ERA CoBioTech

ERA CoBioTech (ERA-Net Cofund on Biotechnologies)

ACHEMA2018

Kick-off session: "Biotechnology for a sustainable bioeconomy"

Sustainable Production of Added Value Chemicals from SynGas-derived Methanol Through Systems and Synthetic Biology Approaches

Project acronym: **BIOMETCHEM**

Name: **Nigel P Minton**

SynBio SBRC Nottingham
a BBSRC/EPSRC Synthetic Biology Research Centre

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

Frankfurt am Main, 12.06.2018

Project Partners

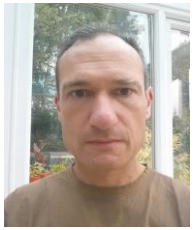
Nigel Minton

Jon McKechnie

Volker Müller

Philippe Soucaille

Peter Dürre



Stephen Poulston

- Total project budget: **€1,823,000.**
- Project start: **1st May 2018**

The Challenge

- **Greatest challenges** facing industry and society are the future **sustainable production** of chemicals and fuels from **non-food resources** while at the same time **reducing GHG emissions & pollution**
- To date, the focus has been on the use of lignocellulosic biomass feedstocks.
- Reliant on an **energy intensive pre-treatment step**, and thereafter, the **addition of costly exogenous hydrolytic enzymes** needed to convert the partially deconstructed biomass into the sugars needed by the fermentative process organisms.
- The costs involved are making the development of **economic processes** extremely challenging.

