ISSN: 0974-8369

Biology and Medicine

International, Open Access

Available online at: www.biolmedonline.com

Biology and Medicine

Rudakova *et al.*, Biol Med (Aligarh) 2015, 7:2
http://www.biolmedonline.com

Research Article Open Access

Optimization of Cultivation Media for Heterologous Gene Expression of Adamalysin-like Melalloendopeptidase *Bacillus pumilus*

Natalia Leonidovna Rudakova*, Nelly Pavlovna Balaban, Ayslu Mirkasimovna Mardanova, Inna Borisovna Chastukhina, Margarita Rashidovna Sharipova

Kazan Federal University, Kremlevskaya Street 18, Kazan 420008, Russia

Abstract

The paper is dedicated to selecting main components of the nutrient medium and additional sources of nutrients for the maximum production of adamalysin-like metalloproteinase *Bacillus pumilus*. For convenience, the gene of metalloproteinase *MprBp* was cloned in protein-deficient strain *B. subtilis* BG2036. During the study, optimal concentration of peptone, inorganic phosphate, and casein was selected. Besides, it was shown that the enzyme production is stimulated by kasamino acids, leucine, alanine, asparagine, glutamine, as well as calcium and magnesium ions.

Keywords

Metalloproteinase; Adamalysine-like; *Bacillus pumilus*; Productivity; Culture fluid; Optimization

Introduction

Zink-dependent metalloproteinases constitute a diverse group of enzymes within the metzincines clan, which includes both procariotic and eucariotic enzymes. Eucariotic metzinkins are mainly involved in regulatory processes, they execute highly specific proteolysis, activate and inactivate various bioactive molecules, are involved in processing of peptides, in embryonic development and differentiation of tissues [1,2]. Bacterial proteinases – analogs of eucariotic metzincines, which are an ideal model for research, are of particular interest. Searching for these enzymes among procariotes and their characteristics provides wide opportunities in science and in practice. Mainly, these proteins are found in Gram-negative bacteria. Genome sequencing has allowed to identify homologous genes in the genomes of bacilli. Cloning of the gene of metalloproteinase *B. pumilus* MprBp made it possible to identify and clean a corresponding protein. It was found that this enzyme belongs to the family adamalysine-like metalloproteinases of the metzincines clan and is currently the only protein of the bacilli isolated in a homogeneous state [3]. In the native strain of *B. pumilus*, metalloproteinase MprBp is a minor protein and is found in the culture fluid in trace quantities. For a substantial increase in the expression of protein, the gene of *MprBp* was cloned on plasmid pSA1 in protease deficient strain *B. subtilis* BG2036, which made it possible to obtain a sufficient amount of homogeneous enzyme for a detailed study of its physico-chemical, enzymatic, and structural features [3,4].

The purpose of the study was the optimization of the nutritional medium for production of metalloproteinase MprBp with the recombinant strain *B. subtilis*.

Materials and Methods

Erythromycin-resistant strain *B. subtilis* JB 2036 was used as the object of the research, bearing plasmid pSA1 with the gene of metalloproteinase *B. pumilus* 3-19 and deficient in its own extracellular proteolytic enzymes.

Plasmid-free strain *B. subtilis* JB 2036 provided by Professor E. Ferrarri (Genencor Int. Inc., USA) was used as the reference strain.

Initial nutrient medium for strain *B. subtilis* JB 2036 with plasmid pSA1 was the medium with the following composition (g/l): peptone – 17, yeast extract – 10, $CaCl_2 \times 2H_2O$, 0.1, $MgSO_4 \times 7H_2O$ – 0.1, NaCl – 3, NH_4Cl – 0.1, $MnSO_4$ – 0.1, pH of the medium 7.7 [5-7]. The medium was sterilized at 1 atm. Solutions of inorganic phosphate (Na_2HPO_4), casamino acids, amino acids, carbonhydrates, protein supplements (gelatin, albumin, and casein), and salts of divalent metals were sterilized separately and added into the medium before inoculation in indicated concentrations.

The recombinant strain was cultivated on a shaker (B. Braun, Germany) with swing intensity of 200 rpm at 37°C for 30 h. The medium volume to flask volume ratio was 1:7. Seed material was the 16 h inoculate (1% v/v). The culture fluid was separated from cells in a centrifuge for 20 min at 8,000 rpm.

The amount of biomass was determined using nephelometry on a photo-electrocalorimeter KFK-2 at 590 nm in a cuvette 1 cm thick and expressed in units of optical density.

Proteolytic activity of metalloproteinase was determined by splitting azocasein [8]. Measurement was made on a spectrophotometer (Bio-Rad, USA) at 450 nm wavelength. The unit of activity was the amount of enzyme that hydrolyzed 1 μg of substrate for 1 min during the experiment, and was expressed in units/ml or in %. Productivity of the culture was defined as the ratio between the value of proteolytic activity to the unit of biomass, and expressed in arbitrary units (arb. units).

Microsoft Excel and the Statgraphics application were used for analysis of experimental data and for analysis of two-factor experiments data. For describing and comparing characteristics, 95% confidence intervals for the averages were built.

Results and Discussion

Study of the dynamics of growth and accumulation of proteolytic activity of metalloproteinases of the recombinant strain *B. subtilis*

***Corresponding author:** Rudakova NL, Kazan Federal University, Kremlevskaya Street 18, Kazan 420008, Russia; E-mail: natalialrudakova@mail.ru

Received: January 5, 2015; **Accepted:** February 17, 2015; **Published:** April 13, 2015

Citation: Rudakova NL, Balaban NP, Mardanova AM, Chastukhina IB, Sharipova MR (2015) Optimization of Cultivation Media for Heterologous Gene Expression of Adamalysin-like Melalloendopeptidase Bacillus pumilus. Biol Med (Aligarh) 7(2): BM-077-15, 8 pages.

showed that the enzyme activity is manifested in the culture fluid at the 18th hour of growth, its level reaches its maximum at the 29-31 h of growth, which corresponds to the stationary phase of culture growth. Proteolytic activity of protease-free strain *B. subtilis* is found in trace quantities in comparison with the level of activity of the recombinant strain (Figure 1).

