MUNI
FACULTY
OF SCIENCE

MBL Microbial Bioengineering Laboratory

Preliminary data 3: Development of genetic engineering tools for *Caldimonas thermodepolymerans*

Microbial Bioengineering Laboratory (data collected by Dr. Anastasiia Ieremenko)

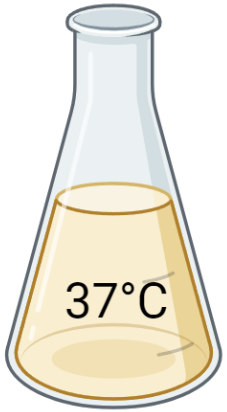
Department of Experimental Biology, Faculty of Science,
Masaryk University, Brno, Czech Republic

MOTIVATION

- Reliable and efficient genetic engineering tools are essential for metabolic engineering of *C. thermodepolymerans* and other attractive Gram-negative thermophilic bacteria.

- Yet unpublished tools, parts and methods we have developed or adopted over the last 18 months are briefly summarised on the following slides.

1. OPTIMISED ELECTROPORATION PROTOCOL

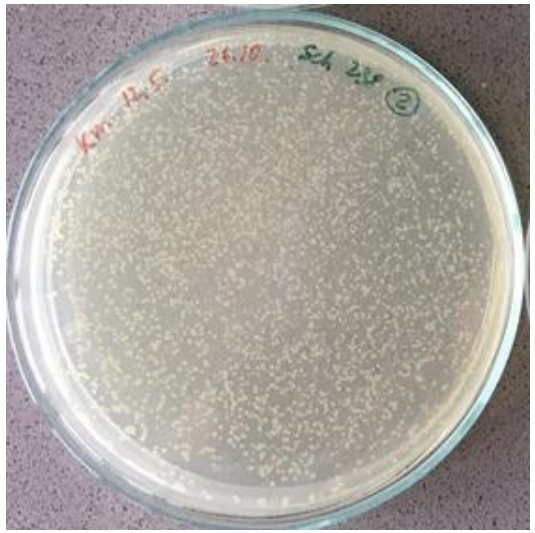


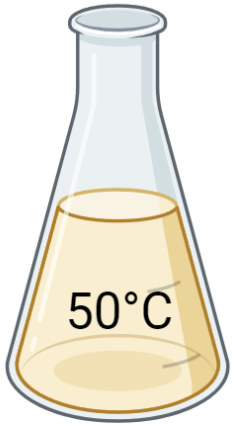
Competent cells preparation

Freezing

Electric pulse

Transformation efficiency
10^3 – 10^5/1µg DNA for
medium and
low copy plasmids



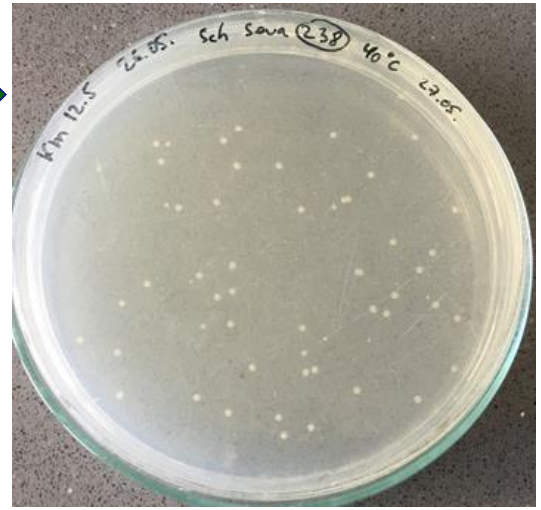


Competent cells preparation

Use immediately

Electric pulse

Transformation efficiency
10^2 – 10^3/1µg DNA for
medium copy plasmids



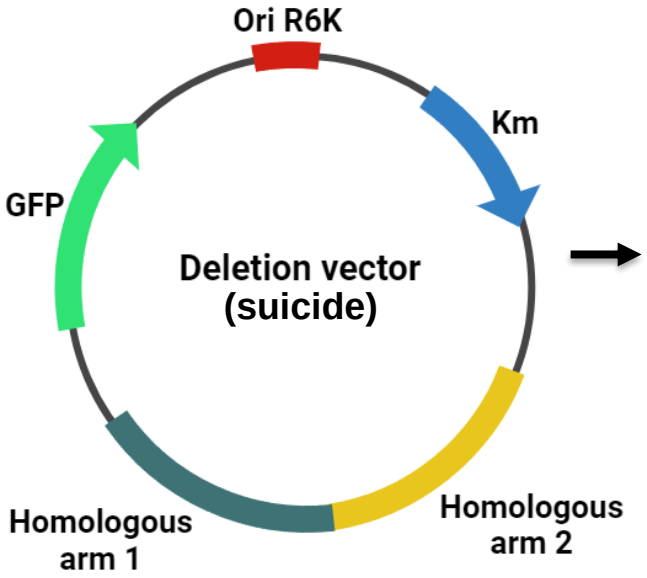
Freezing

Electric pulse

