deoTK

Directed evolution of transketolase to broaden its substrate range for applications in chiral drug synthesis

Programme Blanc Franco-Allemand 2009

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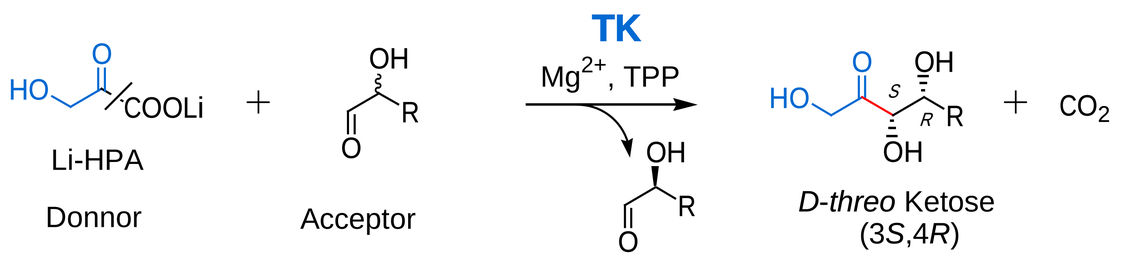
Transketolase (TK) catalyzes **the transfer of a ketol unit** from hydroxypyruvic acid lithium salt (Li-HPA) to an aldehyde to give a **monosaccharide.** Interestingly, the Li-HPA decarboxylation catalyzed by TK renders the reaction irreversible. The newly-formed asymmetric center has the (*S*) configuration. TK is also highly enantioselective towards (2*R*)-hydroxyaldehyde acceptor substrates. Various ***D-threo* (*3S,4R*) ketoses** have been synthetized with TK from yeast and *E.coli.*



The goal of the deoTK project is to improve the catalytic properties of TK, according to two main objectives. First **the production of a thermostable TK**, from a thermophilic micro-organism *Geobacillus stearothermophilus*. Second, the **inversion of the enantioselectivity** towards (2*S*)-hydroxyaldehyde acceptor substrates with *G.stearothermophilus* mutant TK obtained by directed evolution mutagenesis. For the screening of mutant TK libraries, **an efficient and sensitive pH based high-throughput assay** method has been developed.

Production and Characterization of Transketolase from *G. stearothermophilus*

J. Abdoul Zabar, I. Sorel, V. Helaine, F. Charmantray, T. Devamani, D. Yi, V. de Berardinis , D. Louis, P. Marlière, W-D. Fessner, L. Hecquet*, *Adv. Synth. Catal.*, **2012,** *In press*

CLONING AND OVER EXPRESSING

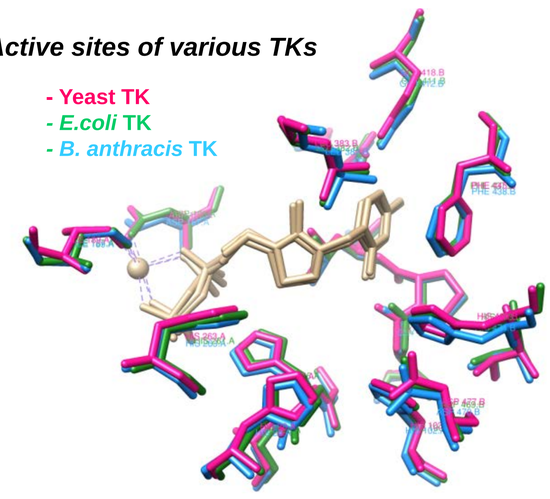
- ***G. stearothermophilus*** (DSM13240) is a thermophilic bacteria widely distributed in soil, hot springs, ocean sediment growing within a temperature range of 30-75 °C.
- **TK from *G. Stearothermophilus* (TK_{gst}) has never been characterized.** This TK presents a high percentage of protein sequence identity with three microbial TKs (yeast, *E.coli* and *B. anthracis*) which 3D structures have already been solved by X-ray.

% of protein sequence identity	Yeast TK	*E.Coli* TK	*B. Anthracis* TK
G. Stearothermophilus TK	48	51	74

- **TK synthetic gene** from *G. stearothermophilus* was cloned in pET47b transformed in an expression *E. coli* strain (BL21 DE3) leading to the production of a large quantity of 6xHistidine-tagged TK (after IPTG induction).

Active sites of various TKs

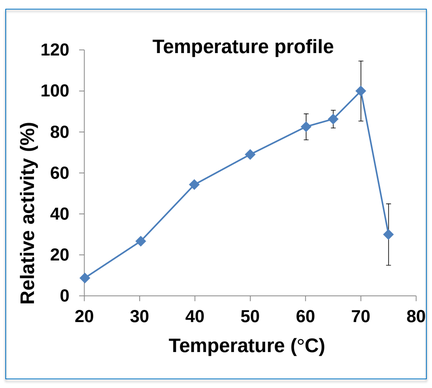
- Yeast TK
- *E.coli* TK
- *B. anthracis* TK



PROPERTIES OF TK_{gst}

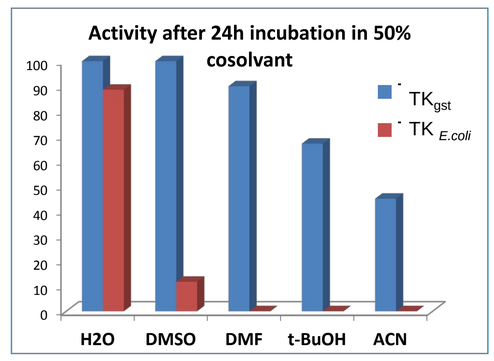
Optimum pH and Temperature

TK_{gst} shows maximum activity at **pH 7.5.**
Its optimum temperature was found at **70° C.** This high temperature enables the rapid and efficient purification of the enzyme by **heat shock treatement.** Furthermore, TK_{gst} can be used in organic synthesis at higher temperatures than the usual TKs (20-37° C)**.**