The synthesis of extracellular enzymes is determined by the composition of the cultivation medium. Induction of proteases occurs in environments with high content of organic compounds (peptone, casein hydrolysate, yeast extract). Also the composition of the medium often includes inorganic phosphorus-containing compounds that are necessary for the growth of the microorganism and for synthesis of essential compounds.

We found that in the original nutrient medium with peptone content of 17 g/l and inorganic phosphate of 0.3 g/l, the enzyme activity and productivity of the recombinant strain *B. subtilis* were insignificant. For the purpose of optimization of the nutrient medium composition, we performed a two-factor experiment where concentration of the main components of the nutrient medium varied in three levels: peptone – 10, 20, and 30 g/l and inorganic phosphate – 0.8, 1.2, and 1.6 g/l (Figure 2).

The results of the two experiments showed that the maximum activity of metalloproteinase was observed at concentration of peptone in the medium of 19 g/l and inorganic phosphate of 1.3 g/l, and the maximum production of the enzyme – at peptone concentration of 20 g/l and that of inorganic phosphate – 1.45 g/l (Figure 2).

Effect of Protein Supplements on Metalloproteinase Production by Recombinant Strain *B. subtilis*

The presence of complex organic substrates in the medium has a stimulating effect on the enzyme biosynthesis. We introduced protein

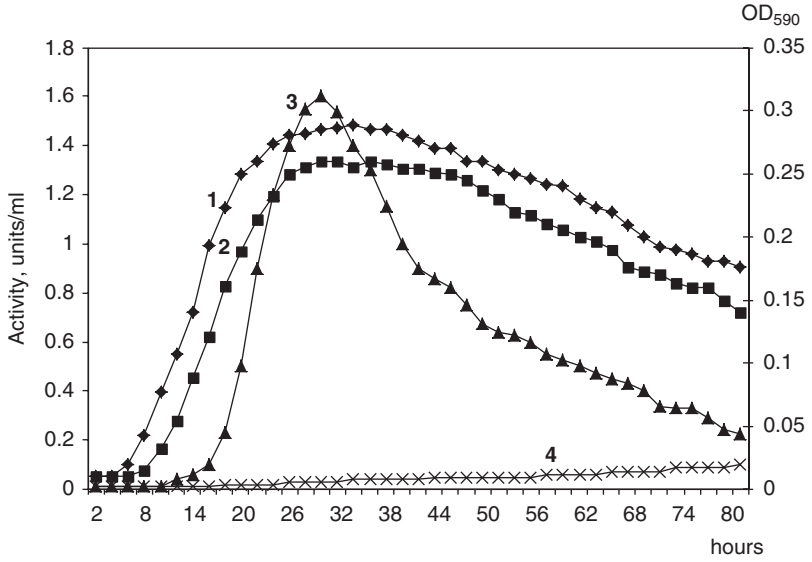

Figure 1: Dynamics of growth and accumulation of proteolytic activity. 1 – growth of the protease-free strain; 2 – growth of the recombinant strain; 3 – proteolytic activity of the recombinant strain; 4 – proteolytic activity of the protease-free strain.

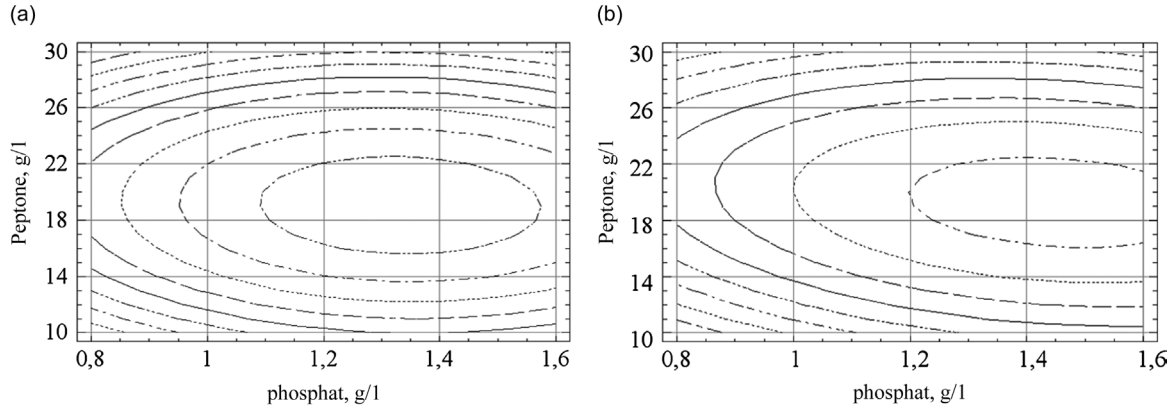

Figure 2: The effect of the concentration of peptone and inorganic phosphate on the activity (a) and productivity (b) of metalloproteinase MprBp.

substrates, such as gelatin, albumin, and casein in concentrations from 0.1 to 2 g/l into the cultural medium with concentration of peptone of 20 g/l and that of inorganic phosphate – 1.45 g/l. The presence of gelatin and albumin in the medium did not increase the enzyme activity and productivity of the recombinant strain *B. subtilis*. Introduction of casein at a concentration of 1 and 2 g/l into the environment increased the enzyme activity by 60%, and the culture production by 30-40% (Figure 3).

