Biochemical Characterization of the \( \alpha-L \)-Rhamnosidase \( \text{DtRha} \) from \( \text{Dictyoglomus thermophilum} \): Application to the Selective Derhamnosylation of Natural Flavonoids

Laure Guillotin,† Hyuna Kim,† Yasmina Traore,† Philippe Moreau,† Pierre Lafite,† Véronique Coquoin,‡ Sylvie Nuccio,‡ René de Vaumas,‡ and Richard Daniellou‡

†Université d’Orléans, CNRS, ICOA, UMR 7311, Rue de Chartres, BP 6759, 45067 Orléans Cedex 2, France
‡Extrasynthese, CS 30062, ZI Lyon Nord, Impasse Jacquard, 69727 Genay Cedex, France

Supporting Information

ABSTRACT: \( \alpha-L \)-Rhamnosidases are catalysts of industrial tremendous interest, but their uses are still somewhat limited by their poor thermal stabilities and selectivities. The thermophilic \( \text{DtRha} \) from \( \text{Dictyoglomus thermophilum} \) was cloned, and the recombinant protein was easily purified to homogeneity to afford 4.5 mg/L culture of biocatalyst. Michaelis–Menten parameters demonstrated it to be fully specific for \( \alpha-L \)-rhamnose. Most significantly, \( \text{DtRha} \) demonstrated to have a stronger preference for \( \alpha(1 \rightarrow 2) \) linkage rather than \( \alpha(1 \rightarrow 6) \) linkage when removing rhamnosyl moiety from natural flavonoids. This selectivity was fully explained by the difference of binding of the corresponding substrates in the active site of the protein.

INTRODUCTION

\( \alpha-L \)-Rhamnosidases (E.C. 3.2.1.40) are ubiquitous enzymes in nature and are responsible for the removal of the \( \alpha-L \)-rhamnose (\( \alpha-L \)-Rha) entity from various glycoconjugates. Indeed, \( \alpha-L \)-Rha is widely distributed in plants and bacteria as polysaccharide residue (pectins, O-antigen of pathogenic bacteria) in glycosylated derivatives (flavonoids and terpenes) or biosurfactants (rhamnolipids). The \( \alpha-L \)-Rha motif is, in addition, widely found in surface glycoproteins of bacteria and therefore participates in the virulence of the pathogen. Conversely, it is rarely described to form a direct bond with proteins. Since many years, rhamnosidases have also been used as biocatalysts in numerous industrial processes. In the food industry, they have been used for production of fruit juice or for the improvement of wine aroma by deglycosylation of terpenes. Thus, \( \alpha-L \)-Rha products can be directly valorized in the chemical industry as chiral precursors in synthesis or for the glycodiversification of products. \( \alpha-L \)-Rhamnosidases are also widely exploited in the pharmaceutical industry for flavonoid derhamnosylation (see Figure 1). Indeed, these compounds are recognized for their beneficial effects on health in humans, and it has been shown that monoglycosylated compounds have better bioavailability than their disaccharide analogues. For example, (1) prunin, the derhamnosylated product of 1 or 2, was recently described to have a strong effect on the inhibition of enzyme systems related to diabetes; (2) diosmetin 7-\( \text{O} \)-glucoside (from 3 and 4) was reported to have cardiovascular and hepatoprotective effects, as well as antioxidant, antiarrhythmic, and anticomplementary activities; (3) quercetin 7-\( \text{O} \)-glucopyranoside (from 5 and 6) has demonstrated its potency in inhibiting the viral RNA polymerase from influenza A and B viruses; and (4) hesperetin 7-\( \text{O} \)-glucoside (from 7 and 8) has been shown to inhibit the growth of \( \text{Helicobacter pylori} \), a causative agent for gastric diseases. Unfortunately, the majority of them have a low occurrence in nature or are difficult to isolate from plant sources, and their chemical syntheses remain tedious, with all consequences leading to expensive natural compounds.

