ERA CoBioTech (ERA-Net Cofund on Biotechnologies)

ACHEMA2018

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

Project name: **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

Frankfurt am Main, 13.06.2018

Project partners

- **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 Chimica del Riconoscimento Molecolare – CNR (Italy)
  - **P4: Felix Müller**, Corporate Innovation, Evonik Industries AG, (Germany)
  - **P5: Elizaveta Bonch-Osmolovskaya**, Federal Research Center of Biotechnology, Russian Academy of Sciences, Winogradsky Institute of Microbiology (Russia)
  - **P6: Jacky Snoep**, Biochemistry, Stellenbosch University (South Africa)

- Total project budget: **1670 k€**
- Project start: **April/July 2018**

Introduction

Project objectives

- Production of **extremolytes**, compounds with medical and personal care application 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

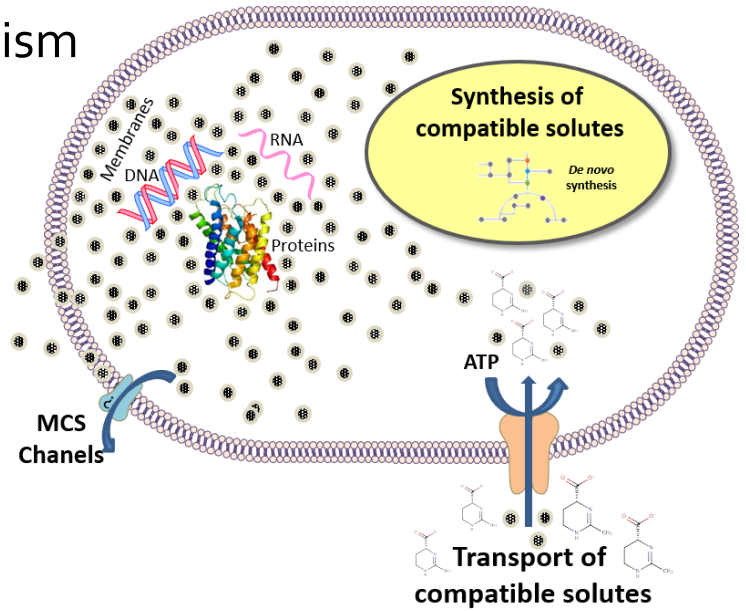
- Synthetic biology, Systems biology
- Development of new products, value-added products and supply service

Compatible solutes

- Response upon environmental stress → cell protection
- Organic low-molecular weight compounds
- Highly soluble
- Accumulated in high concentrations (250 mM – 1.1 M)
- No interference with central metabolism

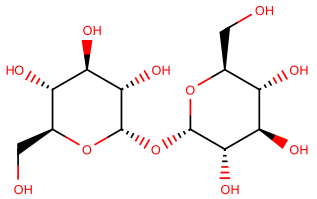
Protection and stabilization

- Cell membrane
- RNA-/ DNA- and protein



Sugars

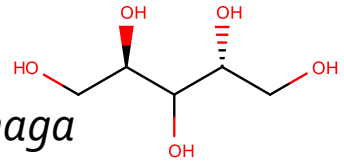
- **Trehalose, Sucrose**
  - ✓ Mesophilic and extremophilic organisms e.g. *E. coli, Sulfolobus acidocaldarius*



Trehalose

Polyols

- **Glycerol, Arabitol, Inositol**
  - ✓ Halophilic fungi and halotolerant plants e.g. *Wallemia ichthyophaga* (fungi)

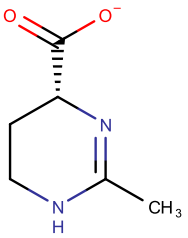


Arabitol

Aminoacids and derivates

- **Prolin, Glutamic acid, Glycinbetain, Ectoin, Hydroxyectoin**
  - ✓ Mesophilic and halophilic bacteria, halotolerant plants, e.g. *E. coli, Halomonas elongata*

