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MOLECULAR GENETIC ANALYSIS OF THE BACTERIUM *ENTEROBACTER KOBEI*

Annotation. This study presents a molecular genetic analysis of a bacterial strain *Enterobacter kobei* isolated from a natural water reservoir, Karasu (West Kazakhstan region). The aim of the research was to identify the isolated microorganism using modern molecular methods, including cultivation, DNA extraction, 16S rRNA gene PCR amplification, and sequencing on the Oxford Nanopore MinION platform. Water samples were inoculated onto Luria–Bertani (LB) nutrient medium, after which the obtained colonies exhibited typical morphological characteristics of the genus Enterobacter. Genomic DNA was extracted using the Monarch Spin gDNA Extraction Kit and evaluated by agarose gel electrophoresis and fluorometric analysis using the Qubit Flex instrument. For molecular identification, a fragment of the 16S rRNA gene was amplified using universal primers 27-F and 1492-R, followed by sequencing of the obtained product. Sequencing results showed that the studied strain most likely belongs to the species *Enterobacter kobei*, which was confirmed by comparative analysis with a reference sequence from the NCBI database (99.8% identity). The obtained data demonstrate the effectiveness of molecular genetic methods for accurate identification of bacteria isolated from natural sources and highlight their importance for microbiological monitoring of aquatic ecosystems.

Keywords: microorganism; *Enterobacteriaceae*; *Enterobacter kobei;* surface waters; Gram-negative bacteria.

Introduction

The genus *Enterobacter* comprises Gram-negative facultative anaerobic bacteria of the family *Enterobacteriaceae*, widely distributed across various ecological niches, including soil, water, plants, and the human body. Representatives of this genus are of considerable importance from both ecological and medical perspectives, as they can act as opportunistic pathogens and contribute to the development of antimicrobial resistance [1, p. 1].

The species *Enterobacter kobei* belongs to the *Enterobacter cloacae* complex and has relatively recently been recognized as a distinct taxon. This microorganism is found both in clinical samples and in environmental objects, including natural water bodies. Its presence in aquatic environments may indicate microbiological contamination, as well as the bacterium's ability to adapt to diverse habitats [2, p. 888].

In recent years, molecular genetic methods such as 16S rRNA gene sequence analysis, multilocus sequence typing (MLST), and whole-genome sequencing have played a key role in the accurate identification and study of phylogenetic relationships among bacteria. The application of these approaches allows not only for clarification of the taxonomic position of

Enterobacter kobei, but also for the identification of genetic determinants of virulence, antibiotic resistance, and adaptive mechanisms [3, p. 5306; 4, p. 2761; 5, p. 3140; 6, p. 309].

The study of *Enterobacter kobei* strains isolated from natural water bodies is of particular interest in the context of assessing the sanitary condition of aquatic ecosystems and potential risks to human health. Despite the growing number of studies devoted to representatives of the genus *Enterobacter*, data on the molecular genetic characteristics of *E. kobei* circulating in natural waters remain limited [4, p. 2761].

The aim of this study is to perform a molecular genetic analysis of a strain of *Enterobacter kobei* isolated from a natural water source using modern methods of genetic identification and comparative analysis.

Materials and methods

As a sample for bacterial isolation, water from a natural reservoir, Karasu, in the West Kazakhstan region, was used. Sterile tubes were used for sample collection. The collected samples were inoculated onto Luria–Bertani (LB) nutrient medium and incubated for 24 hours at 30 °C.

DNA extraction was performed using the commercial Monarch Spin gDNA Extraction Kit [7, p. 1], after which the quality of the samples was assessed by agarose gel electrophoresis on a 1.5% agarose gel in TAE buffer (40 mM Tris, 1 mM EDTA, 17.4 mM CH_3COOH) [8, p. 1]. Quantitative DNA measurement was carried out using a Qubit Flex Fluorometer with the sDNA HS Assay Kit (Thermo Fisher Scientific) according to the manufacturer's protocol [9, p. 1].