The Solution

Household waste

C1 gases are available in high volumes and at low cost throughout the UK/Europe

"C1 gas eating" bacteria

Bioreactor Gas Fermentation

Industrial waste

esp. steel mills

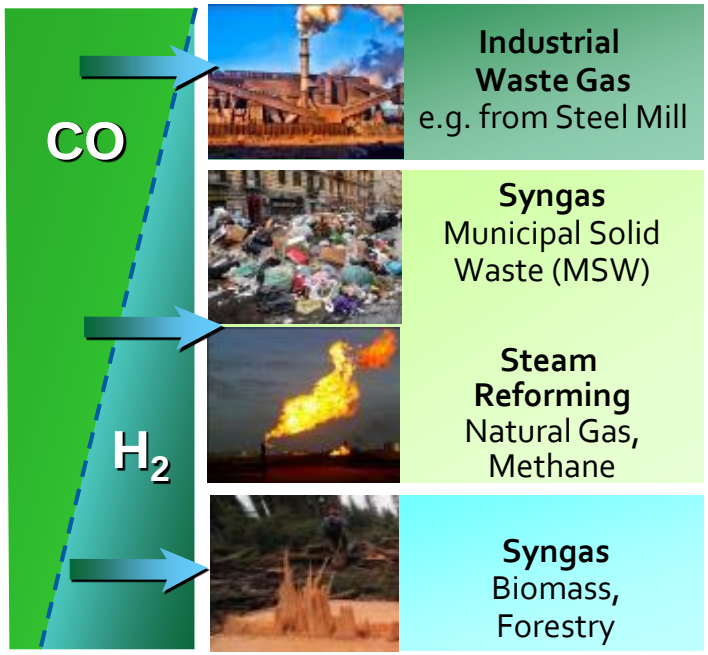
Fuels

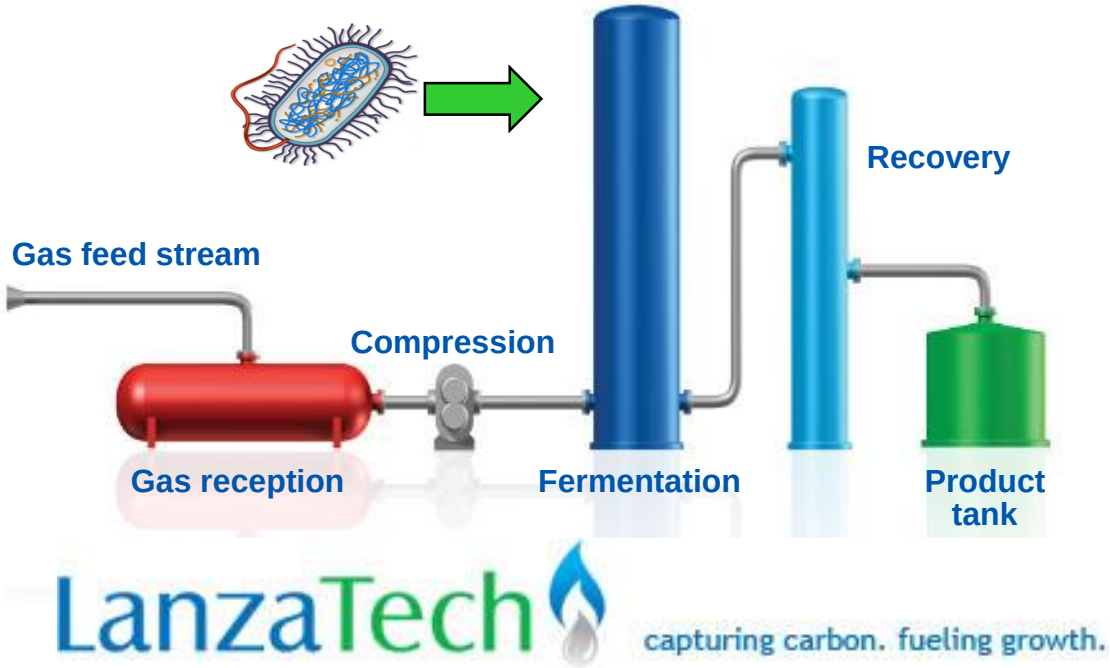
Plastics

Fibres

Cosmetics

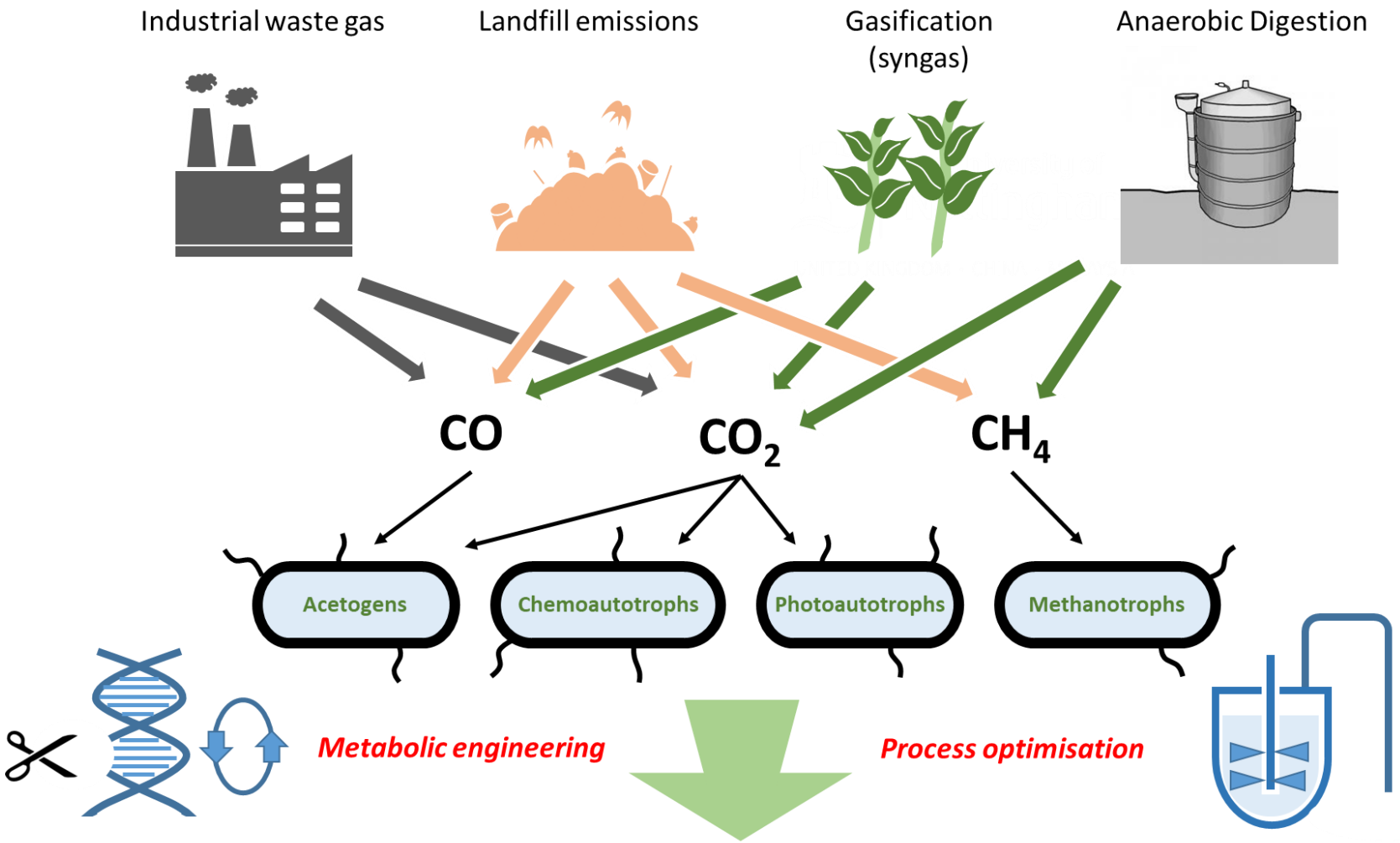
Commercialisation





- LanzaTech has developed a fermentation process using industrial waste gases or syngas as **sole** energy and carbon source for production of fuels and chemicals
- Shougang Group Jingtang Steel Mill (near Beijing, China) became operational on 3rd May 2018 - capacity = 46,000 tons (16 million gallons) of ethanol per year
- **Completely outside of the food value chain**

Routes to Chemicals



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Methanol as Feedstock

SynBio SBRC Nottingham
a BBSRC/EPSRC Synthetic Biology Research Centre

Gas Fermentation is not without Issues

- Gas fermentations are typically limited by the rate of gas-to-liquid mass transfer making them inefficient and requiring expensive reactor designs
- Gas mixtures are potentially explosive, particularly those that combine O_2 with CO_2 or CH_4
- Gas, particularly CO and CO_2, is not necessarily easily stored and transported .