Since the introduction of casein cultivation in concentrations of 1-2 g/l into the medium had a positive influence on accumulation of activity of the extracellular enzyme and productivity of the culture, we performed a two-factor experiment in order to determine the optimal concentrations of casein and inorganic phosphate (Figure 4), Casein and inorganic phosphate was varied in three levels: casein – 0.5, 1.0, and 1.5 g/l and inorganic phosphate – 0.8, 1.2, and 1.6 g/l.

As a result, we found the optimal concentration of casein (1 g/l) in the medium that positively influences enzyme biosynthesis.

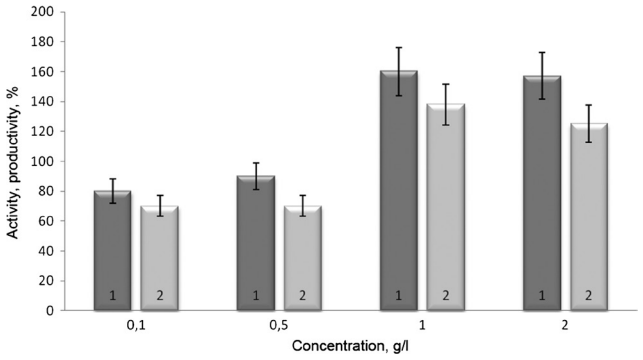

Figure 3: The influence of different concentrations of casein on the activity of metalloproteinase Mugur and productivity of *B. subtilis*. Activity and productivity values were taken for 100% in an environment without introduced protein supplements. 1 – activity of metalloproteinase, 2 – productivity of the culture.

One of the ways of biosynthesis of extracellular enzymes is the regulation using the final product, which can be amino acids and casamino acids. In cultivation of microorganisms, casamino acids can serve as an additional source of both carbon and nitrogen. If casamino acids were introduced into the medium in concentration from 0.1 g/l to 0.5 g/l, the culture growth was at the level of the reference, and the presence of 0.1 g/l of casamino acids increased the activity of the enzyme 2 times, and the productivity 5 times. With increasing concentration of casamino acids to 1-2 g/l, the culture growth was increased 1.5-2 times, respectively; however, the enzyme activity and the culture productivity significantly decreased (Figure 5). The data obtained make it possible to conclude that the synthesis of metalloproteinase MprBp in the presence of large concentrations of casamino acids is sensitive to nitromethabolic repression.

Thus, in order to increase the production of metalloproteinase MprBp, it is appropriate to add 0.1 g/l of casamino acids into the nutrient medium.

Growth and development of microorganisms is stimulated by various supplements containing vitamins, amino acids, polypeptides, trace elements that promote intensive synthesis of proteolytic enzymes. For this purpose, the composition is often contains corn and yeast extracts, extract of flour soybeans and other substrates [9].

We studied the effect of protein additives (gelatin and albumin) on growth and productivity of the culture, and accumulation in the medium of metalloproteinase activity. It was shown that the addition of gelatin in a concentration of 0.1-2% to the cultivation medium significantly reduces enzyme activity and productivity of culture (Figure 6).

Albumin in the same range of concentrations increases the growth in culture of producer by 20% with 0.5% concentration in the environment. Enzyme activity and productivity of the culture with all studied concentrations decrease as compared to the reference. In this regard, it is impractical to introduce albumin and gelatin to the nutrient medium (Figure 6).

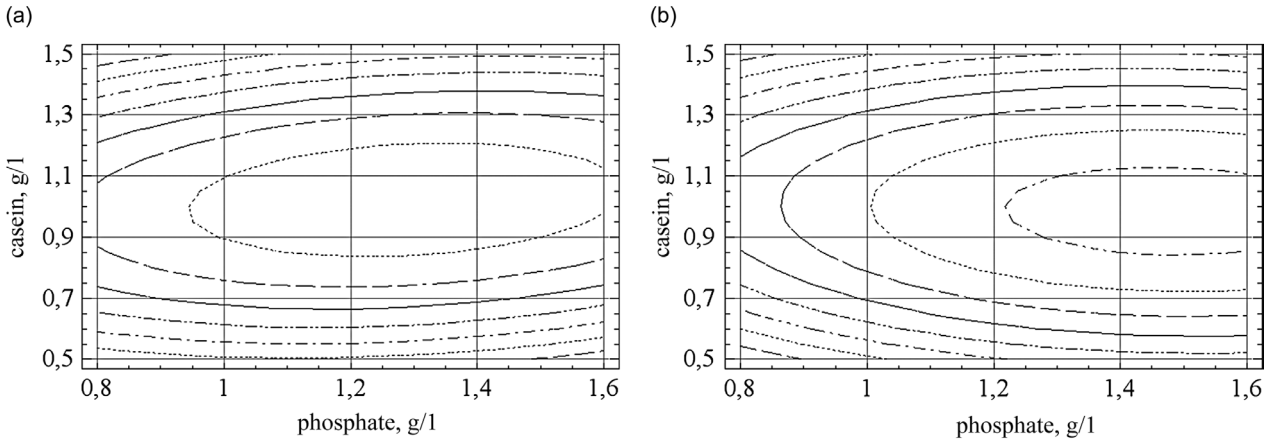

Figure 4: The influence of casein and inorganic phosphate concentration on the activity (a) and productivity (b) of *B. subtilis*.

