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ENZYMATIC SURFACTANT PRODUCTION FROM BIOMASS BYPRODUCTS

In this project, the focus has been to utilize byproducts (or underutilized products in today's industry) from renewable biomass as a starting material for enzymatic elongation of the head groups of selected surfactants of the alkyl glycoside group. Novel enzymes for this purpose have been selected, cloned, produced and analyzed. The first choice of raw material has been wheat bran, which is an agricultural by-product used as low value ingredient in food and animal feed, and large amounts of the bran are due to overproduction, just used as a source of energy (being burnt). The hemicellulose fraction of wheat bran was used as substrate to produce alkyl-glycoside-type surfactants. Thus, enzymes with hemicellulose (mainly xylan) hydrolysis and transfer reactions were developed, produced and tested in the production of alkyl-glycosides. Glycoside hydrolases family 10 (GH10) from plants were identified as potential enzymes for the production of biosurfactants. Indeed, we produced a GH10 from *Eucalyptus globulus* via recombinant technology using *Pichia pastoris* as host (Izzati et al, 2018). Another attractive candidate is a xylanase GH10 from the thermophilic bacterium *Rhodothermus marinus*. The transfer reaction of this enzyme can be improved via protein engineering. The residues involved in the reaction were identified via computational modelling and site-directed mutagenesis (Aronsson, et al. 2017), and use of this enzyme has resulted in transfer products with sugar headgroups composed of two to six xylose-residues (at least two sugar residues are desirable) (results to be published).

The starch fraction is the other component of the wheat bran that can be used as substrate for the production of biosurfactants. Thus, a novel cyclomaltodextrin glucotransferase, CGTase, originating from genomic material found in a terrestrial hotspring was investigated. This enzyme belongs to the GH13 glycoside hydrolase family and acts on starch. The enzyme catalyzes formation of cyclodextrins from linear saccharide chains as one of four reactions catalyzed. The coupling reaction is another of these four reactions, and a combination of cyclization and coupling reactions could be used in the production of surfactants. Computational models of this enzyme were built to pinpoint residues that could influence the substrate binding in the enzyme in order to improve the transfer reaction by site directed mutagenesis. A set of mutants were generated and some of them has shown promising results in the production of surfactants (to be published), improving the production level compared to when the wild type enzyme was used.

References

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