All reported \( \alpha-L \)-rhamnosidases act following a mechanism of inversion of configuration, and they are listed in three \( \text{CAZy} \) glycoside hydrolase (GH) families (GH28, GH78 and GH106, http://www.cazy.org/, accessed November 2018). Unlike GH28 and GH106 families containing \( \alpha-L \)-rhamnosidases that are involved in pectin metabolism, GH78 family is composed of \( \alpha-L \)-rhamnosidases exhibiting valuable activities in natural product synthesis. Indeed, several characterized GH78 \( \alpha-L \)-rhamnosidases have been demonstrated to catalyze the hydrolysis of rhamnosides bound to flavonoid glucosides with a wide diversity of linkage regioselectivity on the glucose: \( \alpha(1 \rightarrow 2) \), \( \alpha(1 \rightarrow 6) \), and more rarely, \( \alpha(1 \rightarrow 3) \). However, if some differences in bond selectivity for hydrolysis were reported in these studies, then no rational understanding of such substrate specificity could be analyzed. This was likely due to the small number of four GH78 structures that are currently available in the Protein Data Bank. The corresponding proteins have been isolated from \( \text{Bacillus sp. GL1} \),

Received: November 14, 2018
Accepted: January 10, 2019
Published: January 24, 2019

DOI: 10.1021/acsomega.8b03186
ACS Omega 2019, 4, 1916–1922
Bacteroides thetaiotaomicron, Streptomyces avermitilis, and Klebsiella oxytoca.

With the objective of using α-L-rhamnosidases as industrial biocatalysts, thermostability is highly advantageous and looked for because it is correlated with a higher resistance of the enzyme toward increased incubation time and temperature, as well as a higher tolerance to nonaqueous solvents. However, few of the characterized ones are from thermophilic microorganisms: Thermomicrobium sp. (RhmA and RhmB), Clostridium stercorarium (RmaA), and Aspergillus terreus. In this context, we have identified a thermophilic bacteria, Dictyoglomus thermophilum, which possesses a single gene on its genome, dicth_0289, coding for potential exo-α-L-rhamnosidase. The corresponding enzyme Dtrha identified by RSYC64 (UniProt identification) has a sequence identity of 35% with the crystallized one from S. avermitilis.

In the course of our ongoing research devoted to the discovery of original and robust biocatalysts for the chemoenzymatic synthesis of natural compounds, we thus got interested in the cloning, overexpression, and purification of this thermophilic Dtrha. Owing to enzymology, 3D structure determination using X-ray diffraction, and modeling experiments, we were able to show the substrate versatility of this α-L-rhamnosidase and to fully demonstrate the requirement for its efficient recognition of glycosylated flavonoid. These experiments led to the development of a cheap and easy access to very expensive flavonoids such as prunin, diosmetin, or hesperetin 7-O-glucopyranoside.

**RESULTS AND DISCUSSION**

**Gene Cloning and Protein Overexpression.** The 2763 bp dtrha gene was amplified from D. thermophilum genomic DNA from the constructed primers to allow insertion between the NheI/SalI restriction sites of the vector pET28a(+). The corresponding recombinant plasmid was transformed in E. coli Rosetta-(DE3), and the protein was expressed after isopropyl β-D-1-thiogalactopyranoside (IPTG) induction of the bacterial culture in the nutrient medium LB/Kan/Cam at 37 °C for 20 h. After lysis, thermal clarification, and purification on a Ni-NTA column, the purified enzyme was analyzed on 1D 10% SDS-PAGE gel (Figure S1). The protein consists of a sequence of 921 acids that represents a theoretical molecular weight of 106 kDa, with the presence of six His motifs (from His-tag), and gel analysis confirmed the purity of the protein because a single band was observed at the level of the 100 kDa reference protein band. The production yield of the protein Dtrha is 4.5 mg/L bacterial culture.

