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TOPIC(s) : Biocatalytic cascade reactions

Development of an enzymatic process for alkyl hydroxycinnamates synthesis

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PURPOSE OF THE ABSTRACT

Alkyl hydroxycinnamates (AHCs) are hydroxycinnamic acid (HCA) esters aliphatic derivatives [1] found in plants associated with suberin and cutin. [2] The presence of the aliphatic moiety on AHCs modulates their hydrophilic/lipophilic balance and improves their integration in oil-based formulations and their biological activities compared to their phenolic acid parents.[3] These features open a broad panel of applications for these molecules as antioxidants, antimicrobials, and UV protectants among others.[1] Lipases are usually used to graft aliphatic moieties on phenolic acids. However, it has been reported that lipase's activity is inhibited in the case of p-hydroxycinnamic acids due to the simultaneous presence of the p-hydroxyl and the double bond near the carboxylic function conjugated to the aromatic cycle on the phenolic acid.[4] Our work seeks to develop an enzymatic process for the synthesis of AHCs by miming their biosynthetic pathway in plants.

We expressed two recombinant enzymes in a 5 L fermenter using transformed *E. coli* competent cells. The first one is a CoA-ligase (4CL) implicated in the activation of p-HCAs, and the second one is an acyltransferase (ACT) responsible for the transfer of the acyl moiety of the activated phenolic acid on an aliphatic moiety (acyl acceptor) for AHCs synthesis (Figure 1). We tested the enzymatic activity of the purified 4CL and ACT on different HCAs and acyl acceptors, respectively. HPLC and LC-MS results showed the successful activation of HCAs and AHCs synthesis. We also optimized the ATP concentration and the reaction time for HCAs activation using design of experiment method to minimize them as much as possible to make the process economically viable. We are currently working on ACT kinetic parameters determination and we aim to scale up the reaction and purify the final product.

FIGURES

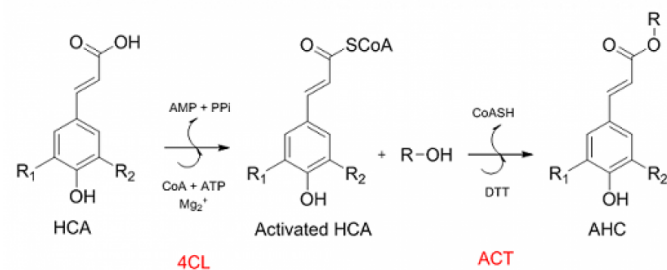


FIGURE 1

Reactions implicated in the enzymatic synthesis of AHCs in plants

4CL: 4-coumarate CoA ligase; ACT: Acyl transferase

FIGURE 2

KEYWORDS

Alkyl hydroxycinnamates (AHCs) | p-hydroxycinnamic acid (HCA) | CoA-ligase (4CL) | Acyl transferase (ACT)

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