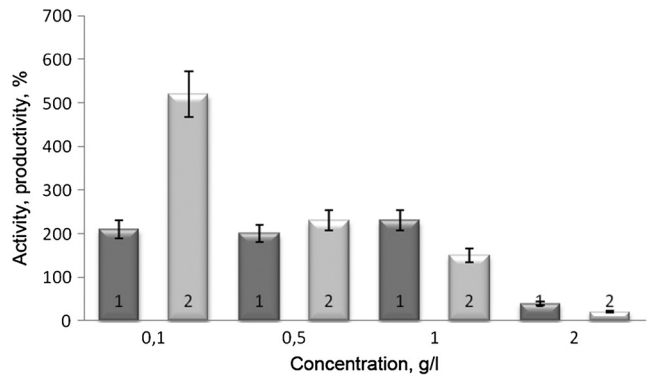

Figure 5: The effect of different concentrations of casamino acids on the activity of metalloproteinase MprBp and productivity of *B. subtilis*. Activity and productivity values were taken for 100% in an environment without introduced casamino acids. 1 – activity of metalloproteinase, 2 – productivity of the culture.

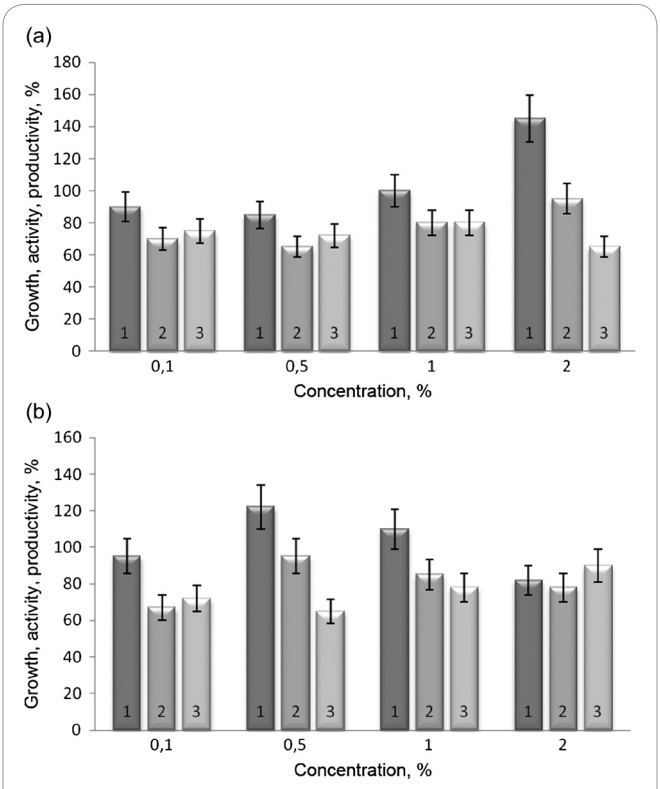

Figure 6: The effect of different concentrations: (a) gelatin on growth (1), activity (2), and productivity (3) (b) of albumin on growth (1), activity (2), and productivity (3) of *B. subtilis*. Activity and productivity values were taken for 100% in an environment without introduced protein supplements.

Effect of Amino Acids on Metalloproteinase Production by Recombinant Strain *B. subtilis*

Amino acids can serve as the source of both carbon and nitrogen and can have various influences on the growth of producer and on the activity of metalloproteinases. It has been noted that synthesis of metalloproteinases of bacilli is stimulated by amino acids such as leucine, isoleucine, tryptophan, glutamic acid, lysine, histidine, arginine added to the nutrient medium in the form of a mixture, or individually [10]. Thus, amino acids are involved in regulation of metalloproteinases synthesis, acting as the stimulant, and as the inducer of the enzyme synthesis.

We studied the influence of some individual amino acids on the growth of the producer and on the activity of metalloproteinase of the recombinant strain *B. subtilis*. Introduction of nonpolar hydrophobic amino acids (alanine, leucine), and aromatic amino acids of tryptophane causes a significant increase in both growth and activity of the enzyme and productivity of the culture. Alanine increases these figures only in the concentration of 0.1%: the activity increases 2 times and the productivity increases 4 times. With increasing concentration of amino acids, the indicators decrease sharply (Figures 7-11).

Leucine in the range of concentrations between 0.1% and 1% stimulates biosynthesis of metalloproteinase 1.4 times, and the productiveness of the culture increases with increasing concentration of amino acids, and reaches 200% with leucine concentration 1%. The effect of triptophane on metalloproteinase biosynthesis has been identified.

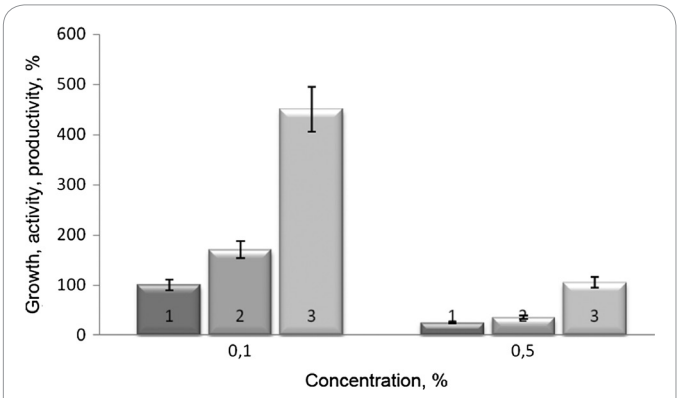

Figure 7: The influence of different concentrations of alanine on growth (1), activity (2), and productivity (3) of *B. subtilis*. Activity and productivity values were taken for 100% in an environment without introduced amino acids.

