

Abstract

Grampositive bacterium *Corynebacterium glutamicum* is an important industrial producer of amido acids and other metabolites. Its genome encodes 7 sigma (σ) subunits of RNA polymerase: primary factor σ^A , primary-like σ^B and five alternative sigma factors, σ^C , σ^D , σ^E , σ^H and σ^M (sigma factors with extracytoplasmic function). This study is focused on revealing so far unknown regulons of stress sigma factors or closer description of regulons whose genes are controlled by σ^D , σ^E , σ^H and σ^M . These factors were partially described for their activity during surface (σ^D and σ^E), heat (σ^E , σ^H and σ^M) and oxidative (σ^H and σ^M) stress response. We assumed that the genes of each regulon are transcribed from promoters of a single class.

For the purpose of detailed promoter analysis, it was necessary to develop methods which can quickly and reliably assign sigma factor to particular promoters and, thus, respective genes. For this purpose, a combination of *in vivo* (two-plasmid system) and *in vitro* (*in vitro* transcription) techniques was developed that allow to specify this assignment. We identified 9 σ^H/σ^E - promiscuous promoters (*PamtR*, *Pcg0378*, *Pcg1121*, *Pcg3309*, *Pcg3344*, *PclgR*, *PdnaJ*, *PdnaK* and *PsigB*), 7 σ^D/σ^H - promiscuous promoters (*Pcg0607*, *Pcg2047*, *Pcmt2*, *PfadD2*, *Plpd*, *PlppS* and *PrsdA*) a 9 σ^H/σ^M - promiscuous promoters (*PbenA*, *PcatA1*, *Pcg0256*, *Pcg0668*, *Pcg0785*, *Pcg2322*, *Pcg2534*, *PsigM C. callunae* and *PsigM C. deserti*) with these methods. Consensus sequences of not yet described σ^E -dependent (-35: GGAAC; -10: GTT) and σ^M -dependent (-35: GGGAACC; -10: GTCNAA) promoters were defined.

Amino acids crucial for specific recognition of σ^H/σ^E - promiscuous promoters (arginine R185 of factor σ^E and methionine M170 of factor σ^H for recognition of -31 nucleic position) and σ^D/σ^H - promiscuous promoters (alanine A60 of factor σ^D and lysine K53 of factor σ^H for recognition of second nucleotide in region -10 or valine V169 with alanine A166 of factor σ^D and serine S171 with threonine T168 of factor σ^H for recognition of second position in region -35) were found by homologous modeling and confirmed by targeted mutagenesis.

Our results show that promiscuous promoters are not an unusual exception in *C. glutamicum* but represent relatively frequent regulatory strategy enabling the organism to effectively cope with complex environmental stresses. The *C. glutamicum* σ^H factor plays evidently the most important role as one of the potential global regulators in regulatory network of sigma factors because it is less specific in recognition of promoters than others sigma factors.

Keywords: *Corynebacterium glutamicum*, sigma factor, promiscuous promoter, transcription