THE PREDICTION OF REGULATION OF SUBTILISIN-LIKE PROTEINASE GENE FROM BACILLUS INTERMEDIUS THROUGH ITS REGULATORY SEQUENCE ANALYSIS

Kazan State University, Kazan, Russia
* Corresponding author: e-mail: airat_kayumov@rambler.ru

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SUMMARY

Motivation: The complicated regulation of the late catabolite genes is actual problem of the modern molecular microbiology. As a model for this research serve extracellular enzymes of Bacilli.

Results: The nucleotide sequence of aprBi gene coding subtilisin-like proteinase from Bacillus intermedius was determined. The sequences recognized by sigma-A-RNAP and translation start site were predicted using BPROM and SignalP programs, respectively. The aprBi promoter analysis revealed the presence of putative sites for interaction with numerous regulatory proteins (Spo0A, DegU, AbrB, CcpA) and sigma factors. Sequences recognized by different operators and transcription sigma factors overlap each other indicating that their contributions in aprBi gene expression control differ in time. The participation of each transcription regulators in aprBi regulation was confirmed using Bacillus subtilis mutant strains.

Availability: Revealing of putative regulation sites in promoter region may serve as a basis for identification of regulation mechanisms that control the gene expression.

INTRODUCTION

The bacterial metabolism efficiency is provided by balance between catabolism and anabolism. Their activation and repression depends on environmental factors. Bacteria have developed mechanisms allowing coordinating metabolism in accordance with nutrients availability. The complicated regulation of the catabolite genes is reflected in their promoter architecture. Analysis of regulatory sites in promoter region allows predicting regulatory mechanisms, which control gene expression (Mironov et al., 1999). As a model for these researches serve microbial extracellular enzymes.

The gram-positive spore-forming bacteria Bacillus intermedius secrete during stationary stage of growth numerous proteinases, in which the major is subtilisin-like proteinase (Sharipova et al., 2002). The enzyme appears in culture liquid at the stage of slowing down of the growth, with maximal levels of the enzyme activity recorded at the 24th and 48th h of growth. Each protein fractions were isolated and characterized. The main properties of these two protein fractions were found to be similar and their N-terminal amino acid sequences appeared to be identical (Balaban et al., 1994, 2004). Proteinase 2 showed higher specific activity against peptide substrate. It was determined, that both enzymes are the products of one gene. However, the mechanisms involved in the
regulation of subtilisin-like proteinases synthesis during the different stages of bacterial life cycle of *B. intermedius* are still unclear.

**METHODS AND ALGORITHMS**

The plasmid pCS9 containing cloned *aprBi* gene was given by prof. Kostrov (IMG RAN, Moscow). The DNA fragments cloned in pCS9 were sequenced by the dideoxy chain-termination method using the T7 (Pharmacia) sequencing kit and a series of synthetic oligonucleotides that primed at intervals of approximately 300 nucleotides. Analysis of the cloned nucleotide sequence performed out using ORF Finder (Open Reading Frame Finder) network server (http://www.ncbi.nlm.nih.gov/gorf). The starting codon was detected using SignalP algorithm (http://www.cbs.dtu.dk/services/SignalP/), which allows predicting the functional activity of potential signal peptides (Bendtsen et al., 2004). The alignment and sequence comparisons with the GenBank database were performed with the enhanced version of the BLAST program (http://www.ncbi.nlm.nih.gov/blast) (Altschul et al., 1997). The DNA sequence preceding the gene for *B. intermedius* proteinase was inspected for the occurrence of the characteristic –35 and –10 boxes of SigA-type promoters (Helmann, 1995) by Softberry BPROM (Prediction of Bacterial Promoters) network server (http://www.softberry.com).

**RESULTS AND DISCUSSION**

The nucleotide sequence of *B. intermedius* subtilisin-like serine proteinase gene has been determined as described above and submitted to the GenBank database under accession number AY754946. The sequence analysis using the ORF Finder program revealed the presence of open reading frame coding for serine proteinase. Three putative start codons (TTG, GTG and ATG) were identified (Fig. 1). Using SignalP algorithm we have established the probability of signal peptides functional activity, starting from each of three supposed translation start sites.

