ERA CoBioTech

BIOTECH 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

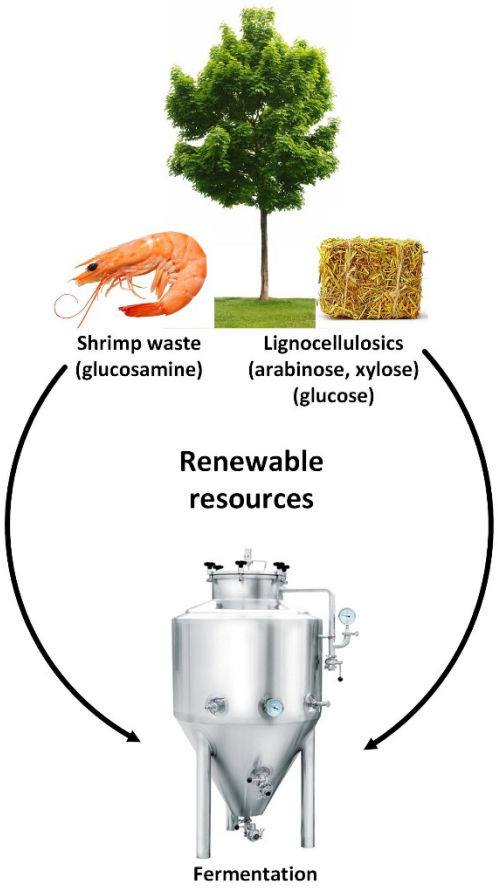
Project partners

- 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)
- 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

