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Whole genome sequence analysis and genomic characterization of *Lactococcus petauri* Ksub4 isolated from rainbow trout

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Abstract:

Lactococcosis is one of the significant bacterial infectious diseases observed in fish worldwide. Etiologically, this disease is primarily associated with *Lactococcus garvieae* and, more recently, the newly identified species *L. petauri*. Traditionally, *L. garvieae* has been recognized as the most important and best-characterized pathogen of this disease. However, recent studies have revealed that the role of *L. petauri* in the epidemiology of the disease is increasingly significant. In this study, an isolate (KSUB4) obtained from a trout farm in the Black Sea region in 2022 and confirmed as *Lactococcus* sp. by Polymerase Chain Reaction (PCR) was identified using Whole Genome Sequencing (WGS) analysis. The genome sequencing of the isolate was performed on the Illumina HiSeqX platform, and the raw sequence reads were assembled using the Unicycler v0.4.8 software after undergoing quality control processes. Annotation of the genome was carried out through the NCBI Prokaryotic Genome Automatic Annotation Pipeline (PGAP). Species-level identification of the isolate was performed using the Type Strain Genome Server (TYGS; https://tygs.dsmz.de/) platform. Virulence factors and antimicrobial resistance (AMR) genes were identified using the Virulence Factor Database (VFDB) and the Comprehensive Antibiotic Resistance Database (CARD), respectively. The analyses revealed that the genome of the KSUB4 isolate was 2,014,614 base pairs (bp) in length with a G+C content of 38.13%. Species identification analyses demonstrated that the KSUB4 isolate exhibited 51% digital DNA-DNA hybridization (dDDH) similarity with *L. garvieae* ATCC 49156T and 85.6% dDDH similarity with *L. petauri* 159469T. In the KSUB4 genome, 46 virulence genes with 35-71% similarity and 12 AMR genes with 53-95% similarity were detected. These findings shed light on the genetic potential of *L. petauri* in fish pathogenicity and provide significant insights into the antimicrobial resistance profile of this species.

Keywords: *Lactococcus petauri*, Whole Genome Sequencing, Virulence Genes, Antibiotic Resistance, Aquaculture

Gökkuşağı Alabalığından İzole Edilen *Lactococcus Petauri* Ksub4'ün Tüm Genom Dizisi Analizi ve Genomik Karakterizasyonu

Özet:

Laktobasilloz, dünya çapında balıklarda görülen önemli bakteriyel bulaşıcı hastalıklardan biridir. Etiyolojik olarak, bu hastalık öncelikle *Lactococcus garvieae* ile ve daha yakın zamanda yeni tanımlanan *L. petauri* türü ile ilişkilidir. Geleneksel olarak, *L. garvieae* bu hastalığın en önemli ve en iyi karakterize edilmiş patojeni olarak kabul edilmiştir. Ancak, son çalışmalar *L. petauri*'nin hastalığın epidemiyolojisindeki rolünün giderek daha önemli hale geldiğini ortaya koymuştur. Bu çalışmada, 2022 yılında Karadeniz bölgesindeki bir alabalık çiftliğinden elde edilen ve Polimeraz Zincir Reaksiyonu (PCR) ile *Lactococcus* sp. olarak doğrulanan bir izolat (KSUB4), Tüm Genom Dizileme (WGS) analizi kullanılarak tanımlanmıştır. İzolatın genom dizilemesi Illumina HiSeqX platformunda gerçekleştirildi ve ham dizi okumaları, kalite kontrol süreçlerinden geçtikten sonra Unicycler v0.4.8 yazılımı kullanılarak birleştirildi. Genomun anotasyonu, NCBI Prokaryotik Genom Otomatik Anotasyon Boru Hattı (PGAP) aracılığıyla gerçekleştirildi. İzolatın tür düzeyinde tanımlanması, Type Strain Genome Server (TYGS; https://tygs.dsmz.de/) platformu kullanılarak gerçekleştirildi. Virülans faktörleri ve antimikrobiyal direnç (AMR) genleri, sırasıyla Virülans Faktörü Veritabanı (VFDB) ve Kapsamlı Antibiyotik Direnç Veritabanı (CARD) kullanılarak tanımlandı. Analizler, KSUB4 izolatının genomunun 2.014.614 baz çifti (bp) uzunluğunda ve %38,13 G+C içeriğine sahip olduğunu ortaya koydu. Tür tanımlama analizleri, KSUB4 izolatının *L. garvieae* ATCC 49156T ile %51 dijital DNA-DNA hibridizasyonu (dDDH) benzerliği ve *L. petauri* 159469T ile %85,6 dDDH benzerliği gösterdiğini ortaya koydu. KSUB4 genomunda, %35-71 benzerliğe sahip 46 virülans geni ve %53-95 benzerliğe sahip 12 AMR geni tespit edildi. Bu bulgular, *L. petauri*'nin balık patojenitesindeki genetik potansiyeline ışık tutmakta ve bu türün antimikrobiyal direnç profili hakkında önemli bilgiler sağlamaktadır.

