-diglycosidases from microorganisms that break down plant diglycoconjugated flavonoids.

Potential substrate for β-diglycosidases

Quercetin 3-*O*-β-rutinoside (rutin), keampferol 3-*O*-β-rutinoside, hesperetin 7-*O*-β-rutinoside (hesperidin), diosmetin 7-*O*-β-rutinoside (diosmin), naringenin 7-*O*-neohesperidoside (naringin), and (S)-linalyl β-primeveroside are the major diglycoconjugated flavonoids of some plants (Fig. 1), mainly buckwheat and tea leaves, and fruits, such as apple, grape, and citrus (Mazzaferro and Breccia 2011). Hydroxynitriles, naphthoquinones, isoflavonoids, and terpenoids also consist of a diglycoside moiety. For instance, β-rutinosidase (6-*O*-α-L-rhamnosyl-β-D-glucosidase; EC 3.2.1.168) cleaves β-rutinose from rutin, hesperidin, and other rutinose (6-*O*-α-L-rhamnopyranosyl-β-D-glucopyranose)-containing glycoconjugates (Fig. 2).

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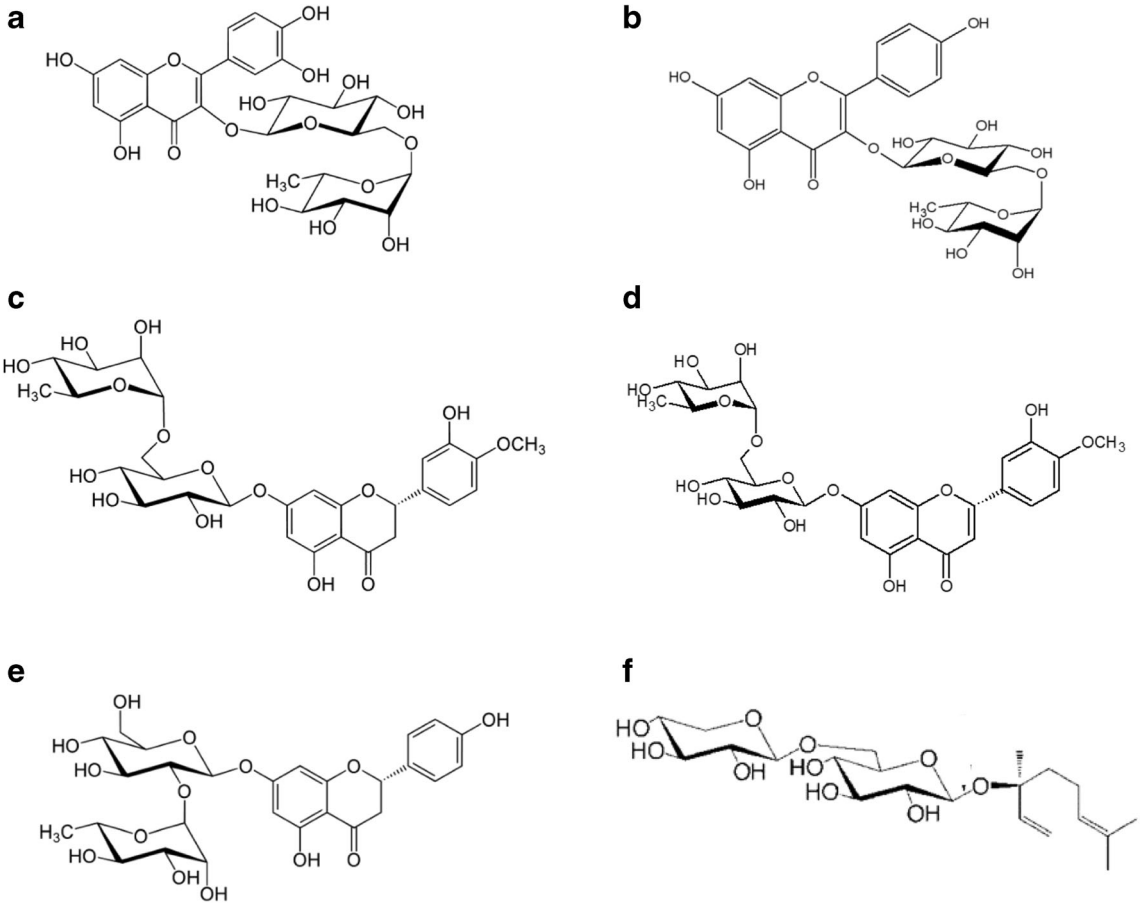

Fig. 1 Chemical structures of potential substrates for β-glycosidase activity. **a** Quercetin-3-*O*-rutinoside (Rutin). **b** Kaempferol-3-*O*-rutinoside. **c** Hesperetin-7-*O*-rutinoside (Hesperidin). **d** Diosmetin-7-*O*-rutinoside (Diosmin). **e** Naringenin-7-*O*-neohesperidoside (Naringin). **f** (S)-Linalyl β-primeveroside

