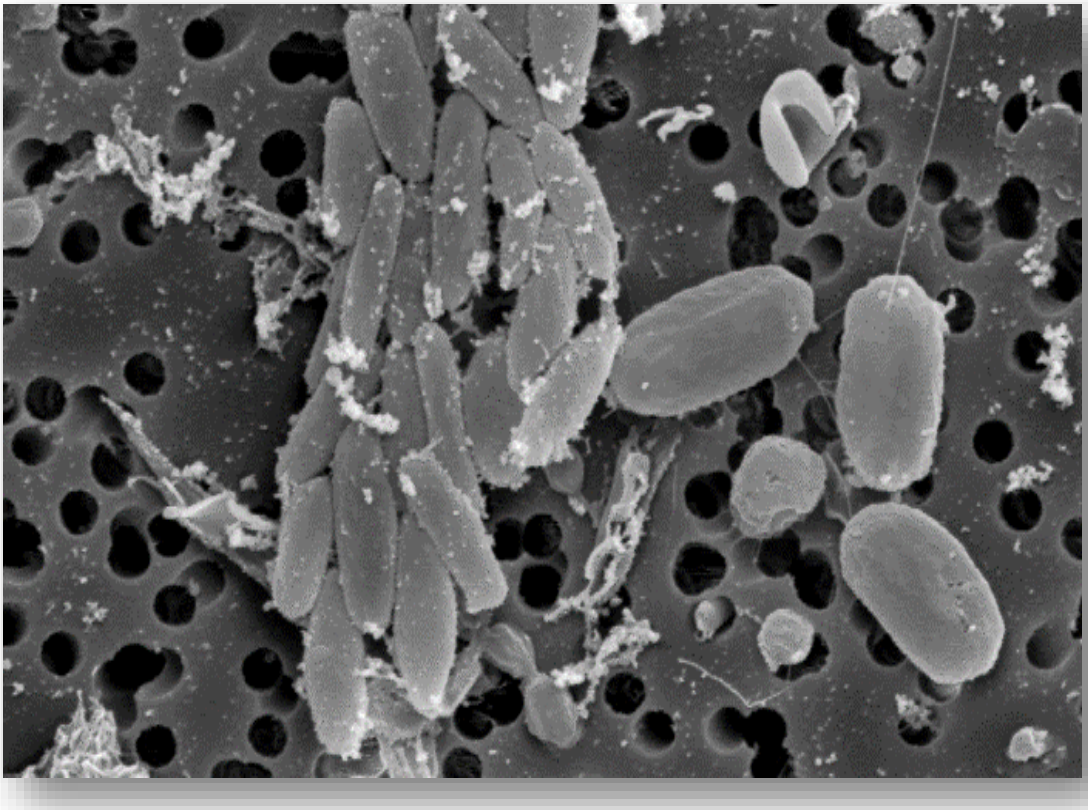
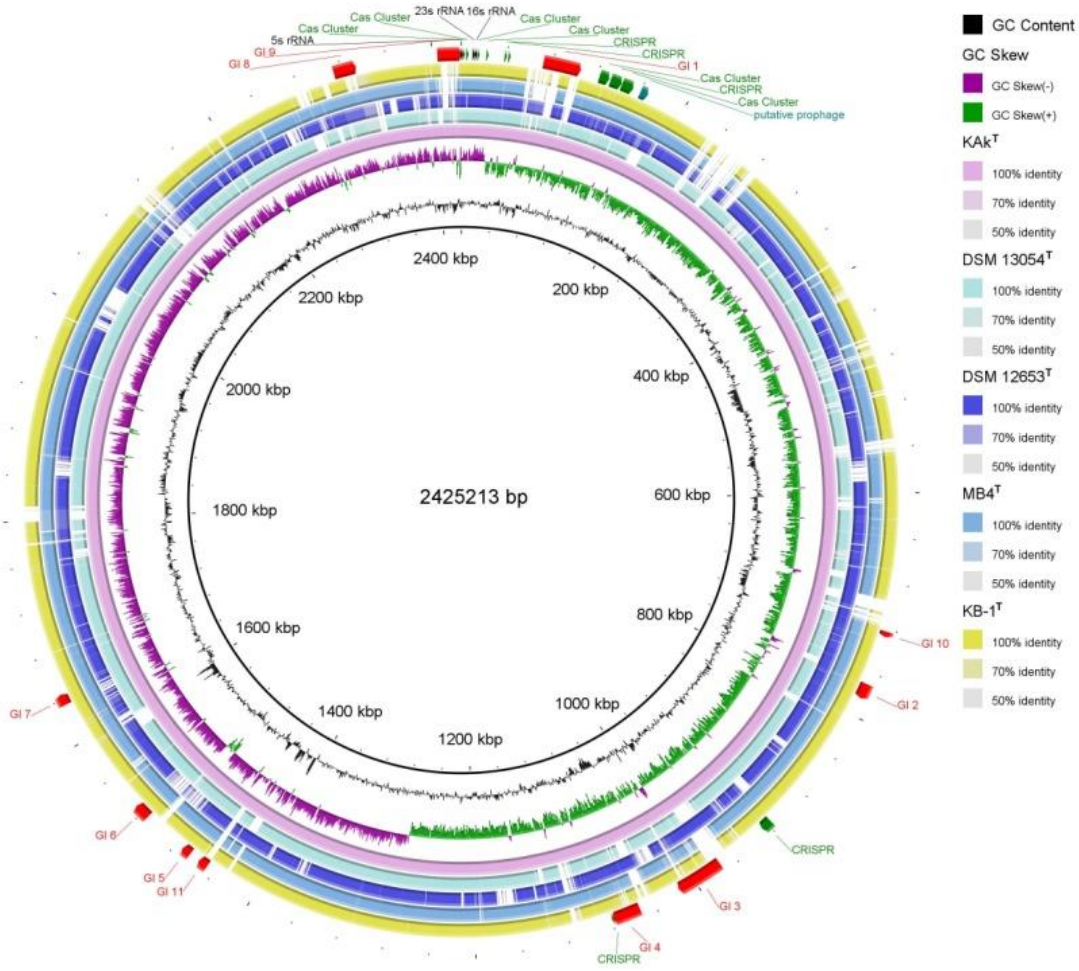
**Brief information about the project**

| | |
|---|---|
| Name of the project | AP14871683 Biotechnology of processing keratin by-products using immobilized thermophilic bacteria. (0122PK00618) |
| Relevance | The idea of the project is the construction and use of a consortium of thermophilic bacteria with keratinolytic activity for the processing of keratin-containing waste from the poultry processing industry. The consortium will be formed from biocompatible strains with proteases of different families (disulfide reductases and keratinases). This will ensure a gradual and more efficient biodegradation of raw materials into a hydrolyzate containing soluble protein, peptides and amino acids. |
| Purpose | Developing a technology for processing keratin by-products of poultry farming into protein hydrolysate by bioconversion with immobilized thermophilic bacteria. |
| Objectives | 1. Screening of keratinolytic thermophilic bacteria.<br>2. Selection of the method of KLBC immobilization into the matrix - bacterial cellulose (BC) and the conditions for its cultivation.<br>3. Obtaining a biological product - a dry hydrolyzate of KBP.<br>4. Semi-industrial approbation and development of technical documentation for a biological product. |
| Expected and achieved results | As a result of the implementation of this project, the following results were obtained:<br>1. Screening of keratinolytic thermophilic bacteria. Screening against the GenBank and RDP-II databases showed that the studied strains belong to the following systematic groups *Bacteria; Firmicutes; Bacilli; Bacillales; Bacillaceae; Bacillus*.<br><br>1.1. The rate of KBP hydrolysis by different strains of thermophiles and their enzymatic activity was determined. The active 8 strains were selected that grew on keratins and were able to almost completely hydrolyze them.<br><br>1.2. A genomic analysis of the similarity of amino acid sequences of proteins of strains with keratinolytic activity was carried out.<br><br>2. An immobilized KLBC has been created that carries out bioconversion of the KBP.<br>The bacteria were identified based on molecular genetic methods of the 16s RNA fragment, and their percentage homology by the nucleotide sequence of the strains was also determined. Technologies for the immobilization of cells into bacterial cellulose have been developed and various methods of modifying the surface of the matrix have been performed to maximize the loading of keratinolytic bacteria. |

