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ScienceDirect
Journal of Biotechnology 126 (2006) 101–110
Journal of BIOTECHNOLOGY
www.elsevier.com/locate/jbiotec

Review

Application of global analysis techniques to *Corynebacterium glutamicum*: New insights into nitrogen regulation

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Received 29 September 2005; received in revised form 24 January 2006; accepted 29 March 2006

Abstract

The regulation of nitrogen metabolism in the amino acid producer *Corynebacterium glutamicum* was subject of research for several decades. While previous studies focused on single enzymes or pathways, the publication of the *C. glutamicum* genome sequence gave a fresh impetus to research, since a global investigation of metabolism and regulation networks became possible based on these data. This communication summarizes the advances made by different studies, in which global analysis approaches were used to characterize the *C. glutamicum* nitrogen starvation response. A combination of bioinformatics approaches, transcriptome and proteome analyses as well as chemostat experiments revealed new insights into the nitrogen control network of *C. glutamicum*.

C. glutamicum reacts to a limited nitrogen supply with a rearrangement of the cellular transport capacity, changes in metabolic pathways for nitrogen assimilation and amino acid biosynthesis, an increased energy generation and increased protein stability. With the aid of chemostat experiments, in which different growth rates were obtained by nitrogen limitation, general starvation effects could be distinguished from specific nitrogen limitation-dependent changes. The core adaptations on the level of transcription are controlled by the master regulator of nitrogen control, the TetR-type protein AmtR. This global regulator governs transcription of at least 33 genes via binding to a palindromic consensus motif (AmtR box). Genes with AmtR box-containing promoters were identified by genome-wide screening and validated, besides by other methods, by transcriptome analyses using DNA microarrays.

Keywords: *Corynebacterium*; DNA microarrays; Nitrogen control; Proteomics; Transcriptome analyses

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doi:10.1016/j.jbiotec.2006.03.039

Contents

1. Nitrogen metabolism and nitrogen control in *Corynebacterium glutamicum*

Nitrogen is an essential component of nearly all macromolecules in a bacterial cell, e.g. proteins, nucleic acids and cell wall components. Accordingly, prokaryotes have developed transport and assimilation systems for a variety of nitrogen sources and sophisticated control mechanisms to provide an optimal supply of nitrogen and to cope with situations of nitrogen limitation.

We are especially interested in nitrogen metabolism and nitrogen regulation in Actinomycetales (for review, see Burkovski, 2003a). This group of bacteria includes producers of antibiotics, e.g. *Streptomyces* species, pathogens such as *Mycobacterium leprae*, *Mycobacterium tuberculosis* and *Corynebacterium diphtheriae* as well as amino acid and nucleotide producers such as *Corynebacterium ammoniagenes*, *Corynebacterium efficiens* and *C. glutamicum*. *C. glutamicum* was isolated by Kinoshita and co-workers in a screening program for L-glutamate-producing bacteria (Kinoshita et al., 1957; Udaka, 1960). Today, large amounts of L-glutamate (more than 1,500,000 tonnes/year) and L-lysine (more than 560,000 tonnes/year) are produced by use of different *C. glutamicum* strains, in addition to smaller amounts of L-alanine, L-isoleucine and L-proline and in addition to different nucleotides (Leuchtenberger, 1996; Hermann, 2003).

Due to the exceptional industrial importance of *C. glutamicum*, the ammonium assimilating, L-glutamate and L-glutamine generating enzymes in this bacterium were studied already in the 1960s. Later, work on other aspects like transport systems or regulatory mechanisms was initiated. As a result of these investigations, detailed information on transport and assimilation of nitrogen sources as well as nitrogen regulation is available on a molecular level for *C. glutamicum* today. Genes encoding uptake systems (Kronemeyer et al., 1995; Siewe et al., 1996; Meier-Wagner et al., 2001; Trötschel et al., 2003; Beckers et al., 2004; Bendt et al., 2004) as well as enzymes (Börmann et al., 1992; Jakoby et al., 1997; Tesch et al., 1999; Beckers et al., 2001; Nolden et al., 2001a; Schulz et al., 2001; Bendt et al., 2004) for ammonium, creatinine, glutamate and urea metabolism are known now. Additionally, the key components of signal transduction and nitrogen control were identified (Jakoby et al., 1999, 2000; Nolden et al., 2001b).

Although obviously successful, these studies had the disadvantage to be typically focused on distinct transporters, enzymes or regulators. A global analysis without prior assumptions and expectations was impossible in the 1990s. This situation changed completely when the *C. glutamicum* genome was sequenced and published independently by different industry-supported groups (Ikeda and Nakagawa, 2003; Kalinowski et al., 2003). The knowledge of