In Silico evaluation of metabolic pathways for the production of 4'-O-methylnorbelladine in *Saccharomyces cerevisiae*

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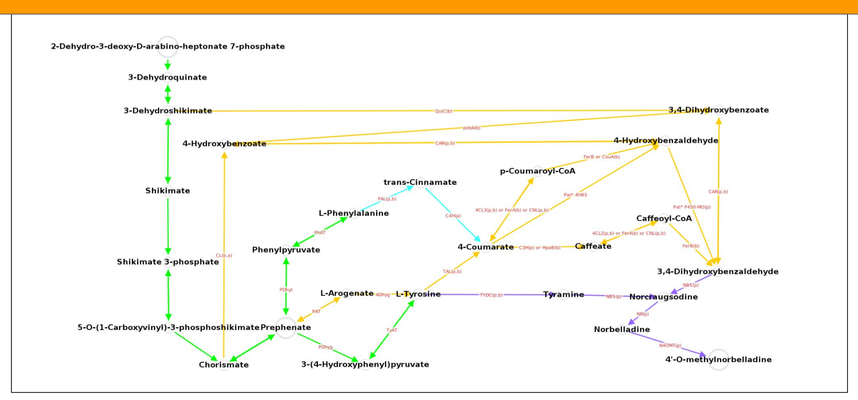
ABSTRACT

Galantamine is an alkaloid used in the treatment of Alzheimer's disease. However, its current method of production requires extraction from plants from *Amaryllidaceae* family, leading to low yields for industrial standards. Its biosynthesis is complex and not fully known, but there are identified pathways to one of its precursors 4'-O-methylnorbelladine. *Saccharomyces cerevisiae* can be a suitable cell host for the heterologous production of 4'-O-methylnorbelladine for its standard usage and tolerance to industrial conditions. In this work, 18 previously identified alternative pathways for 4'-O-methylnorbelladine and the native pathway were assessed against a decision matrix which took into account 4 criteria: metabolic risk, pathway knowledge risk, enzymatic risk and additional requirements risk. The best pathways with the minimum risk score were selected for optimization along with the native pathway for comparison. The Optknock algorithm was used to identify possible knockouts of reaction that would lead to an increased production of 4'-O-methylnorbelladine. The results showed that alternative pathways can provide a better yield of the target metabolite while also having a lesser associated risk in the decision matrix than the native pathway.

INTRODUCTION

- The alkaloid galantamine is an inhibitor of acetylcholinesterase, an enzyme that breaks down the neurotransmitter acetylcholine[1]. For this reason, galantamine is used as a treatment for Alzhaimer's disease[2]. It is naturally produced by members of the *Amaryllidaceae* family[3].
- Its organic synthesis has been a challenge due its chemical complexity, limiting its production to solely plants. As a consequence, crop competition and naturally low production of galantamine in these plants have resulted in yields that are considered low for industrial standards[4].
- The metabolic pathway leading to the biosynthesis of galantamine is not fully known. According to the current literature[5], There is an established pathway leading to 4'-O-methylnorbelladine.
- *S. cerevisiae* is an Eukaryotic model organism that has been extensively researched and is widely used in industrial applications for their tolerance to industrial conditions[6] and it's proven to be a viable host for a cell factory to produce heterologous proteins[7].
- 4'-O-methylnorbelladine can be produced from reactions that derive from the central metabolism of *S. cerevisiae*. Being able to produce this compound in an heterologous microorganism could be a first step to the large-scale synthesis of galantamine.

