Original Article

Recombinant expression and purification of a pathogen-specific murein hydrolase lysin from γ-bacteriophage of *Bacillus anthracis*

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ABSTRACT

High specificity of bacteriophages for infection of their bacterial host species incurs diagnostic and therapeutic values. Usage of bacteriophages to combat bacterial infections has a long history whereas a novel promising area of research is the utilization of the whole bacteriophages or their parts as immunoaffinity reagents to diagnose bacterial infections, e.g. anthrax. Gamma-bacteriophage of *Bacillus anthracis* has an amazing capacity to attack this pathogen which is either in a vegetative state or in the form of spores, and γ-bacteriophage effectively reduces bacterial load. One of the crucial components of γ-bacteriophage, which determines its specificity, is a peptide LKMTADFILQ which resides at the C-terminus of the phage-encoded murein hydrolase (lysin). A recombinant C-terminal fragment of the γ-bacteriophage-encoded N-acetylmuramic-L-alanine amidase (lysin) was produced. A DNA fragment containing lysin gene (PlyG) was synthesized *de novo*. PlyG gene was cloned into expression plasmids pET32 and pET28 and the resulting constructs were transformed into *Escherichia coli* strain BL21(DE3). Recombinant expression products were purified using immobilized metal affinity chromatography (IMAC) and confirmed by denaturing gel electrophoresis and peptide mass-spectrometry. As a result, strains producing the C-terminal fragment of γ-bacteriophage PlyG lysin were obtained. Molecular masses of the expression products were 30 kDa for the pET32-based construct and 16 kDa for the pET28-based construct. Comparison of tryptic peptides' mass-spectra with data from the Swiss Prot database using Mascot software confirmed that the obtained recombinant proteins contain amino acid sequences of the lysin.

KEYWORDS: phage lysin, anthrax, PlyG, murein hydrolase, affinity chromatography, recombinant expression.

INTRODUCTION

Anthrax is a deadly dangerous infectious disease affecting both animals and humans. The causative agent of anthrax is *Bacillus anthracis*, which is a gram-positive bacterium capable of forming highly resistant endospores under adverse environmental conditions [1, 2]. These spores can survive intense treatments (antiseptics, disinfectants, elevated temperatures, UV irradiation) which naturally kill other bacterial species [3, 4].

When evaluating methods for detecting bacterial pathogens in food, clinical or environmental samples, features such as sensitivity, specificity, and rapidity of obtaining results are considered decisive. Methods of microbiology exhibit high specificity, although they are labor-intensive and time-consuming because of a need for long enrichment stages until the development of visible colonies on agar plates. With this regard, it is possible to develop rapid and specific diagnostic tests based on molecular affinity interactions, utilizing bacteriophage-derived components [5].

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The first bacteriophages of *B. anthracis* were described in 1931. In 1951 McCloy characterized an atypical *B. anthracis* strain W the cultures of which contained a bacteriophage capable of lytic infection in 171 strains of *B. anthracis*, and in 2 (out of 54 tested) strains of *B. cereus*. Eighteen other species or variants of bacilli tested by the author were not lysed by the bacteriophage. In subsequent works, a different bacteriophage has been described, consisting of variants with different phenotypes namely β-form and α-mutant [6].

Schuch *et al.* (2002) and Kikkawa *et al.* (2008) established that the protein which participates in the recognition of its host *B. anthracis* by γ-bacteriophage is N-acetylmuramic-L-alanine amidase. This protein (originally named endolysin, PlyG) has an N-terminal domain with catalytic activity and a C-terminal domain with substrate-binding activity [7, 8]. It was shown that PlyG lysine isolated from the *B. anthracis* gamma phage specifically kills *B. anthracis* isolates *in vitro* and *in vivo*. At the same time, both vegetative cells and germinating spores were susceptible. The authors concluded that PlyG is a tool for the treatment and detection of *B. anthracis* [7].

Catalytic domains are highly conserved among various amidases, and the catalytic PlyG site resembles the lysozyme T7 site. Cheng *et al.* (1994) reported that three amino acid residues of lysozyme T7 (His17, Tyr46, and Lys128) are critical for its catalytic activity. Elements of secondary structure in the protein have the following topology: α1-H1-α2-H2-α3-α4-H3-α5-H4-H5-α6-H6-H7-H8-α7-H9-α8-α9-H10-α10, where H, β, and α correspond to helices, β-strands and sheets, respectively [9]. However, little is known about the catalytic mechanism of action of γ-bacteriophage lysin. According to their common protein structure features, phage lysins include three large domains: endo-b-N-acetylglucosaminidase, which acts on sugar fragments, N-acetylmuramidase (lysozyme-like), which cleaves peptide cross-links, and N-acetylmuramic-L-alanine amidase, which hydrolyzes amide bonds [10].

