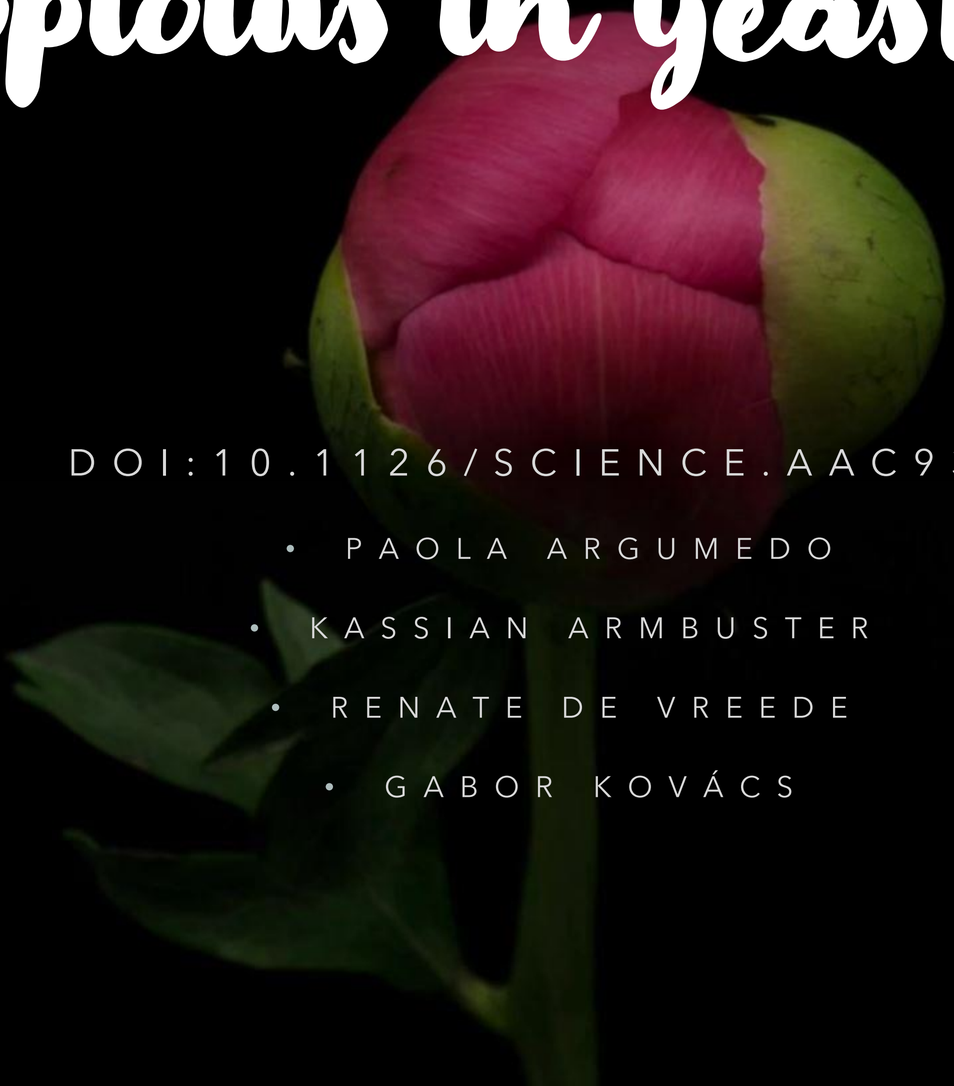
Complete biosynthesis of opioids in yeast



DOI:10.1126/SCIENCE.AAC9373

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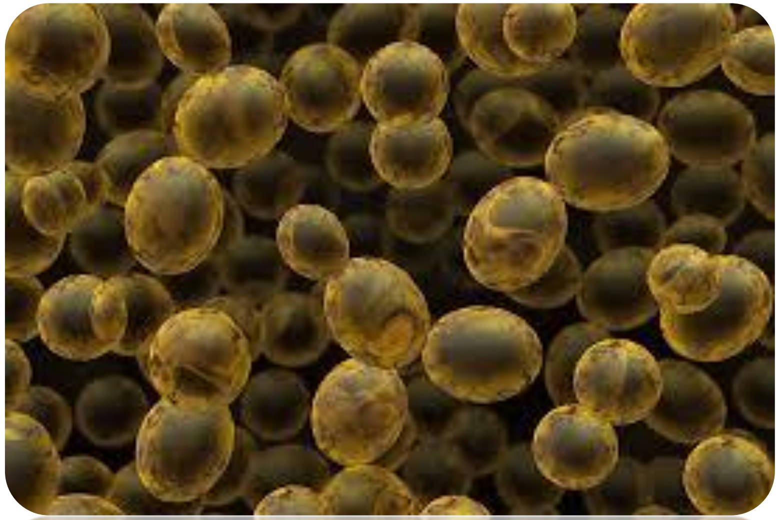
Motivation