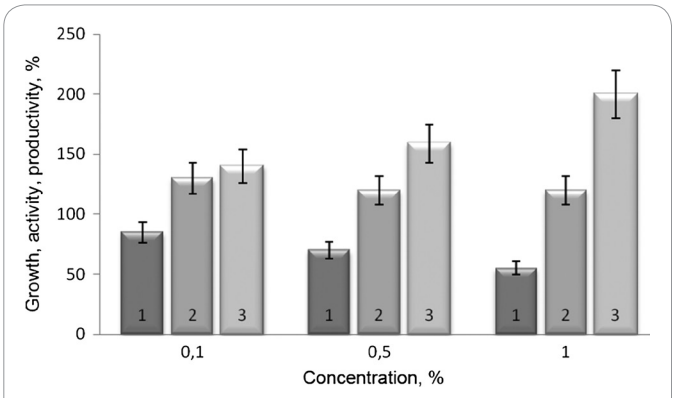

Figure 8: The influence of different concentrations of leucine on growth (1), activity (2), and productivity (3) of *B. subtilis*. Activity and productivity values were taken for 100% in an environment without introduced amino acids.

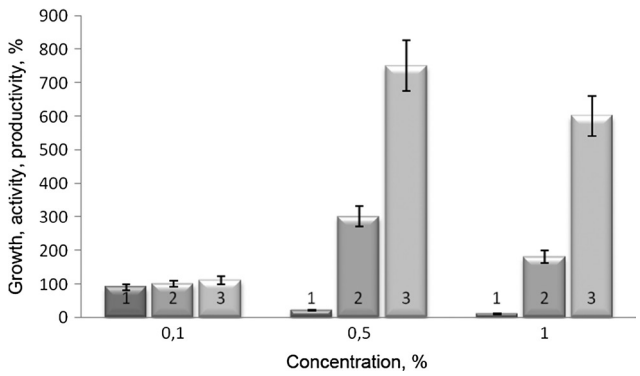

Figure 9: The influence of different concentrations of triptophane on growth (1), activity (2), and productivity (3) of *B. subtilis*. Activity and productivity values were taken for 100% in an environment without introduced amino acids.

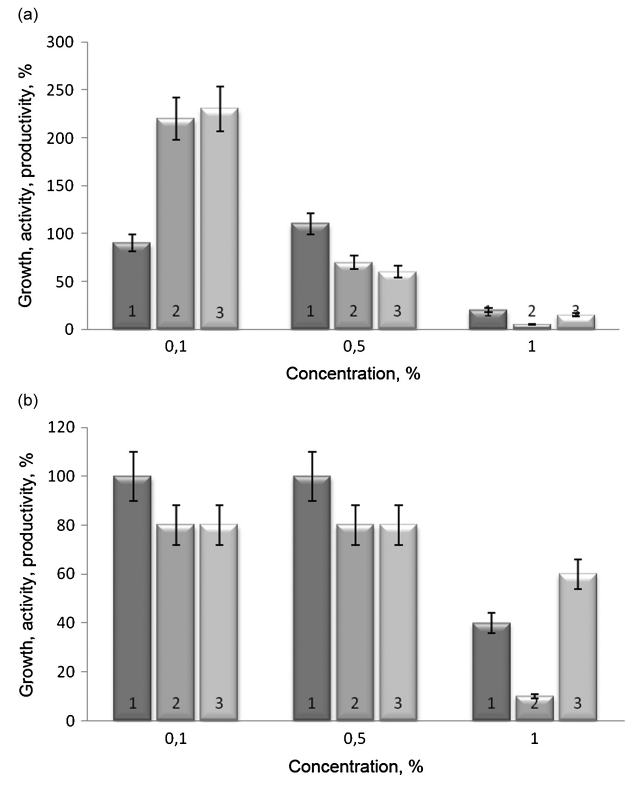

Figure 10: The influence of different concentrations of asparagine (a) and asparagine acid (b) on growth (1), activity (2), and productivity (3) of *B. subtilis*. Activity and productivity values were taken for 100% in an environment without introduced amino acids.

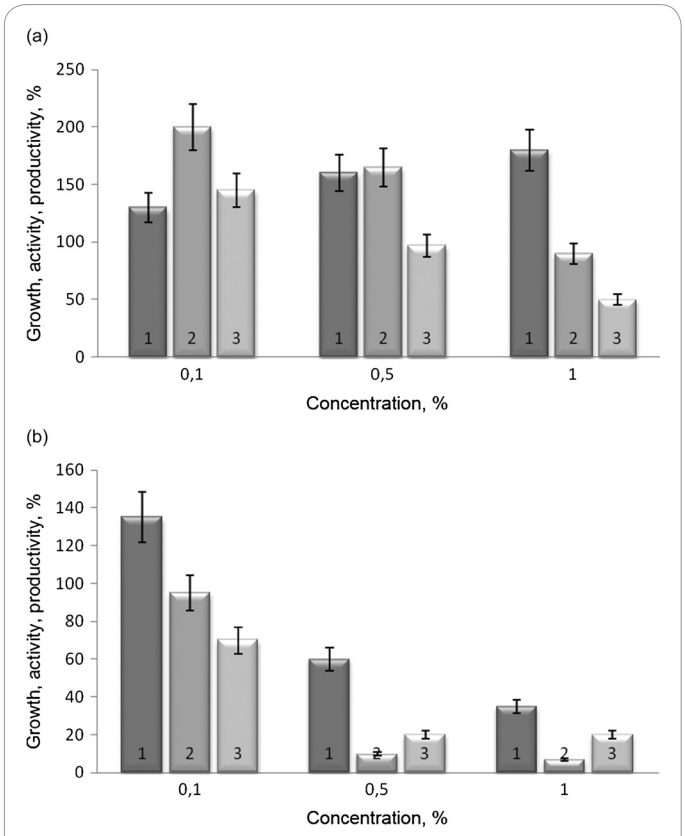

Figure 11: The influence of different concentrations of glutamine (a) and glutamine acid (b) on growth of biomass (1), activity (2), and productivity (3) of metalloproteinase biosynthesis by *B. subtilis*. Activity and productivity values were taken for 100% in an environment without introduced amino acids.

