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endopeptidase in *Bacillus subtilis* cells

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Abstract

The biosynthesis of glutamyl endopeptidase from *Bacillus intermedius 3-19* in recombinant strain of *Bacillus subtil*
tigated. The composition of culture medium, which yielded the maximum glutamyl endopeptidase production by *B. s*
developed, employing response surface methodology. The pathways of regulation of glutamyl endopeptidase synthes
strain in general were found to be similar to those of other serine proteinases and of glutamyl endopeptidase in *B. interm*
sis of glutamyl endopeptidase by recombinant strain was suppressed by easily metabolizable carbon sources. Ions of C
(1 mM), and Co^{2+} (5 mM) stimulated production of the proteinase by *B. subtilis*. In case of Co^{2+} ions strong stimulating e
possibly was due to the release of the membrane-bound enzyme into the culture liquid, according to the mechanism descr
intermedius. The addition of Fe^{2+}, Zn^{2+}, and Cu^{2+} to the medium at concentrations of 1 to 10 mM led to the gradual decr
production by *B. subtilis*. This study has demonstrated a requirement by recombinant strain for excess carbon, nitrog
phosphate for active glutamyl endopeptidase production. In contrast with *B. intermedius*, for the maximum yield of en
subtilis the presence in the culture medium of yeast extract at concentration of 2% and one of the organic substrates of p
or gelatin (1%) was found to be necessary. Our study has revealed the changes in the pathways of secretion of glutam
of *B. intermedius* by *B. subtilis* cells, expressing the gene for glutamyl endopeptidase from the plasmids: the part of th
remained bound to the cell wall.
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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 α-carboxyl groups of glutamyc and aspartic acid [1]. Glutamyl endopeptidases have been isolated from *Staphylococcus*, *Streptomycetes* 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 representative of Glu, Asp-specific proteases is V8
Staphylococcus aureus [6]. So, structure a
bacterial glutamyl endopeptidases are well
whereas their biological role is still uncle
is currently known about the mechanisms
biosynthesis of these enzymes. Thus, furthe
biosynthesis of bacterial glutamyl endopept
desirable.

Recently we isolated and characterize
dopeptidase from streptomycin-resistant
intermedius 3-19 [7]. The pathways of its b
ulation and the location of the enzyme in
cells were described [8]. The gene enc
tamyl endopeptidase of *B. intermedius 3-1*
B. subtilis. Two recombinant plasmids
tained: *pV* and *Δ58.21*, differing in the siz

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