



Phenotypic and phylogenomic characterization of *Lactococcus garvieae* isolates from rainbow trout (*Oncorhynchus mykiss*) in Türkiye

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Abstract

Lactococcosis is an important bacterial disease of farmed fish and causes substantial economic losses in rainbow trout (*Oncorhynchus mykiss*) aquaculture. In this study, *Lactococcus garvieae* isolates recovered from rainbow trout farms in Türkiye were characterized using phenotypic, molecular, and phylogenomic methods. Among 32 presumptive *Lactococcus* isolates recovered from 127 dead rainbow trout, four were confirmed as *L. garvieae* and exhibited identical biochemical characteristics, Pulsed Field Gel Electrophoresis (PFGE) profiles, and broad growth tolerance across different pH, salinity, and temperature conditions. All isolates were presumptively classified as resistant to ciprofloxacin and florfenicol, while remaining susceptible to tetracycline and penicillin. Based on the AMR profiles, strain LG2, which exhibited the most susceptible antimicrobial profile among the isolates, was selected for whole-genome sequencing (WGS). WGS of the representative isolate LG2 generated a single 2,214,687-bp chromosomal contig with 38.5% GC content and 99.0% BUSCO completeness. In silico PCR assigned LG2 to serotype I, and the genome contained an intact capsule-associated *cps/kps* locus. The chromosomal *lsa(D)* determinant and an *mdt(A)*-like efflux-associated gene were detected, whereas no plasmid replicons or acquired quinolone or florfenicol resistance genes were identified, indicating discordance between the phenotypic and genomic AMR results. Taxonomic verification of 236 publicly available *Lactococcus* assemblies yielded 41 verified public *L. garvieae* genomes, which, together with LG2, formed a 42-genome within-species dataset. LG2 was most closely related to the Turkish isolate OS-37, sharing 99.96% ANI and differing by three core SNPs; both belonged to ST109, whereas the other Turkish isolates belonged to ST139. cgMLST identified a conserved genomic backbone, while pan-genome analysis identified 5,655 gene clusters and an open pan-genome characterized by a large cloud-gene fraction. These findings demonstrate the importance of species verification in *Lactococcus* population genomics and reveal substantial accessory-genome diversity within *L. garvieae*. The genomic features of LG2 provide a basis for future pathogenicity and immunogenicity studies, although experimental validation is required. Overall, these findings highlight the importance of local genomic surveillance for understanding *L. garvieae* population structure and provide a genomic framework for future region-specific vaccine research.

Keywords *Lactococcus garvieae* · Whole-genome sequencing (WGS) · Phylogenomics · Rainbow trout · *Oncorhynchus mykiss* · Türkiye

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Introduction

Lactococcosis is an emerging disease that causes significant economic losses globally in both marine and freshwater aquaculture, particularly when water temperatures exceed 15 °C (Austin and Austin 2007). The primary etiological agent of the disease is *Lactococcus garvieae*, which is one of the most frequently isolated bacterial pathogens and causes high mortality (50–80%) in rainbow trout (*Oncorhynchus mykiss*). Initially described as a causative agent of bovine mastitis, it was later recognized as an important pathogen of intensive rainbow trout farming worldwide (Collins et al. 1983; Diler et al. 2002; Duman et al. 2020; Meyburgh et al. 2017; Vendrell et al. 2006).

L. garvieae was first reported in rainbow trout farms in Spain in 1988 (Palacios et al. 1993). Thereafter, the pathogen has become a major threat to aquaculture in numerous countries, particularly rainbow trout farming, in Europe, Africa, Asia, the Middle East, Australia, and countries such as South Africa and Türkiye (Shahi et al. 2018; Vendrell et al. 2006). In Türkiye, the first outbreak was reported in 2001, leading to mortality rates of up to 80% (Diler et al. 2002). Since then, it has been recognized as one of the most challenging pathogens in Turkish trout farms during the summer.

L. garvieae has been isolated from various sources, such as humans, animals, and food products, which suggests its zoonotic potential (Miyachi et al. 2012). Another potential zoonotic agent, given that it has been isolated from humans, other warm-blooded animals, and dairy products, is *Lactococcus petauri*, which is ecologically and genotypically similar to *L. garvieae* (Goodman et al. 2017; Huang et al. 2021; Li et al. 2024; Martinovic et al. 2021; Ou et al. 2020). *L. petauri* has also been linked to lactococcosis in fish, with some isolates previously identified as *L. garvieae* now reclassified as *L. petauri*. Underpinned by their close genetic and evolutionary relationship, both species cause similar clinical signs in fish and impose significant economic losses in aquaculture due to high mortality, reduced growth, and high treatment costs (Altinok et al. 2022; Martinovic et al. 2021).

The routes of transmission are horizontal via contaminated water or direct contact with infected fish (Eynogor et al. 2004; Muzquiz et al. 1999). Subclinical carriers, such as rainbow trout, can harbor *L. garvieae* for up to a year, thereby facilitating disease spread (Algöet et al. 2009; Zlotkin et al. 1998). Vectors like birds and wild fish can act as mechanical vectors for prolonged periods, thereby facilitating, under favorable conditions, bacterial transmission to farmed populations (Algöet et al. 2009; Shahin et al. 2022).

Clinical signs of lactococcosis include anorexia, melanosis, exophthalmia, and hemorrhages in the periorbital

and intraocular regions, including at the base of the fins. Affected fish may also exhibit abdominal distention and anal prolapse (Bekker et al. 2011; Eldar and Ghittino 1999).

L. garvieae has also been implicated in a range of human infections, including blood, bone, urinary tract, and skin infections, pneumonia, and endocarditis. Cases have been reported in various countries, such as the USA, Canada, France, and the UK, in both immunocompromised and healthy individuals (Vendrell et al. 2006).

Two capsule phenotypes, capsulated (KG−) and non-capsulated (KG+), have been examined to determine their relationship with *L. garvieae* pathogenicity in rainbow trout. Capsulated strains (KG−) are generally more virulent than non-capsulated strains (KG+) (Bastardo et al. 2012; Ture and Altinok 2016; Vendrell et al. 2006). However, capsule production alone does not fully explain pathogenicity. Although the non-capsulated Lg-per strain was previously reported to be highly pathogenic, this strain has since been reclassified as *L. petauri* (Altinok et al. 2022). In contrast, the non-capsulated *L. garvieae* ATCC 49,156 strain has also been reported to cause high mortality in experimentally infected rainbow trout, suggesting that factors other than capsule production may contribute to pathogenicity (Ture and Altinok 2016).

While lactococcosis is mostly treated with antibiotics, most strains have gained resistance (Raissy and Ansari 2011; Ture and Boran 2015; Yesiltas et al. 2019). Notably, *L. garvieae* infection in fish can be controlled by vaccination (Rao et al. 2020). The most common vaccine administration routes are oral, injection, and immersion. While injection and immersion offer strong protection, routine implementation in large-scale aquaculture is limited by the need for extensive fish handling and high labor costs (Halimi et al. 2020).

The present study aimed to isolate and characterize *L. garvieae* strains from rainbow trout farms in Türkiye using phenotypic, molecular, and comparative genomic approaches to investigate the phenotypic characteristics and genomic context of locally circulating *L. garvieae* isolates and to assess the relatedness of the representative strain LG2 within the available species-verified genome collection.

Materials and methods

Isolation of *Lactococcus* strains

A total of 127 rainbow trout suspected of lactococcosis were collected from four commercial rainbow trout farms located along the Manavgat River in Antalya Province, Türkiye. Farm A ($n=35$), Farm B ($n=28$), Farm C ($n=31$) and Farm D ($n=33$) contributed to the sampling. The fish



Fig. 1 Lactococcosis-related necropsy findings 1- *Exophthalmos*, 2- *Abdominal swelling*, 3- *Hemorrhage*

Table 1 Primers used for glycosyltransferase family 4 protein gene

Primer	Sequence (5' to 3')	Product Size (bp)	Reference
LG_IBS-F	ACAGAGCATGGGACGACCTA	132	Saticioglu et al. 2023
LG_IBS-R	CTGCTTCTTGAGAGACGCCA		

were transported under cold chain conditions to the Ankara University Faculty of Veterinary Medicine, Department of Microbiology. Necropsies were performed on lactococcosis-suspected fish, and kidney and liver tissues were collected for bacterial isolation (Fig. 1). The samples were cultured on tryptic soy agar (TSA; Oxoid) and 5% defibrinated sheep blood agar and incubated for 24–96 h at 28 °C. After incubation, the colonies were Gram-stained. Gram-positive, cocci-shaped colonies were tested for catalase and oxidase activity. Presumptive *Lactococcus* isolates were stored in glycerol-based medium at –80 °C until further analysis.

