Magdalena Miklaszewska
Supervisor: Prof. dr hab. Antoni Banaś

Substrate specificity of selected fatty acyl-CoA reductases and wax synthases

Wax esters are long-chain esters of fatty acids and fatty alcohols produced by a wide variety of organisms. They fulfill numerous biological functions, including energy storage, chemical communication, sound transmission, buoyancy regulation as well as protection against water loss, UV light and pathogens. In industry wax esters are used as high pressure lubricants and components of pharmaceuticals, cosmetics and printing inks. Wax esters are mainly produced via chemical-catalyzed method or obtained from *Simmondsia chinensis* (jojoba), which stores wax esters in its seeds as an energy source. As both methods are cost intensive, possibilities of producing wax esters using microorganisms and plants are investigated. Transferring the wax ester biosynthetic pathway to oilseed crops might provide renewable sources of different kinds of wax esters.

Fatty acyl-CoA reductases (FAR) and wax synthases (WS) are two main types of enzymes involved in wax ester biosynthesis. Alcohol-forming FARs produce fatty alcohols from fatty acyl-CoAs, while WSs catalyze the esterification reaction of a fatty acyl-CoA with a fatty alcohol.

The properties of wax esters, such as melting temperature and oxidation stability, are dependent on the chemical structure of the fatty acid and the fatty alcohol components. Therefore, the selection of FAR and WS enzymes with proper substrate specificities is the key step of the design of the biotechnological production of wax esters.

In this study, the substrate specificity of fatty acyl-CoA reductases and wax synthases derived from *Arabidopsis thaliana* (AtFAR5), *Simmondsia chinensis* (SchFAR, SchWS), *Mus musculus* (MmFAR1, MmWS) and *Marinobacter hydrocarbonoclasticus* (MhWS2) was characterised. The genes encoding tested enzymes were heterologously expressed in *Saccharomyces cerevisiae* wild type strain BY4742 and the quadruple mutant strain H1246, deficient in triacylglycerol and sterol ester synthesis. Both *in vivo* and *in vitro* substrate specificity of tested enzymes was determined. The activity of FAR and WS enzymes towards substrates with different carbon chain lengths, different saturation degrees and different side chains was characterised in *in vitro* assays using microsomal fractions isolated from transgenic yeast.

Fatty acyl-CoA reductase from *M. musculus* preferred C16 and C18 acyl-CoAs, while plant reductases (SchFAR and AtFAR5) exhibited rather narrow substrate specificity and produced mainly 18:0-OH.

Wax synthases from *M. musculus* and *M. hydrocarbonoclasticus* synthesised wax esters using wide range of substrates with explicit preference towards C12-C18 fatty acids and fatty alcohols. *S. chinensis* wax synthase produced the highest amount of wax esters using certain combinations of C14-C18 substrates. The enzyme also exhibited high activity towards 20:1-CoA.

The obtained data on the substrate specificity of tested FARs and WSs suggest that characterised enzymes may be used for the biotechnological production of wax esters with properties tailored to industrial applications.