Anahtar kelimeler: *Lactococcus petauri*, Tam Genom Dizileme, Virülans Genleri, Antibiyotik Direnci, Su Ürünleri Yetiştiriciliği

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Introduction

Lactococcosis is a bacterial disease characterized by hemorrhagic septicemia in fish, leading to high mortality rates and significant economic losses, particularly in the rainbow trout industry (Palacios et al., 1993; Vendrell et al., 2006). Until recently, *L. garvieae* was known as the sole etiological agent of the disease. Initially named *Streptococcus garvieae*, this bacterium was first isolated from a bovine mastitis case in the United Kingdom, and this isolate was designated as the reference strain for the species (ATCC 43921) (Collins et al., 1983). In aquaculture, it was isolated from infections in yellowtail fish in 1991 and named *Enterococcus seriolicida* (Kusuda et al., 1991). Subsequently, *L. garvieae* was isolated from various fish species and has shown a wide global distribution. In Turkey, the disease was first reported in 2001 following an outbreak at a rainbow trout farm and has since spread nationwide (Ringo and Gatesoupe, 1998; Vendrell et al., 2006; Diler et al., 2002).

Recent studies have revealed that the etiological agent of lactococcosis is not solely *L. garvieae* but also involves *L. petauri*, which was isolated from a sugar glider in 2017 (Goodman et al., 2017). In aquaculture, *L. petauri* was first identified in rainbow trout during a lactococcosis outbreak (Kotzamanidis et al., 2020). Investigations demonstrated that this isolate is genomically closely related to the *L. garvieae* ATCC 43921 reference strain, showing 95.86% sequence similarity for the 16S rRNA gene and 98.08% similarity for the rpoB gene sequence. Whole genome sequence analyses revealed that the isolate shares 93.54% average nucleotide identity (ANIb) and 50.7% dDDH value with the genome of the *L. garvieae* type strain. Based on these findings, the isolate was designated as *L. petauri* sp. nov. (Goodman et al., 2017).

Recent studies have focused on developing rapid and reliable methods for distinguishing between *L. garvieae* and *L. petauri*. To date, methods targeting Matrix Assisted Laser Desorption/ Ionization Time-of-Flight Mass Spectrometry (MALDI-TOF MS), 16S rRNA gene sequencing, or 16S–23S rRNA gene sequencing have been reported to fail in distinguishing between L. *garvieae* and *L. petauri* (Egger et al., 2023; Martinovic et al., 2021; Vela et al., 2024). However, the most recent studies have demonstrated that differentiation can be achieved through sequencing analysis of the DNA gyrase subunit (gyrB) gene region and whole genome sequencing analysis (Abraham et al., 2023; Heckman et al., 2022). In 2011, the *L. garvieae* Lg-per strain, isolated from rainbow trout in the Black Sea, was reclassified as *L. petauri* following whole genome sequencing analysis (Altinok et al., 2022). Similarly, a strain isolated in Greece in 2007 was also identified as *L. petauri* through whole genome analysis (Kotzamanidis et al., 2020). Additionally, studies using species-specific primers revealed that 137 out of 177 isolates previously identified as *L. garvieae* in our country were, in fact, *L. petauri* (Satıcıoğlu et al., 2023). These developments indicate that isolates previously identified as *L. garvieae* in our country are most likely *L. petauri*.

Bioinformatics analyses have further clarified the presence of virulence factors and antibiotic resistance genes associated with *L. petauri*. Comparative genomic studies have identified several genes that may contribute to its pathogenicity, including those involved in hemolytic activity and capsule formation, which are critical for evading host immune responses (Altun et al., 2025; Chan et al., 2024). Additionally, the presence of antimicrobial resistance genes in *L. petauri* strains raises concerns regarding treatment options in both aquaculture and clinical settings. The identification of multidrug resistance profiles in *L. petauri* isolates underscores the need for ongoing surveillance and the development of targeted antimicrobial strategies (Rosário et al., 2024).

In this study, whole genome sequencing analysis was performed on the KSUB4 isolate, which was obtained from a trout farm in the Black Sea Region in 2022 and confirmed as *Lactococcus* sp. by PCR. The obtained genomic data were subjected to comprehensive bioinformatics analyses, allowing for detailed characterization of the isolate's genomic features.

Material and Methods

Bacterial Isolate

In this study, the KSUB4 strain, preserved in the Samsun Ondokuz Mayıs University Culture Collection and isolated during an outbreak at a commercial rainbow trout (Oncorhynchus mykiss) farm in the Black Sea Region in 2022, was used. The isolate was included in the culture collection after being confirmed as *Lactococcus* sp. using the PCR method. Prior to the study, the isolates stored at -20°C in media containing 15% glycerol were revived by subculturing in Trypticase Soy Broth (TSB) medium and incubating at 25°C for 24 hours.