Microbial β-diglycosidases

The β-diglycosidases acting on glycoconjugated flavonoids have been predominantly reported in plants. Recently, β-diglycosidases were also described from microorganisms such as fungi, bacteria, and archae (Narikawa et al. 2000; Yamamoto et al. 2002; Tsuruhami et al. 2006; Nam et al. 2012; Šimčíková et al. 2015; Neher et al. 2016; Ishikawa et al. 2018). These diglycosidases hydrolyze β-rutinoside (6-*O*-α-L-rhamnopyranosyl-β-D-glucopyranoside) or β-primeveroside (6-*O*-β-D-xylopyranosyl-β-D-glucopyranoside). It was found that *Acremonium* sp. SES201, *Penicillium rugulosum*

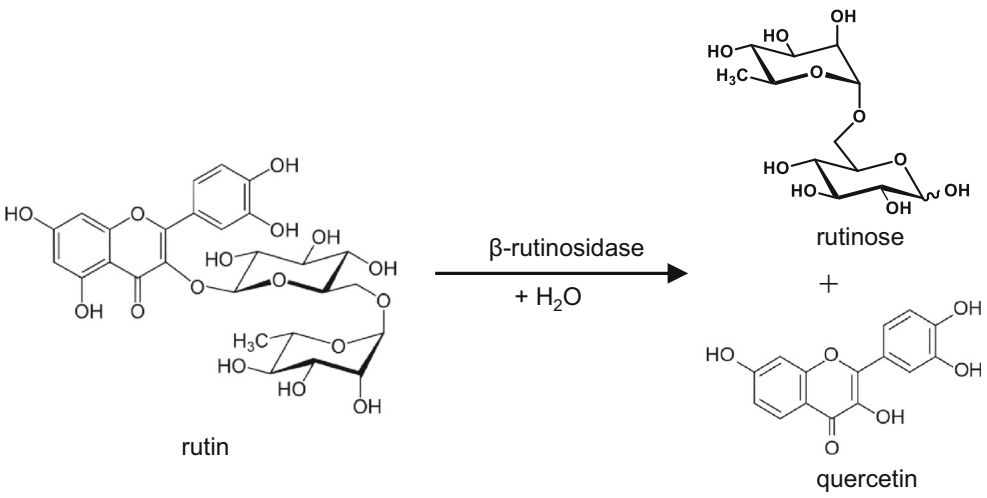

Fig. 2 Reaction scheme of the rutin hydrolysis catalyzed by β-rutinosidase

IFO7242, and *Aspergillus niger* K2 produced β-rutinosidase when 0.5% hesperidin, 2% rutin, 0.5% rutin, respectively, were used as the sole carbon source (Mazzaferro et al. 2010, 2011b; Narikawa et al. 2000; Šimčíková et al. 2015). It has also been reported that the production of *Aspergillus oryzae* β-1,3-exoglucanase (ExgA) was highest when *A. oryzae* was grown with a carbon source containing flavonoids, such as quercetin and rutin (Riou et al. 1998). Moreover, β-primeverosidase from *Aspergillus fumigatus* AP-20 and *Penicillium multicolor* IAM7153 are extracellular inducible enzymes that uses β-primeveroside-containing substances as the carbon source and inducer (Yamamoto et al. 2002; Tsuruhami et al. 2006). At first, the β-rutinosidase (6-*O*-α-rhamnopyranosyl-β-glucosidase)-encoding gene was identified from *A. niger* K2 (Šimčíková et al. 2015). However, this gene was annotated in GenBank as having a putative exo-β-1,3-glucanase-encoding open reading frame. Proximately, the β-rutinosidase-encoding gene was also reported in *A. oryzae* RIB40 (Ishikawa et al. 2018). The β-rutinosidase from *A. oryzae* showed high degree of sequence similarity to the β-rutinosidase from *A. niger* K2 (70%) (Šimčíková et al. 2015) and the β-primeverosidase of *Penicillium multicolor* TS-5 (58%) (Tsuruhami et al. 2006). Based on the amino acid sequence similarity, the CAZy database (Lombard et al. 2014) classifies fungal β-diglycosidases into the GH5-subfamily 23 (GH5_23) of the glycoside hydrolases (Aspeborg et al. 2012). Twelve sequences of only fungal origin have been deposited in the CAZy database in GH5_23 section (Fig. 3). Meanwhile, sequences for exo-β-1,3-glucanases, which are fungal cell wall modifying enzymes, have been deposited in the GH5_9. The β-rutinosidases from *A. niger* (AnRutA) and *A. oryzae* (AoRut) showed low similarities with the exo-β-1,3-glucanases from *A. oryzae* (ExgA and Exg1) and *Lentinula edodes* (Exg1) (Tamano et al. 2007; Sakamoto et al. 2005). A thermostable β-glucosidase/β-rutinosidase from the archaea *Pyrococcus furiosus* (Nam et al. 2012) involved in the production of quercetin from rutin belongs to GH1, similar to the β-diglycosidases from plants. Moreover, the gene encoding the GH55 family member 6-*O*-α-L-rhamnopyranosyl-β-D-glucosidase was identified from *Actinoplanes missouriensis* 431^{T} genome. Biochemical analyses of the corresponding recombinant protein purified from *Escherichia coli* showed specificity for 7-*O*-rutinosylated flavonoids (Neher et al. 2016). Exo-β-1,3-glucanases from fungi including the genera *Aspergillus* and *Penicillium* have also been deposited in the GH55 section of the database.