| | |
|---|---|
| | 2.1. Selected active strains were identified and their biocompatibility was determined.<br>Identification of microorganisms based on molecular genetic methods of the 16s RNA fragment. Their percentage homology was determined by the nucleotide sequence of the strains.<br>2.2. A method for immobilizing KLBC into the BC matrix has been selected.<br>The selection of a method for immobilizing keratinolytic bacteria into a bacterial cellulose matrix was carried out for the effective bioconversion of feather and lint waste.<br>2.3. The dependence of the degree of keratin hydrolysis (ratio of amine to total nitrogen, peptides, amino acids) on temperature, pH, and fermentation time was determined.<br>The optimal parameters for the bioconversion of feather and down waste were selected using immobilized KLBC at a temperature of 65°C, at a pH of 7.1-7.5, fermentation periods lasted from 72 hours to 144 hours.<br>Expected results:<br>3. Technologies will be developed for obtaining the target product - dry protein hydrolyzate KPB. A package of technological documents will be developed.<br>3.1. The influence of the parameters of spray drying of the hydrolysate (temperature, aspiration, rate of solution supply to the installation) on the physical qualities of the hydrolysate (mass fraction of moisture, particle size) will be studied.<br>3.2. Parameters of physiological effectiveness (in vitro digestibility) and the safety of the hydrolysate will be determined according to microbiological indicators (pathogenic, conditionally pathogenic and sanitary-indicative microorganisms) and the content of toxic elements (copper, lead, zinc and cadmium).<br>3.3. Production testing will be carried out in a mini-production environment. A process for obtaining dry KPB hydrolysate will be developed. Technical and regulatory documentation will be developed. |
