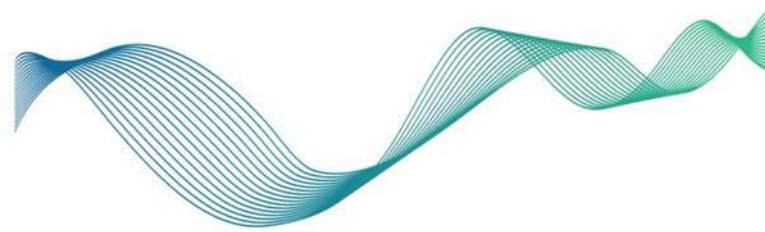


MarineBiotech



ThermoFactories

Thermophilic cell factories for efficient conversion of brown algae biomass to high-value chemicals

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ERA-MBT Meeting Lysaker

21.11.2017



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THE CONSORTIUM



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Project period: 01.02.2016 to 31.01.2019

Aims and Objectives

- **Brown algae** - promising and challenging raw material for industrial biotechnology!
- **ThermoFactories goal** - develop thermophilic microbial cell factories to convert macroalgal carbohydrates into high-value products!



Aims and Objectives

- Four metabolically different environmental bacteria - all inherently suitable to harsh process conditions - are **genetically engineered** for production of 1,2-propanediol, cadaverine, propanol and terpenes:
 - *Thermoanaerobacterium AK17*
 - *Lactobacillus reuterii*
 - *Rhodothermus marinus*
 - *Bacillus methanolicus*
- Why thermophiles:
 - Reduced risk of contamination
 - Increased solubility of sugars (laminaran and alginate), reduced viscosity

Aims and Objectives

- Minimal pre-treatment of the brown algae for **feedstock development**
- **Strain constructions and process engineering**