Whole Genome Sequencing Analysis

DNA extraction from the isolate was performed using a commercial DNA extraction kit (Invitrogen, Canada) based on spin column and filtration principles, following the manufacturer's instructions. In addition to the kit components, a lysozyme lysis buffer containing 0.06 g Tris-HCl, 0.15 g EDTA, 240 µl Triton X-100, and 0.4 g lysozyme in 20 ml was prepared for Gram-positive bacteria.

The quality control of the extracted DNA was conducted using spectrophotometric and fluorometric methods. The quantity and purity of the DNA were determined using a NanoDrop 2000 (Thermo Fisher) device. Prior to whole genome sequencing analysis, the initial concentration of the samples was determined to be >50 ng/µl (10 µl), and absorbance ratios of ~1.8 (260/280) and >1.8 (260/230) were used as reference values for purity assessment.

Library Preparation and Sequencing

TruSeq PCR-free libraries were used during the library preparation stage. Sequencing was performed using the Illumina HiSeq X next-generation sequencing (NGS) platform. Quality control of the data obtained after sequencing was conducted using the FASTQC software (https://www.bioinformatics.babraham.ac.uk).

Assembly and Annotation

The assembly of the raw sequence reads from the isolate was performed using the Unicycler v0.4.8 software available within the PATRIC Web Server 3.6.12 (https://www.patricbrc.org/) (Wick et al., 2017). Genome annotation was carried out using the RAST Tool Kit (RASTtk) (Brettin et al., 2015). Low-quality bases in the Fastq format read data were trimmed to prevent errors in subsequent analysis steps. Quality filtering and trimming processes were performed using the TrimGalore software integrated into the PATRIC Web Server.

Bioinformatics Analysis

Genetic similarities at the species level were determined using ANI analyses, and calculations were performed using the OrthoANIu algorithm available on the EzBioCloud platform (Yoon et al., 2017). To support species differentiation, dDDH analyses and whole genome-based taxonomic analyses of the isolate were conducted using the TYGS (https://tygs.dsmz.de/), a free bioinformatics platform (Meier-Kolthoff and Göker, 2019). Genome-based phylogenetic analyses were performed on the TYGS and Bacterial and Viral Bioinformatics Resource Center (BV-BRC) servers (Meier-Kolthoff and Göker, 2019; Wattam et al., 2017). The identification of antibiotic resistance genes in the isolate's genome was carried out using the CARD (https://card.mcmaster.ca) (McArthur et al., 2013). Virulence genes were identified using the VFDB (http://www.mgc.ac.cn/VFs) (Liu et al., 2022). A phylogenomic tree was constructed using genome sequences of various *L. petauri* strains isolated from different hosts and countries, retrieved from GenBank via the Bacterial and Viral Bioinformatics Resource Center (BV-BRC). The tree was generated using the BV-BRC bacterial genome tree pipeline, following the methodology described by Olson et al. (2023).

Based on all obtained results, the KSUB4 isolate was identified as L. petauri, and the whole genome sequence of the isolate was deposited in the GenBank database under the accession number JAWJYK000000000.

Results

Whole Genome Sequencing Analysis

The isolate KSUB4 was assembled using Unicycler. It was determined that KSUB4 contains 11 contigs, has a genome length of 2,014,641 bp, and an average G+C content of 38.13%. The assembly details of the isolate KSUB4 are provided in Table 1.

The genome of the KSUB4 isolate was annotated using the RAST Tool Kit (RASTtk). It was determined that the genome contains 1910 protein-coding sequences, 41 transfer RNA (tRNA) genes, and 4 ribosomal RNA (rRNA) genes. The annotation features of the genome are provided in Table 2.

Table 1. Assembly Details of the KSUB4 Genome

Number of Contigs	11
GC Content	38.13
Number of Plasmids	0
Contig L50	2
Genome Length	2,014,641 bp
Contig N50	496,485
Number of Chromosomes	0

Table 2. Annotated Genomic Features of the KSUB4 Isolate

CDS	1910
tRNA	41
rRNA	4
Partial CDS	0
Miscellaneous RNA	0
Repeat Regions	0

Bioinformatics Analysis

As presented in Table 3, species-level classification of the KSUB4 isolate was performed using dDDH and ANI analyses. The dDDH analyses revealed that the KSUB4 isolate exhibited 51% similarity with *L. garvieae* ATCC 49156T and 85.6% similarity with *L. petauri* 159469T, which is above the species delineation threshold of 70%.

Table 3. Comparative Genomic Analysis of the KSUB4 Genome with Type Strains and Determination of dDDH Values.

