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GENETIC TRANSFORMATION OF S*ACCHAROMYCES CEREVISIAE* WITH PLANT GENES: PRODUCTION OF CONJUGATES WITH HYDROXYCINNAMATES

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The phenylpropanoid phenolic acids are important components of the antioxidant activity and therapeutic properties displayed by certain plant extracts. The phenylpropanoid pathway catalyzes the conversion of phenylalanine to secondary metabolites, such as hydroxycinnamic acid. Esters formed by the reaction between hydroxycinnamate and particular organic acids (e.g., shikimic and quinic acid) represent a major family of plant phenol acids, with chlorogenic acid as the most naturally abundant molecule.

In plants, the synthesis of phenolic acids can be mediated by the enzymes 4CL (4-coumarate: CoA-ligase) and HCT (hydroxycinnamoyl-CoA:shikimate/quinate hydroxycinnamoyltransferase). The former catalyses the conversion of *p*-coumarate and some related substrates (caffeate and ferulate) to their respective CoA esters; while the latter can use either *p*-coumaroyl-CoA or caffeoyl-CoA as its substrate, and in the presence of either quinate or shikimate, acts to promote the synthesis of either caffeoylquinate (chlorogenic acid), coumaroylquinate, caffeoylshikimate or coumaroylshikimate.

We describe here a yeast-based (*Saccharomyces cerevisiae*) production system of conjugated hydroxycinnamate, founded on the heterologous expression of tobacco 4CL and globe artichoke HCT, we previously isolated. Unlike wild type yeast strains, the transformed yeast cultures metabolized both caffeic and *p*-coumaric acid. Incubation with *p*-coumaric acid produced a detectable level of coumaroylshikimate, the expected reaction end product in the presence of both 4CL and HCT. However, the predominant compounds formed after a 72h incubation with *p*-coumaric and caffeic acid were two highly hydrophobic molecules of molecular mass 298 and 314. We assume that these represent the condensation products between *p*-coumaroyl-CoA and caffeoyl-CoA together with one molecule of 3-hydroxyanthranilic acid. These molecules resemble the avenanthramides, which have anti-carcinogenic and anti-inflammatory activity. To assess the activity of globe artichoke HCT, the relevant cDNA was cloned and heterologously expressed in *E. coli*. This assay confirmed that caffeoyl-CoA can act as a donor molecule in the presence of hydroxyanthranilate.