



ERA CoBioTech

BIO TECH RESEARCH AND INNOVATION HACK 2021

Final seminar of the cofunded projects of ERA CoBioTech



Title: Biotechnological production of sustainable indole

Project acronym: INDIE

Name: dr. Katarina Cankar



This project has received funding from the European Union's Horizon 2020 research and innovation programme under grant 722361

28.09.2021

INDIE PARTNERS

- P1: Wageningen Plant Research, The Netherlands (dr. Katarina Cankar / dr. Dirk Bosch)
 - P2: National Institute of Biology, Slovenia (prof. Kristina Gruden)
 - P3: Bielefeld University, Germany (prof. Volker Wendisch)
 - P4: Wageningen University, The Netherlands (prof. Vitor Martins dos Santos)
 - P5: Axxence GmbH, Germany (dr. Peter van der Schaft)
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- Total project budget: 1.009.000 (total requested funding: 888.000)
 - Project start: 1.5.2018 (SI), 1.9.2018 (NL), 1.11.2018 (DE)
 - Project end date: 31st March 2022

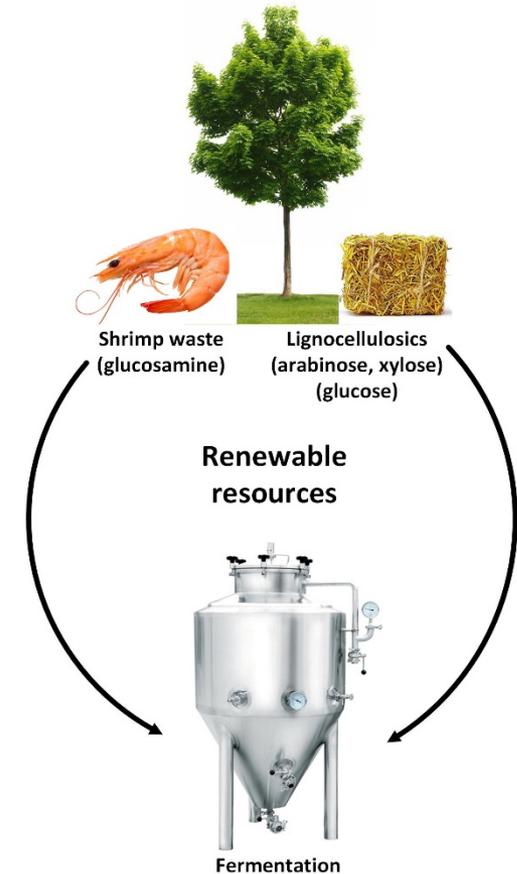
INDIE aim: to produce natural flavors in microbial cell factories

Current indole production

- 30 million €/year
- Synthetic indole is currently produced from coal tar
- Natural indole is produced by a soft chemistry conversion from tryptophan (6000 €/kg)