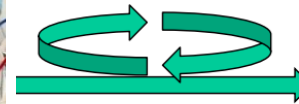
Saccharina latissima



Seaweed extract

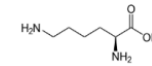


Microbial cultivations at elevated temperatures

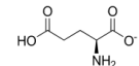


Systems biology,
metabolic
engineering, process
development

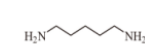
High value products



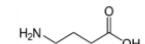
L-lysine¹



L-glutamate²



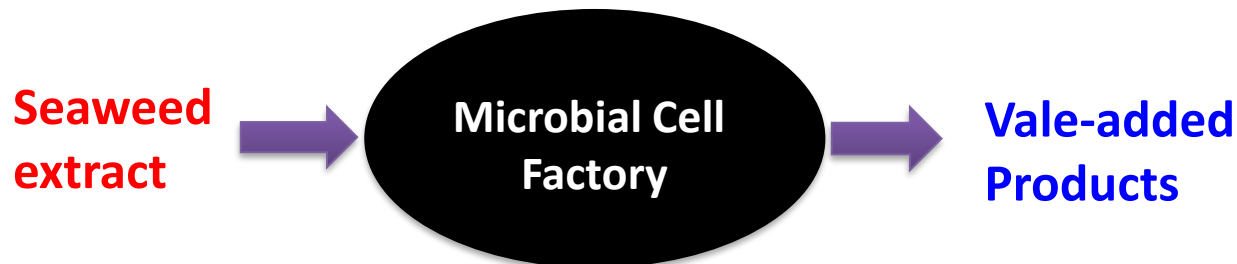
Cadaverine³



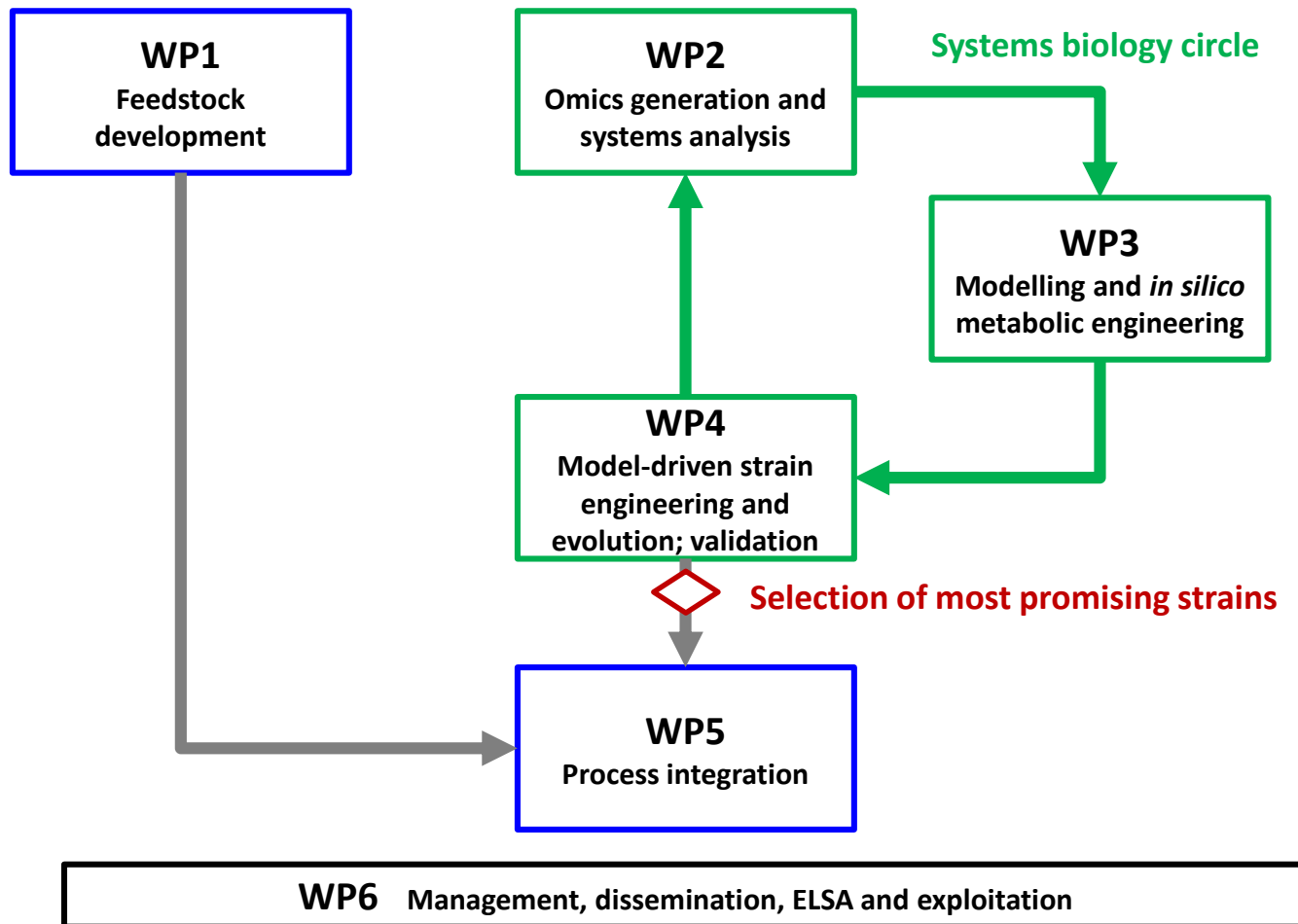
GABA (γ-aminobutyric acid)⁴

Aims and Objectives

- ThermoFactories is a concerted European effort - the work plan consists of three interconnected **technical platforms**:
 - Systems biology
 - Metabolic engineering
 - Process development



Progress to date with achieved results



WP1 – Feedstock development: Macroalgae pre-processing and monosaccharide-rich media preparation



- **Mechanical/chemical pre treatment;** solubilization of major carbohydrates
 - Mannitol, fucose, galactose, glucose, xylose
 - Mannoronic-, guloronic-, glucuronic acids
- **Enzymatic hydrolysis** to oligo- or monomers
 - 3 *Rhodothermus marinus* enzymes recombinantly produced in **WP4**, cellulase (Cel12A), alginate lyase III, and alginate lyase IV

WP1 – Feedstock development: Macroalgae pre-processing and monosaccharide-rich media preparation

Macroalgal processing...:

- *Saccharina latissima*, *Laminaria digitata*



WP2 – Omics generation and systems analysis

- **Genome sequencing** of novel and evolved strains:
 - **24 *Thermoanaerobacter*** wild-type strains; 1,2-propanediol biosynthetic pathway genes + seaweed utilization pathway genes
 - **4 *Lactobacilli reuteri*** strains including mutants growing on elevated temperatures (+46C)
 - **11 *Bacillus methanolicus*** wild-type strains; differences in plasmids and central carbohydrate catabolic pathways

WP2 – Omics generation and systems analysis

Grouping of *Bacillus methanolicus* wild-type strains based on annotated genomes...

