Introduction
Introduction
How rubber is made

CO₂

Citric acid cycle
Acetyl-CoA
Acetoacetyl-CoA

MVA pathway
IPP
DMAPP

HRT2

Rubber
CO₂ → Citric acid cycle → Acetyl-Coa → Acetoacetyl-Coa → MVA pathway → IPP → DMAPP → HRT2 → Rubber
Bacterial rubber

Nutrient

Glycolysis

Pyruvate → GAP

MEP pathway

IPP → DMAPP → HRT2

Rubber
Modeling the MEP pathway

Pyruvate + Glyceraldehyde-3-phosphate → Dxs → 1-Deoxy-D-xylulose-5-phosphate

Vitamin B₁/B₆ → 2-C-methyl-D-erythritol-2,4-cyclodiphosphate

NADPH → IspG → 4-Hydroxy-3-methyl-butenyl-1-diphosphate

IPP ↔ DMAPP
Modeling the MEP pathway

Pyruvate + Glyceraldehyde-3-phosphate → 1-Deoxy-d-xylulose-5-phosphate → 2-C-methyl-d-erythritol-2,4-cyclodiphosphate → 4-Hydroxy-3-methyl-butenyl-1-diphosphate → IPP

Dxs

CO₂

Vitamin B₁/B₆

NADPH

NADP⁺

IspG

IPP ↔ DMAPP
Modeling the MEP pathway

Pyruvate + Glyceraldehyde-3-phosphate

CO$_2$

Dxs

1-Deoxy-β-xylulose-5-phosphate

Vitamin B$_1$/B$_6$

2-C-methyl-β-erythritol-2,4-cyclodiphosphate

NADPH

IspG

NADP$^+$

4-Hydroxy-3-methyl-buteryl-1-diphosphate

IPP

DMAPP
Design of System

Dxs/IspG

HRT2
Dxs/IspG

Diagram showing the relationship between lacl, Plac, dxs, and ispG.
Design of System

Dxs/IspG

HRT2
Expression validation

Expression from lactose  Expression from arabinose
promoter

Promoter
Expression validation

Expression from lactose promoter  Expression from arabinose promoter
% of population above fluorescence threshold

-150  -90   -30   30   90   150

% Parent

Time (min)

+lacI(LVA) -IPTG
+lacI(LVA)+IPTG
+lacI(N) -IPTG
+lacI(N)+IPTG
Expression validation

Expression from lactose promoter

Expression from arabinose promoter
MEP pathway optimization - Characterization

Method validation
Method validation

DMAPP standard curve

$r^2 = 0.9978$

DMAPP $\rightarrow$ H$_2$SO$_4$ $\rightarrow$ Isoprene
MEP pathway optimization
- Characterization

*Method validation*
Results: Rubber production

Table of integrals:

- 3.73 ppm ethanol
- 2.17 ppm acetone
- 1.50 ppm water
- 1.33 ppm ethanol
- 1.26 ppm n-hexane, ethanol
- 0.88 ppm n-hexane

![Diagram of rubber production results with peaks at specific ppm values.](image-url)
Table of impurities
3.73 ppm ethanol
2.17 ppm acetone
1.55 ppm water
1.33 ppm ethanol
1.26 ppm n-hexane, ethanol
0.88 ppm n-hexane

Chemical structure diagram and NMR spectrum.
An intuitive wiki

"Good research is of little value without great exposure"

Introduction
Rubber demand, a new approach, and what we accomplished

Rising global demand for quality rubber calls for innovative ways of satisfying needs without compromising the environment. Natural rubber is produced by draining the rubber tree, and the establishment of new rubber plantations causes deforestation of the rainforest and occupation of arable lands.

We thought of an environmentally friendly approach. Imagine a world where natural rubber is produced by gene-manipulated bacteria; perhaps inside fermentors that can be placed underground or in barren environments unsuitable for agriculture.

We introduced natural rubber-producing abilities from the rubber tree into the widely used production bacteria, *Escherichia coli*. Moreover, we tested whether the manipulated bacteria were in fact capable of producing the natural rubber.

The result? We found strong evidence for proof of concept: rubber producing bacteria. We look forward to presenting this - both throughout this wiki and at the regional jamboree.
Bacteriorganic Rubber Summary

• Constructed a model and used it!
• Built, characterized, and submitted BioBricks, which made rubber!
• Made a wiki for everyone!

Thank you for listening

Also, thanks to
• Our sponsors University of Southern Denmark and Bionordika
• Our great advisor:
  • Ass. professor Jakob Møller-Jensen
• And our fantastic instructors:
  • Kirstine Jacobsen, Ann Zahle, and Tina Kronborg
Thank you for listening

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