Analyzed Strain	Target Strains	dDDH (d0, in %)	C.I. (d0, in %)	dDDH (d4, in %)	C.I. (d4, in %)	dDDH (d6, in %)	C.I. (d6, in %)	G+C content difference (in %)
KSUB4	*L. petauri 159469*	79,6	[75.7 - 83.1]	85,6	[82.9 - 87.9]	83,6	[80.3 - 86.4]	0,46
KSUB4	*Halomonas borealis ATF 5.2*	12,7	[10.0 - 16.0]	73,8	[70.8 - 76.6]	13,1	[10.8 - 15.9]	28,31
KSUB4	*Halomonas niordiana ATF 5.4*	12,7	[10.1 - 16.0]	72,2	[69.2 - 75.0]	13,2	[10.8 - 15.9]	22,96
KSUB4	*L. formosensis subsp. bovis LMG 30663*	67,5	[63.6 - 71.1]	51,2	[48.6 - 53.9]	65,6	[62.2 - 68.8]	0,6
KSUB4	*L. formosensis subsp. bovis DSM 100577*	67,2	[63.3 - 70.8]	51,1	[48.4 - 53.7]	65,3	[61.9 - 68.5]	0,6
KSUB4	*Enterococcus seriolicida ATCC 49156*	78	[74.1 - 81.6]	51	[48.3 - 53.6]	74,3	[70.8 - 77.5]	0,69
KSUB4	*L. garvieae DSM 20684*	74,7	[70.7 - 78.3]	50,8	[48.1 - 53.4]	71,5	[68.0 - 74.7]	0,38
KSUB4	*L. garvieae NBRC 100934*	74,6	[70.6 - 78.2]	50,8	[48.2 - 53.5]	71,4	[68.0 - 74.7]	0,39
KSUB4	*L. formosensis NBRC109475*	73,9	[69.9 - 77.5]	49,3	[46.7 - 51.9]	70,3	[66.9 - 73.6]	0,24
KSUB4	*L. lactis JCM 5805*	13,8	[11.0 - 17.1]	22	[19.7 - 24.4]	14,1	[11.7 - 16.9]	2,94
KSUB4	*L. lactis ATCC 19435*	13,8	[11.0 - 17.1]	21,9	[19.7 - 24.4]	14,1	[11.7 - 16.9]	2,91
KSUB4	*L. cremoris NBRC 100676*	13,7	[10.9 - 17.0]	21,8	[19.6 - 24.3]	14	[11.6 - 16.8]	2,51
KSUB4	*L. cremoris subsp. tructae DSM 21502*	13,7	[10.9 - 17.0]	21,6	[19.3 - 24.0]	14	[11.6 - 16.8]	2,64

Furthermore, OrthoANIu analyses demonstrated the genomic similarity of the KSUB4 isolate with *L. petauri* 159469T (98.44%), L. garvieae ATCC 49156T (93.12%), and *Halomonas borealis* ATF 5.2 (65.50%).

Antibiotic resistance genes associated with the KSUB4 isolate were analyzed using the Resistance Gene Identifier (RGI) tool against the CARD. The analysis revealed the presence of genes suggesting potential resistance to various antibiotic classes. Using the strict algorithm, resistance genes with high similarity percentages, such as *lsa*D (94.57%), *van*T (34.15%), and *van*Y (30.26%), were identified. The *lsa*D gene confers resistance to lincosamides, streptogramins, and pleuromutilins through a target protection mechanism, while the *van*T and *van*Y genes mediate resistance to glycopeptide antibiotics (e.g., vancomycin) by modifying the antibiotic target. In analyses performed using the loose algorithm, the presence of resistance genes such as *eme*A (77.44%), *rpo*B (59.83%), *lmr*D, *pat*A, *gyr*A, and *gyr*B was detected. The *eme*A gene acts as a multidrug and toxic compound extrusion (MATE) transporter, providing resistance to disinfectants, while the *rpo*B gene, found in *Mycobacterium tuberculosis*, contributes to rifampin resistance by inducing mutations in the beta subunit of RNA polymerase.

Analysis of the VFDB (Virulence Factors Database) revealed a total of 46 virulence genes in the KSUB4 genome (with similarity percentages ranging between 35% and 71%) Among the identified virulence genes, adhesion genes such as *pav*A, *ssp*A, and *ssp*B, which support the ability to adhere to and colonize host tissues, were particularly prominent. Additionally, the presence of *spy*A and *sag*A genes, which contribute to tissue damage and the triggering of immune responses by inducing toxic effects in host cells, was noteworthy. Genes associated with capsule synthesis, which facilitate immune evasion, as well as hyl and eno genes, which enhance tissue invasion and energy metabolism, further increase the pathogenic potential of the isolate. Moreover, the presence of the *psa*A gene, which enables the uptake of trace elements essential for bacterial survival, supports the KSUB4 isolate's ability to adapt to environmental conditions and persist within the host.

Phylogenetic Analysis

In this study, the phylogenetic position of the *L. petauri* KSUB4 isolate was determined using 16S rRNA gene sequencing and whole genome-based phylogenetic analyses. Both approaches confirmed that the KSUB4 isolate is positioned within the *L. petauri* species. However, the differences between the phylogenetic trees indicate that whole genome-based phylogenetic analyses provide a more precise resolution of intra- and interspecies relationships.