- Opium is the primary drug in western medicine for pain management
- Still mostly produced from poppy -> cause problems
- New idea: Producing opium from yeast

Past

Future



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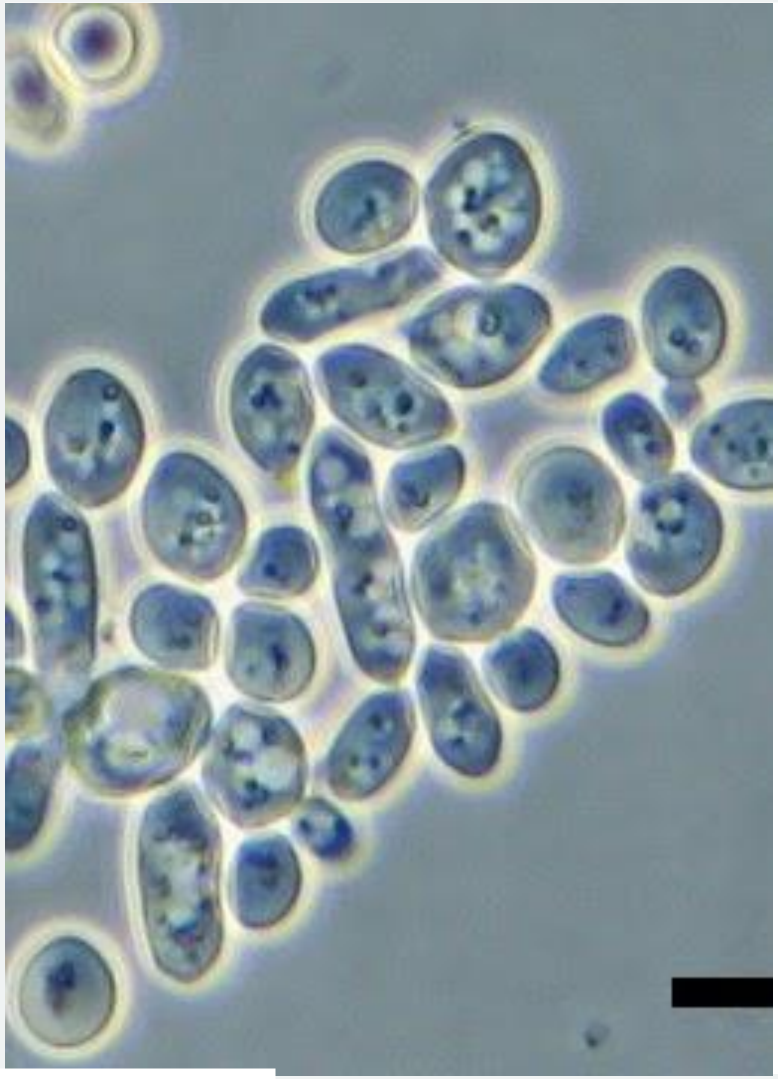
[1] https://www.thenewhumanitarian.org/fr/node/249968
[2] https://www.allaboutfeed.net/animal-feed/feed-additives/are-yeast-cell-wall-products-the-same-from-different-sources/



Methodology

- Map the pathways
- Engineered yeast to produce BIAs
- Improve the production
- Find proteins that will finish the opioid
- Improve the yeast

Module 1

Increase accumulation of L-tyrosine and 4-hydroxyphenylacetaldehyde (4-HPAA)

- 3-deoxy-D-*arabino*-2-heptulosonic acid 7-phosphate (DAHP) synthase
- chorismate mutase (Aro4p^{Q166K}, Aro7p^{T226I})
- phenylpyruvate decarboxylase (Aro10p)

