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Heterologous expression of polyketides in fungi and optimization by an *in silico* guided approach

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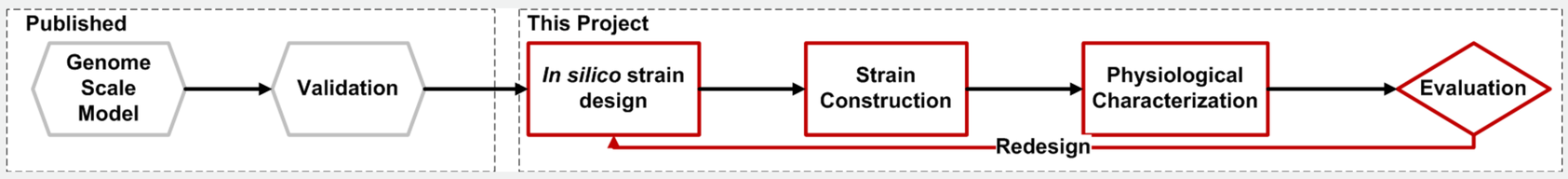
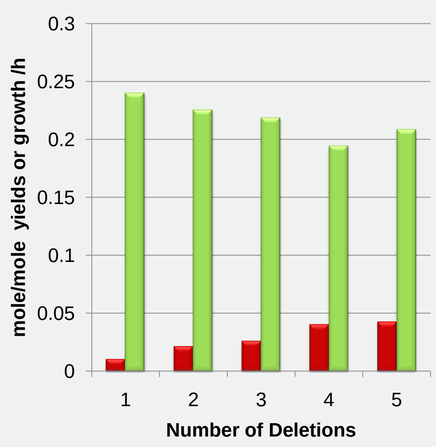
Polyketides are the source of the most potent antibiotics and anticancer agents available today. They constitute a large and diverse group of natural compounds produced by plants, fungi and bacteria. However, the productivity is often very low in the native producer. Thus, expression of polyketide biosynthesis genes in relevant cell factories has a great potential. Towards fulfilling this goal we have chosen the two fungal model species *Saccharomyces cerevisiae* and *Aspergillus nidulans,* where numerous genetic tools are available. The overall aim of the project is to construct microbial super hosts for polyketide production through the use of state of the art genetic engineering and *in silico* modeling tools.

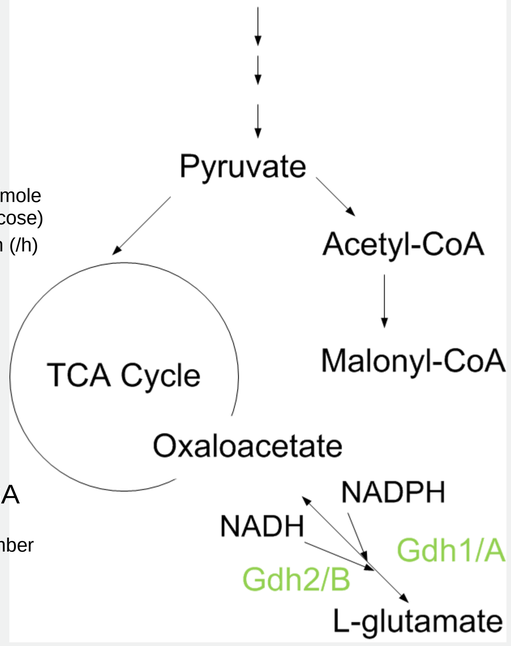
Project strategy



Modeling

By using a stoichiometric model of *S. cerevisiae* and the algorithm OptGene, we were able to predict several gene deletions that should improve the production of 6-methylsalicylic acid (6-MSA)[1].