The 16S rRNA-based phylogenetic tree (Figure 1) shows that the KSUB4 isolate forms a monophyletic group with the *L. petauri* 159469 strain with high bootstrap support (bootstrap = 100). Additionally, the *L. petauri* clade was found to have a close evolutionary relationship with *L. garvieae* and *E. seriolicida*.

In the phylogenetic analysis conducted using whole-genome sequencing data, the KSUB4 isolate was determined to be directly affiliated with *L. petauri* 159469 (Figure 2). However, the phylogenetic placement of *L. garvieae* was classified in a markedly distinct manner compared to the 16S rRNA tree, with this species exhibiting a more pronounced divergence from *L. petauri*. Furthermore, the position of *E. seriolicida* also demonstrated a clear differentiation in the whole-genome analysis.

Phylogenomic analysis placed *L. petauri* strain KSUB4 within a well-supported monophyletic clade that includes strains R21-77-2, 970, 04-8782, and LG6, all of which were isolated from rainbow trout. KSUB4 was most closely related to strain R21-77-2, forming a sister grouping with 100% bootstrap support.

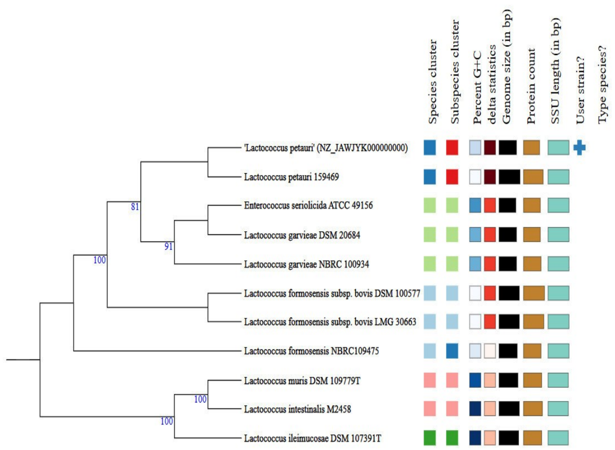

Figure 1. Phylogenetic tree based on the 16S rRNA gene region illustrating the relationship of the KSUB4 strain with closely related type strains. Species and subspecies clusters are color-coded to aid in taxonomic classification. Gradients in GC content and delta (δ) statistics columns facilitate visual comparison across strains. Genome size and predicted protein count are illustrated using bar lengths, with longer bars indicating larger genome assemblies or higher numbers of encoded proteins.

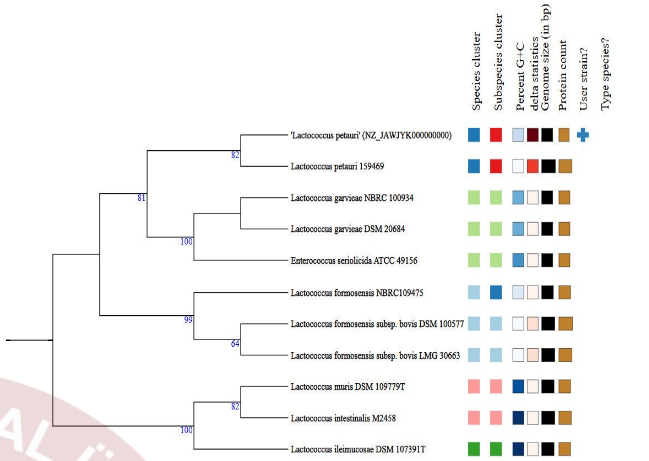

Figure 2. Phylogenetic tree based on whole genome sequence analysis illustrating the relationship of the KSUB4 strain with closely related type strains. Right-hand side columns provide additional genomic information: species and subspecies clusters are represented with different colors for classification purposes. Color gradients in GC content and delta statistics (δ values) columns are for visual comparison. Genome size (bp) and protein count columns are visually represented by bars, where longer bars correspond to larger genomes or higher protein counts.

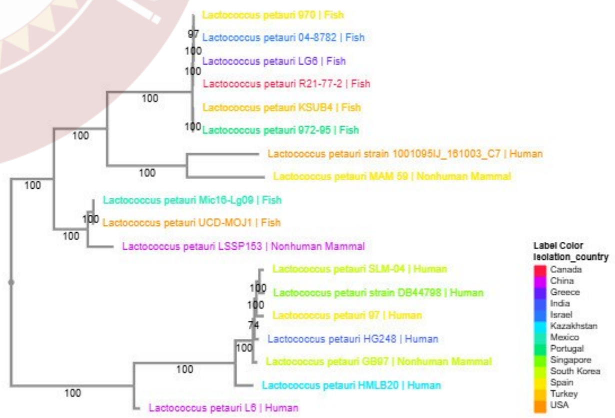

Figure 3. Phylogenomic tree of 18 *L. petauri* strains based on 1000 single-copy core genes. Bootstrap values ≥74 are shown at the nodes. Strain labels include host origin and are color-coded by isolation country.