	MGA3	NIWA	NOA2	N2	HEN9	CFS	DFS2	BVD	TSL32	JCP	SC6	RCP	PB1
MGA3	99.98	94.28	99.02	89.61	90.24	89.27	90.00	73.65	72.03	73.17	73.28	73.79	71.57
NIWA	92.30	99.78	93.96	89.67	90.51	89.42	90.09	72.87	71.60	72.53	72.78	73.72	71.32
NOA2	97.77	90.92	98.25	85.12	85.93	84.59	85.50	65.04	63.82	64.86	65.02	66.89	69.96
N2	90.33	91.88	91.97	99.77	96.17	99.72	99.77	73.16	71.29	72.37	73.10	73.36	71.08
HEN9	89.55	91.66	91.28	95.66	99.73	95.42	95.81	72.82	70.86	72.01	72.45	73.06	70.99
CFS	90.38	91.91	92.00	99.68	96.16	99.68	99.72	73.60	71.80	72.86	73.45	73.70	71.22
DFS2	90.32	91.94	91.92	99.77	96.25	99.75	99.80	73.20	71.29	72.39	73.12	73.39	71.06
BVD	76.54	76.65	74.06	74.68	75.98	74.28	76.14	99.77	99.72	99.76	93.70	95.03	77.41
TSL32	76.47	76.57	73.93	74.63	75.92	74.22	76.06	99.74	99.70	99.74	93.67	95.00	77.37
JCP	76.44	76.55	73.92	74.55	75.85	74.17	76.00	99.74	99.71	99.75	93.66	95.00	77.36
SC6	77.06	76.76	74.81	75.21	76.46	74.91	76.66	92.76	92.41	93.46	99.58	92.56	78.53
RCP	74.44	76.06	73.14	74.12	75.26	73.65	75.46	91.99	92.58	92.71	91.18	99.68	78.42
PB1	72.05	74.29	70.09	72.56	73.56	72.11	73.90	76.12	74.83	75.91	77.13	77.82	99.76

WP2 – Omics generation and systems analysis

- **Transcriptome and metabolome analysis for model refinement**
 - *Lactobacillus reuteri* and *Rhodothermus marinus*:
 - Optimizing media for growth rate and biomass formation, product secretion
 - Data used for the modeling in **WP3**