**Biochemical Characterization of Dtrha.** Purified Dtrha enzyme was further used to assess its catalytic activity. The kinetic parameters were evaluated by monitoring the hydrolytic activity of the enzyme toward 20 different p-nitrophenyl-sugars (pNP-sugars, Figure S2). Potentially cleaved pNP could be easily quantified at 405 nm. Among them, only pNP-α-L-Rha demonstrated to be a substrate, thus confirming the unique
carbohydrate specificity of DtRha. The values of optimum pH and temperature were determined to be 5.0 and 95 °C, respectively (see Figure S3). Indeed, the enzymatic activity is favored for a large range of acidic pH between 4.0 and 7.0, as similarly demonstrated for previously reported α-L-rhamnosidases. The Michaelis–Menten parameters were thus determined at pH 5.0 and at 37 °C. Under these conditions, the $K_M$ value was evaluated at 54.00 ± 0.03 μM and the $k_{cat}$ one at 0.17 ± 0.01 s⁻¹ (Table 1 and Figure S4). Compared to other recombinant α-L-rhamnosidases from the GH78 family, DtRha is one of the best enzymes for the recognition of pNP-α-L-Rha but catalyzes its conversion very slowly at 37 °C (indeed, its relative activity is 17% compared to that at 90 °C). However, a poor activity on unnatural aryl rhamnosides is a not a predictor at all for its enzymatic activity on rhamnosyl flavonoids. Results of the catalyzed hydrolysis for two different concentrations of DtRha are summarized in Table 2. At low concentration, it is noteworthy that $α(1 \rightarrow 2)$-rhamnosylated flavonoids (neo-hesperidiosides) demonstrated to be more efficient substrates than their $α(1 \rightarrow 6)$ counterparts (rutinosides), whatever the aglycone moiety. Moreover, at slightly increased concentration of DtRha, naringin 1, neoe里斯itrin 5 were the most efficiently transformed, with a conversion rate higher than 95% in 3 h under our reaction conditions. On the contrary, even in the case of 10 times higher DtRha concentration, conversion rates were limited to 40% for $α(1 \rightarrow 6)$ derivatives. Therefore, DtRha is specific to $α(1 \rightarrow 2)$ glycosidic linkage rather than $α(1 \rightarrow 6)$ linkage, with ratio going up to 4 in terms of conversion rate. In addition, the aglycone part still seems to have an influence on the recognition and velocity of the enzyme, with the flavonone of naringin 1 being preferred. Flavonoids containing methoxy groups on their aromatic rings were the less favored substrates (see compounds 3, 4, 7, and 8) compared tophenol derivatives (1, 2, 5, and 6). Therefore, the enzymatic preferences in terms of aglycons are in this decreasing order: flavanone, isoflavonoid (phenol group), flavone, and isoflavonoid.

Table 2. Conversion Rate of the Derhamnosylation of Natural Flavonoids Catalyzed by DtRha†

| α(1 → 2)-rhamnosylated flavonoid | conversion rate (%) | α(1 → 6)-rhamnosylated flavonoid | conversion rate (%) |
|----------------------------------|------------------|---------------------------------|------------------|
| compound | 0.04 mg/mL | 0.1 mg/mL | compound | 0.04 mg/mL | 0.4 mg/mL |
| 1       | 44.1       | 97.6       | 2       | 8.5        | 39.4       |
| 3       | 18.1       | 67.9       | 4       | 0.0        | 24.9       |
| 5       | 6.2        | 96.1       | 6       | 2.1        | 22.1       |
| 7       | 19.9       | 58.6       | 8       | 6.0        | 14.5       |

†Reactions were performed according to conditions described in Experimental Procedures and analyzed by reverse-phase HPLC to quantify the conversion rate (percentage of substrate hydrolyzed by DtRha) (Figures S6–S13), and products were characterized by HRMS (Figures S14–S17).
Resolution of the 3D Structure of DtRha. Crystals of DtRha suitable for diffraction were obtained with 30% PEG 1500 using sitting drop vapor diffusion. The structure was refined to 2.7 Å using α-L-rhamnosidase SaRha from S. avermitilis (PDB 3W5M) as the replacement model. Two protein monomers are visible in the asymmetric crystal unit. However, according to PISA server analysis, DtRha is a single monomer, which is supported by size-exclusion chromatography analysis (data not shown). The overall structure of DtRha is similar to those observed for some other reported GH78 α-L-rhamnosidases: SaRha from S. avermitilis (PDB 3W5M and 3W5N) and RhaB from Bacillus sp. GL1 (PDB 2OKX) and B. thetaiotaomicron (3CIH). DtRha is composed of five distinct domains, namely, domain N (1–125), domain
E (126–317), domain F (348–437), catalytic domain A (438–813), and domain C (814–921). The loop 658–694 could not be refined because of poor electron density. When aligning the putative DrRha active site to the SaRha active site, most residues surrounding the active site are conserved, especially the catalytic residues, namely, the catalytic acid E479 (E636 in SaRha) and the catalytic base E782 (E895 in SaRha) (Figure 2).

