ERA CoBioTech (ERA-Net Cofund on Biotechnologies)

ACHEMA2018

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

Project name: Microbial conversion of C1 to value-added products by integrated systems and synthetic biology

Project acronym: C1Pro

Name: Trygve Brautaset, PL

SINTEF

NTNU

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

Frankfurt am Main, 13.06.2018

Project partners

- Partners
  - Partner 1: Trygve Brautaset, NTNU, Norway
  - Partner 2: Volker Wendisch, University of Bielefeld, Germany
  - Partner 3: Stephanie Heux, INSA Toulouse, France
  - Partner 4: Oskar Zelder, BASF, Germany
  - Partner 5: Ingemar Nærdal, SINTEF, Norway
  - Partner 6: Gregor Kosec, Acies Bio, Slovenia
- Total project budget: 1.767.000 Euro
- Project start: 01.03.2018

Introduction

- Project objective (problem to be solved)

C1Pro project aims to establish a sustainable platform for methanol-based production of four value-added products:

1) gamma-aminobutyric acid (GABA)

2) 5-aminovaleric acid (5AVA)

3) L-proline (L-Pro)

4) L-pipecolic acid (L-PA)

with proven industrial applications!

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Scientific approach and project topic area:

- Methanol is an attractive and alternative raw material for biotechnological processes because of its chemical properties, relatively low price and availability from both fossil and renewable sources.

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- Gram-positive, methylotrophic and thermophilic bacterium *Bacillus methanolicus* was chosen as model organism in this project for several reasons:
  - It utilizes methanol as raw material for growth and energy
  - It is thermophilic and grows at elevated temperatures (50 – 55 °C)
  - It naturally overproduces L-glutamate, and its classical mutants have demonstrated a high potential to overproduce L-lysine.

