Protein Expression & Engineering

Dr. Filip Kovacic & Dr. Horst Funken | Institute of Molecular Enzyme Technology | Heinrich-Heine University Düsseldorf Forschungszentrum Jülich GmbH | 52426 Jülich

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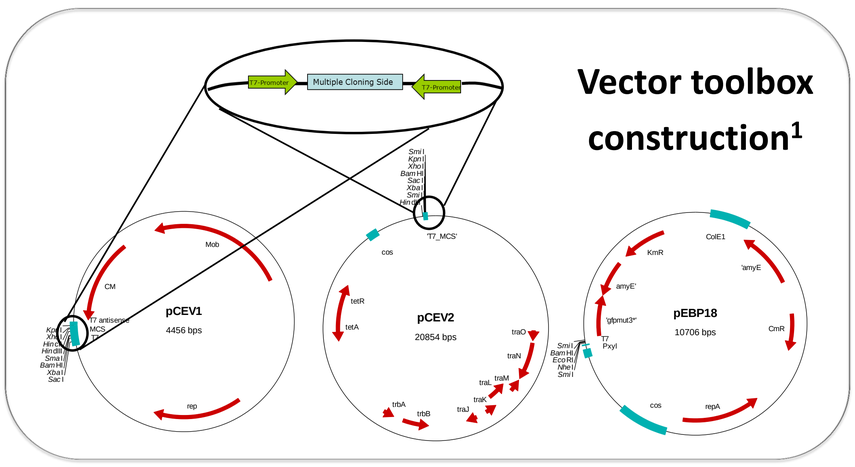
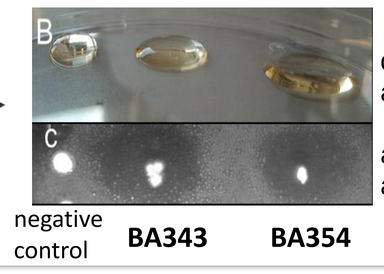
Expression tools for the analysis of metagenomic libraries



pCEV1	pCEV2	pEBP18
• for expression in T7-expression strains	• for expression in T7-expression strains	• for expression in *E. coli, Bacillus, Pseudomonas*
• mobilizable	• mobilizable	• not mobilizable
• insert size up to 10kb	• insert size at least 30kb	• insert size up to 10kb
• no phage packaging	• phage packaging	• phage packaging

- Identification of genes BA343 and BA354 of metagenomic origin responsible for synthesis of biosurfactants

pEBP18-based metagenomic library in *E. coli* DH10b →

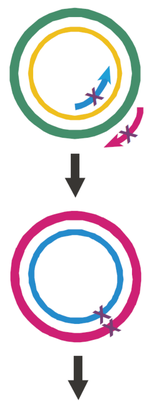


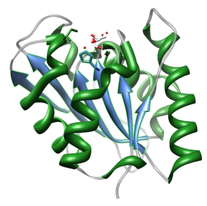
drop collapsing assay

atomized oil assay

Deciphering the detergent resistance of proteins[2]

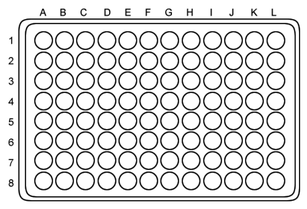
library construction
complete saturation mutagenesis of each position in gene by Quikchange method using NNS-codon



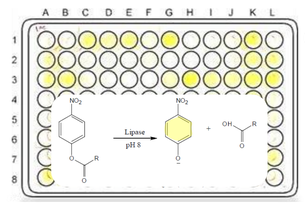


model protein
B. subtilis lipase LipA

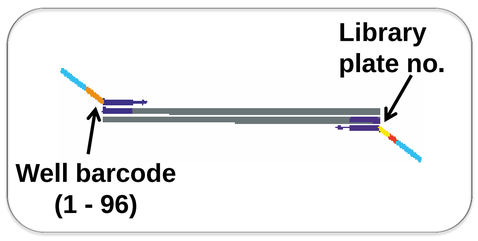
transformation
of *E. coli* BL21(DE3) and prescreening on LB-tributyrin plates



master plate
lipase expression in 96-well plate



enzyme activity assay after incubation with various detergents

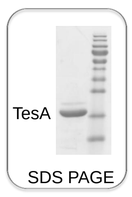


next generation sequencing of each variant

Engineering a novel enzyme activity by rational protein design

- Crystal structure of TesA, a novel lysophospholipase
  - from *Pseudomonas aeruginosa*