| Research team members with their identifiers (Scopus Author ID, Researcher ID, ORCID, if available) and links to relevant profiles | 1. Kistaubayeva A. S. – Candidate of biological sciences, associate professor. h-index 8; Scopus author ID 57197801138; http://orcid.org/0000-0002-9385-7155; ResearcherID F-5542-2013.<br>2. Savitskaya I. S. – Doctor of Biological Sciences, Professor. h- index 10; CiteInd.-353, Scopus author ID 36705310600; https://orcid.org/0000-0003-2417-8463; Researcher ID F-5542-2013.<br>3. Karpenyuk T.A. – Doctor of Biological Sciences, Professor. h- index 3, Scopus Author ID 55550041100, ORCID https://orcid.org/0000-0002-6985-7112. ResearcherID P-7980-2014.<br>4. Goncharova A.V. – Candidate of biological sciences, associate professor. h- index 2, Scopus Author ID |

| | |
|---|---|
| | 56450694300, ORCID https://orcid.org/0000-0002-1217-9613, ResearcherID P-7986-2014.<br>5. Ignatova L.V. – Candidate of biological sciences, associate professor. h- index 6; Scopus Author ID 57216761870.<br>6. Shokatayeva D.Kh. – PhD, h-index 5; Scopus author ID 57197794315.<br>7. Talipova A.B. - Master of Technical Sciences. h-index 3; Scopus author ID 57211535311; https://orcid.org/0000-0001-6874-5760.<br>8. Abdulzhanova M.A. – Master of Technical Sciences. h-index 2; Scopus author ID 57211532475.<br>9. Mashzhan A.S. – Master of Technical Sciences. h-index 2; Scopus Author ID 57211535534; https://orcid.org/0000-0002-9516-5566. |
| List of publications with links to them | 1. А.Ж. Ізмұқан, А.С. Кистаубаева, А.С. Машжан, Н. Биркеланд, И.С. Савицкая. Исследование продуцентов термозимов, выделенных из Жаркентского геотермального источника // Микробиология және вирусология. - 2022, 4(39). – P. 104-122.<br>2. A.Mashzhan, A. Kistaubayeva, R. Javier-López, U. Bissenova, A. Bissenbay, N.Birkeland / International Journal of systematic and evolutionary microbiology. – 2024. DOI 10.1099/ijsem.0.006269. (Q1). |
| Patents | - |




23s rRNA
16s rRNA
5s rRNA
Cas Cluster
Cas Cluster
Cas Cluster
Cas Cluster
Cas Cluster
Cas Cluster
CRISPR
CRISPR
CRISPR
putative prophage
GI 1
GI 2
GI 3
GI 4
GI 5
GI 6
GI 7
GI 8
GI 9
GI 10
GI 11
2425213 bp
200 kbp
400 kbp
600 kbp
800 kbp
1000 kbp
1200 kbp
1400 kbp
1600 kbp
1800 kbp
2000 kbp
2200 kbp
2400 kbp
GC Content
GC Skew
GC Skew(-)
GC Skew(+)
KAk$^T$
100% identity
70% identity
50% identity
DSM 13054$^T$
100% identity
70% identity
50% identity
DSM 12653$^T$
100% identity
70% identity
50% identity
MB4$^T$
100% identity
70% identity
50% identity
KB-1$^T$
100% identity
70% identity
50% identity