PARAGEOBACILLUS THERMOGLUCOSIDASIUS AS A RECOMBINANT HOST: OPTIMIZATION STRATEGIES

Elina Guogytė[1], Rūta Kananavičiūtė[1]

[1]Vilnius University, Life Sciences Center, Institute of Biosciences, Lithuania
elina.guogyte@gmc.stud.vu.lt

Parageobacillus thermoglucosidasius, also known as emerging thermophilic cell factories, is receiving a lot of attention in the field of biotechnology. It is known that *P. thermoglucosidasius* can be an excellent producer of recombinant proteins. The proteins of thermophilic microorganisms are highly valued due to their greater thermostability, structural stability and solubility compared to mesophilic proteins. However, there are still problems with the use of these bacteria in industrial processes. There is a lack of suitable genetic tools, such as stable and high copy number vectors, and the low transformation efficiency of these bacteria [1]. For this reason, in this study, we present strategies that will facilitate the utilization of these bacteria. The main goal of the study is to increase the efficiency of the transformation of *P. thermoglucosidasius* and to expand the set of vectors for the manipulation of these bacteria.

The first strategy was to increase the transformation efficiency of *P. thermoglucosidasius* using differentially methylated plasmid DNA. We used the pG2K shuttle plasmid isolated from 3 *E. coli* strains characterized by different DNA methylation patterns (DH5α (*dam+, dcm+*), RR1 (*dam-, dcm+*) and GM2163 (*dam-, dcm-*) and from the *P. thermoglucosidasius* DSM 2542^T. A "lab-made" electroporator generating square wave pulses was used to transfer the pG2K plasmid into bacteria [2]. The obtained study data show that the *E. coli* RR1 (*dam-, dcm+*) strain and *P. thermoglucosidasius* DSM 2542^T cells are the most suitable for plasmid amplification before transferring them to *P. thermoglucosidasius*.

The second strategy was to expand the selection of plasmid vectors for *P. thermoglucosidasius*. For this, a vector with the minimal pGTG5 plasmid *rep5* replicon [3], isolated from bacteria of the genus *Parageobacillus*, was constructed. The target *rep5* replicon sequence was amplified and used to replace the *repBST1* replicon of the pG1AK-pRpls-GFP vector using restriction and ligation reactions. The resulting pG5AK-GFP construct is expected to be a 3.8 kb shuttle vector containing *ColE1* (origin of replication for *E. coli*), *rep5* (replication initiation protein), *kanR* (kanamycin resistance gene) and MCS (multiple cloning site). Due to its relatively small size, the pG5AK-GFP vector is predicted to exhibit higher bacterial transformation efficiency, consistent with theoretical knowledge that smaller plasmids transform more efficiently.

These strategies may help to adapt *P. thermoglucosidasius* as a recombinant host and expand the spectrum of recombinant proteins already synthesized in these bacteria. Such proteins could be particularly valuable in industrial, medical, and environmental protection field.

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