Module 2

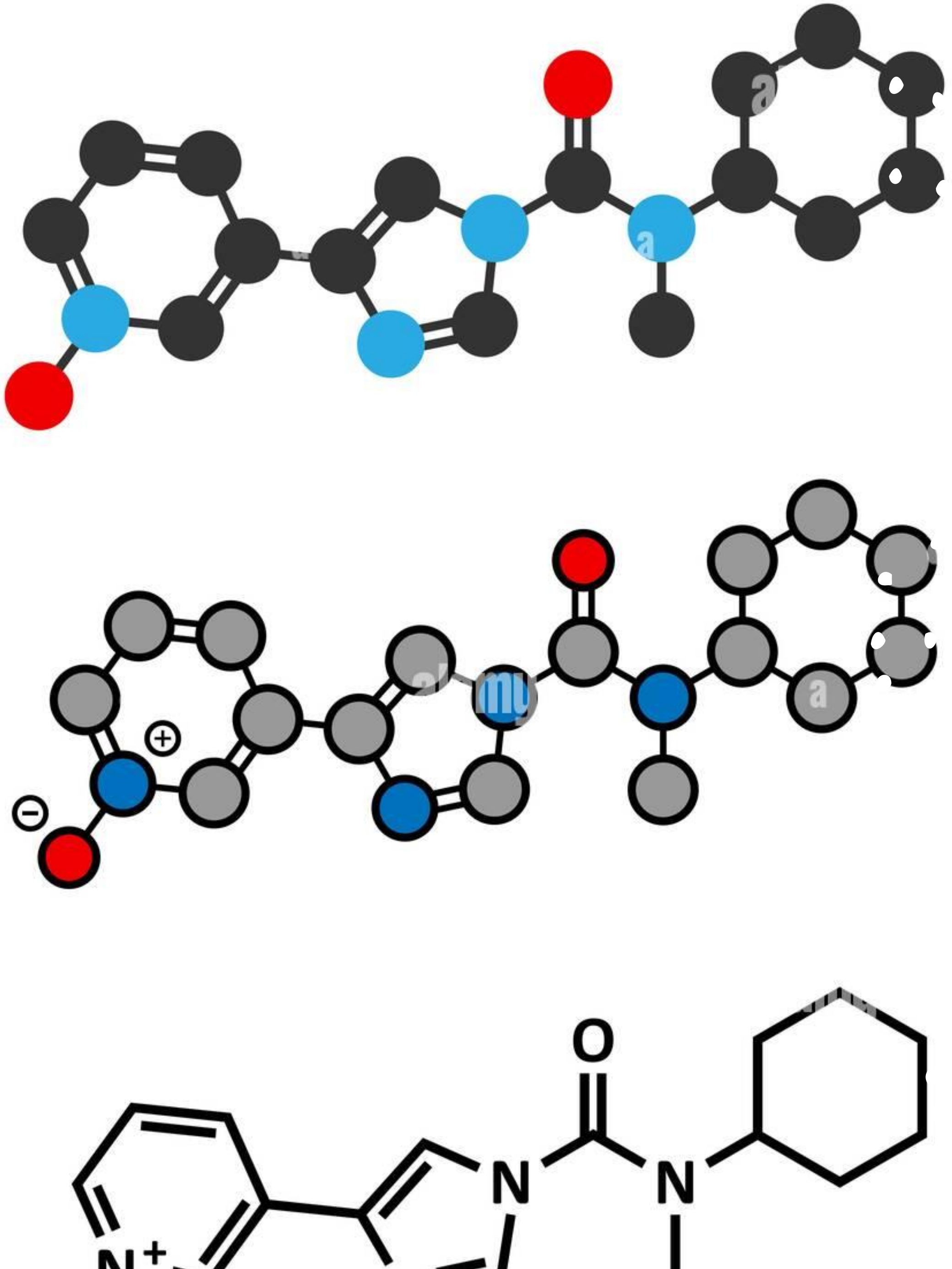
Synthesize and recycle a mammalian redox cofactor

- sepiapterin reductase (SepR)
- 6-pyruvoyl tetrahydrobiopterin synthase (PTPS)
- quinonoid dihydropteridine reductase (QDHPR)
- carbinolamine dehydratase (PCD).

