



ERA CoBioTech

BIO TECH RESEARCH AND INNOVATION HACK 2021

Final seminar of the cofunded
projects of ERA CoBioTech



Thermophilic bacterial and archaeal chassis for extremolyte production



Project acronym: **HotSolute**

Name: **Bettina Siebers**

MEB, University Duisburg-Essen



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

28.09.2021

HotSolute partners

- **P1: Bettina Siebers**, Molecular Enzyme Technology and Biochemistry, University of Duisburg-Essen (Germany)
- **P2: Jennifer Littlechild**, Henry Wellcome Building for Biocatalysis, Biosciences, University of Exeter (The United Kingdom)
- **P3: Daniela Monti**, Consiglio Nazionale delle Ricerche, Istituto di Scienze e Tecnologie Chimiche "G. Natta" – CNR (Italy)
- **P4: Felix Müller**, Corporate Innovation, Evonik Industries AG, (Germany)
- **P5: Elizaveta Bonch-Osmolovskaya**, Winogradsky Institute of Microbiology, Federal Research Center of Biotechnology, RAS, (Russia)
- **P6: Jacky Snoep**, Biochemistry, Stellenbosch University (South Africa)



● Total project budget: **1670 k€**

● Project start: **01/03/2018**

- P1: B. Siebers, July 2018
- P2: J. Littlechild, March 2018
- P3: D. Monti, December 2019
- P4: F. Müller, July 2018
- P5: E. Bonch-Osmolovskaya, December 2019,
- P6: Jacky Snoep, July 2018



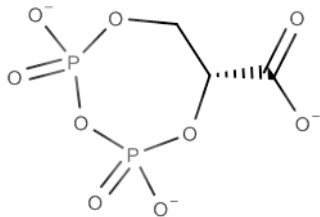
● Project end: **31/12/2021**

Compatible solutes

- Response to environmental stress → cell protection (e.g. protein stabilisation)
- Organic low-molecular weight compounds
- Highly soluble - accumulated in high concentrations (250 mM – 1.1 M)
- No interference with central metabolism
- Compatible solutes from extremophilic microorganisms → **extremolytes**
- Compounds with **medical and personal care application**
- **Pathways known, but so far no suitable production strains available**