DNA extraction and polymerase chain reaction

Genomic DNA was extracted from pure cultures of all the strains included in the study using the HiPurA® Multi-Sample DNA Purification Kit (HiMedia, India) following the manufacturer's instructions.

For molecular diagnosis of *L. garvieae*, the glycosyltransferase family 4 (*gtf4*) protein gene was amplified by PCR using specific primers (Table 1). The PCR conditions were optimized according to Saticioglu et al. (2023). Briefly, the total volume of 25 µl PCR mix consisted of 0.2 µM of each primer, 3 µL MgCl₂ (Thermo Fischer Scientific, USA), 0.2 mM dNTPs (10 mM dNTP mix; Thermo Fischer Scientific, USA), 2.5 µL PCR reaction buffer, 2U Taq DNA polymerase (Thermo Fischer Scientific, USA), and 2 µl template DNA. The PCR conditions were as follows: 94 °C

for 3 min, followed by 30 cycles of 94 °C for 1 min, 54 °C for 1 min, and 72 °C for 1 min, with a final extension at 72 °C for 7 min. The previously characterized *L. garvieae* reference strain OMU_LG_1, confirmed by whole-genome sequencing in a published study, was used as the positive control (Saticioglu et al. 2023). Isolates showing sucrose-positive phenotypes were further examined using the *L. petauri*-specific LP_IBS primers according to Saticioglu et al. (2023). PCR products were examined through electrophoresis on a 1.5% agarose gel (Promega Corporation, USA) and visualized using a UV transilluminator.

Sugar fermentation and biochemical tests of *Lactococcus* isolates

All presumptive *Lactococcus* isolates stored at –80 °C were re-cultured on TSA, then incubated aerobically at 28 °C for 24–96 h. Biochemical characterization, including the sucrose fermentation test used for differentiation of *L. garvieae* and *L. petauri*, was performed according to Saticioglu et al. (2023) using the API Rapid ID 32 Strep kit (bioMérieux, France) in accordance with the manufacturer's instructions. The *L. garvieae* strain OMU_LG_1 was included as the positive control in all biochemical tests.

Phenotypic characterization

The following phenotypic analyses were performed exclusively on the four isolates confirmed as *L. garvieae* to evaluate their growth under different pH, salinity, and temperature conditions.

The ability of *L. garvieae* strains to grow at different pH values was tested using tryptic soy broth (TSB; Oxoid) at three pH levels: 5.0, 6.0, and 9.6. Bacterial growth was observed under aerobic conditions at 28 °C for 24–96 h. Salinity tolerance was evaluated in TSB containing five

different NaCl concentrations: 0%, 0.5%, 2.5%, 4.5%, and 6.5%. The media were incubated under aerobic conditions at 28 °C for 24–96 h (Ortega et al. 2020). The ability of *L. garvieae* strains to grow at different temperatures was tested on TSA at 4, 18, 25, 37, and 45 °C for 96 h. Growth ability and duration were monitored daily during incubation (Ortega et al. 2020), and optimal conditions were maintained throughout the experiment.

Antimicrobial susceptibility tests

Antimicrobial susceptibility was tested using the Kirby-Bauer disk diffusion method. Bacterial suspensions were adjusted to 0.5 McFarland standard and inoculated onto Mueller-Hinton agar (Oxoid) supplemented with 5% sheep blood. Antibiotic discs containing enrofloxacin (5 µg), florfenicol (30 µg), oxytetracycline (30 µg), penicillin (10 U), erythromycin (15 µg), amoxicillin (10 µg), tetracycline (30 µg), gentamicin (10 µg), ciprofloxacin (5 µg), and trimethoprim-sulfamethoxazole (25 µg) were applied, and the plates were incubated at 35 °C ± 2 °C for 20–24 h. Since Clinical and Laboratory Standards Institute (CLSI) species-specific breakpoints are not available for *Lactococcus* spp., zone diameters were interpreted using surrogate criteria for Viridans group streptococci and Group B streptococci, as previously described by Malek et al. (2019). These results should therefore be considered presumptive and interpreted with caution. Breakpoint limits were determined according to CLSI (CLSI 2026). Quality control was performed using *Staphylococcus aureus* ATCC 25,923 in accordance with CLSI recommendations.

Genotypic characterization

Genotypic characterization was performed on the four *L. garvieae* isolates.

Pulsed-field gel electrophoresis

PFGE of the isolates was performed according to Vela et al. (2000). Bacterial suspensions were prepared from colonies grown on TSA at 37 °C to an A_{610} of approximately 0.6. Cells were collected by centrifugation (13,000 × g, 5 min, 4 °C) and washed twice with buffer containing 10 mM Tris-HCl, 10 mM EDTA, 10 mM EGTA, and 1 M NaCl (pH 8). Agarose plugs were prepared by mixing the cell suspension with an equal volume of 2% low-melting-point agarose. The plugs were incubated for 24 h at 37 °C in a lysis buffer of 6 mM Tris-HCl, 100 mM EDTA, 1 M NaCl, 0.5% (wt/vol) Brij 58, 0.2% (wt/vol) sodium deoxycholate, 0.5% (vol/vol) lauroyl sarcosine, and lysozyme at 5 mg/ml. Subsequently, the plugs were treated at 56 °C for 50 h with an

equal volume of a solution containing 0.25 M EDTA, 20 mM NaCl, 1% lauroyl sarcosine (pH 9), and proteinase K at 2.5 mg/ml. The plugs were then washed three times with Tris-EDTA buffer for 1 h at 4 °C. Restriction digestion was carried out using *Apal* (Promega Co.) enzyme according to the manufacturer's instructions.

Electrophoresis was performed on 1% PFGE agarose gels using a CHEF-DR III System (Bio-Rad) according to the following parameters: running time, 21 h at 14 °C; voltage gradient, 6 V/cm; included angle, 120°, with an initial pulse time of 0.1 s and a final pulse time of 25 s. The band patterns were evaluated by GelCompar II 6.6.11 software (Applied Maths, Sint-Matenslatem, Belgium). The similarity between patterns was calculated using the Dice coefficient with 1% tolerance, while the dendrogram was generated using the Unweighted Pair-Group Method with Arithmetic Mean (UPGMA) method. For comparison, one representative *L. petauri* isolate identified in this study was included in the PFGE analysis. The lambda ladder PFGE marker (Boehringer Mannheim) was used to determine molecular size.

Whole-genome sequencing

Following PFGE analysis, phenotypic characterization, and antimicrobial susceptibility testing, strain LG2 was selected for whole-genome sequencing because it exhibited the most susceptible overall antimicrobial profile and was considered the most appropriate candidate for further genomic characterization. DNA purity (A260/280), quantity, and integrity were assessed via spectrophotometry, Qubit fluorometry (Thermo Fisher), and agarose gel electrophoresis, respectively. Library preparation was performed using the Ligation Sequencing Kit (SQK-LSK114) for sequencing on the MinION platform (Oxford Nanopore Technologies, ONT, UK).

The prepared libraries were sequenced on MinION flow cells (FLO-MIN114, R10.4.1), with basecalling via Dorado v.1.0.0 (ONT). Raw reads were quality-filtered using fastp (Chen et al. 2018) and filtlong (Wick 2021) retaining reads with a minimum length of 1,000 bp. Read quality was assessed using FastQC v0.11.9 (Andrews 2010) and MultiQC v.1.26 (Ewels et al. 2016); reads with Phred < 10 were excluded. *De novo* assembly was performed with Flye v. 2.9.2 (*-nano-raw -genome-size 2 m*) (Kolmogorov et al. 2019). The draft assemblies were polished with four rounds of Racon (Vaser et al. 2017), followed by consensus correction using Medaka v2.2.1 (ONT, 2026). The draft genome was assessed with QUAST v.5.3.0 (Gurevich et al. 2013), and genome completeness was evaluated using BUSCO v5.2.2 (Manni et al. 2021) against the lactobacillales_odb10 lineage dataset ($n=402$). The draft genome was compared

to GenBank using local blastn v.2.12.0+ (Camacho et al. 2009) and annotated with Bakta (Schwengers et al. 2021). Plasmid content was investigated using MOB-suite v3.1.7 (Robertson and Nash 2018). Putative horizontally acquired regions were assessed using AlienHunter (Vernikos and Parkhill 2006).