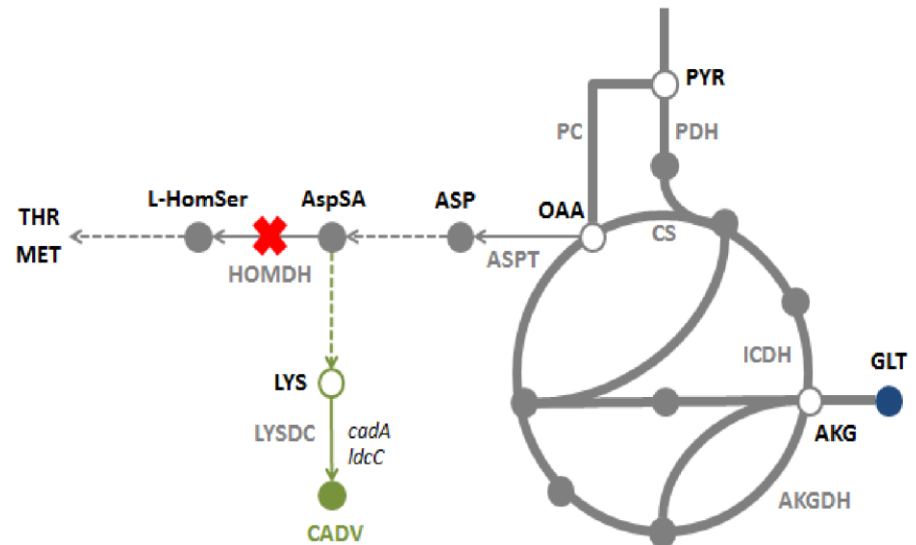
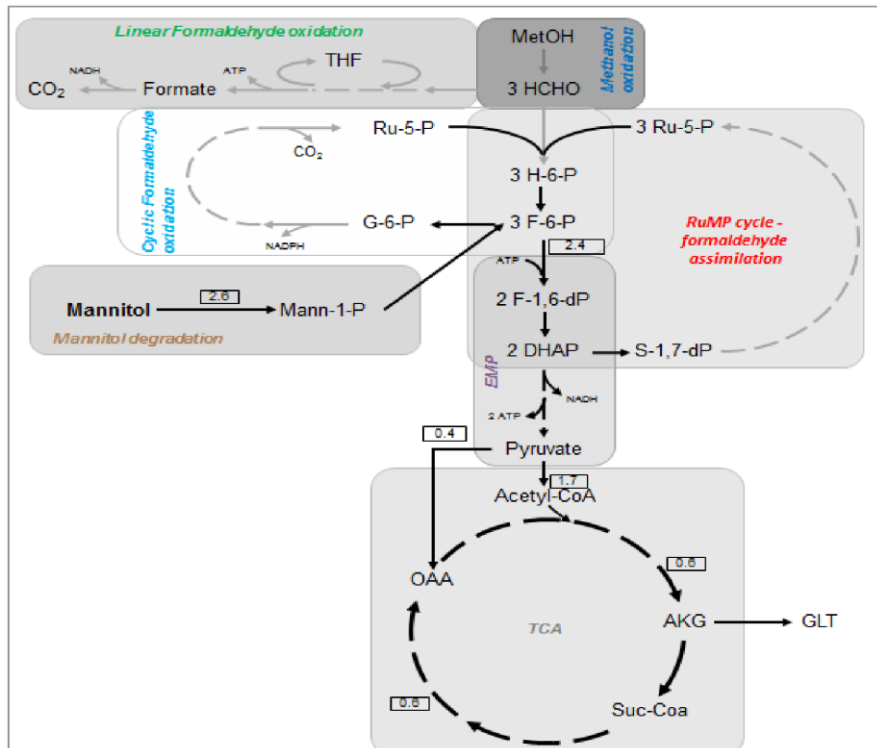
WP3 – Genome-scale modelling and in-silico metabolic engineering

Summary statistics for the Thermofactories metabolic network reconstructions:

Metabolic reconstruction	Bacillus methanolicus	Lactobacillus reuteri	Rhodothermus marinus	Thermoanaerobacterium AK17(*)
Genome size (Mbp)	3.4	2.0	3.3	2.4
Number of genes	425	549	510	(179)
Number of enzymatic reactions	637	521	735	(284)
Number of unique metabolites	425	574	718	(258)
Number of transport reactions	121	114	85	(14)

WP3 – Genome-scale modelling and in-silico metabolic engineering

Bacillus methanolicus genome-scale modelling:

