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АМИЛОИДОГЕННЫЕ И АНТИБАКТЕРИАЛЬНЫЕ ЭФФЕКТЫ ПЕПТИДОВ, СИНТЕЗИРОВАННЫХ НА ОСНОВЕ ПОСЛЕДОВАТЕЛЬНОСТИ РИБОСОМНОГО БЕЛКА S1

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Антибиотикорезистентность

Antimicrobial and Amyloidogenic Activity of Peptides Synthesized on the Basis of the Ribosomal S1 Protein from Thermus Thermophilus

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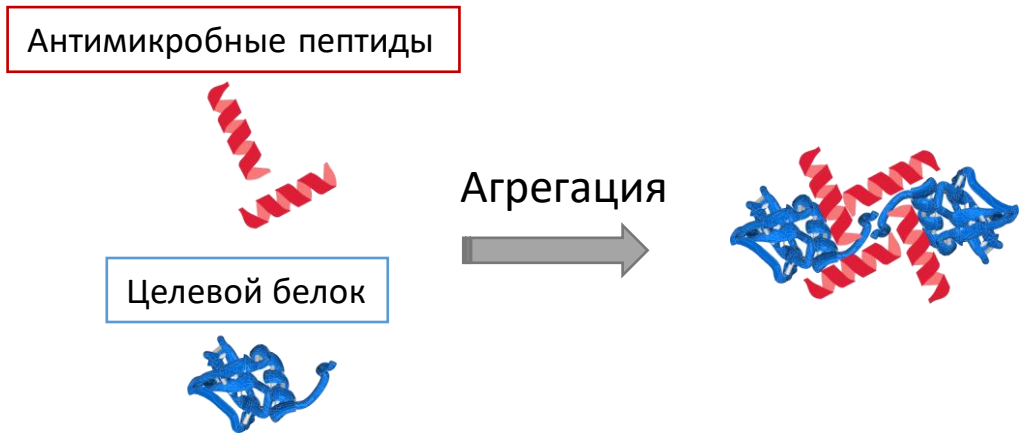


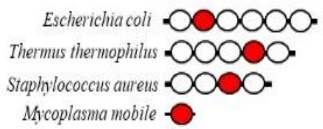
нарушение функции целевого белка

↓

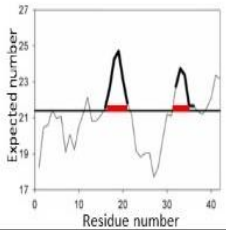
антимикробное действие

Creation of new antibacterial peptides based on targeted protein aggregation

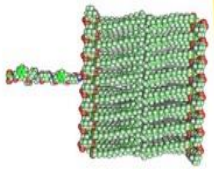
Theoretical methods



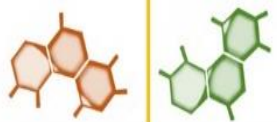
Search for unique domains in the bacterial proteins



Bioinformatics search for amyloidogenic regions

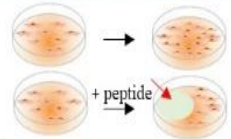


Molecular dynamics modeling of the penetration of peptides through the lipid layer

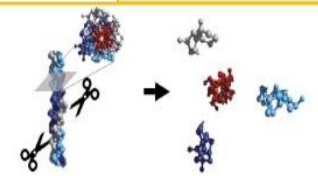


Design and synthesis of the amyloidogenic/antibacterial peptides

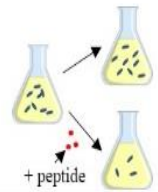
Practical methods



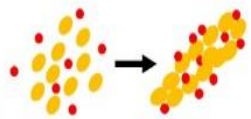
Toxicity testing of peptide again fibroblasts



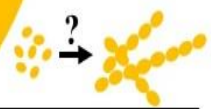
Identification of protein regions in the backbone of amyloid fibrils by mass spectrometry analysis



Determine the antimicrobial activities of peptide



Co-aggregation of amyloidogenic peptide and protein-target



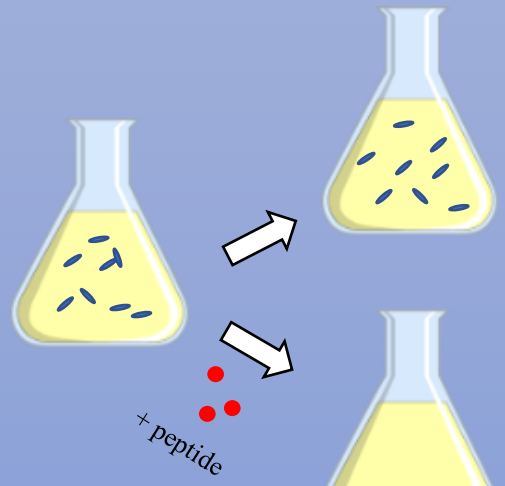
Studies of the process of fibril formation by AMPs

Методология

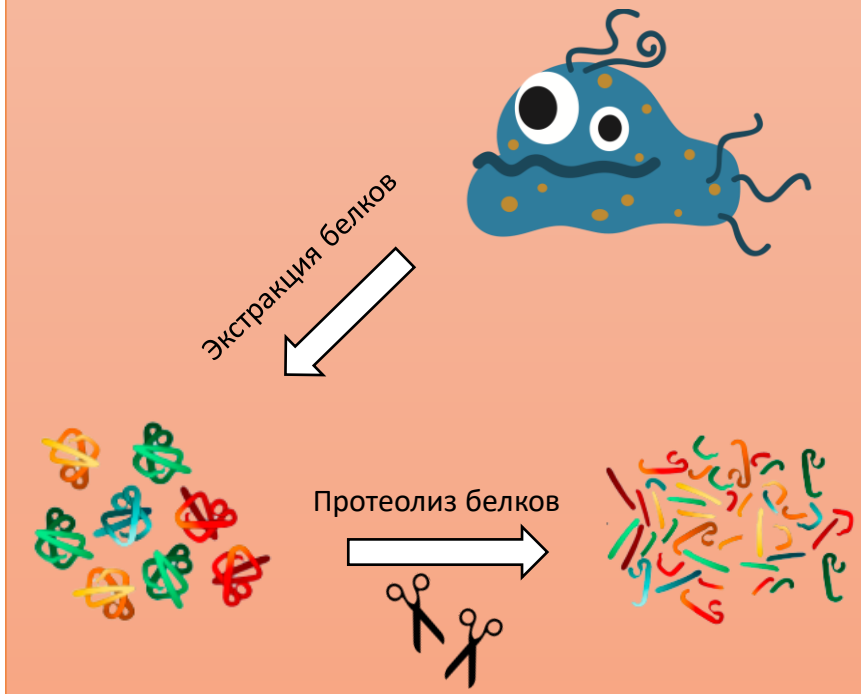
1. Подготовительный этап

- Выбор антимикробных веществ
  - ✓ пептидной природы
  - ✓ **не**пептидной природы
- Определить ингибиторные и прединги биторные концентрации антимикробных веществ