Antimicrobial resistance genes were identified using AMRFinderPlus (Feldgarden et al. 2019) and Abricate (Seemann 2018) with the CARD (Jia et al. 2017), ResFinder (Zankari et al. 2012), NCBI, and ARG-ANNOT (Gupta et al. 2014) databases. Virulence genes were screened using Abricate against VFDB (Chen et al. 2016). The presence of capsule biosynthesis loci was confirmed by BLASTn comparison against the reference *cps* cluster of *L. garvieae* strain FDAARGOS_929 (GenBank NZ_CP065637.1). Molecular serotype was determined in silico by electronic PCR of the LG2 assembly with the serotype-specific LGD-F/LGD-R primers (Ohbayashi et al. 2017), using EMBOSS primersearch (Rice et al. 2000), whereby a 285-bp product denotes serotype I and a 1,285-bp product serotype II. ANI was calculated using FastANI (Jain et al. 2018). Genome visualization and evaluation were performed using Proksee (Grant et al. 2023; Stothard and Wishart 2005).

Phylogenetic analysis

For comparative genomic analysis, publicly available genomes initially annotated as *Lactococcus* spp. were retrieved from NCBI and PubMLST together with available metadata. The initial comparative panel consisted of 236 publicly available *Lactococcus* spp. assemblies. To avoid species-level misclassification, all genomes were first subjected to taxonomic verification using Mash-based screening and PubMLST species assignment. Genomes that were low quality, incomplete, or assigned to species other than *L. garvieae* were excluded from downstream within-species analyses and annotated with Bakta. In addition, one genome initially assigned to *L. garvieae* (strain DCC43, GenBank GCA_000300795.2) was excluded from all within-species analyses because it shares only 82.8% ANI with the *L. garvieae* type strain and is classified as *Lactococcus garvieae*_A in GTDB ("Inconclusive" in the NCBI taxonomy-check), yielding a species-verified set of 42 *L. garvieae* genomes. The multilocus sequence type (MLST) of the *L. garvieae* LG2 strain was determined with the gene regions *als*, *atpA*, *galP*, *gapC*, *gyrB*, *rpoC*, and *tuf* to assign ST and PubMLST scheme. For the pangenome analyses, the GFF files were first quality checked using the RefSeq Mash database. Panaroo v.1.3.2 was then used for pangenome construction (Tonkin-Hill et al. 2020). The core genome files were aligned with MAFFT v.7.471 (Katoh and Standley 2013) and a phylogenetic tree was generated using IQ-TREE2 with the

best-fit model (Minh et al. 2020). Following the gene presence/absence analysis, the SNP differences were assessed using Parsnp (Treangen et al. 2014), with a focus on Turkish isolates. To identify and mask recombinant regions prior to phylogenetic inference, Gubbins v3.0 was applied to the core genome alignment (Croucher et al. 2015). Recombination masked maximum likelihood phylogeny of 42 *L. garvieae* genomes rooted on the *L. petauri* Lg-per outgroup (Altinok et al. 2022). Core genome multilocus sequence typing (cgMLST) was performed using chewBBACA v3.3.10 (Silva et al. 2018). A *de novo* schema was generated from all *Lactococcus* spp. genomes, and core loci were defined using a 95% presence threshold (cgMLST95). The resulting cgMLST profiles were used to calculate allelic distances between strains. The phylogenetic trees were visualized and annotated with metadata using iTOL and Phandango (Hadfield et al. 2018; Letunic and Bork 2021). Trees were visualised in R v4.6.0 with the ggtree, ggtreeExtra, treeio, ggplot2, ggnewscale and patchwork packages. The IQ-TREE phylogeny was outgroup-rooted, ladderised, and annotated with PubMLST-derived country and host metadata.

Results

Bacterial isolation

Gram staining and microscopic examination of the bacterial colonies revealed Gram-positive, cocci-shaped isolates, which were subsequently tested for motility, catalase, oxidase activity and sucrose fermentation. A total of 32 strains that were immotile, catalase-negative, and oxidase-negative were identified as *Lactococcus* spp. Among these, four sucrose-negative isolates were identified as presumptive *L. garvieae*, whereas the remaining 28 isolates were presumptively identified as *L. petauri*.

Molecular diagnosis of *L. garvieae*

The PCR analysis of the glycosyltransferase family 4 protein gene confirmed that four out of the 32 suspected *L. garvieae* strains were indeed *L. garvieae*. These positive samples were designated LG1, LG2, LG3, and LG4 (Fig. 2). The remaining isolates were negative in the *L. garvieae*-specific PCR assay and were therefore excluded from further phenotypic and genomic analyses. These isolates were subsequently identified as *L. petauri* using the species-specific LP_IBS PCR assay. PCR amplicons were not subjected to DNA sequencing.



Fig. 2 PCR results for the identification of *L. garvieae* isolates Lane M: 100 bp DNA ladder (Thermo Scientific); *L. garvieae* ATCC 43,921 (positive control); *L. petauri* LMG 30,040 (positive control); negative

control (molecular-grade water); LG1–LG4: *L. garvieae* isolates; LP1: *L. petauri* isolate. The expected amplicon size was 132 bp for *L. garvieae* and 583 bp for *L. petauri*

Determination of phenotypic characteristics

Biochemical, morphological, and physiological characterisation

The biochemical profiles of the four *L. garvieae* isolates were identical, and the detailed results are presented in Table 2. The sucrose fermentation test, which is critical for differentiating *L. garvieae* from *L. petauri*, was performed on all four strains, all of which tested negative. The results of the biochemical and sugar fermentation tests of the four strains were identical to each other and those of the positive control strain, *L. garvieae* OMU_LG_1.

Growth at varying pH, salt and temperature conditions

The four *L. garvieae* strains exhibited growth in all the tested ranges of pH (5.0, 6.0, and 9.6), salinity (0.5%, 2.5%, 4.5%, and 6.5%), and temperature conditions (4 °C, 18 °C, 25 °C, 37 °C, and 45 °C).

Antimicrobial susceptibility

All the *L. garvieae* strains were resistant to florfenicol (30 µg) and ciprofloxacin (5 µg) but were susceptible to tetracycline (30 µg) and penicillin (10 U). Table 3 presents the

inhibition zone diameters, which indicate the susceptibility of *L. garvieae* strains to various antibiotics.

Determination of genotypic characteristics

PFGE and phylogenetic analysis

The four *L. garvieae* strains exhibited identical PFGE banding patterns (100% similarity), indicating that they belong to the same clonal lineage. In contrast, the *L. petauri* strain displayed a distinct PFGE profile sharing 91% similarity with the *L. garvieae* isolates and clearly differentiating it from them (Fig. 3).

Whole-genome sequencing

WGS of strain LG2 generated a complete genome assembly comprising a single chromosomal contig of 2,214,687 bp (100% of the total assembly). This genome had an N50 of 2,214,687 bp, an overall GC content of 38.50%, and contained no ambiguous bases (0 N's per 100 kbp). Genome completeness was 398 complete BUSCOs (99.0%; 98.5% single-copy and 0.5% duplicated), one fragmented BUSCO (0.2%) and three missing BUSCOs (0.8%), indicating a near-complete and high-quality assembly (Supplementary Table 1). Plasmid content was assessed using MOB-suite

Table 2 API Rapid ID 32 Strep results of *L. garvieae* strains

Tests	Strains				
	PK	LG1	LG2	LG3	LG4
ADH	+	+	+	+	+
βGLU	+	+	+	+	+
βGAR	-	-	-	-	-
βGUR	-	-	-	-	-
αGAL	-	-	-	-	-
PAL	-	-	-	-	-
RIB	+	+	+	+	+
MAN	+	+	+	+	+
SOR	-	-	-	-	-
LAC	-	-	-	-	-
TRE	+	+	+	+	+
RAF	-	-	-	-	-
SAC	-	-	-	-	-
LARA	-	-	-	-	-
DARL	-	-	-	-	-
CDEX	-	-	-	-	-
VP	+	+	+	+	+
APPA	+	+	+	+	+
βGAL	-	-	-	-	-
PyrA	+	+	+	+	+
βNAG	-	-	-	-	-
GTA	-	-	-	-	-
HIP	-	-	-	-	-
GLYG	-	-	-	-	-
PUL	-	-	-	-	-
MAL	+	+	+	+	+
MEL	-	-	-	-	-
MLZ	-	-	-	-	-
MBDG	+	+	+	+	+
TAG	-	-	-	-	-
βMAN	-	-	-	-	-
URE	-	-	-	-	-