![Figure 1. Putative translation start sites in *aprBi* gene. The probabilities of functional activity of corresponding signal peptides are indicated at the bottom.](image)

Concerning analysis results, most probable are TTG (D-value = 0,79) or GTG (D-value = 0,69) (Fig. 1), not ATG (D-value = 0,23). It should be noted, that in *Bacilli* genes 10 % of ORFs are translated from GTG and 12 % start from TTG. The mutagenesis of putative start-codons has showed the translation starts from GTG.

The alignment of the *aprBi* promoter sequence with that of the gene for *B. pumilus* subtilisin-like proteinase showed 91 % identity. On the contrary, the comparative analysis of the *aprBi* and the gene for *B. subtilis* subtilisin (*aprE*) revealed only 61 % identity on extension of 81 bp in the promoter region. We propose that various regulatory pathways are involved in expression of these genes.

The *aprBi* promoter region was analyzed with respect to the putative target sequences for binding to a number of regulatory proteins. Using Softberry BPROM network server, a potential promoter sequence with poor similarity to $\sigma^A$-type –35 (score 22) and –10 (score 52) promoter recognition sequences was found in contrast with *B. subtilis* *aprE* gene (scores 48 and 54, respectively) (Fig. 2). It leads us to conclusion that other regulatory factors for effective *aprBi* transcription are required. The *aprBi* promoter region was examined for...
putative regulatory sites. The sequences sharing 78 %, 75 % and 82 % identity with canonical sequences for interaction with σ\(^{L}\), σ\(^{H}\) and σ\(^{E}\) were found (Fig. 2). The putative operator sequence for binding with carbon catabolite repressor CcpA with 78.6 % identity with canonical sequence (TGWNANCNTNWCA) was found (Fig. 3).

(a) The regulatory region of \(aprBi\) gene. The putative sequences recognized by transcription sigma factors are boxed.

(b) The regulatory region of \(aprBi\) gene. A region showing homology to the consensus sequences for site binding the catabolite repressor, TGWAARCGYTWNCW and the AbrB regulatory protein, WAWWTTTWCAAAAAW are boxed, identical nucleotides are underlined.

Figure 2. The regulatory region of \(aprBi\) gene. The putative sequences recognized by transcription sigma factors are boxed.

Figure 3. The regulatory region of \(aprBi\) gene. A region showing homology to the consensus sequences for site binding the catabolite repressor, TGWAARCGYTWNCW and the AbrB regulatory protein, WAWWTTTWCAAAAAW are boxed, identical nucleotides are underlined.

The \(aprBi\) gene expression was found to be repressing by exogenous glucose conforming its regulation by catabolite repression mechanism. Screening with WAWWTTTWCAAAAAW, a 16-bp consensus sequence based on 20 observed AbrB binding regions, identified a region with 63 % identity (Fig. 3). The data of \(aprBi\) expression in AbrB-Spo0A double mutants have demonstrated the AbrB protein participation in subtilisin-like proteinase gene control. Further, in the \(aprBi\) gene regulatory region nucleotide sequences sharing 72–86 % identity with consensus sequence (AGAA\(_{11-13}\)TTCAG) typical for DegU-regulation were detected (Dartois et al., 1998) (Fig. 4). These sequences appeared to be organized as direct tandem repeats. The regulatory region of \(aprBi\) gene contains also the sequences with structural homology (70–86 %) to specific target site for binding with Spo0A regulatory protein (TGNCAGAA) (Fig. 4). Using DegS-DegU and Spo0A mutant strains was established the positive regulation of \(aprBi\) by these regulatory systems. Interesting, in contrast with \(B.subtilis\) subtilisin gene, DegS-DegU system plays minor regulatory role in \(B.intermedius\) subtilisin-like proteinase gene expression.

The data presented here describe the complex network regulation of \(B.intermedius\) serine proteinase expression, including the action of spo0, degU genes, catabolite repression and AbrB protein. This data confirm the changes of control of enzyme biosynthesis at the different stages of bacterial growth.
Figure 4. The aprBi gene promoter. Potential Spo0A binding sites are boxed. Putative DegU sites are underlined; consensus sequences for recognition by DegU are bold.

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REFERENCES