When applied to the tryptophane medium at the concentration of 0.5-1%, the activity of the enzyme increases 1.5 times, and the productivity of the culture increases 1.6-2 times. However, strong inhibition of culture growth should be noted, which has a significant impact on productivity increase. Hydrophilic uncharged amino acids, amides of asparagine acid (asparagine) and glutamine acid (glutamine) in particular at low concentrations stimulate the synthesis of metalloproteinase. In the presence of asparagine at a concentration of 0.1% increases the activity of metalloproteinase and medium productivity 2.5 times, without compromising the growth of the producer biomass. However, at concentrations of 0.5% and 1%, these indicators reduce by 30% and 90%, respectively. Asparagine acid in concentrations of 0.1-0.5% has almost no effect on the activity and production of the enzyme, at a concentration of 1%, activity and productivity are reduced by 80% and 40%, respectively. The presence of glutamine in the concentration of 0.1% increases growth by 35%, activity and productivity – 2 and 1.5 times, respectively. Addition of glutamine to the cultural medium in a concentration of 1% leads to a decline in the enzyme productivity 2 times. Glutamine acid in a concentration of 0.1% increases the growth of the producer by 40%, while the activity and productivity reduces by 10% and 30%, respectively. With increasing concentration of glutamine acid, the indicators decrease sharply (Figure 13).

Thus, the studied individual amino acids in low concentrations (0.1%) stimulate synthesis of metalloproteinases. With increasing concentration of these amino acids, the enzyme activity and the medium productivity significantly reduce.

The Influence of Carbohydrates on the Biosynthesis of Metalloproteinase by the Recombinant Strain *B. subtilis*

Often for growing producers of metalloproteinases, media that contain carbohydrates in a wide range of concentrations are used [11-14].

Carbohydrates stimulate growth and activity of metalloproteinases of some bacilli. However, this effect is not always the case. It was shown that under the influence of carbohydrates, particularly glucose, either inhibition of growth and biosynthesis of metalloproteinase or suppression only of enzyme synthesis occurs. In addition, it was noted that growth inhibition is sometimes stimulated formation of metalloproteinase [15]. We studied the influence of carbohydrates on the biosynthesis of metalloproteinase by the recombinant strain *B. subtilis*.

Monosaccharides (glucose and galactose) and disaccharides (maltose and lactose) in the range of concentrations between 0.1% and 1% have a positively influence upon the growth of the producer. The largest biomass growth is ensured by glucose in a concentration of 0.5% and 1% to 270% and 260%, respectively. Galactose in a concentration of 1% also increases the growth of the culture to 170% (Figure 12). These data are consistent with the literature data.

In case of glucose concentration of 0.1%, the enzyme activity remains at the reference level, while in the presence of other carbohydrates, activity slightly increased. Galactose in concentrations of 0.5% and 1% increases the activity of the enzyme by 40% and 20%, respectively, and

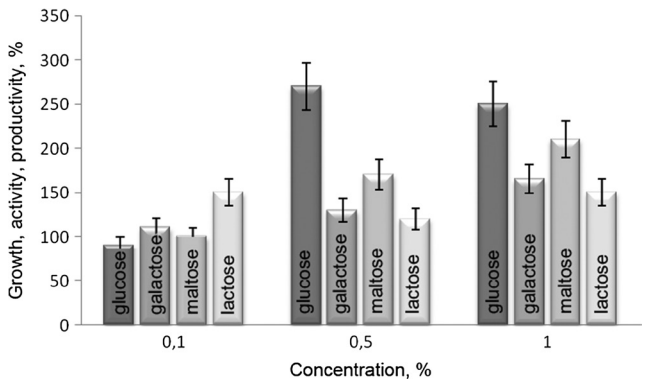

Figure 12: The influence of carbohydrates on the growth of metalloproteinases *B. subtilis* producer. Values of culture growth in the medium without any sugars were taken for 100%.

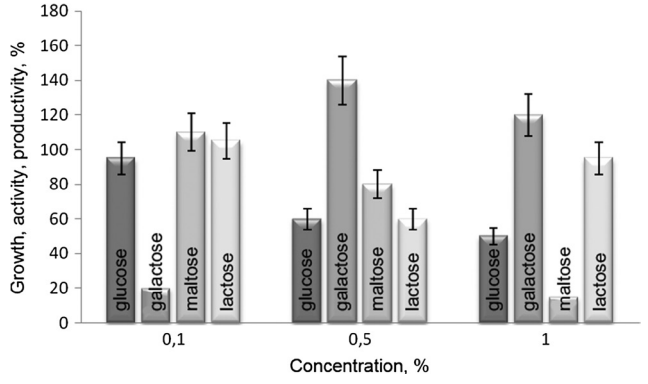

Figure 13: The influence of carbohydrates on the activity of metalloproteinase *B. subtilis*. Values of metalloproteinase activity in the medium without any sugars were taken for 100%.