PK Positive Control, LG 1–4 *L. garvieae* positive samples, ADH L-arginine dihydrolase, βGLU β-glucosidase, βGAR β-galactosidase, βGUR β-glucuronidase, αGAL α-galactosidase, PAL alkaline phosphatase, RIB D-ribose fermentation, ADAM mannitol fermentation, SOR sorbitol fermentation, LAC lactose fermentation, TRE D-trehalose fermentation, RAF D-raffinose fermentation, VP Voges-Proskauer test, APPA L-alanyl-L-phenylalanyl-L-prolyl-beta-naphthylamide, βGAL β-galactosidase, PYRA pyroglutamic acid-β-naphthylamide, β-NAG N-acetyl-β-glucosaminidase, GTA glycyl-tryptophan arylamidase, HIP hippurate hydrolysis, GLYG glycogen fermentation, PUL pullulan fermentation, MAL D-maltose fermentation, MEL D-melibiose fermentation, MLZ D-melezitose fermentation, SAC D-sucrose fermentation, LARA L-arabinose fermentation, DARL D-arabitol fermentation, MBDG methyl-β-D-glucopyranoside fermentation, TAG D-tagatose fermentation, βMAN β-mannosidase, CDEX D-cyclodextrin fermentation, URE urease

v3.1.7; no plasmid replicons were detected, and contig was classified as chromosomal. The LG2 genome was then submitted to the NCBI GenBank and assigned the Project ID: PRJNA1127444 and Biosample ID: SAMN61799678.

AMRFinderPlus identified a single chromosomally encoded resistance determinant, *lsa(D)*, located on contig

Table 3 Antimicrobial susceptibility of 4 *L. garvieae* isolates

Antibiotic	Sensitivity of Strains (mm)			
	LG1	LG2	LG3	LG4
Enrofloxacin	S (25)	I (21)	I (20)	R (11)
Florfenicol	R (17)	R (22)	R (0)	R (15)
Oxytetracycline	R (0)	I (16)	I (17)	R (0)
Penicillin	S (23)	S (22)	S (27)	S (20)
Erythromycin	S (24)	S (26)	I (15)	S (22)
Amoxicillin	R (13)	I (15)	R (13)	R (13)
Tetracycline	S (20)	S (22)	S (22)	S (25)
Gentamicin	S (17)	S (22)	S (20)	I (13)
Ciprofloxacin	R (12)	R (13)	R (13)	R (18)
Trimethoprim-sulfamethoxazole	I (13)	I (11)	R (0)	S (16)

R Resistant, I Intermediate, S Susceptible

1 between positions 1,612,092 and 1,613,579, encoding an ABC-F type ribosomal protection protein conferring resistance to lincosamides, pleuromutilins and streptogramins A (94.96% identity, 99.80% coverage to WP_165713684.1). The same locus was recovered by Abricate against both CARD (91.03% identity) and the NCBI resistance database. In addition, Abricate's ResFinder screen detected *mdt(A)* (1,671,300–1,672,555; 88.94% identity, 99.92% coverage to X92946), encoding a multidrug efflux pump associated with reduced susceptibility to macrolides and tetracyclines. No acquired beta-lactam, aminoglycoside, quinolone or florfenicol resistance genes were detected, and no plasmid replicons were predicted by PlasmidFinder. LG2 carried a chromosomally encoded *lgaQnr* homolog. No canonical resistance-associated substitutions were detected at the major *gyrA*, *gyrB* or *parC*, *parE* QRDR positions. The observed *gyrA* D87E and *parC* E84D substitutions were conservative and were not interpreted as established resistance-associated mutations.

A targeted BLASTn search of the LG2 assembly against the reference capsule cluster of *L. garvieae* strain FDAAR-GOS_929 (GenBank NZ_CP065637.1) (190,000–230,000) revealed the presence of an intact ~40 kb *cps* cluster with 98.02% nucleotide identity and 99.85% query coverage (39,941/40,000 aligned bases). Bakta annotation identified 2,212 coding sequences, 67 tRNA genes, 16 rRNA features, six ncRNAs and one tmRNA; 472 CDSs (21.3%) were annotated as hypothetical proteins. Within the BLAST-confirmed capsule region, genes encoding the complete dTDP-L-rhamnose biosynthesis pathway, multiple glycosyl- and rhamnosyltransferases, and an associated ABC transporter-permease system were identified. Additional putative virulence-associated features included a collagenase-like protease, hemolysin III, a putative adhesin, a BspA-family surface protein, multiple LPXTG-domain surface proteins, and a sortase A-major pilin locus. The genome also carried an accessory Sec glycosylation and secretion cluster comprising a second *secA*, *gtfA*, *gtfB*, *secY2* and the Asp1–Asp4

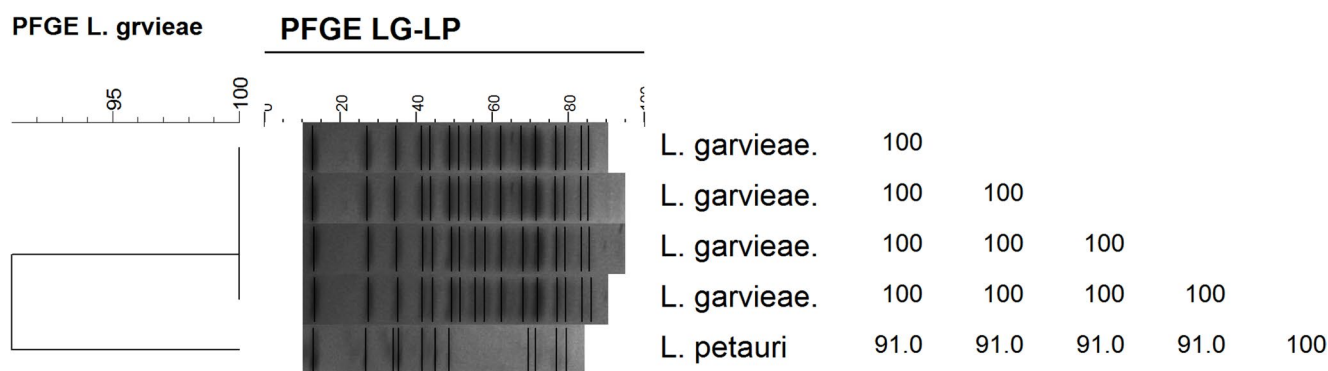


Fig. 3 PFGE profile of *L. garvieae* strains

accessory secretion proteins. Enolase, phosphoglucosylase, glyceraldehyde-3-phosphate dehydrogenase, NADH oxidase, superoxide dismutase, and ferric/ferrous iron-uptake systems (Supplementary Table 2). In addition, In silico serotyping assigned LG2 to serotype I (285-bp LGD amplicon, with no 1,285-bp serotype II product).

Within this dataset, the LG2 (this study) genome showed the highest similarity to the Turkish isolate OS-37 (GCF_022698225.1, Muğla, 2013), sharing 99.96% ANI. Both genomes were assigned to ST109 by MLST, supporting their placement within the same closely related clonal lineage rather than reflecting high overall genomic similarity alone. The remaining *L. garvieae* genomes were distinguishable from this LG2/OS-37 pair, sharing 96.4–98.2% ANI with LG2. Notably, the other Turkish isolates included in the analysis, Lg_ABFT_1 and Lg_ABFT_5, belonged to ST139 and shared only approximately 98.0% ANI with LG2, comparable to values observed for several non-Turkish genomes. This indicates that at least two distinct *L. garvieae* lineages are represented among Turkish field isolates. ANI values between LG2 and non-Turkish genomes ranged from 97.8% to 98.2%, lower than the value observed between LG2 and OS-37 but comparable to those obtained for the Turkish ST139 isolates. These values remain above the commonly used 95% species-delineation threshold but clearly separate these genomes from the LG2/OS-37 ST109 cluster. By contrast, the *L. petauri* outgroup shared only 92.9% ANI with LG2, below the 95% species boundary, confirming its assignment to a separate species (Fig. 4, Supplementary Table 3).