Extremolytes



Ectoin

Trehalose

- Food industry, stabilizer & sweetener
- Cosmetic industry, body lotions & deodorants
- Pharma industry, cryopreservation e.g. sperm cells, stem cells
- Biotech industry, stabilization of biomolecules e.g. DNA, enzymes

NAGASE America

Arguelles, 2000; Higashiyama, 2002; Colaco et al., 1992; Gribbon et al., 1996; Roser, 1991

Ectoine & Hydroxyectoine

- Cosmetic industry, soaps, creams, sun protection and anti-aging
- Pharma industry , allergy products, eye-drops, nose spray, ointments
- Biotech industry, stabilization of biomolecules e.g. antibodies

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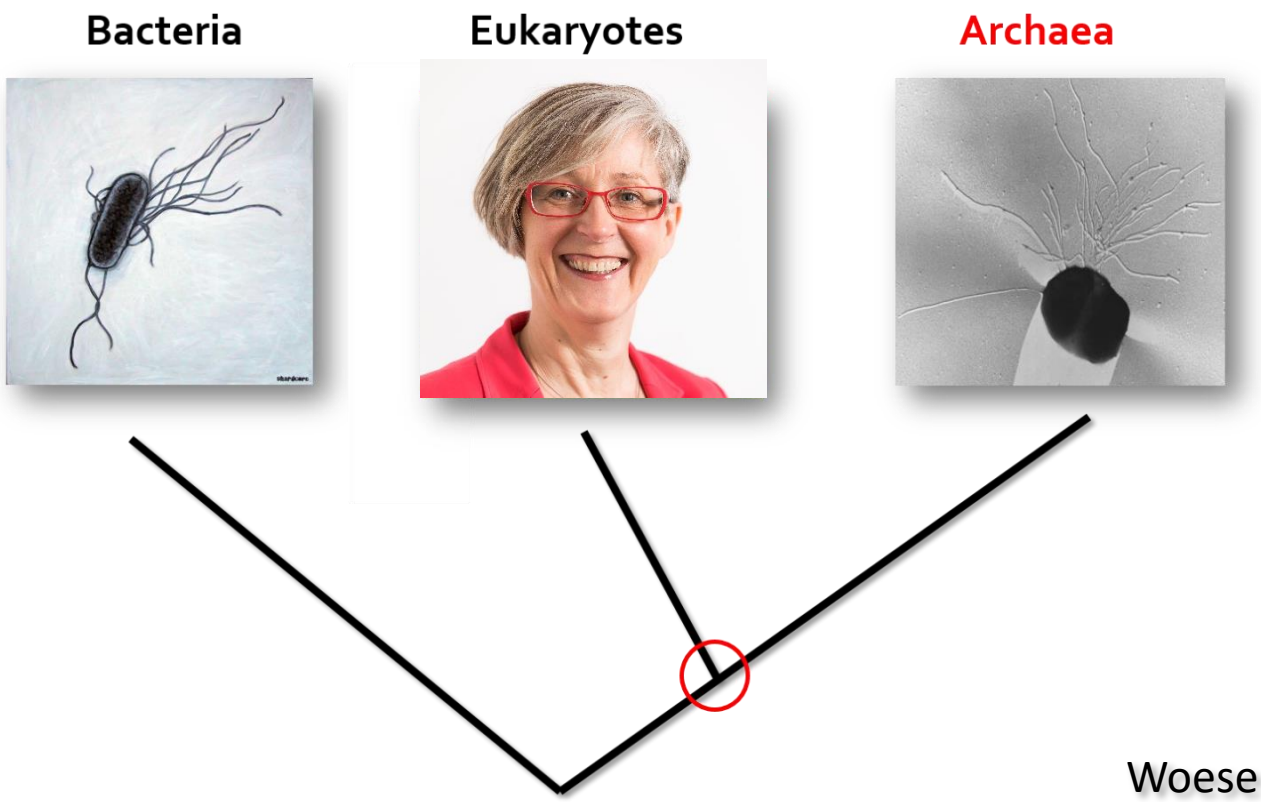
Extremolytes for Life

Bitop AG, Merck AG

Compatible solutes are present in all three domains of life….