Introduction

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

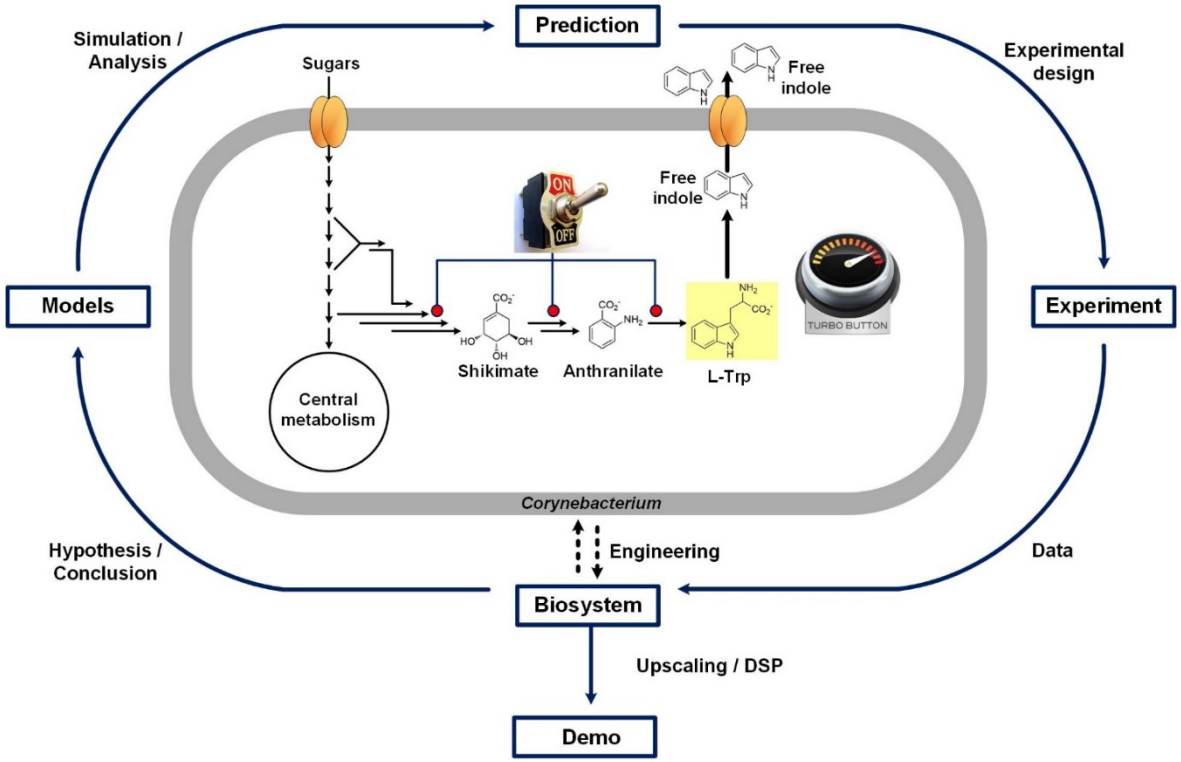


Technical overview

Host microorganism: Corynebacterium glutamicum

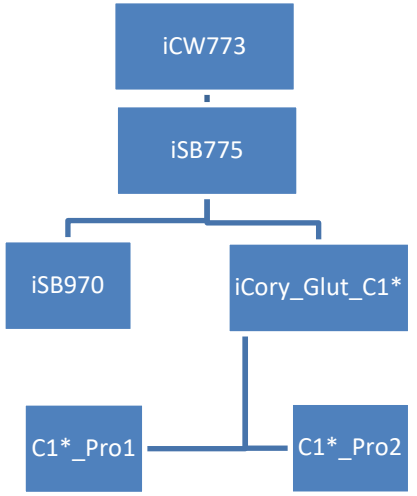
INDIE CONCEPT

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



Project outcomes

- 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 of *C. glutamicum* C1* as a microbial cell factory.
- C1*_Pro1 and C1*Pro2 are specific for Indole production



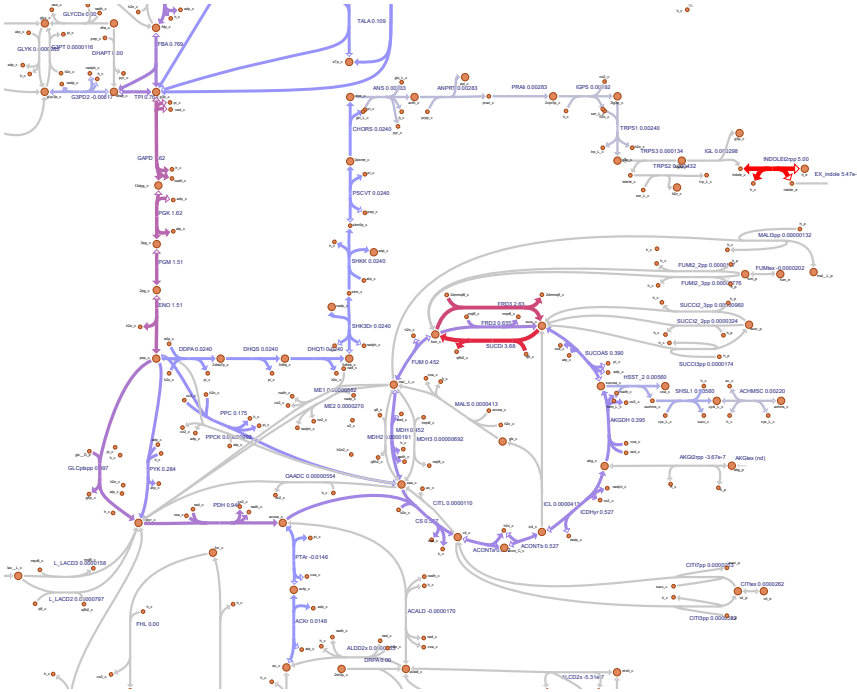
Overview of generated models

	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

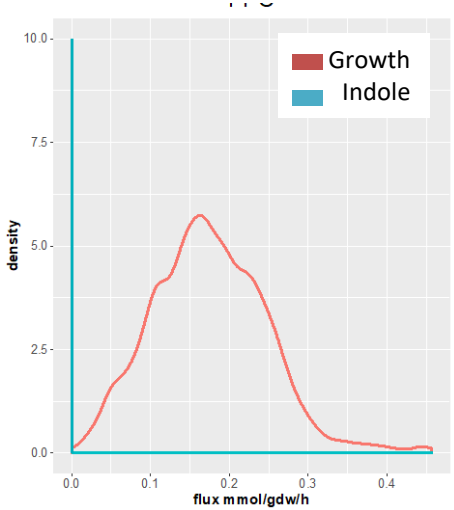
Summary caracteristics of the models

Project outcomes

- 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.