Species-level verification of the comparative panel

Species-level verification of the initially compiled 236 genome *Lactococcus* panel revealed substantial taxonomic misclassification among publicly available assemblies. Of the 236 public assemblies screened, only 42 corresponded to the current species definition of *L. garvieae*, whereas 114 were assigned to *L. petauri* and 80 to *L. formosensis*.

However, one of these putative *L. garvieae* assemblies (strain DCC43, GenBank GCA_000300795.2) shared only 82.8% ANI with the *L. garvieae* type strain, is flagged as “Inconclusive” by the NCBI taxonomy-check and is classified as *L. garvieae_A* in GTDB, and was therefore excluded as a divergent, non-representative lineage, leaving 41 verified public *L. garvieae* assemblies. After exclusion of DCC43, 41 verified public *L. garvieae* genomes remained. Following the addition of LG2, the final within-species dataset comprised 42 genomes. The *L. petauri* reference strain Lg-per was retained only as an outgroup for rooted phylogenetic reconstruction (Supplementary Table 4).

Patristic distance clustering (k=9) resolved the panel into one larger sub-clade (Sub-clade A, $n=15$) together with several mid-sized and minor lineages (Sub-clades B–I). LG2 grouped in a two-member sub-clade together with the Turkish reference strain OS-37, its near-clonal ST109 partner, whereas the USA isolates VC11, VC13 and R22-8 formed a separate lineage. PubMLST derived country annotation (Fig. 5, country track) confirmed that the panel spans diverse geographic origins (China and Hong Kong, USA, Spain, Japan, Italy, India, Vietnam, Thailand and Türkiye) and that individual sub-clades, including the LG2 sub-clade, which contains isolates from Türkiye, Spain and Hong Kong, are internationally distributed rather than strictly geographically structured. Notably, the Turkish isolates were not monophyletic but distributed across several sub-clades, with LG2 grouping most closely with the Turkish reference OS-37, consistent with the ANI results. Host annotation showed that most isolates were derived from fish, with a smaller fraction from human, bovine and other animal sources.

Re-screening AMR genes after species-level verification produced a dramatically different picture from the pooled analysis. Within the 42 confirmed *L. garvieae* genomes only two AMR determinants were detected: the chromosomally encoded lincosamide-streptogramin-pleuromutilin resistance gene *lsa(D)* in 42/42 (100%) genomes, and the tetracycline-resistance gene *tet(S)* in 1/42 (2.4%). All

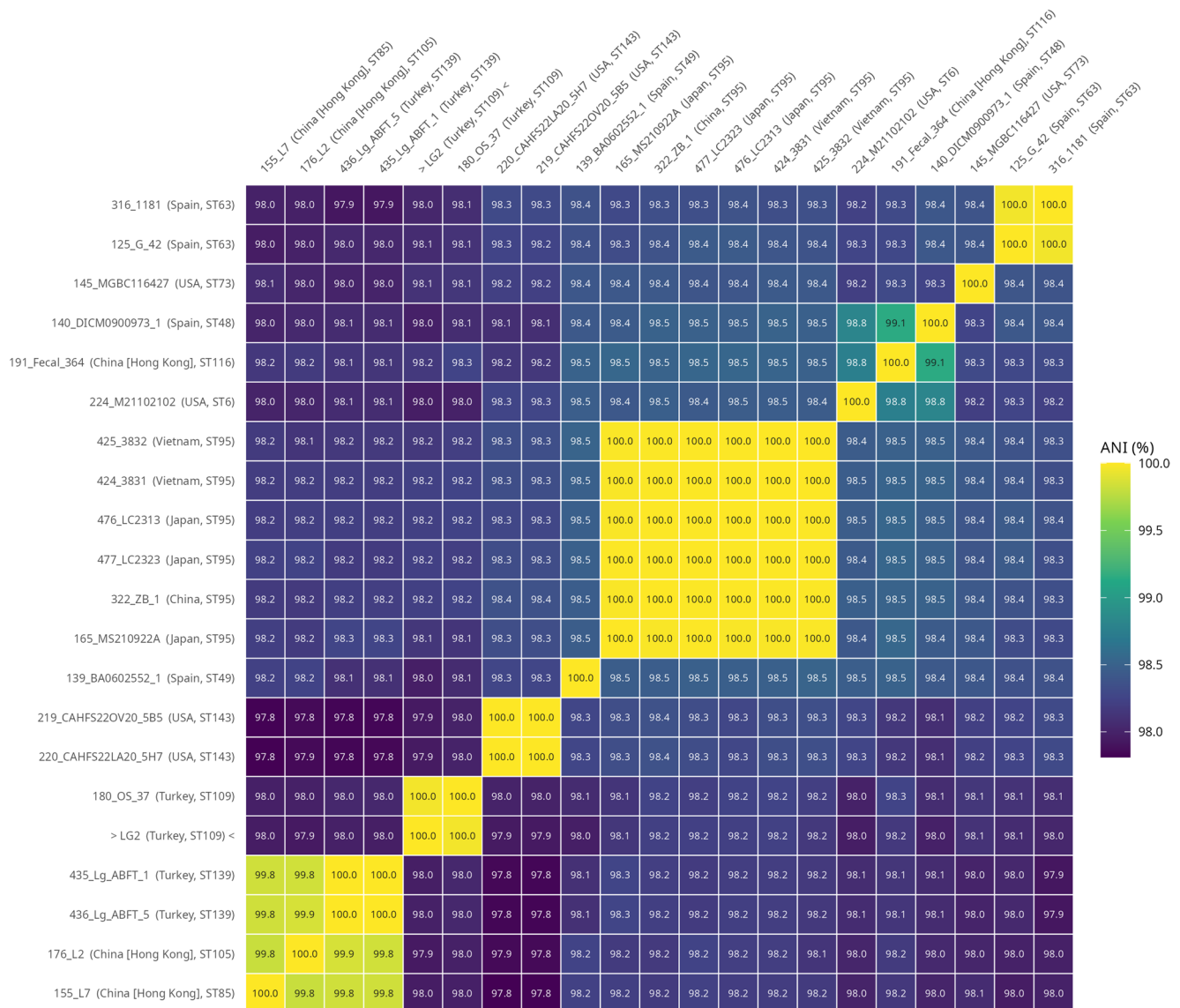


Fig. 4 Pairwise average nucleotide identity heatmap of LG2 and its closest *Lactococcus garvieae* genomes. Pairwise ANI values were calculated using FastANI for LG2 and the 20 species-verified *L. garvieae*

genomes showing the highest ANI to LG2. Strain names are accompanied by country of origin and multilocus sequence type (ST)

other AMR determinants previously reported in the pooled 236 genome panel (*tet(L)*, *erm(B)*, *fexA*, *optrA*, *ant(6)-Ia*, *aac(6')-Ie/aph(2'')-Ia* and others) were exclusively carried by misclassified *L. petauri* or *L. formosensis* isolates and are absent from the true *L. garvieae* population. Therefore, *lsa(D)* appears to represent an intrinsic, chromosomally fixed feature of *L. garvieae* rather than an acquired AMR determinant. Apart from the rare occurrence of *tet(S)*, acquired AMR determinants were uncommon within the verified *L. garvieae* population.