Introduction

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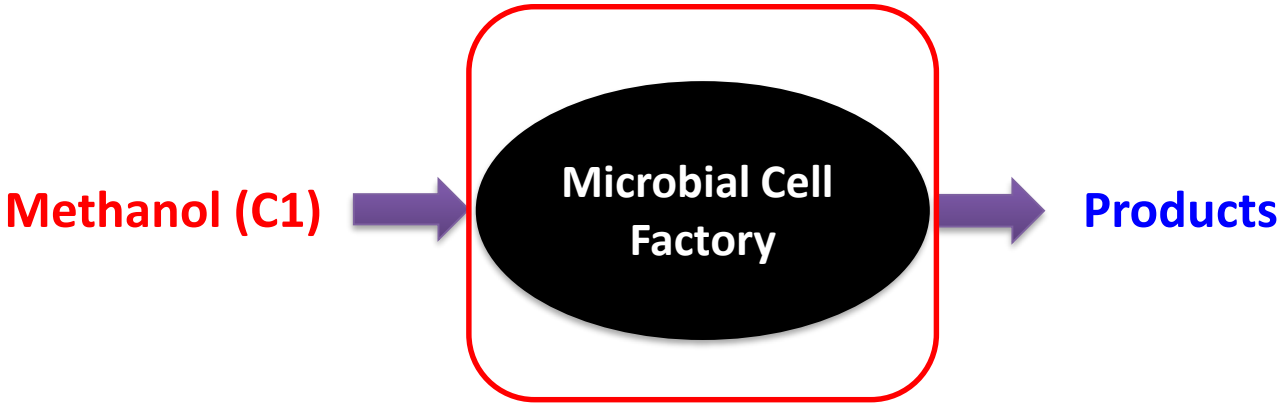
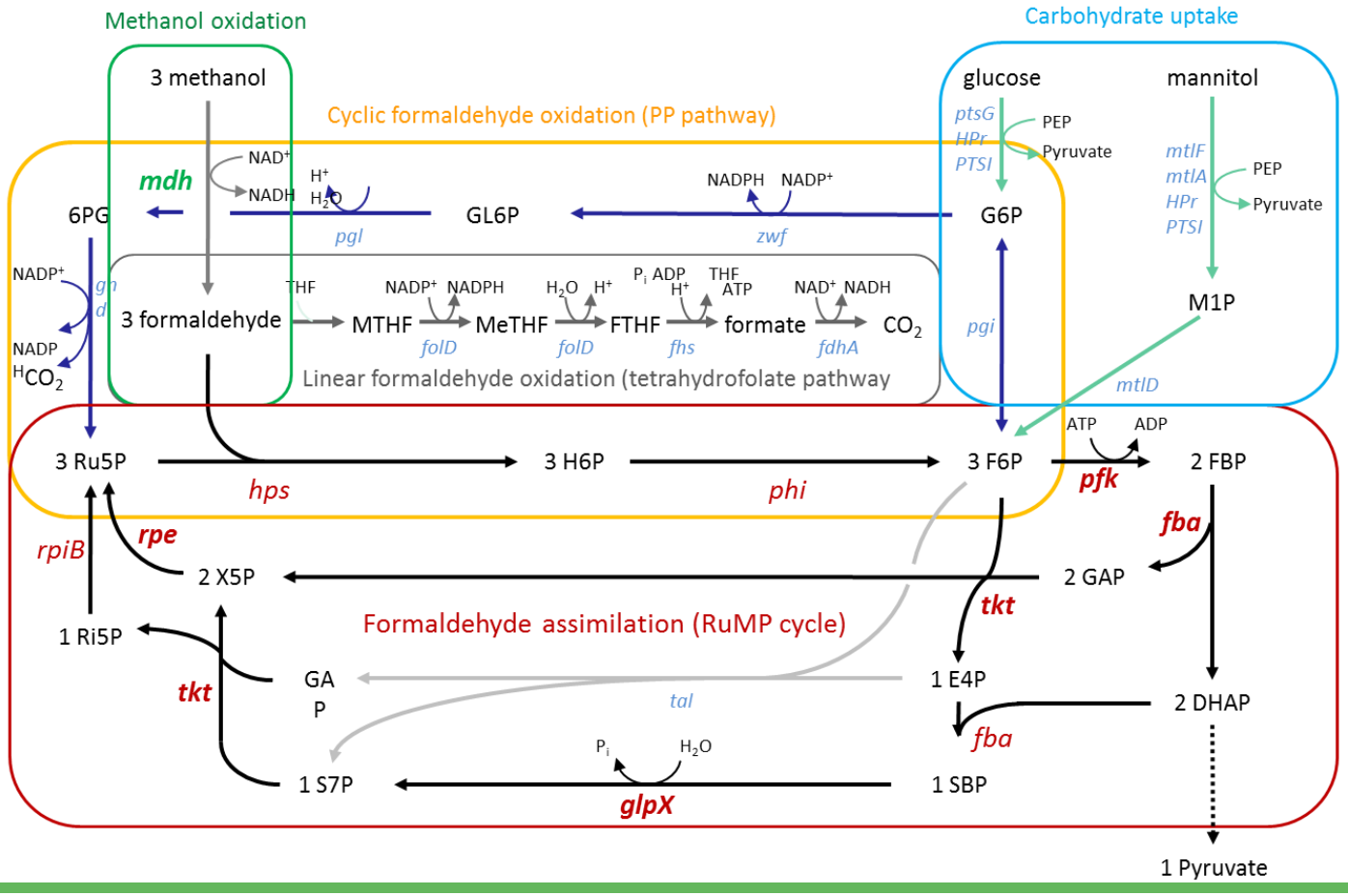
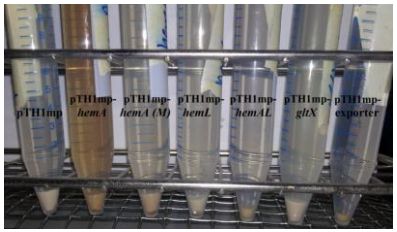


Methylotrophic pathways in *B. methanolicus*



Platform strain for production of amino acids and their derivatives

- L-Glutamate: MGA3, 60 g/L (Heggeset *et al.*, 2012)
  - GABA: MGA3 (pTH1mp-*gad*ST), 9 g/L (Irla *et al.*, 2016a)

