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Salt stress induction of glutamyl endopeptidase biosynthesis in *Bacillus intermedius*

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Summary

Bacteria from the genus *Bacillus* have evolved complicated regulatory networks to be protected from various environmental stresses, including sudden increase in salinity. Among these regulatory mechanisms is the DegS–DegU signal transduction system, which controls degradative enzyme synthesis and is involved in sensing salt stress in *Bacillus subtilis*. We report the study of biosynthesis regulation of *Bacillus intermedius* glutamyl endopeptidase under salt stress conditions. Salt stress during growth in medium containing 1–2.5 M NaCl, KCl or disodium succinate leads to the induction of glutamyl endopeptidase. Analysis of the regulatory region of the gene for *B. intermedius* glutamyl endopeptidase revealed the presence of a tentative target sequence for DegU control, $AGATN_{10}TTGAG$. For the expression of the glutamyl endopeptidase gene, functional DegU protein is required. Thus, we suggest that expression of the gene for *B. intermedius* glutamyl endopeptidase may be controlled by a regulatory system analogous to DegS–DegU two-component system in *B. subtilis*.
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Introduction

Bacteria from the genera *Bacillus* have evolved highly sophisticated regulatory networks for protection against sudden unfavourable environmental changes, including nutrient starvation, changes in temperature and humidity, oxidative stress, sudden elevation in medium salinity and others. *Bacillus subtilis* is able to respond to environmental challenges by spore formation, the uptake of foreign DNA (competence), the production of degradative enzymes, or the induction of a large set of general stress proteins (Sonensein, 2000; Kunst and Rapoport, 1995; Hecker and Volker, 2001; Petersohn et al., 2001).

As a soil bacterium, *B. subtilis* has developed sensing and adaptation mechanisms which allow it

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to cope with salt stress conditions among other environmental stresses. These regulatory mechanisms involve global changes in expression of a large number of genes. At least three distinct mechanisms of salt stress induction have been reported for *B. subtilis*. Transcriptional activation by alternative sigma factor σ^B of the class II heat shock genes, coding for general stress proteins, is the first type of regulation (Volker et al., 1999).

Class II heat shock genes encode also a σ^B-independent group of general stress proteins including FtsH, ClpC and ClpP proteases (Deuerling et al., 1995; Fedhila et al., 2002). Among them, ClpC was proved to be required for the response to salt stress (Fedhila et al., 2002). Notably, these two mechanisms provide non-specific stress resistance. The synthesis of degradative enzymes together with the expression of genetic competence in *B. subtilis* are controlled by a complex regulatory cascade including the ComP–ComA and DegS–DegU two-component systems (Klier et al., 1992; Mader et al., 2002). Recently, expression of several genes that are controlled by the DegS–DegU regulatory pair, including the genes coding for degradative enzymes, was shown to be affected under salt stress conditions (Kunst and Rapoport, 1995; Dartois et al., 1998). In contrast to the first two mechanisms, salt stress response mediated by DegS–DegU two-component system seems to be subjected to salt-specific induction. Besides, the DegS–DegU signal transduction system is known to play a key role in the complex network that governs transition from the exponential to the stationary growth phase under growth-limiting conditions (Mader et al., 2002).

Bacillus intermedius, a gram-positive spore-forming soil bacterium, is known to produce during the stationary phase a wide range of extracellular proteases (Balaban et al., 1993, 1994). Recently, we have isolated and characterized the novel Glu, Asp-specific protease (glutamyl endopeptidase) from *B. intermedius*, strain 3-19 (Leshchinskaya et al., 1997). Glutamyl endopeptidases (EC 3.4.21.19) constitute a newly discovered subfamily within chymotrypsin family of serine proteases. These enzymes possess narrow substrate specificity and split only the peptide bonds formed by α-carboxyl groups of glutamyl and aspartic acid (Rudenskaya, 1998). Glutamyl endopeptidases have been isolated from *Staphylococcus*, *streptomycetes* and *Bacilli* (Drapeau et al., 1972; Yokoi et al., 2001; Ohara-Nemoto et al., 2002; Yoshida et al., 1988; Khaidarova et al., 1989; Kitadokoro et al., 1993; Niidome et al., 1990; Svendsen and Breddam, 1992). Structure and enzymatic properties of glutamyl endopeptidases are thoroughly studied, and the genes for these enzymes in many cases are cloned and sequenced (Demidyuk and Kostrov, 1999). However, their biological role is still unclear, and data available in literature describing the mechanisms that control biosynthesis of glutamyl endopeptidases are very scanty.

We have described earlier the pathways of biosynthesis and the location of glutamyl endopeptidase in *B. intermedius* cells (Gabdrakhmanova et al., 1999). The gene encoding for *B. intermedius* glutamyl endopeptidase was cloned in *B. subtilis* and expression of the gene was analyzed in recombinant strains (Rebrikov et al., 1999; Gabdrakhmanova et al., 2002).

Here we report the study of the regulation of biosynthesis of *B. intermedius* glutamyl endopeptidase under salt stress conditions, underlying the suggestion that expression of the gene coding for this enzyme is controlled by DegU protein.

Materials and methods

Bacterial strains, plasmids and transformation

Streptomycin-resistant *B. intermedius* strain 3-19, obtained from The All-Russia Collection of Industrial Microorganisms (B-3833), was used throughout this study. *B. subtilis* strain *AJ73* (amyE4, npr512, apr73) and *B. subtilis* strain QB4883 (*trpC2 amyE*::(*wapA′-lacZ aphA3*) *ΔdegU::erm*) were used as the plasmid hosts for expression studies. Strain *B. subtilis* AJ73 was kindly supplied by Dr. J. Jomantas (Institute of Molecular Genetics, Moscow, Russia), strain *B. subtilis* QB4883 – by Prof. M. Debarbouille (Institut Pasteur, Paris, France). Plasmid Δ58.21 was constructed by using the pCB22 vector and contained the complete gene for glutamyl endopeptidase from *B. intermedius* conferring resistance to chloramphenicol in *B. subtilis* hosts (Rebrikov et al., 1999). Plasmid isolation was performed as described by Sambrook et al. (1989). Transformation of *B. subtilis* competent cells with plasmid DNA was carried out using standard technique (Sambrook et al., 1989). Transformants were selected by using the chloramphenicol resistance gene linked to the gene construct.

Culture conditions

Initially, *B. intermedius* cells were cultivated in a medium containing (%): peptone, 2.0; $CaCl_2 \cdot 2H_2O$, 0.055; $MgSO_4 \cdot 7H_2O$, 0.05; NaCl, 0.3; $MnSO_4$, 0.01;

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