A. oryzae RIB40 and *Oerskovia* sp. Y1 produce isoprimeverose-producing oligoxyloglucan hydrolase (EC 3.2.1.120), a unique β-diglycosidase, that recognizes isoprimeverose (6-*O*-α-D-xylopyranosyl-β-D-glucopyranoside) units from the non-reducing ends of oligoxyloglucans (Kato et al. 1985; Yaoi et al. 2007; Matsuzawa et al. 2016). The enzyme-encoding genes have been identified (Yaoi and Miyazaki 2012; Matsuzawa et al. 2016). Based on the amino acid sequence, isoprimeverose-producing oligoxyloglucan hydrolase has been classified as a member of the GH3 family.

Properties of microbial β-diglycosidases

Data from the studies cited in Table 1 show that the pH and temperature optima of β-rutinosidase (α-rhamnosyl-β-glucosidase) and β-primeverosidase from fungi ranged from 2.2–5.0 to 45–70 °C, respectively. The β-rutinosidases from *P. rugulosum* and *A. niger,* and β-primeverosidase from *A. fumigatus* are extreme acidophiles (Narikawa et al. 2000; Šimčíková et al. 2015; Yamamoto et al. 2002). Meanwhile, the optimal pH of α-rhamnosyl-β-glucosidase from *A. missouriensis* belonging to the GH55 family was 7.0 (Neher et al. 2016). α-Rhamnosyl-β-glucosidase from *Acremonium* sp. and isoprimeverose-producing oligoxyloglucan hydrolase from *A. oryzae* are thermophilic enzymes (Mazzaferro et al. 2010, 2011b; Kato et al. 1985). Especially, β-glucosidase/β-rutinosidase from archaea *P. furiosus* is an extremely thermostable enzyme (Nam et al. 2012). However, the specific activity for rutin of β-glucosidase/β-rutinosidase from *P. furiosus* was approximately 900- and 15,700-fold lower, respectively, than those for isoquercitrin and *p*-nitrophenyl-β-D-glucoside.

Furthermore, β-rutinosidase hydrolyzes several rutinose-containing glycoconjugates including flavonoids, such as hesperidin, rutin, kaempferol-3-*O*-rutinoside, and hesperidin methylchalcone. However, the enzyme does not hydrolyze neohesperidose (2-*O*-α-L-rhamnopyranosyl-β-D-glucopyranose)-conjugated flavonoids, such as naringin. The β-rutinosidases from *P. rugulosum* and *Arthrobacter* sp. show specificity for 3-*O*-linked rutinosides such as rutin, a 3-*O*-rutinosylated flavonol (Narikawa et al. 2000; Song-Joon et al. 1990). The catalytic activity of β-rutinosidase from *A. niger* was almost ten times higher for rutin hydrolysis than that for hesperidin, a 7-*O*-rutinosylated flavanone (Šimčíková et al. 2015). Meanwhile, among the three substrates examined, the catalytic activity of β-rutinosidase from *A. oryzae* was highest for kaempferol-3-*O*-rutinoside, which is a 3-*O*-rutinosylated flavonol, moderate for rutin, and lowest for hesperidin (Ishikawa et al. 2018). In contrast to this, α-rhamnosyl-β-glucosidase from *Acremonium* sp. and *A. missouriensis* hydrolyzes 7-*O*-linked rutinosides only, such as hesperidin, but not 3-*O*-linked rutinosides such as rutin (Mazzaferro et al. 2010, 2011b Neher et al. 2016). However, no activity of β-rutinosidases from *A. missouriensis* and *A. oryzae* was determined toward diosmin, a 7-*O*-rutinosylated flavone. This suggests that the structure of flavonoids also determines enzyme specificity.