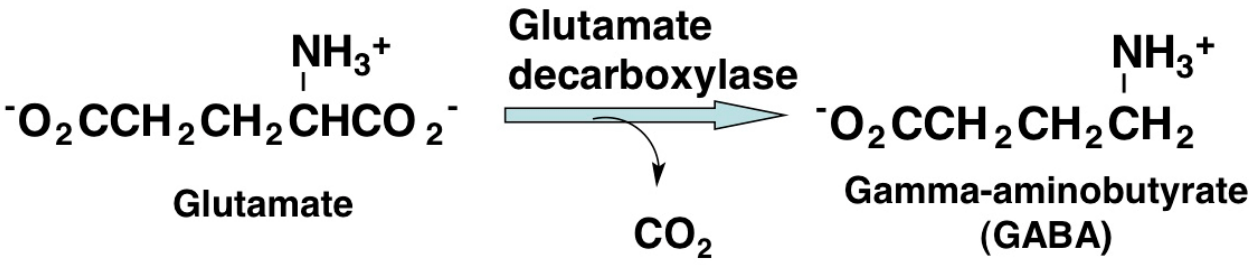


- L-Lysine: MGA3 (pTH1yclM), 11 g/L

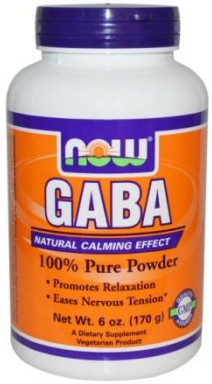
(Nærdal *et al.*, 2011)

  - Cadaverine: MGA3 (pTH1mp-*cadA*), 11 g/L (Nærdal, Pfeifenschneider *et al.*, 2015)

Production of γ-aminobutyric acid (GABA) from methanol



Expression of heretologous glutamate decarboxylase (*gad*) genes in *B. methanolicus*