Methanol as Feedstock

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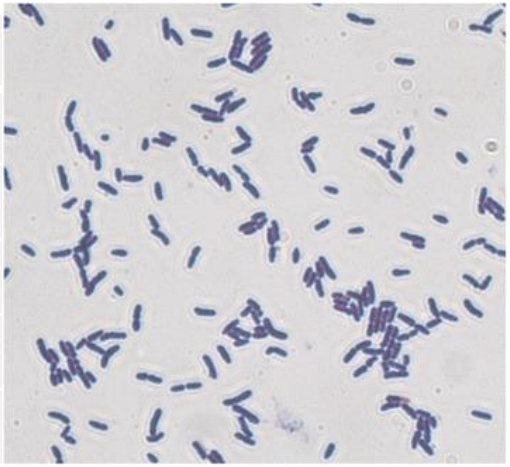
Methanol as a Feedstock – a liquid

- does not suffer from mass transfer issues associated with C1 gases such as CO and CO_2 in fermenters
- overcomes possibility of explosive gas mixtures
- easily stored and transported.
- can be made from many sustainable feedstocks, including biomass, MSW, biogas and waste CO_2
- there is currently a glut of methanol available around the world

The Chassis

Eubacterium limosum

Phylum:	Firmicutes
Class:	Clostridia
Order:	Clostridiales
Family:	Eubacteriaceae
Genus:	*Eubacterium*
Species:	*limosum*



Kelly et al. 2016

- Gram positive - Rod shaped - Obligate anaerobe
- Chemolithoautotrophic acetogen
- Produces C4 (butyrate/butanol) in addition to C2 (acetate/ethanol)
- In addition to CO and CO_2, it grows well on **methanol**

Objective

Our overall objective is to establish *Eubacterium limosum* as a chassis for producing high added value chemicals from biomass-derived methanol.

- Products will be:
  - **γ-aminobutyric acid, GABA**, (useful in the pharmaceutical and food additive industries) and
  - **1,4-butanediol, BDO** (a platform chemical).
- This will be accomplished by a combination of interdisciplinary methodologies:-
  - **systems biology** (INSA, University of Toulouse);
  - **synthetic biology** (UNOTT, University of Nottingham);
  - **metabolic engineering** (ULM, University of Ulm);
  - **enzymology** (UFRA, University of Frankfurt), and;
  - **methanol fermentation development** (All Partners).

Transformation & Plasmid Compatibility

Rob Mansfield

- Developed and optimised transformation methodology

Efficiency: $5x10^5$ CFU/µg

plasmids of >11 kb

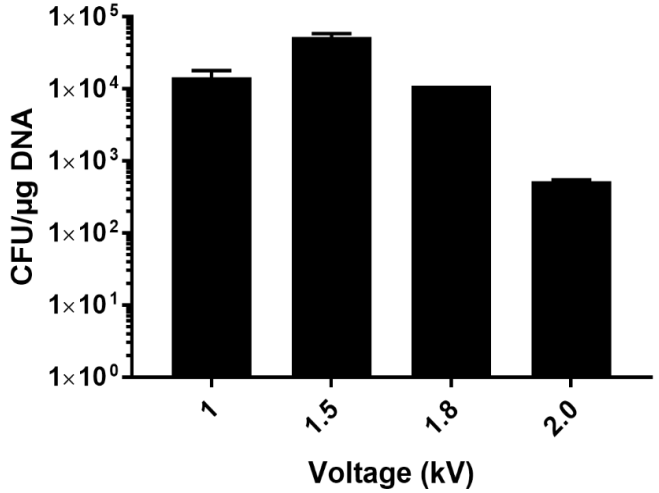


Figure 3.3: Transformation efficiency of plasmid pMTL87151 into *E. limosum* at varying voltages