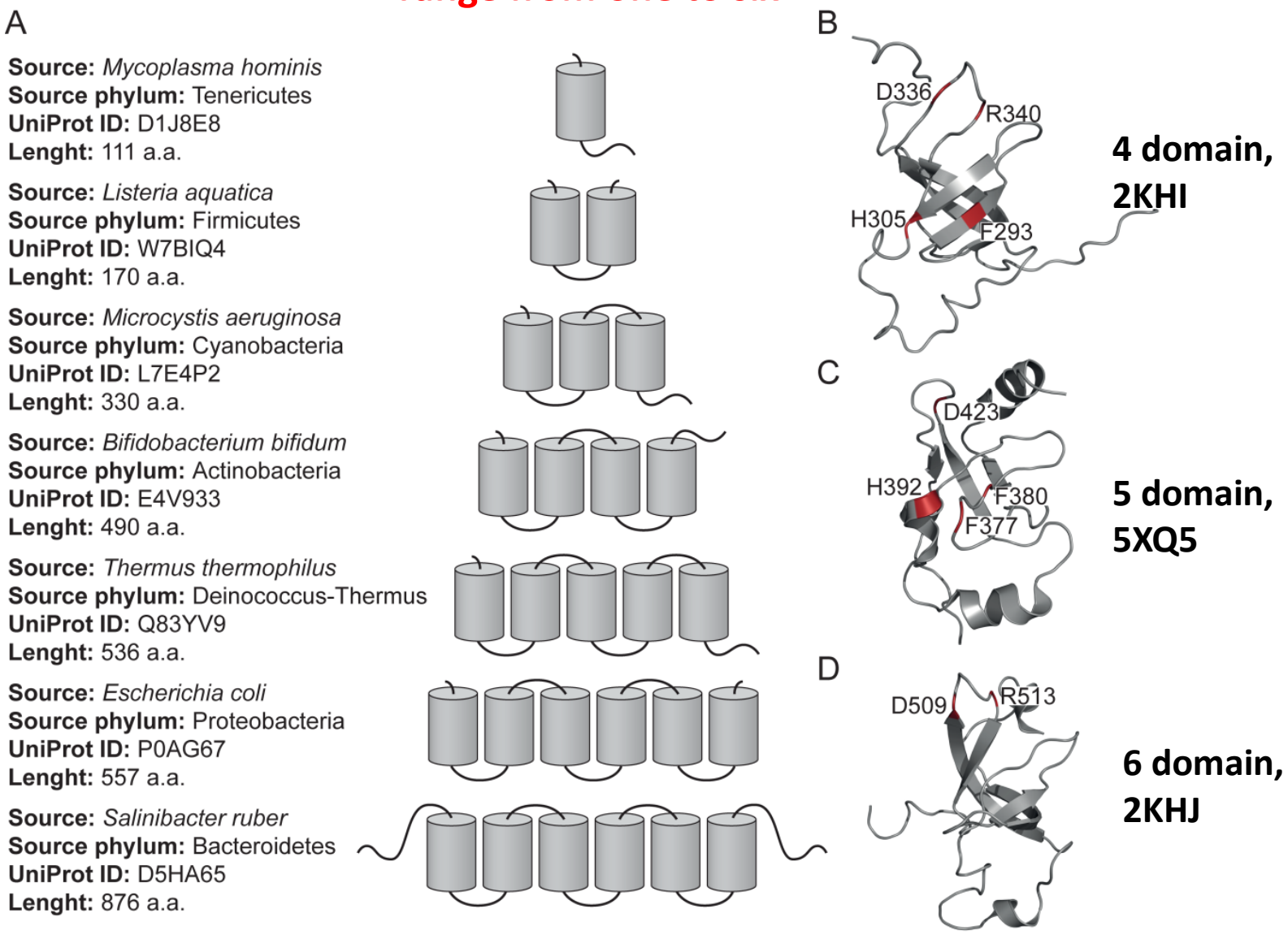
2. Воздействие антимикробными пептидами на бактерии