Module 3

Synthesize the first BIA backbone molecule

- Mutant of tyrosine hydroxylase ($TyrH^{WR}$)
- Dihydrofolate reductase (DHFR)
- DOPA decarboxylase (DoDC)
- Norcoclaurine synthase (NCS)



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Module 4

Synthesize the key BIA branchpoint molecule

- Norcoclaurine 6-*O*-methyltransferase (6OMT)
- Coclaurine-*N*-methyltransferase (CNMT)
- 4′-*O*-methyltransferase (4′OMT)
- Cytochrome P450 reductase (CPR)
- *N*-methylcoclaurine hydroxylase (NMCH)

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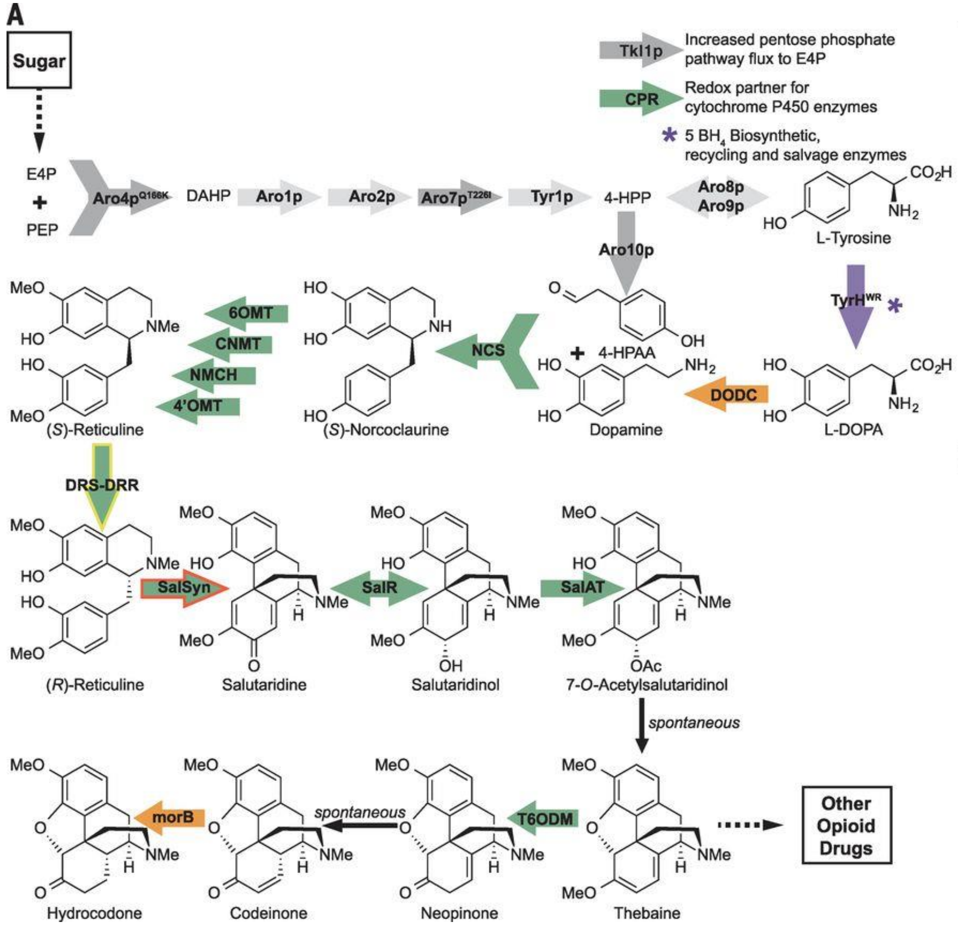
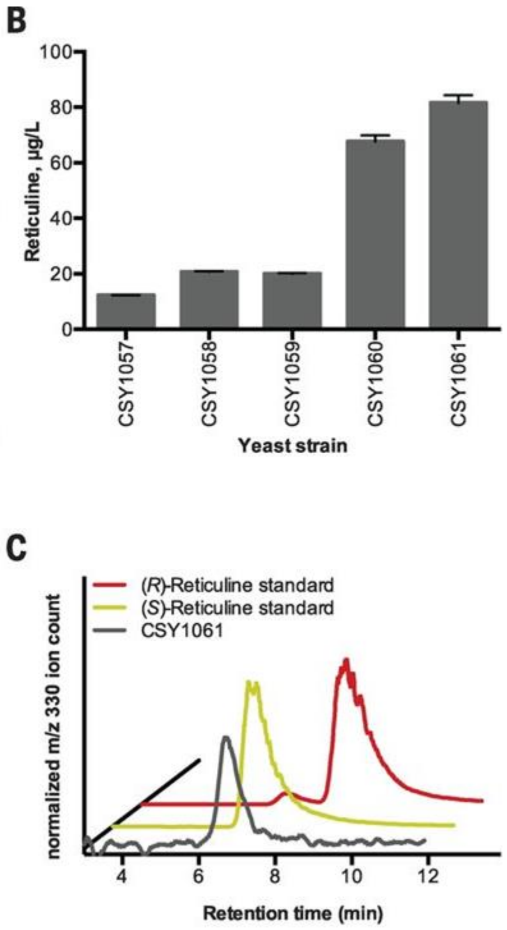