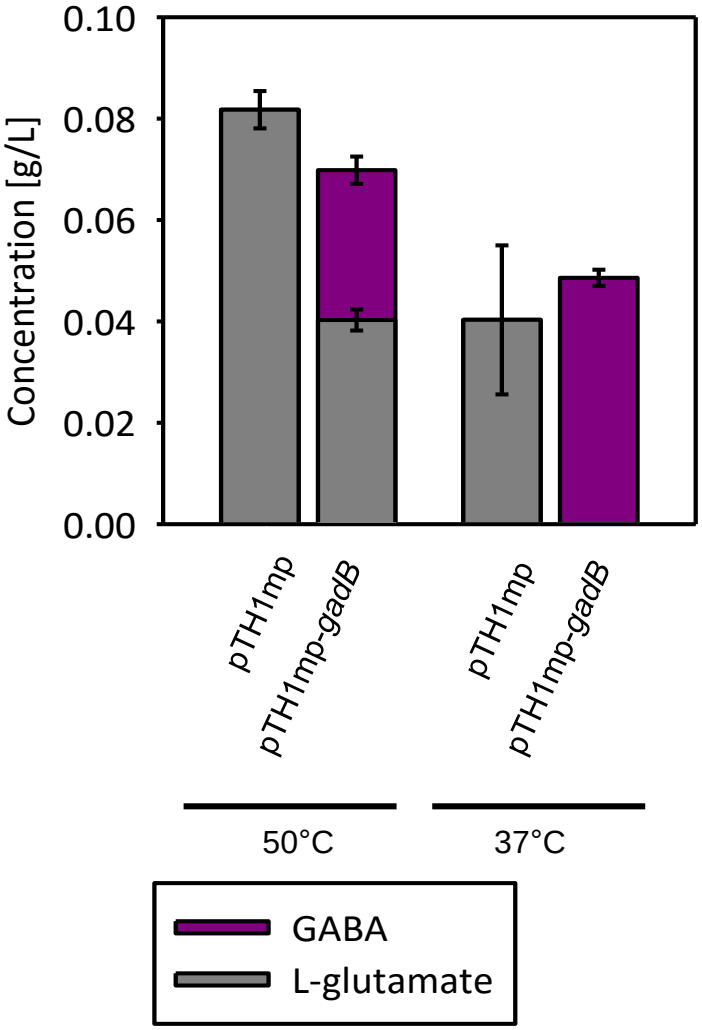


Evaluation of GABA producing *B. methanolicus* strains in small scale

Strain of *B. methanolicus*	Donor organism	Characterization of GAD in literature		GABA [g/L][5]
		Optimal pH	Optimal T [°C]	
MGA3(pTH1mp-*gadB*)	*E. coli* [1]	4.6	37	0.03±0.00
MGA3(pTH1mp-*gad*St)	*Sulfobacillus thermosulfidooxidans*	Not characterized		0.03±0.00
MGA3(TH1mp-*gad*^{Bm})	*B. megaterium*	Not characterized		Not detected
MGA3(pTH1mp-*gad*^{Ct})	*Corynebacterium terpenotabidum*	Not characterized		Not detected
MGA3(pTH1mp-*gadB1*^{Lb})	*L. brevis*[2]	4.0-5.2	37-50	Not detected
MGA3(pTH1mp-*gadB2*^{Lb})	*L. brevis*[3]	4.2-5.0	30	Not detected