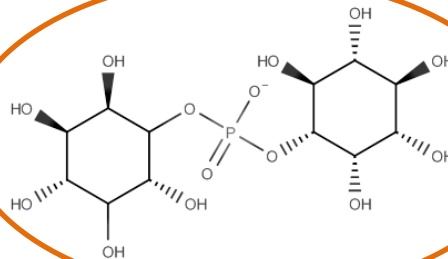
Cyclic-2,3-diphosphoglycerate

cDPG



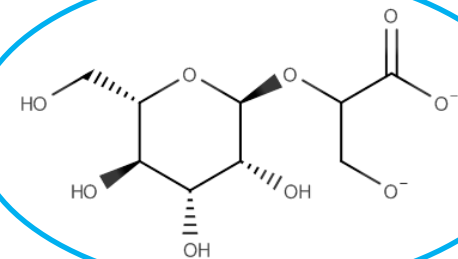
Di-myo-inositolphosphate

DIP



Mannosylglycerate

MG

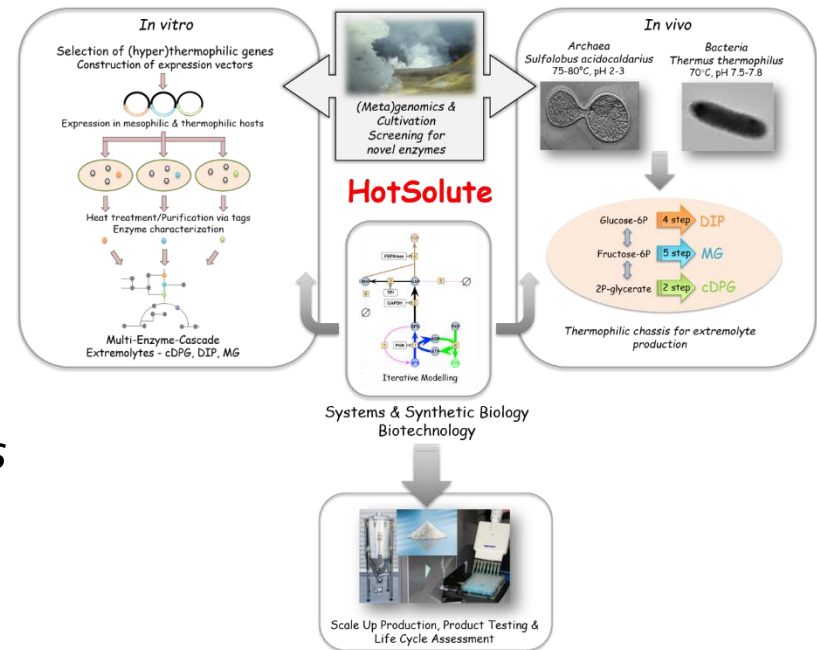


Project objectives

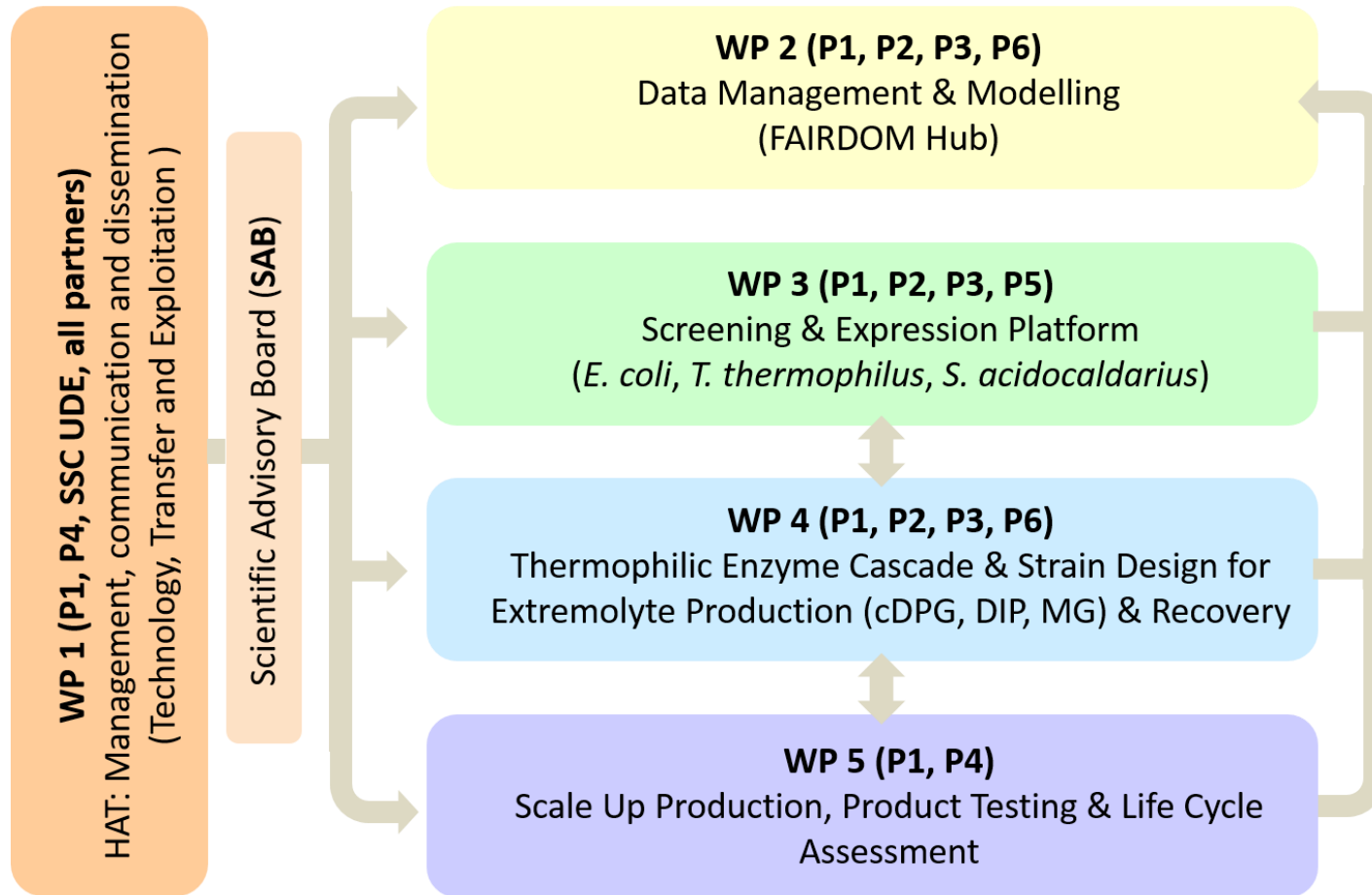
- Production of **extremolytes** by
 - ✓ **Thermophilic enzyme cascades**
 - ✓ **Two thermophilic 'cell factories'**
 - Bacterium *Thermus thermophilus* (*Tth*, 70°C, pH 7.5-7.8)
 - Archaeon *Sulfolobus acidocaldarius* (*Saci*, 75-80°C, pH 2-3)