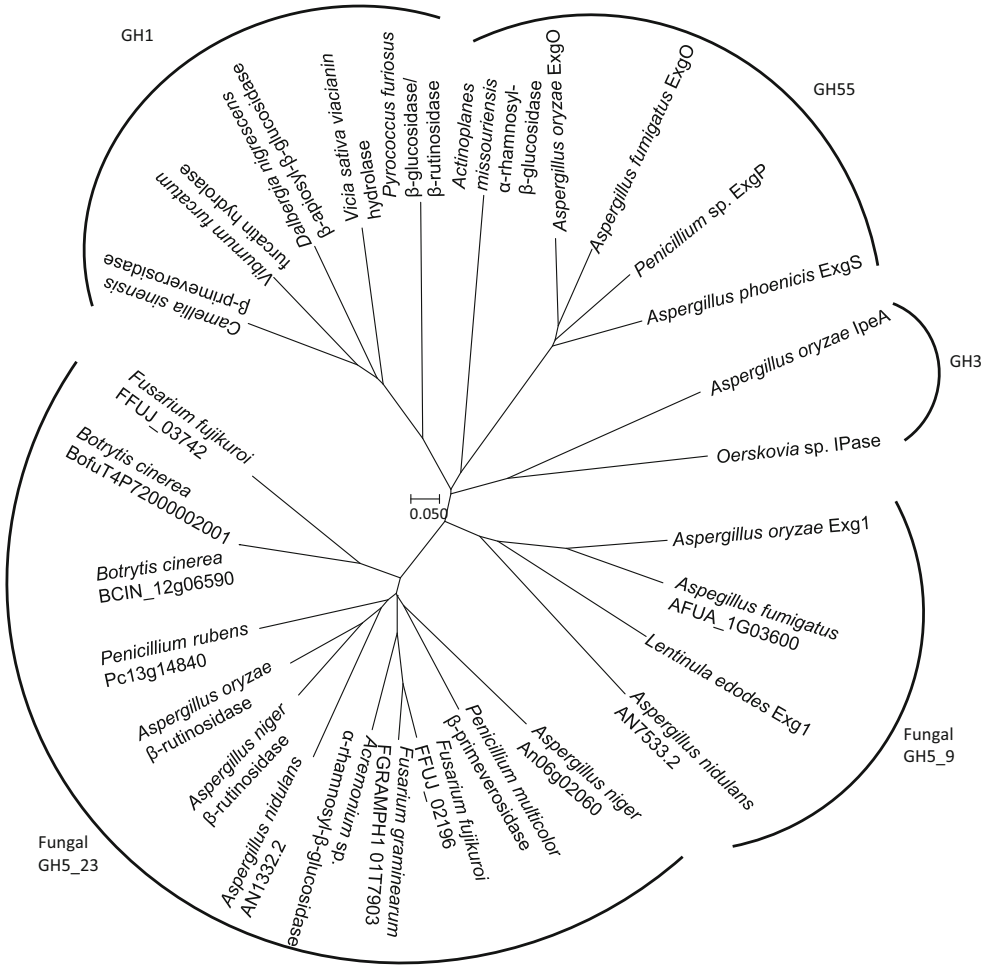

Fig. 3 Phylogenetic trees among β-diglycosidases and exo-β-1,3-glucanases classified as GH1, GH3, GH5_9, GH5_23, and GH55 in the CAZy database. An amino acid sequence alignment was performed using ClustalW (Thompson et al. 1994), and the phylogenetic tree was constructed using molecular evolutionary genetics analysis software version7.0 (Tamura et al. 2007). The accession numbers are as follows: *Acremonium* sp. α-rhamnosyl-β-glucosidase (AMD11613.1), *Actinoplanes missouriensis* α-rhamnosyl-β-glucosidase (BAL86042.1), *Aspergillus fumigatus* ExgO (CAF32160.1), *Aspergillus oryzae* ExgA (CAC07551.1), *Aspergillus oryzae* ExgO (BAB92972.1), *Aspergillus oryzae* IpeA (BAE62006.1), *Aspergillus oryzae* β-rutinosidase (BAE61018.1), *Aspergillus nidulans* AN1332.2 (EAA65515.1), *Aspergillus niger* β-rutinosidase (CAK39791.1), *Aspergillus niger* An06g02060 (CAK48049.1), *Aspergillus phoenicis* ExgS (BAB83607.1), *Botrytis cinerea* BofuT4P72000002001 (CCD33736.1), *Camellia sinensis* β-primeverosidase (BAC78656.1), *Dalbergia nigrescens* β-apiosyl-β-glucosidase (A3RF67.1), *Fusarium fujikuroi* FFUJ_02196 (CCT65263.1), *Fusarium fujikuroi* FFUJ_03742 (CCT64988.1), *Lentinula edodes* Exg1 (BAD97445.1), *Oerskovia* sp. IPase (BAM08953.1), *Penicillium multicolor* β-primeverosidase (BAG70961.1), *Penicillium rubens* Pc13g14840 (CAP925533.1), *Penicillium sp.* ExgP (BAH69264.1), *Pyrococcus furiosus* β-glucosidase/β-rutinosidase (AAC25555.1), and *Viburnum furcatum* furcatin hydrolase (BAD14925.1). The bar represents 0.05 amino acid substitutions per site

The hydrolytic activity of the β-primeverosidase from *A. fumigatus* on the *p*-nitrophenyl β-gentiobioside was greater than that from *P. multicolor* (Yamamoto et al. 2002; Tsuruhami et al. 2006). The substrate specificity of these fungal enzymes also differed appreciably from that of tea β-primeverosidase.