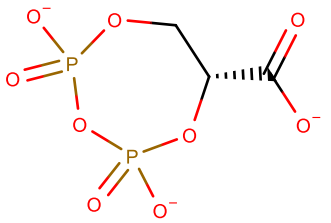


Woese & Fox 1977

Spang et al. 2015

Cyclic 2,3-Diphosphoglycerate

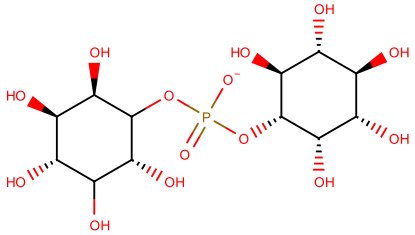
- **Methanogenic (hyper)thermophilic archaea e**.g. *Methanothermus fervidus*, *Methanopyrus kandleri*, *Methanothermobacter thermoautotrophicus*



Cyclic-Diphosphoglycerate

Di-*myo*-inositolphosphate

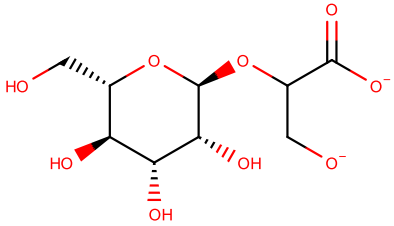
- **Hyperthermophilic archaea and hyperthermophilic bacteria** e.g. *Pyrococcus woesii*, *Thermococcus kodakaraensis*, *Archaeoglobus fulgidus*, *Thermotoga maritima*



Di-*myo*-inositolphosphate

Mannosylglycerate

- **Hyperthermophilic archaea and bacteria; mesophilic bacteria and eukarya** e.g. *Pyrococcus horikoshii*, *Pyrococcus furiosus*, *Thermococcus litoralis*, *Thermus thermophilus*, *Rhodothermus marinus*, red algae



Mannosylglycerate

Cyclic-Diphosphoglycerate

- Thermoadaption (*M. kandleri* 1.1 M cDPG; Hensel & König. 1988; Lehmacher et al., 1990; Shima et al., 1998)
- Stabilization of plasmid DNA (*in vitro*) (Lentzen & Schwarz, 2006)
- Protection of DNA against reactive oxygen species (*in vitro*) (Lentzen & Schwarz, 2006)

Di-*myo*-inositolphosphate

- Heat stress and osmoadaption (*P. woesii* and *P. furiosus* 0.4 -0.6 M DIP; Scholz & Hensel,1992; Esteves & Santos, 2014)
- Stabilization of plasmid DNA (*in vitro*) (Lentzen & Schwarz, 2006)
- Protection of DNA against reactive oxygen species (*in vitro*) (Lentzen & Schwarz, 2006)

