Terpenoid engineering in *Pichia pastoris*

Harald Pichler1,2, Sandra Moser1,2, Tamara Wriessnegger2, Gernot A Strohmeier2,3, Melanie Hirz1, Anita Emmerstorfer-Augustin1,2, Erich Leitner2,4, Matthias Engleder2, Monika Müller5, Martin Schürmann5, Daniel Mink5, Koenraad Vanhessche6, Thomas J Plocek6, Michael Haertlein7, Günther Zellnig8

1Graz University of Technology, Institute of Molecular Biotechnology, NAWI Graz, BioTechMed Graz, Graz, Austria
2acib (Austrian Centre of Industrial Biotechnology) GmbH, Graz, Austria
3Graz University of Technology, Institute of Organic Chemistry, Graz, Austria
4Graz University of Technology, Institute of Analytical Chemistry and Food Chemistry, Graz, Austria
5InnoSyn B.V., Geleen, The Netherlands
6ACS International S.A. Plan-les-Ouates, Switzerland
7Institut Laue-Langevin, Grenoble, France
8University of Graz, Institute of Plant Sciences, NAWI Graz, Graz, Austria

*Corresponding author: harald.pichler@tugraz.at*

*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.
