Abstract

Corynebacterium glutamicum is a Gram-positive non-sporulating soil bacterium which is used in biotechnology as a producer of amino acids, nucleotides, biofuels and alcohols. The aim of this thesis was to create a hybrid σ factor of RNA polymerase which would be able to recognize a matching hybrid promoter without effect on expression of the host genes. Based on the σ^{D} and σ^{H} amino acid sequence, two types of hybrid factors, σ^{DH} and σ^{HD}, were designed by the sequence combination of *sigD* and *sigH*. As an alternative approach, based on the *in silico* homology modeling, mutations of wild-type σ^{H} in the region recognizing the -35 promoter element of the σ^{H}-dependent promoter were introduced. Hybrid promoters were constructed by combining the -35 and -10 promoter regions that were derived from the σ^{D}- and σ^{H}-dependent promoters. Promoter activity was determined by using *gfp*uv reporter gene under the control of hybrid promoter. The expression of *gfp*uv in strains with hybrid sigma factors σ^{DH}/ σ^{HD} and hybrid promoters was rather low compared to strains that carried wild-type σ factor and the respective promoter. The aim of the thesis was achieved by using one of the mutant σ^{H} factor (σ^{mutH_6A}) with alterations in the region recognizing the -35 element of the σ^{H}-dependent promoter. This mutant σ factor selectively drove transcription of the reporter gene from the hybrid P*sigDH* promoter.

Keywords: *Corynebacterium glutamicum*, sigma factor, promoter