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# EXPRESSION, PURIFICATION AND BIOCHEMICAL CHARACTERIZATION OF RECOMBINANT PHOSPHOHYDROLASE APPA IN *ESCHERICHIA COLI*

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**ABSTRACT**

**Development of fodder and increase of feed efficiency is an urgent task for livestock and poultry in Kazakhstan. An important characteristic of feed is the content of bioavailable phosphorus. Traditional source of phosphorus in feed is the mineral phosphate. New and cost-effective way to improve the quality of feed is to mobilize bound phosphate from indigestible plant components.**

**Development of pig and poultry production has increased the cost of traditional sources of organic phosphorus feed (fish and meat and bone meal). At the same time, the limitations of the world's reserves of phosphorus-containing minerals will lead to the progressive increase of the price of animal feed with mineral additives.**

**Relatively new and cost-effective way to address the shortage of bioavailable phosphorus for livestock, poultry and fish farming is the use of feed additives - phytase. Phytase - a group of enzymes phosphohydrolases cleaving the ester bond in the molecule of phytic acid and releasing one or more residues of phosphoric acid.**

**In this work gene *appA* of *Escherichia coli* BL21 (DE3) was cloned into plasmid vector under T7 promoter control and was expressed resulting in high purity recombinant phytase AppA. Optimal cultivation temperature and induction conditions, cell lysis conditions, purification conditions of recombinant enzyme AppA were determined. Phytase activity assay was performed for recombinant enzyme.**

**Work has absolute novelty for the biotechnology industry in Kazakhstan.**

**Keywords: phytase, polymerase chain reaction, *Escherichia coli*, recombinant protein.**

## INTRODUCTION

The study of enzymes began in the XVIII century after discovery of the digestive enzymes. Enzymes have been isolated from biological objects: horseradish peroxidase, α-amylase from grain, etc. After the study the mechanism of action of enzymes it was suggested that enzymes form complexes with substrates. The theory of enzyme-substrate complex was developed and the first mathematical model for the description of enzyme kinetics was offered. The methods of protein engineering, based on knowledge of dependence between amino-acid sequence, three-dimensional structure and catalytic activity of enzymes, allow to successfully modify enzymes for improvement of their technological properties [1, 2]. Changing the mode of action and substrate specificity of enzymes is achieved, for example, by deletion of loops blocking the active center of exohydrolases that convert them into related endohydrolases [3]. At the end of the last century methods of genome's modification of the microorganisms, based on achievements of genetic engineering, were developed. Use of this approach allowed to arrange industrial production of microorganisms-producers of recombinant enzymes.

The first product containing phytases, appeared in the market of feed additives in 1991 and was made by the company Gist Brocades. Later products with similar action appeared from other companies. Mainly they were fungi phytases. Recently there is the increasing number of publications in the scientific press, focused on producing of bacterial recombinant phytases by a secreted expression, both in bacteria and in yeast [4-10].

Биотехнология. Теория и практика. 2014, №3, стр. 61-65

DOI: 10.11134/btp.3.2014.9

The phosphohydrolase gene *phyN* (1455 bp) from *Neosartorya spinosa* BCC 41923 was cloned in *Pichia pastoris* yeast with use of a shuttle vector of pPIC9K. Glycosylated recombinant protein was received as extracellular enzyme with a molecular weight of 52 kDa [11].

Mayer with coauthors used genetically modified strain of *Hansenula polymorpha* containing several copies of a phytase gene from *Aspergillus terreus*, two variants of a gene from *Aspergillus fumigates*. Using glucose as a carbon source, authors could receive culture with high concentration of target protein – more than 13.5 g/L [12].

Level of a secreted expression in bacteria usually is low. In particular, plasmid expression of phytase PhyC from *Bacillus subtillis* VTT E-68013 in *Lactobacillus plantarum* 755 to which was fused in the genetically engineered way by alpha-amylase signal peptide. It was shown that recombinant protein mainly located in intracellular space [13].

The phytase gene *appA* was found in a genome of a bacterium of *Buttiauxella* sp. GC21 isolated from an intestinal tract of grass carp. Recombinant protein AppA was secreted and purified and showed phytase activity [14]. Chong with coauthors could achieve an expression of a gene bacterial phytase AppA from *Escherichia coli* in *Pichia pastoris* yeast [15]. Rodriguez with coauthors extracted from a rectum of a pig *Escherichia coli* strains containing acid phosphatase/phytase Appa2. After sequencing, authors expressed Appa2 in *Pichia pastoris* yeast, using vector pPICZalphaA [16]. The gene *appA* from *Escherichia coli* was cloned and expressed in a secreted form in *Streptomyces lividans* and *Pichia pastoris*. Recombinant phytase expressed in *Streptomyces lividans* was not glycosylated. Recombinant enzyme purified from yeast showed higher temperature stability at high temperatures: from + 65°C to + 75°C [17].

It is known that level of expression of a bacterial gene in eukaryotic organisms can be raised if to carry out a codon optimization. For example, level of an expression of the synthesized and codon optimizied of gene *appA* in yeast grew, and heat stability increased by 40% at a temperature + 80°C within 10 minutes [18]. Eliminating the main defect of bacterial phytase AppA– residual low heat stability, authors, using a method of a site specific mutagenesis, received the modified forms with mutations [19].