MGA3(pTH1mp-*gadB3*^{Lb})	*L. brevis*	Not characterized		Not detected
MGA3(pTH1mp-*gadB1*^{Ao})	*Aspergillus oryzae*[4]	5.5	60	Not detected
MGA3(pTH1mp-*gadB3*^{Ao})	*Aspergillus oryzae*	Not characterized		Not detected

Overexpression of *E. coli* and *S. thermosulfidooxidans*-derived *gad* led to accumulation of GABA in *B. methanolicus*

Influence of temperature on GABA production in strains expressing the *E. coli gadB* gene



Decrease of temperature leads to full conversion of L-glutamate to GABA within 24 hours

Irla *et al*., 2016a

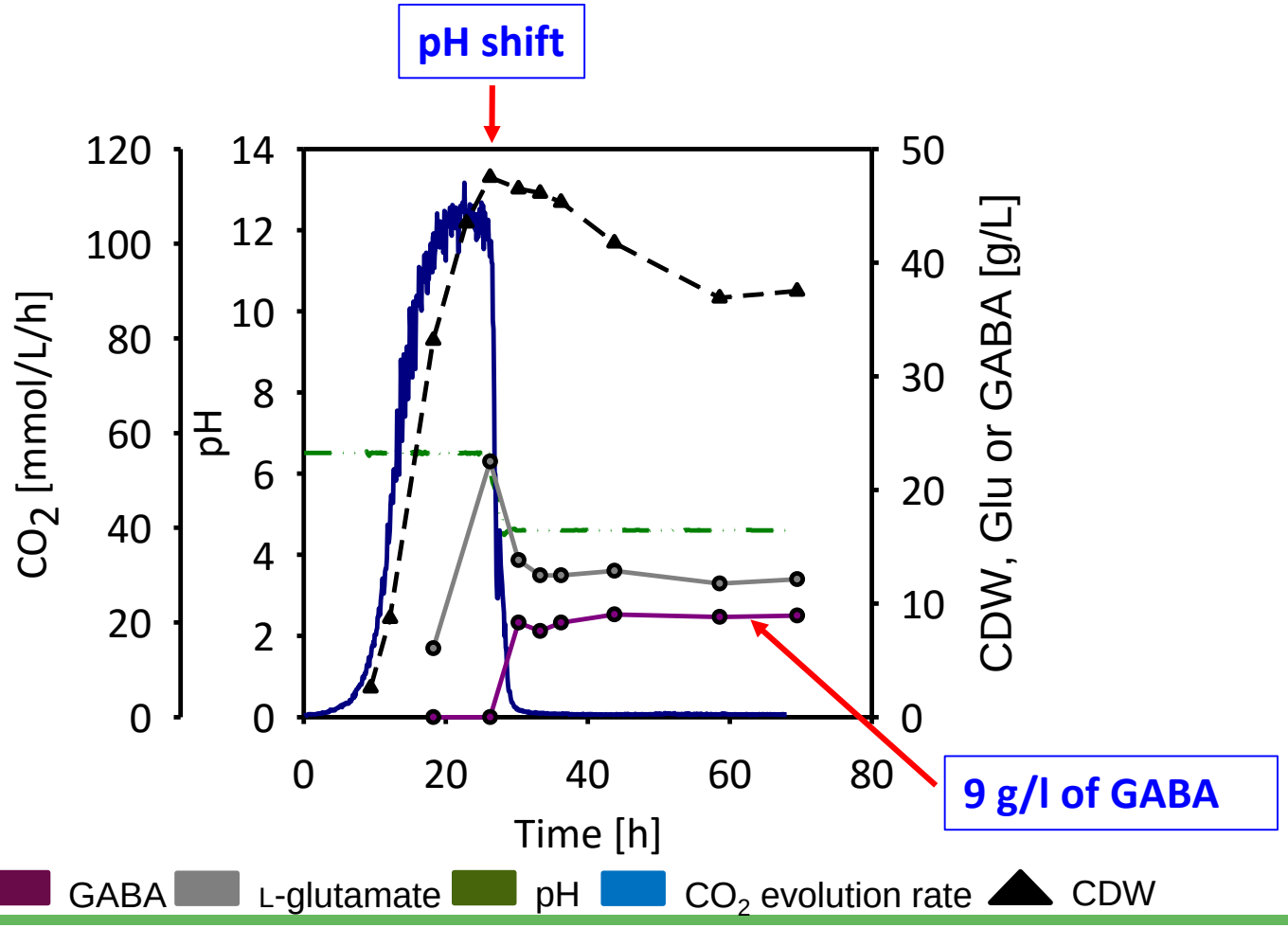
Strain evaluations under methanol controlled fed-batch fermentation