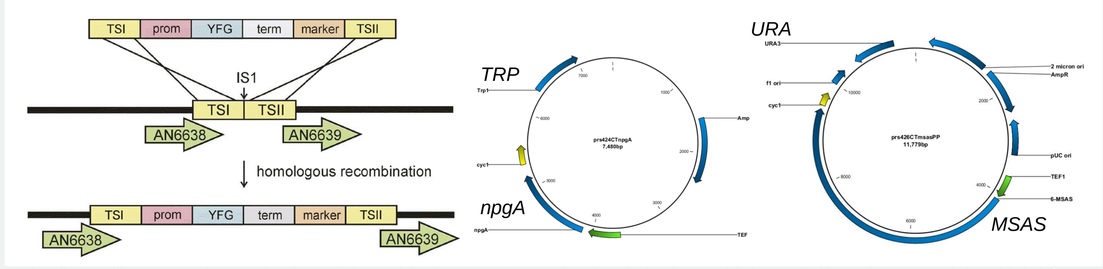


Acetyl-CoA + 3 Malonyl-CoA + NADPH → 6-MSA

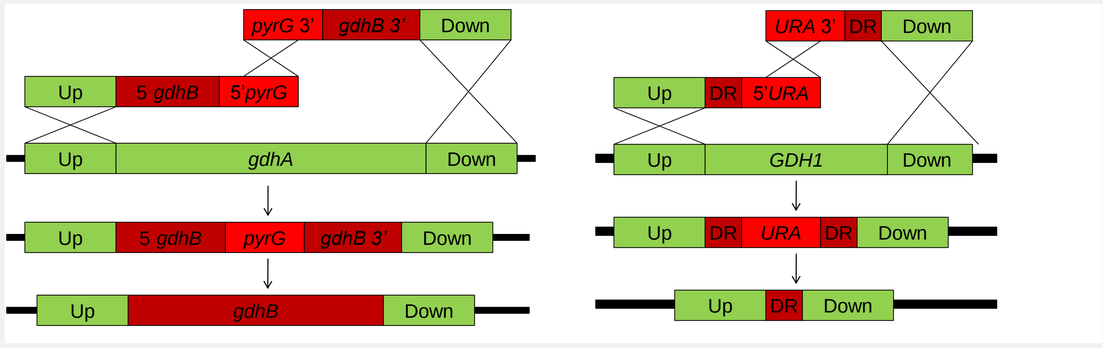
The predicted improvement in yield of 6-MSA as a function of the number of deletions. The stoichiometric reaction for 6-MSA and a view of metabolism related to the predicted deletions.

Strain Construction

6-MSA production was established in *S. cerevisiae* and *A. nidulans* followed by the predicted deletion of the gene encoding glutamate dehydrogenase 1 (*GDH1 and gdhA*, respectively). This was coupled to the insertion of the respective *GDH2* and *gdhB* [2, 3].



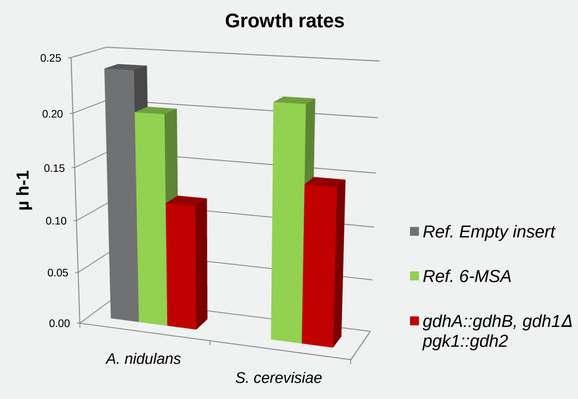
The figure illustrates the method used for achieving 6-MSA production in both *A. nidulans* (left) and *S. cerevisiae (*right) [2, 4].



The gene targeting method used for deleting *gdhA* and *GDH1* in *A. nidulans* and *S. cerevisiae*, respectively [3]. For *A. nidulans* the deletion was combined with the insertion of *gdhB* whereas GDH2 was overexpressed by a promoter exchange in *S. cerevisiae.*

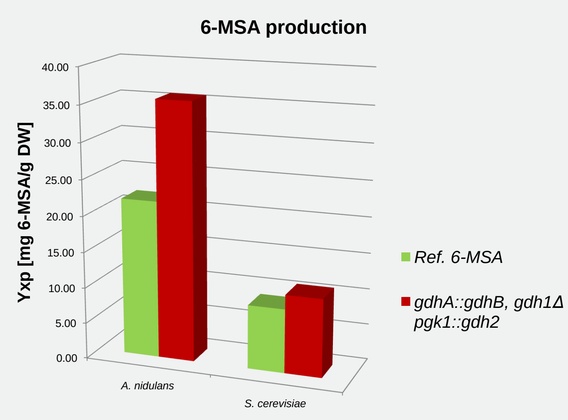
Physiological Characterization

Removal of Gdh1 (or GdhA) decreased the growth rate of both *S. cerevisiae* and *A. nidulans*. However the yield of 6-MSA was improved. In *A. nidulans* the yield was increased by 63%.



	A. nidulans	*S. cerevisiae*
Ref. Empty insert	*0.24*	
Ref. 6-MSA	*0.20*	*0.22*
gdhA::gdhB, gdh1Δ pgk1::gdh2	*0.12*	*0.15*

The effects of the deletion of Gdh1/A on growth rate of both *S. cerevisiae* and *A. nidulans*.



	A. nidulans	*S. cerevisiae*
Ref. 6-MSA	*21.91*	*8.90*
gdhA::gdhB, gdh1Δ pgk1::gdh2	*35.69*	*10.90*

The improved yield of 6-MSA due to the deletion of Gdh1/A.

Conclusion

The predictions obtained by modelling were validated in both *S. cerevisiae* and *A. nidulans*. The yield of 6-MSA was improved for both strains. Thus it has been shown that this method has great potential for the future design of efficient cell factories.

References

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[3] Nielsen, J.B., Nielsen, M.L., Mortensen, U.H, **Transiently disrupted non-homologous end joining pathway in Aspergillus nidulans allows simple and efficient gene targeting. A 'Technological Advancement,** Fungal Genetics Biology, 2008, 45: 165-170..

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