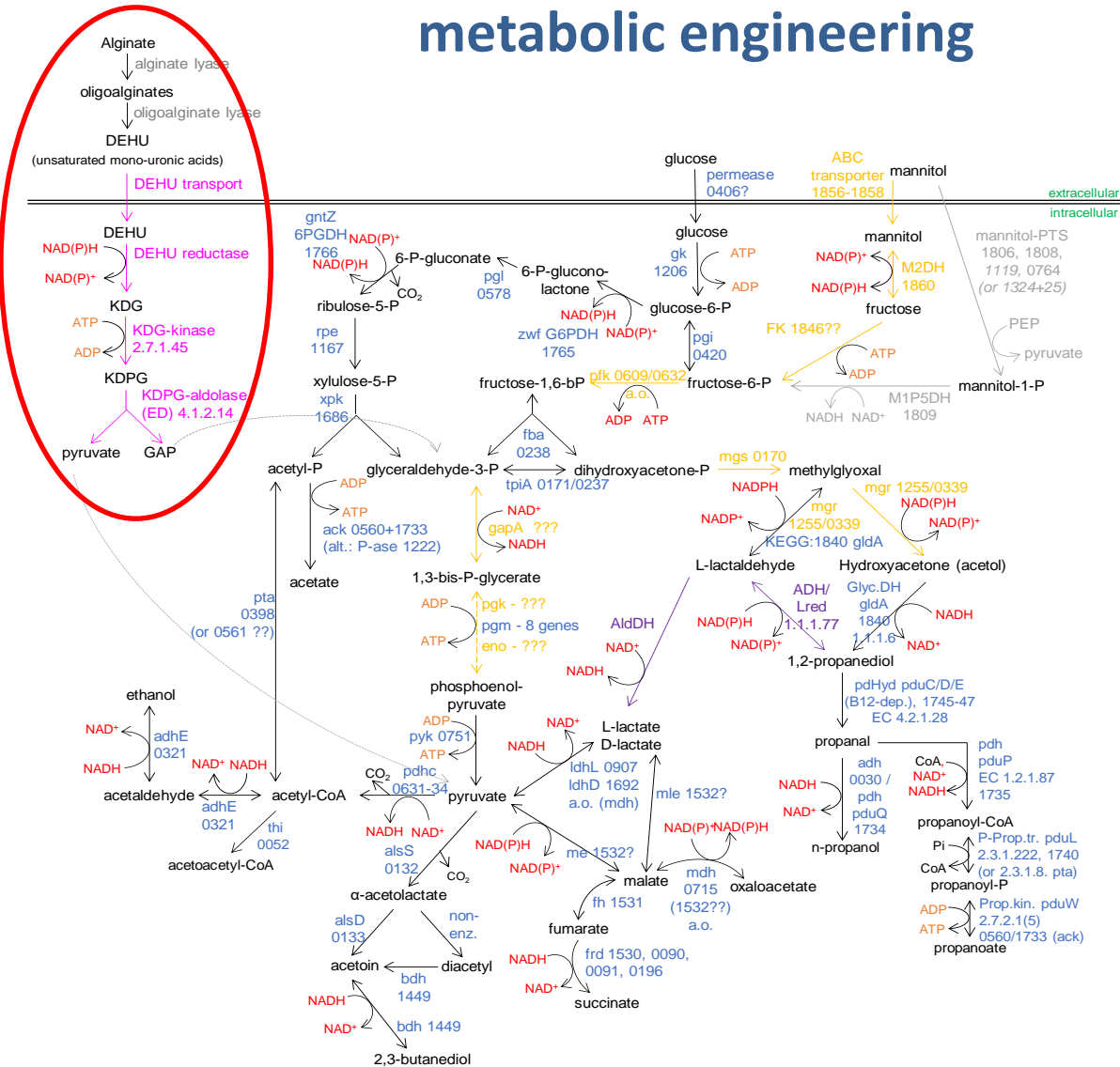


PC – pyruvate arboxylase
 PDH – pyruvate dehydrogenase
 CS – citrate synthase
 ICDH – isocitrate dehydrogenase
 AKGDH – 2-oxoglutarate dehydrogenase
 ASPT – aspartate transaminase
 HOMDH – homoserine dehydrogenase
 LYSDC – lysine decarboxylase

Predicted flux distributions for growth on mannitol (**left**) and *in silico* design for cadaverine production (**right**)

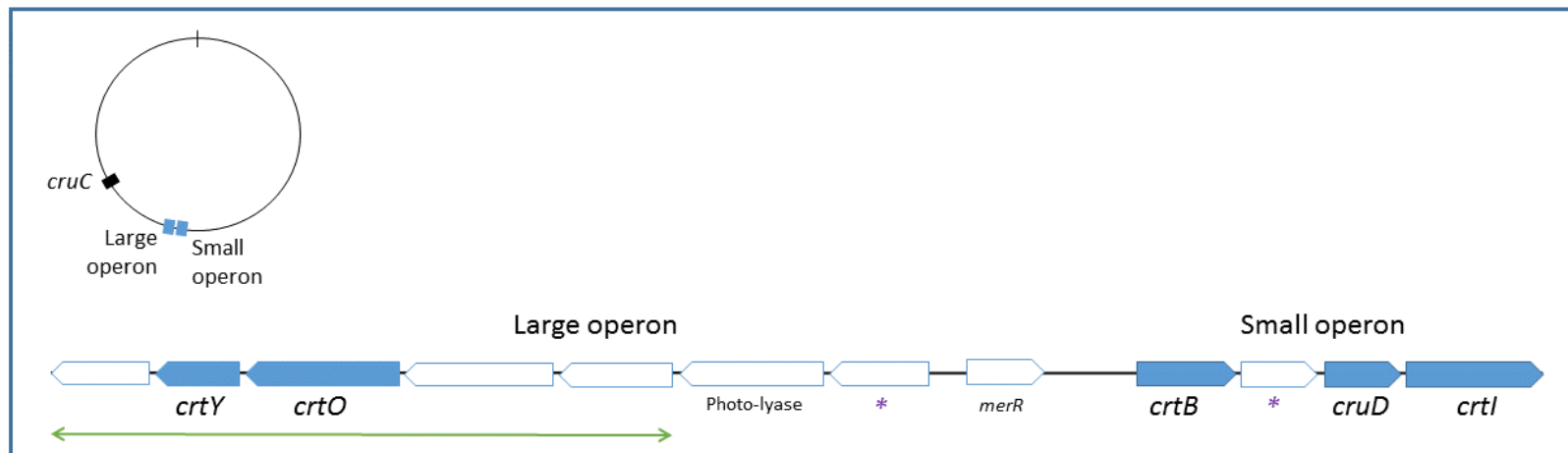
WP3 – Genome-scale modelling and in-silico metabolic engineering