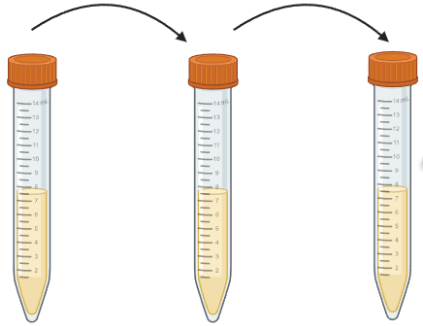
Cells are not viable

2. HOMOLOGOUS-RECOMBINATION-BASED GENE DELETION

Deletion of *phaC** gene



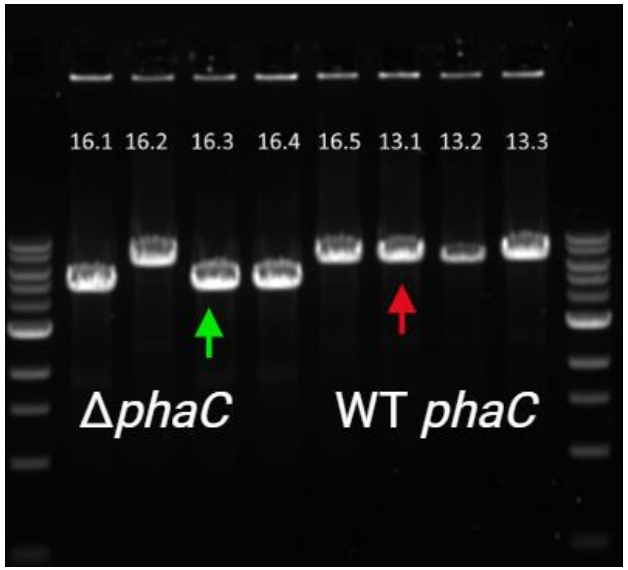
~ 0.05 % single crossovers



~ 0,3 % double crossovers

33 % mutant double crossovers

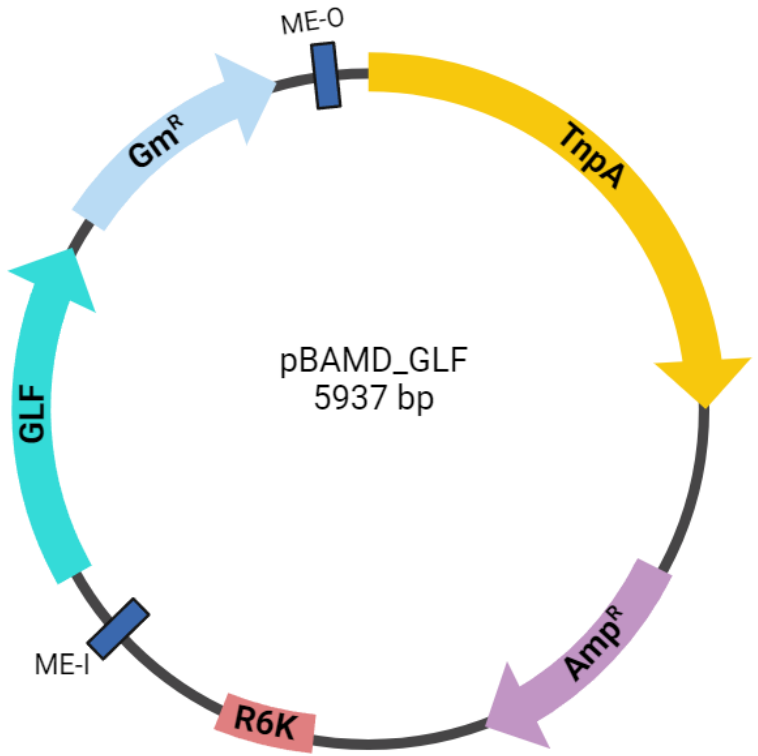
67 % WT revertant double crossovers



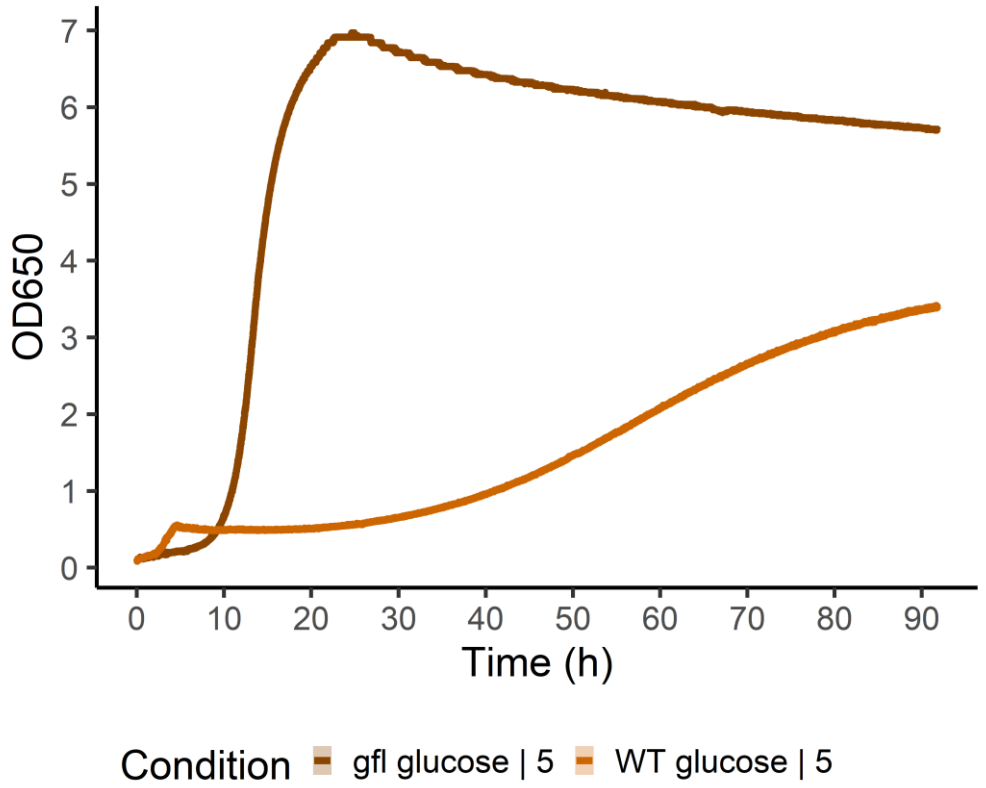
* encodes *C. thermodepolymerans* PHA synthase

3: RANDOM INTEGRATION INTO THE CHROMOSOME WITH pBAMD

A. Integration of high-capacity glucose facilitator Glf from *Zymomonas mobilis* for **better growth of *C. thermodepolymerans* on a glucose**