INDIE aims to produce natural indole

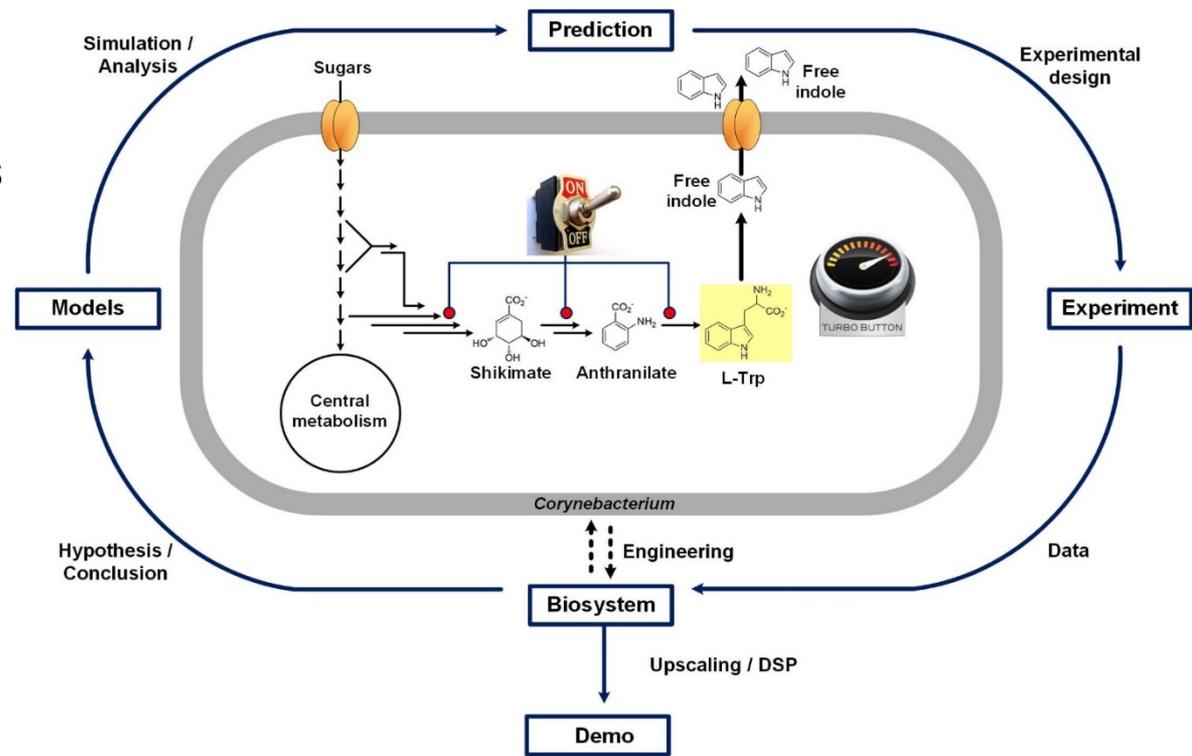
- De novo production of indole via fermentation
- Sustainable feedstocks: lignocellulose components (arabinose, xylose, glucose), glucosamine
- Safe host microorganism: GRAS bacterium *Corynebacterium glutamicum*
- Development of fermentation protocols to obtain food-grade indole



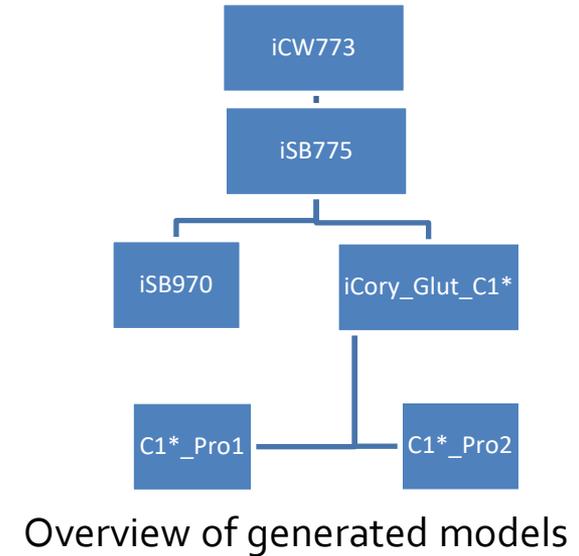
INDIE CONCEPT

- WP1: production of indole precursors
- WP2: conversion of L-trp to indole
- WP3: use of regulatory switches
- WP4: systems biology analysis
- WP5: DSP and optimisation of the fermentation process