Essential Tools in Place

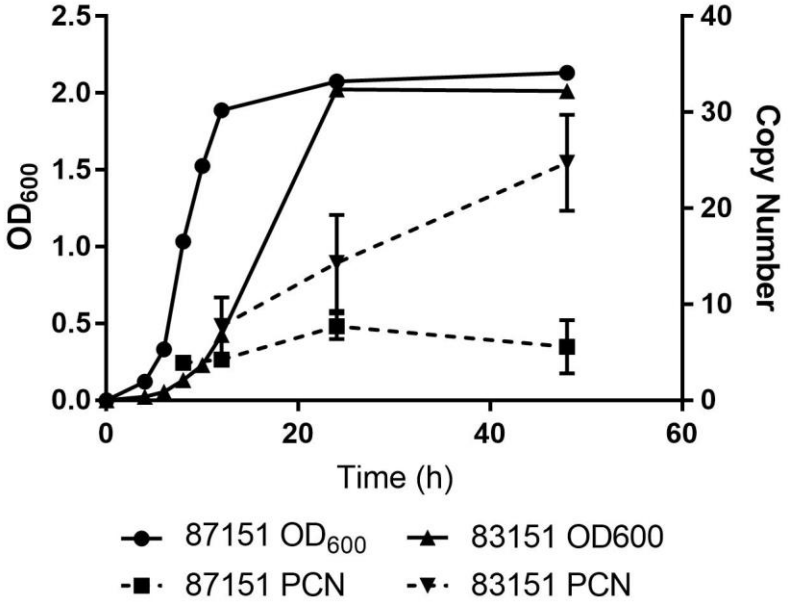
Transformation & Plasmid Compatibility

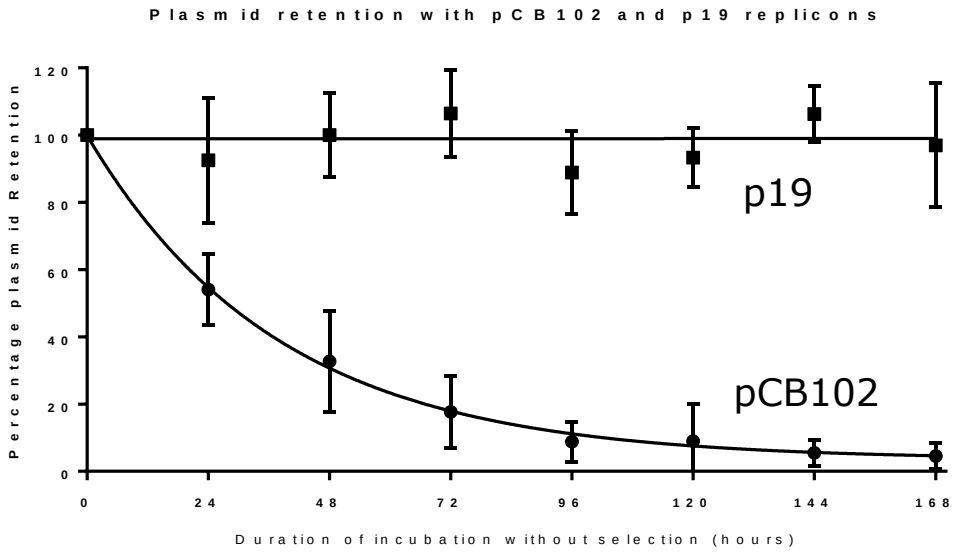
James Millard

- Developed and optimised transformation methodology
- Identified stable and unstable plasmid replicons

Replicons

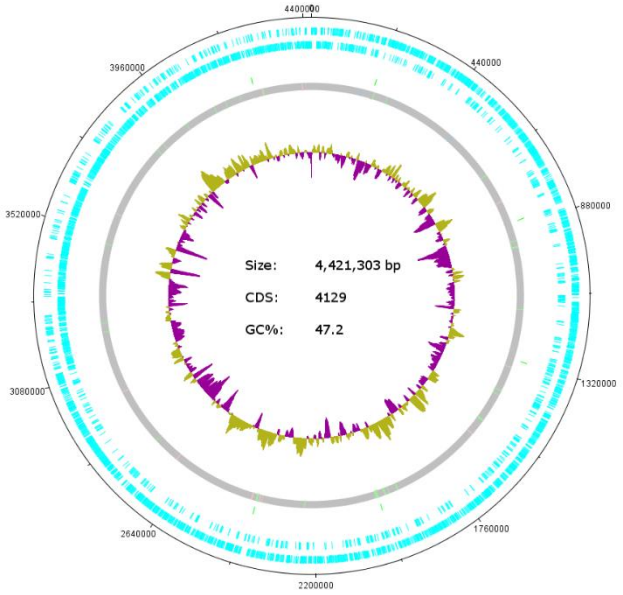
- pCB102: Rep-deficient, high-copy
- p19: Very stable, low-copy



