Brief information about the project

Name of the project	AP09259735 «Development and evaluation of bacteriophage chimeric endolysins to combat multidrug-resistant gram-negative pathogens of sturgeon fish» (0121PK00302)
Relevance	Sturgeon fish belong to the most ancient fish species, they generally long-lived and slow to mature and they have significant economic value as an animal protein source, including caviar and meat. To date, due to large amounts of illegal catches, water pollution and destruction of habitat sturgeon population *are on the verge* of *extinction. Sturgeon aquaculture would be the solution for the decline in the sturgeon population and restore the almost interrupted life cycle of the species. However, the rapid development of aquaculture has been accompanied by outbreaks of disease caused by bacterial infection that lead to high mortality and catastrophic economic losses in sturgeon aquaculture. The most severe bacterial disease in farmed sturgeons is Aeromonas* and *Pseudomonas infections.* Currently, due to the widespread and often uncontrolled use of antibiotics, number of antibiotic-resistant bacteria has increased dramatically and represent a leading cause of morbidity and mortality. This phenomenon may not only cause failure of antimicrobial therapy but also raise safety concerns on fish products. For this reason, novel strategies to combat with these multidrug-resistant pathogens are urgently needed. Currently, endolysin therapy is considered as a very promising alternative for the treatment of complex infections. Endolysins are phage-encoded enzymes, which have peptidoglycan hydrolase activity and are therefore able to degrade the bacterial cell wall, allowing the progeny virions to escape the host cell after replication. As opposed to antibiotics and bacteriophage, bacterial strains do not develop resistance to endolysins. The endolysin research field has undergone a significant acceleration in the last decade. Some endolysins, primarily against gram-positive and gram-negative pathogens of human and animals, developed by various companies, are currently at the stage of preclinical and clinical trials. However, potential of endolysins as antibacterial agents has not been studied yet in aquaculture field, although cultured fish, like other animals and humans, are constantly threatened by microbial attacks. Our research project focuses on the development of novel effective chimeric endolysins with extended lytic activity against Gram-negative and antibiotic resistant bacterium that primary cause of disease in sturgeon aquaculture.
Purpose	Investigation of the therapeutic potential of parental and chimeric endolysins against the Gram-negative and antibiotic resistant bacterium *P. fluorescens, P. putida, A. hydrophila*, *A. salmonicida* and *A. sobria.*
Objectives	1. Isolation and *physiological*, biochemical, molecular identification of *P. putida, P. fluorescens, A. hydrophila, A. salmonicida* and *A. sobria* bacterial pathogens from infected sturgeon fish.

	2. Construction of a chimeric endolysins with extended lytic activity against bacterial pathogens causing of sturgeon fish diseases in aquaculture. 3. Characterization *in vitro* and *in vivo* antibacterial activity of parental and constructed novel chimeric endolysins.
Expected and achieved results	According to research results bacterial isolates were recovered and biochemically characterized . By sequencing the 16S rRNA and gyrB genes bacterial isolates were identified as *A. hydrophila, A. salmonicida, A. veronii, A. bestiarum, P. parafulva* and *P. protegens*. Experimental infection with *A. hydrophila* and *A. salmonicida* at a concentration of 10^8 and 10^{10} CFU/ml in *O. niloticus* and *A. baerii* resulted in 100% mortality. Histopathological changes in experimentally challenged fish were investigated and resulted to pronounced clinical signs and gross pathological lesions. We have constructed 4 novels chimeric endolysins by swapping domains by using synthetic and codon-optimized endolysin genes heterologous origin to modulate specificity and to enhance antibacterial activity. We found a novel chimeric endolysin, Gp110 / LysPA26, which showed enhanced lytic activity against bacteria of the Aeromonas genus compared to its parental endolysin forms. The results obtained in vitro were confirmed by *in vivo* assays because the survival of infected *O. niloticus* was better when *O. niloticus* individuals were treated with endolysin Gp110 or Gp110 / LysPA26 than when treated with the other endolysins. Furthermore, effects of intramuscular injection of Gp110 on wound-healing progression were evaluated in *Acipenser baerii* naturally affected by aeromonosis. The percentage of wound closure in the fish treated with Gp110 was 41.8% on the 6th day, 79% on the 12th day, and 95.7% on the 25th day. Our results show that Gp110 and Gp110 / LysPA26 is a promising candidate for the development of therapeutics against *Aeromonas* infections in aquaculture.
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List of publications with links to them	1. Bakiyev S.S., Bissenbaev A.K. Diseases caused by bacteria of the Aeromonas and Pseudomonas genus when reared fish in controlled systems // Experimental biology. – 2021. № 2. – P.4-16. – DOI: 10.26577/eb.2021.v87.i2.01.
	
