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Enzymatic fructosylation: an eco- friendly tool to enhance the water solubility and stability of the natural aglycon phloretin

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PURPOSE OF THE ABSTRACT
Phloretin (2',4',6'-trihydroxy-3-(4-hydroxyphenyl)-propiophenone) is a natural dihydrochalcone widely distributed in the leaves, bark and fruit of apple trees. Phloretin exhibits several pharmacological properties, such as antidiabetic, antioxidant, anti-inflammatory, and antitumor activities. As other aglycons, phloretin is poorly soluble in water and consequently its bioavailability is low. In the plant, glucosylation of phloretin at the 2' position is the last step in phlorizin (phloretin-2'-β-D-glucopyranoside) biosynthesis. Phloretin glucosides have also been synthesized in acceptor reactions using sucrose phosphorylase1, amylosucrase2 or UDP-glycosyltransferase3. The in-vivo or in-vitro synthesis of phloretin fructosides has not been reported so far.

Microbial and plant enzymes from the glycoside hydrolase (GH) families 32 and 68 (clan GH-J) initiate de novo fructan synthesis by transferring the fructosyl moiety from one sucrose molecule (donor) to another sucrose molecule (acceptor). The fructosyl moiety can be also released to water resulting in substrate hydrolysis. The transfructosylation/hydrolysis (T/H) ratio, the regio-selectivity, and the polymerization capacity are intrinsic properties that vary among clan GH-J enzymes of different origins.

In a previous study, enzymatic fructosylation was successfully used to enhance the water solubility and stability of phlorizin4. In this study, five clan GH-J enzymes of different catalytic properties were assayed for transferring the fructosyl residue of the natural substrate sucrose to the aglycon phloretin. The enzymes sucrose:sucrose 1-fructosyltransferase from the plant Schedonorus arundinaceus (Sa_1-SST, EC 2.4.1.99, GH32), and the Lactobacillus reuteri levansucrase (Lr_Lev, EC 2.4.1.10, GH68) and inulosucrase (Lr_Inu, EC 2.4.1.9, GH68) failed to fructosylate the non-sugar acceptor. The Thermotoga maritima β-fructosidase (Tm_BfrA, EC 3.2.1.26, GH32) synthesized a unique phloretin mono-fructoside, but the conversion efficiency was below 10%. More interestingly, the Gluconacetobacter diazotrophicus levansucrase (Gd_LsdA) yielded short-chain series of phloretin fructosides including two positional mono-fructoside isomers with maximal acceptor conversion of 83 %. Due to its high activity and wide acceptor promiscuity, Gd_LsdA is an attractive catalyst for biotech application in the fructosylation of different phenolic compounds, including the apple dihydrochalcones phlorizin and phloretin.

FIGURES

FIGURE 1

FIGURE 2

KEYWORDS

Levansucrase | Phenolic compounds | Transfructosylase

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