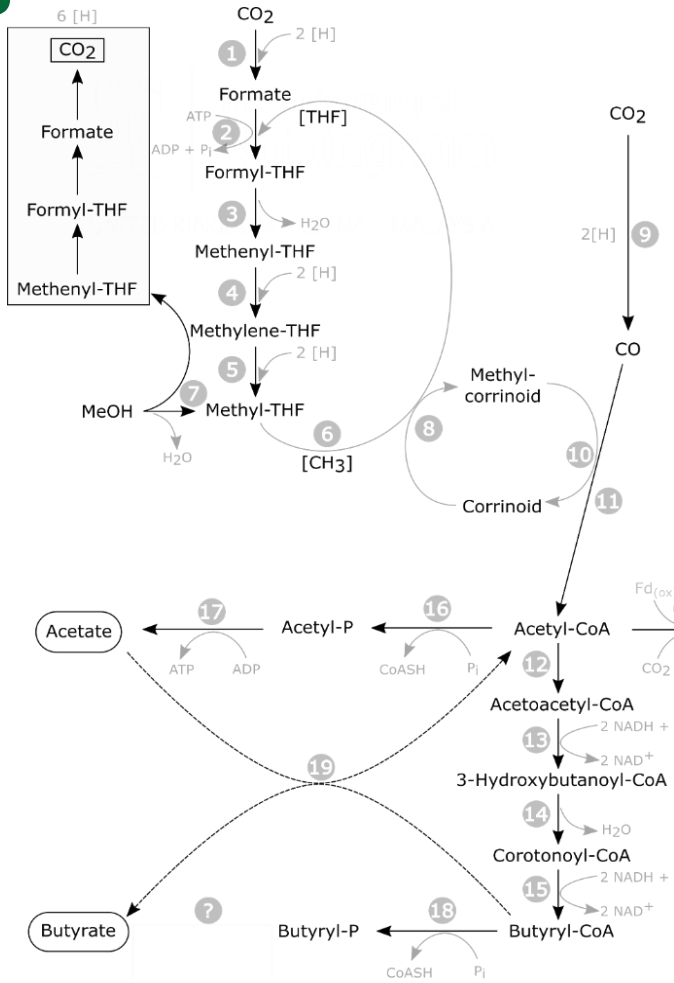


Essential Tools in Place

Genome Strain ATCC 8486



Sequence: PacBio + Illumina
Annotation: JGI (IMG/M)