	2. Bakiyev S.S., Tilvaldiyeva S.V. Реттелетін жүйелер жағдайында өсірілетін бекіре тұқымдас балықтардың ауруын тудыратын Aeromonas sobria бактериясын биохимиялық және молекулалық-генетикалық идентификациялау [Kaz: Retteletïn jüyeler jağdayında ösiretin bekire tuqımdas balıqtardıñ awrwın twdıratın Aeromonas sobria bakterï bïoxïmïya jäne molekwla-genetïka ulınıñ ïdentïfïkacïyası] // International scientific conference of students and young scientists “Farabi Alemi”. - 2021. – P.260.
	
	3. Bakiyev S.S., Bissenbaev A.K. *Aeromonas hydrophila* from Siberian sturgeon (Acipenser *baerii*) // The 5th Symposium on EuroAsian Biodiversity. – 2021. - P.304.
	
	4. Bakiev S.S., Bisenbaev A.K. Biochemical and molecular genetic identification of the bacterium Pseudomonas putida causing disease in sturgeon fish farmed in regulated systems [Rus: Biokhimicheskaya i molekulyarno-geneticheskaya identifikatsiya bakterii Pseudomonas putida vyzyvayushchaya zabolevaniye osetrovykh ryb, vyrashchivayemykh v usloviyakh reguliruyemykh sistem] // VIII International conference "Modern biotechnology for science and practice". - 2021. – C. 7-8.
	
	5. Bakiyev S., Smekenov I., Zharkova I., Kobegenova S., Sergaliyev N., Absatirov G., Bissenbaev A. Isolation, identification, and characterization of pathogenic Aeromonas hydrophila from critically endangered Acipenser baerii // Aquaculture Reports. – 2022. – Vol. 26. - 101293. - DOI: https://10.1016/j.aqrep.2022.101293 (Web of science: Q1, Scopus: Q1, procentile – 84%).
	
	6. Bakiyev S. S., Smekenov I.T., Baltakhozha N. B., Kauysbekov A., Bissenbaev A.K. Isolation, identification and physiological growth characteristics of Pseudomonas parafulva from diseased Acipenser baerii // International Journal of Biology and Chemistry. – 2022. – Vol. 15, № 2. - P. 18-24. – DOI: 10.26577/ijbch.2022.v15.i2.03.
	
	7. Bakiev S.S. Biology of the bacterium Aeromonas hydrophila isolated from diseased sturgeon fish grown in recirculating water supply installations (Rus: Biologiya bakterii Aeromonas hydrophila vydelennoy iz bol'nykh osetrovykh ryb, vyrashchivayemykh v ustanovkakh zamknutogo vodosnabzheniya (UZV)) // International

	scientific conference of students and young scientists “Farabi Alemi”. - 2022. – P.18. 8. Tilvaldiyeva S.V., Bakiyev S.S. Identification and physiological analysis of the causative agent of sturgeon fish - the bacterium *Aeromonas veronii* based on biochemical and molecular genetic characteristics [Kaz: Bekire tuqımdas balıqtarınıñ patogeni – *Aeromonas veronii* bakterïyasın bïoxïmïyalıq jäne molekwlalıq-genetïkalıq sïpattamaları negizinde ïdentïfïkacïyalaw men fïzïologïyalıq taldaw] // International scientific conference of students and young scientists "Farabi World".- 2022. – P.314. 9. Baltakhozha N.B., Kauysbekov A.Zh., Bakyiev S.S. Isolation, identification and analysis of antibiotic resistance of the sturgeon pathogen *Pseudomonas parafulva* [Bekire tuqımdas balıqtarınıñ patogeni *Pseudomonas parafulva* bakterïyasın bölip alw, ïdenfïkacïyalaw jäne antïbïotïkterge tözimdiligin taldaw] // International scientific conference of students and young scientists "Farabi World». - 2022. – P.283. 10. Bakiyev S., Smekenov I., Zharkova I., Kobegenova S., Sergaliyev N., Absatirov G., Bissenbaev A. Characterization of atypical pathogenic Aeromonas salmonicida isolated from a diseased Siberian sturgeon (Acipenser baerii) // Heliyon. – 2023. – Vol. 9. – P. 1-17. – DOI: 10.1016/j.heliyon.2023.e17775 (Web of science: Q2, Scopus, procentile – 86%). 11. Bakiyev S., Smekenov I., Bissenbaev A. Comparative analysis of potential effects of three phage endolysins against antibiotic-resistant bacteria from the genus Aeromonas //International Aquatic Research. – 2023. – Vol. 15. – P. 249-262. – DOI: 10.22034/IAR.2023.1988163.1454 (Web of science: Q3, Scopus: procentile – 57%). 12. Kauysbekov A.Zh., Bakieyv S.S. Пептидогликан-байланыстырушы ExeA домені бар эндолизиннің химерлі конструкциясын құрастыру және бактерияға қарсы белсенділігін тексеру [Kaz:] // International scientific conference of students and young scientists "Farabi World". – 2023. – P.259.
Patents	-