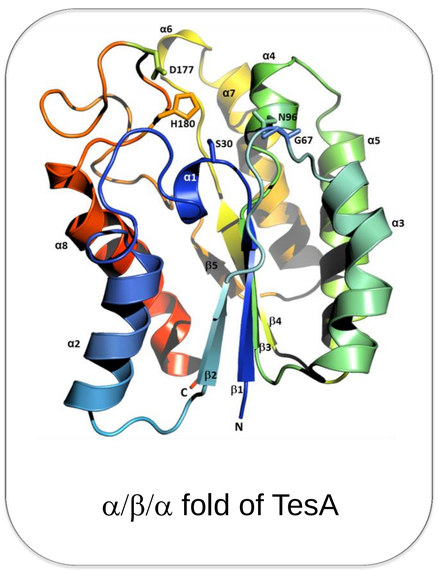
TesA expression
E. coli BL21(DE3)
LB medium, 37°C



purification
via IMAC
40mg TesA/l culture

crystallization
20% (w/v) PEG 3350, 50 mM sodium-citrate buffer (pH 4,5)

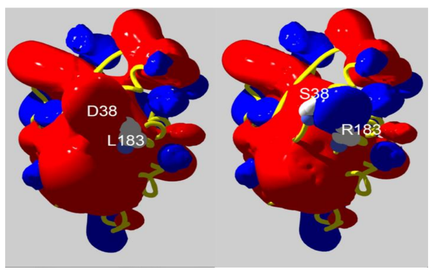
structure determination
1.9 Å resolution



α/β/α fold of TesA

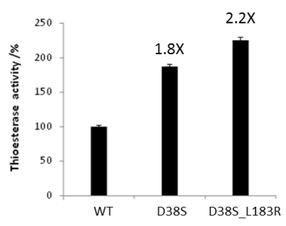
- TesA shows low thioesterase activity but mutations in putative Coenzyme A binding site increase its thioesterase activity for 2.2 fold

TesA wt | TesA D38S+L183R



Electrostatic potential maps showing the field which propagates into the solvent

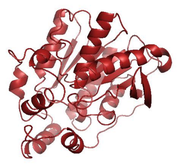
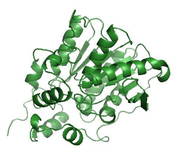
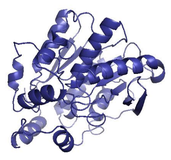
Blue: +1.8 kT/e Red: -1.8 kT/e

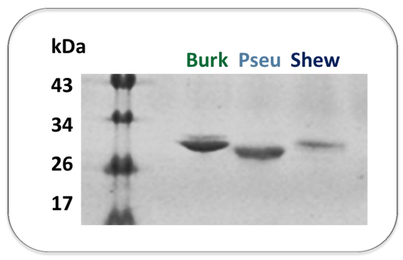


Thioesterase activity of TesA measured with lauroyl-CoA

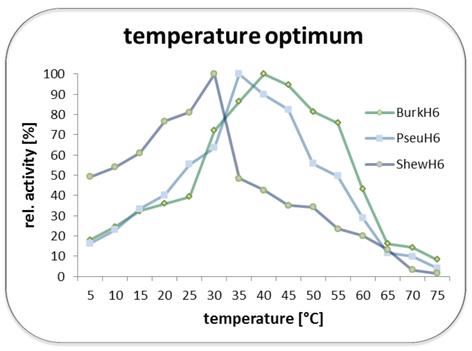
Understanding the temperature adaptation of extremophilic enzymes

comparative study of lipolytic enzymes from thermophilic, mesophilic, psychrophilic, and psychrotrophic bacteria

X-ray structure[4]	3D models of unknown enzymes with sequence homology to AliEst[5]		
			
Esterase from *Alicyclobacillus acidocaldarius*	Esterase from *Burkholderia thailandensis*	Esterase from *Pseudomonas sp. B11-1*	Esterase from *Shewanella halifaxensis*
Growth temperature 70°C	37°C	20°C	15°C
Seq. identity	45%	47%	38%



- expression and purification of *B. thailandensis*, *P. sp. B11-1* and *S. halifaxensis* esterases



- temperature optimum of these three enzymes correlate with the optimal growth temperature of the strain

1. Troeschel SC, Thies S, Link O, Real CI, Knops K, Wilhelm S, Rosenau F, Jaeger KE. (2012) J Biotechnol.
2. in cooperation with Ulrich Schwaneberg group from RWTA Aachen-
3. F. Kovačić, J. Granzin, S. Wilhelm, F. Rosenau, B. Kojić-Prodić, R. Batra-Safferling, K.-E. Jaeger. to be submitted
4. Mandrich L, Menchise V, Alterio V, De Simone G, Pedone C, Rossi M, Manco G. (2008) Proteins.(71)1721-31.
5. in cooperation with Brigit Strodel group from FZ Juelich, ICS-6.

CLIB Graduate Cluster Industrial Biotechnology
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MARIE CURIE ACTIONS