Host microorganism: *Corynebacterium glutamicum*



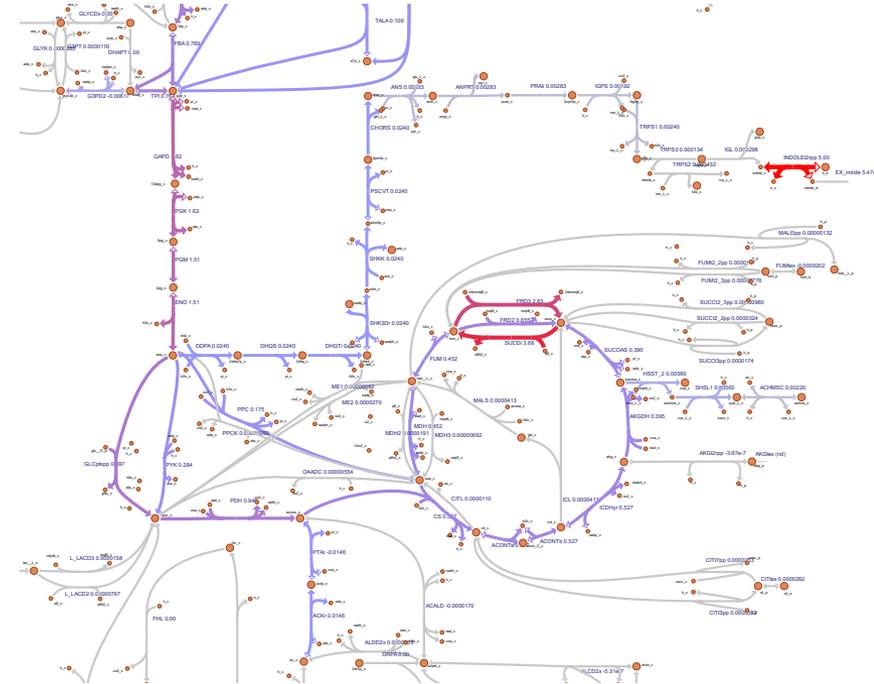
- Reduced metabolic model of *C. glutamicum* C1* strain established and curated with INDIE generated data.
- iCory_glut_C1* a general purpose model enhancing the potential of *C. glutamicum* C1* as a microbial cell factory.
- C1*_Pro1 and C1*_Pro2 are specific for Indole production



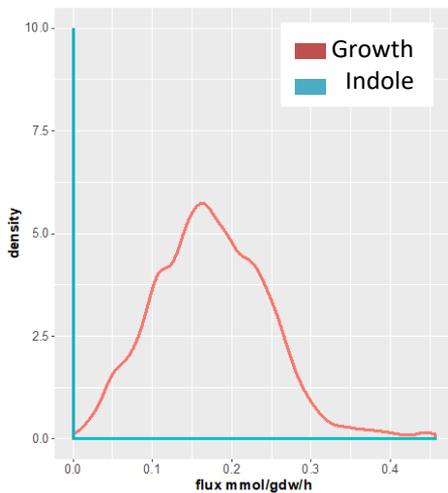
	iCW773	iSB775	iCory_glut_C1*	C1*_Pro1	C1*_Pro2
Strain	ATCC13032	ATCC13032	C1*	C1*	C1*
# genes	773	775	753	753+4	753+4
# reactions	1203	1207	1199	1199	1199
# metabolites	945	950	950	951	951

Summary characteristics of the models

- Production routes for indole modelled and evaluated:
 - Maps of metabolism of *C. glutamicum* show clear differences between growth and production conditions.
 - Comparisons of fluxes to evaluate strategies to increase production (knock out, over expression)



Simulations of fluxes through central metabolism for indole production



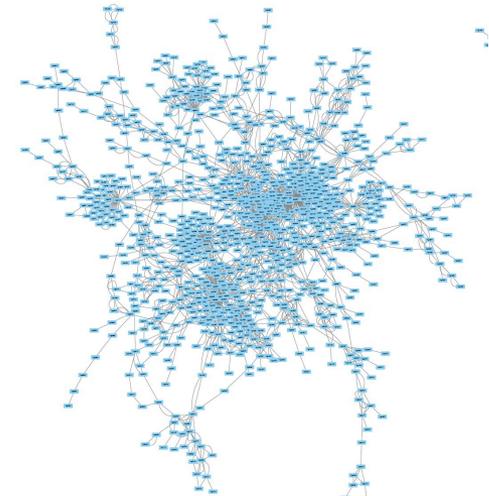
Distribution of flux through the reaction in conditions of maximal growth (red) 0.18 mmol gDW⁻¹ h⁻¹ and maximal production (blue) 0.0 mmol gDW⁻¹ h⁻¹ suggest this reaction as an excellent candidate for knock out.