Use of the Tat-mediated secretion allows to obtain secreted forms of recombinant proteins in bacterial culture. In particular, authors obtained phytase AppA in *Bacillus subtilis* bacteria using proteins as a part of a Tat-operon [20]. The correct folding of polypeptide and formation of disulfide bonds is very important for stabilization of tertiary structure of some globular proteins. Authors studied this process on the example of secreted protein AppA in a periplasmatic membrane of *Escherichia coli* [21].

Thus, by efforts of many laboratories, researches, directed on purification of phytase enzymes, continue to be conducted.

The purpose of this work was to purify recombinant phytase AppA from *Escherichia coli* BL21 (DE3).

## MATERIALS AND METHODS

### Bacterial strains , DNA, plasmids and reagents

In this study *Escherichia coli*: JM109, DH5α, BL21(DE3), ArcticExpress(DE3)RP, Rosetta(DE3) strains were used. Plasmid pET-28c(+) (Novagen, UK) was used for cloning and expression.

*Escherichia coli* cells with the plasmid were cultured at +37°C in LB medium containing kanamycin at a concentration of 50 μg/ml. Enzyme Phusion High-Fidelity DNA Polymerase (Thermo Scientific, USA) was used for the amplification of the target gene. Restriction enzymes and T4 DNA ligase were purchased from Thermo Scientific (USA).

For protein purification we used HiTrap Chelating HP 1 ml column (GE Healthcare, USA) reactivated with ions $Ni^{2+}$.

Биотехнология. Теория и практика. 2014, №3, стр. 61-65

DOI: 10.11134/btp.3.2014.9

**Oligonucleotides**

In this paper we used oligonucleotides presented in table 1.

**Table 1.** List of oligonucleotides that were used in this work

| Oligonucleotide | Sequence |
|---|---|
| appA-fr | 5'-ATGAAAGCGATCTTAATCCC-3' |
| appA-rv | 5'-CAAACTGCACGCCGG-3' |
| NcoI-appA | 5'-CATGCCATGGGGATGAAAGCGATCTTAATCCC-3' |
| NotI-appA | 5'-TTTTCCTTTTGCGGCCGCCAAACTGCACGCCGG-3' |
| appa-f240 | 5'-CGGACATTACCAACGCCAGCG-3' |
| appa-r501 | 5'-GAGGATCGCGTCAGTCACGTT-3' |
| appa-f821 | 5'-CGCAATTTTATTTGCTACAACGCACG-3' |
| appa-r1101 | 5'-GCTGTTATCGCTTAGCCGACG-3' |
| T7Promoter | 5'-TAATACGACTCACTATAGGG-3' |
| T7Terminator | 5'-GCTAGTTATTGCTCAGCGG-3' |

**Media**

Different versions of Luria Bertani broth were used: normal saline for cultivation of *E. coli* cells (1% tryptone, 0.5% yeast extract, 1% NaCl) and low-salt (1% tryptone, 0,5% yeast extract, 0,5% NaCl) for protein expression. For incubation of the transformed cells, was used SOC medium (2% tryptone, 0,5% yeast extract, 0,05% NaCl, 2,5 mM KCl, 20 mM $MgSO_4$, 20 mM glucose, pH 7,5). Preparation of media was carried out according to the protocol of Maniatis [22].

**Cloning of a *appA* gene into expression vector**

*AppA* gene was PCR amplified from DNA of *Escherichia coli* BL21 (DE3) (100 ng). Other components were 1 µl (10 µM) of each oligonucleotide (5'- NcoI-appA primer, and 3'- NotI-appA primer), 4 µl (10 mM total) dNTPs, 10 µl of 5x PCR buffer (containing 7.5 mM $MgCl_2$) and 0.5 µl of Phusion Hot Start II DNA Polymerase (2 U/µl). PCR was performed in thermocycler Mastercycler nexus gradient (Eppendorf, Germany). The size of amplified product is 1326 bp. The amplified gene was ligated into the expression vector pET-28c(+) digested by NcoI and NotI restriction enzymes. Ligation was performed with T4 DNA ligase at +23°C for 1 hour. Isolation of plasmids from the positive clones was performed using a kit MiniPrep (Promega, USA) according to the manufacturer's protocol. Check of plasmids for the presence of phytase gene was performed using restriction analysis. Genetic constructs were confirmed by DNA sequencing with list of primers: T7Promoter, T7Terminator, appa-f240, appa-r501, appa-f821, appa-r1101 and tested for compliance with the required sequence of genomic data bank (GenBank: AM946981.2, *Escherichia coli* BL21(DE3), complete genome) using Vector NTI software Advance (TM) 11.0 (Invitrogen, USA).

Constructed genetic expression vector pET-28c (+)/appA, which later received the name pAppA, was accumulated in the cells of *E.coli*.

**Optimization of culture conditions of the strain**

Cells were transformed with the three strains of *Escherichia coli*: BL21(DE3), ArcticExpress(DE3)RP and Rosetta(DE3).

Since the concentration of the inductor, depending on the protein can vary in the range from 0.05 mM to 1 mM, experiments were conducted on the selection of the optimal concentration of IPTG. It was determined the optimum optical density of the culture. It was considered three options with $OD_{600}$ = 0.4, 0.6 and 0.8. Also, the culture incubation time was determined for production of recombinant protein.