Scientific approach and project topic area

- Synthetic biology, Systems biology
- Development of new products, value-added products and supply service



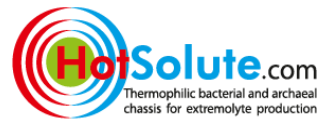
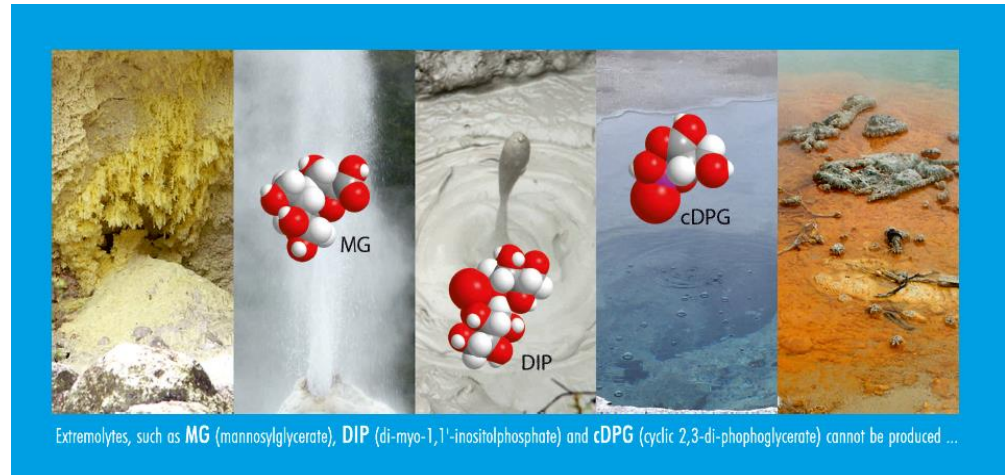
Work Packages



WP1 Communication and dissemination strategy

WP 1 (P1, P4, SSC UDE)

HAT: Management, communication and dissemination (Technology, Transfer and Exploitation)



The **HotSolute** project aims to produce small molecules 'extremolytes' for biotechnological applications. These molecules stabilise cellular components in organisms that grow at high temperatures and thereby protecting the cells from stress conditions.

Extremolytes cannot be produced in the available mesophilic production hosts and therefore two thermophilic hosts, *Thermus thermophilus* and *Sulfolobus acidocaldarius* of bacterial and archaeal origin, respectively, will be established as novel platform organisms.

The stabilisation effect of Extremolytes have many applications in industrial biotechnology, especially for the cosmetic and healthcare markets, and display therefore a high market product.

Professor Bettina Siebers

Biofilm Centre, Faculty of Chemistry,
University of Duisburg-Essen, Germany

Professor Jennifer Littlechild

Biocatalysis Centre,
University of Exeter, UK

Dr. Daniela Monti

Istituto di Chimica
del Ricicciamento Molecolare - CNR, Italy

Dr. Felix Müller


Vice President European Research Policy,
Evanik Industries AG, Innovation Networks, Germany

Professor Elizaveta Bonch-Osmolovskaya

Winogradsky Institute of Microbiology,
Federal Research Center of Biotechnology RAS, Russia

Professor Jacky L. Snoep

Department of Biochemistry,
Stellenbosch University, South Africa

 This project has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No [722361].