Biosynthetic scheme for production of thebaine and hydrocodone from sugar

Experimentation



Performed in a wild-type haploid strain CEN.PK2

- CYS1057 (m II-IV) - production rate 12.3 mg/L
- CYS1058 (m I-IV) - production rate 20.7 mg/L
- CYS1059 (m I-IV+Aro10p) production rate 20 mg/L

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Module 5

Bottleneck module

Over expression of $TyrH^{WR}$, 4′OMT, and NCS

Experimentation

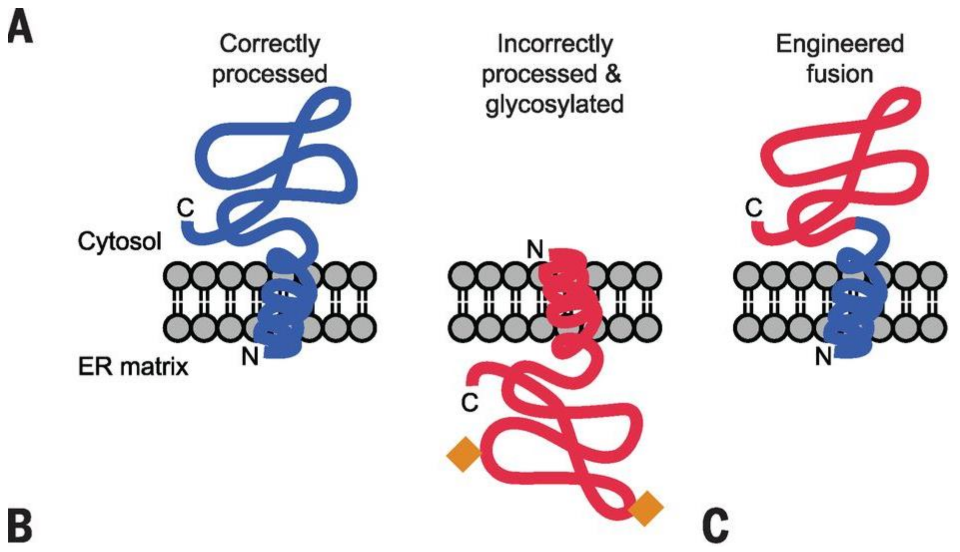
CYS1060 (just over expression)