METHODS



18 PATHWAY CANDIDATES + NATIVE PATHWAY

DECISION MATRIX

METABOLIC RISK	PATHWAY KNOWLEDGE RISK	ENZYMATIC RISK	ADDITIONAL REQUIREMENTS RISK

MINIMIZE RISK ASSESSMENT

OPTKNOCK OPTIMIZATION

RESULTS

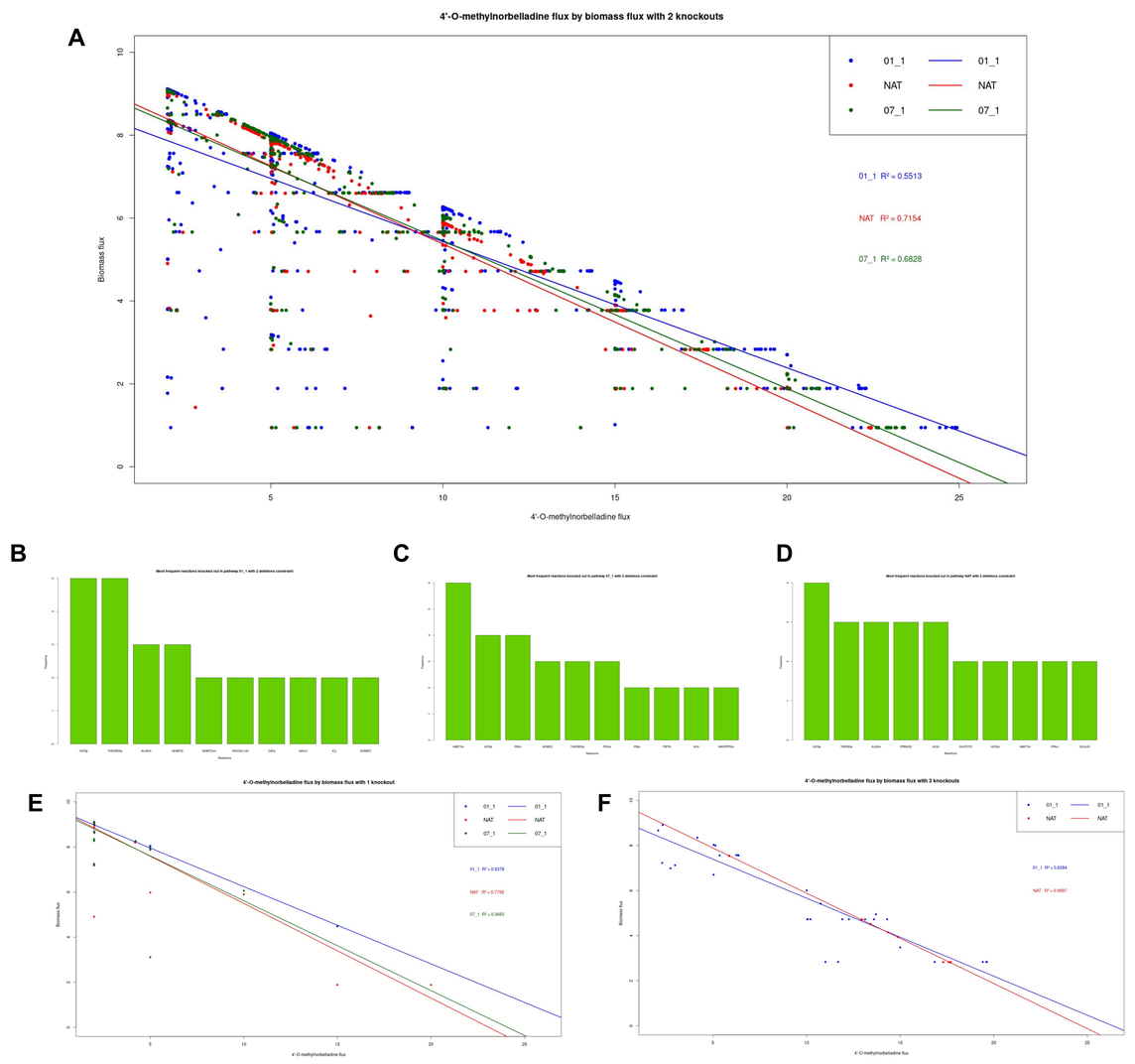


Figure 1. A) 4'-O-methylnorbelladine flux by biomass flux of pathways 01_1, 07_1 and NAT with 2 deletions constraint. **B) C) D)** Frequency of most knocked reactions in pathway 01_1, 07_1 and NAT, respectively with 2 deletions and greater than 20 4'-O-methylnorbelladine flux constraint. **E)** 4'-O-methylnorbelladine flux by biomass flux of pathways 01_1, 07_1 and NAT with 1 deletion constraint. **F)** 4'-O-methylnorbelladine flux by biomass flux of pathways 01_1 and NAT with 3 deletions constraint.

CONCLUSIONS

- Different pathways present a different biomass/target tradeoff.
- Knockout optimization is dependent on pathway. Some can present more different solutions than others given the same set of constraints. The abundance of knocked reactions vary between pathways.
- Alternative pathways can provide a better yield of 4'-O-methylnorbelladine.
- Alternative pathways can have less risk associated with its construction.
- It is worth to search for alternative pathways as they can provide more viable implementations.

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