WP2 Data management & Modelling

WP 2 (P1, P6)
Data Management & Modelling
(FAIRDOM Hub)

- FAIRDOMHub/SEEK (Jacky Snoep)
- Fairdom Hub workshop in Essen (30.04.2019, 31.05.2021) by Olga Krebs, HITS, Heidelberg (GER)

The screenshot shows the FAIRDOM HUB search results for 'HotSolute', displaying 90 items. Below the search results is a detailed diagram of the Weimberg pathway optimization workflow, divided into three main stages: Experiment, Model, and Application.

Experiment: Includes Recombinant expression and enzyme purification, Initial rate kinetics, Single step cascades, One pot cascade: Reference state, One pot cascade: Perturbations, and One pot cascade: Optimisation.

Model: Includes Model construction, Model validation, and Model application.

Application: Includes Metabolic engineering: Cell extracts and Fine chemical production (mg to g scale).

The diagram illustrates the integration of experimental data with mathematical models to optimize the Weimberg pathway. It shows the flow from experimental data to model construction, validation, and application, leading to the production of fine chemicals.

Article Information:
 nature COMMUNICATIONS
 Shen L, Kohlhaas M, Enoki J, et al. *Nat Commun.* 2020;11(1):1098.
 doi:10.1038/s41467-020-14830-y
 ARTICLE
<https://doi.org/10.1038/s41467-020-14830-y> OPEN
 A combined experimental and modelling approach for the Weimberg pathway optimisation
 Lu Shen¹, Martha Kohlhaas², Junichi Enoki³, Roland Meier⁴, Bernhard Schöenberger⁴, Roland Wohlgemuth^{4,5}, Robert Kourist^{3,6}, Felix Niemeyer², David van Niekerk⁷, Christopher Bräsen¹, Jochen Niemeyer^{2,8}, Jacky Snoep^{2,8,9} & Bettina Siebers^{1,8}

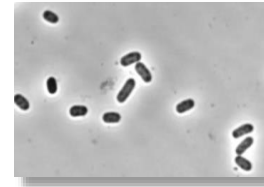
WP3 Screening of reported enzymes for expression

WP 3 (P1, P2, P3, P5)

Screening & Expression Platform
(*E. coli*, *T. thermophilus*, *S. acidocaldarius*)

- Pathways for extremolyte production reported previously
- Screenings and expression of previously reported candidates established
- Recombinant expression in chassis strains confirmed (DIP, MG, cDPG in *Eco* and *Saci*, cDPG (DIP ongoing) in *Tth*)

WP3 Expression hosts



Mesophilic Bacterium
E. coli
30-42°C, pH 7-7.5



Extremophilic Archaeon
S. acidocaldarius
75-80°C, pH 2-3



Thermophilic Bacterium
T. thermophilus
70°C, pH 7.5-7.8

WP3 Screening for novel candidates

WP 3 (P1, P2, P3, P5)

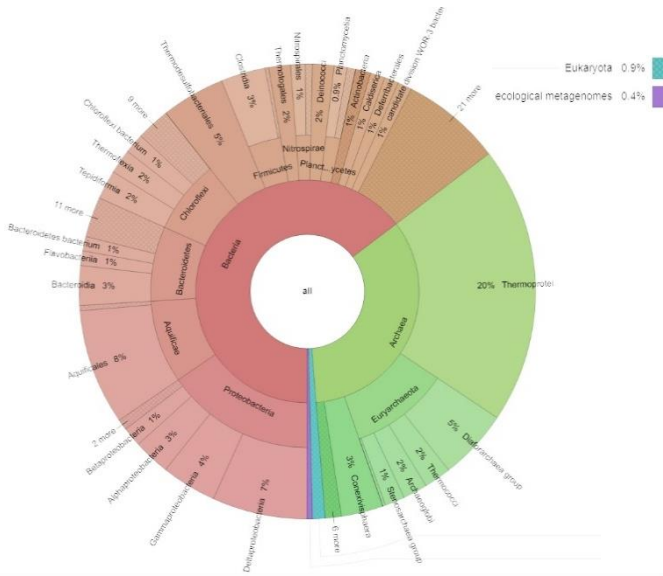
Screening & Expression Platform

(*E. coli*, *T. thermophilus*, *S. acidocaldarius*)