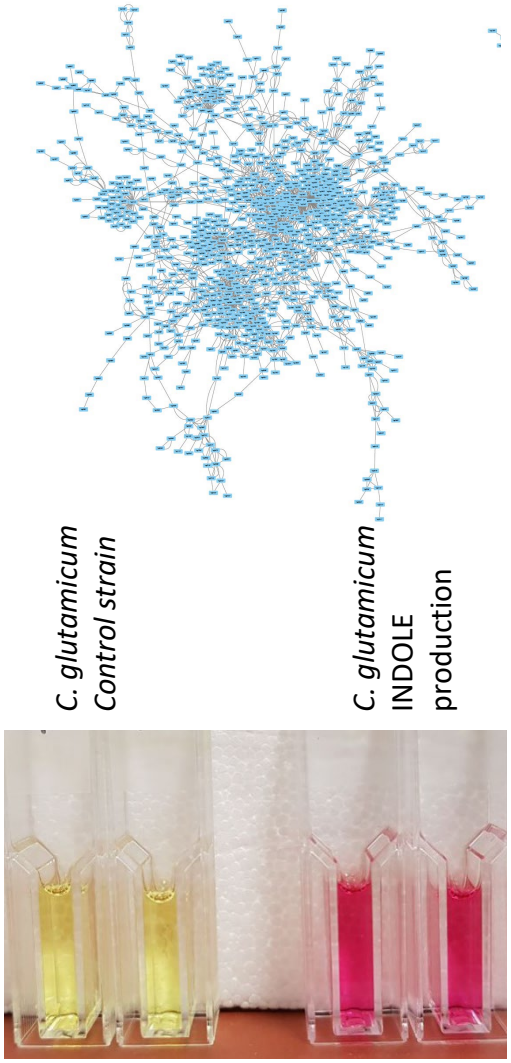
Project outcomes

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

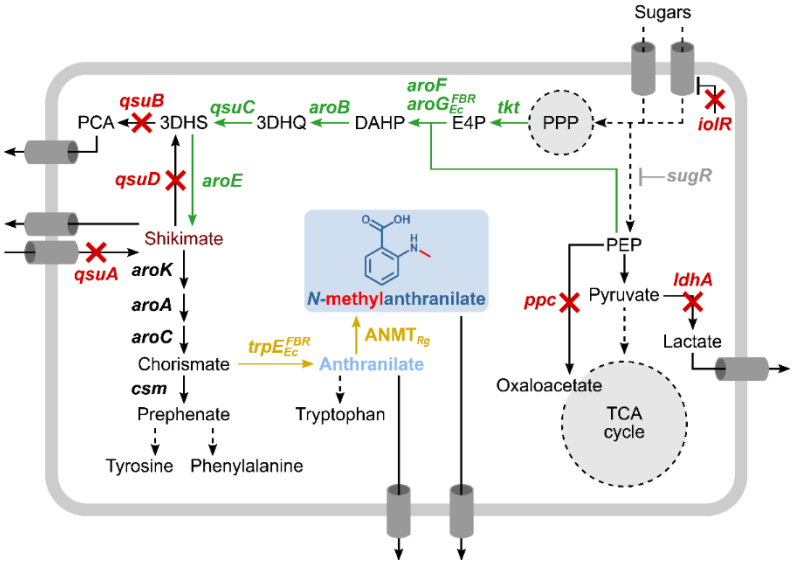
- (i) enhancing the carbon flux to tryptophan via the shikimate and tryptophan pathway by overexpressing endogenous and heterologous genes, feedback deregulation of key enzymes
- (ii) preventing by-product formation, especially of other aromatic compounds
- (iii) enhancing precursor supply by overexpressing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

