Artemisinic Acid: From plant to yeast

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Introduction

- Artemisinic acid (AA) is a precursor of the antimalarial drug artemisinin, which is originally extracted from *Artemisia annua*.
- In plants it is present in short supply and its chemical production is costly and difficult.
- Climate dependence could be avoided if the metabolic pathway was moved from *A. annua* to *Saccharomyces cerevisiae*.

Goals

- Evaluate different modifications carried out to convert *S. cerevisiae* into a cell factory for artemisinic acid production.
- Examine whether those modifications could be broadened to produce other sesquiterpenes.

Materials

- Reviews (*PubMed*).
- Scientific research publications (*PubMed).*
- Patents (*Espacenet).*
- Company's Websites.
- Comments from experts.

Results

Original metabolic engineering strategy

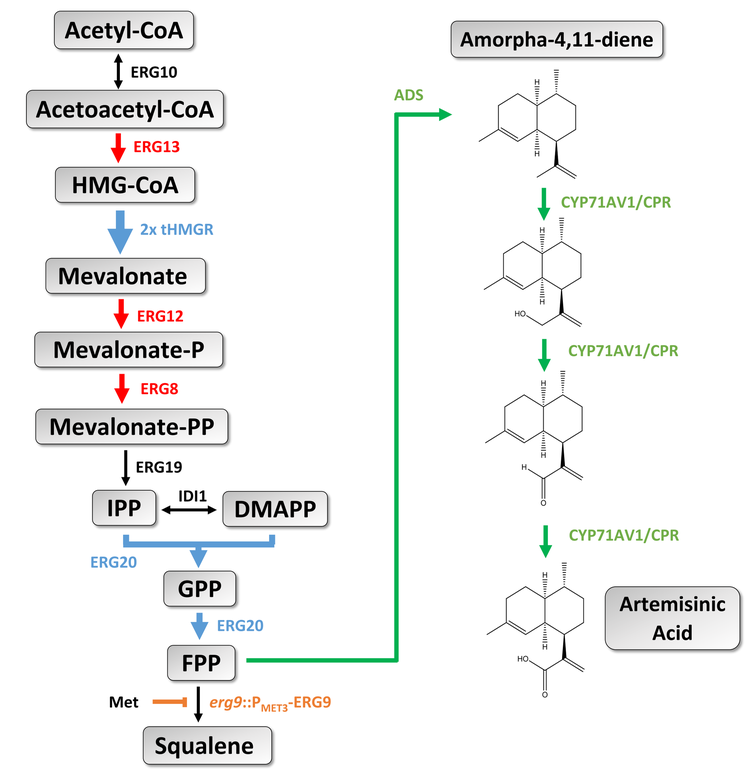


Figure 1. *Overview of the genetic modifications carried out in the first artemisinic acid – producing S. cerevisiae strain[1] (Adapted from Ro et al).*

1. FPP overproduction:

Direct overexpression of tHMGR and ERG20 by GAL induction

Indirect expression by UPC2-1 overexpression

ERG9 repression by methionine addition

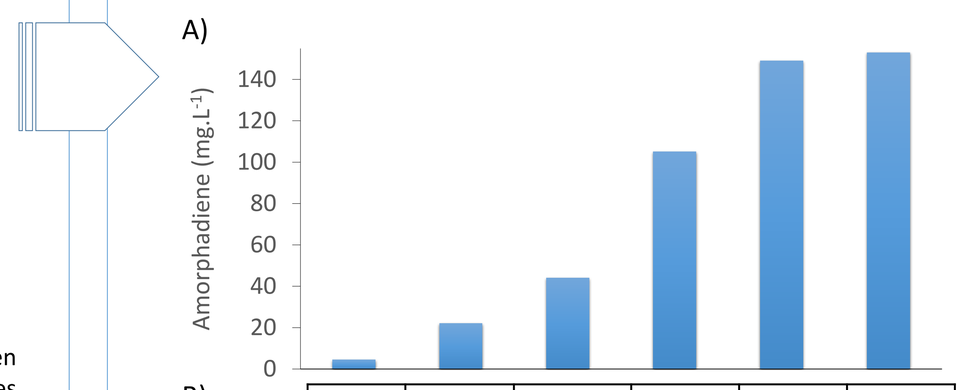
2. Artemisinic Acid production:

Heterologous expression of codon-optimised ADS

Heterologous expression of CYP71AV1 and CPR1

Genetic engineering tools

- **Plasmids.** Yeast Integrative plasmids (YIp) have been used for integration of constructions containing genes from endogenous mevalonate pathway. On the other hand, Yeast Episomal plasmids (YEp) have been used for high-level expression of heterologous enzymes (ADS, CYP71AV1 and CPR).
- **Markers.** Auxotrophic genetic markers have been used for strain selection (LEU2, URA3 and HIS3).
- **Promoters**. Galactokinase promoters (P_{GAL1} and P_{GAL10}) have been used for gene overexpression and the native promoter of the *erg9* gene has been substituted by a methionine-repressible promoter for ERG9 repression.



