ERA CoBioTech

BioTech Research & Innovation Hack

2021

ERA CoBioTech Funded Projects at A Glance: BIOMETCHEM

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

PART OF EUROPEAN BIOTECH WEEK

INNOVATION IS IN OUR GENES

BIOMETCHEM

Establishment of a microbial chassis for the production of chemicals from methanol

To meet NetZero targets, biological conversion processes for chemical production from sustainable sources need to be developed. This project seeks to develop the anaerobe Eubacterium limosum as a production chassis using sustainably sourced methanol.

Systems and biochemical characterisation of metabolism and methanol utilisation in *E. limosum*, accompanied by the development and exploitation of synthetic biology tools for metabolic engineering

One of the greatest challenges facing society is the future sustainable production of chemicals and fuels from non-petrochemical resources while at the same time reducing greenhouse gas emissions. The recalcitrance of lignocellulose to deconstruction for feedstock purposes is making the economic development of biologically-based processes extremely challenging. This has led to the concept of using low-cost, abundant one-carbon (C_1) feedstocks. Here the focus has been on using C_1 gases, such as CO/CO_2 and CH_4, sourced as a waste from industrial processes, anaerobic digestion or deliberately formed as synthesis gas (syngas) through the gasification of any waste containing biomass (agricultural/ forestry residues and municipal solid waste) or by the reformation of shale gas. Such an approach is not without its issues. The mass transfer of gases into the liquid phase in reactors places constraints on reactor design and performance, while in the case of aerobic chassis the additional presence of H_2, and O_2 is potentially explosive. In contrast, as a liquid, methanol does not suffer from mass transfer issues in fermenters and is more easily stored and transported. It can be made from many sustainable feedstocks, including biomass, MSW, biogas, waste CO_2, and even renewable electricity.
BIOMETCHEM has developed and exploited synthetic biology tools for the manipulation of *Eubacterium limosum* enabling the derivation of engineered strains able to produce chemical products (butanol and acetone) currently derived from fossil fuels using biomass derived methanol as a feedstock. Objectives were achieved through a combination of interdisciplinary methodologies, including systems biology synthetic biology, metabolic engineering, enzymology and methanol fermentation development. Responsible Research Innovation (RRI) practices were embedded within the programme of work through the participation of dedicated Social Scientists, ensuring the outputs are socially acceptable.

Transcriptomics, proteomics, metabolomics; genome-scale modelling; gene deletion; Transposon-directed insertion sequencing (TraDIS); enzyme purification and characterisation; metabolic engineering.

By combining resources and expertise, BIOMETCHEM connects research partners in three different European countries (France, Germany and UK) with different but complementary scientific and technological expertise, thereby maximising resources and sharing the risks, costs and skills. The participants represent some of Europe's leading experts in anaerobic metabolism, and in particular single carbon (C_1) feedstocks, and have capitalised on their expertise to develop the anaerobe *Eubacterium limosum* as a platform for the production of chemicals from sustainable methanol. In parallel approaches they have used a system approach to derive a quantitative genome scale model (informed through the application of transcriptomics, proteomics and metabolomics) while at the same time using classical biochemistry and enzymology to identify and characterise the principal steps and corresponding enzymes involved in methanol utilisation. To confirm their essential role in the assimilation of this feedstock, inactivation of the encoding genes using a pre-existing CRISPR/Cas9 system was not successful. Accordingly, a novel gene knock-out system was devised based on a toxin/anti-toxin, suicide system native to *E. limosum*. This has proven to be highly effective for mutant generation. In parallel, a novel transposon system was used in Transposon-directed insertion sequencing (TraDIS) to identify those genes essential to growth on methanol. The use of an oxygen-independent fluorescent FAST reporter (fluorescent activating and absorption shifting tag) reporter was exploited to facilitate the production of the targeted products butanol and acetone. Life Cycle and Technoeconomic Analysis was used to compare the production of model products from fossil and renewable methanol. To facilitate reflection on values and ideas of responsibility in BIOMETCHEM, and to explore and compare scientific and public perspectives on the project, a series of interactive workshops were held.

