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ИСПОЛЬЗОВАНИЕ *BACILLUS SUBTILIS* В КАЧЕСТВЕ НОСИТЕЛЯ ОРАЛЬНОЙ ВАКЦИНЫ ПРОТИВ *STREPTOCOCCUS SUIS*

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Из-за прогрессирующего роста бактерий, вызванного широким применением антибиотиков, лечение стрептококкоза становится все более сложной задачей. Необходима надежная вакцинация против *Streptococcus suis.* Современные возможности молекулярной диагностики и генной инженерии создают перспективы для прямого клонирования протективных эпитопов гена Lmb местного штамма *S. suis* в предложенную систему доставки антигена иммунной системы свиней. Среди носителей оральных вакцин *Bacillus subtilis* признана относительно экологически чистым носителем с эффективной системой секреции белка и адаптивным метаболизмом, способная продуцировать споры в относительно жестких условиях. Это свойство спор может использоваться для повышения стабильности и возможности повторного использования вакцин. Изучена возможность использования протективных эпитопов Lmb *S. suis* в составе *B. subtilis* в качестве носителя оральной рекомбинантной вакцины против *Streptococcus suis*. Нуклеотидные последовательности *S. suis* получены в базе данных GenBank после предварительного анализа литературных данных об известных протективных антигенах *S. suis* различных серотипов. Анализ нуклеотидных последовательностей проводили с использованием программного обеспечения Unipro UGENE v. 43.0. Для поиска T (CTL и Th) и B зависимых эпитопов гена Lmb использовали The Immune Epitope Database (IEDB). Приведено описание сконструированной на основе компьютерного дизайна вакцины, в которой спрогнозирована локализация CTL, B и Th эпитопов. Описаны результаты клонирования последовательности антигенно-активного эпитопа белка *S. suis* Lmb в *B. subtilis* для последующего перорального введения и изучения изменений иммунологических реакций и побочных реакций у животных. Выявлена возможность клонировать эпитопы рекомбинантного белка Lmb *S. suis* в полилинкер вектора pBE-S. В перспективе представляется возможным создать новую недорогую и удобную в эксплуатации вакцину против *S. suis,* не требующую инъекционного введения.

Ключевые слова: *Streptococcus suis,* оральная вакцина, *Bacillus subtilis*, эпитоп, Lmb

USING *BACILLUS SUBTILIS* AS AN ORAL VACCINE CARRIER AGAINST *STREPTOCOCCUS SUIS*

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Due to the progressive growth of the bacteria caused by the widespread use of antibiotics, treatment of streptococcosis is becoming increasingly difficult. Reliable vaccination against *Streptococcus suis*

is necessary. Modern molecular diagnostic and genetic engineering capabilities create prospects for direct cloning of the protective epitopes of the Lmb gene of the local *S. suis* strain into the proposed delivery system of the pig immune system antigen. Among oral vaccine carriers, *Bacillus subtilis* is recognized as a relatively environmentally friendly carrier with an efficient protein secretion system and adaptive metabolism capable of spore production under relatively harsh conditions. This spore property can be used to increase the stability and reusability of vaccines. The possibility of using the protective Lmb epitopes of *S. suis* in *B. subtilis* as a carrier of an oral recombinant vaccine against *Streptococcus suis* was studied. The nucleotide sequences of *S. suis* were obtained from the GenBank database after a preliminary analysis of literature data on the known protective antigens of *S. suis* of various serotypes. Nucleotide sequence analysis was performed using Unipro UGENE v. 43.0. The Immune Epitope Database (IEDB) was used to search for T (CTL and Th) and B dependent epitopes of the Lmb gene. A computer-designed vaccine in which localization of CTL, B, and Th epitopes is predicted is described. The results of cloning the sequence of the antigenically active epitope of the *S. suis* Lmb protein in *B. subtilis* for subsequent oral administration and study of changes in immunological reactions and adverse reactions in animals are described. The possibility to clone the epitopes of recombinant *S. suis* Lmb protein into the pBE-S polylinker vector was revealed. In the long term, it seems possible to create a new inexpensive and easy-to-use vaccine against *S. suis* that does not require injection.

Keywords: *Streptococcus suis,* oral vaccine, *Bacillus subtilis*, epitope, Lmb

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INTRODUCTION

Streptococcus suis (S. suis) is a gram-positive coccus [1] that can be divided into 35 serotypes according to different antigens of the *Streptococcus* capsular polysaccharide *[2]*. Due to the progressive increase in bacterial resistance caused by the widespread use of antibiotics, the treatment of *S. suis* is becoming increasingly difficult, leading to an increasing need for effective vaccination against it *[3]*.