Amplification of the 16S rRNA gene fragment was performed using primers 27-F and 1492-R according to the classical bacterial 16S rRNA gene amplification method [10, p. 698], in a 25 μL PCR reaction containing Taq DNA polymerase, buffer, dNTPs, primers, and template DNA. The program included 30 cycles with denaturation at 94 °C, annealing at 55 °C, and extension at 72 °C, followed by a final extension step of 10 minutes.

Library preparation and sequencing were performed on the MinION Mk1B platform using the SQK-RBK114.96 kit [11, p.1], including barcoding, purification, adapter ligation, and flow cell loading. Sequencing was carried out using MinKNOW, followed by data processing (basecalling and barcode trimming) using Dorado v7.4.12 [12, p. 1].

Research results

During inoculation on Luria–Bertani nutrient medium, bacterial colonies were observed, which were characterized by a size of 1.5–2 mm (Figure 1).

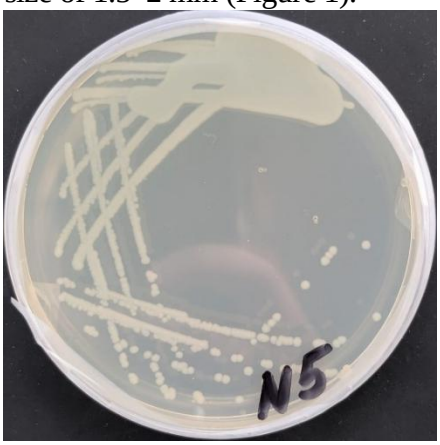

Figure 1 – Growth of bacterial colonies on nutrient medium

Colonies have a circular shape with smooth edges, a smooth and shiny surface, and a slightly convex elevation; the consistency is creamy and soft. The colonies are cream-white in color, without pigmentation.

As a result of the performed sequencing analysis of the 16S rRNA gene nucleotide sequence, it was determined that the isolated bacterium belongs to the species *Enterobacter kobei* (Table 1).

Table 1 – Nucleotide sequence of the 16S rRNA gene

№	Species name	Nucleotide sequence 5′–3′
1	*Enterobacter kobei*	ATWYATYGTYACGACTTCACCCCAGTCATGAATCACAAAGTG GTAAGCGCCCTCCCGAAGGTTAAGCTACCTACTTCTTTTGCAA CCCACTCCCATGGTGTGACGGGCGGTGTGTACAAGGCCCGGG AACGTATTCACCGTAGCATTCTGATCTACGATTACTAGCGATT CCGACTTCATGGAGTCGAGTTGCAGACTCCAATCCGGACTAC GACGCACTTTATGAGGTCCGCTTGCTCTCGCGAGGTCGCTTCT CTTTGTATGCGCCATTGTAGCACGTGTGTAGCCCTACTCGTAA GGGCCATGATGACTTGACGTCATCCCCACCTTCCTCCAGTTTA TCACTGGCAGTCTCCTTTGAGTTCCCGGCCNTAACCGCTGGCA ACAAAGGATAAGGGTTGCGCTCGTTGCGGGACTTAACCCAAC ATTTCACAACACGAGCTGACGACAGCCATGCAGCACCTGTCT CAGAGTTCCCGAAGGCACCAAAGCATCTCTGCTAAGTTCTCT GGATGTCAAGAGTAGGTAAGGTTCTTCGCGTTGCATCGAATT AAACCACATGCTCCACCGCTTGTGCGGGCCCCCGTCAATTCAT TTGAGTTTTAACCTTGCGGCCGTACTCCCCAGGCGGTCGACTT AACGCGTTAGCTCCGGAAGCCACGCCTCAAGGGCACAACCTC CAAGTCGACATCGTTTACGGCGTGGACTACCAGGGTATCTAA TCCTGTTTGCTCCCCACGCTTTCGCACCTGAGCGTCAGTCTTT GTCCAGGGGGCCGCCTTCGCCACCGGTATTCCTCCAGATCTCT ACGCATTTCACCGCTACACCTGGAATTCTACCCCCCTCTACAA GACTCTAGCCTGCCAGTTTCGAATGCAGTTCCCAGGTTGAGCC CGGGGATTTCACATCCGACTTGACAGACCGCCTGCGTGCGCTT TACGCCCAGTAATTCCGATTAACGCTTGCACCCTCCGTATTAC CGCGGCTGCTGGCACGGAGTTAGCCGGTGCTTCTTCTGCGGGT AACGTCAATTGCTGCGGTTATTAACCACAACACCTTCCTCCCC GCTGAAAGTACTTTACAACCCGAAGGCCTTCTTCATACACGC GGCATGGCTGCATCAGGCTTGCGCCCATTGTGCAATATTCCCC ACTGCTGCCTCCCGTAGGAGTCTGGACCGTGTCTCAGTTCCAG TGTGGCTGGTCATCCTCTCAGACCAGCTAGGGATCGTCGCCTA GGTGAGCCATTACCCCACCTACTAGCTAATCCCATCTGGGCAC ATCTGATGGCAAGAGGCCCGAAGGTCCCCCTCTTTGGTCTTGC GACGTTATGCGGTATTAGCTACCGTTTCCAGTAGTTATCCCCC TCCATCAGGCAGTTTCCCAGACATTACTCACCCGTCCGCCACT CGTCACCCGAGAGCAAGCTCTCTGTGCTACCGTTCGACTTGCA TGTGTTAGGCCTGCCGCCAGCGTTCAATCTGAG