Docking of Rhamnosylated Flavonoids in DrRha Active Site. Using the DrRha crystal structure as a starting model, the missing 658–694 loop was rebuilt by several cycles of molecular dynamics, followed by energy minimization. Once a full DrRha model was available, three ligands were chosen to identify the structural interactions that lead to differences in enzymatic activity. Naringin 1 was chosen as a representative efficient substrate, bearing a neohesperidose glycan unit containing an α(1 → 2)-linked rhamnose on a naringenin aglycon moiety. Narirutin 2, bearing the same aglycon—but linked to rutinose disaccharide—exhibiting an α(1 → 6)-linked rhamnose, was also chosen as a weak substrate. Eriocitrin 6, a quasi-ineffective substrate in low enzymatic concentration, also bearing a rutinoside moiety on eriodictyol aglycon, was selected in docking analyses. All three substrates were docked using soft-restrained molecular dynamics, as previously reported for other enzymes,25 and minimized final snapshots are depicted in Figure 3. Narirutin and eriocitrin exhibited similar interactions in the active site, involving some H-bonding between rhamnoside hydroxyls O2 and O3 with carboxylic residues (namely, D473, E479, and E782). On the other hand, naringin is tightly bound to the DrRha active site. Rhamnoside O3 is H-bonded to NE, from W538, and O2 is H-bonded to D473, R477, and E782. The latter residue also bridges O3 from the glucoside moiety, strongly stabilizing the disaccharide in the active site. Two other stabilizing H-bonds between R783 and O3 and O4 from glucose are visible, which strongly increase the stabilization of the complex. Finally, the aglycon conformation enables the H-bond between the hydroxyl and the YS87 residue and π-stacking between F144 and the aromatic ring of the naringin. These differences in H-bond networks between naringin and narirutin (as well as eriocitrin) can be explained by the nature of the glycosidic bond between rhamnose and glucose. In other words, neohesperidoside conformation helps to interact with the DrRha active site, whereas rutinoside does not provide a conformation that leads to H-bonds. A similar analysis based on molecular dynamics and computational studies on Ram2 α-l-rhamnosidase from Pedicioccus acidilactici was used to demonstrate the peculiar specificity of this enzyme for rhamnose-bound glucose compared to aryl aglycons such as pNP-α-l-Rha, correlated to a difference in substrate binding and orientation in the active site.25

### CONCLUSIONS

The search for efficient, thermostable, and robust biocatalysts is still an ongoing quest in the biotechnological field for numerous industrial applications. An original α-l-rhamnosidase was cloned from D. thermophilum and purified to homogeneity with good yields. It proved to be very efficient up to 90 °C and in complex solvents’ mixture. Despite its moderate efficiency toward the hydrolysis of pNP-α-l-Rha, we were able to demonstrate its advantage for the enzymatic synthesis of expensive and natural compounds with biological properties. Of tremendous interest, for the first time, we report here, to our knowledge, the structural requirements that explain why DrRha possesses an α(1 → 2) toward α(1 → 6) preference during the hydrolysis of α-l-Rha from flavonoids. In addition, this particular enzyme proves to be very versatile toward the aglycone moiety. Besides these fundamental observations, such enzymatic properties of DrRha pave the way for the industrial development of high-scale preparation of prunin, diosmetin, or hesperetin 7-O-glucopyranoside.

