

Random Mutagenesis of *atfA* and Screening for *Acinetobacter baylyi* Mutants with an Altered Accumulation of Wax Esters and Triacylglycerols

Röttig, A., Steinbüchel, A., Institute for Molecular Microbiology and Biotechnology,
Münster, Germany

Wax esters, composed of a long-chain acyl and a fatty alcohol residue, are valuable ingredients of many commercial products. However, natural sources for high-quality wax esters are currently mainly restricted to the expensive oil of the jojoba plant. Among bacteria, the Gram-negative *Acinetobacter baylyi* is remarkable as it synthesizes and stores large amounts of wax esters with a composition similar to that of jojoba-oil. This opens up a possibility for a sustainable biotechnological production of wax esters with desired chemical compositions and properties not only to be processed in cosmetics, medical products or lubricants, but also to be used as biodiesel replacement as fatty acid ethyl esters (FAEE). The key enzyme wax ester synthase/acyl-CoA:diacyl-glycerol acyltransferase (WS/DGAT or AtfA) for wax ester and triacylglycerol synthesis in *A. baylyi* serves as a model enzyme for a new class of bacterial acyl-CoA acyltransferases with an impressively broad substrate range. However, its three-dimensional structure and exact biochemical mechanism could not be elucidated, yet. Therefore, an efficient combination of random mutagenesis, screening and sequencing of *atfA* variants in *A. baylyi* was developed in order to gain detailed information about distinct amino acids or regions of AtfA having a crucial impact on its activity and/or substrate range. Employing *in silico* enzyme modeling, these insights into the relation between sequence, structure and biochemistry build a basis for further optimization of the enzyme in consideration of its promising biotechnological applications.