OPTIMIZATION OF CONDITIONS FOR GENETIC TRANSFORMATION OF THERMOPHILIC BACTERIA

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Thermophilic bacteria are considered to be useful biotechnological objects, since this type of bacteria has a lot of possible uses in various industrial fields, where these microorganisms serve as a source of many thermostable enzymes as well as biotechnologically produced metabolites. However, due to the lack of reliable genetic transformation systems for thermophiles, industrial application remains a challenging approach. Thus, optimization of genetic transformation of thermophilic bacteria is highly required.

The aim of this research was to broaden current knowledge of genetic manipulation of thermophilic bacteria in order to find the method of transforming the three bacterial strains - *Geobacillus stearothermophilus* 15, *Aeribacillus pallidus* 8 and *Parageobacillus toebii* DSM 14590^T. Shuttle vectors used in this study were methylated and non-methylated, containing replication initiation proteins from different species of thermophilic bacteria. Unfortunately, we did not obtain any transformants, which confirms that transformation efficiency is not strongly dependent on these factors.

Further studies concentrate on the vector methylation using recombinant restriction-modification (R-M) systems encoded by the thermophilic bacteria strains. Using the Restriction Enzyme Database (REBASE), we identified the R-M systems in the thermophiles and transferred these genes to the *Escherichia coli* strains. The extracted shuttle-vectors are later used for genetic transformation of thermophiles. We hope that using the heterologous expression of R-M system genes from thermophilic bacteria will allow exogenous DNA to avoid restriction by the host R-M system.

Overall, we believe that this work has a great potential and could be useful in future researches associated with genetic engineering of thermophilic microorganisms.