An overview of the central metabolism of *Lactobacillus reuteri* together with a proposed pathway for alginate catabolism.



WP4 – Model-driven metabolic engineering and evolution of biorefinery strains and evaluation

- Consolidated production of carotenoids in *Rhodothermus marinus*
 - Rhodothermus marinus* is inherently suitable for bioconversions in consolidated processes.
 - It produces and secretes several **hydrolases** and **lyases** active on macroalgal polysaccharides, including alginate.

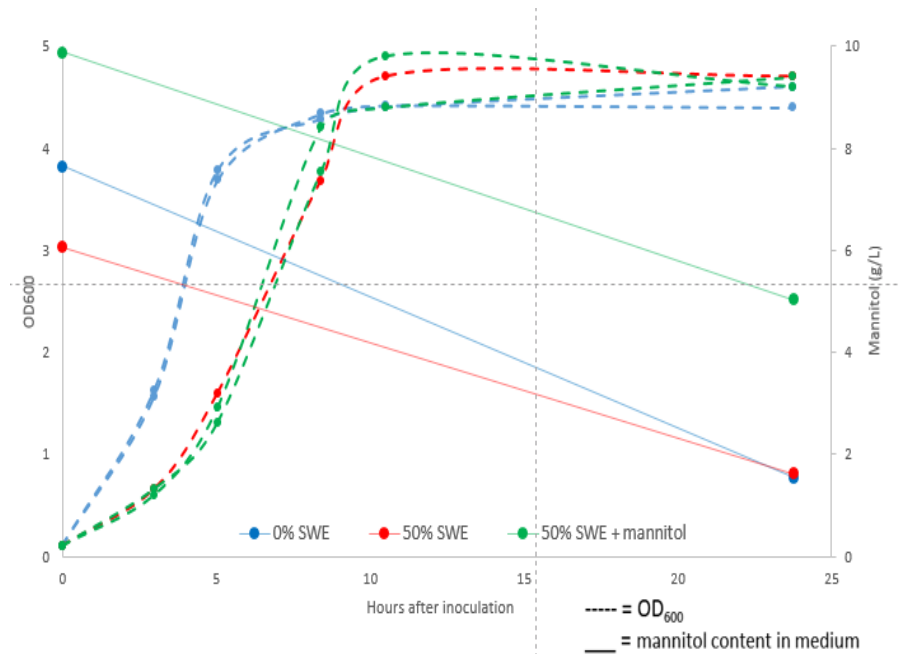


Carotenoid gene cluster:

- Green arrow shows the genes that have been knocked out
- Purple stars show genes which are planned to be knocked out

WP4 – Model-driven metabolic engineering and evolution of biorefinery strains and evaluation

Conversion of seaweed into cadaverine by *Bacillus methanolicus*



Shakeflask cultivations

mg/L

MGA3 (pBV2mp-cadA)

MGA3 (pTH1mp-cadA)

