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Cascade transformations of Leloir glycosyltransferase on solid support: continuous production of the natural C-glycoside nothofagin

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PURPOSE OF THE ABSTRACT

Glycosyltransferases (GTs) are an ubiquitous group of enzymes that synthesize oligosaccharides, polysaccharides, glycoconjugates and their analogues via catalytic formation of glycosidic bond using sugar donors.[1] Nothofagin, the 3'-C-beta-D-glucoside of phloretin, is a prominent antioxidant that is widely applicable for food additives, cosmetic ingredients, as well as nutraceuticals.[2] The glycosyltransferase cascade reaction has been successfully reported for the synthesis of nothofagin, involving a C-glycosyltransferase from rice (Oryza sativa; OsCGT) and sucrose synthase from soybean (Glycine max; GmSuSy).[3] The overall reaction was 3'-C-beta-glycosylation of the polyphenol phloretin from uridine 5'-diphosphate (UDP)-glucose that was released in situ from sucrose and UDP. Our aim in this study is to make a fundamental improvement in process intensification for the biocatalytic synthesis of C-glycoside nothofagin.

Continuous-flow may itself be commonly regarded as a mean for process intensification, to improve the control over reaction performance and to achieve higher productivity.[4] In bio-catalysis applied to chemical synthesis, competitive process technologies build on highly active enzyme preparations that are incorporated efficiently into scalable bioreactors for continuous operation. Generally, immobilizing the soluble enzyme(s) on a solid carrier remains in the center of attention for development. Packed bed reactor was extensively utilized in continuous catalytic and chemical process, which offers numerous advantages with higher conversion per unit mass of catalyst, recyclability, continuous operation, and minimum product inhibition.[5]

Therefore, in our study, we present an integrated process technology for flow synthesis with co-immobilized sugar nucleotide-dependent glycosyltransferases using efficient glycosylation from sucrose via the internally recycled UDP-glucose. The result shows continuous production of nothofagin (1.8 g; 90 ml) in 90 reactor cycles (2.3 h/cycle) with a space-time yield of ~11 mg/(ml h), reaching a total turnover number of up to 2.9 × 103 mg product/mg immobilized enzyme used. This provides a basis from engineering science to promote glycosyltransferase applications for natural product glycosides and oligosaccharides.

FIGURES

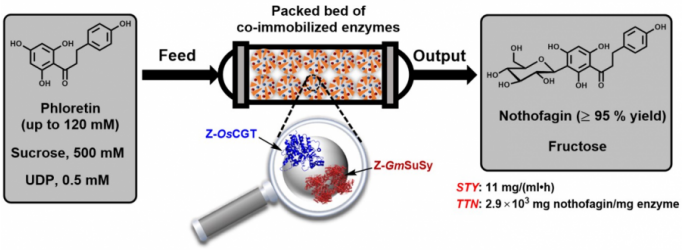

FIGURE 1

Continuous flow synthesis of nothofagin through a packed-bed reactor with co-immobilized glycosyltransferases.

Continuous flow synthesis of nothofagin through a packed-bed reactor with co-immobilized glycosyltransferases.

FIGURE 2

KEYWORDS

continuous production | natural product glycosides | process intensification | sugar nucleotide?dependent glycosyltransferase

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