This clade was clearly separated from other *L. petauri* strains of human and pig origin. The phylogenomic tree was constructed using 1000 single-copy core genes across 18 genomes, aligned with MAFFT, and inferred using RAxML with rapid bootstrapping. All major branches, including the clade containing KSUB4, received high bootstrap values, supporting the robustness of the inferred evolutionary relationships (Figure 3).

Discussion

L. petauri has emerged as a significant pathogen in aquaculture, particularly playing a role in the etiology of fish diseases such as lactococcosis. This disease causes substantial economic losses in fish populations, underscoring the importance of understanding the pathogenic mechanisms of *L. petauri*. Initially isolated from a sugar glider (Petaurus *breviceps*), *L. petauri* has since been reported to exhibit pathogenic potential in both fish and mammals (Goodman et al., 2017; Kotzamanidis et al., 2020; Sciuto et al., 2024; Stoppani et al., 2023). Whole-genome sequence analyses have revealed that the 159469T strain exhibits an ANIb of 93.54% and a dDDH value of 50.7% compared to the type strain of *L. garvieae*. This high genetic similarity suggests that some strains previously identified as *L. garvieae* may, in fact, belong to *L. petauri* (Altınok et al., 2022; Kotzamanidis et al., 2020). In this study, an isolate (KSUB4) obtained from a trout farm in the Black Sea region in 2022 and confirmed as *Lactococcus* sp. by PCR was subjected to whole-genome sequencing for species identification. ANI and dDDH analyses demonstrated that the KSUB4 isolate exhibited 93.12% and 51% similarity, respectively, with the *L. garvieae* ATCC 49156T strain, and 98.44% and 85.6% similarity with the *L. petauri* 159469T strain. These results confirm that the KSUB4 isolate belongs to the species *L. petauri*.

Phylogenetic analyses, based on both 16S rRNA gene sequences and whole-genome approaches, confirmed that the KSUB4 isolate clusters within the *L. petauri* clade. However, it was observed that 16S rRNA-based analyses are insufficient in reflecting intraspecies genetic variations. Specifically, the 16S rRNA gene lacks the necessary discriminatory power to differentiate closely related species such as *L. petauri* and *L. garvieae*. This finding supports the hypothesis that the isolate exhibits high genetic similarity with *L. garvieae* and that some strains previously classified as *L. garvieae* may, in fact, belong to *L. petauri*. Furthermore, whole-genome analyses demonstrate a clearer resolution of genetic relationships between species (Goodman et al., 2017; Altınok et al., 2022). Additionally, Satıcıoğlu et al. (2023) reported that phenotypic tests, such as sucrose fermentation, and the design of species-specific primers have proven highly effective in distinguishing these two species.

The phylogenomic positioning of *Lactococcus petauri* KSUB4 within a distinct clade comprising exclusively rainbow trout-derived isolates suggests a potential host-associated lineage within the species. Its closest relationship to strain R21-77-2, along with consistent clustering with strains 970, 04-8782, and LG6, all originating from salmonid hosts, points to a possible evolutionary adaptation of this group to fish. The high bootstrap values observed throughout the clade reinforce the genetic cohesion of these piscine isolates and may indicate clonal expansion or selective pressures unique to the aquaculture environment. In contrast, the clear phylogenetic separation from strains isolated from humans and pigs implies host-driven genomic divergence within *L. petauri*. These findings support the hypothesis that strain KSUB4 represents part of a specialized sublineage adapted to fish, warranting further investigation into specific genomic features and potential virulence mechanisms that contribute to host specificity and pathogenicity in aquaculture systems.