Potential industrial applications

In the citrus processing industry, the deglycosylation of flavonoids plays an important role in improving the product quality, such as in the reduction of bitterness, clarification of juices, and in enhancing the aroma in wine and tea (Günata et al. 1998; Hemingway et al. 1999; Wang et al. 2001; Ma et al. 2001; Genovés et al. 2005). This process is also used for controlling aroma compounds, such as monoterpene alcohols, in sweet potato shochu which is a traditional Japanese distilled spirit (Sato et al. 2018). A simple enzymatic-spectrophotometric method for hesperidin quantification in citrus-based foods was developed by means of *Acremonium* sp. α-rhamnosyl-β-glucosidase (Mazzaferro and Breccia 2012a).

Rutinose-containing compounds have been demonstrated to have interesting pharmaceutical and medicinal

Table 1 Properties of β-diglycosidases from microorganisms

Organism	Enzyme	EC number	Molecular mass (kDa)	pHopt	Topt (°C)	pHsta	Tsta (°C)	Main substrate	Accessions	Reference
Fungi										
Acremonium sp. DSM24697	α-rhamnosyl-β-glucosidase	3.2.1.168	46	5.0	70			hesperetin 7-*O*-β-rutinoside	AMD11613.1	Mazzaferro et al. 2010; 2011
Aspergillus fumigatus AP20	β-primeverosidase-like endo-manner β-glycosidase	3.2.1.149	47	2.5–3.0	55	7.0–8.0		*p-nitrophenyl* β-primeveroside		Yamamoto et al. 2002
Aspergillus oryzae RIB40	β-rutinosidase	3.2.1.168	65–75	4.0	45		45	kaempferol-3-*O*-β-rutinoside	BAE61018.1	Ishikawa et al. 2018
Aspergillus oryzae RIB40	isoprimeverose-producing oligoxyloglucan hydrolase	3.2.1.120	115	5.0	60	5.0–7.0	50	oligoxyloglucan	BAE62006.1	Kato et al. 1985; Matsuzawa et al. 2016
Aspergillus niger K2	β-rutinosidase	3.2.1.168	~ 75	3.0	50			quercetin-3-*O*-β-rutinoside	CAK39791.1	Šimčíková et al. 2015
Penicillium multicolor IAM7153	β-primeverosidase-like enzyme	3.2.1.149	50	4.5–5.5	55			*p-nitrophenyl* β-primeveroside	BAG70961.1	Tsuruhami et al. 2006
Penicillium rugulosum NBRC7242	β-rutinosidase	3.2.1.168	65	2.2	50	2.0–11.0	40	quercetin-3-*O*-β-rutinoside		Narikawa et al. 2000
Bacteria										
Actinoplanes missouriensis 431^T	α-rhamnosyl-β-glucosidase	3.2.1.168	62	7.0	55		50	hesperetin 7-*O*-β-rutinoside	BAL86042.1	Neher et al. 2016
Arthrobactor sp.	β-rutinosidase	3.2.1.168	42					quercetin-3-*O*-β-rutinoside		Song-Joon et al. 1990
Oerskovia sp. Y1	isoprimeverose-producing oligoxyloglucan hydrolase	3.2.1.120	105	4.5	55	3.5–7.5	45	oligoxyloglucan	BAM08953.1	Yaoi et al. 2007; 2012
Archaea										
Pyrococcus furiosus DSMZ3638	β-glucosidase/β-rutinosidase	3.2.1.21		5.0	95			*p-nitrophenyl* β-D-glucoside	AAC25555	Nam et al. 2012

applications (Robinson et al. 2004; Knaup et al. 2007). Rutinose-containing flavonoids have been shown to be absorbed in he intestines only after rhamnose hydrolysis and catalyzed by human gut microflora (Nielsen et al. 2006). The transglycosylation potential of the fungal β-rutinosidase has been explored. The biocatalyst has been shown to have broad acceptor specificity toward aliphatic, aromatic, and arylalkyl alcohols using α-rhamnosyl-β-glucosidases from *Acremonium* sp. (Minig et al. 2011; Mazzaferro et al. 2012b) and *A. niger* (Šimčíková et al. 2015; Bassanini et al. 2017). Bassanini et al. (2017) have developed two-step two-enzymatic synthesis of coniferin using the α-rhamnosyl-β-glucosidase and *A. terreus* α-L-rhamnosidase. It has also been reported that rutinosylation of various phenolic acids can increase their antiviral activity against feline calicivirrus, more than the respective aglycone (Katayama et al. 2013).

Conclusions

In conclusion, we present here the biochemical characteristics and potential industrial applications of β-diglycosidases from archaea, bacteria, and fungi that breakdown plant diglycoconjugated flavonoids. Eukaryotic β-diglycosidases are effective catalysts when food technology for aroma modulation and pharmaceutical and medicinal applications are envisioned. Because of their retaining mechanism, transglycosylation activity is to be expected. The synthetic potential of β-diglycosidases from fungi and plants has also been demonstrated, which can glycosylate alkylic, phenolic, and arylalkyl alcohols and phenolic acids in vitro (Mazzaferro et al. 2012b; Šimčíková et al. 2015; Katayama et al. 2013; Bassanini et al. 2017).