A C-terminal fragment of bacteriophage lysin is of significant interest because it provides for a specific interaction between the lysin and its substrate. Sainathrao *et al.* (2009) conducted studies on diagnostic characterization of short synthetic peptides containing only the fragment-derived amino acid sequence LKMTADFILQ. Such short synthetic peptides may be useful in developing new methods for the detection of *B. anthracis* and other variants of this group, such as the vaccine strain *B. anthracis* (Sterne, 34F2) and strain *B. cereus*-4342 susceptible to γ-bacteriophage. One advantage of short synthetic peptides is that they can be synthesized in large quantities. In the cited paper six synthetic peptides derived from PlyG were tested for the ability to bind to the bacterial cell wall. Three synthetic peptides were identified which bind to the cell walls of *B. cereus-4342* and *B. anthracis* (Sterne 34F2) cells [11].

According to one research, the lysin C-terminal domain binds with high affinity to secondary polysaccharides of the cell wall, which are known to be specific for sporulated and for vegetative forms of *B. anthracis* as well as for particular strains of *B. cereus* [12].

MATERIALS AND METHODS

Bacterial strain, plasmids, and antibodies

E. coli strains DH5α and BL21(DE3) (Novagen), attenuated STI vaccine strain *B. anthracis* (Russia), *B. cereus* (ATCC, USA), and plasmids pGEM-T Easy (Promega, USA), pET28, and pET32 (Novagen, USA) were used. *E. coli* were maintained in lysogeny broth (LB) medium. Peptidoglycan of *Bacillus subtilis* was purchased from Sigma-Aldrich. Mouse monoclonal antibody against 6xHis-tag conjugated to peroxidase (Sigma-Aldrich) was used for western blotting and enzyme-linked immunosorbent assay (ELISA).

Gene synthesis

Oligonucleotides were synthesized in the laboratory for organic synthesis in the National Center for Biotechnology (Astana, Kazakhstan). The oligonucleotides used as polymerase chain reaction (PCR) primers are listed in Table 1. DNA fragments encoding the PlyG C-end fragment were assembled *de novo* from synthetic oligonucleotides. Gene synthesis was performed using a two-round PCR in the presence of Phusion High-Fidelity DNA Polymerase (Thermo Fisher Scientific). For sequencing confirmation of the synthetic gene BigDye Terminator v3.1 Cycle Sequencing Kit (Thermo Fisher Scientific) was used. The PlyG gene

Table 1. Oligonucleotides for synthesis of a C-terminal fragment of PlyG gene.

#	Name	Nucleotide sequence	Length (bp)
1	PlyG C-end 1	GGATCCGAATTCCCATGGGAATGCTGGCGGAAGGCC	36
2	PlyG C-end 2	CGCCACGTTGCCGTTTTTCACTTTCTGAATAAACGCG CCCCAGCGGCCTTCCGCCAGCAT	60
3	PlyG C-end 3	AAAACGGCAACGTGGCGACCACCAGCCCGACCAAA CAGAACATTATCCAGAGCGGTGCGT	60
4	PlyG C-end 4	CTGGTCAGCGCGCCCATCACATCCGGGGTTTCATAC GGGCTAAACGCACCGCTCTGGATA	60
5	PlyG C-end 5	GGGCGCGCTGACCAGCCTGAAAATGACCGCGGATTT TATTCTGCAGAGCGATGGCCTGAC	60
6	PlyG C-end 6	GCTTTCAGCTGCGCATCGCTGGTCGGTTTGCTGATAA AATAGGTCAGGCCATCGCTCTGC	60
7	PlyG C-end 7	CGATGCGCAGCTGAAAGCGATGAAAGAATATCTGGA TCGCAAAGGCTGGTGGTATCATCA	60
8	PlyG C-end 8	AAGAAGCTTCTCGAGTTAATGATGATGATGATGATG ATACCACCAGCCTTTG	52
9	PlyG C-end start	GGATCCGAATTCCCATGGGAATG	23
10	PlyG C-end stop	AAGAAGCTTCTCGAGTTAATG	21

C-end fragment was cloned into expression plasmids pET28 and pET32 (Novagen).