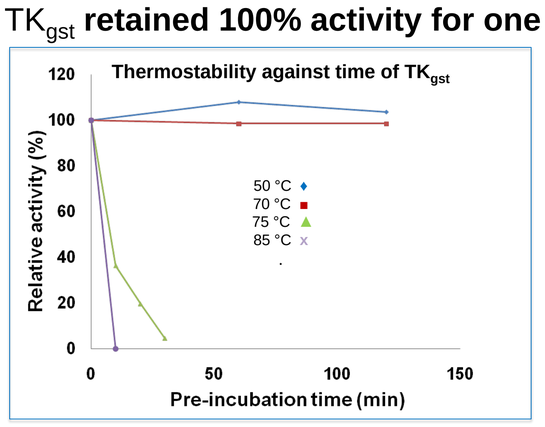
Tolerance towards cosolvents

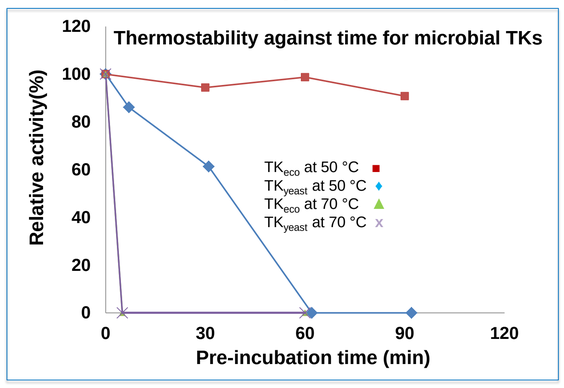
TK_{gst} showed a tolerance to cosolvent up to 50% after 24 h incubation at 20° C, in contrary to $TK_{E.coli}$ which lost all activity at this cosolvent concentration. This property will enable the study of activity towards substrates poorly soluble in water.



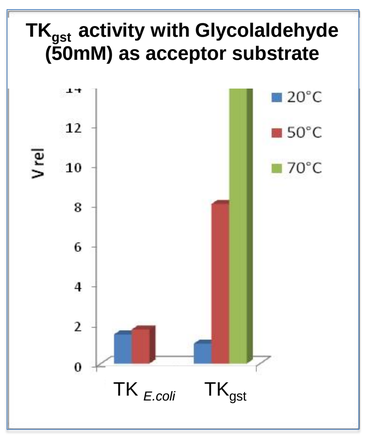
Thermostability over time

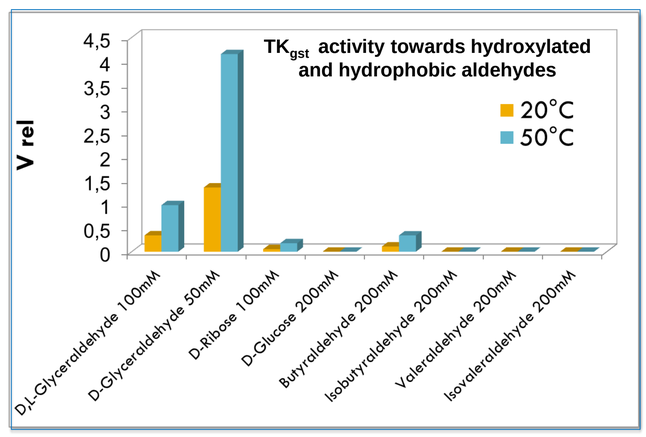
TK_{gst} **retained 100% activity for one week at 50° C** and 3 days at 70° C.





Substrate specificity





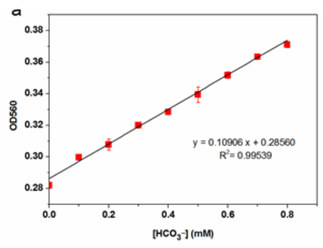
Colorimetric assay for mutant TK_{gst} screening

D. Yi, T. Devamani, J. Abdoul Zabar, F. Charmantray, V. Helaine, L. Hecquet, W-D.Fessner*, *ChemBioChem*, **2012**, *In press*

The method is based on the titration of carbon dioxide released by the TK- reaction using Li-HPA in the presence of **phenol red as pH indicator**. The reaction progress causes a pH increase that can be monitored thanks to the color change of the pH indicator (OD 560 nm).

Phenol red color transition with pH: 6,8 → 8,2

In the presence of low buffer concentration (2 mM triethanolamine, pH 7.5) the method is highly sensitive and allows a continuous monitoring for the quantitative determination of TK activity.



Calibration curve : OD560 as a function of the HCO_3^- concentration

Sccreening of a TK_{gst} mutants library With Li-HPA and L-glyceraldehyde as acceptor

Conclusion and Prospects

The processes at elevated temperature can offer many opportunities to improve and extend TK biocatalysis applications, such as hydrophobic aldehyde acceptor substrate solubility and tolerance towards non-conventional media, which we will be investigating using free and immobilized enzyme. Other avenue of research is the improvement of its biocatalytic properties such as inversion of enantioselectivity by mutagenesis which is in progress.

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