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Compliance with ethical standards

Conflict of interest The authors declare that they have no competing interests.

Ethical approval This article does not contain any studies with human participants or animals performed by any of the authors.

References

Ahn YO, Mizutani M, Saino H, Sakata K (2004) Furcatin hydrolase from *Viburnum furcatum* Blume is a novel disaccharide-specific acuminosidase in glycosyl hydrolase family 1. J Biol Chem 279: 23405–23414. https://doi.org/10.1074/jbc.M311379200

Ahn YO, Saino H, Mizutani M, Shimizu B, Sakata K (2007) Vicianin hydrolase is a novel cyanogenic β-glycosidase specific to β-vicianoside (6-*O*-α-L-arabinopyrannosyl-β-D-glucopyranoside) in seeds of *Vicia angustifolia*. Plant Cell Physiol 48:938–947. https://doi.org/10.1093/pcp/pcm065

Aspeborg H, Coutinho PM, Wang Y, Brumer H III, Henrisaat B (2012) Evolution, substrate specificity and subfamily classification of glycoside hydrolase family 5 (GH5). BMC Evol Biol 12:186. https://doi.org/10.1186/1471-2148-12-186

Bassanini I, Krejzová J, Panzeri W, Monti D, Křen V, Riva S (2017) A sustainable one-pot, two-enzyme synthesis of naturally occurring arylalkyl glucosides. ChemSusChem 10:2040–2045. https://doi.org/10.1002/cssc.201700136

Baumgertel A, Grimm R, Eisenbeiß W, Kreis W (2003) Purification and characterization of a flavonoid 3-*O*-β-heterodisccharidase from the dried herb of *Fagopyrum esculentum* Moench. Phytochemistry 64: 411–418. https://doi.org/10.1016/S0031-9422(03)00418-7

Chuankhayan P, Hua Y, Svasti J, Sakdarat S, Sullivan PA, Ketudat Cairns JA (2005) Purification of an isoflavonoid 7-*O*-β-apiosyl-glucoside β-glycosidase and its substrates from *Dalbergia nigrescens* Kurz. Phytochemistry 66:1880–1889. https://doi.org/10.1016/j.phytochem.2005.06.024

Genovés S, Gil JV, Vallés S, Casas JA, Manzanares P (2005) Assessment of the aromatic potential of palomino fino grape must using glycosidases. Am J Enol Vitic 56:188–191

Günata Z, Blondeel C, Vallier MJ, Lepoutre JP, Sapis JC, Watanabe N (1998) An endoglycosidase from grape berry skin of cv *M Alexandria* hydrolyzing potentially aromatic disaccharide glycosides. J Agric Food Chem 46:2748–2753. https://doi.org/10.1021/jf980084j

Hemingway KM, Alston MJ, Chappell CG, Taylor AJ (1999) Carbohydrate-flavour conjugates in wine. Carbohydr Polym 38: 283–286. https://doi.org/10.1016/S0144-8617(98)00103-9

Imaseki H, Yamamoto T (1961) A furcatin hydrolyzing glycosidase of *Viburnum furcatum* blum. Arch Biochem Biophys 92:467–474

Ishikawa M, Kawasaki M, Shiono Y, Koseki T (2018) A novel *Aspergillus oryzae* diglycosidase that hydrolyzes 6-*O*-α-L-rhamnosyl-β-D-glucoside from flavonoids. Appl Microbiol Biotechnol 102:3193–3201. https://doi.org/10.1007/s00253-018-8840-9

Katayama S, Ohno F, Yamaguchi Y, Kato M, Makabe H, Nakamura S (2013) Enzymatic synthesis of novel phenol acid rutinosides using rutinose and their antiviral activity *in vitro*. J Agric Food Chem 61: 9617–9622. https://doi.org/10.1021/jf4021703

Kato Y, Matsushita J, Kobodera T, Matsuda K (1985) A novel enzyme producing isoprimeverose from oligoxyloglucans of *Aspergillus oryzae*. J Biochem 97:801–810. https://doi.org/10.1093/oxfordjournals.jbchem.a135120

Knaup B, Kahle K, Erk T, Valotis A, Scheppach W, Schreier P, Richling E (2007) Human intestinal hydrolysis of phenol glycosides-a study with quercetin and *p*-nitrophenol glycosides using ileostomy fluid. Mol Nutr Food Res 51:1423–1429. https://doi.org/10.1002/mnfr.200700036

Lizotte PA, Poulton JE (1988) Catabolism of cyanogenic glycosides by purified vicianin hydrolase from squirrel's foot fern (*Davallia Trichomanoides* Blume). Plant Physiol 86:0322–0324. https://doi.org/10.1104/pp.86.2.322