Expression of recombinant PlyG

Competent cells of *E. coli* strain DH5α were transformed with pET28/PlyG or pET32/PlyG following the method of D. Hanahan [13]. Transformation of electrocompetent cells of the expression strain BL21(DE3) with the above plasmid constructs was performed using a MicroPulser (BioRad) under the following conditions: 100 ng of plasmid per 50 μL of cell suspension, 2.5 kV, 25 μF, 200 Ω. Electroporation duration was 5.2 ms. The transformed cells were incubated in 950 μL 2YT medium at 37 °C for 40 minutes with shaking at 150 rpm. Then 50 μL of the cells were seeded on LB agar plates with an appropriate antibiotic as a selection factor and grown at 37 °C for 16 hours. Single colonies of the transformants were cultivated in LB broth containing antibiotic at 37 °C, 150 rpm. In the middle of the logarithmic growth phase (absorbance at λ = 600 nm, OD600 = 0.6) 5 mL of the bacterial culture was taken as negative control. To the rest of the bacterial culture 0,2 mM of the inducer, isopropyl-β-D-1-galactopyranoside (IPTG) was added and the culture was incubated at 25 °C. After 2, 4, 6 and 18 hours upon addition of IPTG,

aliquots (5 mL) of the cell culture were collected to determine induction of protein expression. The cells were precipitated by centrifugation at 6,000 rpm, 4 °C, 10 min.

Chromatographic purification of recombinant proteins

Cells were resuspended in TNE buffer (20 mM Tris pH 7.5; 1 mM EDTA; 100 mM NaCl) and lysed using UP200S ultrasonic disintegrator at 24 kHz in a pulsating mode (20 pulses for 1.5 min at 2-minute intervals). Recombinant proteins were purified using immobilized metal affinity chromatography on Ni^{2+} ions with the use of 1-mL HisTrapTM HP column (GE Healthcare). Column equilibration and loading of cells' lysate were performed in accordance with the manufacturer's protocol. A stepwise imidazole gradient with buffer A (500 mM NaCl, 20 mM HEPES pH 7.5, 20 mM imidazole) and buffer B (500 mM NaCl, 20 mM HEPES pH 7.5, 500 mM imidazole) was used to define the optimal concentration of imidazole in an elution buffer. For fast protein liquid chromatography (FPLC) AKTA Purifier 10 (GE Healthcare) was used. Elution of proteins was monitored at λ = 280 nm. Protein concentrations were determined using the Bradford assay [14]. Electrophoretic separation of

proteins was performed by the Leammli method in a denaturing polyacrylamide gel (11% SDS-PAGE) [15].

Western blotting

Gel-fractionation of rPlyG was performed by electrophoresis using 11% SDS-PAGE according to the Laemmli method using a Bio-Rad electrophoresis apparatus. Antigens were blotted onto nitrocellulose membranes using an immunoblotting device (Transblot, Bio-Rad).

For immunochemical detection of specific antigens, nitrocellulose membranes were first incubated in 1% bovine serum albumin (BSA) solution overnight at 4 °C and then washed thrice in buffer 1 containing 137 mM NaCl, 10 mM Na_2HPO_4, pH 7.4, and buffer 2 (137 mM NaCl, 10 mM Na_2HPO_4, pH 7.4, Tween-20). Blots were incubated for 1.5 h at 37 °C in a solution of mouse monoclonal antibody anti-His6-peroxidase (used at 1:100) in buffer 2. The substrate solution contains 0.01 g of 4-chloro-naphthol (Sigma) dissolved in 2 mL of methanol and mixed with 18 mL of buffer 1; 0.01 mL of 3% (v/v) hydrogen peroxide was added to the solution prior to use. The substrate solution was applied to blot membranes to color immunoreactive protein bands. The membranes were stained for 15 min at room temperature in the dark. Stained bands were photographed using GelDoc XR imager (Bio-Rad).