**Isolation, purification and identification of recombinant phytase activity**

Transformation of electrocompetent cells by a plasmid vector pAppA performed by electroporation using MicroPulser (BioRad, USA) under the following conditions: 100 ng of

Биотехнология. Теория и практика. 2014, №3, стр. 61-65

DOI: 10.11134/btp.3.2014.9

plasmid per 50 µl cell, voltage – 2,5 kV, electric capacity – 25 µF, resistance – 200 Ohms. Transformed cells were incubated in 950 µl of SOC at +37°C for 1 hour with vigorous shaking. Then 50 µl of cells were plated on LB agar with kanamycin and grown at +37°C for 16 hours. Single colonies-transformants were cultured in LB broth with kanamycin in a volume of 400 ml.

The recombinant protein AppA after lysis of induced cells ArcticExpress(DE3)RP/AppA present in water-soluble and water-insoluble fractions of AppA where aggregates in inclusion bodies. A metal-purification was performed from the soluble fraction by column chromatography using 1 ml HiTrap Chelating/$Ni^{2+}$ and preparative chromatograph AKTA Purifier 10.

For chromatographic purification of the protein induced culture was accumulating in volume of 400 ml. Cells were harvested by centrifugation on a preparative Beckman centrifuge with JA10 rotor at the following conditions: 6000g, +4°C, 7 min. The precipitated cells were resuspended in 12 ml buffer 20 mM NaCl, 20 mM Hepes-NaOH (pH 7,5) at the rate of 5 ml buffer per 1 gram of cells. The resulting suspension was frozen at -20°C, thawed and re-frozen to increase the efficiency of lysis. After the second thawing, cells were lysed by ultrasonic sonication in the pulsed mode for 40 minutes. During lysis PMSF was added to a final concentration of 0,2 mM for the inhibition of serine proteases. Further, the lysate was centrifuged in a centrifuge using Beckman JA20 rotor at the following conditions: 40000g, +4°C for 1 hour. Clarified soluble lysate was applied to the column, previously activated with ions of $Ni^{2+}$. Elution of the recombinant protein was carried out on the imidazole gradient from 20 mM to 500 mM with the following buffers: Low Imidazole (500 mM NaCl, 20 mM Imidazole, 20 mM Hepes-NaOH to pH 7,5) and High Imidazole (500 mM NaCl, 500 mM Imidazole, 20 mM Hepes-NaOH to pH 7,5). Collected fractions were analyzed by electrophoresis. Electrophoretic separation of proteins was performed by the method of Laemmli [23] in 12% polyacrylamide gel under denaturing conditions. Quantitative determination of the protein concentration in the fractions was determined by the Bradford assay using bovine serum albumin as a standard [24].

**Purification from inclusion bodies**

Purification of recombinant protein AppA from inclusion bodies was performed by dissolving the protein in urea or guanidinium hydrochloride differing from the concentration of urea (from 1 M to 8 M) and guanidinium hydrochloride (from 1 M to 6 M).

**Phytase activity assay**

The phytase activity was assayed by standard method based on phytase ability to release inorganic phosphate and subsequent reaction of inorganic phosphate with vanadium-molybdenum reagent with formation a yellow complex with maximum absorbance at 410 nm.

A sample of the enzyme was diluted in 100 mM acetate buffer pH=5,5. Reaction was carried out at standard condition pH=5,5, substrate concentration (sodium phytate) 5 mM at +37°C for 15 min. The reaction was stopped by addition of coloring reagent (vanadium-molybdenum reagent containing 10 mM ammonium molybdate, 2,5 mM ammonium metavanadate and 1.1M nitric acid). After 10 min incubation with coloring reagent at room temperature, absorbance of the solution was determined at 700 nm, which was used to calculate the concentration of $PO_4$ from a standard curve made using potassium phosphate monobasic solution in the concentration range 0.25-2.0 mM . One unit of phytase activity was defined as the amount of enzyme that releases 1 µmol phosphate per minute at +37°C.

## RESULTS AND DISCUSSION

**Genetic construction**

Previously constructed genetic construction pET-28c/appA showed its lack of effectiveness in obtaining purified recombinant protein AppA. In order to obtain a more efficient construction *appA* gene was cloned in the same vector pET-28c(+) but by other sites and thus resulting protein AppA was fused with hexahistidine tag on C-terminus end not on N-terminus, as in the

Биотехнология. Теория и практика. 2014, №3, стр. 61-65

DOI: 10.11134/btp.3.2014.9

previous construction. The complete amino acid sequence of the recombinant protein AppA has 445 amino acid residues with a calculated mass of 48.5 kDa.

**Culture conditions**

All three transformed strains demonstrated the ability to accumulate in the cells of the recombinant protein of AppA (figure 1).