### EXPERIMENTAL PROCEDURES

**Materials.** All p-nitrophenyl monosaccharides (pNP-sugars) were purchased from Carbosynth (Compton, U.K.). All chemicals used were of analytical reagent grade. Phusion High-Fidelity DNA polymerase was purchased from Thermo Fisher and pET-28a(+) from Novagen.

**DrRha Cloning, Expression, and Purification.** The open reading frame encoding DrRha (DICTH_0289) was amplified from D. thermophilum DSM 3960 genomic DNA (DSMZ Institute, Germany) by PCR using Phusion High-Fidelity DNA polymerase (Thermo Fisher) using the following primers: 5′-ttgctagctagtcaatatttcacctttc-3′ and 5′-ttgctagcaatatttctacctcc′-3′. These primers respectively enabled the insertion of Nhel and SalI restriction sites up-and-downstream of the gene. After enzymatic digestion, PCR amplicon was directly ligated into pET-28a(+) vector using T4 DNA Ligase (Rapid DNA ligation kit, Fermentas), and the final construct pDrRha-28 was transformed in E. coli DH5α for amplification. pDrRha-28 plasmid was then transformed in Rosetta(DE3) expression strain. After selection on antibiotics, a single colony of transformed cells was used to inoculate LB broth medium containing the appropriate antibiotics. Cells were grown at 37 °C with 250 rpm shaking until OD600 reached 0.6, and IPTG (200 μM final concentration) was added before overnight incubation at 37 °C. Cells were harvested, resuspended in lysis buffer (50 mM Tris (pH 8.0), 200 mM NaCl, and 0.1% Triton X-100), incubated with lysozyme (0.1 mg/mL) at 4 °C for 20 min, and lysed by several freeze–thaw cycles, followed by ultrasonication. After the centrifugation step (32,000g for 20 min), clarification of supernatant was carried out by heat treatment (15 min at 70 °C) to remove nonthermostable proteins. Final centrifugation (32,000g for 20 min) was done, and supernatant was used for chemoenzymatic synthesis.

**Enzyme Activity Assays.** DrRha activity was assayed at 37 °C in 200 μL reaction mixtures containing substrates (0.01–10 mM), 0.425 μg enzyme, and Tris buffer (200 mM, pH 6.0). Residual spontaneous hydrolysis of the substrate was determined on a sample containing H2O instead of enzyme. For p-nitropheno1 (pNP)-containing substrates, after 30 min reaction, 100 μL sodium carbonate (1 M) was added, and produced pNP was quantified by absorbance measurement at 405 nm. All kinetics parameters were calculated by fitting of saturation curves (as the mean of triplicate measurements) with standard Michaelis–Menten equation using Prism 6 (GraphPad).

**Effect of pH and Temperature on DrRha Activity.** The optimum pH for DrRha was determined by measuring pNP-α-l-Rha hydrolysis under several pH values ranging from pH 4.0 to 10.0. The buffers used were as follows: acid citric/sodium phosphate buffer (200 mM) for pH 4.0 to 6.0, imidazole/HCl buffer (200 mM) for pH 7.0, Tris/HCl buffer (200 mM) for pH 8.0, tris base buffer (200 mM) for pH 9.0, and sodium carbonate buffer (200 mM) for pH 10.0. One hundred
microliter samples containing 1 mM pNP-α-L-Rha, 10 μL buffer, and 5 μL diluted DrRha were incubated for 5 min at 37 °C. Then, reactions were quenched by adding 50 μL Na₂CO₃ (1 M), and absorbance was measured at 405 nm. The dependence of the enzyme activity on temperature was determined by measuring the hydrolysis of pNP-α-L-Rha for several temperatures ranging from 40 to 100 °C. Samples were prepared following the same protocol as the pH dependence study but only in 20 mM imidazole-HCl buffer at pH 6.0. After 5 min incubation, reaction was quenched by adding 50 μL Na₂CO₃ (1 M), and relative activity was calculated according to absorbance measured for each temperature at 405 nm.