Indirect ELISA

Aliquots (100 μL) of solutions of peptidoglycans from *B. anthracis STI*, or *B. cereus* or *B. subtilis* were dispensed into wells of 96-well polystyrene plates. The microbial peptidoglycans were used as trapping antigens. Each species-specific peptidoglycan was distributed in 3 rows of the plates. To prepare the solutions, 50 μg of peptidoglycan was dissolved in 5 ml of coating buffer (50 mM Na2CO3, pH 9.6). Plates were incubated overnight at 4 °C and blocked with a solution of 1% BSA in phosphate-buffered saline (PBS) for 30 min at room temperature (RT). Upon three washes with PBS containing 0.1% Tween 20 (PBST), the diluted rPlyG protein was added to wells. A set of serial dilutions starting from 10 μg/mL was prepared for testing the reactivity of the rPlyG with the peptidoglycans in ELISA. Plates were incubated for 1 h at RT followed by three washes in PBST. A conjugated antibody (anti-His6-peroxidase) was added at 1:1000 dilution.

Plates were incubated for 30 min at RT followed by three washes in PBST. Next, the solution of o-phenylenediamine dihydrochloride (Sigma) was added to wells to produce a color reaction. The reaction was stopped after 15 min by addition of 2M sulfuric acid. Optical absorbance values (OD) in wells were determined at 490 nm using a plate reader.

NanoLC and tandem mass spectrometry (nanoLC-MS/MS)

Purified recombinant PlyG was separated by electrophoresis in 11% SDS-PAGE. Gels were stained with Coomassie blue. Protein bands were excised precisely and transferred to Eppendorf tubes. The excised gel slices were minced into small fragments, <1×1 mm. To remove the gel stain (Coomassie blue), 100 μL of 100 mM ammonium bicarbonate in acetonitrile (1:1) was added; the gel pieces were incubated at 37 °C for 30-40 min. After removing the supernatant, 5 mM dithiothreitol (DTT) was added to each tube and the tubes were incubated at 60 °C for 10 min. The solution of DTT was removed and 100 μL of 100 mM iodoacetamide was added to alkylate cysteine residues; the tubes were incubated at 37 °C for 15 min. Liquid reagents were removed and the gel pieces were washed twice in 100 μL of 50 mM ammonium bicarbonate. For removal of residing iodoacetamide, the gel pieces were subjected to two repeated cycles of dehydration with 200 μL of 100% acetonitrile and saturated with 50 mM ammonium bicarbonate in water. The gel pieces were again dehydrated in 100% acetonitrile for 3-5 min to reduce the size of the gel pieces; acetonitrile was removed, and pellets were dried for 5 min. Finally, 2 μL of 100 ng/μL trypsin and 50 μL of 50 mM ammonium bicarbonate were added, and the samples were incubated overnight at 37 °C to digest the eluted protein. Upon digestion, supernatants containing digestion products were transferred to new tubes. Additional extraction of the tryptic peptides was performed by washing the remaining gel pieces with 50 μL of 50 mM ammonium bicarbonate, followed by incubation for 15-20 min, and the supernatants were combined. Contents of the tubes were evaporated to dryness using a vacuum concentrator at 45 °C for 30-60 min. After complete removal of solvents, the solid residue containing peptides was dissolved in 10 μL of 0.1% trifluoroacetic acid (TFA), and soluble peptide mixtures were desalted using the Zip-tip

kit (Millipore Ziptips Micro-C18, 0.2 µL bed, Z720003-96EA).

The resulting mixtures of trypsin-digested peptides were separated using high-performance liquid chromatography (HPLC) and analyzed by in-line tandem mass spectrometry. For LC-MS/MS, an Acclaim™ PepMap™ 100 C18 pre-column (5 mm × 300 cm; 5 µm particles; Thermo Fisher Scientific) was used along with a Dionex HPLC pump (Ultimate 3000 RSLCnano System, Thermo Fisher Scientific). A peptide mixture was separated on an Acclaim™ Pep-Map™ RSLC column (15 cm × 75 µm, 2 µm particles; Thermo Fisher Scientific) using a 75-min multistage acetonitrile gradient (buffer A, 0.1% formic acid; buffer B, 90% acetonitrile/10 % H$_2$O in 0.1% formic acid) at a flow rate of 0.3 µL/min. The gradient program for buffer B was: 0 min-2%, 10 min-2%, 58 min-50%, 59 min-99%, 69 min-99%, 70 min-2.0%, 75 min-2.0%. The unmodified CaptiveSpray ion source (Capillary 1300 V, dry gas 3.0 L/min, dry temperature 150 °C) was used to interface the chromatography system to the Impact II (Bruker). Subjecting the mixture of digested peptides to chromatography ensured removal of low-molecular-weight impurities. The tandem MS/MS conditions were as follows: two of the most intense precursor ions, to obtain sample data, were selected for subsequent fragmentation with a full-time cycle of 3 s. The mass range was from 150 to 2,200 m/z under the positive ion mode.