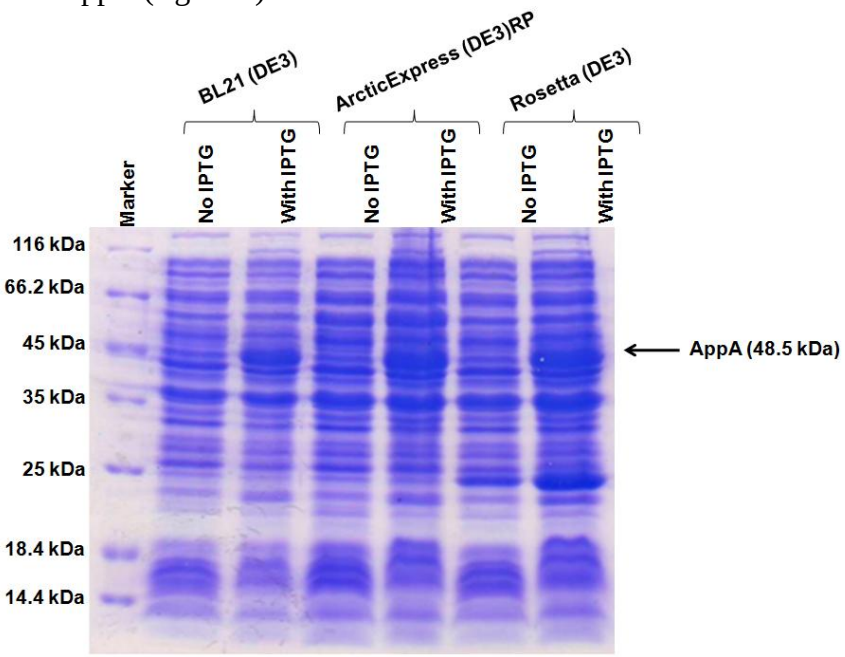



**Fig. 1.** The accumulation of protein AppA in cell lysates of strains

Compared to other strain ArcticExpress(DE3)RP/pAppA has slightly higher producing ability, it is clearly seen in the analysis of total protein lysates.

Experiments on the selection of the optimal concentration of IPTG showed almost the same level of expression for different concentrations of inducer IPTG. It is sufficient to induce protein 0,05 mM IPTG. This concentration was used in further experiments to optimize the culture conditions.

Experiments with culturing temperature showed that the recombinant strains doesn't have strong temperature dependence. Optimal temperature regime is cultivated at +30°C … +37°C, which is very typical for *E. coli*. Since the target protein AppA is a protein of *Escherichia coli* and then it does not cause the accumulation of any toxic effects for bacteria.

The obtained data suggest the following culture conditions producing strain of recombinant phytase AppA-His-tag: the cultivation temperature and induction of +30°C … +37°C under continuous aeration; protein induction should be carried out when the optical density of $OD_{600}$ = 0,8; optimal final concentration of IPTG in the culture was 0,05 mM, time incubation of induced culture is 16 hours. These conditions allow obtaining the maximum amount of recombinant protein per unit volume of bacterial culture.

**Purification of recombinant phytase**

AppA-His protein was isolated from the culture of 400 ml by metal affinity chromatography on a column of 1 ml HiTrap Chelaning activated ions $Ni^{2+}$ by a linear gradient of imidazole from 20 mM to 500 mM (figure 2).

Биотехнология. Теория и практика. 2014, №3, стр. 61-65
DOI: 10.11134/btp.3.2014.9

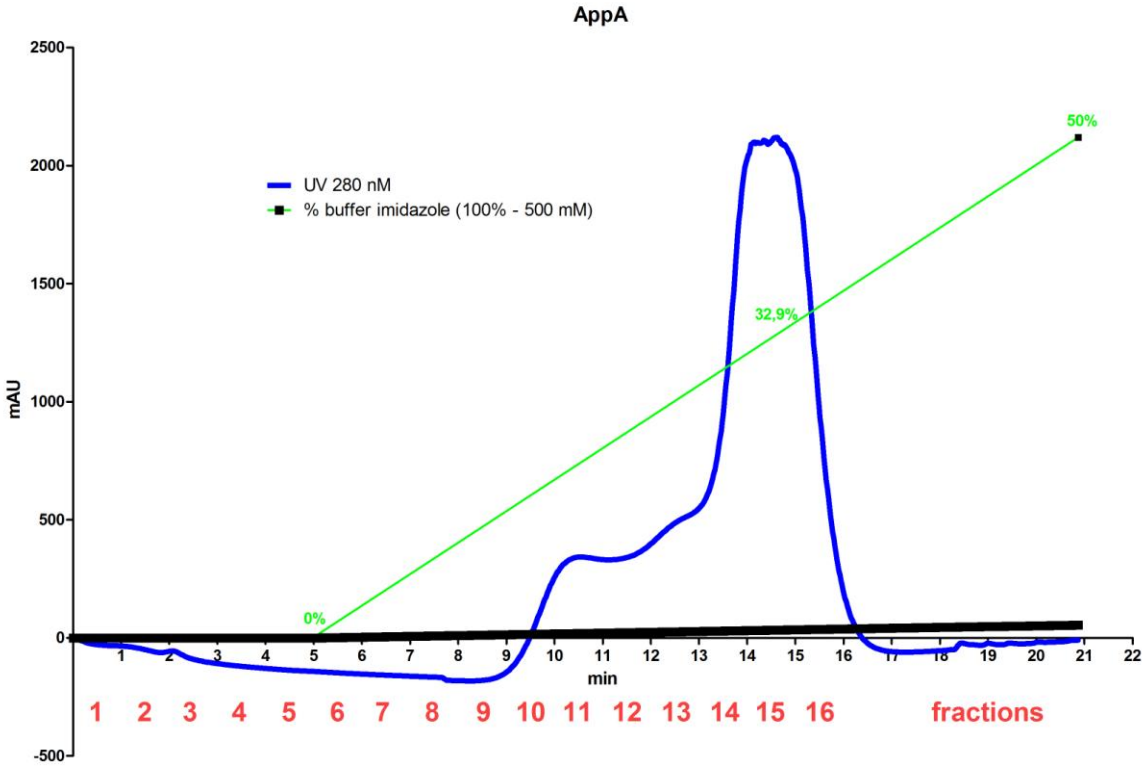



**Fig. 2.** Chromatogram of AppA protein purification from the soluble fraction

From the chromatogram it could be seen that fractions 10-16 contain proteins. For identification of these proteins SDS-PAGE electrophoresis was performed. Figure 3 shows the result of electrophoresis of the protein of clarified lysate before application on the column, after the column, and fractionated eluates 12-16 (each fraction 500 µl).