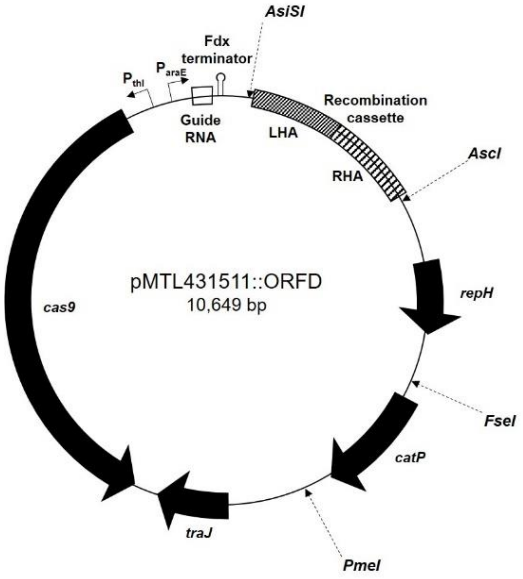
Thomas Millat

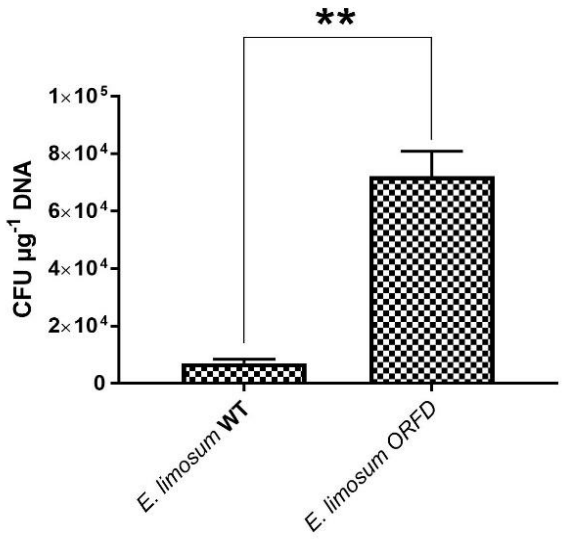
Rob Mansfield

Essential Tools in Place

CRISPR/Cas9 Type I RM Knock-out

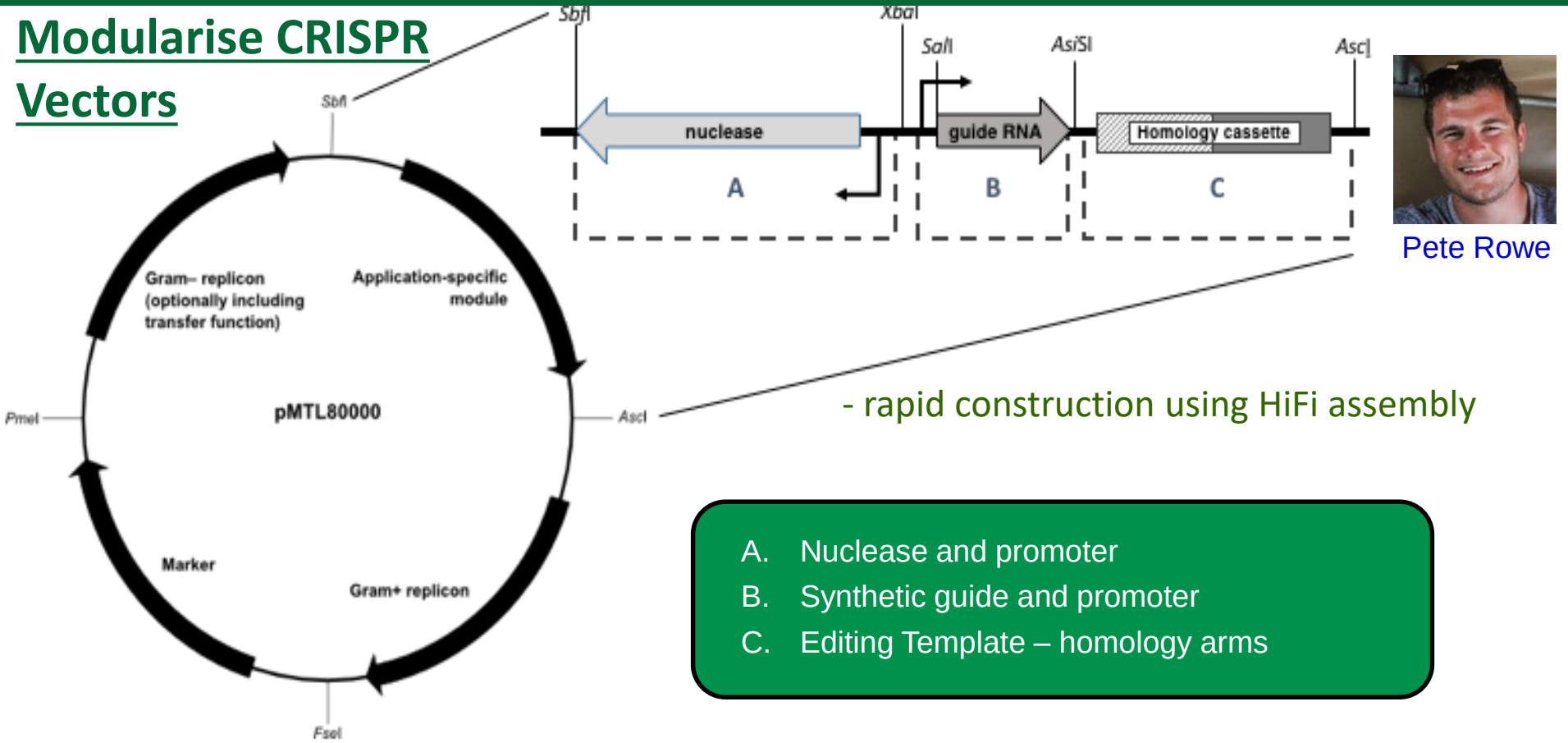
- Two likely active RM systems in *E. limosum* ATCC 8486
- Type I system deleted via CRISPR Cas9
- 10-fold increase in DNA transfer efficiency observed.





Essential Tools in Place

Modularise CRISPR Vectors



Pete Rowe

- rapid construction using HiFi assembly

A. Nuclease and promoter
B. Synthetic guide and promoter
C. Editing Template – homology arms

- Huang H, Chai C, Li N, Rowe P, Minton NP, Yang S, Jiang W, Gu Y. CRISPR/Cas9-Based Efficient Genome Editing in *Clostridium ljungdahl*ii, an Autotrophic Gas-Fermenting Bacterium. *ACS Synthetic Biology* 2016; **5(12):** 1355-1361.
- Heap JT, Pennington OJ, Cartman ST, Minton NP. A modular system for *Clostridium* shuttle plasmids. *Journal of Microbiological Methods*. 2009; **78(1):** 79-85.