B)

Strain	EPY201	EPY208	EPY225	EPY213	EPY219	EPY224
tHMGR Expression	No	Yes				
ERG9 Repression	No		Yes			
UPC2-1 Expression	No			Yes		
tHMGR Expression	No				Yes	
ERG20 Expression	No					Yes

Figure 2. *Amorphadiene production in shake-flask cultures by different engineered strains (A) and sequential steps to engineer the artemisinic acid-producing strain[1] (B) (Adapted from Ro et al).*

Overall production: Up to **100 mg·L^{-1} of artemisinic acid**

Optimised metabolic engineering strategy

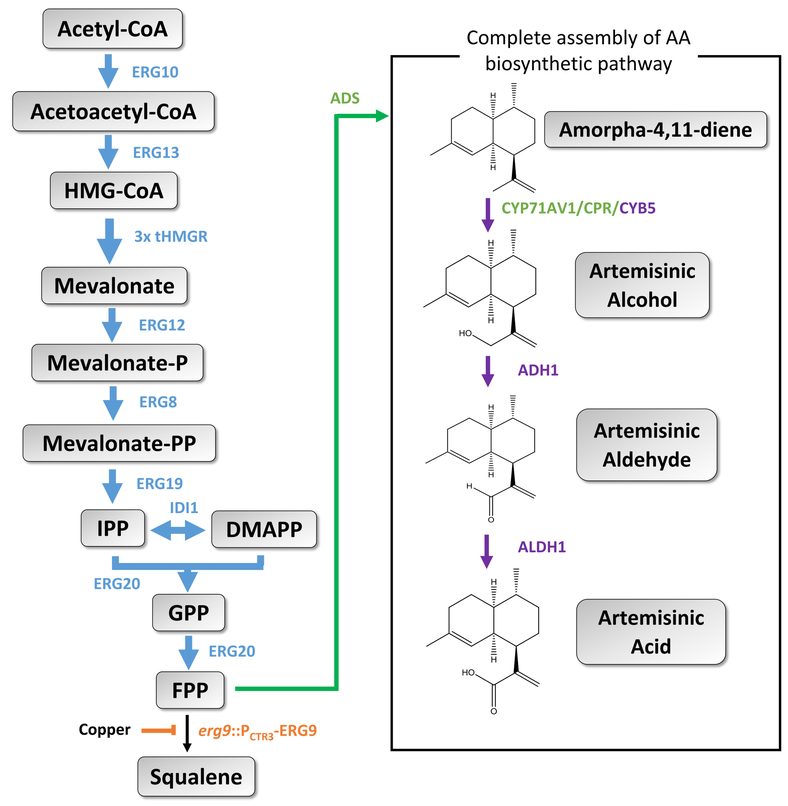


Figure 3. *Overview of the genetic modifications to optimise AA production[2].*

1. FPP overproduction

Direct overexpression of all genes from the mevalonate pathway

ERG9 repression by copper addition

2. Artemisinic Acid production

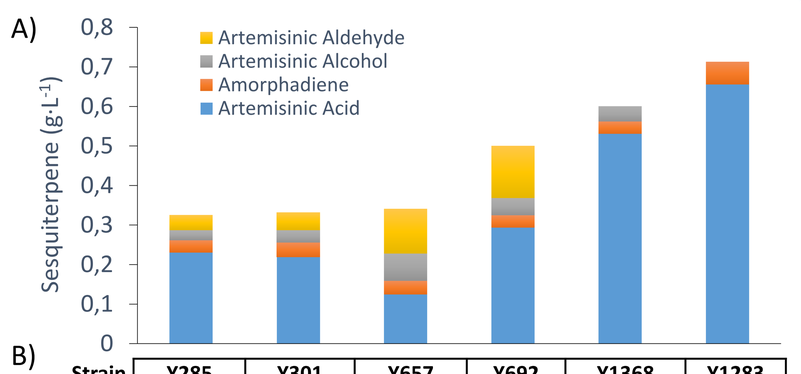
Heterologous expression of codon-optimised ADS and CYP71AV1

Integration and repression of codon-optimised CPR1

Integration and overexpression of three additional codon-optimised plant enzymes (CYB5, ADH1 and ALDH1)

Genetic engineering tools

- **Cassettes.** Expression cassettes were obtained with overlapping PCR. These cassettes contained homologous fragments for targeted integration.
- **Markers.** Drug resistance markers (*dsdA*, *natA*, *kanA* and *hphA*) and auxotrophic markers (LEU2, URA3 and HIS3) have been used for strain selection.
- **Promoters.** P_{GAL1} and P_{GAL10} have been used for the overexpression of all genes from the mevalonate pathway. A strong promoter (P_{GAL7}) induced CYB5, ALDH1 and ADH1 overexpression and a weaker promoter (P_{GAL3}), reduced CPR expression. Deletion of *gal80* allowed constitutive expression of all galactose-regulated enzymes. The MET3 promoter was replaced with the copper-regulated CTR3 promoter.