Lombard V, Golaconda RH, Drula E, Coutinho PM, Henrissat B (2014) The carbohydrate-active enzymes database (CAZy) in 2013. Nucleic Acids Res 42:D490–D495. https://doi.org/10.1093/nar/gkt1178

Ma SJ, MIzutani M, Hiratake J, Hayashi K, Yagi K, Watanabe N, Sakata K (2001) Substrate specificity of β-primeverosidase, a key enzyme in aroma formation during oolong tea and black tea manufacturing. Biosci Biotechnol Biochem 65:2719–2729

Matsuzawa T, Mitsuishi Y, Kameyama A, Yaoi K (2016) Identification of the gene encoding isoprimeverose-producing oligoxyloglucan hydrolase in *Aspergillus oryzae*. J Biol Chem 291:5080–5087. https://doi.org/10.1074/jbc.M115.701474

Mazzaferro LS, Breccia JD (2011a) Fuctional and biotechnological insights into diglycosidases. Biocat Biotrans 29:103–112. https://doi.org/10.3109/10242422.2011.594882

Mazzaferro LS, Breccia JD (2012a) Quantification of hesperidin in citrus-based foods using a fungal diglycosidase. Food Chem 134:2338–2344. https://doi.org/10.1016/j.foodchem.2012.03.107

Mazzaferro LS, Piñuel L, Minig M, Breccia JD (2010) Extracellular monoenzyme deglycosylation system of 7-*O*-linked flavonoid β-rutinosides and its disaccharide transglycosylation activity from *Stibella fimetaria*. Arch Microbiol 192:383–393 https://doi.org/10.1007/s00203-010-0567-7

Mazzaferro LS, Piñuel L, Minig M, Breccia JD (2011b) Erratum to: Extracellular monoenzyme deglycosylation system of 7-O-linked flavonoid β-rutinosides and its disaccharide transglycosylation activity from Stibella fimetaria. Arch Microbiol 193: 461. https://doi.org/10.1007/s00203-010-0709-6

Mazzaferro LS, Piñuel L, Erra-Balsells R, Giudicessi SL, Breccia JD (2012b) Transglycosylation specificity of *Acremonium* sp. α-L-rhamnopyranosyl-β-D-glucopyranosidase and its application to the synthesis of the new fluorogenic substrate 4-methylumbelliferyl-rutinoside. Carbohydr Res 347:69–75. https://doi.org/10.1016/j.carres.2011.11.008

Minig M, Mazzaferro LS, Erra-Baisells R, Petroselli G, Breccia JD (2011) α-L-Rhamnopyranosyl-β-D-glucopyranosidase-catalyzed reactions for analysis and biotransformations of plant-based foods. J Agric Food Chem 59:11238–11242. https://doi.org/10.1021/jf202412e

Mizutani M, Nakanishi H, Ema J, Ma SJ, Noguchi E, Inohara-Ochiai M, Fukuchi-Mizutani M, Nakao M, Sakata K (2002) Cloning of β-primeverosidase from tea leaves, a key enzyme in tea aroma formation. Plant Physiol 130:2164–2176

Nakanishi F, Nagasawa Y, Kabaya Y, Sekimoto H, Shimomura K (2005) Characterization of lucidin formation in *Rubia tinctorum* L. Plant Physiol Biochem 43:921–928. https://doi.org/10.1016/j.plaphy.2005.08.005

Nam HK, Hong SH, Shin KC, Oh DK (2012) Quercetin production from rutin by a thermostable β-rutinosidase from *Pyrococcus furiosus*. Biotechnol Lett 34:483–489. https://doi.org/10.1007/s10529-011-0786-2

Narikawa T, Shinoyama H, Fujii T (2000) A β-primeverosidase from *Penicillium rugulosum* IFO7242 that is a peculiar flavonoid glycosidase. Biosci Biotechnol Biochem 64:1317–1319

Neher B, Mazzaferro LS, Kotik M, Oyhenart J, Halada P, Křen V, Breccia JD (2016) Bacteria as source of diglycosidase activity: *Actinoplanes missouriensis* produces 6-*O*-α-L-rhamnopyranosyl-β-D-glucopyranosidase active on flavonoids. Appl Microbiol Biotechnol 100:3061–3070. https://doi.org/10.1007/s00235-015-7088-x

Nielsen ILF, Chee WSS, Poulsen L, Offord-Cavin E, Rasmussen SE, Frederiksen H, Enslen M, Barron D, Horcajada M, Williamson G (2006) Bioavailability is improved by enzymatic modification of the citrus flavonoid hesperidin in humans: a randomized, double-blind, crossover trial. J Nutr 136:404–408

Ogawa K, Ijima Y, Guo W, Watanabe N, Usui T, Dong S, Tong Q, Sakata K (1997) Purification of a β-primeverosidase concerned with alcoholic aroma formation in tea leaves (Cv. *Shuixian*) to be processed to oolong tea. J Agric Food Chem 45:877–882. https://doi.org/10.1021/jf9605431