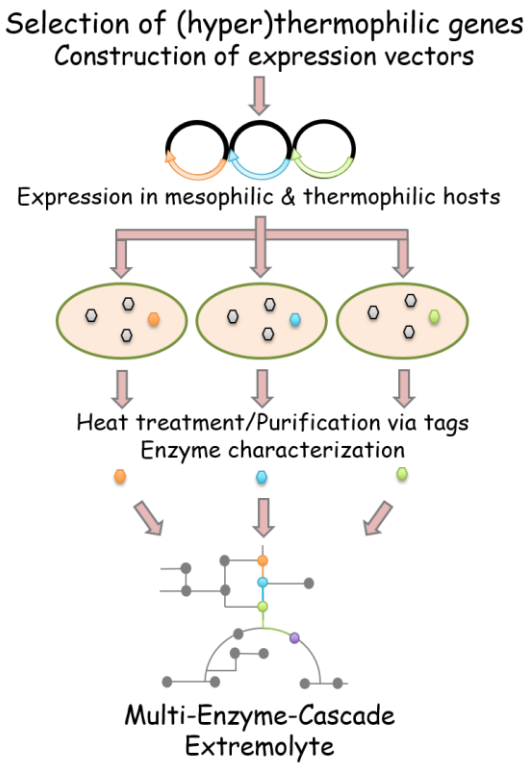
Mannosylglycerate

- Osmoadaption (*P. furiosus* 0.25 M MG; Martins & Santos,1992)
- Stabilization of proteins (*in vitro*) (Borges & Santos, 2001)
- Protein protecting properties; yeast model of Parkinson´s disease (Faria & Santos, 2013)

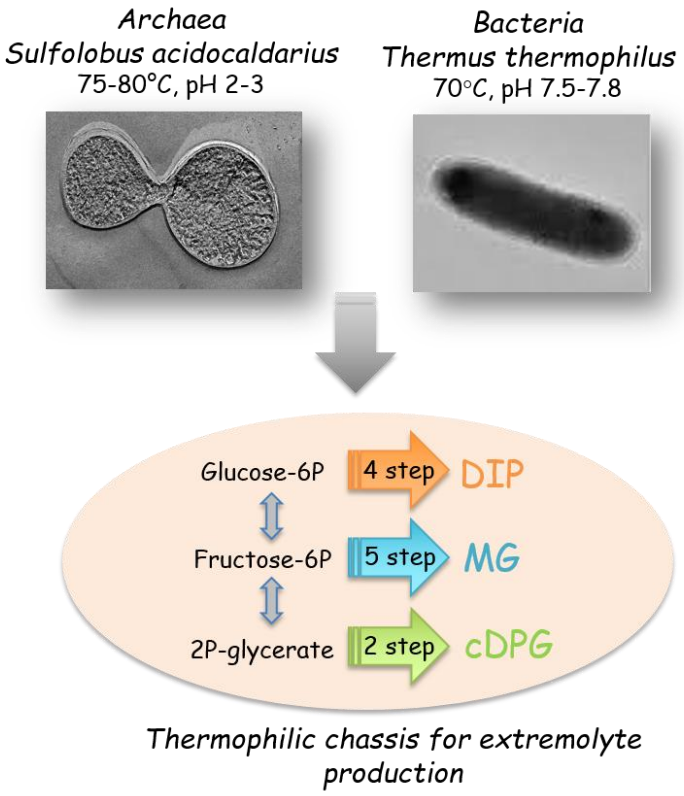
→ **NO suitable production systems or production strains**

→ The synthesis pathways and enzymes are known !

Thermophilic enzyme cascades „in vitro"