In the present study, pairwise SNP distances of LG2 differed from the Turkish isolate OS-37 (ST109) by only three core SNPs; the two genomes were therefore essentially identical and consistent with their 99.96% ANI and shared

sequence type, represent a single clonal lineage. In contrast, LG2 differed from all other *L. garvieae* genomes by several thousand core SNPs (approximately 6,400–9,600 for most of the collection, rising to ~19,400 for the most divergent isolates); the next-closest genome to LG2 after OS-37 differed by 6,447 core SNPs. These larger absolute counts reflect the substantially larger recombination-filtered core-genome alignment recovered once the divergent DCC43 lineage was excluded (43,855 versus 4,672 parsimony-informative sites) and do not affect the relative relationships. Notably, the remaining Turkish isolates in the dataset (Lg_ABFT_1 and Lg_ABFT_5, ST139) differed from LG2 by 9,225 SNPs, within the same range as the international genomes, indicating that they belong to a distinct Turkish

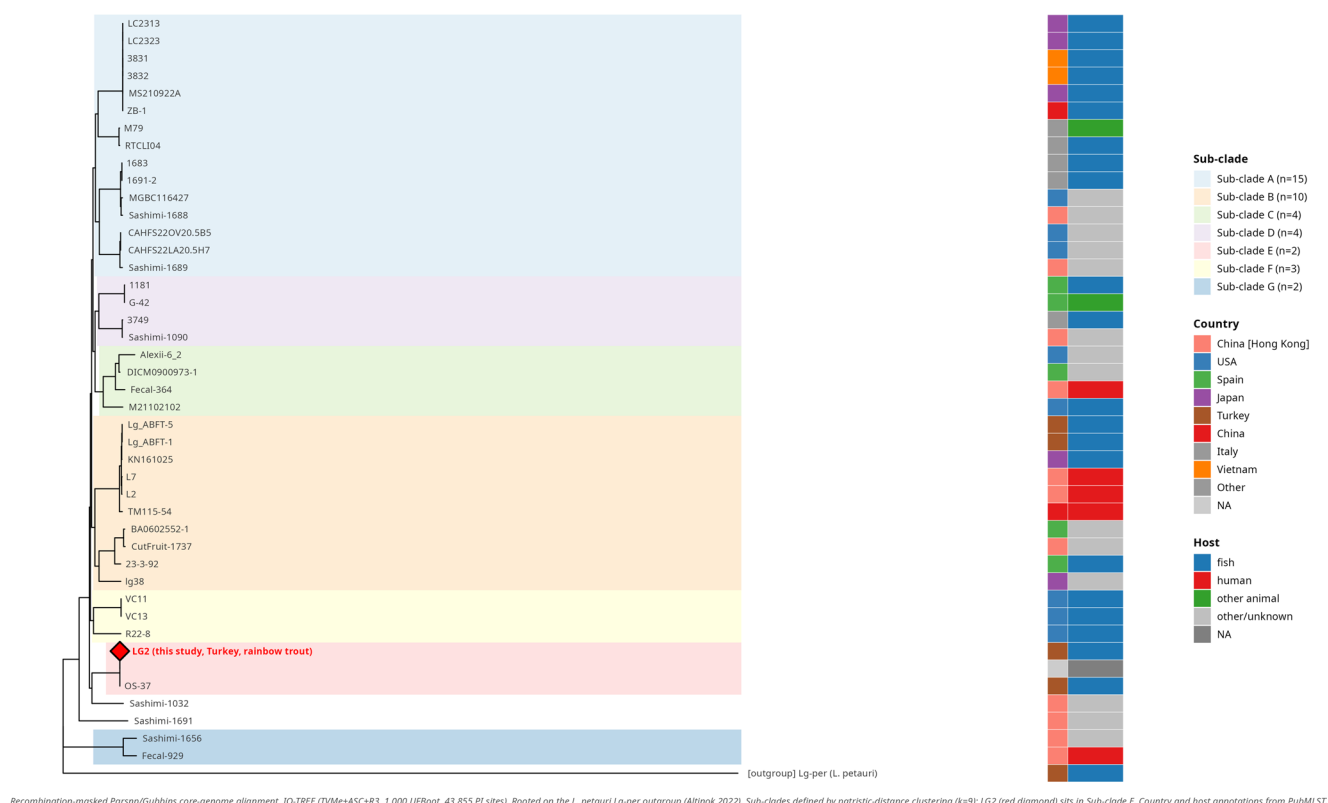


Fig. 5 Maximum-likelihood phylogeny of species-verified *Lactococcus garvieae* genomes. The tree was inferred with IQ-TREE 2 from a recombination-masked core-genome alignment of 42 species-verified *L. garvieae* genomes. The *L. petauri* strain Lg-per was included solely as an outgroup (Altınok et al. 2022). Branch support was assessed

using 1,000 ultrafast bootstrap replicates under the TVM+F+ASC+R3 substitution model. Patristic-distance clustering with average linkage ($k=9$) identified six multi-isolate groups (A–F), which are indicated by background shading; the three singleton lineages are shown without shading. Country and host metadata are displayed alongside the tree

lineage rather than to the LG2/OS-37 clone. Representative geographically distant genomes, such as the Chinese ST95 isolate ZB_1 and the USA ST6 isolate M21102102, differed from LG2 by 9,631 and 9,418 SNPs, respectively, whereas the *L. petauri* outgroup differed by 60,085 SNPs, far exceeding the within-species distances. Together with the phylogenetic placement of LG2, these SNP distances confirm that LG2 forms a near-identical clonal pair with the Turkish ST109 isolate OS-37, clearly separated both from the co-circulating Turkish ST139 lineage and from the more divergent international *L. garvieae* populations.

cgMLST results showed that the compact 1,169-locus 100%-present core indicates an unusually stable, conserved genome for the species (Fig. 6). Schema files are provided in GitHub repo (Ünal 2026) to enable adoption of the scheme for future *L. garvieae* surveillance. Panaroo analysis of 42 species-verified *L. garvieae* genomes identified 5,655 gene clusters, 1,467 core (25.9%), 92 soft-core (1.6%), 544 shell (9.6%) and 3,552 cloud genes (62.8%). Gene accumulation curves from 30 random permutations did not plateau, and Heaps' law $\alpha=0.35$ confirmed an open pan-genome. The large cloud-gene fraction indicates substantial intra-species gene-content diversity, supporting the need for

region-specific vaccine formulations based on locally circulating strains such as LG2 (Fig. 7).

Discussion

L. garvieae remains an important bacterial pathogen in rainbow trout aquaculture, particularly in regions where intensive farming and elevated water temperatures favour lactococcosis outbreaks. In the present study, *Lactococcus* suspected isolates recovered from rainbow trout farms in Antalya, Türkiye, were subjected to phenotypic, molecular and genomic characterization. Among 32 *Lactococcus*-like isolates, 4 were confirmed as *L. garvieae* by species-specific PCR and biochemical differentiation, highlighting the importance of accurate species-level identification within the closely related *L. garvieae*-*L. petauri* complex.

Previous studies have demonstrated phenotypic and genotypic variation among *L. garvieae* strains from different geographical origins (Altun et al. 2013). Such heterogeneity may influence both epidemiological interpretation and the effectiveness of preventive measures, including vaccination. Autogenous vaccines are used against lactococcosis

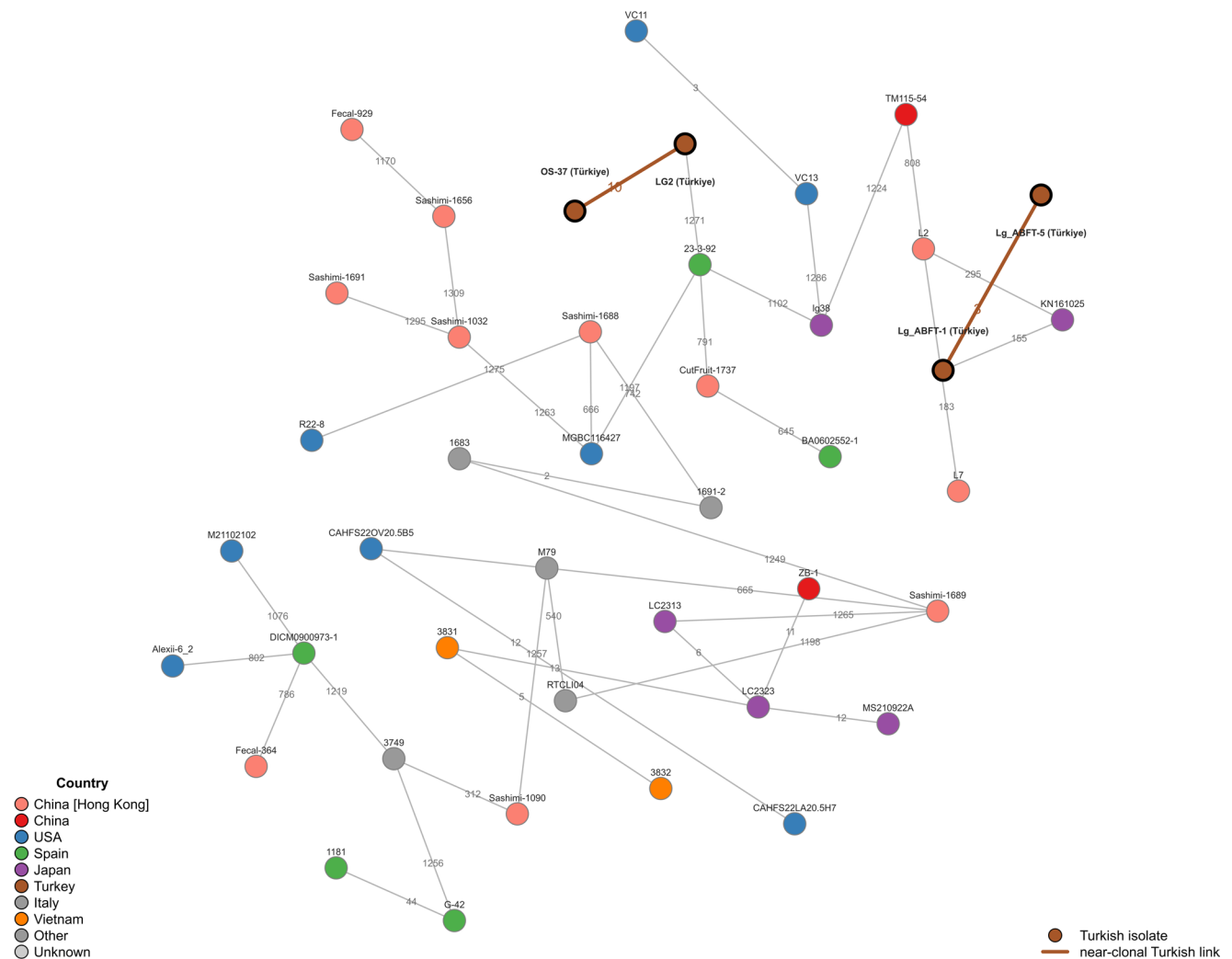
Turkey-focused cgMLST minimum spanning tree (42 *L. garvieae*; edge = allelic differences)