The Mascot software was used to search the SwissProt 2016_10 database (552,884 sequences; 197,760,918 residues). Search parameters included variable modifications, including cysteine carbamidomethylation and methionine oxidation, fragment ion mass tolerance of 0.6 Da, and a mass tolerance of the parent ion at 1.20 Da.

RESULTS

Design of and *de novo* synthesis of PlyG gene

To produce a recombinant C-terminal fragment of γ-bacteriophage lysin, a sequence (89 amino acids) from the GenBank (ABC40469.1) was used (Figure 1). This fragment is the C-terminal portion of lysin of γ-bacteriophage comprising amino acid residues 141 to 230 which contains a sequence GALTSLKMTADFILQSDGLT known for highly specific binding to peptidoglycan of *B. anthracis*.

Starting from the amino acid sequence, codon optimization for expression in *E. coli* was performed and oligonucleotides for gene synthesis were designed. As a result, two constructs based on pET28 and pET32 vectors were devised. Sequencing of an assembled DNA fragment (326 bp-long) confirmed the absence of errors in accordance to the planned sequence. The synthetic gene PlyG was ligated into pET28 using restriction sites BamHI and XhoI, and also into pET32 using restriction sites NcoI and XhoI.

Development of producer strains

The expression constructs were transformed by electroporation into BL21(DE3) expression strain and seeded onto LB medium containing an appropriate antibiotic. To detect protein expression, transformed cells were cultured in LB medium in the presence of 0.2 mM IPTG. After the addition of IPTG,


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                        1                                          50
                        101                                        150
PlyG full    (101)  YYKAEDNAVDVVRQLMSMYNIPIENVRTHQSWSGKYCPHRMLAEGRWGAF
PlyG C-and    (1)   -----------------------------------------MLAEGRWGAF
Consensus    (101)                                           MLAEGRWGAF
                        151                                        200
PlyG full    (151)  IQKVKNGNVATTSPTKQNIIQSGAFSPYETPDVMGALTSLKMTADFILQS
PlyG C-and    (11)  IQKVKNGNVATTSPTKQNIIQSGAFSPYETPDVMGALTSLKMTADFILQS
Consensus    (151)  IQKVKNGNVATTSPTKQNIIQSGAFSPYETPDVMGALTSLKMTADFILQS
                        201                          233
PlyG full    (201)  DGLTYFISKPTSDAQLKAMKEYLDRKGWWYEVK
PlyG C-and    (61)  DGLTYFISKPTSDAQLKAMKEYLDRKGWWY---
Consensus    (201)  DGLTYFISKPTSDAQLKAMKEYLDRKGWWY
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Figure 1. Amino acid sequence of the C-terminal fragment of γ-bacteriophage lysin PlyG.

aliquots from the growing cultures were taken at various time points, bacteria were collected, destroyed by sonication and released proteins were analyzed in SDS-PAGE. The SDS-PAGE gels showed that recombinant lysin (rPlyG) was expressed after 2 h upon addition of IPTG (Figure 2, lane 2), and the observed molecular masses of the expression products are: 30 kDa for the pET32-based construct and 16 kDa for the pET28-based construct. The results are consistent with the theoretically estimated molecular masses. Both recombinant proteins reached the highest yields at 4 hours of incubation with IPTG and the yields do not increase during further 12 hours of incubation (Figure 2, lane 3-5).

Purification of rPlyG

Purification of the recombinant proteins was performed by metal chelate chromatography on Ni-Sepharose column. Buffers with various concentrations of imidazole were used for elution of the recombinant antigen. For chromatography, the buffers containing 8 M urea were used. As a result, it was found that the optimal concentration of imidazole is 200 mM, which allows to effectively elute the recombinant protein from the column (Figure 3). Western blotting confirmed the presence of the hexahistidine tag in a protein with a molecular mass of ~30 kDa, which corresponds to the predicted molecular mass of rPlyG.