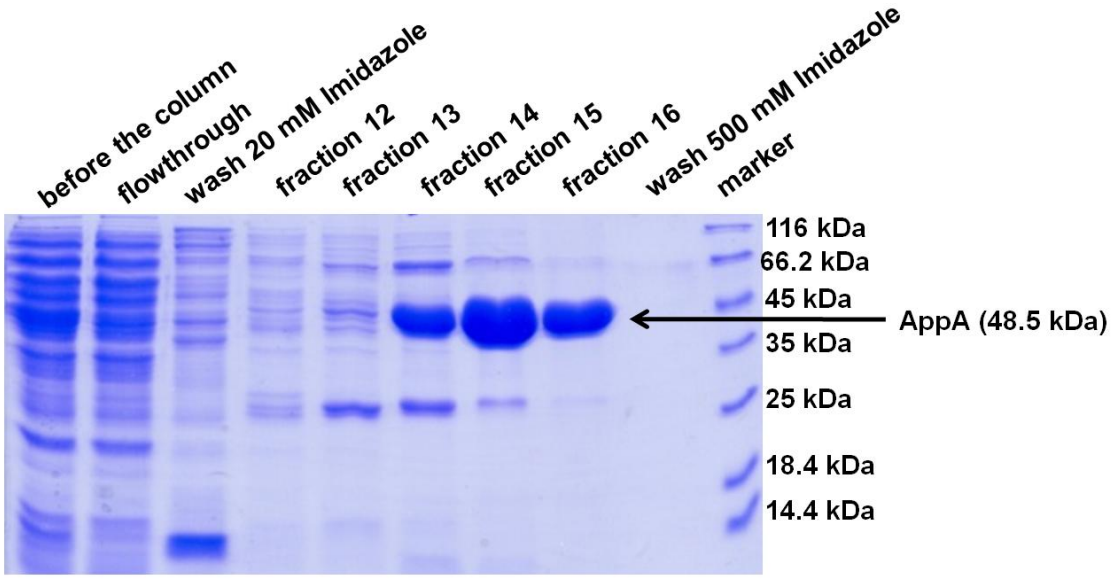



**Fig. 3.** SDS-PAGE results of purification from the water-soluble fraction

Analysis of the chromatogram and SDS-PAGE showed that the target protein AppA is adsorbed on a column and elution started when imidazole concentrations was 185 mM.

Measurement of the biochemical activity of the purified fractions indicated that fractions 14-16 are highly active (table 2).

Биотехнология. Теория и практика. 2014, №3, стр. 61-65
DOI: 10.11134/btp.3.2014.9

**Table 2.** Biochemical activity of the fractions

| Sample | Protein concentration, mg/ml | Activity, U (μmol $PO_4^{3-}$* $min^{-1}$) | Specific activity on 1 mg of protein, U/mg |
|---|---|---|---|
| fraction 14 | 1.24 | 2608 | 2103 |
| fraction 15 | 1.85 | 6052 | 3271 |
| fraction 16 | 1.17 | 3818 | 3263 |

**Table 3.** Biochemical activity of the fractions (AppA) in comparison with negative control

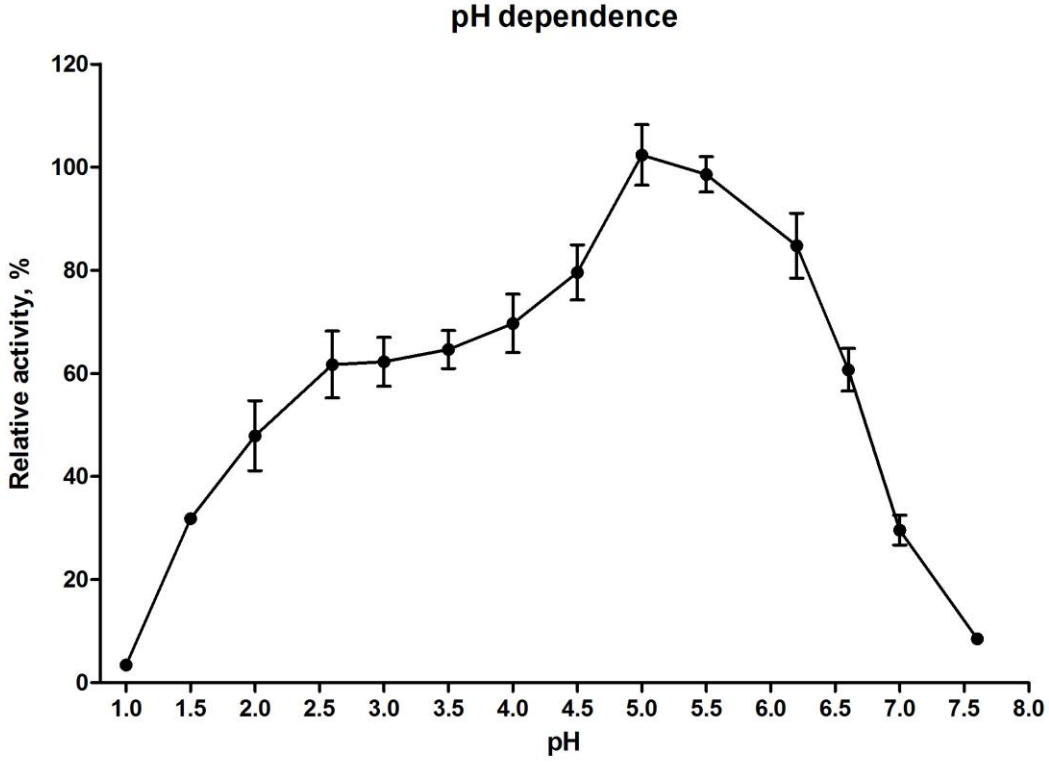
| Sample | U (μmol $PO_4^{3-}$* $min^{-1}$) | % |
|---|---|---|
| AppA/400 ml (fractions 14-16) | 6219,19 | 99,7 |
| Negative control without recombinant phytase/400 ml | 19,2 | 0,3 |

