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PREPARATION OF RECOMBINANT ALPHA-HEMOLYSIN *STAPHYLOCOCCUS AUREUS*

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Abstract: *This work is devoted to the development of methods for obtaining recombinant alpha-hemolysin Staphylococcus aureus. The results include sections devoted to individual research tasks, the preparation of the plasmid construction of the hla gene, the expression of the recombinant pTZ57R-hla construct, the preparation, purification and study of the specificity of the recombinant alpha-hemolysin protein.*

Keywords: *Staphylococcus aureus, alpha-hemolysin, hla gene, pTZ57R, cloning, pQE-30 plasmid, recombinant protein, mouse.*

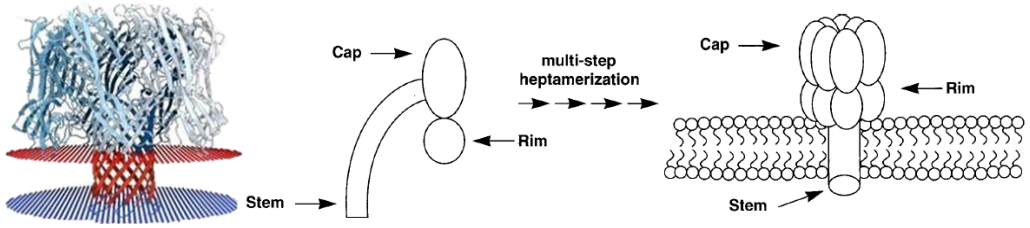
Introduction: In the structure of diseases caused by conditionally pathogenic bacteria, *Staphylococcus aureus* occupies about 50%. Staphylococcal infection is one of the causes of endocarditis, peritonitis, pneumonia, mastitis, keratitis and sepsis. The introduction of antibiotics into the practice of healthcare has led to a temporary decrease in morbidity. However, the emergence of multidrug resistance with the formation of so-called methicillin-resistant strains has returned this indicator to its previous level, which makes it expedient to develop antistaphylococcal vaccines and immunoglobulins. Alpha-hemolysin is one of the main factors

of *S. aureus* pathogenicity and has high immunogenic activity. Therefore, it is used for the development of protective immunity and the production of specific immunoglobulins. The most effective method for obtaining this antigen is to create its recombinant form using a bacterial producer based on *Escherichia coli*.

The purpose of the work: Cloning of the gene encoding *S. aureus* alpha-hemolysin and obtaining the corresponding recombinant protein.

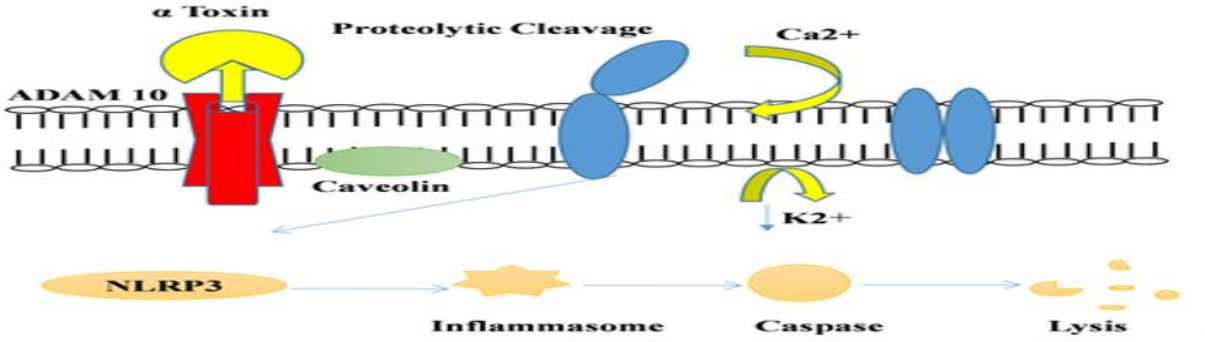
Materials and methods: The *hla* gene encoding the alpha-hemolysin protein was obtained by PCR using *S. aureus* FDA 209-P (ATCC 6538-P) genomic DNA as a matrix. The following primers were used for PCR: for forward primer: 5`-GGA TCC GCA GAT TCT GAT ATT AAT ATT AAA ACC G and for reverse primer: 5`-AAG CTT AAT TTG TCA TTT CTT CTT TTT CCC AAT C.

The straight primer corresponded to the beginning of the *hla* gene and included an additional restriction site *Bam*HI, and the reverse primer was complementary to the nucleotides flanking the end of the *hla* gene and included an additional restriction site *Hind*III. The amplified *hla* gene was cloned using the InsT/Aclone PCR Product Cloning Kit (Fermentas). As a result, it was embedded in the pTZ57R plasmid. The selection of recombinant clones was carried out by restriction analysis and sequencing. Next, the cloned *hla* gene was embedded in the pQE-30 plasmid at the restriction sites *BamH*I and *Hind*III. The expression of the recombinant gene was carried out using IPTG in the strain *E. coli* M15. Proteins were analyzed in a 12% polyacrylamide gel using the Lammley method. The recombinant protein was purified in a column with Ni-sepharose in an 8 M buffer solution of urea. For dialysis, a 50 mM solution of Tris-HCl pH 9.0 was used. The activity of recombinant alpha-hemolysin was evaluated *in vitro* on rabbit erythrocytes and *in vivo* on white mongrel mice weighing 14-16 g, injecting the drug intraperitoneally.