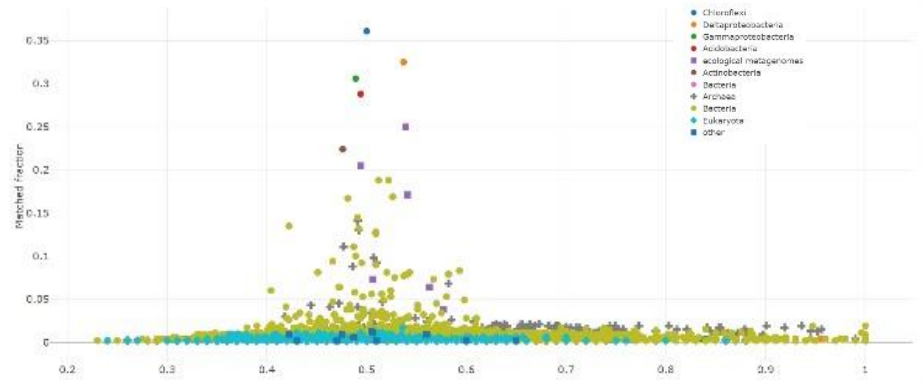


Kamchatka (2019)

Taxonomic profile DIP & cDPG



Taxonomic profile DIP & cDPG



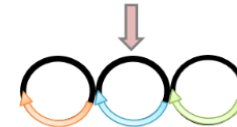
WP4 Summary

WP 4 (P1, P2, P3, P6)

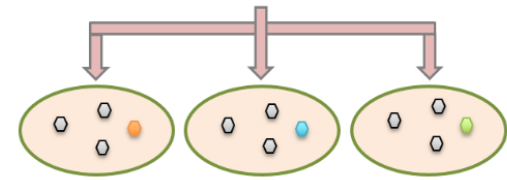
Thermophilic Enzyme Cascade & Strain Design for Extremolyte Production (cDPG, DIP, MG) & Recovery

- First “*in vitro*” enzyme cascade (*Eco*) established
- *Tth* and *Eco* cDPG production strains
- Construction of trehalose deficient *Saci* deletion strain for extremolyte production (MG, cDPG, DIP)
- Optimization of production strains (MG, cDPG, DIP) ongoing
- Development of analytical detection methods via LC-MS (MG) and NMR (cDPG) has been developed

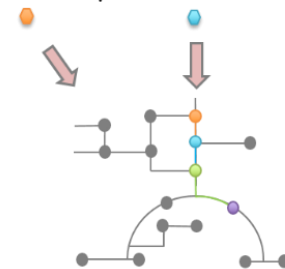
Selection of (hyper)thermophilic genes
Construction of expression vectors