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Project duration:

01 May 2018 - 31 December 2021

Total budget: 1.8 €M

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Main results

BIOMETCHEM has achieved a number of 'firsts' in the field. Through purification and painstaking characterisation, the key role of a number of enzymes involved in methanol assimilation in *E. limosum* have been established. For instance, purified FDH (formate dehydrogenase) and bifurcating Hydrogenase (HYD) enzymes form a functional complex and both have been shown to be bifurcating, which is in contrast to the two putative Methylene-THF reductases present. The essential role of these proteins in assimilating methanol has either been confirmed by the inactivation of the encoding genes using the newly developed toxin/antitoxin system, through transcriptomic data obtained when cells are grown on methanol and from the demonstration of essentiality using TraDIS. The mutant library created in the latter procedure comprised some 180,000 unique insertion mutants, representing one insertion every 25 base pairs. Through high throughput sequencing of mutant pools passaged on appropriate media, 560 genes essential for growth on glucose, and an overlapping set of 713 genes essential for growth on methanol, were identified. Moreover, the abundance of sequencing reads attributable to a specific mutant can be tracked over passages, giving an indication of the fitness contribution (positive or negative) of each mutation. A genome scale model was developed in collaboration with KAIST (South Korea) and chemostat cultures on methanol and glucose performed. The transcriptomic, proteomic and fluxomic data generated have been used to improve the accuracy of the genome scale model. In vivo translation rates for each gene as well as in vivo turnover rates for each enzyme are currently being calculated. In terms of metabolic engineering, the first reporter use of FAST in *E. limosum*, facilitated the implementation of the pathways needed to make butanol and acetone. This represents the first demonstration of the production of these chemicals from methanol.

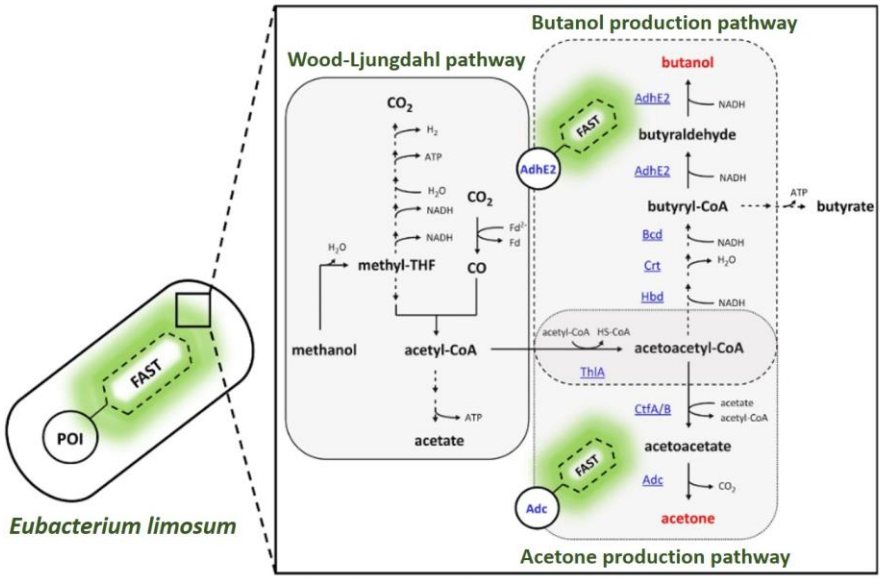

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Figure 1: Schematic overview of the Wood-Ljungdahl pathway coupled with the recombinant butanol and acetone production pathway in E. limosum.