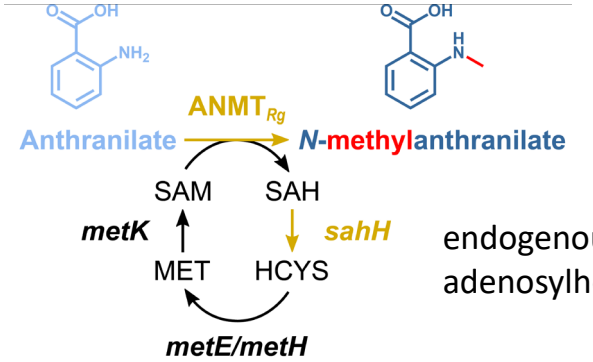


INDOLE precursor: **SAM-dependent *N*-alkylation**

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

C. glutamicum engineered for overproduction of L-anthranilate





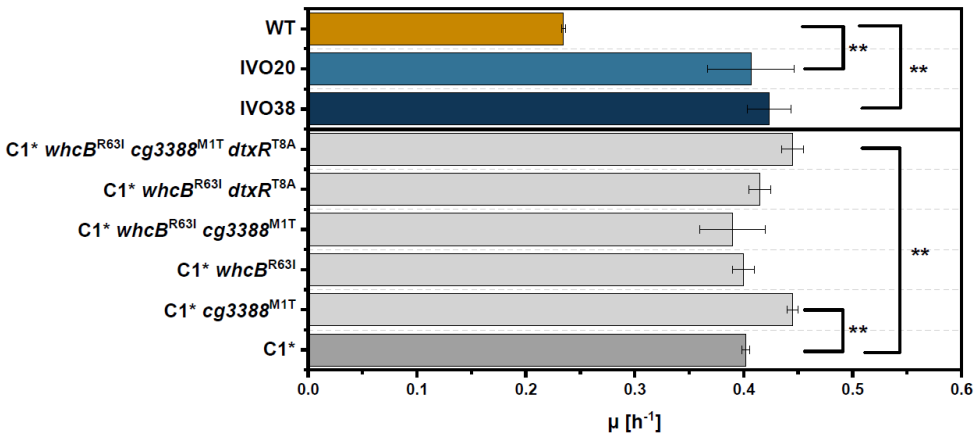
endogenous adenosylhomocysteinase

Walter, Al Medani, Burgardt, Cankar, Ferrer, Kerbs, Lee, Mindt, Risse, Wendisch (2020) *Microorganisms* **8:** 866

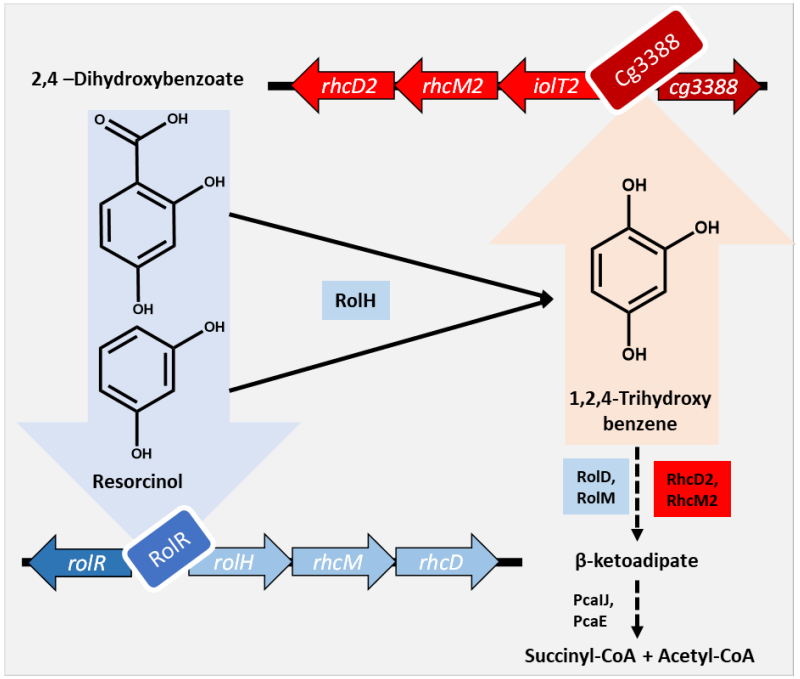
INDOLE tolerance: **Adaptive Laboratory Evolution**

