

Production of α -Cuprenene in *Xanthophyllomyces dendrorhous*

Elena Melillo* and Oliver Kayser

Terpenoids play a major role as natural products in pharma, food and cosmetics. Among these terpene-based natural products play a major role, especially for drugs like paclitaxel, artemisinin and steroids, terpenoids are a structural source in the drug discovery process. Most natural products are produced in low quantities only why alternative production systems like micro-organisms are of high interest. *Xanthophyllomyces dendrorhous* is well known as high producer for asthaxanthin as orange dye in salmon. Due to high biosynthetic rates for corresponding precursor *X. dendrorhous* was studied for its capability to accept heterologous genes for biosynthesis of non carotenoids like sesquiterpenoids.

By metabolic engineering, approaches knock out mutants have been constructed to accumulate farnesyl diphosphate as central precursor towards α -cuprenene as model compound (Figure 1).

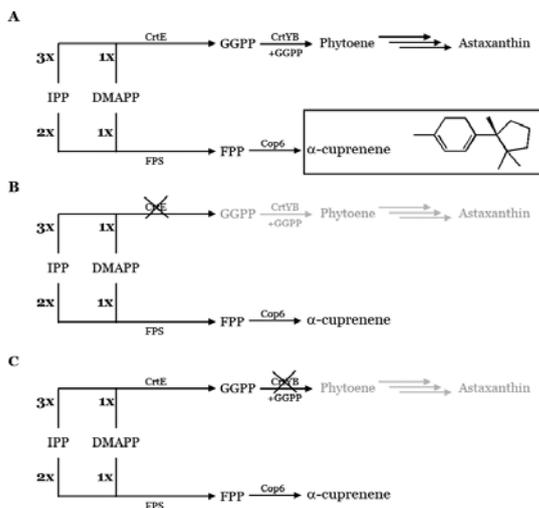


Figure 1. Schematic representation of *X. dendrorhous* mutant strains. (A) In the mutant *XdCop6*, the native astaxanthin pathway has not been modified but the gene *Cop6* has been integrated in the rDNA of the yeast allowing the mutant to produce both astaxanthin and α -cuprenene. (B) In the strain ΔE -*Cop6* the *Cop6* gene has been inserted in the *crtE* gene causing the disruption of the carotenoid production at the GGPP synthesis level. (C) When *Cop6* is inserted in the *crtYB* gene, the ΔYB -*Cop6* strain is created.

The heterologous gene (*Cop6*) derived from the fungus *Coprinus cinereus* was cloned into *X. dendrorhous*. *Cop6* produces, starting from FPP, the sesquiterpene α -cuprenene, which is the basic structure for the formation of lagopodin A, an antimicrobial sesquiterpene quinon. We compared the production of α -cuprenene in *E. coli*, *S. cerevisiae* and with main focus on three different mutants obtained for *X. dendrorhous* to identify the organism that could accumulate the highest concentration of sesquiterpene (Figure 2).

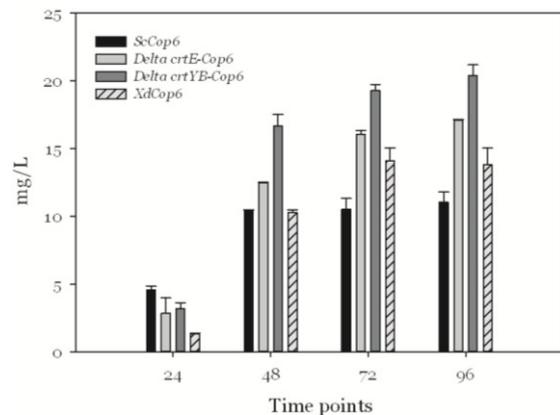


Figure 2. Production of α -cuprenene in minimal medium in *X. dendrorhous*

α -cuprenene was successfully identified by GC-MS and structure elucidated (Figure 3). For the first time a heterologous gene was cloned and expressed successful in *X. dendrorhous*, which may give a start for genetic and biochemical exploration of this important technical strain as terpenoid platform organism in the future.

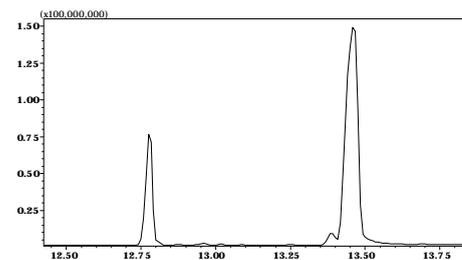


Figure 3. Chromatograms of the diluted dodecane from *X. dendrorhous* and ΔE -*Cop6*. The peak at 13.5 minutes is the -hexadecane used as internal standard. The α -cuprenene has a retention time of approximately 12.8 minutes

e.melillo@rug.nl
oliver.kayser@bci.tu-dortmund.de

Collaborator: Prof. Dr. Wim. J. Quax (w.j.quax@rug.nl), Pharmaceutical Biology, Rijksuniversiteit Groningen (RUG), NL.

*Dr. E. Mellilo carried out the practical work at the RUG.

Publications:

- ✓ Melillo, E., Setroikromo, R., Quax, W.J., Kayser, O. (2013) Production of α -cuprenene in *Xanthophyllomyces dendrorhous*: a step closer to a potent terpene biofactory. *Microbial Cell Factory* 12:13-17
- ✓ Melillo, E., Muntendam, R., Quax, W.J., Kayser, O. (2012) Heterologous expression of pentalenene synthase from *Streptomyces* UC5319 in *Xanthophyllomyces dendrorhous*. *Journal of Biotechnology* 161: 302-307
- ✓ Muintendam R., Mellilo, E., Ryden, A., Kayser, O. (2009) Perspectives and limits of engineering the isoprenoid metabolism in heterologous hosts. *Applied Microbiology and Biotechnology* 84:1003-1019