

# Optimization of *Bacillus intermedius* glutamyl endopeptidase production by recombinant strain of *Bacillus subtilis* and localization of glutamyl endopeptidase in *Bacillus subtilis* cells

L.A. Gabdrakhmanova<sup>a,\*</sup>, N.P. Balaban<sup>a</sup>, M.R. Sharipova<sup>a</sup>, S.V. Kostrov<sup>a</sup>,  
T.V. Akimkina<sup>b</sup>, G.N. Rudenskaya<sup>c</sup>, I.B. Leshchinskaya<sup>a</sup>

<sup>a</sup> Department of Microbiology, Kazan State University, Kremliovskaya str. 18, 420008 Kazan, Russia

<sup>b</sup> Institute of Molecular Genetics, Moscow, Russia

<sup>c</sup> Moscow State University, Moscow, Russia

Received 24 July 2001; accepted 12 March 2002

## Abstract

The biosynthesis of glutamyl endopeptidase from *Bacillus intermedius* 3-19 in recombinant strain of *Bacillus subtilis* was investigated. The composition of culture medium, which yielded the maximum glutamyl endopeptidase production by *B. subtilis* was developed, employing response surface methodology. The pathways of regulation of glutamyl endopeptidase synthesis in recombinant strain in general were found to be similar to those of other serine proteinases and of glutamyl endopeptidase in *B. intermedius*. Synthesis of glutamyl endopeptidase by recombinant strain was suppressed by easily metabolizable carbon sources. Ions of Ca<sup>2+</sup> (1 mM), and Co<sup>2+</sup> (5 mM) stimulated production of the proteinase by *B. subtilis*. In case of Co<sup>2+</sup> ions strong stimulating effect possibly was due to the release of the membrane-bound enzyme into the culture liquid, according to the mechanism described for *B. intermedius*. The addition of Fe<sup>2+</sup>, Zn<sup>2+</sup>, and Cu<sup>2+</sup> to the medium at concentrations of 1 to 10 mM led to the gradual decrease of production by *B. subtilis*. This study has demonstrated a requirement by recombinant strain for excess carbon, nitrogen and phosphate for active glutamyl endopeptidase production. In contrast with *B. intermedius*, for the maximum yield of endopeptidase in *B. subtilis* the presence in the culture medium of yeast extract at concentration of 2% and one of the organic substrates of proteinase or gelatin (1%) was found to be necessary. Our study has revealed the changes in the pathways of secretion of glutamyl endopeptidase of *B. intermedius* by *B. subtilis* cells, expressing the gene for glutamyl endopeptidase from the plasmids: the part of the enzyme remained bound to the cell wall.

© 2002 Elsevier Science Inc. All rights reserved.

**Keywords:** Glutamyl endopeptidase; Biosynthesis; Localization; Recombinant strain; Optimization

## 1. Introduction

Glutamyl endopeptidases (Glu, Asp-specific proteases; EC 3.4.21.19) constitute a new, recently discovered subfamily within chymotrypsin family of serine proteases. These enzymes possess narrow substrate specificity and split only the peptide bonds formed by  $\alpha$ -carboxyl groups of glutamic and aspartic acid [1]. Glutamyl endopeptidases have been isolated from *Staphylococcus*, *Streptomyces* and *Bacilli* [2–4]. These are secreted proteins of 18–29 kDa, their pI varying in a wide range of pH values. Enzymatic properties of glutamyl endopeptidases are thoroughly studied [5]. The most known commercially available representa-

tive of Glu, Asp-specific proteases is V8 from *Staphylococcus aureus* [6]. So, structure and function of bacterial glutamyl endopeptidases are well known, whereas their biological role is still unclear. It is currently known about the mechanisms of biosynthesis of these enzymes. Thus, further study of biosynthesis of bacterial glutamyl endopeptidase is desirable.

Recently we isolated and characterized glutamyl endopeptidase from streptomycin-resistant strain of *B. intermedius* 3-19 [7]. The pathways of its biosynthesis and the location of the enzyme in recombinant cells were described [8]. The gene encoding glutamyl endopeptidase of *B. intermedius* 3-19 was cloned into *B. subtilis*. Two recombinant plasmids were obtained: pV and  $\Delta$ 58.21, differing in the size

\* Corresponding author.

E-mail address: leila.gabdrakhmanova@ksu.ru (L.A. Gabdrakhmanova).