CYS1061(eliminate *zwf1*) - production rate 82 mg/L

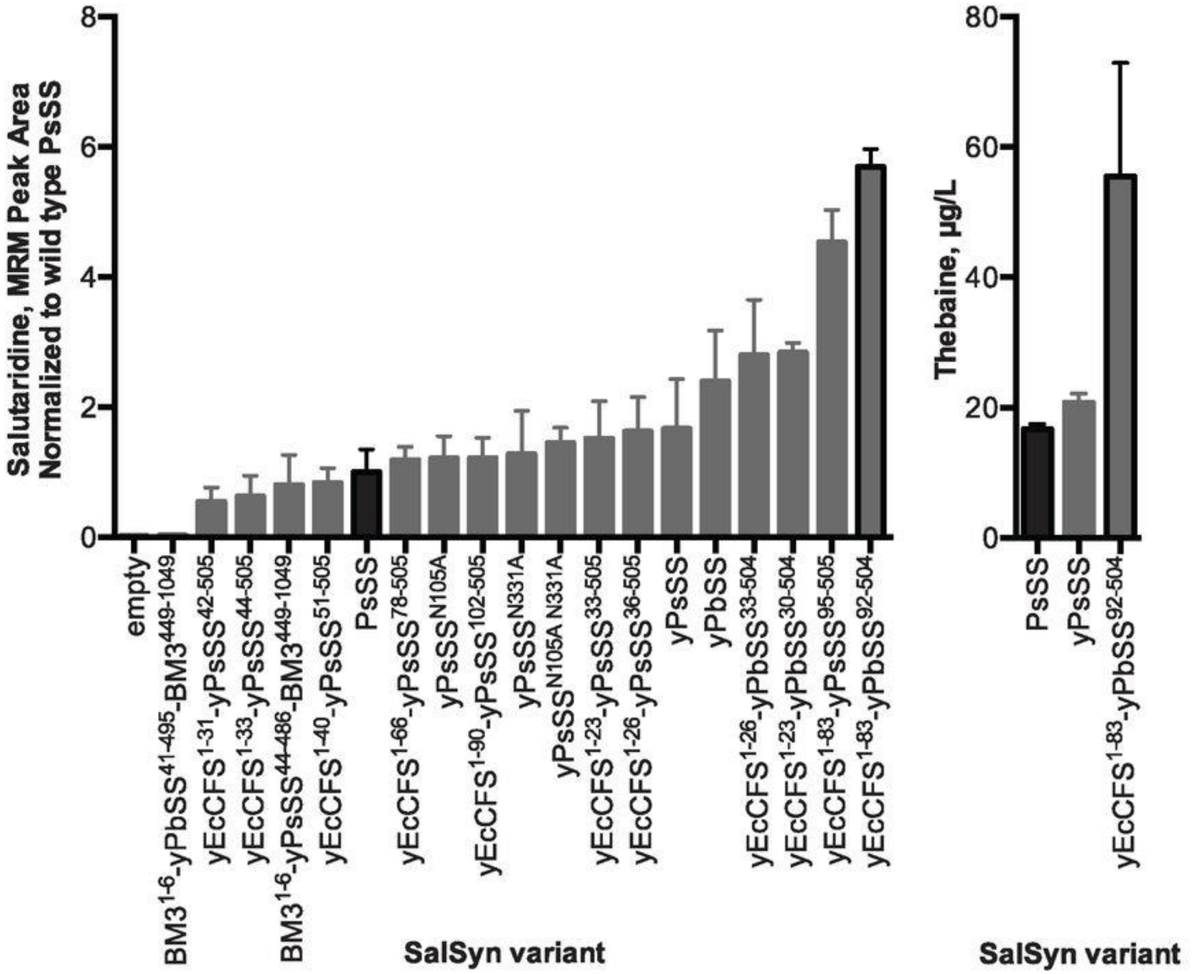
The protein

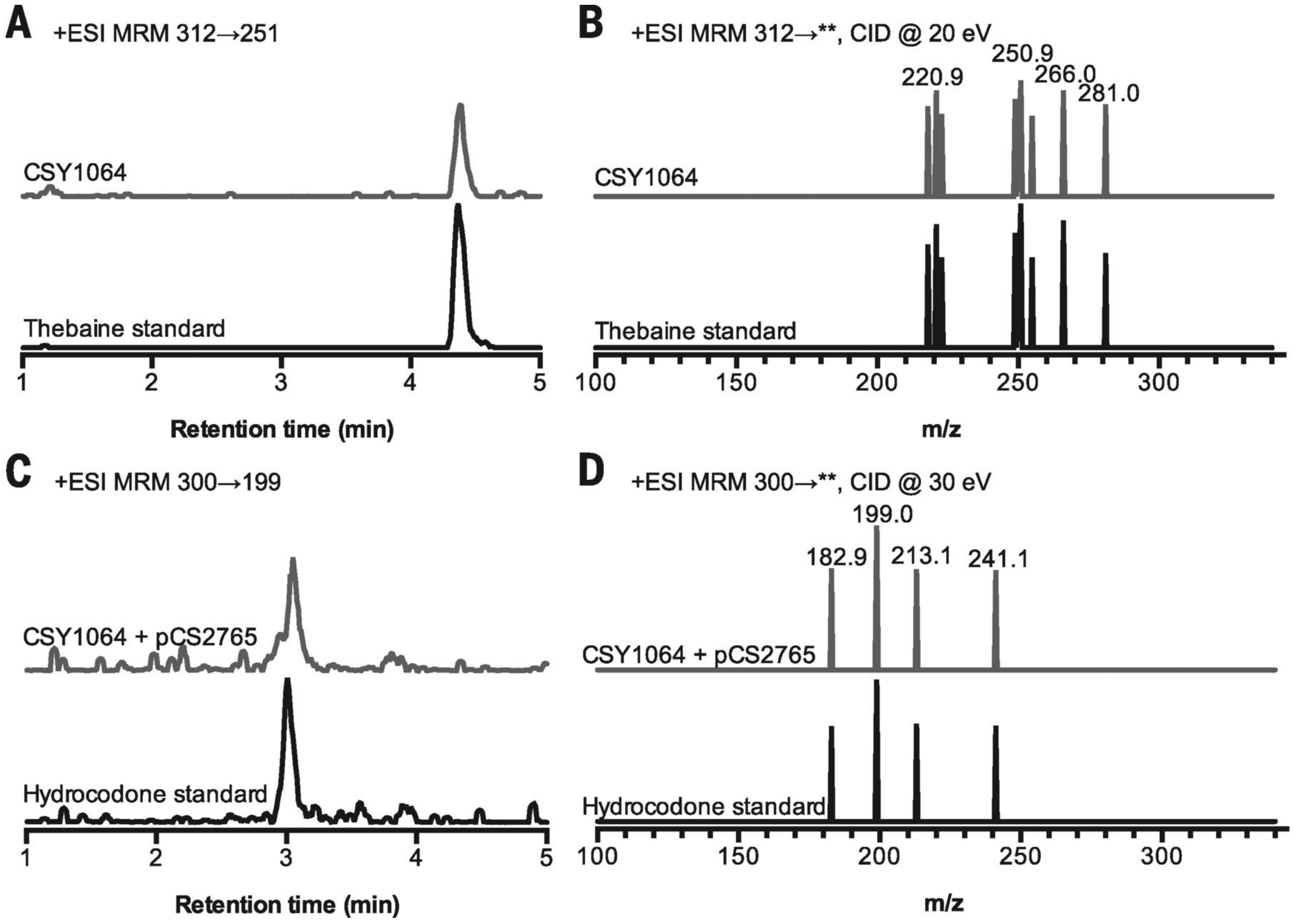
1,2-dehydroreticuline synthase (DRS) and 1,2-dehydroreticuline reductase (DRR) enzymes

CYS1071 - 17 µg/L (thebaine) when cultured with 1 mM *rac*-norlaudanosoline for 96 hours



Comparison of salutaridine produced and Comparison of thebaine produced