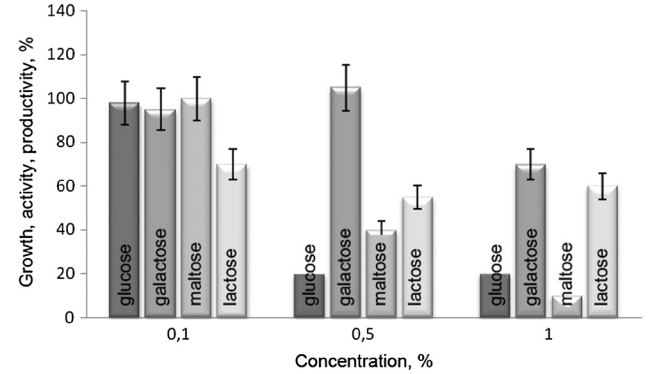

Figure 14: The influence of carbohydrates on the productivity of *B. subtilis*. Values of culture productivity in the medium without any sugars were taken for 100%.

the presence of other carbohydrates in the medium reduces enzyme activity (Figure 13). It should be noted that medium productivity in the presence of the studied carbohydrate in a concentration of 0.1% remains at the reference level, and in the presence of lactose, productivity falls by 40%. The increase in the concentration of carbohydrates in the medium to 1% leads to a drop in the metalloproteinase production by recombinant strain (Figure 14).

Thus, it was found that the studied carbohydrates can both stimulate synthesis of the enzyme (galactose) and call it repression, and with increasing concentration of carbohydrates, this effect increases.

The Influence of Divalent Metals Ions on Metalloproteinase Biosynthesis by Recombinant Strain *B. subtilis*

For cell growth and biosynthesis of enzymes, the presence of small amounts of bivalent metals (Mg^{2+}, Ca^{2+}) ions, which are needed by microorganisms as electrolytes and for different catalytic reactions, is required in the medium [16]. In addition, some bacteria require ions of Fe^{2+}, Zn^{2+}, Mn^{2+}, Co^{2+}, Cu^{2+}, Ni^{2+} in trace quantities, and higher concentrations (mM) have a toxic effect on growth and biosynthesis of the enzyme. In the literature, there is information about positive effect of Ca^{2+}, Mg^{2+}, and Mn^{2+} ions on the production of proteolytic enzymes [17,18]. Thus, there is a reason to believe that these ions are involved in the mechanism of proteases secretion at the stage of release from the membrane into the medium [19]. In this regard, we studied catalytic activity of the enzyme depending on a concentration of bivalent metals ions added to the cultivation medium (Figure 15).

Mg^{2+} ions have positive effect on enzyme activity. At a concentration of 7 mM, they increase enzyme activity by 25%. Ca^{2+} ions at a concentration of 4-18 mm have no significant effect on metalloproteinase activity. Probably, calcium is involved in ensuring the stability of the enzyme molecule. Mn^{2+} at a concentration of 2 mM considerably reduces enzyme activity. With concentration increasing, the inhibiting effect of Mn^{2+} increases. Fe^{2+} ions in a concentration of 0.5-2 mM considerably inhibits activity enzyme (Figure 15).

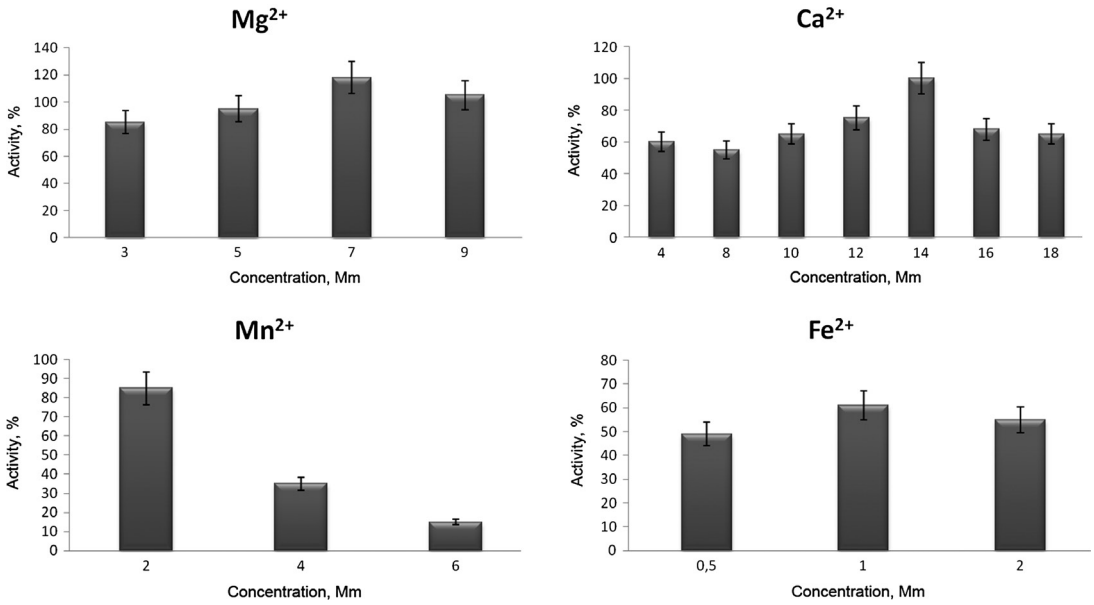

Figure 15: The influence of divalent metals ions on the activity of metalloproteinase *B. subtilis*. Values of metalloproteinase activity in the medium without metal ions were taken for 100%.

Conclusions

So, it was found during the experiment that, for the maximum production of metalloproteinases MprBp, it is practical to introduce into the production medium of recombinant strain of *B. subtilis* peptone at a concentration of 20 g/l and inorganic phosphate in a concentration of 1.45 g/l as main components, and casamino acid (0.1 g/l), leucine (1%), alanine (0.1%), asparagine or glutamine (0.1%), and Ca^{2+} and Mg^{2+} ions at a concentration of 7 mM and 14 mM, respectively, as additional components. Introduction of gelatin, albumin, tryptophan, aspartic and glutamic acids, carbohydrates, ions Fe^{2+} and Mn^{2+} into the cultural medium is impractical.

