Biosynthesis of hernandulcin derivatives, a natural sweetener, through synthetic biology approach

Arthur Sarrade-Loucheur\(^1\), Magali Remaud-Simeon\(^2\), and Gilles Truan\(^3\)

\(^1\)Laboratoire d’Ingénierie des Systèmes Biologiques et des Procédés (LISBP) – Institut National des Sciences Appliquées (INSA), Institut National des Sciences Appliquées [INSA] – 135 Avenue de rangueil 31077 Toulouse cedex 04, France

\(^2\)Laboratoire d’Ingénierie des Systèmes Biologiques et des Procédés (LISBP) – Institut National des Sciences Appliquées [INSA], Institut National de la Recherche Agronomique - INRA, Centre National de la Recherche Scientifique - CNRS, Institut national de la recherche agronomique (INRA) – 135 Avenue de rangueil 31077 Toulouse cedex 04, France

\(^3\)Laboratoire d’Ingénierie des Systèmes Biologiques et des Procédés (LISBP) – CNRS : UMR5504, INSA - Institut National des Sciences Appliquées, Institut national de la recherche agronomique (INRA) – INSA Toulouse 135 Avenue de Rangueil 31077 Toulouse Cedex 4, France

Abstract

Lippia dulcis, a plant from Mexico, naturally produces hernandulcin whose sweetness potency is 1000 times higher than sucrose. However, its commercial use is impeded by a low production in planta and its chemical synthesis remains difficult. Hernandulcin also suffers from low solubility and from a bitter taste. One possibility would be to target the production of new molecules close to hernandulcin. Yeast metabolic engineering can be used to produce hernandulcin and/or derivatives, potentially generating new molecules. Bisdabolol, the precursor of hernandulcin, is produced by bisdabolol synthase (BS) starting from farnesyl pyrophosphate. The gene encoding BS from Lippia dulcis has been identified and produced in yeast. However, the enzymatic step converting bisdabolol to hernandulcin is unknown, although the mechanism (oxydation) resembles those of cytochrome P450 reactions. Using a bisdabolol producing yeast we identified human and plant cytochromes P450 that can recognize bisdabolol as a substrate and lead to the production of new molecules. Promiscuous enzymes, recognizing derivatives of farnesyl pyrophosphate were also identified. In vitro enzymatic assays validated their activities. The chemical structures of the new compounds as well as their sweetness potency is now under investigation.

Keywords: Synthetic biology / Metabolic engineering / In vivo screening

*Speaker