Substrate Specificity of Recombinant α-L-Rhamnosidase DrRha. An assay mixture composed of 100 μL citric acid-sodium phosphate buffer (0.1 M, pH 6.0), 1 mM substrate, and 0.05 mg/mL DrRha was heated at 50 °C. At respective times, the reaction mixture was subjected to HPLC analysis after filtration through a 0.22 μm filter. An Agilent 1220 Infinity II LC coupled with an Agilent Zorbax Eclipse XDB-C18 column (4.6 × 150 mm, 3.5 μm) and a diode array detector (DAD) was used to determine the concentrations of the substrates and products. Following an injection of 20 μL, the column was eluted with a gradient elution at 30 °C and 1220 mbar (0.1 M, pH 6.0), 1 mM Rha. Following an injection of 20 μL, the column was eluted with a gradient elution at 30 °C and kept the flow rate at 0.8 mL/min. The mobile phase was composed of H₂O/0.1% TFA (A) and CH₃CN/0.1% TFA (B). The gradient procedure began with A/B = 90:10 within 0–4 min. This procedure was followed by a linear change to A/B = 20:80 within 4–23 min, maintaining A/B = 20:80 within 23–24 min, and then a linear return to A/B = 90:10 within 24–30 min. The target compounds were captured in DAD at 280 nm, and samples were analyzed by HRMS.

DrRha Crystallogenesis and Structure Determination. DrRha was further purified by immobilized-metal-affinity chromatography (IMAC) and gel filtration before being concentrated in 20 mM Tris at 10 mg/mL before being used for crystalllogenesis assays. Crystals suitable for diffraction were obtained by sitting drop vapor diffusion when using 30% PEG 1500 as the crystallization agent. Crystals were cryprotected using 25% methylpentane-2,4-diol before being frozen in liquid nitrogen. Data were collected on beamline Proxima-1 at Soleil Synchrotron Facility, integrated, reduced, and scaled with the X-Ray Detector Software (XDS).31 S. avermitilis α-L-rhamnosidase (PDB 3W3M)17 was then used as a template for molecular replacement using the PHASER program.32 Refinement and model building were conducted using Phenix33 and COOT.34 Model quality was assessed at every refinement step using MolProbity.35 The structure of DrRha was deposited to the Protein Data Bank (PDB code 6I60). Data and final refinement statistics are listed in Table S1.

Homology Modeling of DrRha and Docking of Ligands. The structure of DrRha was used as a starting template, and loop 681–712, which was missing in the structure, was built using COOT. The resulting model was then prepared with AmberTools36 and then equilibrated using the NAMD software37 and AMBER-FB15 force field.38 Docking of rhamnosylated substrates was done by first applying AM1-BCC charges on the ligand.39 Then, the ligand was placed 10 Å away, facing the active site (according to PDB 3W3M and 3WSN). Then, the DrRha–substrate complex was formed using steered molecular dynamics at 100 K using the structural alignment of rhamnose in its binding pocket as the final orientation, according to published procedures.40 DrRha backbone was kept constrained during the whole procedure. Finally, protein–ligand complex models were equilibrated by releasing substrate constraints and applying several cycles of energy minimization (10,000 steps, steepest descent), followed by molecular dynamics (100 K, 1 ns). Final complex models were obtained by final energy minimization. All structural figures were drawn using PyMOL Molecular Graphics system 1.6 (www.pymol.org).

## Associated Content

### Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acsomega.8b03186.

SDS-PAGE gel, chemical formulae of substrates, pH and T profiles, Michaelis–Menten plot, stability of DrRha, HPLC chromatograms, HRMS spectra, and data collection and refinement statistics for DrRha crystal structure (PDF).

## Author Information

### Corresponding Author

*E-mail: richard.daniellou@univ-orleans.fr.

### ORCID

Richard Daniellou: 0000-0001-7296-3736

## Acknowledgments

This work was partially funded by La Région Centre-Val de Loire of France (APR IR Glycopese). We acknowledge SOLEIL for the provision of synchrotron radiation facilities (proposal ID BAG20160782) in using Proxima beamlines.

