

Potential Utilisation of Wax Synthases from *Simmondsia chinensis* and *Marinobacter hydrocarbonoclasticus* for Production of Wax Esters in Plants

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Wax esters are esters of long chain fatty alcohols and fatty acids produced by a wide variety of organisms including bacteria, plants and animals. Wax esters are major components of the plant cuticle. In mammals wax esters constitute approximately 30 % of sebum. Wax esters are used as high pressure lubricants, replacing hydraulic oil, and in the pharmaceutical, cosmetic, printing and leather industries. As obtaining high amounts of wax esters is very expensive, possibilities of producing wax esters using plants are investigated. The modification of oilseed crops using genes of enzymes involved in wax esters biosynthesis might enable to obtain different kinds of wax esters.

Wax synthases (fatty acyl-CoA:fatty alcohol acyltransferases) are membrane-associated enzymes which catalyze esterification reaction of a fatty acyl-CoA with a long chain fatty alcohol.

Wax synthases from *Simmondsia chinensis* and *Marinobacter hydrocarbonoclasticus* were expressed heterologously in *Saccharomyces cerevisiae*. The *in vivo* substrate specificity of tested enzymes was investigated by measuring the amount of accumulated wax esters in the yeast cells after feeding with a fatty alcohol. The *in vitro* substrate specificity was determined in microsomal fractions isolated from yeast expressing wax synthase genes using radiolabelled fatty acyl-CoAs and fatty alcohols.

The expression of both wax synthases in yeast fed with 16:0 and 18:0 alcohol resulted in accumulation of wax esters in the cells. *In vitro* assays using nine [¹⁴C] acyl-CoAs in combination with 17 fatty alcohols with different carbon chain lengths as a substrates enabled to determine which of 153 different wax esters are produced by each enzyme with the highest efficiency.

This work was supported by the European Commission through the FP7 ICON project