Bioconversion

- Strains for bioconversion of L-tryptophan to indole
- Bioprospecting for most efficient enzymes and pathways for indole production from plants and bacteria

De novo production of indole

- (i) enhancing the carbon flux to tryptophan via the shikimate and tryptophan pathway by overexpressing endogenous and heterologous genes, feedback deregulation of key enzymes
- (ii) preventing by-product formation, especially of other aromatic compounds
- (iii) enhancing precursor supply by overexpressing genes from the pentose phosphate pathway



C. glutamicum
Control strain

C. glutamicum
INDOLE
production

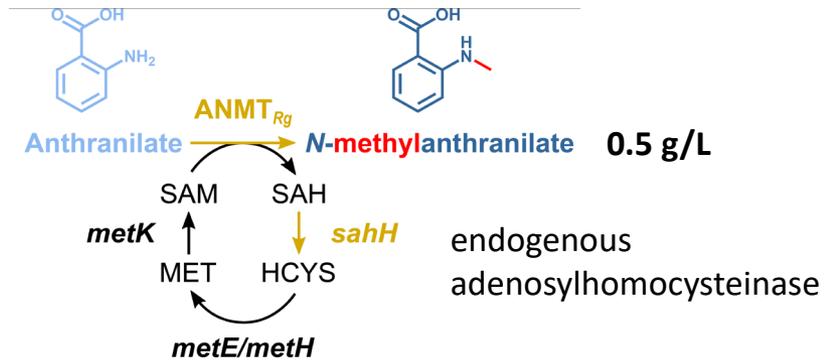
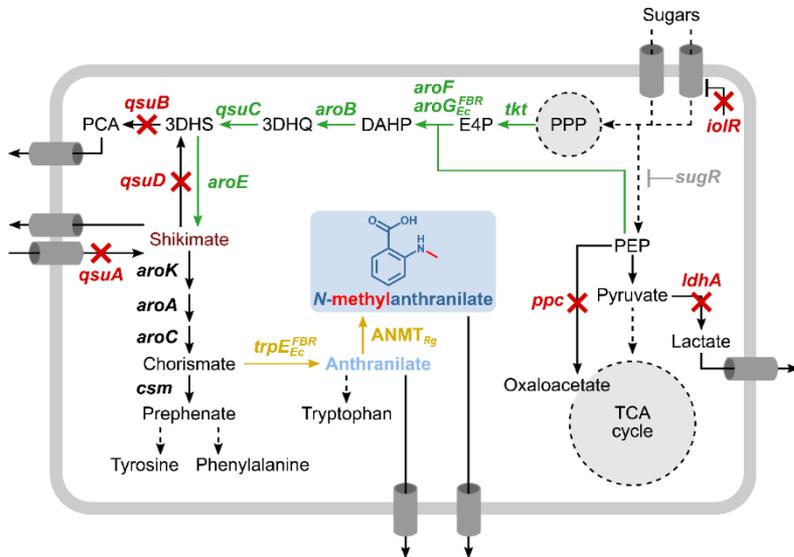


INDOLE precursor: SAM-dependent N-alkylation



SAM-dependent anthranilate N-methyltransferase from *Ruta graveolens* (acidone alkaloid synthesizing plant herb-of-grace)

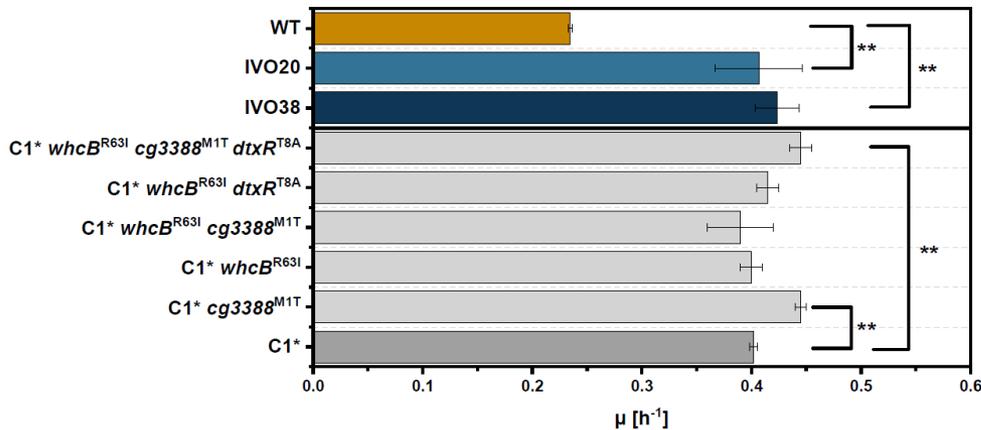
C. glutamicum engineered for overproduction of L-anthranilate