The route of transmission of *S. suis* in pigs is usually as follows: piglets become infected in the mother (vertical transmission) or from other animals in the herd (horizontal transmission) [4]. Despite the fact that contact with infected meat or animals through wounds is insignificant, the predominant route of *S. suis* transmission to humans is the consumption of undercooked, infected pork. In both pigs and humans, *S. suis* can cause meningitis, septicemia, and other diseases. This infection causes an acute zoonotic infectious disease [5, 6].

Over the years, scientists have studied various new vaccines. In our study, it was decided to choose an oral vaccine with live bacteria as the carrier. We believe that oral administration can be simple and easy to implement in practice and can also stimulate mucosal immunity [7].

Among oral vaccine applicators, the bacterium *Bacillus subtilis* (*B. subtilis*) is recognized as a relatively environmentally friendly host with an efficient protein secretion system and adaptive metabolism. This microorganism can also produce spores under relatively harsh conditions [8]. This property of spores can be used to increase the stability of vaccines under relatively unfavorable conditions of storage and application [9]. The technology of using spore-forming bacteria has been successfully applied in various industries, including vaccine production [10]. Thus, *B. subtilis* is an optimally suitable carrier of vaccine antigens.

After selecting a strain as a vaccine vector, we need to develop a vaccine. Lmb is an extracellular protein first discovered in *S. agalactiae* in 1999 [11], then in various *Streptococcus* species [12, 13]. Subsequent studies have shown that Lmb protein may have protective ability against streptococcal infection (see Fig. 1).

Lmb is a surface protein involved in the absorption of zinc ions (possibly a zinc receptor). These proteins associated with bacterial adhesion belong to the lipoprotein receptor family. Immunization of mice has shown that specific antibodies produced by the Lmb protein can effectively resist streptococcal infection[1].

The purpose of the study was to investigate the possibility of using the protective Lmb epitopes of *S. suis* in *B. subtilis* as a carrier of an oral recombinant vaccine against *S. suis*.

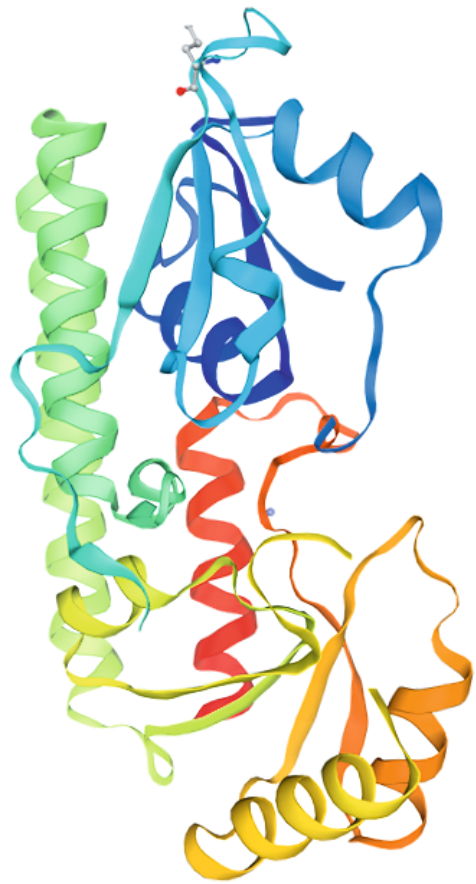

Рис. 1. Lmb: поверхностный белок, связывающий ламинин

Fig. 1. Lmb: surface laminin binding protein

MATERIAL AND METHODS

The work was performed at the Pharmacogenomics Laboratory of the Institute of Chemical Biology and Fundamental Medicine SB RAS, as well as in the Molecular Biology Sector of the Siberian Federal Center of Agro-BioTechnologies RAS. The *S. suis* nucleotide sequences were obtained in the GenBank database after preliminary analysis of the literature data on the known protective antigens of *S. suis* of various serotypes. The nucleotide sequences were analyzed using Unipro UGENE v. 43.0. The Immune Epitope Database (IEDB) was used to search for T (CTL and Th) and B dependent epitopes of the Lmb gene (see footnote 1).

Primer design was performed to clone and model the cloning process of Lmb gene regions containing T (CTL and Th) and B dependent epitopes by ligation at the BamH I, HindIII restriction sites into the shuttle vector pBE-S polylinker. *S. suis* DNA was isolated by silicosorption method from field isolates provided by Alexis LLC. PCR was performed using generally accepted methods on a Tercik amplifier ("DNA Technology" LLC, Russia).

