

My thesis is focused on characterization of *Corynebacterium glutamicum* promoters responding to increase of temperature. *C. glutamicum* is the most important representative of a gram-positive, non-pathogenic, amino-acid producing bacteria. Its strains are industrial producers of glutamic acid, lysine, threonine, tryptophane and phenylalanine (Liebl *et al.*, 1991). Amino acids produced by microbial fermentation are used in medicine (as infusion liquids or therapeutics), chemical industry (as detergents and cosmetics compounds), food industry (as additives), agriculture and stockbreeding (as growth-regulator additives to the animals feed) (Hermann, 2003).

Although knowledge of organizations of its genes involved in biosynthesis of various amino acids increased considerably during last years, still little is known about molecular signals controlling gene expression. Since regulation of gene expression on the level of transcription plays crucial role, understanding the structure and function of transcription initiation signals (promoters) is of primary importance. Promoters, which can be easily switched on and off during fermentations by external factors may ensure the controlled overexpression of the chosen genes. Therefore, the *C. glutamicum* promoters responding to increase of temperature have been isolated.

Two different approaches for isolation of the promoters were employed. First approach was isolation of the promoters of heat-shock proteins based on knowledge of whole genome sequence of *C. glutamicum* (Kalinowski *et al.*, 2003; Ikeda a Nakagawa, 2003). According to data in the literature (Muffler *et al.*, 2002), three promoters of *clpB*, *clpC* and *sigE* were chosen for analysis in different reporter systems. Gene *clpB* is coding for heat-shock protein ClpB with chaperon activity which is together with DnaK/DnaJ/GrpE complex involved in protein folding and prevention of protein aggregation (Motohashi *et al.*, 1999). ClpB is

necessary for optimal growth and cell survival (Squires *et al.*, 1991). Gene *clpC* is coding for ClpC protein with ATPase activity and is essential for renaturation or degradation of misfolded or denatured proteins that accumulate in the cell upon exposure to stress conditions (Schirmer *et al.*, 1996). Gene *sigE* is coding for alternative sigma subunit of RNA polymerase which is induced by external stress factors, mostly by extreme temperature (Manganelli *et al.*, 2001).

Construction of *C. glutamicum* promoter library and screening for heat shock responding promoters was an alternative approach. Clone harbouring promoter for ncRNA (non-coding RNA) responding to increase of temperature was obtained by this approach. Last part of the thesis deals with detailed analysis of the first described ncRNA in *C. glutamicum*, which probably participate on response to heat shock.

Isolation and characterization of promoters responding to increase of temperature can be used for construction of amino acid producer strains, where temperature will up-regulate the activity of promoters and provide higher gene expression after heat shock.