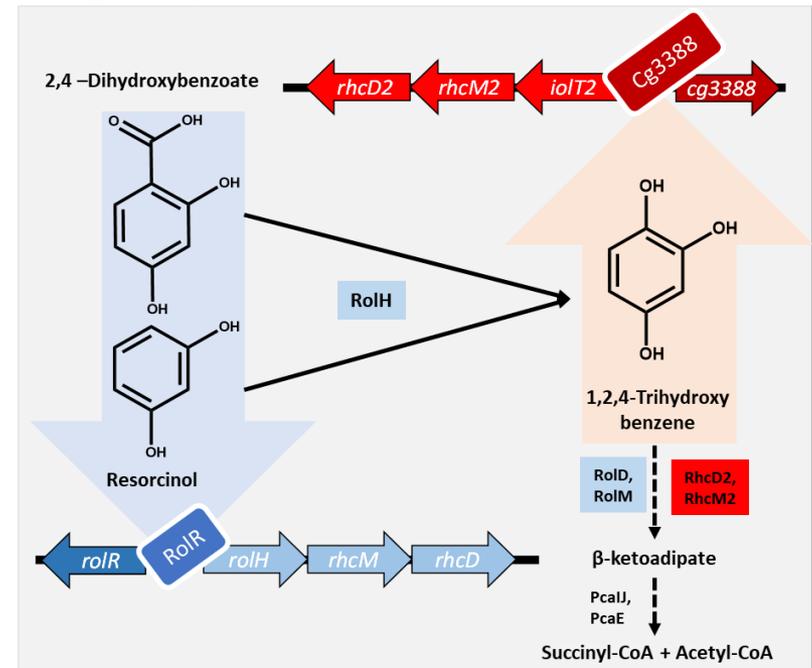
INDOLE tolerance: Adaptive Laboratory Evolution



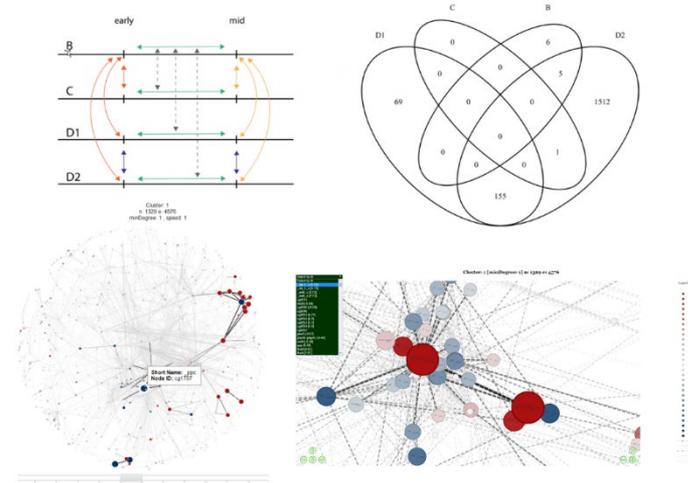
- ➔ IVO20 & IVO38 tolerated indole better
- ➔ Genome sequencing identified candidate mutations
- ➔ Reverse genetics revealed that 3 mutations (*dtxR*, *whcB* and *cg1388*) caused increased tolerance



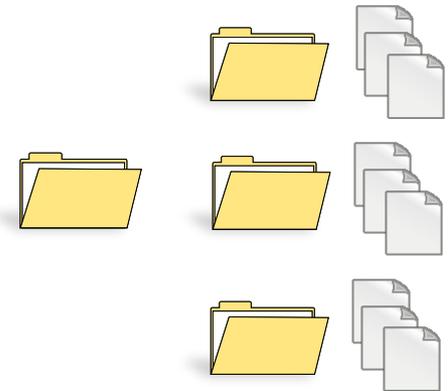
- ➔ *Cg1388* identified as regulator of aromatic degradation
- ➔ Regulatory mechanism characterized