Riou C, Salmon JM, Vallier MJ, Günata Z, Barre P (1998) Purification, characterization, and substrate specificity of a novel highly glucose-tolerant β-glucosidase from *Aspergillus oryzae*. Appl Environ Microbiol 64:3607–3614

Robinson MA, Charlton ST, Garnier P, Wang XT, Davis SS, Perkins AC, Frier M, Duncan R, Savage TJ, Wyatt DA, Watson SA, Davis BG (2004) LEAPT: Lectin-directed enzyme-activated prodrug therapy. Proc Natl Acad Sci U S A 101:14527–14532. https://doi.org/10.1073/pnas.0303574101

Saino H, Shimizu T, Hiratake J, Nakatsu T, Kato H, Sakata K, Mizutani M (2014) Crystal structure of β-primeverosidase in complex with disaccharide amidine inhibitors. J Biol Chem 289:16826–16834. https://doi.org/10.1074/jbcM114.553271

Sakamoto Y, Irie T, Sato T (2005) Isolation and characterization of a fruting body-specific exo-beta-1,3-glucanase-encoding gene, *exg1*, from *Lentinula edodes*. Curr Genet 47:244–252

Sato Y, Han J, Fukuda H, Mikami S (2018) Enhancing monoterpene alcohols in sweet potato shochu using the diglycoside-specific β-primeverosidase. J Biosci Bioeng 125:218–223. https://doi.org/10.1016/j.jbiosc.2017.08.012

Šimčíková D, Kotik M, Weignerová L, Halada P, Pelantová H, Adamcová K, Křen V (2015) α-L-Rhamnosyl-β-D-glucosidase (rutinosidase) from *Aspergillus niger*: characterization and synthetic potential of a novel diglycosidase. Adv Synth Catal 357:107–117. https://doi.org/10.1002/adsc.201400566

Song-Joon L, Omori T, Kodama T (1990) Purification and some properties of rutinosidase from *Arthrobacter* sp. J Appl Microbiol Biotechnol 18:360–367

Suzuki T, Honda Y, Funatsuki W, Nakatsuka K (2002) Purification and characterization of flavonol 3-glucosidase, and its activity during ripening in tartary buckwheat seeds. Plant Sci 163:417–423

Tamano K, Satoh Y, Ishii T, Terabayashi Y, Ohtake S, Sano M, Takahashi T, Koyama Y, Mizutani O, Abe K, Machida M (2007) The β-1,3-exoglucanase gene *exgA* (*exg1*) of *Aspergillus oryzae* is required to catabolize extracellular glucan, and is induced in growth on a solid surface. Biosci Biotechnol Biochem 71:926–934. https://doi.org/10.1271/bbb.60591

Tamura K, Dudley J, Nei M, Kumar S (2007) MEGA4: molecular evolutionary genetics analysis (MEGA) software version 4.0. Mol Biol Evol 24:1596–1599. https://doi.org/10.1093/molbev/msm092

Thompson JD, Higgins DG, Gibson TJ (1994) CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. Nucleic Acids Res 22:4673–4680

Tsuruhami K, Mori S, Amarume S, Sarawatari S, Murata T, Hirakake J, Sakata K, Usui T (2006) Isolation and characterization of a β-primeverosidase-like enzyme from *Penicillium multicolor*. Biosci Biotechnol Biochem 70:691–698

Wirth J, Guo W, Baumes R, Günata Z (2001) Volatile compounds released by enzymatic hydrolysis of glycoconjugates of leaves and grape berries from *Vitis vinifera* Muscat of Alexandria and Shiraz cultivars. J Agric Food Chem 49:2917–2923. https://doi.org/10.1021/jf0013981

Yamamoto S, Okuda M, Usui T, Sakata K (2002) Isolation and characterization of a β-primeverosidase-like endo-manner β-glycosidase from *Aspergillus fumigatus* AP-20. Biosci Biotechnol Biochem 66:801–807

Yaoi K, Miyazaki K (2012) Cloning and expression of isoprimeverose-producing oligoxyloglucan hydrolase from Actinomycetes species, *Oerskovia* sp. Y1. J Appl Glycosci 59:83–88. https://doi.org/10.5458/jag.jag.JAG-2011_023

Yaoi K, Hiyoshi A, Mitsuishi Y (2007) Screening, purification and characterization of a prokaryotic isoprimeverose-producing oligoxyloglucan hydrolase from *Oerskovia* sp. Y1. J Appl Glycosci 54:91–94

Yasuda T, Nakagawa H (1994) Purification and characterization of the rutin-degrading enzymes in tartary buckwheat seeds. Phytochemistry 37:133–136. https://doi.org/10.1016/0031-9422(94)85012-7

Wang D, Kurasawa E, Yamaguchi Y, Kubota K, Kobayashi A (2001) Analysis of glycosidically bound aroma precursors in tea leaves. 2. Changes in glycosidic contents and glycosidase activities in tea leaves during the black tea manufacturing process. J Agric Food Chem 49:1900–1903. https://doi.org/10.1021/jf001077+