ELISA

Since PlyG peptides exhibited specific binding to *B. cereus* 4342 and *B. anthracis*-Sterne in ELISA (Sainathrao *et al.* 2009) we decided to evaluate the ability of the resulting rPlyG protein to bind to peptidoglycan of various bacterial species. The
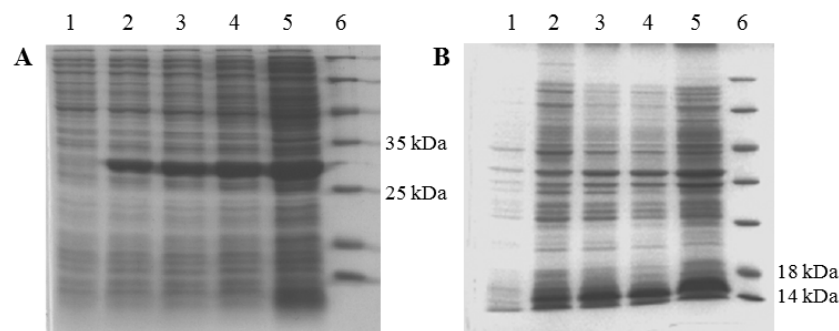

Figure 2. SDS-PAGE of cell lysates from producer strains BL21/pET32/PlyG (panel A) and BL21/pET28/PlyG (panel B). Lanes: 1, without IPTG; 2-5, in the presence of 0.2 mM IPTG; 2, 2 hours incubation; 3, 4 hours; 4, 6 hours; 5, 12 hours. Lane 6, molecular mass marker.
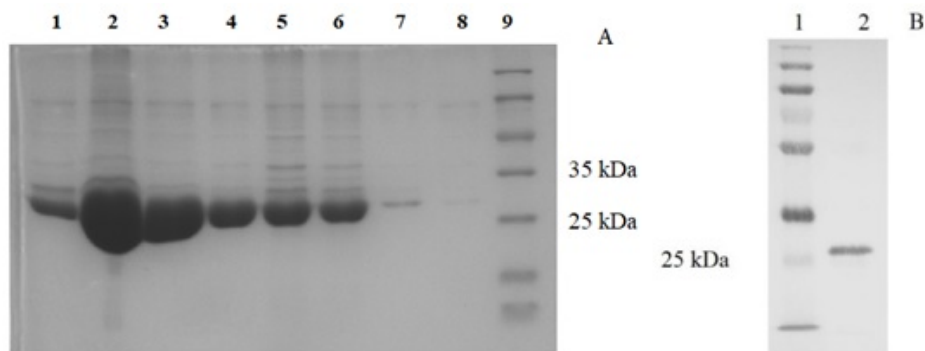

Figure 3. Panel A, SDS-PAGE of rPlyG protein. Lanes: 1-4, protein fractions eluted with 200 mM imidazole; 5-8, protein fractions eluted with 500 mM imidazole; 9, molecular mass marker. Panel B, western blot. Lanes: 1 - molecular mass marker; 2 - protein fraction eluted with 500 mM imidazole.
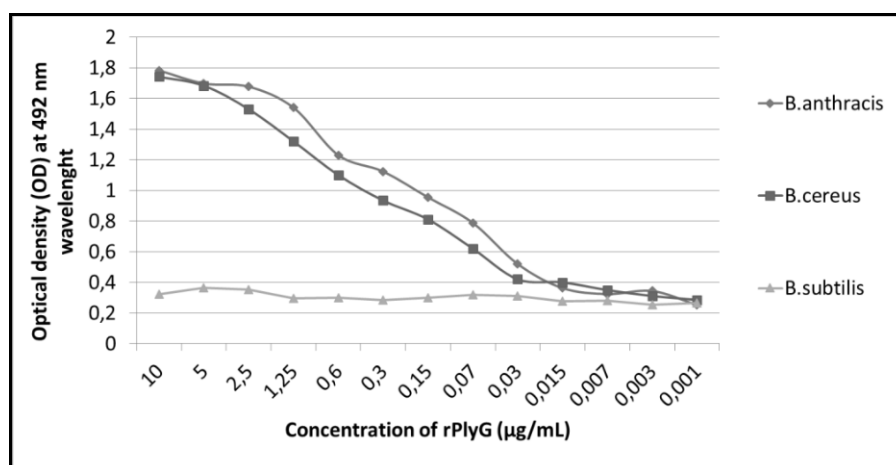
Figure 4. Results of ELISA, measuring the binding of rPlyG to peptidoglycans from *B. anthracis*, *B. cereus* and *B. subtilis*.

results of an indirect ELISA demonstrate that the rPlyG binds to peptidoglycans from *B. cereus* and *B. anthracis* even at low concentrations of the binding target. It is also worth mentioning that rPlyG does not bind to peptidoglycan from *B. subtilis* (Figure 4).