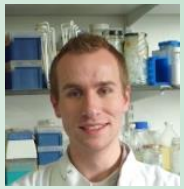
SBRC Chassis Exemplification

Clostridium autoethanogenum

Production chassis of the US gas fermentation company LanzaTech

Knockouts generated in over a dozen genes

90-100% efficiency




Chris Humphreys Pete Rowe

Clostridium difficile

Major cause of healthcare and antibiotic associated diarrhoea

Successfully knock-out *pyrE, ermB* and *hsdR*

80-90% efficiency

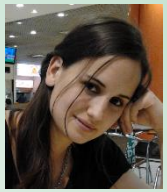

Parick Ingle Daphne Groothuis

Clostridium botulinum

Causative agent of botulism - select agent

Precise removal of neurotoxin genes in several strains

100% efficiency




Raquel Rodriques D Groothuis

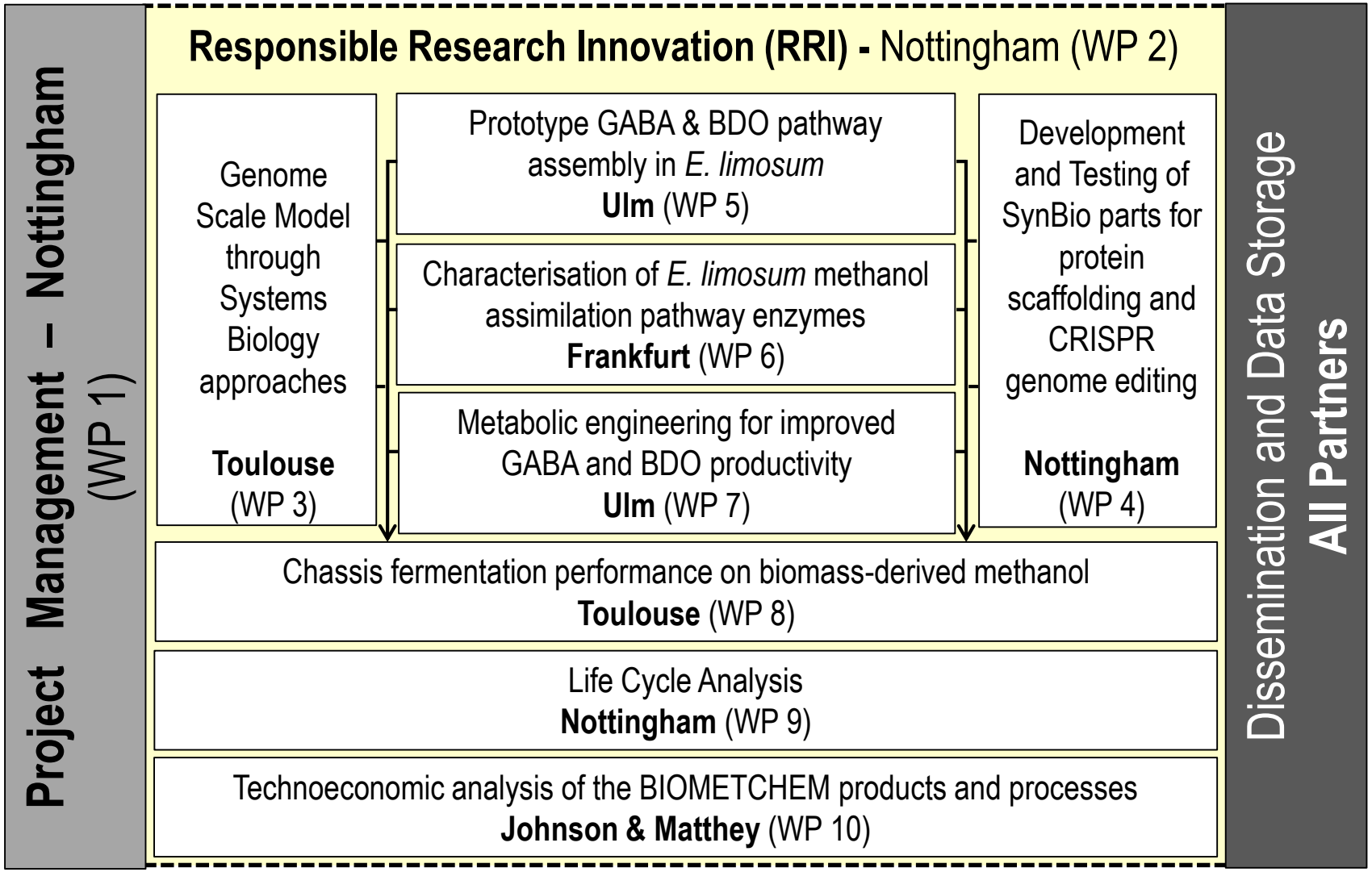
- also demonstrated in *Clostridium pasteurianum, Clostridium sporogenes, Geobacillus, Clostridium beijerinckii, Clostridium saccharoperbutylacetonicum, Clostridium*

Essential Tools in Place

SUMMARY

- high frequency electroporation – **5 x 10^5 /10^6 CFU/µg**
- stable and unstable replicons identified
- allelic exchange gene Knock-out using *pyrE* counter selection and CRISPR/Cas9
- annotated genome sequence
- random mutagenesis using *mariner* transposon
- orthogonal expression system base on TcdR sigma factor

Means of Delivery



RRI Activities

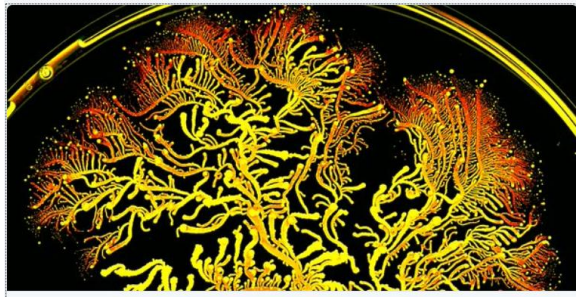
Outputs

- Regular blog posts.
- Interdisciplinary journal articles.
- International conference papers.
- Internal and external reports.
- Workshops will be held to educate and inform project team members, the wider community, industry and the general public.
  - *Planning workshop - 2 day*
  - *Stage gate Workshop – 1 day*
  - *Final workshop – 2 day*


