Секция «Микробиология»

Preparation of recombinant alpha-Hemolysin of Staphylococcus aureus

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Introduction: *Staphylococcus aureus* occupies about 50 % of the structure of diseases caused by opportunistic bacteria. Staphylococcal infection is one of the causes of endocarditis, peritonitis, pneumonia, mastitis, keratitis and sepsis. The introduction of antibiotics into public health practice has led to a temporary decrease in the incidence of diseases. However, the emergence of multiple drug resistance with the formation of so-called methicillin- resistant strains (MRSA) has returned this indicator to its previous level, which makes it advisable to develop anti-staphylococcal vaccines and immunoglobulins. Alpha-Hemolysin is one of the main factors of *S. aureus* pathogenicity and has a 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 the genomic DNA of *S. aureus* FDA 209-P (ATCC 6538-P) as a matrix. The following primers were used for PCR: 5‘ - GGA TCC GCA GAT TCT GAT ATT AAT ATT AAA ACC G and 5‘ - AAG CTT AAT TTG TCA TTT CTT CTT TTT CCC AAT C. The forward primer corresponded to the beginning of the hla gene and included an additional restriction site BamHI, and the reverse primer was complementary to the nucleotides flanking the end of the hla gene and included an additional restriction site HindIII. 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 performed by restriction analysis and sequencing. Next, the cloned hla gene was embedded in the pQE-30 plasmid at the restriction sites BamHI and HindIII. The recombinant gene was expressed using IPTG in the E. coli M15 strain. The 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 urea solution. 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 red blood cells and in vivo on white mongrel mice weighing 14-16 g, injecting the drug intraperitoneally.

Results: As a result of restriction analysis and sequencing of recombinant constructs, the cloning of the hla gene was confirmed, and its sequence was identical to four of the twelve reference sequences from the GenBank database (CP020741, NBSI01000003, CP019563, MTFQ010000 which were used for the selection of primers. As a result of the expression of the hla gene embedded in the pQE-30 plasmid vector under the control of the modified prokaryotic T5 promoter, 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 the recombinant protein in the amount of 0.88 μg effectively destroyed rabbit red blood cells obtained from 50 μl 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 hair, lethargy, extensive skin ulcers 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.

Illustrations

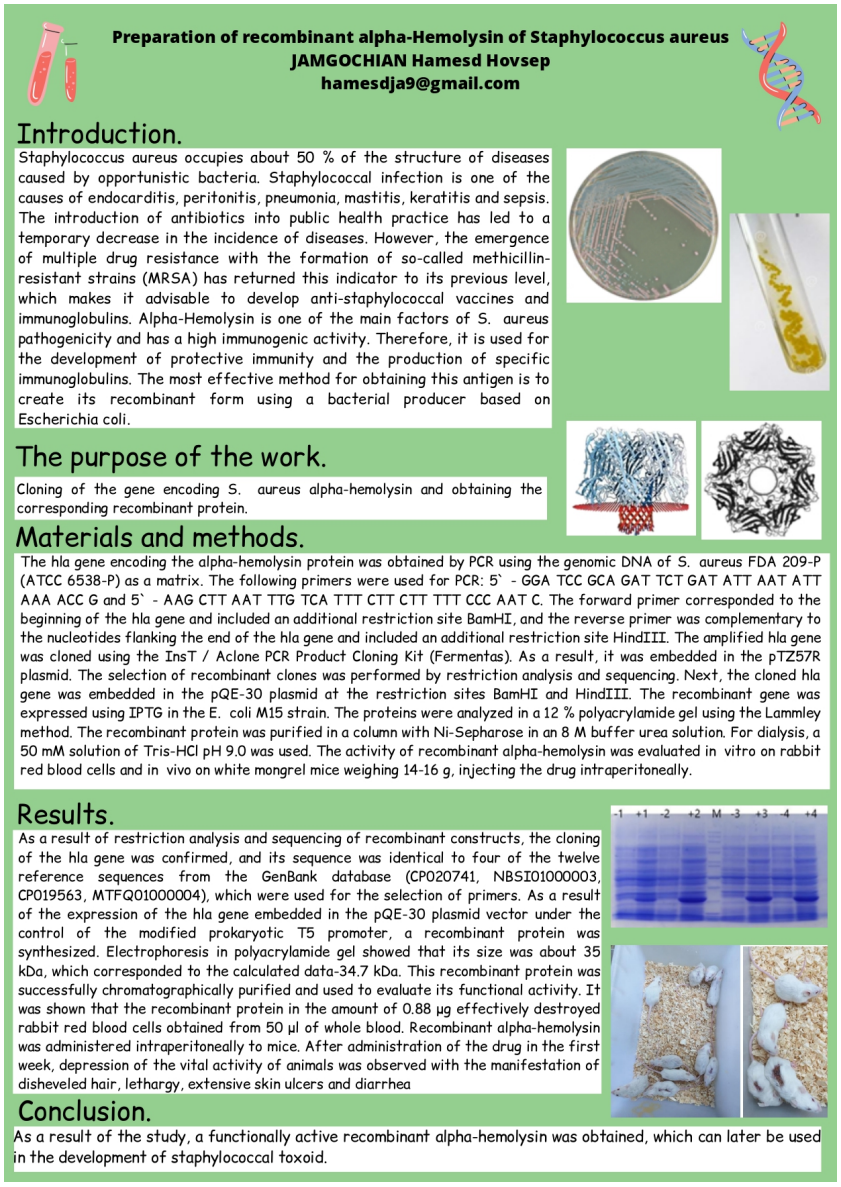

Рис. 1. internet and web of science