3. Подготовка проб для масс-спектрометрического анализа методом bottom-up

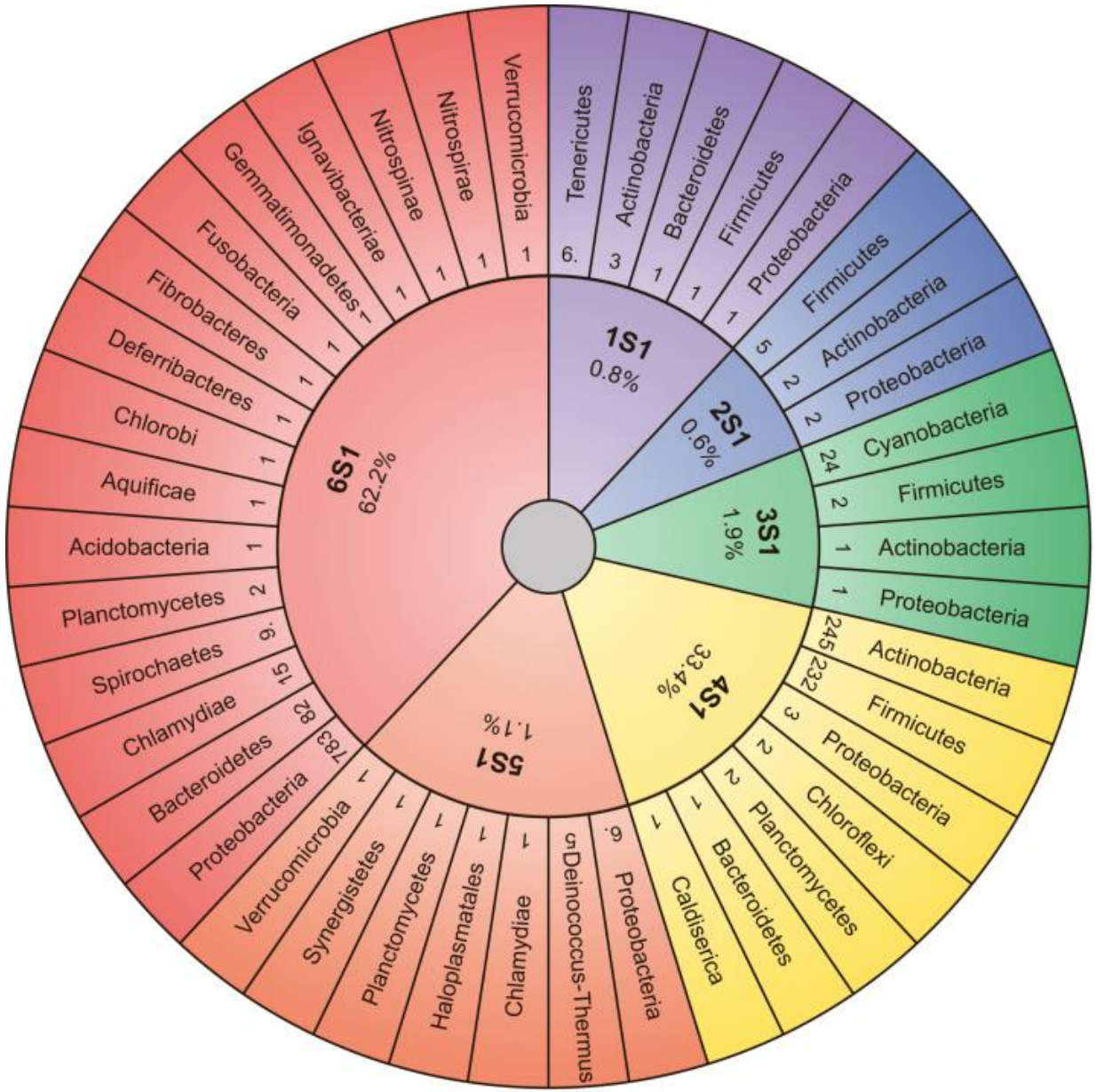


Number of structural S1 domains in bacteria changes strictly within a limited range from one to six



(A) Number of structural S1 domains in different bacteria (according to the SMART database). (B), (C), (D) NMR structures of the fourth (2KHI), fifth (5XQ5) and sixth (2KHJ) S1 domains from E.coli.

The 1453 sequences of S1 **were identified in 25** (from 30) different phyla (except candidate phyla). All studied phyla of bacteria and the number of domains S1 found in them are shown in the sunburst chart



a) **1S1** - **0.8%** from all investigated ribosomal proteins S1. The most represented in this group is the phylum **Tenericutes**

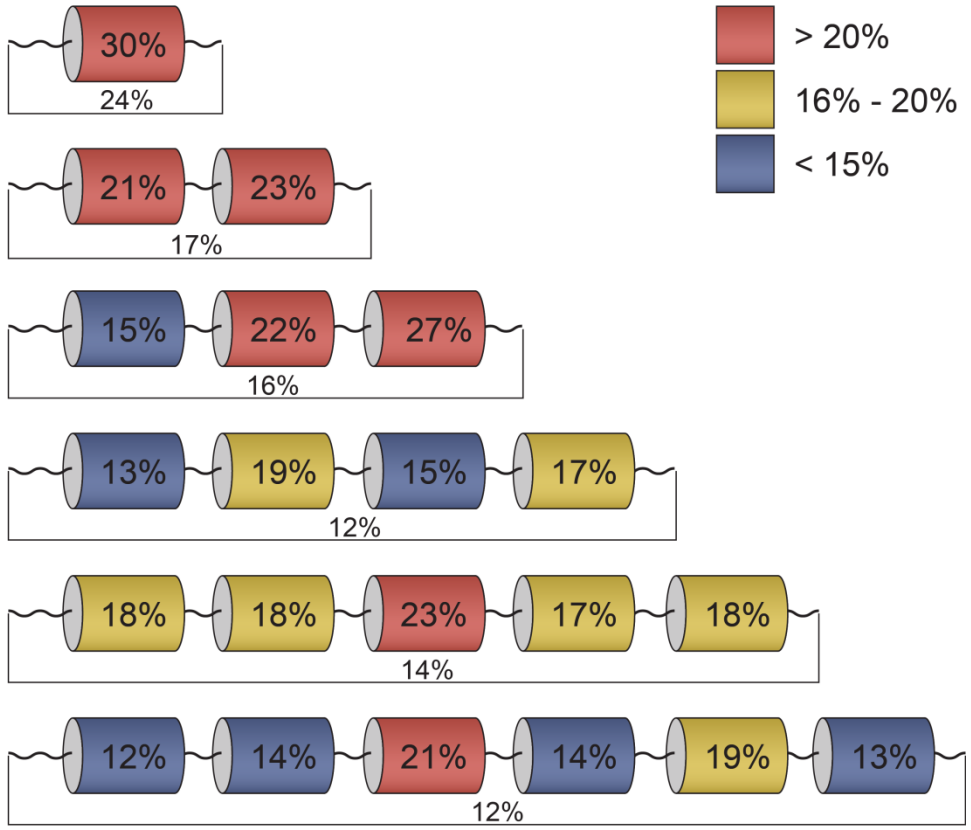
b) **Cyanobacteria** has **3 S1** domains; also three domains have some representatives of phyla Firmicutes, Actinobacteria and Proteobacteria.

c) **4S1** domains were identified in **33%** cases. Almost all analyzed bacteria in this group relate to phyla **Actinobacteria** (50% from all four-domain S1 proteins) and **Firmicutes** (47% from all four-domain S1 proteins).

d) **About 62% of all records** are identified as **6-domain S1** proteins, which belong to phylum **Proteobacteria**

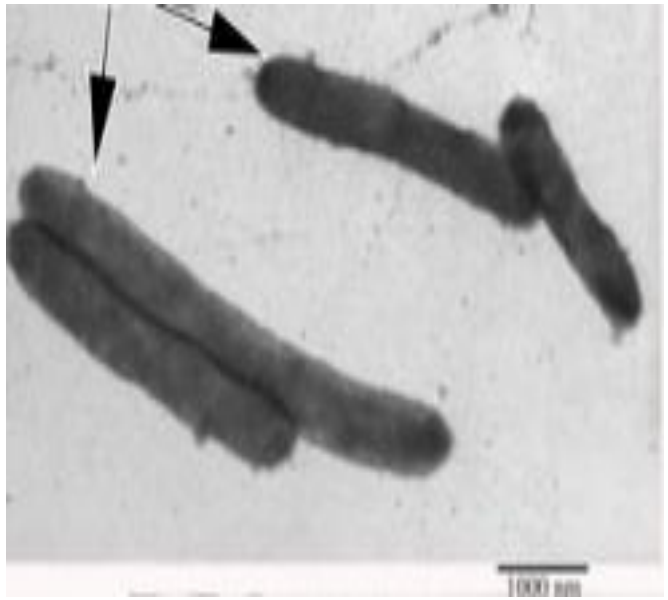
Analysis of amyloidogenicity of the S1 proteins and its domains

foldamyloid



http://bioinfo.protres.ru/fold-amyloid/ Bioinformatics. 2010 Feb 1;26(3):326-32.