Carmen McLeod


Brigitte Nerlich


Louise Dynes



Bacteria, scientists and stewardship

Bacteria have fascinated scientists for centuries and still do. One of the first to see bacteria under the microscope was "probably the Dutch

blogs.nottingham.ac.uk

ELSEVIER

Energy Research & Social Science

Volume 30, August 2017, Pages 35-42

Original research article

Working with bacteria and putting bacteria to work: The biopolitics of synthetic biology for energy in the United Kingdom

Carmen McLeod [a, b], Brigitte Nerlich [b], Alison Mohr [b]

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https://doi.org/10.1016/j.erss.2017.06.017 Get rights and content

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4 Methane Eaters - Microbiologists #4 5:18 nottinghamscience
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Game of Fuels

GAME OF FUELS
PLAY > LEARN > SAVE

Microbiology Dance!

DANCE equation
The University of Nottingham UNITED KINGDOM · CHINA · MALAYSIA
ignite!
sfam society for applied microbiology
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FESTIVAL of SCIENCE & CURIOSITY Stemcity

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SynBio SBRC Nottingham
a BBSRC/EPSRC Synthetic Biology Research Centre

New Scientist Live: ExCel, London - October 2017

Expected Outcomes

Engineered *Eubacterium limosum* strains able to convert biomass-derived methanol into:

- **γ-aminobutyric acid, GABA**, (a relatively high value chemicals, 7$ per kg, useful in the pharmaceutical and food additive industries) and
- **1,4-Butanediol, BDO** (a lower value chemical, 2$ per kg but with a multibillion $ market, mainly used for the production of polyesters) at high yield on methanol (at least 70% of the theoretical yield).

The targeted yield on methanol will be higher than the theoretical yield on glucose or xylose making these engineered strains/process on methanol very attractive.

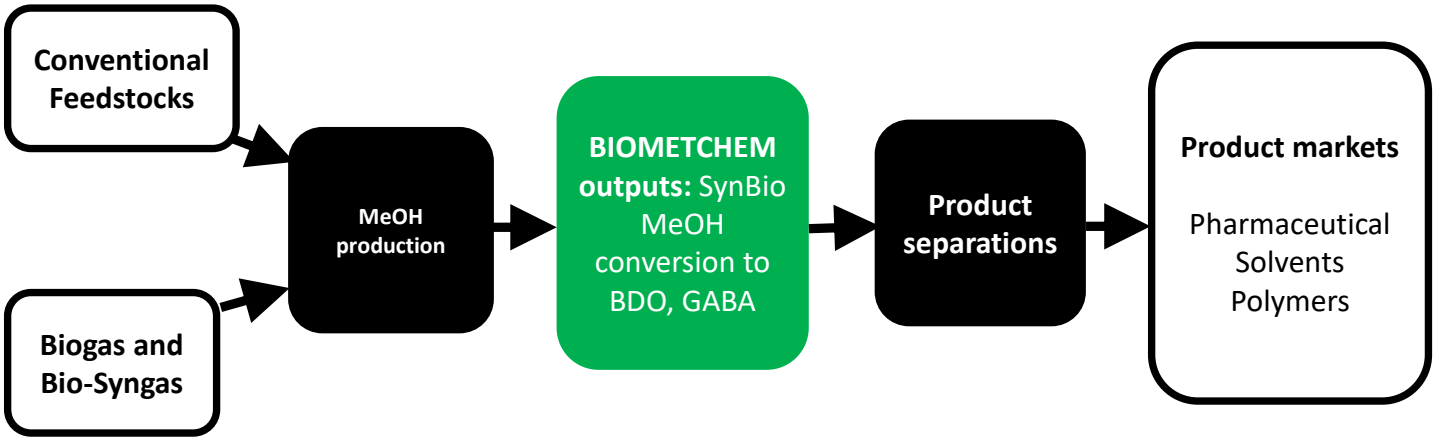
Look towards commercialisation in collaboration with our industry partner Johnson Matthey

Johnson Matthey

LCA and TEA

Life Cycle Analysis will quantify overall environmental impacts of manufacturing 1,4-BD and GABA from methanol. It will encompass a cradle-to-gate scope and focus on:

- Methanol production from conventional (coal, natural gas) and biomass sources; appropriate allocation to consider waste methanol as a feedstock
- SynBio production of 1,4-butanediol and GABA from methanol, including product separation/ purification and treatment of any wastes arising in the process.



TechnoEconomic Analysis extends the process simulation models to consider full production costs, product markets, and implications of ongoing technology development on the viability of SynBio conversion of methanol to 1,4 BD and GABA.

Contact Details

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