Also, experiments were performed on purification of recombinant phytase from inclusion bodies. The recombinant AppA protein in inclusion bodies has an extremely low solubility in the presence of sufficiently strong agents such as 8M urea, 6M guanidine hydrochloride. Slightly successful was in addition to the buffer containing 8M urea and 1% Triton X-100 and 10 mM still β-mercaptoethanol, but its use can disrupt globular structure of AppA protein and this could affect for enzymatic activity.

Obtained findings suggest that the most promising strategy is to separate the protein from the soluble fraction of AppA. This is justified by the high yield of recombinant protein and high specific activity. Using this data, and have accumulated 10 mg of purified recombinant phytase AppA from the bacterial culture ArcticExpress(DE3)RP/pAppA.

**pH and temperature dependence of recombinant phytase AppA**

It was analyzed the optimal hydrolytic conditions for the recombinant phytase by performing activity assays in various pH buffers and reaction temperatures (figure 4).



Биотехнология. Теория и практика. 2014, №3, стр. 61-65

DOI: 10.11134/btp.3.2014.9

**Fig. 4.** Diagram of determination of optimal pH

As can be seen from the picture 4, the highest activity of enzyme was at pH 5,0-5,5.

Also it was performed experiment to determine the temperature optimum of recombinant AppA. The highest activity of enzyme was +65°C … +70°C, and then activity decreased rapidly when incubated at a higher temperature (figure 5).

