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Recombinant FAD-dependent monooxygenase used for precise flavonoid functionalization

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PURPOSE OF THE ABSTRACT

The potential of natural products, particularly those derived from plants and other biological sources, as a source of new pharmaceuticals is unlimited [1]. Despite technological and societal changes, natural products remain a consistent source of new medicines. In particular, plant secondary metabolites show potent antioxidant activity, which is closely linked to the prevention and treatment of cardiovascular disease and cancer [2]. Their health-promoting properties are mainly related to the presence and position of the hydroxyl group and conjugated double bonds [3]. Although there have been reported advances in methods to obtain hydroxylated polyphenols using hydrogen peroxide and metal catalysts, such as vanadium, palladium, TiO2, or chemical oxidation in supercritical carbon dioxide, these methods are still limited in their selectivity and efficiency. Complex, multi-step processes, such as the Hock process, are still mainly used to obtain hydroxylated polyphenols [4].

Enzyme catalysis is an excellent alternative to these methods. However, most of the identified enzymes for this purpose belong to the cytochrome P-450 monooxygenases, which depend on a compatible reductase [5], and often have very low yields or do not provide sufficient regioselectivity. In contrast, flavoprotein monooxygenases (FMOs), which belong to group B of flavin-dependent monooxygenases, can directly use NADPH as an electron donor [6], and exhibit excellent regioselectivity. Nevertheless, to date, there are limited data on FMOs active against flavonoids [7].

The application of microorganisms or isolated enzymes as biocatalysts is a well-established strategy for the synthesis of high-value natural compounds. This approach provides environmentally friendly and efficient production of compounds that were previously beyond the reach of science [8]. Furthermore, the modification of natural compounds by recombinant protein expands our understanding of their origin, stability, or activity. This offers the potential for their industrial use in the development of new classes of drugs or nutraceuticals that provide social and environmental benefits based on the latest knowledge.

The reported here work concerns the heterologous expression and complete characterization of fdeE from H. seropedicae SmR1, the first selective ortho-hydroxylase of isoflavones and flavonols at the C-8 position. Furthermore, a clever maneuver with the reaction environment modification allowed the stable production of hydroxylation products, which exhibited extreme instability in both in vivo and in vitro assays. This approach yielded an 8-hydroxyquercetin titer of 0.16 g/L, which, once optimized, can be widely used for large-scale production of these compounds.

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FIGURES

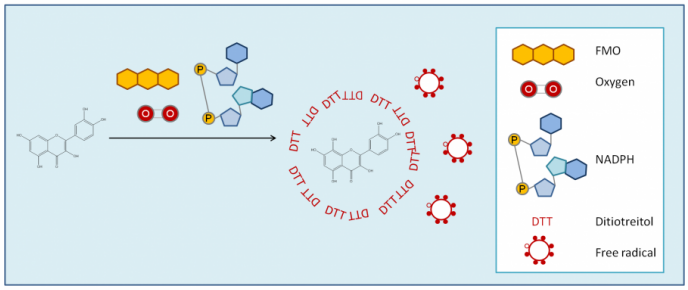

FIGURE 1

Mechanizm of stable in vitro hydroxylation of flavonoids by FMO.

The legend is included in the figure.

FIGURE 2

KEYWORDS

monooxygenase | recombinant protein | ortho-hydroxylation | enzymatic cascade

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