Terpenoid engineering in *Pichia pastoris*

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Pichia pastoris is a methylotrophic yeast having been developed into a superb host for recombinant, microbial protein production. *P. pastoris* is genetically amenable – offering a multitude of genetic tools including CRISPR technology - and grows to several-fold higher cell-densities than *S. cerevisiae* or other conventional yeasts. Thus, *P. pastoris* is a well-suited host to obtain also lipids on an industrial scale. Though, there is a substantial lack in biochemical and cell biological knowledge in this yeast.

We have shown that *P. pastoris* is a highly interesting host for the production of sesqui- and triterpenoids. Employing a fortified farnesyl pyrophosphate (FPP) pool, a gold-standard valencene synthase and cytochrome P450-based functionalization of (+)-valencene to (+)-nootkatone yielded in the first metabolic pathway to generate this aroma compound in yeast *de novo* [1]. Another fragrance, ambrox, can be derived from (+)-ambrein, which we obtain through sterol pathway and enzyme engineering in *P. pastoris* [2]. Cholesterol-producing yeasts can be employed to study mammalian membrane protein structure-function relationships and are used to obtain deuterated cholesterol for biophysical studies. Moreover, cholesterol-producing *P. pastoris* showed an astounding phenotype in electron microscopy that prompted us to follow up as of yet uncharacterized function(s) of specific sterol structures.

Seminal studies on engineering *P. pastoris* for sesqui- and triterpenoid biosynthesis highlight the promising position of this methylotrophic yeast, beside *S. cerevisiae* and oleaginous yeasts, for industrial lipid production. It is still a long way to go, but it will be worthwhile to carve out the differences between *P. pastoris* and the much better characterized yeasts, in terms of lipid metabolism and its regulation.

[1] Wriessnegger T., Augustin P., Engleder M., Leitner E., Müller M., Kaluzna I., Schürmann M., Mink D., Zellnig G., Schwab H., Pichler H. (2014) Production of the sesquiterpenoid (+)-nootkatone by metabolic engineering of *Pichia pastoris*. *Metabolic Engineering* 24:18-29

[2] Moser S., Strohmeier G.A., Leitner E., Plocek T.J., Vanhessche K., Pichler H. (2018) Whole-cell (+)-ambrein production in the yeast *Pichia pastoris*. *Metabolic Engineering Communications* 7:e00077