Frankfurt 2013

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References

Frankfurt: Night of Science

Berlin: Biotechnology 2020+

London: SB 6.0

Brussels: IDTNDNA

Economics

- Assumptions
  - Yeast: 0.138 g per l cells of GGOH after 100 h
  - -> per 31 g 19 l cells of GGOH after 3 months (12x 100 h)
- Stevia plant: 31 g per kg leaves after 3 months
- BioEthics: shift of wealth to first world countries
- IP: 2 patents by Evolva and Jim Brandle
international Genetically Engineered Machine competition

Biosynthesis of a Steviolglycoside

- Few calories
- No delay of satiety
- Anticariogenic
- Antidiabetic activity
- Anti-hyperglycemic
- pH-stable
- Heat-stable
First step of overexpression in the mevalonate pathway:
1.) HMG1
2.) Mevalonate pathway
3.) GGOH

Overproduction of GGOH by metabolically engineered S. cerevisiae
Tokuhiro 2009
Two at one blow:
Bifunctional GGPP/CPP Cyclase

- Two domains
- CPP synthase activity & Ent-kaurene synthase activity
- Present in Gibberellin producing fungi and some liverworts

Fungi: Gibberella fujikuroi [1,3,4], Phaeosphaeria spp., Neurospora crassa [2], Esilinöe brasiliensis [3] (Syn. Sphaceloma manihoticola);

Liverworts: Jungermannia subulata [5], Physcomitrella patens [6]

References:
Stevia KO1 has complete catalytic activity [in S. cerevisiae] for the three step conversion of ent-kaurene to ent-kaurenoic acid. Humphrey, 2006

Alternative: Expression of a different KO from plant or fungal origin (GA producing fungi)
ent-Kaurenoic acid
13-hydroxylase

"Molecular cloning of a cytochrome P450 ent-kaurenoic acid-13-hydroxylase involved in steviol biosynthesis in Stevia rebaudiana (Bertoni)."
Kumar H., Kumar S., Ahuja P.S.
Submitted (MAY-2008) to the EMBL/GenBank/DDBJ databases

Expression in S. cerevisiae patented by J. Brandle
"Compositions having ent-kaurenoic acid 13-hydroxylase activity and methods for producing same"
4 glycosylation steps

It appears that the many plant UGTs that now exist have evolved from a single ancestral sequence and through time were recruited to new functions (Paquette et al., 2003).

- Repeat EST profiling (Richman et al. 2005)
- Try out UGTs of different organisms, but might be highly specific

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Steviol

UGT85C2

Steviolmonoside

UGT74G1

Steviolbioside

Steviobioside

Stevioside

UGT76G1

Rebaudioside A
Methods

Assembly of second plasmid

PCR of
- copalylprophosphate synthase
- ent-kaurene oxidase
- ent-kaurenoic acid hydroxylase

Construction of a BioBrick

Digest with EcoRI and PstI
- ent-kaurenoic acid hydroxylase
- pSB1C3 plasmid

Ligation

Transformation

Plasmid preparation

Restriction digest

Sequencing

Analytics

Yeast transformation

Cell lysis with glassbeats

Dephosphorylation of GGPP

Hexane extraction

Derivatisation

Analytics with GC-MS

Plasmid preparation

E.coli transformation

Plasmid preparation

Restriction digest

Sequencing

Assembly through homologue recombination in yeast

Wrong digest result
Results

PCR fragments of steviol plasmid

Control digest of GGPP Plasmid

Analytics of GGPP with GC-MS

Fig. 1: Rapid GC-MS HR analysis of transgenic leaves for γ-lactone decomposition. (+)-trans-β-lactone, (+)-trans-γ-lactone; (−)-trans-β-lactone, (−)-trans-γ-lactone. (C135) 1.341 g, 1.241 g, 1.341 g; (C134) 1.241 g, 1.341 g, 1.241 g. See Table 1 for further analysis.
Economics

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Human practice

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London: SB 6.0

Brüssel: IDTDNA
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References

Pia Dahm. Metabolic engineering of the Taxolbiosynthese in Saccharomyces cerevisiae. 2011