Thermus thermophilus as a model organism for molecular biotechnology research



EM image of cells *T. thermophilus*

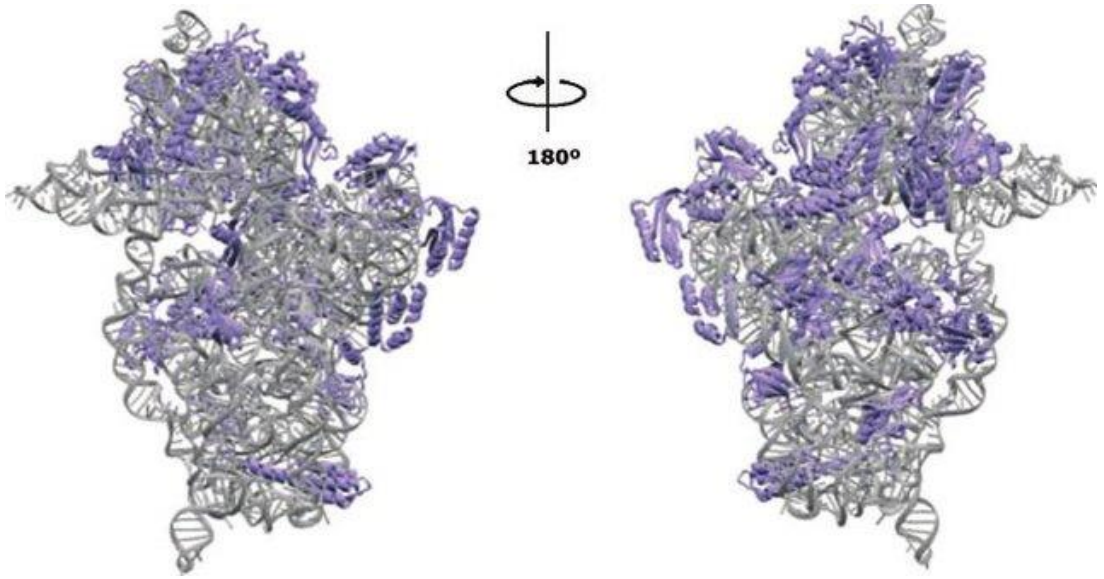


Figure 50S ribosome subunit
T. thermophilus

(https://www.researchgate.net/publication/41058475, 2009)

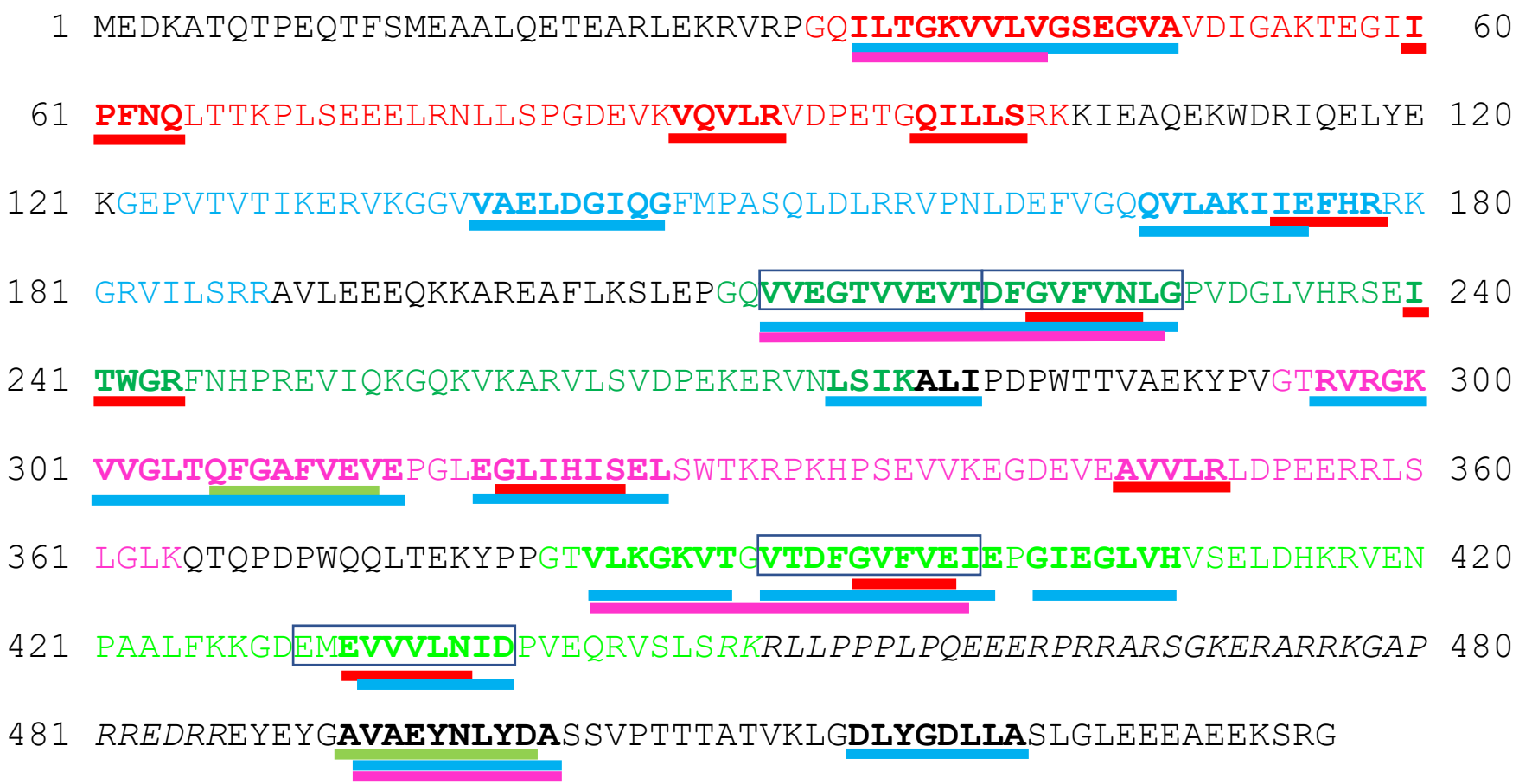
Objective: Development of antibacterial peptides based on amyloidogenic regions of the S1 protein from *T. thermophilus*

Tasks:

1. **Search for amyloidogenic regions of S1 protein from *T. thermophilus*.**
2. **Synthesize peptides based on the identified amyloidogenic sites.**
3. **Check the amyloidogenic and antibacterial properties of the obtained peptides.**

AMYLOIDOGENIC AND ANTIBACTERIAL EFFECTS OF PEPTIDES SYNTHESIZED BASED ON THE SEQUENCE OF S1 PROTEIN FROM *THERMUS THERMOPHILUS*