MprBp is a unique bacillary homologue of eucariotic adamalysines. A detailed study of its structure will help to learn more about evolution of these enzymes that are important for human health. The cultivation medium received in course of this work will make it possible to obtain the metalloproteinase MprBp in quantities that are sufficient for such studies.

Acknowledgments

This work was performed in accordance with the Russian Government Program of Competitive Growth of the Kazan Federal University. This work was supported by the subsidy allocated to the Kazan Federal University for the state assignment in the sphere of scientific activities.

References

1. Gomis-Rüth F-X (2003) Structural aspects of the metzincin clan of metalloendopeptidases. Molecular Biotechnology 24: 157-202.
2. Sterchi EE, Stöcker W, Bond JS (2008) Meprins, membrane-bound and secreted astacin metalloproteinase. Molecular Aspects of Medicine 29: 309-328.
3. Sabirova AR, Rudakova NL, Balaban NP, Ilyinskaya ON, Demidyuk IV, *et al.* (2010) A novel secreted metzincin metalloproteinase from *Bacillus intermedius*. FEBS Letters 584(21): 4419-4425.
4. Rudakova NL, Balaban NP, Danilova YV, Rudenskaya GN, Sharipova MR (2010) Characteristics of a novel secreted zinc-dependent endopeptidase of *Bacillus intermedius.* Biochemistry (Moscow) 75(10): 1294-1301.
5. Shakirov EV, Gabdrakhmanova LA, Balaban NP, Sharipova MR, Rudenskaya GN, *et al.* (2000) The influence of the components of the nutrient medium on the accumulation of glutamyltranspeptidase in the culture fluid of *Bacillus intermedius* 3-19. Microbiology (Moscow) 69(1): 29-33.
6. Malikova LA, Mardanova AM, Sokolova EA, Balaban NP, Rudanskaya GN, *et al.* (2007) Conditions of biosynthesis of extracellular subtilizyn-like proteases of *Bacillus pumilus* KMM 62. Microbiology (Moscow) 76(3): 313-320.
7. Balaban NP, Mardanova AM, Malikova LA, Il'inskaya ON, Sharipova MR (2008) Biosynthesis *Bacillus amyloliquefaciens* H2 subtilizyn-like proteases and biological properties of the enzyme. Uchenye zapiski Kazanskogo Universitetata 150(2): 81-90.
8. Demidyuk IV, Kalashnikov AE, Gromova TY, Gasanov EV, Safina DR, *et al.* (2006) Cloning, sequencing, expression and characterization of protealysin, a novel neutral proteinase from *Serratia proteamaculans* representing a new group of thermolysin-like proteases with short N-terminal region of precursor. Protein Expression and Purification 47: 551-561.
9. Zhu MJ, Cheng JR, Chen HT, Deng MC, Xie WH (2013) Optimization of neutral protease production from *Bacillus subtilis*: using agroindustrial residues as substrates and response surface methodology. Biotechnology and Applied Biochemistry 60(3): 336-342.
10. Ramnani P, Gupta R (2004) Optimization of medium composition for keratinase production on feather by *Bacillus licheniformis* RG1 using statistical methods involving response surface methodology. Biotechnology and Applied Biochemistry 40(2): 191-196.
11. Adinarayana K, Ellaiah P (2002) Response surface optimization of the critical medium components for the production of alkaline protease by a newly isolated *Bacillus* sp. Journal of Pharmacy and Pharmaceutical Sciences 5(3): 272-278.
12. He GQ, Chen QH, Ju XJ, Shi ND (2004) Improved elastase production by *Bacillus* sp. EL31410 – further optimization and kinetics studies of culture medium for batch fermentation. Journal of Zhejiang University Science B 5(2): 149-156.

13. Joo YS, Choi JW (2012) Purification and characterization of a novel alkaline protease from *Bacillus horikoshii*. Journal of Microbiology and Biotechnology 22(1): 58-68.

14. Tiwary E, Gupta R (2010) Medium optimization for a novel 58 kDa dimeric keratinase from *Bacillus licheniformis* ER-15: biochemical characterization and application in feather degradation and dehairing of hides. Bioresource Technology 101(15): 6102-6110.

15. Tsaplina IA (1979) Synthesis of the neutral protease by microorganisms. Biosynthesis of Nucleases and Proteases by Microorganisms, Imshenetski AA (Ed.). Moscow: Mir, pp. 197-244.

16. Jayakumar R, Jayashree S, Anapurna B, Seshadri S (2012) Characterization of thermostable serine alkaline protease from an alkaliphilic strain *Bacillus pumilus* MCAS8 and its applications. Applied Biochemistry and Biotechnology 168(7): 1849-1866.

17. Kirillova YM, Mikhailova EO, Balaban NP, Mardanova AM, Rudenskaya GN, *et al.* (2006) Terms of culture growth and biosynthesis of subtilisin-like *Bacillus intermedius* serine protease by recombinant strain of *Bacillus subtilis*. Microbiology (Moscow) 75(2): 172-178.

18. Chu WH (2007) Optimization of extracellular alkaline protease production from species of *Bacillus*. Journal of Industrial Microbiology and Biotechnology 34(3): 241-245.

19. Sharipova MR, Shakirov EV, Balaban NP, Gabdrakhmanova LA, Shilova MA, *et al.* (2000) Localization of proteolytic enzymes in *Bacillus intermedius* cells. Microbiology (Moscow) 69(5): 660-667.

Citation: Rudakova NL, Balaban NP, Mardanova AM, Chastukhina IB, Sharipova MR (2015) Optimization of Cultivation Media for Heterologous Gene Expression of Adamalysin-like Melalloendopeptidase *Bacillus pumilus*. Biol Med (Aligarh) 7(2): BM-077-15, 8 pages.