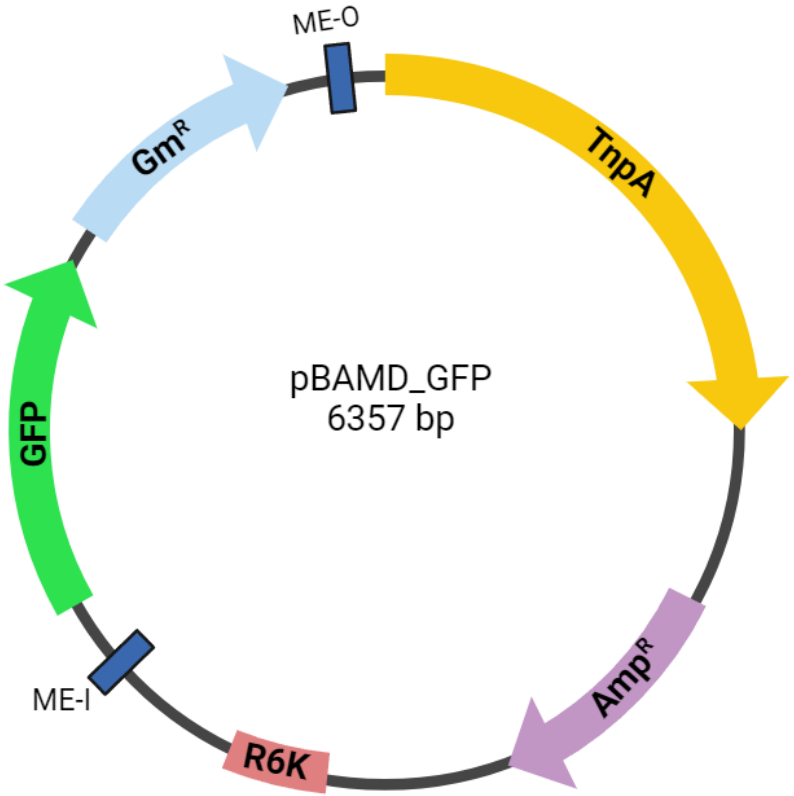


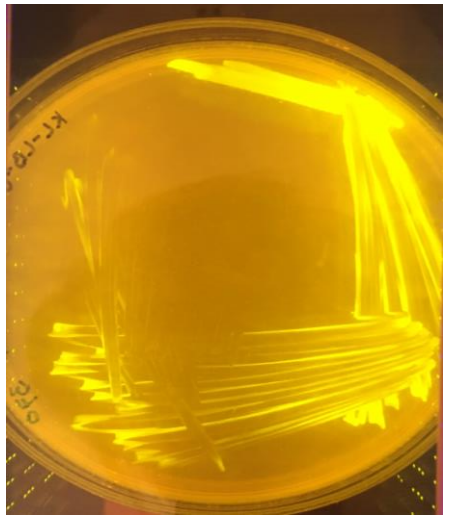
Growth of *C.t.* WT and one of the mutants with integrated *glf* on 5 g/L D-glucose in Chi.Bio reactor at 42°C



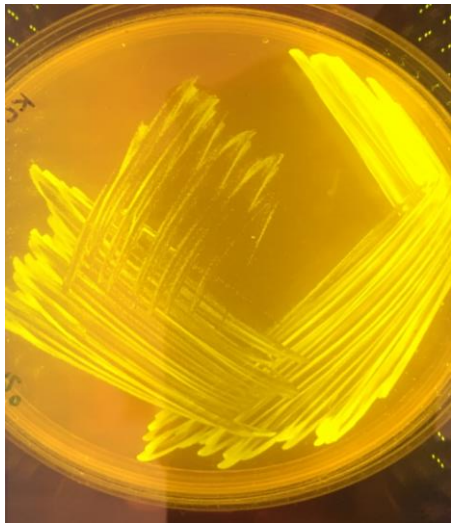
3: RANDOM INTEGRATION INTO THE CHROMOSOME WITH pBAMD

B. Random insertion of promotorless *gfp* and screening for native *C. thermodepolymerans* promoters, verification of sfGFP stability at 50°C