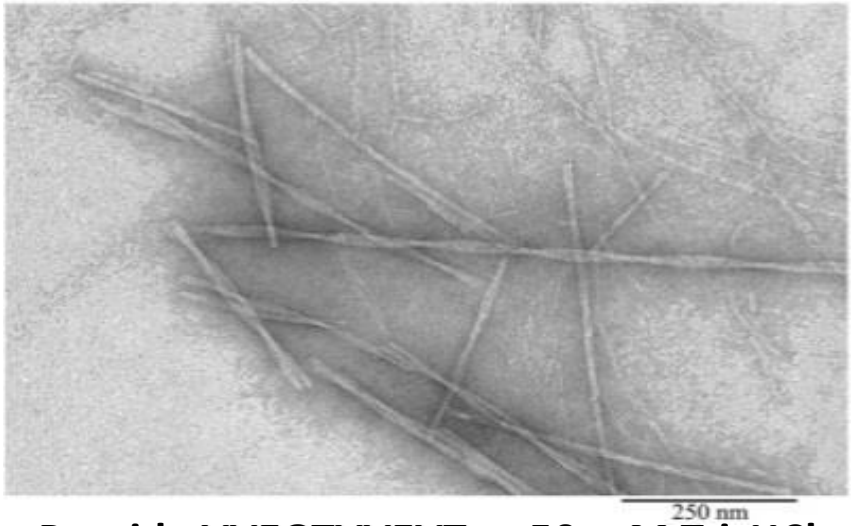
S1 from *T. thermophilus*



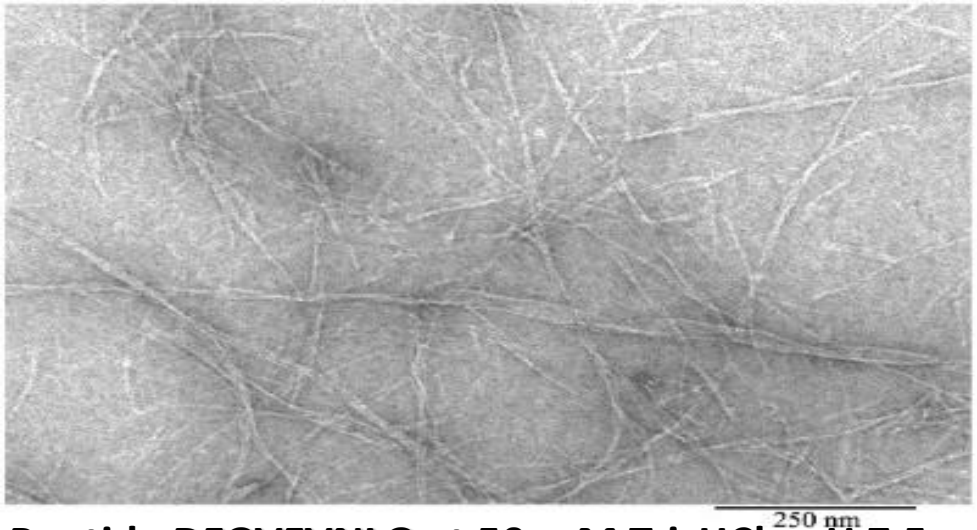
Амилоидогенные участки:
FoldAmyloid
Waltz
AGGRESCAN
PASTA 2.0

1 домен
2 домен
3 домен: 1) VVEGTVVEVT (211-220) и 2) DFGVFVNLG (221-229)
4 домен
5 домен: 3) VTDFGVFVEI (391-400) и 4) EMEVVVLNID (430-439)

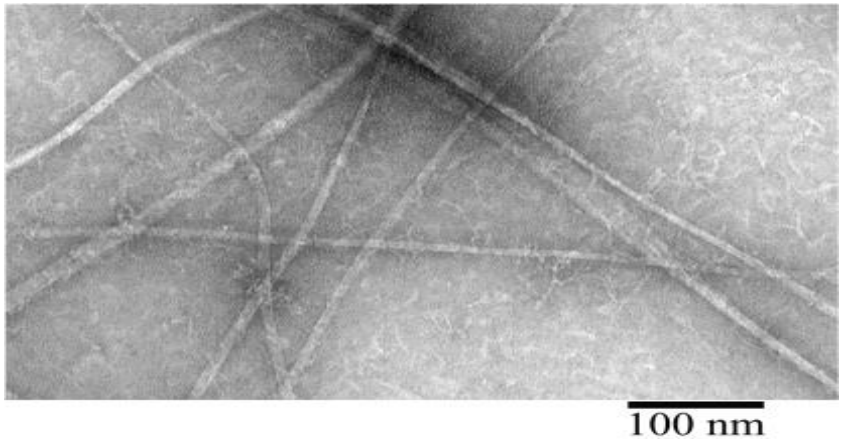
Amyloidogenic properties of four peptides synthesized from the predicted amyloidogenic sites of S1



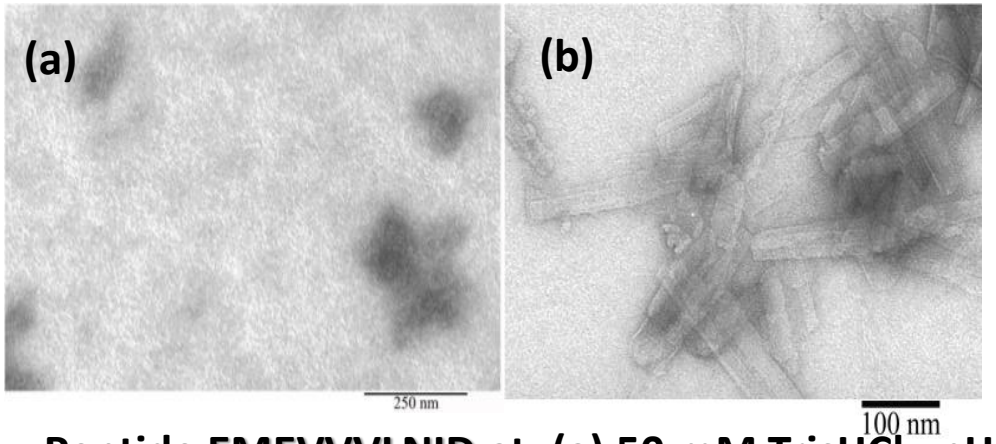
Peptide **VVEGTVVEVT** at 50 mM TrisHCl, pH 7,5; 150 mM NaCl, 5 hours.