Butanol and acetone production can be achieved with FAST-tagged AdhE2 and Adc fusion proteins, respectively. ThlA, thiolase; CtfA/B, acetoacetyl-CoA:acetate/butyrate-CoA transferase; Adc, acetoacetate decarboxylase; Hbd, 3-hydroxybutyryl-CoA dehydrogenase; Crt, crotonase; Bcd, butyryl-CoA dehydrogenase; AdhE2, bifunctional acetaldehyde/alcohol dehydrogenase.

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Future prospect

During this project a novel gene knockout system was developed, based on the use of a repurposed toxin-antitoxin system as a counter-selective marker. This strategy is enabled by the efficient transfer of DNA to *E. limosum*, which allows for the use of non-replicating plasmids. This in turn minimises the risk of phenotypic reversion in mutants, which has previously been observed in *E. limosum* mutants generated using replicative CRISPR/Cas9 knockout vectors. The gene knockout system is currently being used to validate those genes identified as being essential for growth on methanol using TraDIS – many of which are hypothetical proteins. The system has proven to applications in other organisms, in particular as a system for inducing plasmid loss in a range of Gram positive and Gram negative chassis.

FAST expands the molecular toolbox of *E. limosum*. Moreover, FAST might be suited as a reporter protein in other anaerobic bacteria as well, which includes several industrial relevant acetogens like *C. autoethanogenum*. Therefore, FAST might be the reporter protein of choice for applications in anaerobic environments in the future. In addition, the production of value-added products from methanol is feasible with *E. limosum*, which enables new opportunities for the sustainable production of butanol and acetone.

The developments made represent a important step in the path towards new sustainable route to the production of chemicals and fuels. Methanol represents an ideal feedstock as it does not suffer from mass transfer issues in fermenters, is easily stored and transported and me be derived from biomass, MSW, biogas, waste CO_2, and even renewable electricity.

The novel mutagenesis method drafted for publication. The Genome Scale model and systems biology to be published with KAIST (South Korea) - joint review already published:

Jin S, Bae J, Song Y, Pearcy N, Shin J, Kang S, Minton NP, Soucaille P, Cho BK. Synthetic Biology on Acetogenic Bacteria for Highly Efficient Conversion of C1 Gases to Biochemicals. Int J Mol Sci. 2020 Oct 15;21(20):7639. doi: 10.3390/ijms21207639.

BIOMETCHEM metabolic engineering outputs presented as posters and talks at two international conferences and published:

Flaiz M, Ludwig G, Bengelsdorf FR, Dürre P. Production of the biocommodities butanol and acetone from methanol with fluorescent FAST-tagged proteins using metabolically engineered strains of *E. limosum*. Biotechnol Biofuels. 2021. 14, 1-20. doi: 10.1186/s13068-021-01966-2.

RRI outputs being submitted to Journal of Responsible Innovation and presented at numerous events including an online event on 'Responsible Innovation in Industrial Biotechnology and Engineering Biology' on 25th Jan 2021 with 225 participants from 19 countries and sectors including higher education, industry, policy/ government, research funding, civil society.

Hadley Kershaw, E. Raising reflexivity: Science and Technology Studies intervention in international scientific governance and practice. Society for Social Studies of Science (4S) Annual Conference 2019, New Orleans, US, Sept 2019.

Hadley Kershaw, E. Invited participant. 3rd Social Lab workshop on RR H2020 'Leadership in enabling and industrial technologies' (New HoRRIzon Consortium). Wageningen University and Research, NL, (online) Apr 2020.

Hadley Kershaw, E., McLeod, C., de Saille, S. 'RRI in ERA CoBioTech: Challenges and opportunities of using Lego Serious Play', European Biotechnology and Society Online Seminar Series 1, 14th Oct 2020. Hadley Kershaw, E. 'RRI in European Biotechnology: Looking back and forward', European Biotechnology and Society Online Seminar Series 2, 7th Jul 2021.

Website: https://sbrc-nottingham.ac.uk/associated-projects/biometchem.aspx

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