LC-MS/MS analysis of rPlyG

LC-MS/MS was used to confirm the molecular identity of rPlyG. MS/MS spectra of peaks corresponding to fragmented ions of peptides derived from trypsin-digested rPlyG were identified following SDS-PAGE, trypsin digestion, and chromatographic separation. Trypsin-digested peptides have Lys or Arg residues at their C-termini. The MS/MS spectra were converted to mgf files using the DataAnalysis program. These files were submitted to the Mascot search engine, which compares the experimental data with theoretical mass spectra using available sequence databases of amino acids, such as NCBI or SwissProt.

As a result, the Mascot output included 13 of the most probable proteins, corresponding to cumulative MS/MS spectra. The highest score (5,762) corresponded to only one protein, N-acetylmuramic-L-alanine amidase of *B. cereus*. Representative MS/MS spectra of peptides QNIIQSGAFSPYET PDVMGALTSLK and MTADFILQSDGLTYFIS KPTSDAQLK, which are products of rPlyG digestion, and their fragmentation ions are presented in Figure 5.

DISCUSSION

A C-terminal region of phage lysin PlyG comprising amino acid residues 156-233 is sufficient for specific binding to *B. anthracis*. Mutants that lack amino acids at positions 190-199 (LKMTADFILQ) in PlyG have lost the ability to bind to bacteria, which suggests that this short region imparts binding activity to the PlyG polypeptide. Further mutational analysis showed that residues L190 and Q199 of the sequence LKMTADFILQ are important for the active binding of PlyG to peptidoglycan of *B. anthracis*. In these studies, larger PlyG fragments served as probes for the detection of *B. anthracis* [11].

Novel technology of producing protein-encoding sequences using *de novo* gene synthesis is becoming a powerful tool in biotechnology. The ability to synthesize genes with desired sequences provides nearly unlimited research possibilities. Recently, significant advances have been made in the *de novo* synthesis of genes utilizing a PCR as a method of assembly of synthetic oligonucleotides into larger sequences. The PCR-based assembly approach is a fast and accurate method that provides researchers with desired DNA fragments [16, 17, 18].

PCR-based methods may suffer from the intrinsic propensity of thermophilic polymerases to introduce errors during copying of DNA strands. Thus, DNA fragments obtained using PCR has to be sequence-confirmed. A possible way to diminish
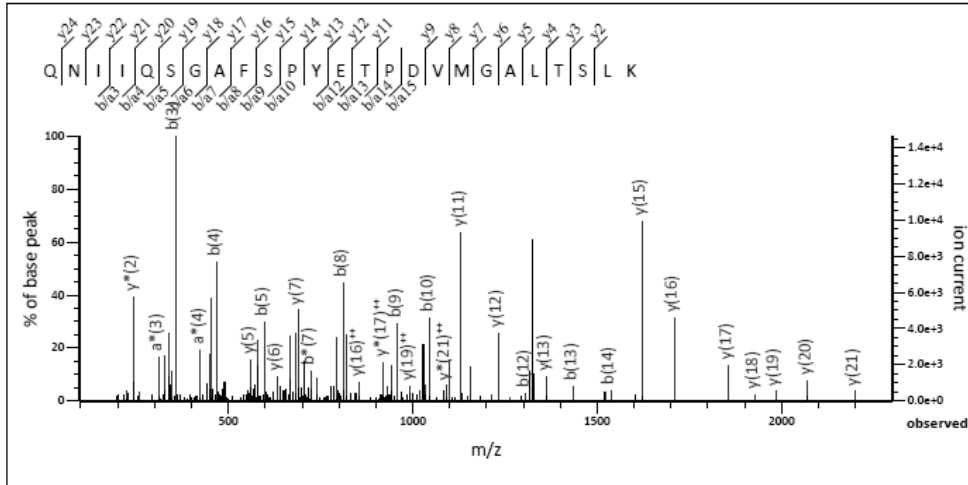
MATRIX SCIENCE Mascot Search Results

Peptide View

MS/MS Fragmentation of QNIIQSGAFSPYETPDVMGALTSLK
Found in gi|487909423 in NCBInr, N-acetylmuramoyl-L-alanine amidase [Bacillus cereus]