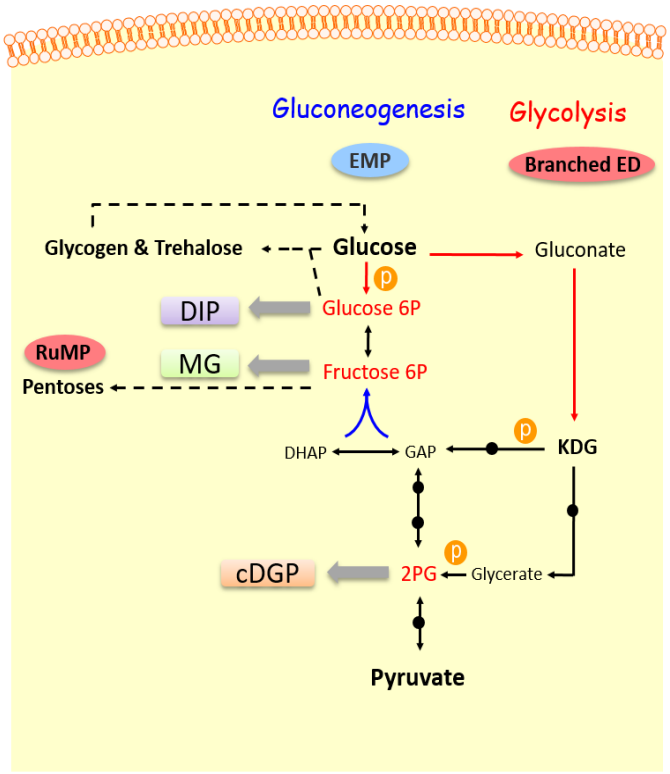


Thermophilic expression hosts *„in vivo"*

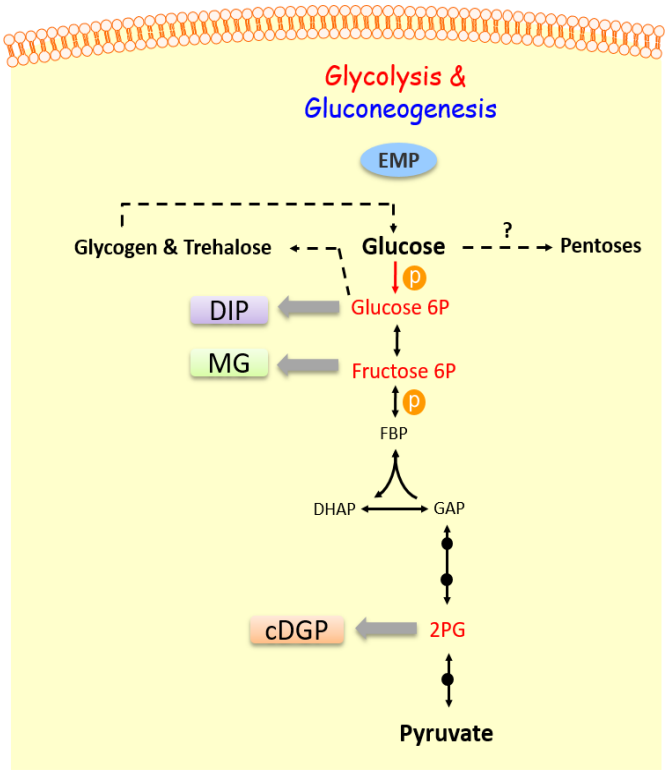


Thermophilic chassis for extremolyte production

- Central metabolism well established
- Genetic systems available

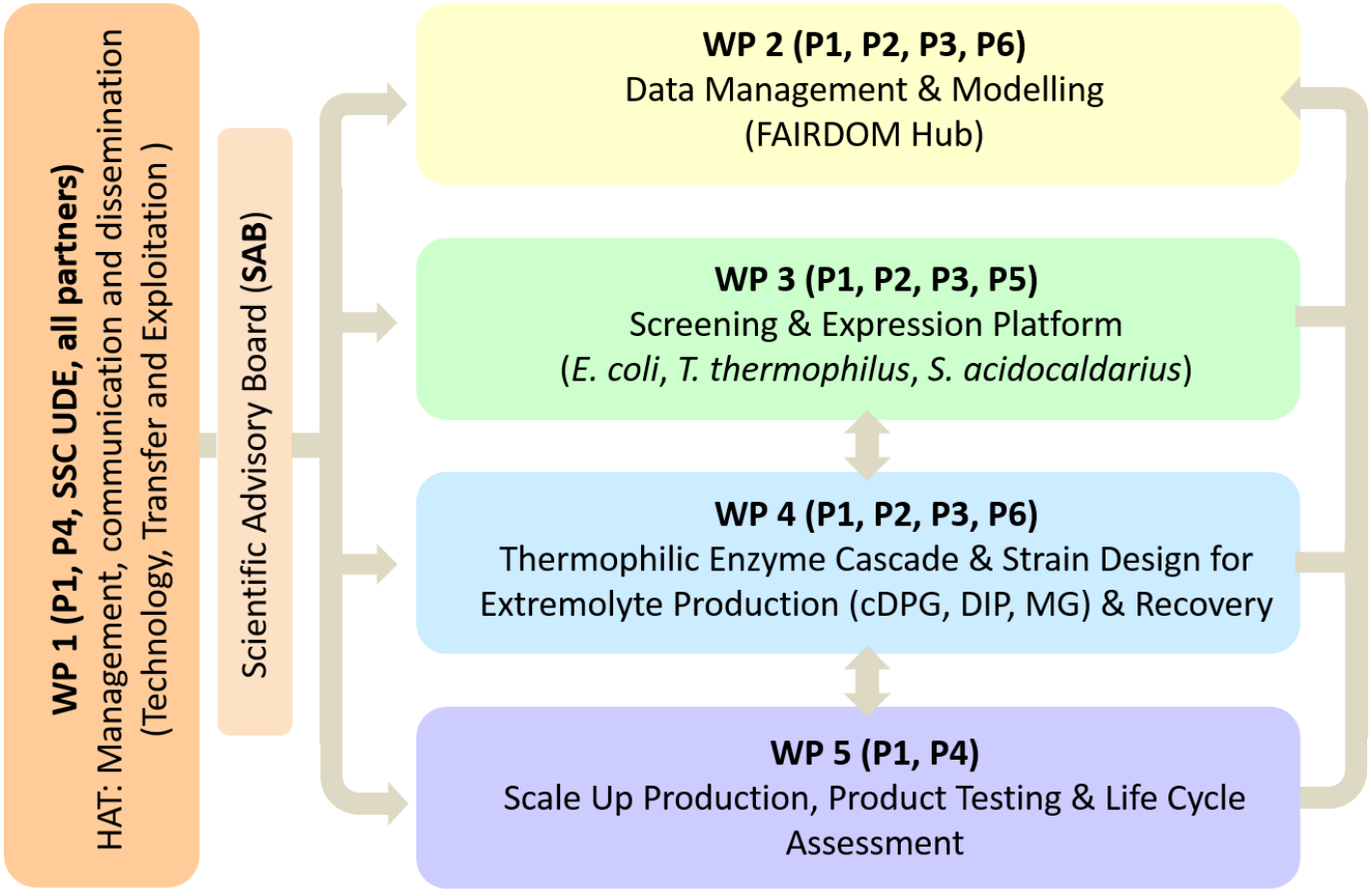


Sulfolobus acidocaldarius (Archaea)



Thermus thermophilus (Bacteria)

Work Packages



Project plan

Datamanagement

- FAIRDOMHub/SEEK (Jacky Snoep)

Communication strategy

- Industry
- Scientific community
- Wider public community/consumers

Responsible research and innovation

- Novel thermophilic enzyme cascades
- Novel thermophilic archaeal and bacterial chassis
- Production of three high-value product ‘extremolytes’

Expected outcomes

- *This project will develop the current applications of thermophilic enzyme cascades and micro-organisms for the industrial production of **three small molecule extremolytes** which have both medical and healthcare applications.*

- *Planed implementation and exploitation of results*
  - *Scale-up and Application*
  - *Industrial partners*