Fig. 6 Türkiye-focused cgMLST minimum spanning tree (MST) The minimum-spanning tree was constructed from pairwise allelic distances across 1,358 loci present in at least 95% of the 42 species-verified *L. garvieae* genomes (cgMLST95 scheme). Each node represents

one genome, node colours indicate country of origin, and isolates from Türkiye are highlighted in brown. Numbers on connecting edges represent allelic differences between genomes

in several countries, while the inactivated commercial vaccine Ichtiovac-Lg is authorized for use in Türkiye (Meyburgh et al. 2017). However, its reported protective efficacy is below 50% (Altinok et al. 2022; Satıcıoğlu et al. 2023). A mismatch between the vaccine strain and locally circulating populations could potentially contribute to this limited protection; however, this hypothesis cannot currently be evaluated because no whole-genome sequence or publicly available genomic data exist for the commercial vaccine strain. In the present analysis, LG2 was assigned to ST109, whereas the other available Turkish genomes belonged to ST139, indicating that more than one genomic background is represented among isolates associated with Türkiye. This observation should be interpreted cautiously because of the

limited number of available Turkish genomes. Nevertheless, genomic characterization of locally circulating strains such as LG2 may provide a basis for evaluating population diversity and selecting isolates for future region-specific vaccine studies. Genomic characteristics alone, however, are insufficient to establish LG2 as a vaccine candidate, and its suitability must be assessed through antigenic profiling, immunogenicity testing and controlled challenge experiments.

The *L. garvieae* strains were incubated at a range of temperatures (4 °C, 18 °C, 25 °C, 37 °C, and 45 °C). The growth results were consistent with previous studies reporting that *L. garvieae* is capable of growth over a broad temperature

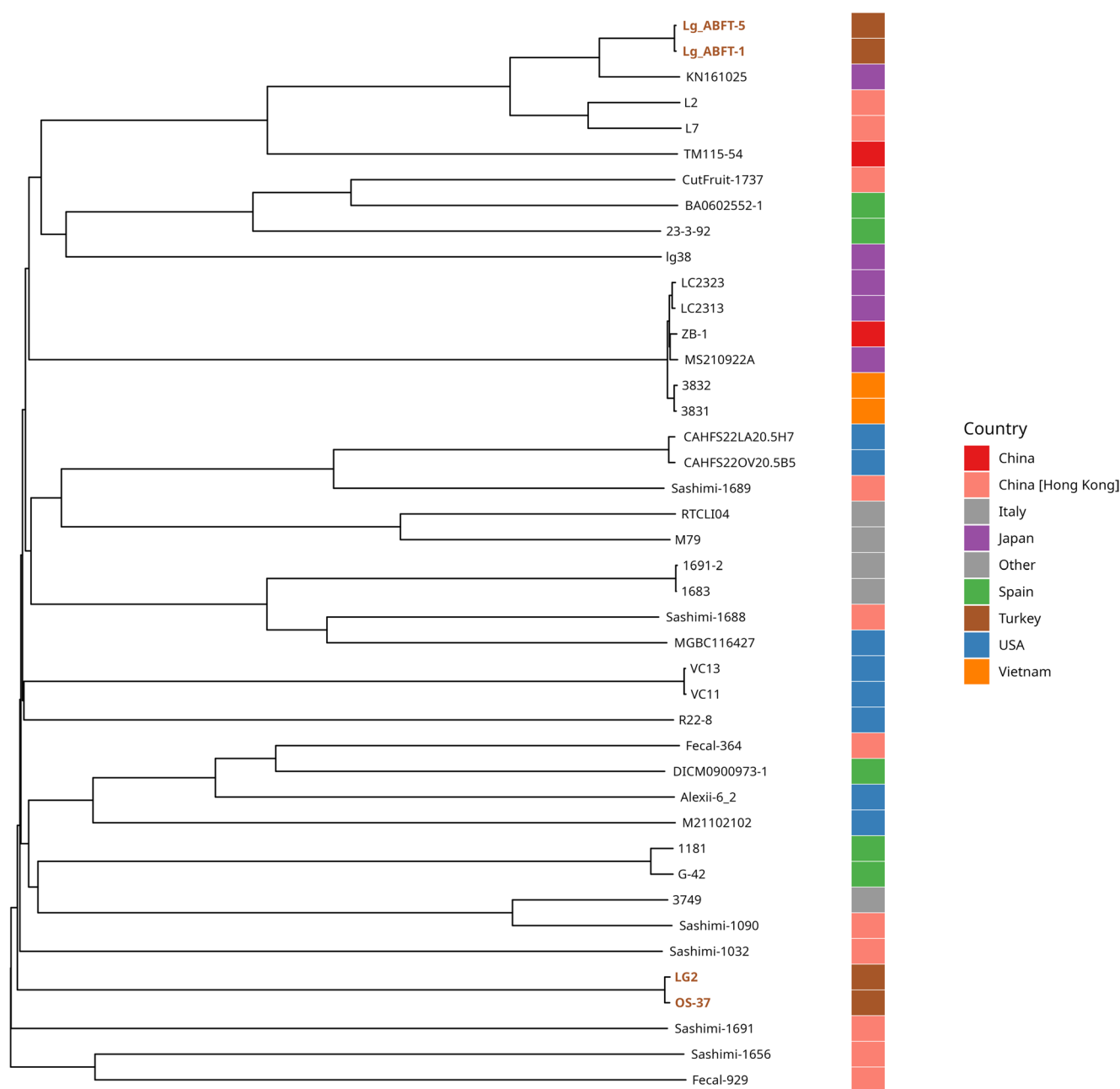


Fig. 7 Allelic-distance tree based on the strict-core cgMLST scheme of *Lactococcus garvieae*. The tree was reconstructed from pairwise allelic distances across 1,169 loci present in all 42 species-verified *L. garvieae* genomes. The genome sequenced in this study (LG2) and

the other available Turkish genomes (OS-37, Lg_ABFT_1 and Lg_ABFT_5) are highlighted in bold orange. Coloured squares at the tips indicate the country of origin of each genome

range (4–45 °C) (Diler et al. 2002; Karami et al. 2019; Kubilay et al. 2005; Öztürk et al. 2013).

Growth was also detected in media with NaCl concentrations of 0.5%, 2.5%, 4.5%, and 6.5%. These results align well with previous reports. For example, Kubilay et al. (2005) found that *L. garvieae* strains can grow in media containing 0%, 3%, 6%, and 6.5% NaCl, while Öztürk et al. (2013) observed growth at 0%, 2.5%, and 6.5% salinity. However, both studies reported no growth at 8% NaCl

(Kubilay et al. 2005; Öztürk et al. 2013). It should be noted that these studies were conducted before the recognition of *L. petauri* as a distinct species; therefore, isolates reported as *L. garvieae* may have included *L. petauri* (Altınok et al. 2022).