B)

Strain	Y285	Y301	Y657	Y692	Y1368	Y1283
ERG9 Repression	PMET3			PCTR3		
CPR1 Expression	High			Low		
CYB5 Expression	No			Yes		
ALDH1 Expression	No				Yes	
ADH1 Expression	No					Yes

Figure 4. *Production of artemisinic acid and other pathway intermediates in shake-flask cultures by different engineered strains (A) and sequential steps to obtain each engineered strain[2] (B) (Adapted from Paddon et al*).

Overall production: **25 g·L^{-1} of artemisinic acid**

Sanofi announced in April, 2013 the launch of large-scale production of semisynthetic artemisinin. They plan to produce, on average, from **50 to 60 tons** from 2014 onwards in order to guarantee enough supply for up to **150 million ACT treatments.**

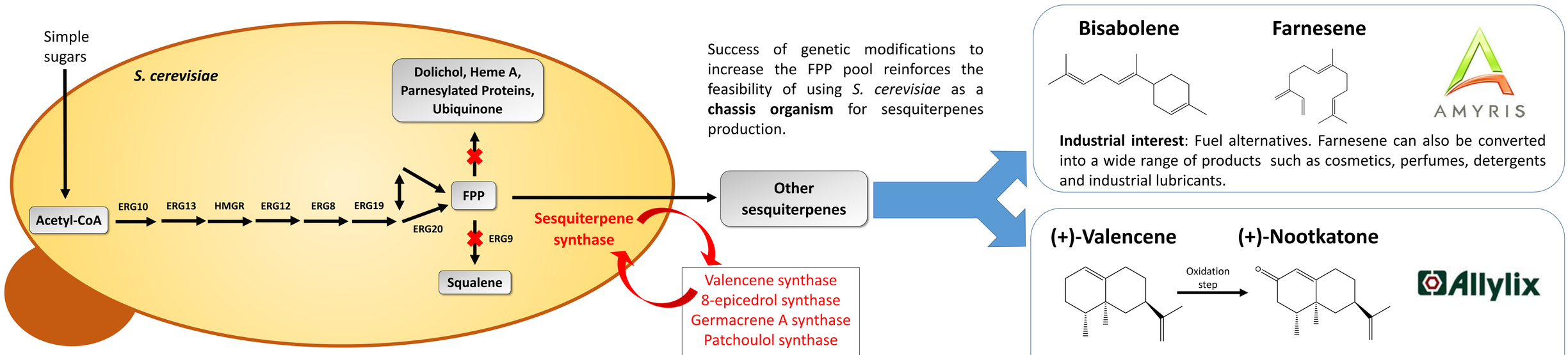


Figure 5. *Schematic representation of S. cerevisiae as a chassis organism for sesquiterpenes production.*

Success of genetic modifications to increase the FPP pool reinforces the feasibility of using *S. cerevisiae* as a **chassis organism** for sesquiterpenes production.

Bisabolene **Farnesene**

AMYRIS

Industrial interest: Fuel alternatives. Farnesene can also be converted into a wide range of products such as cosmetics, perfumes, detergents and industrial lubricants.

(+)-Valencene **(+)-Nootkatone**

Allylix

Industrial interest: Flavours, fragrances and insect repellents.

Conclusions

- Different strategies and tools have been employed to engineer the mevalonate pathway of *S. cerevisiae* for FPP overproduction and artemisinic acid production. Furthermore, they have increased the efficiency of amorphadiene conversion to artemisinic acid and have paved the way for a viable large-scale production of artemisinin.
- Use of synthetic biology and metabolic engineering have allowed the assembly of the complete pathway for artemisinic acid production in a *S. cerevisiae* strain.
- Similarities in genetic modifications between valencene, bisabolene, farnesene and artemisinic acid strain producers support that *S. cerevisiae* could be used as a *cell factory platform* for sesquiterpenes production.

Bibliography

[1] Ro D-K, Paradise EM, Ouellet M, et al. Production of the antimalarial drug precursor artemisinic acid in engineered yeast. *Nature*. 2006;440:940–3.

[2] Paddon CJ, Westfall PJ, Pitera DJ, et al. High-level semi-synthetic production of the potent antimalarial artemisinin. *Nature*. 2013;496:528–32.