ERA CoBioTech

BioTech Research & Innovation Hack

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ERA CoBioTech Funded Projects At A Glance: HotSolute

Thermophilic bacterial and archaeal chassis for extremolyte production

HotSolute

Establishment of biotechnological extremolyte production

Researchers within the EU-funded HotSolute project have established thermophilic in vitro enzyme cascades as well as two new chassis, the thermophilic bacterium Thermus thermophilus (Tth, 65-75°C, pH 7.0) and the thermoacidophilic archaeon Sulfolobus acidocaldarius (Saci, 75-80°C, pH 2-4) as new thermophilic, bacterial and archaeal platforms for the production of novel high added value products, i.e. extremolytes.

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Project duration:

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Total budget: 1.67 €M

Provision of extremolytes for the application

Thermophilic organisms belong to both bacterial and archaeal kingdoms. The enzymes isolated from these species and from other extreme habitats are more robust to temperature, organic solvents and proteolysis. They often have unique substrate specificities and originate from novel metabolic pathways. Thermophiles as well as their stable enzymes ('thermozymes or extremozymes') are receiving increased attention for biotechnological applications. In the HotSolute project we have established thermophilic i*n vitro* enzyme cascades as well as two new chassis, the thermophilic bacterium *Thermus thermophilus* (Tth, 65-75°C, pH 7.0) and the thermo-acidophilic archaeon *Sulfolobus acidocaldarius* (Saci, 75-80°C, pH 2-4), as new thermophilic, bacterial and archaeal platforms for the production of novel high added-value products, i.e. 'extremolytes'. Extremolytes are small molecular compatible solutes found naturally in the cells of thermophilic species that accumulate in the cell in response to multiple environmental stresses and stabilize cellular components (including proteins and membranes). Extremolytes offer an amazing so far unexploited potential for biotechnological applications in the food, health, consumer care and cosmetic industries. However, their production in common mesophilic organisms (such as yeast or *Escherichia coli*) is hampered by the hyperthermophilic origin of the respective metabolic pathways which supported the idea of developing new thermophilic cell factories. Within the HotSolute project we have developed two newly designed 'cell factories' for the production of two extremolytes, cyclic 2,3 di-phosphoglycerate (cDPG) and mannosyl-glycerate (MG) in Tth and Saci, respectively. These extremolytes (with few exceptions for MG) are exclusively found in hyperthermophiles and have not been produced in a mesophilic host to date.

A Potpourri of new approaches

In the HotSolute project, international partners from academic and industrial environments have joined forces to pursue new ways of providing extremolytes for industrial applications. In order to avoid the existing bottlenecks for the production of extremolytes in classic mesophilic expression hosts, thermophilic representatives of the archaea (Saci) and bacteria (Tth) have been established as expression platforms. For this purpose, the some existing genetic manipulation systems have been used and further developed. Furthermore, *in vitro* enzyme cascades for the synthesis of extremolytes were established. This has involved the cloning and over-expression of the enzymes involved in the natural thermophilic cascades optimised for expression in both *Escherichia coli* and in the bacterial and archaeal thermophilic hosts. The individual enzymes in the respective pathways have been purified and biochemically and structurally characterised. Homologues of the enzymes have been identified from newly identified hyperthermophilic genomes and metagenomes to add to the 'tool box' of available enzymes for future industrial applications of extremolyte production.

Main results

In the HotSolute project, the metabolic pathways for extremolyte production could be introduced into the thermophilic host strains. Different enzymes derived from public databases as well as metagenomes were expressed and tested for activity in *E. coli*, Tth and Saci. The whole cell synthesis of cDPG was established in the bacterial representative Tth. The two enzymes involved are found naturally in a pathway for extremolyte production in another hyperthermophilic archaeon (*Methanothermus*). The two enzymes involved in this pathway are unique and no sequence or structural homologues have been reported. The crystal structure of the enzyme (cDPG synthetase) involved in this pathway has been solved to high resolution and the other enzyme (2PGK) has been crystallised and the structural solution is close to completion.

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A trehalose-deficient Saci strain was established and optimized for the synthesis of MG. A patent for the *in vitro* synthesis of extremolytes is in preparation. The platform organisms produced in HotSolute and the enzyme cascade are an important contribution to making extremolytes available for everyday products for the general public use. The work in the HotSolute project thus provides an important starting point for future applications of extremolytes in health and consumer care. Further work on upscaling of extremolyte production will be carried out in future projects in close cooperation with the industrial partner.

Future prospect

Compatible solutes are ubiquitous and are found in all three domains of life. They accumulate to high concentrations in cells in response to diverse environmental stresses (e.g. heat, cold, osmotic stress, and desiccation) without interfering with cellular metabolism and allow their hosts to survive harsh environmental conditions by stabilizing and protecting biomolecular structures and the cellular conformation. Because of their protective effect on biological structures such as enzymes, DNA, membranes and whole cells, the compatible solutes have found commercial applications in different industrial fields such as food, health and consumer care and cosmetics. So far, extremolytes - unusual compatible solutes from extremophiles - could not be produced by conventional production strains. The provision of thermophilic production strains and enzyme cascades is therefore an essential step for future applications. In general, the provision of thermophilic expression platforms and extremozymes from the domain of bacteria and archaea is of great biotechnological importance. The utilization of extremophiles and their enzymes for high temperature processes offers clear benefits for industrial biotechnology such as increased reaction rates and improved substrate accessibility, reduction of microbial contamination at higher temperatures, reduced energy input for cooling steps and process cost reduction and ease of volatile product separation and recovery. In addition, the use of thermozymes provides improved substrate solubility at higher temperatures and the potential for use of organic solvents without enzyme denaturation. Because of these advantages, the HotSolute project is also of particular importance for the development of new, future-oriented, environmentally friendly and renewable technologies.

In the beginning of the project HotSolute presented for example a project poster at the International Extremophile meeting in Ischia (Italy, Sep 2018) and several posters were presented by HotSolute postdocs and PhD students at different meetings. Notably, S. De Rose was awarded the poster prize for his poster entitled "'*Thermus thermophilus* as a Whole Cell Factory for the Production of Extremolytes" at the International Thermophiles 2019 meeting (Kyusuh University, Japan, Sep. 2019). Due to the COVID pandemic the options for live presentations at meetings have been limited. However, virtual conferences have been initiated. The HotSolute project was presented at the European Society of Applied Biocatalysis (ESAB) Webinar (28th May 2021) and a poster at the MECP 2020+ meeting in September 2021. Two manuscripts have already been published: one review about the potential of Saci as novel platform organism for biotechnology (DOI:10.1016/j.copbio.2019.02.012) and one original research paper about the construction of the trehalose deficient Saci strain for extremolyte production (doi: 10.1128/AEM.01565-20). In addition, three manuscripts are in preparation:

1) 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.),
2) 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.), and
3) Mannosylglycerate production in *Sulfolobus acidocaldarius* (Meyer B., Siebers B. et al.).

A patent is currently being prepared for the synthesis of extremolytes. To reach a broader 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' (23rd June 2021) and Bettina Siebers was engaged with an online discussion event organized by the German Minister on Science and Education Anja Karliczek (https://www.wissenschaftsjahr.de/2020-21/veranstaltungen/karliczek-impulse/karliczek-impulse-biotechnologie-machts-moeglich).

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Figure 1: HotSolute kick-off meeting Hanau (Germany) 2018

Figure 2: Hot spring collage by Bettina Siebers

Website: **http://www.HotSolute.com**
Twitter: **@HotSolute**

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