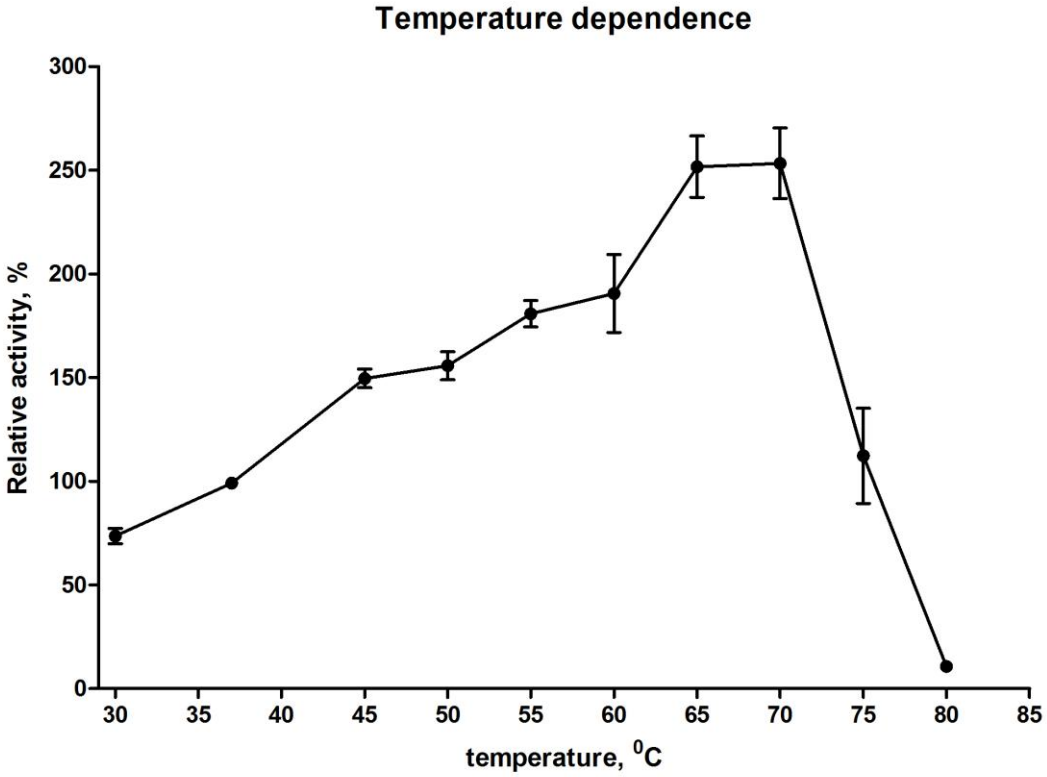



**Fig. 5.** Determination of optimum temperature

## CONCLUSION

In this study, we have obtained genetic construction pAppA comprising a gene *appA* cloned under T7 promoter control, and comprising an active hexameric histidine tag on the C-terminus. Transformation of *Escherichia coli* cells by vector pAppA resulted in recombinant strain ArcticExpress(DE3)RP/pAppA efficiently producing phytase. It was optimized the culture conditions of recombinant-producing strain, optimized parameters of induction of the recombinant protein of AppA. It was found that the optimal conditions are a temperature of cultivation and induction of +30°C … +37°C under continuous aeration; protein induction should be carried out when the optical density is $OD_{600}$ = 0,8; optimal final concentration of IPTG in the culture was 0,05 mM, incubation time was 16 hours induced culture. It was optimized conditions for purification of recombinant enzyme AppA from soluble fraction of the lysate and inclusion bodies. It has been established that it is effective isolation of the water soluble fraction of bacterial lysates using the metal chelate chromatography with $Ni^{2+}$ ions. Purification of recombinant proteins from inclusion bodies by urea and guanidine hydrochloride is ineffective. Using of these agents leads to a denaturation of the protein AppA which strongly affected its biochemical activity. Attempts to renaturate and return to the normal protein folding by dialysis were unsuccessful. Thus, we can conclude that the most promising is a protein purification from water-soluble fraction of AppA, where he is in a biochemically active. It was determined enzymatic activity of recombinant protein to the phytic acid derivative with the

release of inorganic phosphate $PO_4$. The activity of the purified fractions 2608 – 6052 ml * $PO_4$ $mol^{-1}$ * $min^{-1}$ which is very high.

The obtained data are well agreed with the similar data described in literature [6, 9]. Found temperature optimum in +65°C … +70°C, also doesn't contradict references [4] and is promising from the technological point of view. Moreover, it is already established that a number of insignificant modifications in structure of AppA will allow to increase thermostability to 80°C [25, 26] that considerably will improve properties of AppA as enzyme preparation. We consider that continuation of researches in this direction is perspective and promising. This suggests that recombinant AppA is suitable for animal feed additives.

Producing strain of recombinant hydrolase ArcticExpress(DE3) RP/AppA was deposited in the collection of microorganisms “Kazakh Research Institute of Food and Processing Industry”, No. B-494.

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***REFERENCES***

*1. Tucker G., Woods L. Enzymes in Food Processing. 2nd ed. Glasgow, Academic and Professional, 1995, 319 p.*

*2. Godfrey T., West S. Industrial Enzymology – 2nd ed. Basingstoke: Macmillan Press and New York: Stockton Press, 1996, 609 p.*

*3. Rabinovich M., Melnik M., Bolobova A. Cellulose of microorganisms. Applied biochemistry and molecular biology, 2002, vol. 38, no. 4, pp. 355-373.*

*4. Haefner S., Knietsch A., Scholten E., Braun J., Lohscheidt M., Zelder O. Biotechnological production and applications of phytases. Appl Microbiol Biotechnol., 2005, vol. 68, no. 5, pp. 588-597. doi: 16041577.*

*5. Miksch G., Kleist S., Friehs K., Flaschel E. Overexpression of the phytase from Escherichia coli and its extracellular production in bioreactors. Appl Microbiol Biotechnol., 2002, vol. 59, pp. 685–694. doi: 12226725.*

*6. Vohra A., Satyanarayana T. Phytases: microbial sources, production, purification, and potential biotechnological applications. Crit Rev Biotechnol., 2003, vol. 23, no. 1, pp. 29-60. doi:12693443.*

*7. Polaina J., MacCabe A.P. Industrial Enzymes: Structure, Function and Applications. Springer, 2007, 642 p.*

*8. Yao M.Z., Zhang Y.H., Lu W.L., Hu M.Q., Wang W., Liang A.H. Phytases: crystal structures, protein engineering and potential biotechnological applications. J Appl Microbiol., 2012, vol. 112, no. 1, pp. 1-14. doi: 22017627.*

*9. Xin Gen Lei, Jesus M. Porres. Phytase enzymology, applications, and biotechnology. Biotechnology Letters, 2003, vol. 25, pp. 1787-1794. doi: 14677699.*

*10. Phillippy B.Q., Mullaney E.J. Expression of an Aspergillus niger Phytase (phyA) in Escherichia coli. J Agric Food Chem., 1997, vol. 45, pp. 3337-3342. Available at:http://dx.doi.org/10.1021/jf970276z.*

*11. Pandee P., Summpunn P., Wiyakrutta S., Isarangkul D., Meevootisom V.A. Thermostable phytase from Neosartorya spinosa BCC 41923 and its expression in Pichia pastoris. J Microbiol., 2011, vol. 49, no. 2, pp. 257-64. doi: 21538247.*

Биотехнология. Теория и практика. 2014, №3, стр. 61-65

DOI: 10.11134/btp.3.2014.9

*12. Mayer A.F., Hellmuth K., Schlieker H., Lopez-Ulibarri R., Oertel S, Dahlems U., Strasser A. van Loop AP. An expression system matures: a highly efficient and cost-effective process for phytase production by recombinant strains of Hansenula polymorpha. Biotechnol Bioeng., 1999, vol. 63, pp. 373-381. doi: 10099617.*

*13. Kerovuo J., Tynkkynen S. Expression of Bacillus subtilis phytase in Lactobacillus plantarum 755. Lett Appl Microbiol., 2000, vol. 30, no. 4, pp. 325-329. doi: 10792656.*

*14. Pengjun S., Huoqing H., Wang Y., Huiying Luo, Bo Wu, Kun Meng, Peilong Yang, Bin Yao A novel phytase gene appA from Buttiauxella sp. GC21 isolated from grass carp intestine. Aquaculture, 2008, vol. 275, no. 1-4, pp. 70-75. Available at:http://dx.doi.org/10.1016/j.aquaculture.2008.01.021.*