Wendisch Lab

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



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

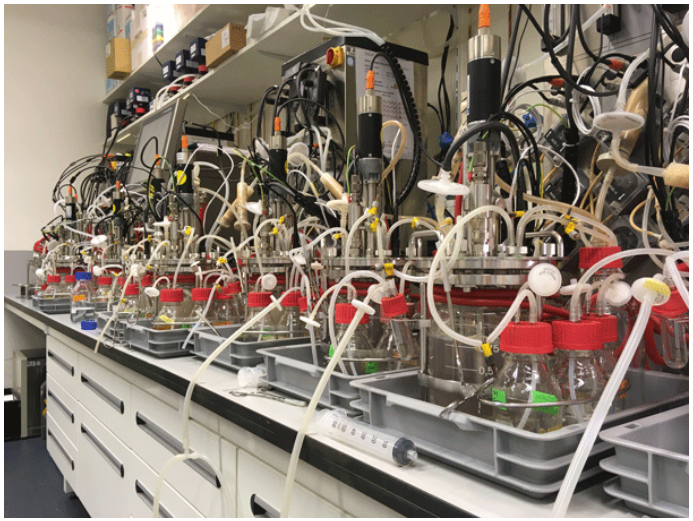


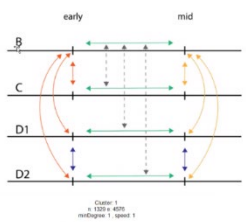
WAGENINGEN UNIVERSITY & RESEARCH
UNIVERSITÄT BIELEFELD

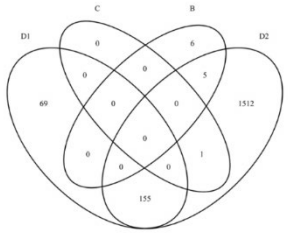
Walter, Veldmann, Götker, Busche, Rückert, Kashkooli, Paulus, Cankar, Wendisch (2020) *Microorganisms* **8:** 1945

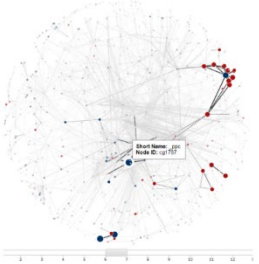
Project outcomes

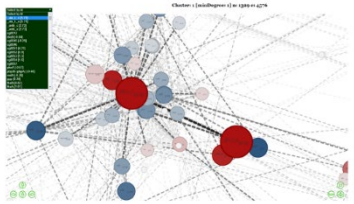
- Systems biology analysis of indole production
  - Best bioconversion and *de novo* production strains tested
  - Indole production in 1L parallel bioreactors
  - Metabolomics and transcriptomics for systems biology evaluation
  - Identification of pathway bottlenecks
- Regulation of the indole biosynthesis
  - 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





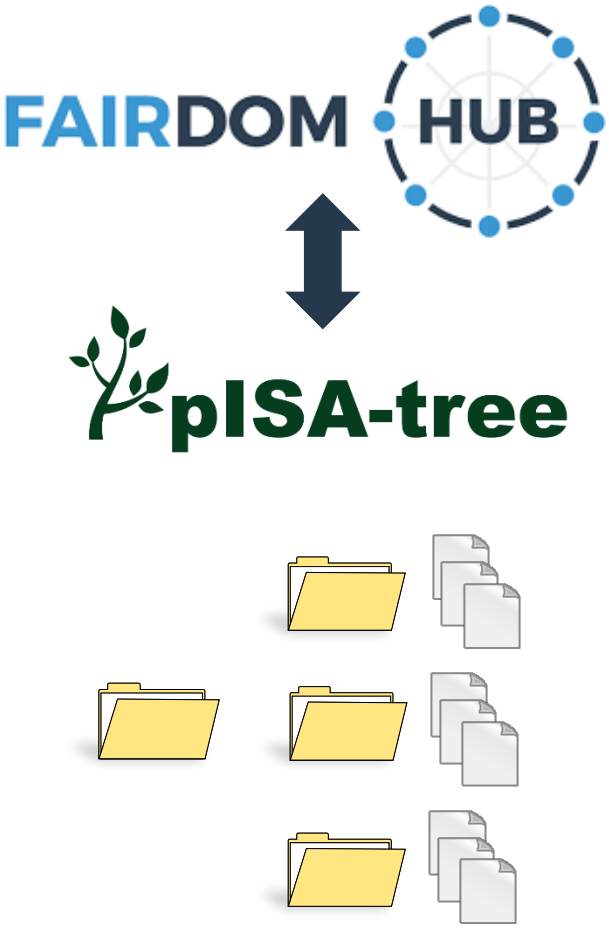






Data management

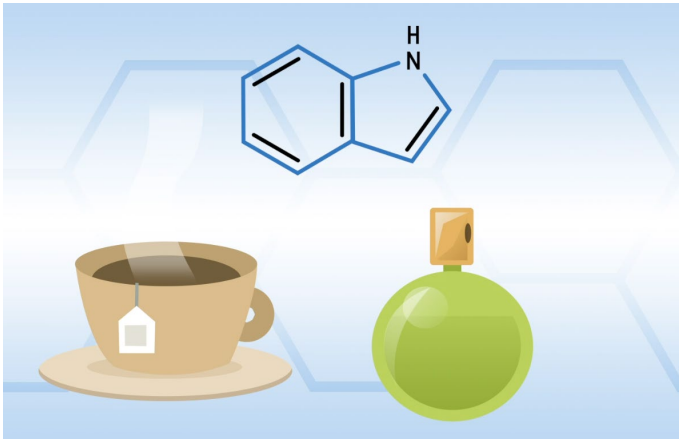
- FAIR data management workshops organised
- Project established on FAIRDOM-Hub
- Systems biology and biotechnology data managed locally using pISA-tree tool
- Synthetic biology parts managed in a common cloud spreadsheet
- Publication of the data management approach by partner NIB