Expression in mesophilic & thermophilic hosts



Heat treatment/Purification via tags
Enzyme characterization



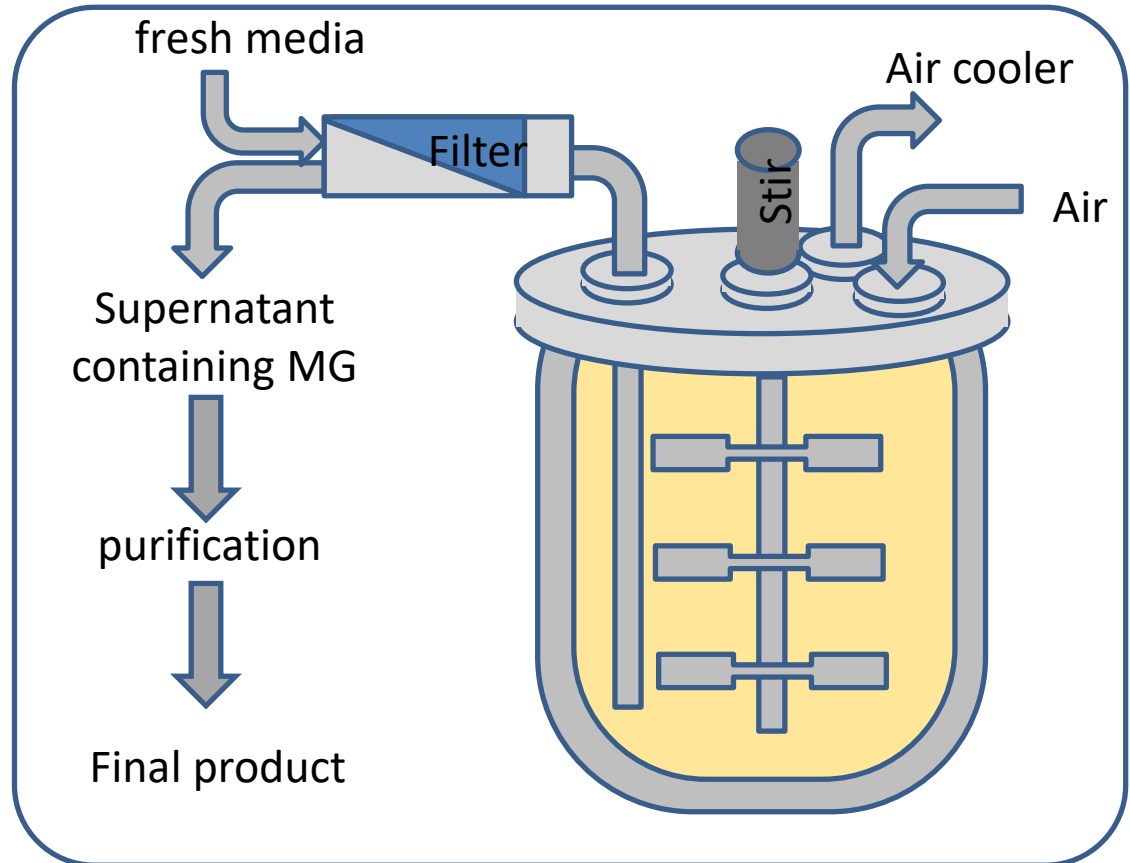
Multi-Enzyme-Cascade
Extremolyte

WP5 Testing & Life cycle assessment, Scale up

WP 5 (P1, P4)

Scale Up Production, Product Testing & Life Cycle Assessment

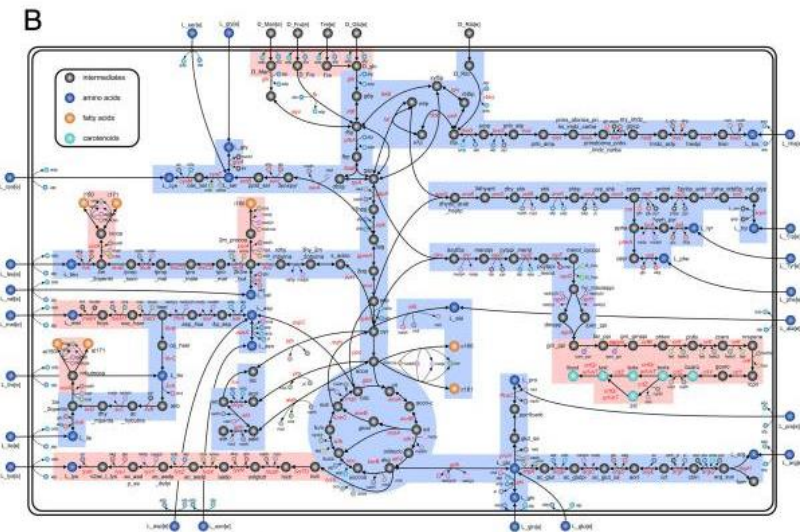
- Establishment of detection methods (NMR, LC-MS)
- Overproduction strains
- Product purification
- Toxicity tests (skin and tissue models)
- Life cycle assessment