*15. Chen C.C., Wu P.H., Huang C.T., Cheng K.J. A Pichia pastoris fermentation strategy for enhancing the heterologous expression of an Escherichia coli phytase. Enzyme Microb Technol., 2004, vol. 35, pp. 315-320. Available at:http://dx.doi.org/10.1016/j.enzmictec.2004.05.007.*

*16. Rodriguez E., Han Y., Lei X.G. Cloning, sequencing, and expression of an Escherichia coli acid phosphatase/phytase gene (appA2) isolated from pig colon. Biochem Biophys Res Commun., 1999, vol. 257, no. 1, pp. 117-123. doi: 10092520.*

*17. Stahl C.H., Wilson D.B., Lei X.G. Comparison of extracellular Escherichia coli AppA phytases expressed in Streptomyces lividans and Pichia pastoris. Biotechnol Lett., 2013, vol. 25, no. 10, pp. 827-831. doi: 12882015.*

*18. Yao M.Z., Wang X., Wang W., Fu Y.J., Liang AH. Improving the thermostability of Escherichia coli phytase, appA, by enhancement of glycosylation. Biotechnol Lett., 2013, vol. 35, no. 10, pp. 1669-1676. doi: 23794051.*

*19. Fei B., Xu H., Zhang F., Li X., Ma S., Cao Y., Xie J., Qiao D., Cao Y. Relationship between Escherichia coli AppA phytase's thermostability and salt bridges. J Biosci Bioeng., 2013, vol. 115, no. 6, pp. 623-627. doi: 23333035.*

*20. Gerlach R., Pop O., Müller J.P. Tat dependent export of E. coli phytase AppA by using the PhoD-specific transport system of Bacillus subtilis. J Basic Microbiol., 2004, vol. 44, no. 5, pp. 351-359. doi: 15378526.*

*21. Berkmen M., Boyd D., Beckwith J. The nonconsecutive disulfide bond of Escherichia coli phytase (AppA) renders it dependent on the protein-disulfide isomerase, DsbC. J Biol Chem., 2005, vol. 280, no. 12, pp. 11387-11394. doi: 15642731.*

*22. Maniatis T., Fritsch E.E., Sambrook J. Molecular cloning. A laboratory manual. New York, Cold Spring Harbor Laboratory, 1982, 545 p.*

*23. Laemmli U.K. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature, 1970, vol. 227, pp. 680-685. doi: 5432063.*

*24. Bradford M.M. Rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal. Biochem., 1976, vol. 72, pp. 248-254. doi: 942051.*

*25. Fei B., Xu H., Cao Y., Ma S., Guo H., Song T., Qiao D., Cao Y. A multi-factors rational design strategy for enhancing the thermostability of Escherichia coli AppA phytase. Journal of Industrial Microbiology & Biotechnology, 2013, vol. 40, Issue 5, pp. 457-464. doi: 23494709.*

26. *Fei B., Cao Y., Xu H., Li X., Song T., Fei Z., Qiao D., Cao Y. AppA C-terminal plays an important role in its thermostability in Escherichia coli. Curr Microbiol., 2013, vol. 66, issue 4, pp. 374-378. Available at: http://dx.doi.org/10.1007/s00284-012-0283-4.*

## ТҮЙІН

Мал азығын дамыту мен азықпен қамтамасыз ету тиімділігін арттыру –Қазақстандағы мал шаруашылығы және құс шаруашылығы үшін өзекті мәселе. Азықпен қамтамасыз етуде маңызды шарт – ол биоөнімді фосфордың болуы. Азықпен қамтамасыз етудің

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сапасын көтеру үшін және де оны рентабелді етудің негізгі тәсілі өсімдіктің қорытылмайтын құрамдастарынан байланысқан фосфорды пайдалану болып табылады.

Шошқа шаруашылығы мен құс шаруашылығының дамуы органикалық фосфордың дәстүрлі көздері (балық және сүйек-ет ұны) құнының өсуіне әкелді. Бұған қоса, фосфор қосылған минералдардың әлемдік қорының шектеулілігінен азыққа минералды қоспаларды қосу бағасы прогрессивті түрде өсуде.

Салыстырмалы түрде, мал шаруашылығы, құс шаруашылығы және балық шаруашылығы үшін биоөнімді фосфордың тапшылығынан құтылу үшін бірден бір жол азық қоспасын, фитаза қолдану. Фитазалар- фитин қышқылы молекуласындағы күрделі эфирлі байланысты ыдыратып, бір не одан да көп фосфор қышқылы қалдығын босатып шығаратын, фосфогидролаза ферменттер тобы.

Осы жұмыста appA гені T7 промоторы бақылауындағы векторға клондалып, жоғары тазалықтағы рекомбинантты AppA гидролазасы экспрессияланды. Рекомбинантты AppA ферментінің оңтайлы дымқылдандыру температурасы мен индукция, жасушаны лизистеу және тазалау шарттары анықталды. Фитазаның белсендігін анықтау жұмыстары рекомбинантты гидролаза үшін орындалған.

Орындалған жұмыс Қазақстанның өндірістік биотехнологиясы үшін мүлде жаңа болып табылады.

**Кілтті сөздер:** фитаза, полимеразды тізбекті реакция, *Escherichia coli*, рекомбинантты нәруыз.