The presented nucleotide sequence, with a length of 1485 bp, corresponds in its composition and structure to a fragment of the 16S rRNA gene, which is widely used for bacterial identification and phylogenetic analysis. The sequence contains both highly conserved regions characteristic of Gram-negative bacteria of the family *Enterobacteriaceae* and variable regions that enable taxonomic differentiation at the species level. The presence of ambiguous nucleotides (W, Y, N) indicates positions with possible mixed interpretation of

sequencing signals or polymorphic sites, which may be associated with read quality or intraspecies variability.

Based on conserved motifs (in particular, GC-rich regions and typical conserved domains of the 16S rRNA gene), the sequence demonstrates high homology with members of the genus *Enterobacter*, including the *Enterobacter cloacae* complex, to which *E. kobei* belongs. Overall, the analysis indicates that the studied sample belongs to Gram-negative enterobacteria with a high probability of identification as *Enterobacter kobei*, which requires confirmation through comparison with reference sequences in databases (NCBI BLAST). A 99.8% identity was observed with a previously characterized strain deposited in the NCBI database under accession number CP017181.1.

Conclusion

As a result of the conducted study, a bacterial strain *Enterobacter kobei* isolated from the natural Karasu reservoir was obtained and identified. Morphological analysis of the colonies and subsequent molecular genetic investigation confirmed the affiliation of the isolate to the family *Enterobacteriaceae*. Sequencing of the 16S rRNA gene revealed a high degree of homology (99.8%) with the reference strain of *E. kobei* deposited in the NCBI database.

The obtained results demonstrate the effectiveness of an integrated approach combining microbiological and molecular genetic methods for accurate bacterial identification. The detection of *E. kobei* in a natural water body indicates a potential microbiological load in the aquatic ecosystem and highlights the need for further monitoring of such environments.

Thus, the study expands the understanding of the distribution and molecular characteristics of *Enterobacter kobei* in the natural environment and may serve as a basis for further research in environmental microbiology and epidemiological surveillance.

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nimipressuralis comb. nov. and Lelliottia amnigena comb. nov., respectively, E. gergoviae and E. pyrinus into Pluralibacter gen. nov. as Pluralibacter gergoviae comb. nov. and Pluralibacter pyrinus comb. nov., respectively, E. cowanii, E. radicincitans, E. oryzae and E. arachidis into Kosakonia gen. nov. as Kosakonia cowanii comb. nov., Kosakonia radicincitans comb. nov., Kosakonia oryzae comb. nov. and Kosakonia arachidis comb. nov., respectively, and E. turicensis, E. helveticus and E. pulveris into Cronobacter as Cronobacter zurichensis nom. nov., Cronobacter helveticus comb. nov. and Cronobacter pulveris comb. nov., respectively, and emended description of the genera Enterobacter and Cronobacter / C. Brady, I. Cleenwerck, S. Venter [et al.] // Systematic and Applied Microbiology. – 2013. – Vol. 36, № 5. – P. 309–319. – https://doi.org/10.1016/j.syapm.2013.03.005.