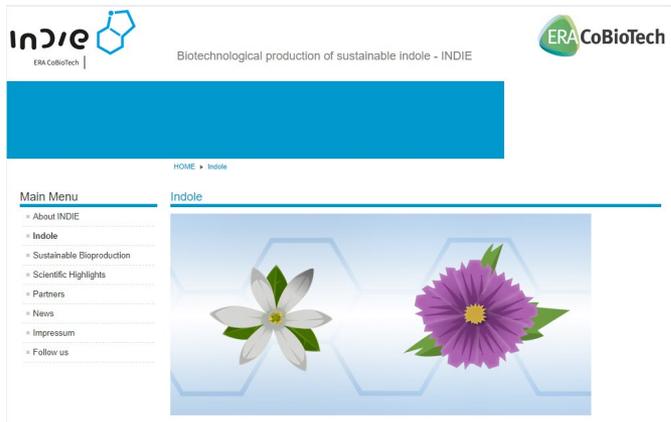


- Systems biology analysis of indole production
 - Best bioconversion and *de novo* production strains tested
 - Indole production in 1L parallel bioreactors
 - Metabolomics and transcriptomics for systems biology evaluation
 - Identification of pathway bottlenecks
- Regulation of the indole biosynthesis
 - Prevention of indole toxicity
 - Design of regulatory switches for 2nd generation of indole producing strains
- Downstream processing & TEA (Axxence)
- Series of high-quality joint scientific publications on various aspects of the project



- FAIR data management workshops organised
- Project established on FAIRDOME-Hub
- Systems biology and biotechnology data managed locally using pISA-tree tool
- Synthetic biology parts managed in a common cloud spreadsheet
- Publication of the data management approach by partner NIB

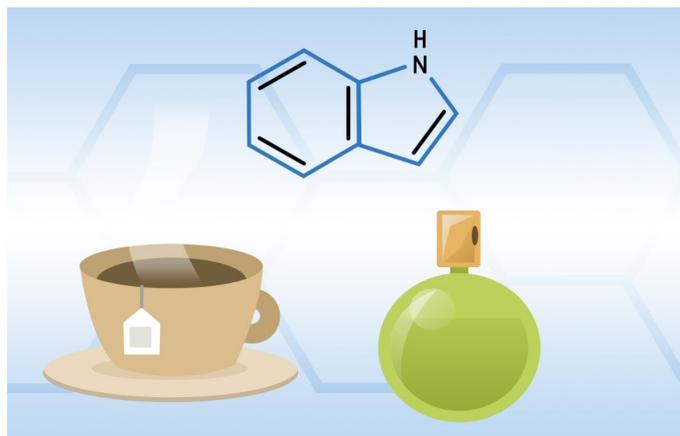




INDIE website, Twitter, LinkedIn



9th International CeBiTec Research Conference at Bielefeld University



INDIE movie @ Global Bioeconomy Summit 2020



Teaching activities: TeutoLab Academy (Bielefeld)



Online videos for students

UNIVERSITÄT BIELEFELD
Faculty of Biology

CeBiTec

Fermentative production of precursors of acridone and indole alkaloids

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¹Wageningen University and Research, Wageningen, The Netherlands; ²Nature Institute of Biology, Ljubljana, Slovenia; ³Institute of Biotechnology, University of Ljubljana, Ljubljana, Slovenia; ⁴Institute of Biotechnology, University of Ljubljana, Ljubljana, Slovenia; ⁵University of Bielefeld, Bielefeld, Germany

Abstract: Acridone alkaloids, such as N-methyl-acridone from *Ruta graveolens*, are plant-based heterocyclic bioactive compounds with cytotoxic, anticancer, anti-dish or antimicrobial properties and are therefore used as pharmaceuticals and therapeutics. Indole alkaloids are a large class of phytochemicals with pharmacological interest as well and often found in fungi like *Psilocybe azuleii*. For both substance classes the aromatic compound anthranilate is the common precursor. Here, we investigated the potential of the biotechnological relevant amino acid producing *Corynebacterium glutamicum* for the production of anthranilate as well as N-methyl-anthranilate as precursor for indole alkaloids by (i) enhancing the carbon flux to anthranilate via the shikimate and tryptophan pathway by overexpressing endogenous genes, introducing heterologous genes, feedback deregulation of key enzymes and preventing export of pathway intermediates, (ii) preventing byproduct formation by deleting genes or by reducing their transcription, and (iii) enhancing precursor supply by overexpressing genes from the pentose phosphate pathway.