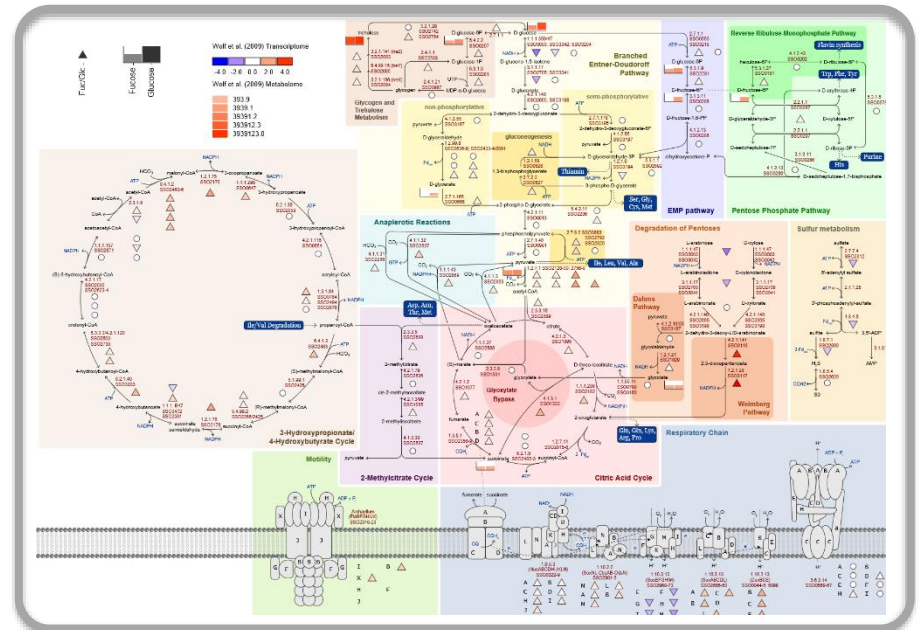
WP2 Data management & Modelling

WP 2 (P1, P6)
Data Management & Modelling
(FAIRDOM Hub)

- Genome scale models *Tth* and *Saci*
- Metabomaps, BRENDA (HotSysAPP)
<https://metabomaps.brenda-enzymes.org/maps.php?svg=1&org=1>



Lee NR, Lakshmanan M, Aggarwal S, et al. Genome-scale metabolic network reconstruction and in silico flux analysis of the thermophilic bacterium *Thermus thermophilus* HB27. *Microb Cell Fact.* 2014;13:61. Published 2014 Apr 28. doi:10.1186/1475-2859-13-61



Helmecke J, Schomburg D and Neumann-Schaal M. MetaboMAPS: Pathway sharing and multi-omics data visualization in metabolic context. (<https://doi.org/10.12688/f1000research.23427.1>)

How do you improve interactions between your research and society?

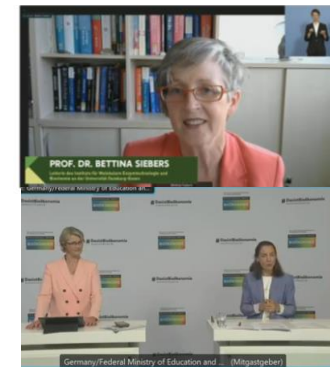
Due to the corona pandemic the direct interaction to the public was restricted, allowing only the interaction via online platforms, e.g. twitter, websites.

To reach a **broader public audience**

- ✓ Dr Felix Müller (Evonik) presented a talk in the ERA CoBioTech Biotechnology & Society seminar series 'HotSolute: Sustainability from the view of the chemical industry' (June 2021).
- ✓ Prof. Siebers joint the online discussion event organized by the German Minister on Science and Education Anja Karliczek „Biotechnology makes it possible - innovations for more sustainability“
- ✓ **Pupil visits** in the lab (now continued)

To reach the **scientific audience** the project was presented at

- ✓ The International Thermophile Meeting, Japan (Sep 2019)
- ✓ The European Society of Applied Biocatalysis (ESAB, May 2021)
- ✓ The MECP 2020+ meeting (Sep 2021)



How do you consider gender/diversity/culture dimension in your project?

The HotSolute consortium is composed of an equal distribution of female and male (7/9) researchers.

How do you manage your data with the all consortium?

