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Growth-based selection of glycoside hydrolases in the extreme thermophile *Thermus thermophilus*

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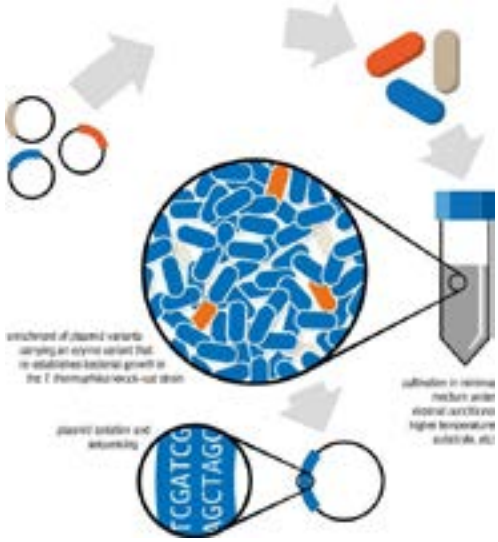
The constant depletion of fossil energy sources and the raising demand for more eco-friendly alternatives challenges science to develop new technologies. The utilization of plant biomass is a promising substitute for conventional systems by being a rather inexpensive energy carrier and of high sustainability at the same time. Its availability through cultivation of energy crops and by usage of agricultural/forestry waste account for great industrial value. In particular, the valorisation of waste by converting it to fermentable substrates has the advantage not to threaten food security. Since plants themselves consist primarily of lignocellulosic fibres (a mixture of cellulose, lignin and hemicellulose), the first step in the process of fuel production is the hydrolysis of this bigger structures into smaller, more soluble sugars. Its depolymerization into glucose monomers is often facilitated by using cellulases – an enzyme group consisting of endoglucanases (EC 3.2.1.4), cellobiohydrolases (EC 3.2.1.91) and β-glucosidases (EC 3.2.1.21) which work synergistically together. With increasing market share of new bio fuels, the future demand for (novel) cellulases – which today already make up 8 % of worldwide industrial enzyme demands – will therefor rise as well constantly. In this work we developed a system to select for thermostable glycoside hydrolase enzymes (GH) using the extremely thermophilic bacterium *Thermus thermophilus*. Unlike mesophilic bacteria (e.g. *E. coli*), *T. thermophilus* provides an overall more suitable enzymatic background and thereby greater potential to express thermostable recombinant proteins properly. This increases the probability of detecting novel thermostable cellulases when transformed in this organism; compared to the commonly used host *E. coli*. As an advantage, growth-based selection approaches already result in favourable enzyme variants, compared to traditional screening methods which require testing of every single clone. In order to obtain a GH-negative strain, we constructed a *T. thermophilus* knock-out strain which lacks four glycosidases. As confirmed by para-nitrophenol (pNP) enzyme assays and incubation of cell extract with X-Gal and X-Glu, these deletions reduced the hosts ability to cleave β-glycosidic and β- galactosidic bonds to a minimum. Without these GHs, the knock out strain is not able to grow in minimal medium. Complementation with the hosts own β-glucosidase via the shuttle vector pMK18 re-established growth of the knock out strain. For purpose of following system verification, cglT – a glycosyl hydrolase belonging to GH family 1 from the thermophilic bacterium *Thermoanaerobacter brockii* – was transformed in the *T. thermophilus* knock-out strain. This novel approach of complementation-based selection in an extreme thermophilic organism is a promising tool to look through big meta genomes or mutagenesis libraries, selecting for enzyme variants of higher thermostability and/or other substrate specificity in a highly efficient manner.



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2. Leis B.*, Held C.*, Bergkemper F., Dennemarck K., Steinbauer R., Reiter A., Mechelke M., Moerch M., Graubner S., Liebl W., Schwarz WH., Zverlov VV. Comparative characterization of all cellulosomal cellulases from Clostridium thermocellum reveals high diversity in endoglucanase product formation essential for complex activity. Biotechnol Biofuels. 2017 Oct 23;10:240 *These authors contributed equally to this work.

Biography

Matthias Moerch has completed his Masters in Molecular Biotechnology at the Technical University of Munich in 2014, comparing different protein digestion methods for shotgun proteomics in his Master's Thesis. Since 2015 he pursues his doctorate at the Department of Microbiology at the Technical University of Munich establishing the extreme thermophilic bacterium *Thermus thermophilus* as an alternative host for metagenome analysis.

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