Strain of *B. methanolicus*	Conditions of fermentation	CDW [g/L]	Glu [g/L]	GABA [g/L]
MGA3(pTH1mp-*gadB*)	Control conditions	45.5	37.9	0.0
MGA3(pTH1mp-*gadB*)	Shift to 37 °C	31.0	25.5	0.0
MGA3(pTH1mp-*gadB*)	Shift to pH 4.6	34.2	24.3	0.0
MGA3(pTH1mp-*gad*St)	Control conditions	31.6	28.8	0.1
MGA3(pTH1mp-*gad*St)	Constant pH 6.0	41.0	31.7	0.3
MGA3(pTH1mp-*gad*St)	Shift to pH 4.6	47.5	12.9	9.0

The condition optimization leads to 90-fold increase of GABA accumulation in comparison to control conditions

Irla *et al*., 2016a

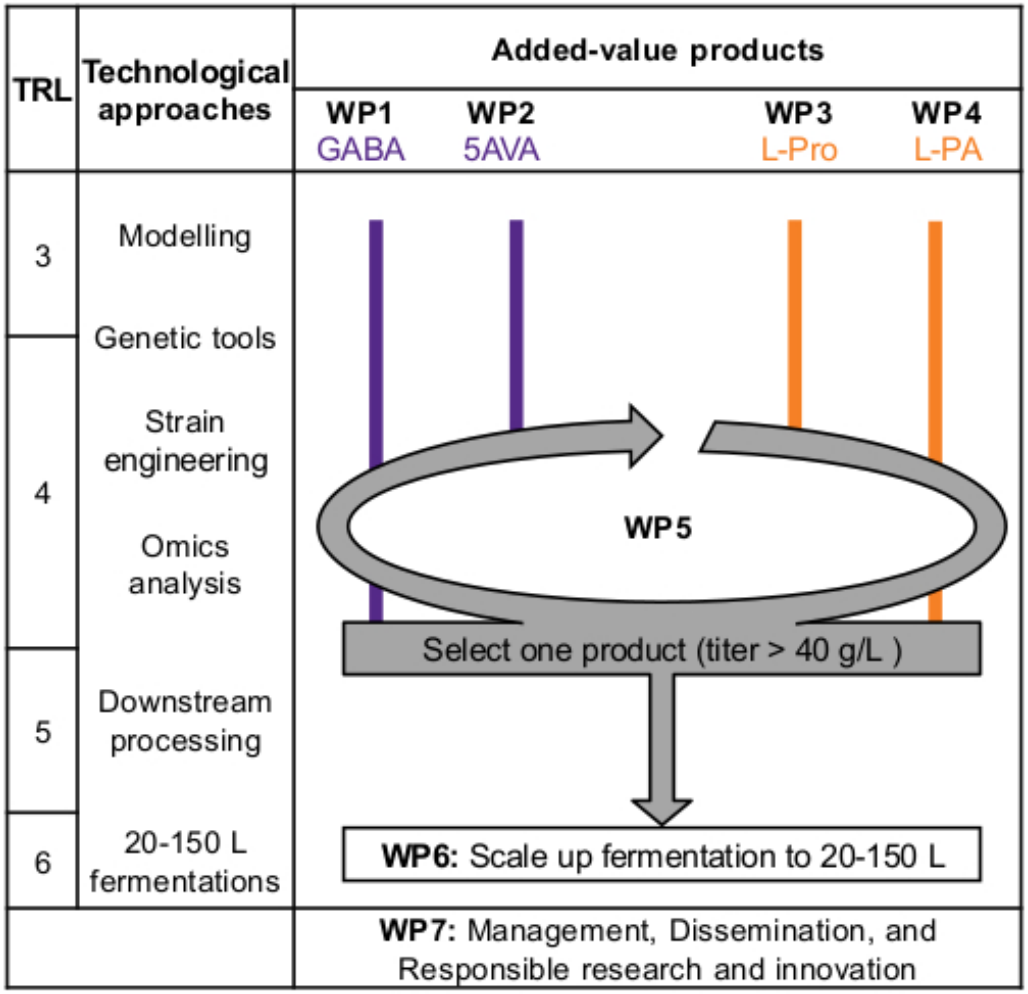
Methanol controlled fed batch fermentation



Project plan

- ***C1Pro consist of 7 interlinked Work packages (WPs):***
  - ***WP1:*** *Establishment of technological platform for GABA production and downstream processing*
  - ***WP2:*** *Development of 5AVA production strains and application of synthetic regulatory circuits*
  - ***WP3:*** *Development of L-Pro production strains and genetic tools for engineering industrial production strains*
  - ***WP4:*** *Development of L-PA production strains and application of synthetic regulatory circuits*
  - ***WP5:*** *System-based analysis for strains design and optimization*
  - ***WP6:*** *Scale up to pilot scale of fermentation process with highest industrial potential.*
  - ***WP7:*** *Management, communication and dissemination, and Responsible Research and Innovation*