To clone a fragment of the Lmb gene, the following primers were developed:

– Fwd Lmb: 5'-GAGGGATCCGCGATGTTAAAGAAAGTGATAAG-3';

– Rev Lmb: 5'-GACAAGCTTGGGTAAAAGTTCACCAATCGC-3'.

[1]URL: http://www.iedb.org/.

RESULTS AND DISCUSSION

We used a computer-based vaccine design to predict the candidate epitopes (i.e., antigenic determinants) obtained using the algorithms provided on the website (see footnote 1). After screening the laminin-binding protein (Lmb) was selected as the vaccine antigen (see Fig. 2).

According to bioinformatic analysis, one CTL epitope was found at the positions 12-20 of Lmb amino acid residues; four B-cell epitopes were found at the positions 65-75, 131-141, 179-189 and 279-287 of Lmb amino acid residues, respectively. Epitopes 1 Th were found at the positions 12-20 of Lmb amino acid residues (see Table 1).

The study applied techniques to analyze a possible other epitope of the protein sequence and then used the pBE-S vector to transform the com-position containing the protective Lmb epitope in *B. subtilis*. Enteroinvasive *B. subtilis* strain 53 IHBFM (isolated and studied earlier) was used as a vector strain.

The pBE-S vector is capable of expressing the recombinant protein in *B. subtilis* cells and has an additional source of replication (origin) in *Escherichia coli* cells. The primers we developed have additional Bam H1 and Hind III restriction sites, which makes it possible to clone the epitopes found in the polylinker of this vector, including their amplification from primary *S. suis* isolates and pathological material (see Fig. 3).

Bioinformatic analysis of S. suis Lmp gene revealed antigenic epitopes which are promising for recombinant vaccine creation: one CTL epitope was found at the positions 12-20 of Lmb amino acid residues, four B-cell epitopes - at positions 65-75, 131-141, 179- 189 and 279-287 amino acid residues Lmb, respectively. Th 1 epitopes were found at the positions 12-20 of

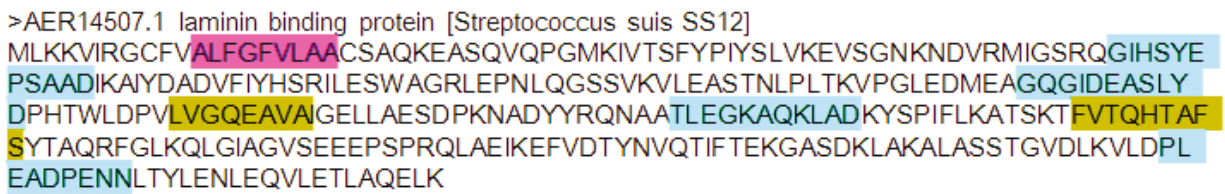

Рис. 2. Локализация эпитопов ЦТЛ и В-клеток Lmb по данным биоинформатического анализа.

Примечание. Эпитоп ЦТЛ (цитотоксические Т-клетки, цитотоксические Т-лимфоциты) отмечен фиолетовым, сегменты В-клеточного эпитопа – синим, эпитоп Th – зеленым.

Fig. 2. Localization of CTL epitopes and B-cells Lmb according to bioinformatics analysis

Note. The CTL epitope (cytotoxic T-cells, cytotoxic T-lymphocytes) is marked in purple, the B-cell epitope segments are marked in blue, the Th-epitope is marked in green.

Табл. 1. Аминокислотные и нуклеотидные последовательности CTL, B и Th эпитопов Lmb белка *S. suis* (SS12)

Table 1. Amino acid and nucleotide sequences of CTL, B, and Th epitopes of *Lmb* of *S. suis (SS12)* protein

№	Nucleotide sequence	Amino acid sequence
	Predicted epitope B	
1	GGCATACACTCTTATGAACCATCGGCTGCGGAC	GIHSYEPSAAD
2	GGTCAAGGGATTGATGAAGCTAGTTTATATGAC	GQGIDEASLYD
3	ACTTTAGAGGGAAAGGCGCAAAAGTTGGCAGAC	TLEGKAQKLAD
4	CCTCTTGAAGCAGATCCAGAAAATAAT	PLEADPENN
	Predicted epitope CTL	
1	GCCTTATTCGGTTTTGTTTTAGCAGCT	ALFGFVLAA
	Predicted epitope Th	
1	TTGGTTGGTCAGGAAGCTGTTGCGATT	LVGQEAVAI
2	TTTGTCACTCAACACACAGCCTTCTCT	FVTQHTAFS