Figure 1: Metabolic engineering of central aromatic amino acid metabolism in *C. glutamicum* for the production of anthranilate and N-Me-anthranilate. Green arrows and gene names indicate genes used for overexpression, blue arrows and gene names indicate gene deletions. Shaded boxes are the genes which inhibit gene expression. Construction was performed by replacement of a reporter gene and, hence, conversion of *araC* by *araC* promoter and *araC* gene.

• *araC* promoter by *P_{araC}*
• *araC* gene by *araC* gene
• *araC* promoter by *P_{araC}*
• *araC* gene by *araC* gene

Results:

A. Metabolic engineering of *C. glutamicum* C1* for N-Me-anthranilate production (Figure 1)

- Overexpression of feedback-deregulated anthranilate synthase *trpE* from *E. coli* led to an accumulation of 9 mM anthranilate.
- Deregulation of the committed step of the shikimate pathway and prevention of aromatic byproduct and lactate formation by inserting feedback-resistant *aroPHE* gene from *E. coli* into the locus of *aroA* and deleting *aroA* further increased anthranilate to 15 mM (Figure 2)
- In several consecutive steps of metabolic engineering the final strain **AR003(pEKEx3-*trpE*)²+** (AR003) was constructed and achieved an anthranilate titer of 22.0 ± 1.6 mM (3.1 g L⁻¹) after 48 h of shake flask cultivation (Figure 2).
- Heterologous expression of the anthranilate-N-methyltransferase gene *amnt* from *Ruta graveolens* enabled production of N-methyl-anthranilate, which was improved by optimizing SAM recycling.

B. Fermentation of N-Me-anthranilate producing strain NMA025 in fed-batch in a 2 L bioreactor reached a final titer of 0.5 g L⁻¹ with a volumetric productivity of 0.00 g L⁻¹ h⁻¹ and a yield of 4.8 mg g⁻¹ glucose (Figure 3).

Outlook: Expand metabolic network of the anthranilate and N-Me-anthranilate producing *C. glutamicum* strains developed here for fermentative production of indole and acridone alkaloids.

Figure 2: Production of anthranilate (mM) and biomass (OD₆₀₀) over time (h) for strains Cx C1* and AR003 in 250 mL shake flasks. Error bars represent standard deviation.

Figure 3: N-methyl-anthranilate production by *C. glutamicum* strain NMA025 in fed-batch bioreactor in serum medium. The left y-axis shows glucose (g L⁻¹) and the right y-axis shows NMA (mM). Error bars represent standard deviation.

Online conferences

Očarljivi poskusi

Z rastlinami

Urednice:
Maruša Pompe Novak
Spela Baebler
Marina Dermastia

Experiment book for children

- *How to improve interactions between research and society?*
 - *Visualisation of science (videos, social media, book for children)*
 - *Dialogue (with students, organizing panel discussion with industrial participants, organizing a scientific conference)*
- *How to consider gender/diversity/culture dimension in a project?*
 - *Not a part of research questions*
 - *Gender balance in consortium (10F, 5M)*
- *How to manage data within the consortium?*
 - *All the project data managed using the same tools (pISA-tree and a cloud spreadsheet – linked to FAIRDOM hub) → easier sharing and integration*

Production of indole:

- Achieved both via biotransformation and *de novo* production
- DSP protocols and medium optimizations ongoing in collaboration with industrial partner Axxence Aromatics
- TEA ongoing by industrial partner

Application to other aromatic compounds:

- Models and systems biology tools developed for production of aromatic compounds in *Corynebacteria*
- *Corynebacteria* as a synthetic biology chassis for *à la carte* production of aromatic compounds
- Production and regulatory modules available for future use

- *Industrial microbiology and Systems and synthetic biology applied for aromatic production in Corynebacteria*
- *Sustainable biobased production of indole was successfully established*
- *Focus on output in communication and dissemination*

- *Benefits of the international collaboration*
 - *Complementary expertise*
 - *Exchange of materials, strains and data [whole is more than the sum of the parts]*
 - *Large common experiments possible*
 - *Academic and industrial partners working together along the value chain*
 - *Insight into different scientific cultures (by country, academia vs company)*

- *Comments, feedback to ERA CoBioTech*
 - *Establish a follow-up of this successful program for the sustainable production of fragrances and flavors*

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