Communication activities

INDIE website, Twitter, LinkedIn

9th International CeBiTec Research Conference at Bielefeld University

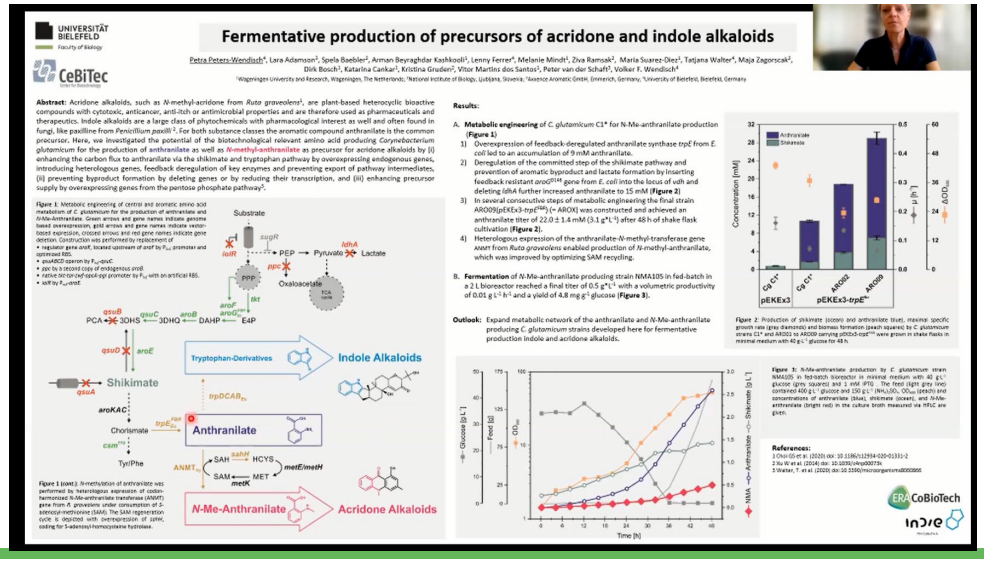


INDIE movie @ Global Bioeconomy Summit 2020

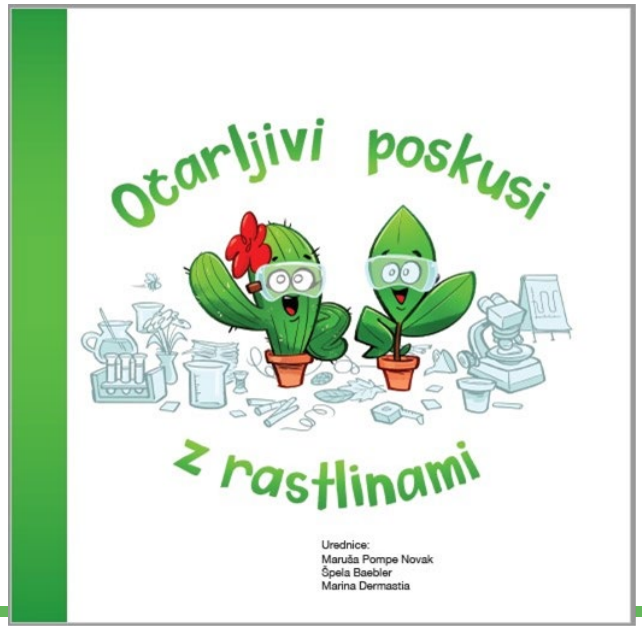
Teaching activities: TeutoLab Academy (Bielefeld)

Moving on in the times of pandemic…

Online conferences



Online videos for students



Experiment book for children

RRI aspects

- *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*

Technology Transfer and market potential

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

Summary

- *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*

General Evaluation

- *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*

Contact details

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