Project plan



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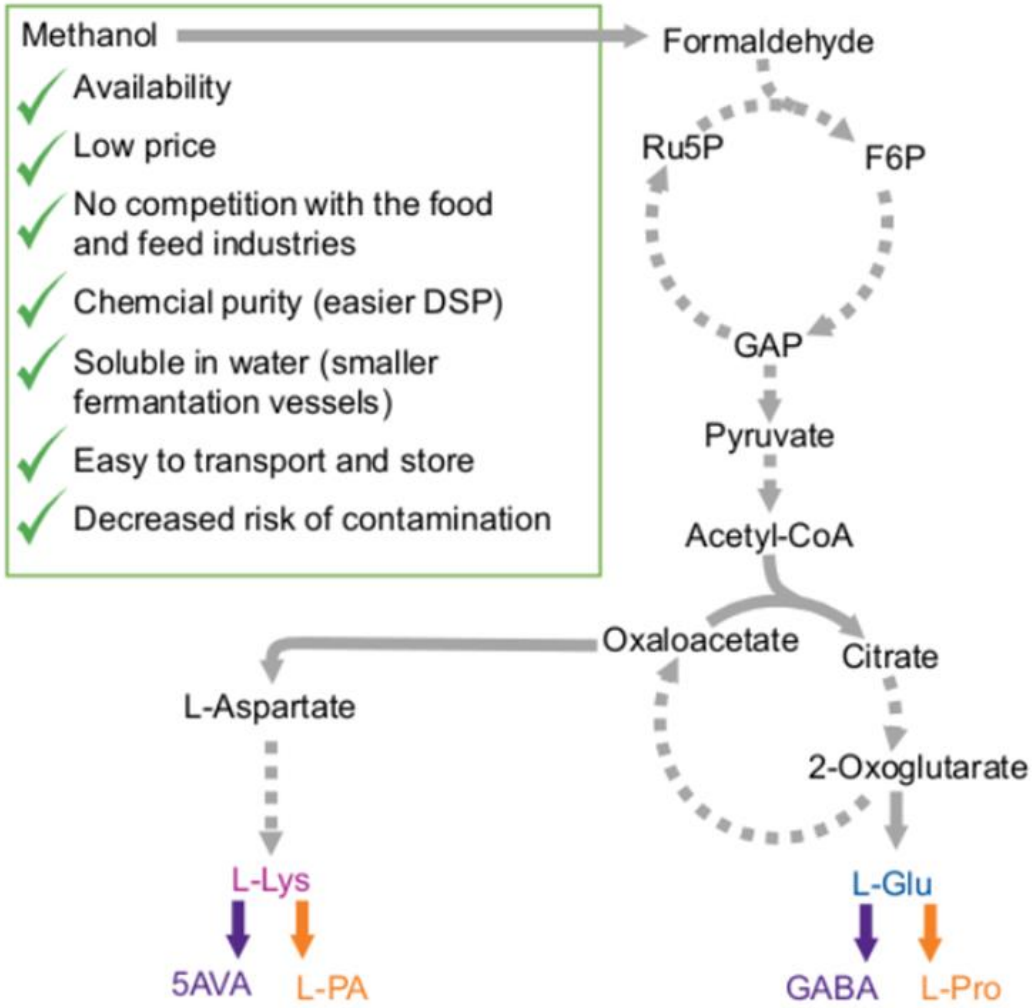

Data Management (DM) Plan

- C1Pro will generate experimental data (meta-data, physiologic and fermentation data, product/substrate/metabolite analytic data from HPLC/GC/MS/NMR experiments, transcriptomics data from RNAseq experiments and fluxomics data from 13C-labeling experiments)

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- The DM plan builds on our previous experience in the ERASysAPP project MetApp (partners NTNU, SINTEF, UNIBI, INSA)
  - DMP-representative: Prof. Volker Wendisch (UNIBI)
  - PAL-Modeller: Dr. Stephanie Heux (INSA)
  - PAL- Experimental: Dr. Marta Irla (NTNU)

Data Management (DM) Plan

- Long term storage of the processed data intended for dissemination together with metadata and scripts in interlinked form is planned in FAIRDOMHub

- Data-sharing within the project will be organized via a project-own cloud maintained at UNIBI and via SEEK

Communication and Dissemination (CD) strategy

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- C1Pro communication addresses as stakeholders:
  - The general public
  - Professionals active in chemical/ biotech industry
  - Policy makers in the order reflecting priority ranking

Responsible Research & innovation (RRI)

- Scientists should increasingly reflect on their visions and presumptions, including positive and negative impacts of their work on society
- An effective process of learning about making research and innovation responsible to the needs of society is supposed to emerge through processes of anticipation, reflection, and inclusion:

What future should biotech enable?

- **Anticipatory** – plausible impacts & implications
- **Reflexive** – how do I know what I now?
- **Inclusive** – who might be affected by my research?
- **Responsive** – adapt practices & governance to lessons learned

Builds on RRI framework

RRI crosscutting activity

Expected outcomes

Outcomes to be achieved; planned implementation and exploitation of results?

- Industrially interesting production strains and technology
- New Knowledge
- Scale-up and demonstration
- IPR and comercialization

Summary

What is proposed; what should be achieved?

- *Combined synthetic and systems biology*
- *Transdisciplinarity*
- *Strong industry collaboration*
- *Industrially relevant project strains and products*
- *Training arena for PhD, Postdoc and Master candidates*

Contact details

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