The emergence of *L. petauri* as a pathogen in fish, particularly in aquaculture environments, raises significant concerns due to its notable antibiotic resistance profiles (Rosário et al., 2024; Egger et al., 2023). In this study, whole-genome analysis of the KSUB4 *L. petauri* isolate identified various antibiotic resistance genes (ARGs) and resistance mechanisms using the CARD. According to the findings, the *lsa*D gene exhibits a "target protection" mechanism against lincosamides, streptogramins, and pleuromutilins, while the *eme*A gene demonstrates an "antibiotic efflux" mechanism, facilitating the expulsion of multiple drugs and toxic compounds. Mutations in *rps*E and *fus*A confer resistance to spectinomycin and fusidic acid through "target alteration," whereas *lmr*D and *pat*A genes play roles in lincosamide and fluoroquinolone resistance. Additionally, *rpo*B mutations exhibit both "target alteration" and "target replacement" mechanisms against rifamycins. The *gyr*B and *mur*A genes confer resistance to fluoroquinolones and fosfomycin, respectively, while the *van*Y and *van*F clusters provide resistance to glycopeptide antibiotics. The diversity of these genes and their resistance mechanisms highlights the strong multidrug resistance potential of *L. petauri*. The high multidrug resistance profile identified in this study aligns with findings from other studies in the literature. For instance, Rosário et al. (2024) reported that *L. petauri* exhibits resistance rates of up to 87.5% against antibiotics such as trimethoprim/ sulfamethoxazole and norfloxacin. Similarly, the data obtained in this study support the presence of strong resistance to fluoroquinolones. The identification of fluoroquinolone-resistant gyrB and patA genes suggests that the use of this antibiotic class in aquaculture may be limited. Öztürk et al. (2024) reported a wide range of ARGs in *L. petauri* and *L. garvieae* isolates from different species and countries. ARGs were found in 97.3% of the isolates, with the most prevalent resistance genes including *gyr*A (78.2%) and *qnr*A (80.9%), contributing to quinolone resistance, and *str*B (42.7%), associated with aminoglycoside resistance. Tetracycline (*tet*B, *tet*C, *tet*G) and macrolide (*erm*B, *erm*C) resistance genes were also identified. In terms of geographical distribution, *gyr*A and *qnr*A genes were prevalent in isolates from Turkey and Spain, while similar resistance profiles were observed in isolates from Italy and Greece. These findings underscore the resistance potential of *L. petauri* and *L. garvieae* isolates, particularly against quinolones, aminoglycosides, and macrolides. In another study, Satıcıoğlu et al. (2023) detected the presence of various ARGs in *L. petauri*. Notably, they reported the prominence of the RF0133 ribosomal protection protein, responsible for tetracycline resistance, and a subclass B1 beta-lactamase gene conferring resistance to penicillins, cephalosporins, and carbapenems. Additionally, the MFS efflux pump contributed to quinolone and tetracycline resistance, while the chloramphenicol acetyltransferase (CAT) gene played a role in chloramphenicol resistance. These findings demonstrate the potential of *L. petauri* to develop resistance to a broad spectrum of antibiotics. The implementation of effective control measures and the development of targeted antimicrobial therapies depend on accurate identification. The resistance genes in *L. petauri* not only reduce treatment efficacy but also pose a concerning risk of horizontal gene transfer to other bacteria via mobile genetic elements. This could facilitate the rapid spread of resistant strains in aquatic environments and contribute to the expansion of environmental resistance reservoirs. In addition to its impact on aquaculture, *L. petauri* has also been implicated in human infections, further emphasizing the importance of understanding its resistance mechanisms. Studies have documented cases of urinary tract infections caused by *L. petauri*, highlighting its potential as an opportunistic pathogen in humans (Colussi et al., 2023). This dual role as both a fish and human pathogen underscores the need for comprehensive studies on its resistance profiles and zoonotic transmission potential. The application of advanced genomic techniques has provided detailed insights into the phylogenetic relationships among *Lactococcus* species, revealing their genetic diversity and potential for horizontal gene transfer. Such studies are crucial for elucidating the evolutionary pathways contributing to the development of antibiotic resistance in *L. petauri* and its relatives.

Initially classified as *L. garvieae*, *L. petauri* has been reclassified based on genomic analyses, revealing significant genetic similarities while also uncovering distinct pathogenic traits that warrant further investigation into its virulence genes and pathogenicity mechanisms (Mahmoud et al., 2023; Egger et al., 2022; Egger et al., 2023). Understanding the virulence factors of *L. petauri* is crucial, as it plays a role in lactococcosis, a serious disease affecting various fish species and causing significant economic losses in aquaculture (Egger et al., 2023; Meyburgh et al., 2017). In this study, the presence of 46 virulence genes identified in the KSUB4 isolate supports the pathogenic potential of *L. petauri*. The detection of adhesion genes such as *pavA*, *sspA*, and *ssp*B, which facilitate attachment to and colonization of host tissues, underscores the bacterium's ability to initiate infections. Additionally, the *spyA* and *sag*A genes identified in this study may contribute to tissue damage and the triggering of immune responses by inducing toxic effects in host cells. Similarly, Satıcıoğlu et al. (2023) confirmed the presence of these toxin genes in *L. petauri* strains and suggested that these genes may enhance the bacterium's invasive capabilities. Notably, the presence of genes associated with capsule synthesis indicates the bacterium's ability to evade the immune system and develop resistance to phagocytosis.