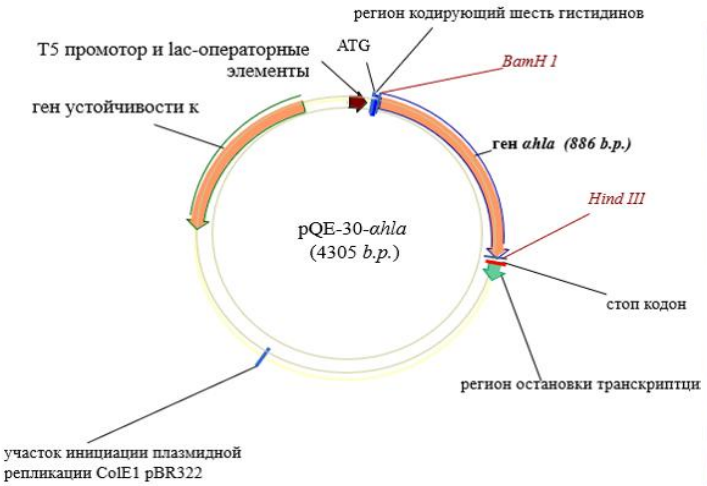
(1)

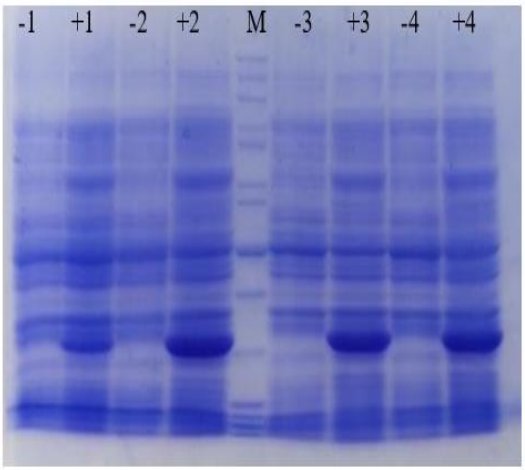
(1) *S. aureus* alpha-hemolysin protein (molecular weight 35 kDa) and pattern of pore formation.



(2)

(2) The figure shows a diagram of the formation of pores in the cell membrane by cytolytic toxins. The rim domain of the toxin adheres to the membrane, and the intertwined regions of the trunk are responsible for the formation of a pore with an exclusion radius of 14 Aº.

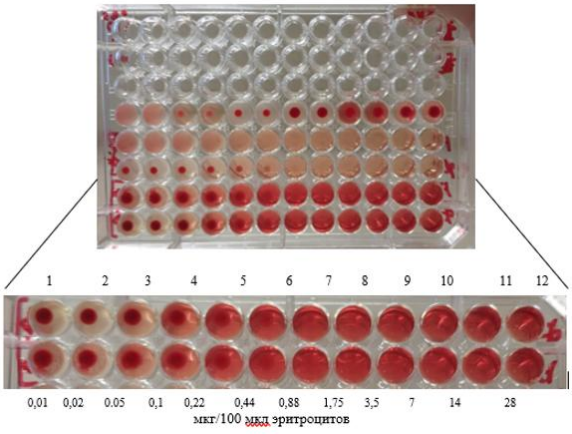




(3) (4)

(3) Scheme of genetic engineering design for the production of recombinant alpha-hemolysin protein in *E. coli* cells (strain M15). The selection of recombinant clones was carried out by restriction analysis and sequencing. The cloned *hla* gene was embedded in the pQE-30 plasmid at the restriction sites *BamH*I and *Hind*III. (Vector construction, author's scheme).

(4) Analysis of protein products derived from *hla* gene expression by Polyacrylamide gel 12% by the Lammley method (SDS-PAGE painted Coomassie R250). (+) producer proteins with induction, (-) Progenitor proteins without induction.



(5) (6)

(5) Lysis of rabbit erythrocytes by recombinant alpha-hemolysin *in vitro.*

(6) Condition of mice one week after intraperitoneal injection of recombinant alpha-hemolysin.

Results: As a result of restriction analysis and sequencing of recombinant constructs, the cloning of the *hla* gene was confirmed, and its sequence turned out to be identical to four of the twelve reference sequences from the GenBank database (CP020741, NBSI01000003, CP019563, MTFQ01000004), which were used for the selection of primers. As a result of the expression of the *hla* gene embedded in the plasmid vector pQE-30 under the control of the modified prokaryotic promoter T5, a recombinant protein was synthesized. Electrophoresis in polyacrylamide gel showed that its size was about 35 kDa, which corresponded to the calculated data – 34.7 kDa.

This recombinant protein was successfully chromatographically purified and used to evaluate its functional activity. It was shown that recombinant protein in the amount of 0.88 mcg effectively destroyed rabbit erythrocytes obtained from 50 mcl of whole blood. Recombinant alpha-hemolysin was administered intraperitoneally to mice. After administration of the drug in the first week, depression of the vital activity of animals was observed with the manifestation of disheveled, lethargy, extensive ulcers on the skin and diarrhea.

Conclusion: As a result of the study, a functionally active recombinant alpha-hemolysin was obtained, which can later be used in the development of staphylococcal toxoid.

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ОСВОЕНИЕ НАВЫКОВ НА УРОКАХ РКИ С ПОМОЩЬЮ ИГРОВЫХ УПРАЖНЕНИЙ

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Аннотация*: На сегодняшний день тема включения игр в процесс обучения иностранного языка является актуальной, так как данный вид упражнений помогает не только освоить необходимый учебный материал, но*