FAIRDOMHub for the exchange of data and the data management



TRL level reached? Bottlenecks?

- Desired TRL are delayed
- Good Progress
 - Expression of the enzymes producing the extremolytes was extremely challenging, we were able to **express all required enzymes, development of new tools for extremophiles**
 - **Productions strains for all extremolytes MG, cDPG** (DIP in progress) have been developed, either in the bacterial or archaeal thermophilic platform
- Technological bottleneck lies in the optimization of the production strains “**upscaling**” to increase the production of extremolytes
 - Project start of partners

● *Benefits of international collaboration*

- Complementary expertise
- Exchange of knowledge, materials, strains etc.
- Involvement of industrial partner

● *Publications*

- ✓ **Thermoacidophilic *Sulfolobus* species as source for extremozymes and as novel archaeal platform organisms** (Schocke L. , Bräsen C. and Siebers B.)
- ✓ **Salt Stress Response of *Sulfolobus acidocaldarius* Involves Complex Trehalose Metabolism Utilizing a Novel Trehalose-6-Phosphate Synthase (TPS)/Trehalose-6-Phosphate Phosphatase (TPP) Pathway** (Stracke C. , Siebers B. *et al.*)

Manuscripts in preparation:

- ✓ **First crystal structure of a novel thermophilic cyclic 2,3 diphosphoglycerate synthetase enzyme involved with extremolyte production** (De Rose, S, Isupov, M, Littlechild, J *et al.*)
- ✓ **The use of *Thermus thermophilus* as a host cell system for the production of the extremolyte cyclic 2,3 diphosphoglycerate** (De Rose, S, Isupov, M, Harmer, N, Littlechild, J. *et al.*)
- ✓ **Mannosylglycerate production in *Sulfolobus acidocaldarius*** (Meyer B., Siebers B. *et al.*).

● *Exchange of researchers*

- *COVID pandemic*

- *Biotechnology will contribute to 2030 Agenda for Sustainable Development*

- *Recommendations for political measures*

- “Upscaling”
- Improve exchange between academics and industry but also and may be most important the acceptance of consumers

“Communication”

- *Pertinent results and findings based on your research activities*

- Biodiversity
- Extremophiles “novel process techniques”
- ERA CoBioTech great opportunity
“adapt partner starts”



Department of Economic and Social Affairs
Sustainable Development



University of Duisburg-Essen

Bettina Siebers

Christopher Bräsen

Benjamin Meyer

Christina Stracke

University of Exeter, Exeter, UK

Jennifer Littlechild

Nic Harmer

Simone De Rose

Michail Isupov

Istituto di Scienze e Tecnologie Chimiche, Milan, Italy

Daniela Monti

Erica Ferrandi

Elenora Dore

Evonik Technology & Infrastructure GmbH, Essen, Germany

Felix Müller

Winogradsky Institute of Microbiology, Moscow, Russia

Elizaveta Bonch-Osmolovskaya

Kublanov Ilya

Sergey Gavrilov

Stellenbosch University, Stellenbosch, South Africa

Jacky Snoep



Bettina Siebers (Co-ordinator)

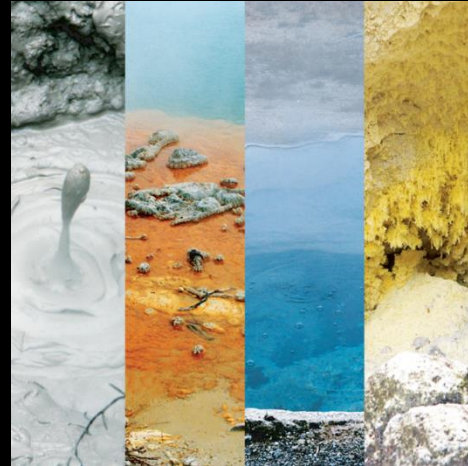
Molecular Enzyme Technology and Biochemistry (MEB),
Environmental Microbiology and Biotechnology (EMB),
University of Duisburg-Essen, Essen, Germany
bettina.siebers@uni-due.de



<https://twitter.com/hotsolute>



Thank you for your attention !



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Ministry of Science and Higher Education of the Russian Federation



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