According to studies in the literature, Altun et al. (2025) conducted a comparative analysis of virulence genes in *L. garvieae* and *L. petauri* isolates. Genomic analyses revealed that both species possess various adhesion and virulence factors, including hemolysin III-related proteins, collagenases, platelet-binding proteins (adhesin Pav), LPXTG surface proteins, internalin-like proteins, sortase A, and mucus adhesins. However, it was found that *L. petauri* isolates carry a greater number of virulence genes compared to *L. garvieae*. Notably, unlike the *L. garvieae* Lg2 strain, which possesses a capsule gene cluster, all isolates examined in this study were found to lack the capsule gene cluster. These findings explain the increasing pathogenic role of *L. petauri* in fish lactococcosis. In their study, Kotzamanidis et al. (2020) performed a genomic analysis of the *L. petauri* LG_SAV_20 strain, revealing the presence of various virulence factors that may play a role in the pathogenesis of fish lactococcosis. Genes associated with host cell adhesion and invasion mechanisms, such as fibronectin-binding proteins, LPATG motif-containing surface proteins, hemolysin, and NADH oxidase, were identified in the genome. Additionally, potential virulence factors, including siderophore carrier genes related to iron uptake and a collagenase gene facilitating extracellular matrix binding, were also detected. These findings indicate that the *L. petauri* LG_SAV_20 strain shares similar virulence mechanisms with fish pathogens like *L. garvieae* and may play a significant role in the etiology of lactococcosis.

Chan et al. (2024) conducted a comparative analysis of virulence gene profiles in 212 isolates of *L. petauri*, *L. formosensis*, and *L. garvieae*. A total of 68 virulence genes were identified, including 29 adhesion genes, 9 iron uptake genes, 3 hemolysin genes, 7 enzyme-related virulence genes, 15 capsular polysaccharide (cps) cluster genes, and 5 biofilm-enhancing genes (bee locus). Among the adhesion genes, 12 (*adhCI, adhCII, adhPavA, adhPsaA, CLase, srt2, LPxTG-5, LPxTG -6, pili-1, srtC, pili-2, pili-3*) were commonly found in all three species, while 14 adhesion genes (sp-CnaB, sp-COG4713, srt3, WxLsp, adh, CnaB1, inl, LPxTG-1, LPxTG-2, LPxTG-3, LPxTG-4, MucAd1, MucAd2, pilsAg) showed significant differences among the species. Seven iron uptake genes (*fecB, fecD, fecE, feoA, feoB, fepB, fep*D) were present in all isolates, while the bsh1 gene was significantly more prevalent in *L. petauri* compared to the other species. Capsular polysaccharide cluster genes were detected in only 21 isolates (10%). These findings highlight significant differences in virulence gene composition among species and sources, providing important insights into host specificity and pathogenicity mechanisms of these bacteria. In another study, Sciuto et al. (2024) reported the presence of virulence factors in *L. petauri*, including adhesins (AdhCl, AdhPsaA, AdhPar), superoxide dismutase (Sod), hemolysins (Hly2, Hly3), and surface proteins (LpxTg2, LpxTg3). Adhesins facilitate bacterial attachment to host cell surfaces and evasion of mucosal secretions, thereby supporting colonization, while superoxide dismutase (Sod) protects the bacterium against oxidative stress. Hemolysins enhance pathogenicity by causing lysis of erythrocytes and leukocytes, and LPxTG surface proteins contribute to bacterial virulence by binding to the peptidoglycan layer. The study also identified differences in virulence genes between human- and dog-derived isolates; for example, enolase (eno), phosphoglucomutase (pgm), and NADH oxidase genes were only detected in human isolates. These findings suggest that L. petauri's virulence mechanisms may adaptively vary across different host species, and the role of these genes in pathogenicity may be host specific. Our findings are largely consistent with the literature. The hasC and EF0818 genes identified in the KSUB4 isolate are thought to contribute to increased pathogenicity by interacting with extracellular matrix components. Additionally, the clpP gene has been shown to play a critical role in managing stress responses and enabling adaptation to environmental conditions. The virulence genes identified in the KSUB4 isolate provide valuable insights into the pathogenicity and infection potential of *L. petauri*. Compared to other studies in the literature, the KSUB4 isolate demonstrates a strong potential in adhesion and invasion mechanisms.

In this study, the whole genome sequence analysis of the KSUB4 isolate obtained from a trout farm in the Black Sea Region was performed, and its genomic characteristics were examined in detail using bioinformatic methods. The obtained results clearly demonstrated that the KSUB4 isolate exhibits high genetic similarity (98.44% ANI, 85.6% dDDH) and belongs to the species *L. petauri*. Virulence gene analysis identified the presence of 46 different virulence genes in the KSUB4 isolate, highlighting genes such as *pavA, sspA, spyA, and sagA*, which contribute to host tissue adhesion, immune system evasion, and environmental adaptation capabilities. Antibiotic resistance analysis revealed the presence of genes such as *lsaD, vanT*, and *vanY* in the KSUB4 isolate, which confer resistance to glycopeptide and lincosamide antibiotics. This indicates that the isolate carries potential multidrug resistance, necessitating careful consideration in treatment management.

This study highlights the pathogenic potential of *L. petauri* in aquaculture and its possible zoonotic risks. The findings provide significant contributions to both the protection of aquatic ecosystems and public health. Furthermore, the study underscores the critical role of genomic analyses in accurate species identification and the development of targeted treatment strategies.

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