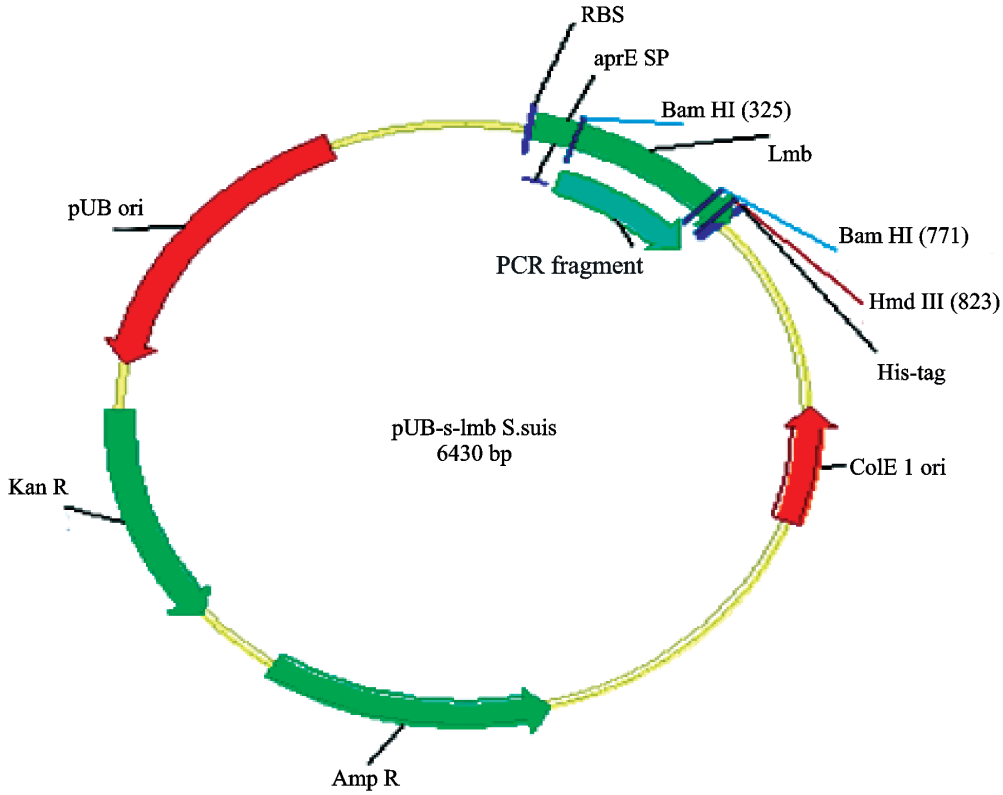

Рис. 3. Карта плазмидного вектора pBE-S со вставкой *S. suis* Lmb:

pUB ori – ориджин для рода *Bacillus* (сайт старта начала репликации плазмиды); ColE 1 ori – ориджин для *E. coli*; Kan R – ген устойчивости к канамицину (экспрессируется в бактериях рода *Bacillus*); Amp R – ген устойчивости к ампициллину (экспрессируется в *Escherichia coli*); His-tag – гистидиновый хвост на никелевых колонках для выделения рекомбинантного белка; Hind lll (823), Bam Hl (771), Bam Hl (325) – сайты рестрикции; Lmb – фрагмент антигена *S. suis;* aprE Sp; RBS – промотерная область для экспрессии белка у микроорганизмов рода *Bacillus*

Fig. 3. Map of plasmid vector pBE-S with *S. suis* Lmb insert:

pUB ori - origin for *Bacillus* genus (plasmid replication start site); Col E1 ori - origin for *E. coli*; Kan R, kanamycin resistance gene (expressed in *Bacillus* bacteria); AmpR, ampicillin resistance gene (expressed in *Escherichia coli*); His-tag, histidine tail on nickel columns for recombinant protein isolation; Hind lll (823), Bam Hl (771), Bam Hl (325), restriction sites; Lmb, fragment of *S. suis;* aprE Sp; RBS, promoter region for protein expression in microorganisms of the genus *Bacillus*

Lmb amino acid residues. The proposed delivery system for recombinant protective antigens of *S. suis* includes the plasmid vector pBE-S as part of the enteroinvasive strain *B. subtilis* B53 IHBFM.

CONCLUSIONS

1. The possibility to clone the epitopes of recombinant *S. suis* Lmb protein into the pBE-S vector polylinker was revealed.
2. In the long term, it seems possible to create a new inexpensive and easy-to-use vaccine against *S. suis* that does not require injection.

Modern possibilities of molecular diagnostics and genetic engineering create perspectives for direct cloning of protective epitopes of Lmb gene of local *S. suis* strain into the proposed system of antigen delivery to pig immune system.

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