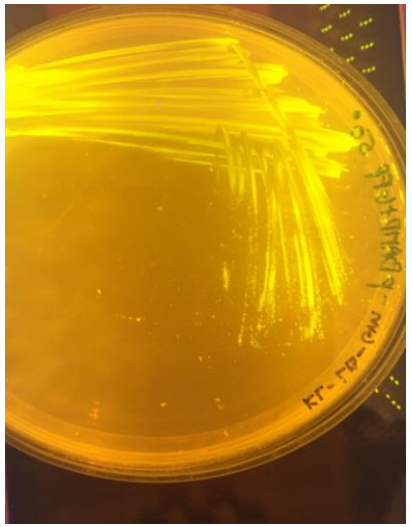




37 °C



42 °C

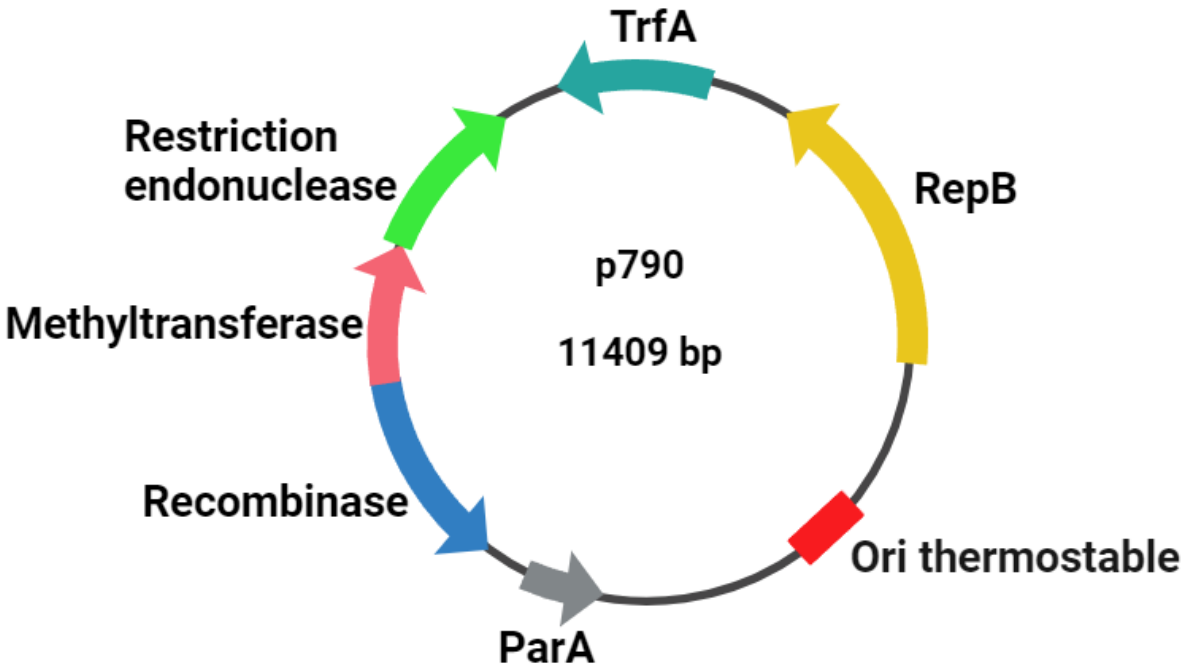


50 °C

4: ASSEMBLY OF THERMOSTABLE EXPRESSION VECTOR

Search for the suitable thermostable origin of replication

Native plasmid isolated from related bacterium *C.hydrothermale* (14 species screened for native plasmids), sequenced (map below) and its parts are being used for the construction of expression plasmid usable for *C. thermodepolymerans*



Cultures of Gram- bacteria
Chelatococcus hermostellatus
Chelatococcus composti
Caldimonas hydrothermale
Caldimonas taiwanensis
Caldimonas manganoxidans
Thermomonas hydrothermalis
Tepidiphilus thermophilus
Tepidomonas taiwanensis
Tepidomonas fonticaldi
Pseudomonas thermotolerans
Chelatococcus daeguensis
Chelatococcus sambhunathii
Caldimonas meghalayensis
Tepidomonas fonticaldi

CONCLUSIONS

- An electroporation protocol and basic gene deletion/insertion protocols have been developed for *C. thermodepolymerans*.
- All protocols need further optimisation to improve their efficiency.
- The growth of *C.t.* on glucose can be improved with inserted glucose transporter.
- Monomeric superfolder GFP can be used as a reporter in *C.t.* at 50°C.
- Expression plasmid for *C.t.* is under construction.