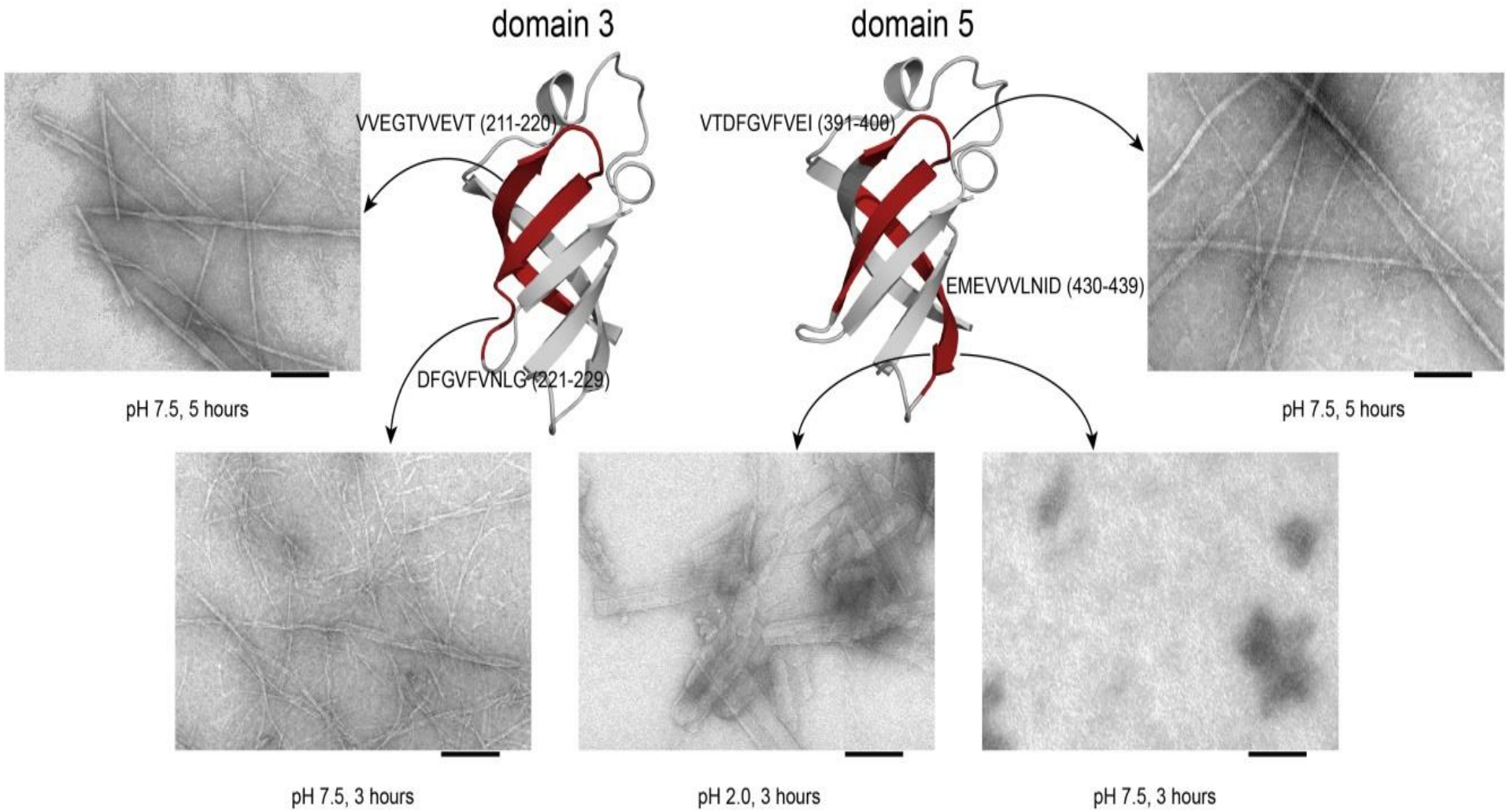
Peptide **DFGVFVNLG** at 50 mM TrisHCl, pH 7,5; 150 mM NaCl, 3 hours.



Peptide **VTDFGVFVEI** at 50 mM TrisHCl, pH 7,5; 150 mM NaCl, 5 hours.



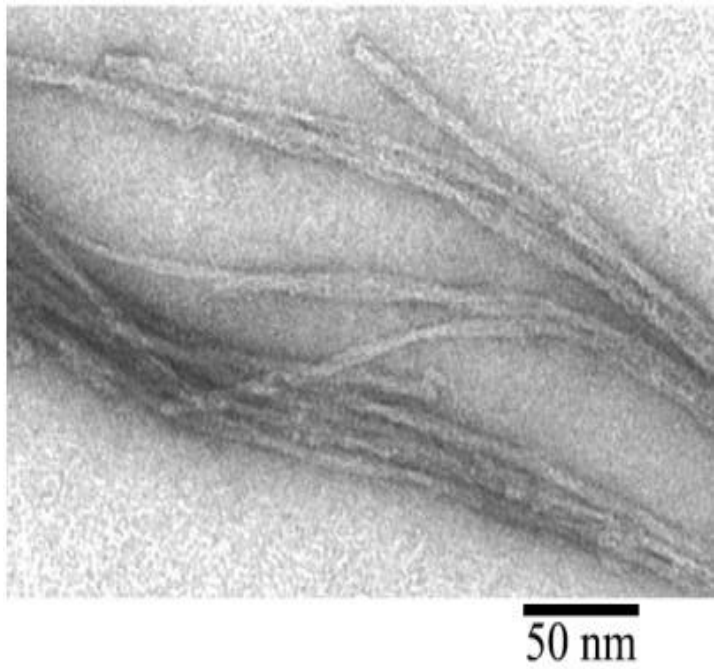
Peptide **EMEVVVLNID** at: (a) 50 mM TrisHCl, pH 7,5; 150 mM NaCl, 3 hours (b) 20% acetic acid, pH 2.0, 150 mM NaCl, 8 hours.



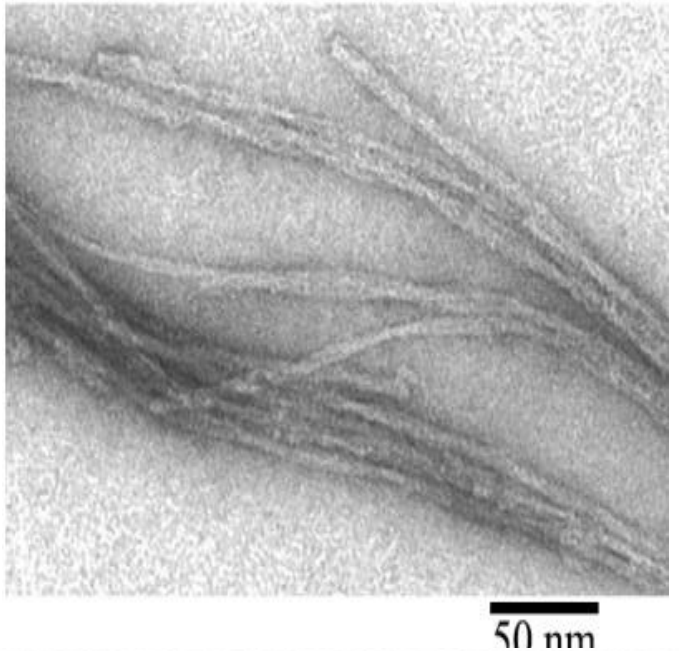
3D structures of the S1 domains from *T. thermophilus*. Experimentally studied amyloidogenic regions (position and sequence are given) are highlighted with red color. 3D structures of domains 3 and 5 were predicted using the Robetta server. Scale bar = 100 nm.