## References

1. Cui, Z.; Maruyama, Y.; Mikami, B.; Hashimoto, W.; Murata, K. Crystal Structure of Glycoside Hydrolase Family 78 α-L-Rhamnosidase from Bacillus Sp. GL1. J. Mol. Biol. 2007, 374, 384–398.
2. Lafite, P.; Daniellou, R. Rare and Unusual Glycosylation of Peptides and Proteins. Nat. Prod. Rep. 2012, 29, 729–738.
3. Thibodeaux, C. J.; Melançon, C. E., III; Lii, H.-W. Natural-Product Sugar Biosynthesis and Enzymatic Glycodiversification. Angew. Chem., Int. Ed. 2008, 47, 9814–9859.
4. Rabausch, U.; Ilmberger, N.; Streit, W. R. The Metagenome-Derived Enzyme RhaB Opens a New Subclass of Bacterial B Type α-L-Rhamnosidases. J. Biotechnol. 2014, 191, 38–45.
5. Weignerová, L.; Marhol, P.; Gerstorferová, D.; Křen, V. Preparatory Production of Quercetin-3-β-D-Glucopyranoside Using Alkali-Tolerant Thermostable α-L-Rhamnosidase from Aspergillus terreus. Bioresour. Technol. 2012, 115, 222–227.
6. Jung, H. A.; Paudel, P.; Seong, S. H.; Min, B.-S.; Choi, J. S. Structure-Related Protein Tyrosine Phosphatase 1B Inhibition by Naringenin Derivatives. Bioorg. Med. Chem. Lett. 2017, 27, 2274–2280.
7. Zhao, M.; Du, L.; Tao, J.; Qian, D.; Shang, E.-x.; Jiang, S.; Guo, J.; Liu, P.; Su, S.-I.; Duan, J.-a. Determination of Metabolites of Diosmetin-7-O-Glucoside by a Newly Isolated Escherichia coli from Human Gut Using UPLC-Q-TOF/MS. J. Agric. Food Chem. 2014, 62, 11441–11448.
8. Gasnikh, E.; Kazibwe, Z.; Pandurangan, M.; Judy, G.; Kim, D. H. Probing the Impact of Quercetin-7-O-Glucoside on Influenza Virus Replication Influence. Phytochemicals 2016, 23, 958–967.
9. Lee, Y.-S.; Huh, J.-Y.; Nam, S.-H.; Moon, S.-K.; Lee, S.-B. Enzymatic Bioconversion of Citrus Hesperidin by Aspergillus Sojae Naringinase: Enhanced Solubility of Hesperetin-7-O-Glucoside with
in Vitro Inhibition of Human Intestinal Maltase, HMG-CoA Reductase, and Growth of Helicobacter Pylori. Food Chem. 2012, 133, 2253–2259.

(10) Lombard, V.; Golaconda Ramulu, H.; Drula, E.; Coutinho, P. M.; Henri ssat, B. The Carbohydrate-Active Enzymes Database (CAzY) in 2013. Nucleic Acids Res. 2014, 42, D490–D495.

(11) Ichinose, H.; Fujimoto, Z.; Kaneko, S. Characterization of an α-L-Rhamnosidase from Streptomyces avermitilis. Bioch. Biotechnol. Biochem. 2013, 77, 213–216.

(12) Manzanoares, P.; van den Broeck, H. C.; de Graaff, L. H.; Visser, J. Purification and Characterization of Two Different α-L-Rhamnosidases, RhaA and RhaB, from Aspergillus aculeatus. Appl. Environ. Microbiol. 2001, 67, 2230–2234.

(13) Tamayo-Ramos, J. A.; Flippini, M.; Pardo, E.; Manzanoares, P.; Orejas, M. L-Rhamnose Induction of Aspergillus nidulans α-L-Rhamnosidase Genes is Glucose Repressed via a CreA-Independent Mechanism Acting at the Level of Inducer Uptake. Microb. Cell Fact. 2012, 11, 26.

(14) Beekwilder, J.; Marcozzi, D.; Vecchi, S.; de Vos, R.; Janssen, P.; Francke, C.; van Hylckama Vlieg, J.; Hall, R. D. Characterization of Rhamnosidases from Lactobacillus Plantarum and Lactobacillus Acidophilus. Appl. Environ. Microbiol. 2009, 75, 3447–3454.