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Әліқул А.Б.

ENTEROBACTER KOBEI БАКТЕРИЯСЫНЫҢ МОЛЕКУЛАЛЫҚ-ГЕНЕТИКАЛЫҚ ТАЛДАУЫ

Аңдатпа. Бұл жұмыста Батыс Қазақстан облысы, Қарасу табиғи су қоймасынан бөлініп алынған *Enterobacter kobei* бактерия штаммының молекулалық-генетикалық талдауы ұсынылған. Зерттеудің мақсаты – заманауи молекулалық әдістерді, соның ішінде культивирлеу, ДНҚ бөліп алу, 16S рРНҚ генін ПТР арқылы амплификациялау және Oxford Nanopore MinION платформасында секвенирлеу арқылы бөлінген микроорганизмді идентификациялау болды. Су үлгілері Луриа–Бертани (LB) қоректік ортасына егілді, кейін алынған колониялар *Enterobacter* туысына тән морфологиялық белгілерді көрсетті. Геномдық ДНҚ Monarch Spin gDNA Extraction Kit көмегімен бөлініп алынып, агарозды гель электрофорезі және Qubit Flex флуориметрі арқылы бағаланды. Молекулалық идентификация үшін 27-F және 1492-R әмбебап праймерлерімен 16S рРНҚ генінің фрагменті амплификацияланып, алынған өнім секвенирленді. Секвенирлеу нәтижелері зерттелген штаммның *Enterobacter kobei* түріне жатуы ықтимал екенін көрсетті, бұл NCBI деректер базасындағы референстік тізбегімен салыстырмалы талдау арқылы (99,8% сәйкестік) расталды. Алынған нәтижелер табиғи көздерден бөлінген бактерияларды дәл идентификациялауда молекулалық-генетикалық әдістердің тиімділігін көрсетеді және су экожүйелерінің микробиологиялық мониторингіндегі маңыздылығын айқындайды.

Кілт сөздер: микроорганизм; *Enterobacteriaceae*; *Enterobacter kobei*; жерүсті сулары; грам-теріс бактериялар.

Әліқул А.Б.

МОЛЕКУЛЯРНО-ГЕНЕТИЧЕСКИЙ АНАЛИЗ БАКТЕРИИ *ENTEROBACTER KOBEI*

Аннотация. В данной работе представлен молекулярно-генетический анализ штамма бактерии *Enterobacter kobei*, выделенного из природного водоёма Карасу (Западно-Казахстанская область). Цель исследования заключалась в идентификации выделенного микроорганизма с использованием современных молекулярных методов, включая культивирование, экстракцию ДНК, ПЦР-амплификацию гена 16S рРНК и секвенирование на платформе Oxford Nanopore MinION. Образцы воды были посеяны на питательную среду Луриа–Бертани, после чего выделенные колонии характеризовались типичными морфологическими признаками представителей рода *Enterobacter*. Геномная ДНК была выделена с использованием набора Monarch Spin gDNA Extraction Kit и оценена методом агарозного электрофореза и флуориметрии на приборе Qubit Flex. Для молекулярной идентификации был амплифицирован фрагмент гена 16S рРНК с универсальными праймерами 27-F и 1492-R, после чего проведено секвенирование полученного продукта. Результаты секвенирования показали, что исследуемый штамм с высокой вероятностью относится к виду *Enterobacter kobei*, что подтверждается сравнительным анализом с референсной последовательностью в базе данных NCBI (идентичность 99,8%). Полученные данные подтверждают эффективность использования молекулярно-генетических методов для точной идентификации бактерий, выделенных из природных источников, и подчеркивают их значение для микробиологического мониторинга водных экосистем.

Ключевые слова: микроорганизм; *Enterobacteriaceae*; *Enterobacter kobei*; поверхностные воды; грамотрицательные бактерии.