Match to Query 17084: 2666.342768 from(1334.178660,2+) intensity(756748.0000) rtinseconds(3052.14) scans(MS: 14110 MSMS: 14114) index(12620)
Title: Cmpd 12632, +MS2(1334.1787), 65.0eV, 50.9 min #14114
Data file ProteinAnalysisResultsF1.mgf

MATRIX SCIENCE Mascot Search Results

Peptide View

MS/MS Fragmentation of MTADFILQSDGLTYFISKPTSDAQLK
Found in gi|487909423 in NCBInr, N-acetylmuramoyl-L-alanine amidase [Bacillus cereus]

Match to Query 17826: 2889.463842 from(964.161890,3+) intensity(523174.0000) rtinseconds(2936.28) scans(MS: 13538 MSMS: 13542) index(12090)
Title: Cmpd 12102, +MS2(964.1619), 48.2eV, 48.9 min #13542
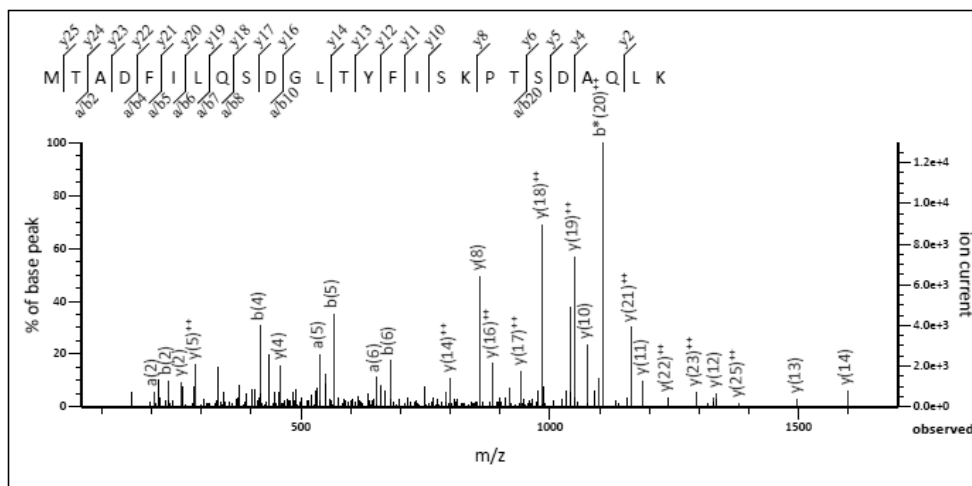Data file ProteinAnalysisResultsF1.mgf

Figure 5. MS/MS spectra of fragmented peptides derived from trypsin-digested rPlyG protein.

the inaccuracy problem is to use the last generation polymerases which have the base-editing capacity. Authors Dolgova and Stukolova (2017) tested the fidelity of three different DNA polymerases (Taq, Pfu, and Phusion). Average error rates for DNA-amplification for Pfu and Phusion polymerases were 2.2 ± 0.837 and 0.6 ± 0.548, respectively. Based on the results, the authors concluded that Phusion polymerase is three times more accurate than Pfu polymerase and supposed that this is because Phusion inherited the accuracy from a Pyrococcus-derived DNA polymerase [19].

Producer strains BL21/pET28/PlyG and BL21/pET32/PlyG accumulate a recombinant C-terminal fragment of γ-bacteriophage lysin PlyG. Similar results were obtained by Sainathrao *et al.* (2009), although a smaller fragment (10 amino acids) was cloned in the cited source. Specific binding of the produced peptide to peptidoglycan from the cell wall of *B. anthracis* has also been demonstrated [11]. A method of identification of *B. anthracis* isolates by using mass-spectrometry was developed, which allowed analyzing geographical distributions of the isolates.

CONCLUSION

In this paper assembly of a gene encoding a C-terminal fragment of γ-bacteriophage lysin PlyG and engineering of recombinant expression constructs are described. Transformed expression strains produce recombinant proteins (16 kDa and 30 kDa) which were analyzed by MS-MS spectrometry and were found to contain sequences of the γ-bacteriophage-encoded N-acetylmuramic-L-alanine amidase.

ACKNOWLEDGMENTS

CONFLICT OF INTEREST STATEMENT

On behalf of all authors, the corresponding author states that there is no conflict of interest.

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