(15) Zverlov, V. V.; Hertel, C.; Brunnenmeyer, K.; Hroch, A.; Kellermann, J.; Schwarz, W. H. The Thermostable α-L-Rhamnosidase RamA of Clostridium Stercorarium: Biochemical Characterization and Primary Structure of a Bacterial α-L-Rhamnose Hydrolase, a New Type of Inverting Glycoside Hydrolase. Mol. Microbiol. 2000, 35, 173–179.

(16) Koseki, T.; Mese, Y.; Nishibori, N.; Masaki, K.; Fujii, T.; Hanada, T.; Yamane, Y.; Shi no, Y.; Murayama, T.; Iefuji, H. Characterization of an α-L-Rhamnosidase from Aspergillus Kowa oichi and Its Gene. Appl. Microbiol. Biotechnol. 2008, 80, 1007–1013.

(17) Fujimoto, Z.; Jackson, A.; Michikawa, M.; Maehara, T.; Momma, M.; Henri ssat, B.; Gilbert, H. J.; Kaneko, S. The Structure of a Streptomyces avermitilis α-L-Rhamnosidase Reveals a Novel Carbohydrate-Binding Module CMB67 within the Six-Domain Arrangement. J. Biol. Chem. 2013, 288, 12376–12385.

(18) O' Neill, E. C.; Stevenson, C. E. M.; Paterson, M. J.; Rejzek, M.; Chauvin, A.-L.; Lawson, D. M.; Field, R. A. Crystallization of a Novel Two Domain GH78 Family α-Rhamnosidase from Klebsiella oxytoca with Rhamnose Bound. Proteins: Struct., Funct., Bioinf. 2015, 83, 1742–1749.

(19) Birgisson, H.; Hreggvidsson, G. O.; Fridjónsson, O. H.; Mort, A.; Kristjansson, J. K.; Mattiasson, B. Two New Thermostable α-L-Rhamnosidases from a Novel Thermophilic Bacterium. Enzyme Microb. Technol. 2004, 34, 561–571.

(20) Saiki, T.; Kobayashi, Y.; Kawago e, K.; Beppu, T. Dictyoglycosum Thermophilum Gen. Nov., Sp. Nov., a Chemoorganotrophic, Anaerobic, Thermophilic Bacterium. Int. J. Syst. Evol. Microbiol. 1985, 35, 253–259.

(21) Coil, D. A.; Badger, J. A.; Forberger, H. C.; Riggs, F.; Madupu, R.; Fedorova, N.; Ward, N.; Robb, F. T.; Eisen, J. A. Complete Genome Sequence of the Extreme Thermophile Dictyoglycosum Thermophilum H-6-12. Genome Announc. 2014, 2, No. e00109-14.

(22) Chlubnova, I.; Králova, B.; Dvořáková, H.; Spivok, V.; Filipp, D.; Nuges-Chauvin, C.; Daniellou, R.; Ferrière, V. Biocatalyzed Synthesis of Difu ranosides and Their Ability to Trigger Production of TNF-α. Bioorg. Med. Chem. Lett. 2016, 26, 1550–1553.

(23) Ati, J.; Lafite, P.; Daniellou, R. Genetic Synthesis of Glycosides: From Natural O- and N-Glycosides to Rare C- and S-Glycosides. Beilstein J. Org. Chem. 2017, 13, 1857–1865.

(24) Guillotin, L.; Cancellieri, P.; Lafite, P.; Landemarre, L.; Daniellou, R. Chemo-Enzymatic Synthesis of 3-O-(β-D-Glucopyrano syl)-sn-Glycerols and Their Evaluation as Preservative in Cosmetics. Pure Appl. Chem. 2017, 89, 1295–1304.

(25) Grandits, M.; Michlmayr, H.; Sygmund, C.; Oostenbrink, C. Calculation of Substrate Binding Affinities for a Bacterial GH78 Rhamnosidase through Molecular Dynamics Simulations. J. Mol. Catal. B Enzym. 2013, 92, 34–43.