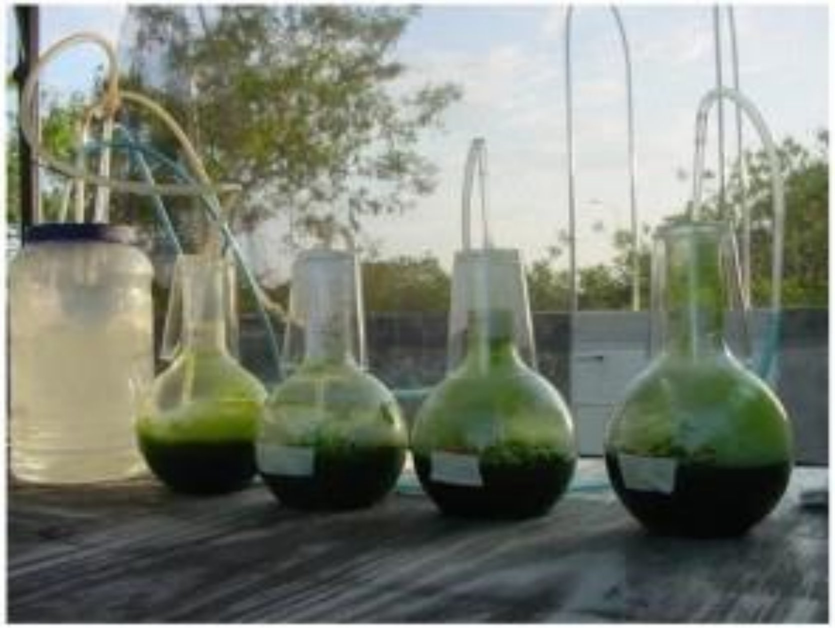
Chromatograms of thebaine and hydrocodone in CSY1064(+pCS276)

The results

- Thebaine: 6.4 ± 0.3 μg/liter
- Hydrocodone: ~0.3 μg/liter
- Target: 5 g/liter

What about homebrewing?

- Feasibility
- Not just a matter of scaling up



Novel research

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Future outlook

Thank you for your attenttion