EVONIK INDUSTRIES

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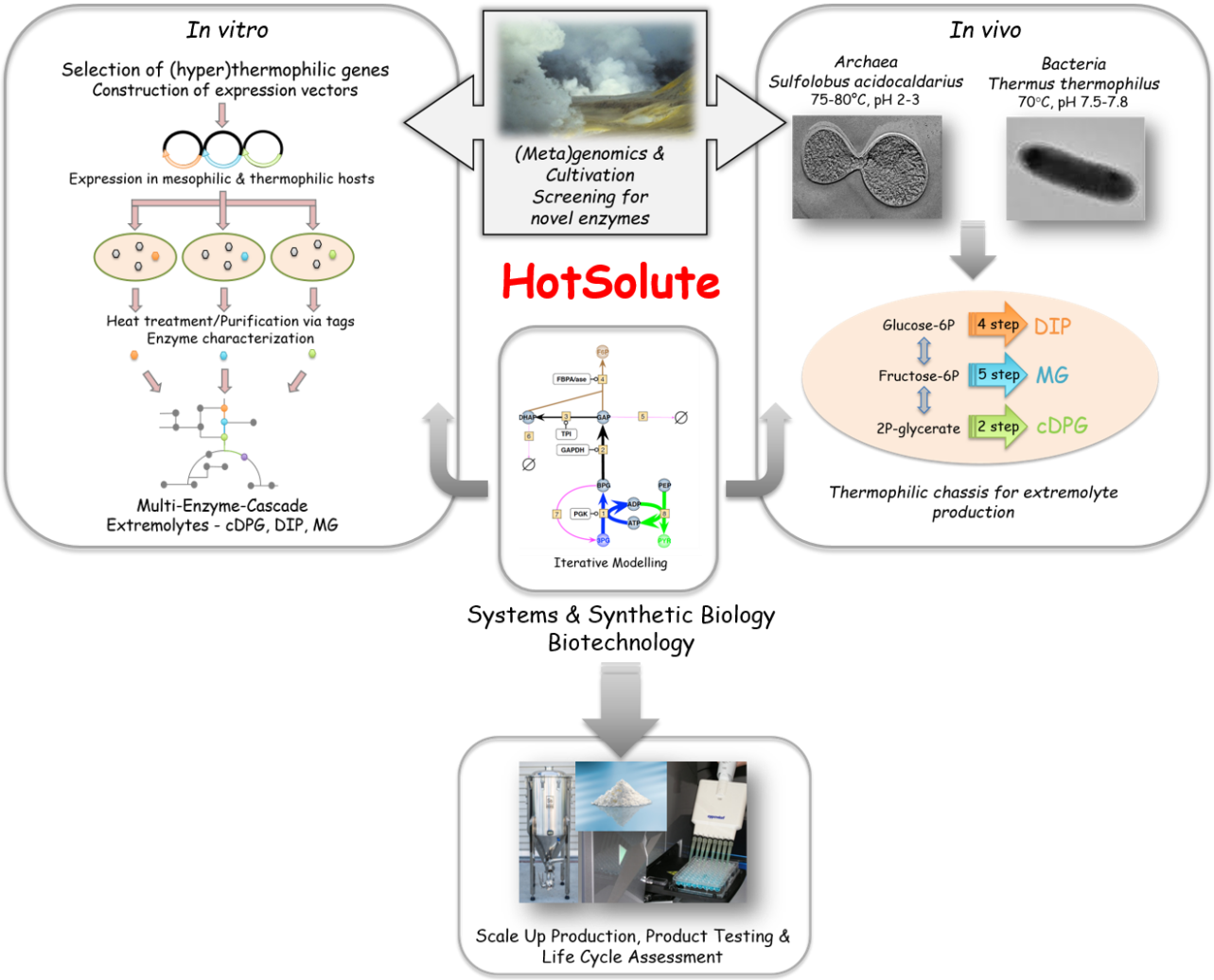
Extremolytes for Life

Summary


In vitro
Selection of (hyper)thermophilic genes
Construction of expression vectors
Expression in mesophilic & thermophilic hosts
Heat treatment/Purification via tags
Enzyme characterization
Multi-Enzyme-Cascade
Extremolytes - cDPG, DIP, MG
(Meta)genomics &
Cultivation
Screening for
novel enzymes
HotSolute
Iterative Modelling
In vivo
Archaea
Sulfolobus acidocaldarius
75-80°C, pH 2-3
Bacteria
Thermus thermophilus
70°C, pH 7.5-7.8
Glucose-6P
4 step
DIP
Fructose-6P
5 step
MG
2P-glycerate
2 step
cDPG
Thermophilic chassis for extremolyte
production
Systems & Synthetic Biology
Biotechnology
Scale Up Production, Product Testing &
Life Cycle Assessment