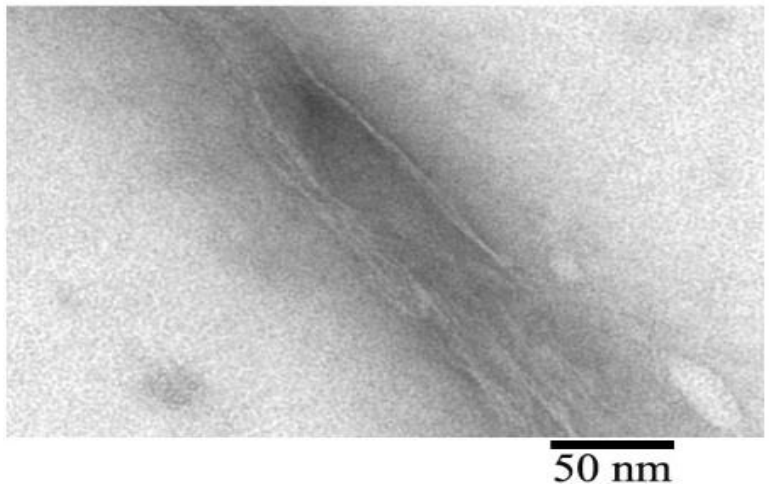
Coagregation of amyloidogenic peptides and ribosomal S1 protein

S1:V10T=1:5 (0.5 mg/ml and 2.5 mg/ml)



S1:R23T=1:5 (0.5 mg/ml and 2.5 mg/ml)





It was similarly verified that coaggregation of the V10T peptide and the S1 protein at 5:1 ratio led to the formation of fibrils.

Antibacterial properties of peptides synthesized based on the predicted amyloidogenic sites of S1

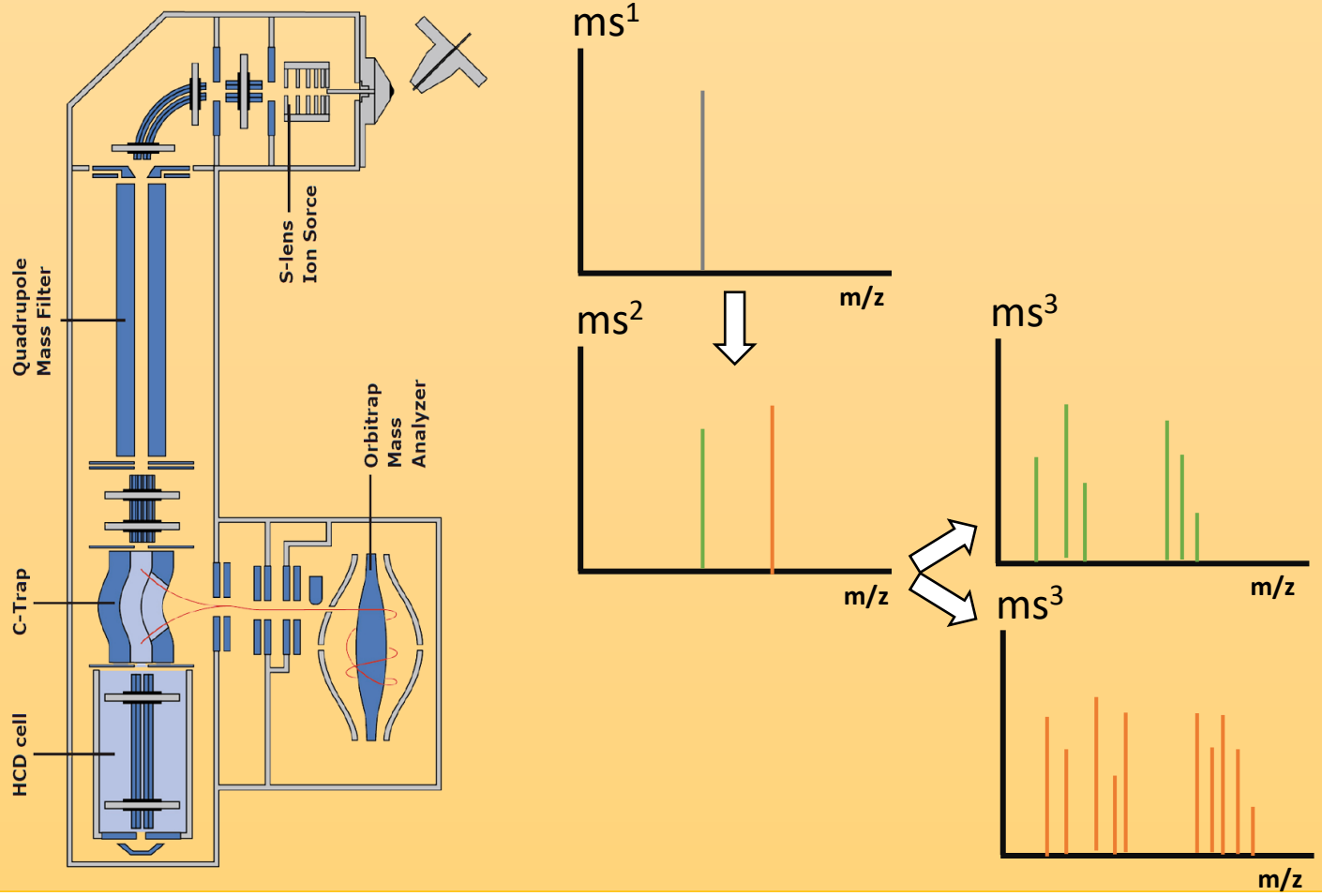
$$Э = 1 - \frac{A\,(Experiment)}{A\,(Control\,)} \quad (1)$$

The evaluation of the antibacterial effect (E) was carried out according to formula (1), where A is the light absorption of the liquid culture of *T. thermophilus* after 24 hours of incubation. An E value greater than 0.5 indicates an antibacterial effect.