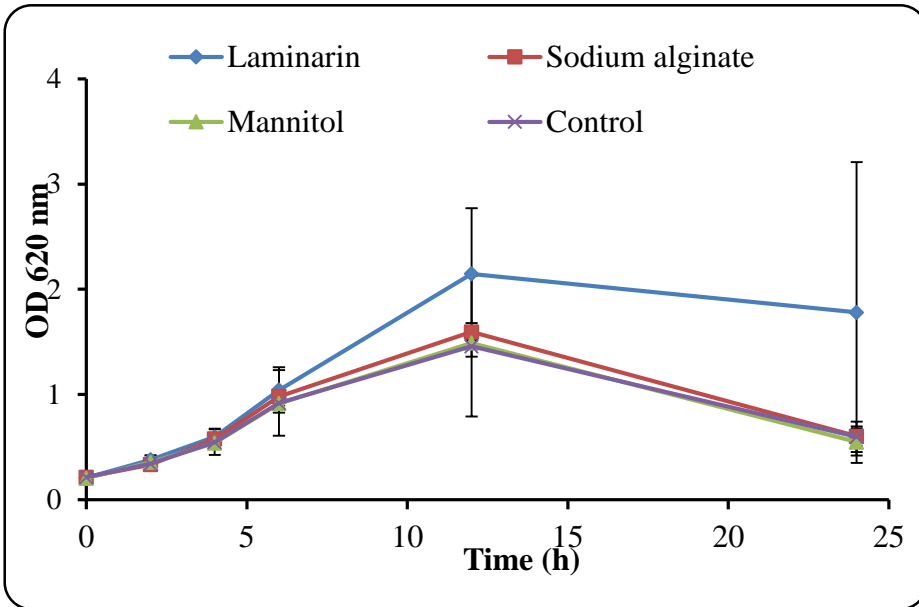
	MeOH ₂₀₀	Man ₁₀	SWE
MGA3 (pBV2mp-cadA)	407*	572	486
MGA3 (pTH1mp-cadA)	19	14	15

For the first time:

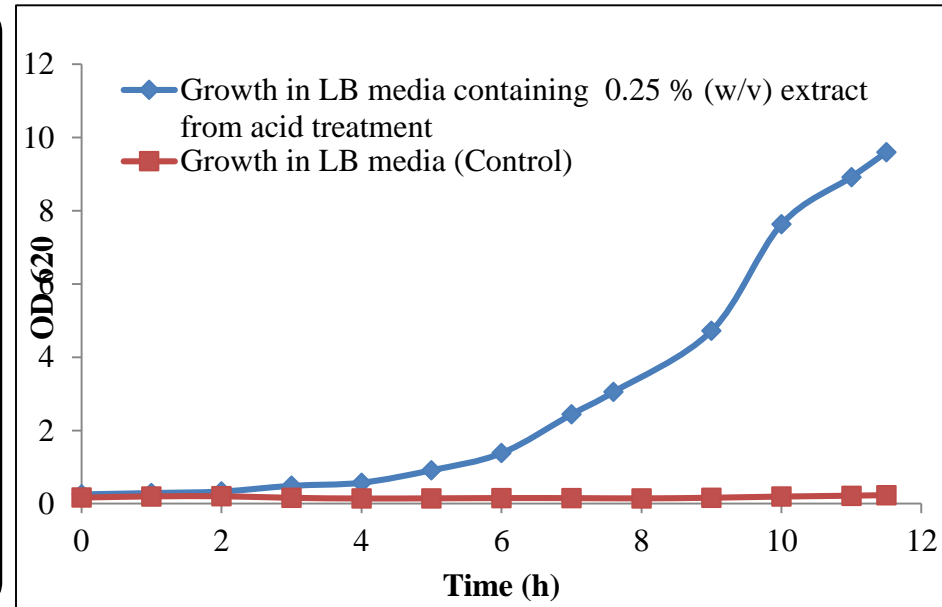
- Growth of *Bacillus methanolicus* on seaweed raw material!
- Cadaverine production from seaweed raw material!

WP5: fermentation and product recovery – Process integration

Simultaneous saccharification and fermentation of algae biomass using *Rhodothermus marinus* and its enzymes



Shake flasks



Fermentor

Progress to date with achieved results - summarized



- Integrated project – collaboration at all levels; sharing knowledge, biological materials, data & results
- Complete marine biorefinery pipeline: harvesting – processing – microbial cultivations – strain constructions – modeling & omics – production – upscaling

Potential objectives and impact



- **Research-related**
 - European trans-disciplinary collaboration
 - Academia + industry!
 - ...
- **Innovation-related**
 - Marine biorefinery
 - Industrial biotechnology; product oriented
 - Broad approach – new inventions and discoveries
 - ...
- **Societal-related**
 - Natural marine resources
 - Bioeconomy; sustainability
 - ...

Future directions and needs/gaps in this area



- Systems-level metabolic engineering for cell factories constructions; promising and also time consuming approach; establish trans-disciplinary consortia - joint creativity!
- Industry participation is good and stimulating
- Very good learning platforms for PhDs, postdocs and researchers

Thank you for listening!