Contact details

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Open-Minded

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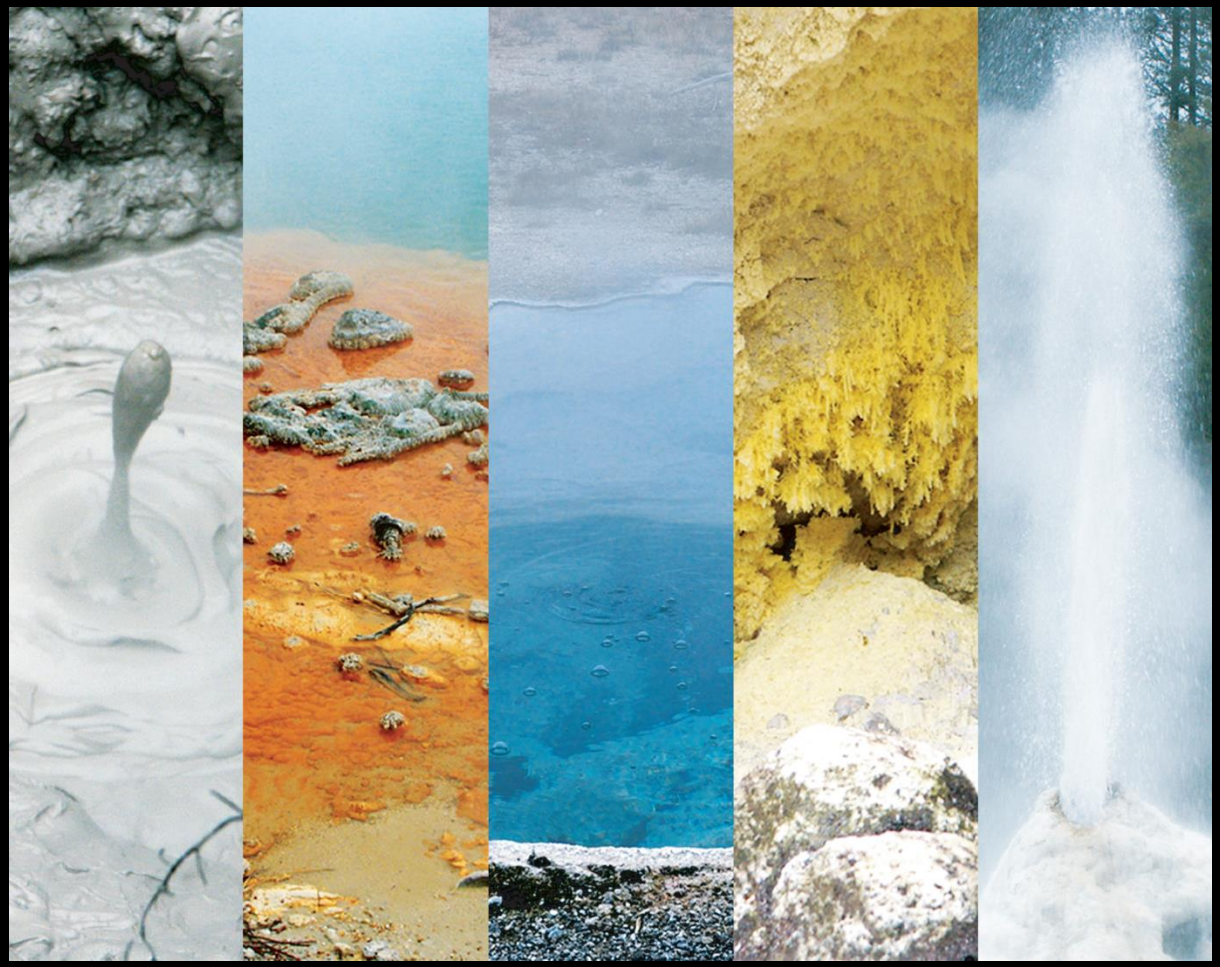
In the heart of Europe
43,089 students





Thank you for your attention !



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