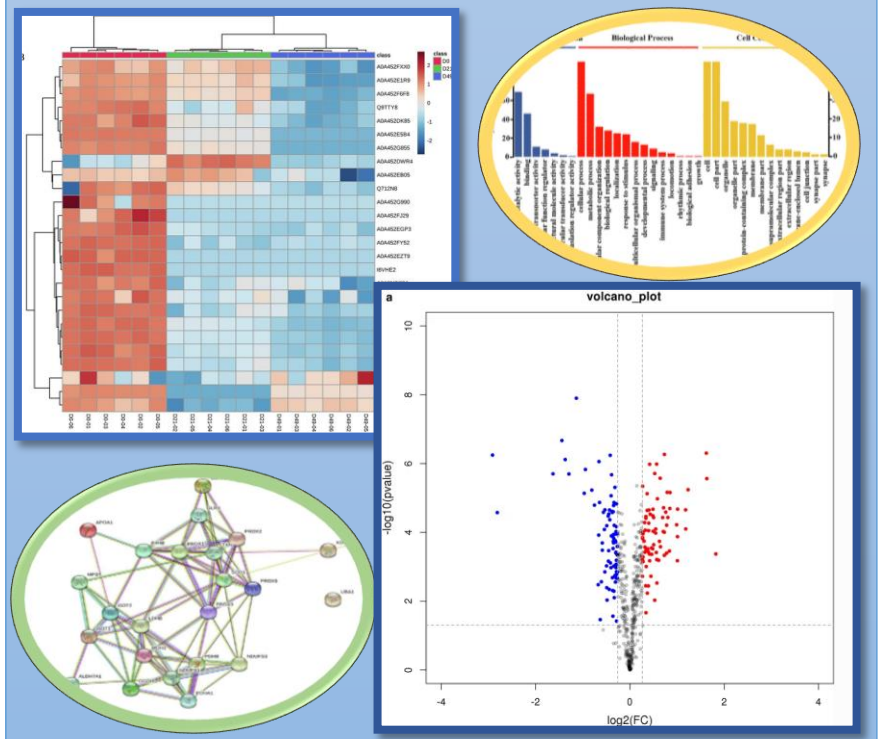
Sequence peptide	Peptide concentration and presence (+) or absence (-) antibacterial effect					
	1 µg/ml	10 µg/ml	50 µg/ml	100 µg/ml	500 µg/ml	1000 µg/ml
DFGVFVNLG	–	–	–	–	–	–
EMEVVVLNID	–	–	–	–	–	–
VTDFGVFVEI	–	–	–	–	–	+
VVEGTVVEVT	–	–	–	–	–	–
RKKRRQRRRGGSarG VTDFGVFVEI	–	–	+	+	+	–
RKKRRQRRRGGSarG VVEGTVVEVT	–	–	–	–	+	+

Методология

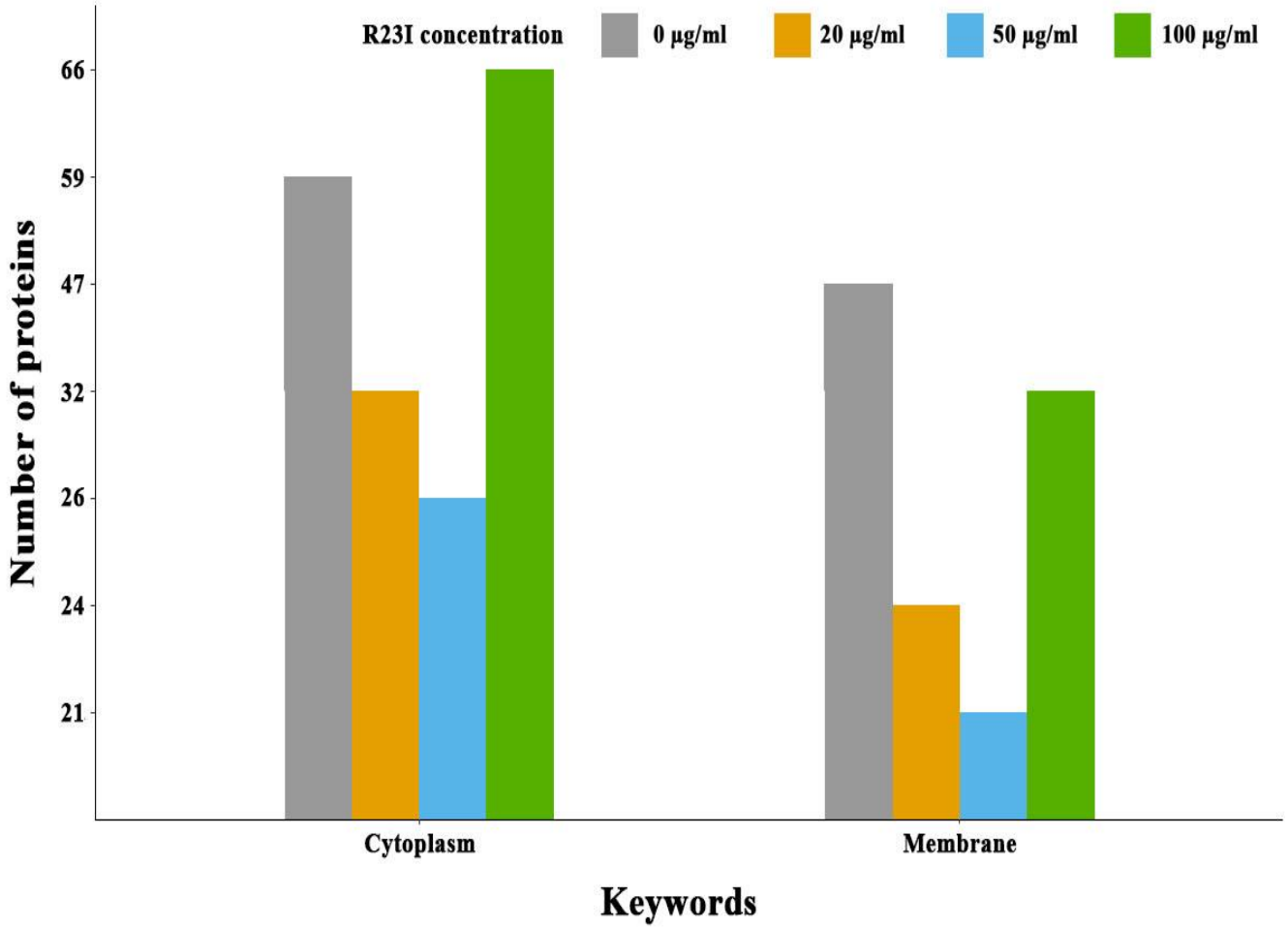
4. Масс-спектрометрический анализ



5. Идентификация белков,
статистический анализ,
аннотация белков



Study of the proteome of intact and peptide-treated *T. thermophilus* cells



Number of annotated cytoplasmic and membrane proteins depending on the R23I concentration in the cellular environment.

Antimicrobial and Amyloidogenic Activity of Peptides Synthesized on the Basis of the Ribosomal S1 Protein from Thermus Thermophilus. Int J Mol Sci. 2020, 21(17):6382.

Conclusions and Plans

Predicted and synthesized amyloidogenic and antibacterial peptides based on the S1 protein sequence from *T. thermophilus*. Among the studied peptides, only the R23I peptide proved to have the highest antimicrobial activity comparable with commercial antibiotics. The minimum inhibitory concentration (MIC) of the R23I peptide is 50 μg/ml.

The antimicrobial effect of amyloidogenic peptides can be increased by modifying the original peptides and creating hybrid peptides of the "amyloidogenic + antibacterial amino acid site" type.

In the future, it is planned to use algorithms for searching for promising antimicrobial peptides based on amyloidogenic sequences of the S1 protein from pathogenic organisms (*P. aeruginosa, S. aureus)*.

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Russian Science Foundation 18-14-00321.