All the strains exhibited detectable growth at three pH levels (5.0, 6.0, and 9.6). Similarly, Kubilay et al. (2005) and Karami et al. (2019) reported growth of *L. garvieae*

strains at pH levels of 5.0, 6.0, and 9.6, and from 5.0 to 9.5, respectively.

PFGE is recognized as a higher resolution method than other molecular techniques, making it capable of distinguishing strain-level variations within a species (Rao et al. 2022; Ture et al. 2015). We also used PFGE to analyse four *L. garvieae* strains and one *L. petauri* strain to determine their clonal relationship, based on their phenotypic growth profiles. The PFGE band patterns of all the *L. garvieae* strains indicated genetic homogeneity with 100% similarity. Rao et al. (2022) analysed 45 *L. garvieae* strains, identified with Zlotkin et al. (1998) PCR method, for clonal relationships through the PFGE method using the *ApaI* restriction enzyme. They revealed genetic heterogeneity between the strains. However, this genetic diversity may be because the PCR primers developed by Zlotkin et al. (1998) had insufficient specificity to accurately differentiate *L. garvieae* from *L. petauri*. In addition, the identical PFGE profiles observed in the present study may also reflect circulation of a closely related clonal lineage among the sampled farms.

Reviewing national and international studies reveals that *L. garvieae* strains have diverse phenotypic and genotypic AMR profiles (Altun et al. 2013; Diler et al. 2002; Duman et al. 2020; Hancı and Onuk 2018; Karami et al. 2019; Kubilay et al. 2005; Maki et al. 2008; Meyburgh et al. 2017). Ciprofloxacin resistance may arise through several mechanisms (Gibello et al. 2014; Shariati et al. 2022). One mechanism is plasmid-mediated, involving quinolone resistance genes such as *qnr*, *aac(6')-Ib-cr*, *oqxAB*, *qepA*, and *qacBIII*, which are associated with low-level quinolone resistance (Celik et al. 2023; Shariati et al. 2022). Another mechanism involves chromosomal mutations in the *gyrA* and *gyrB* genes encoding DNA gyrase, or in other quinolone resistance-determining regions (QRDRs), which can also reduce quinolone susceptibility (Fàbrega et al. 2009; Gibello et al. 2014; Zhao and Dang 2012). In the present study, however, no plasmid sequences were detected in either the raw sequencing reads or the assembled genome, indicating that the ciprofloxacin resistance observed in LG2 is unlikely to be plasmid-mediated. Instead, BLAST analysis identified a chromosomally encoded *lgaQnr* homolog, consistent with the possible mechanism previously described by Gibello et al. (2014). Although no canonical resistance-associated mutations were detected in the *gyrA* or *parC* quinolone resistance-determining regions, the presence of the chromosomal *lgaQnr* homolog hypothetically may contribute to the reduced ciprofloxacin susceptibility observed in LG2. Nevertheless, additional functional studies are required to confirm its contribution to the resistance phenotype.

Another concern is florfenicol, a broad-spectrum antibiotic commonly used in aquaculture and structurally similar to thiamphenicol and chloramphenicol. Florfenicol

resistance genes (e.g., *floR*, *fexA*, and *cfr*) may be located on either plasmids or chromosomes (Guo et al. 2024). From their analysis of AMR and the plasmid content of *L. garvieae* strains, Ture and Boran (2015) found that although 83.3% were susceptible to florfenicol, 46.6% harbored the *floR* gene, which they attributed to excessive florfenicol use. Similarly, Duman et al. (2020) found that although none of the 137 field isolates of *L. garvieae* carried the *floR* gene, 25 isolates were phenotypically resistant. Thus, to avoid environmental contamination and potential human or animal health risks, such genes should be confined to research purposes only and not released into the environment (Alcolea et al. 2019). In our study, despite the phenotypic resistance of LG2 to ciprofloxacin and florfenicol, no acquired quinolone or florfenicol resistance determinants and no plasmid replicons were detected. This phenotype-genotype discordance suggests that the observed resistance may be associated with mechanisms not captured by routine acquired AMR gene screening, such as chromosomal mutations, regulatory changes, intrinsic reduced susceptibility, or efflux-related mechanisms. Therefore, further genomic surveillance and targeted functional studies are needed to better understand AMR determinants and resistance mechanisms in *L. garvieae*. In addition, the susceptibility classifications should be interpreted with caution because they were based on surrogate breakpoints. The use of alternative interpretive criteria or future *Lactococcus*-specific breakpoints could potentially alter the resistant/susceptible categorization of some isolates.

Comparative analysis of virulence-associated loci indicated strain-level variation in capsule biosynthesis within *L. garvieae*. Unlike the RTCLI04 strain, which was reported to lack the capsule gene cluster (Shahi and Mallik 2020), LG2 carried an intact capsule-associated locus containing genes involved in dTDP-L-rhamnose and polysaccharide biosynthesis. Capsule production has previously been associated with resistance to host immune clearance and increased virulence in fish-pathogenic *L. garvieae* (Morita et al. 2011; Miyauchi et al. 2012). However, the presence of this locus alone does not establish capsule expression or greater pathogenicity, as virulence is multifactorial and can also occur in non-capsulated strains. Therefore, the difference between LG2 and RTCLI04 should be interpreted as strain-level variation rather than evidence of geographical structuring or differential virulence. Phenotypic confirmation of capsule production and controlled infection studies are required to determine its functional significance in LG2.

MLST and cgMLST further complement this approach by assigning isolates to defined sequence types, while whole-genome SNP analysis provides greater discriminatory power for resolving relationships within species. Such genome-based approaches have also been widely used for

source tracing and outbreak investigation (Bush et al. 2020; Deurenberg et al. 2017; Labbé et al. 2021). Together with the phylogenetic placement of LG2, these SNP distances confirm that LG2 forms a near-identical possible clonal pair with the Turkish ST109 isolate OS-37, separated both from the circulating Turkish ST139 lineage and from the more divergent international *L. garvieae* populations. However, because few genomes from Türkiye are currently available, these findings are insufficient to infer co-circulation patterns or the broader national population structure of *L. garvieae*.

The pan-genome analysis further demonstrated substantial gene-content diversity within *L. garvieae*. Together with the estimated Heaps' law exponent of 0.35, these findings support an open pan-genome in which additional genomes are expected to contribute new accessory genes. Similar patterns have been associated with recombination, horizontal gene transfer and adaptation to different hosts or ecological niches in previous comparative genomic studies of *L. garvieae* (Miyauchi et al. 2012; Ferrario et al. 2013; Francés-Cuesta et al. 2022). However, gene presence or absence alone does not demonstrate a functional effect and should be evaluated experimentally.

A limitation of the present study is that whole-genome sequencing was performed on a single representative *L. garvieae* isolate (LG2). Consequently, the comparative genomic analyses presented here reflect this representative isolate and may not capture the full genomic diversity of *L. garvieae* in Türkiye. Future studies including a larger number of sequenced isolates are needed to validate and expand these findings.

In conclusion, this study provides a high-quality genome for the Turkish *L. garvieae* isolate LG2 and places it within a species-verified phylogenomic framework. LG2 was closely related to the Turkish ST109 isolate OS-37, whereas the available ST139 isolates represented a distinct genomic background. Taxonomic curation demonstrated that acquired AMR is uncommon within verified *L. garvieae* genomes, while cgMLST and pan-genome analyses revealed a conserved core genome accompanied by substantial accessory-gene diversity. These findings provide a foundation for genomic surveillance and experimental investigation of locally circulating *L. garvieae* strains.

Supplementary Information The online version contains supplementary material available at <https://doi.org/10.1007/s11259-026-11508-6>.

Author contributions İ.B.M., G.Ü., and H.K.M. performed the laboratory experiments, collected the data. G.Ü. performed the bioinformatic and comparative genomic analyses and contributed to manuscript preparation. H.K.M. conceived and supervised the study. E.E.O. contributed to sample collection and interpretation of the results. İ.B.M. and G.Ü. wrote the main manuscript text. All authors reviewed and approved the final manuscript.

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Data availability All supplementary datasets and analysis files supporting this study are publicly available in the *Lactococcus_paper_data* GitHub repository. The repository includes LG2 genome assembly and annotation records, supplementary tables covering genome statistics, functional annotation, ANI-based species verification, and metadata for 237 species-verified *Lactococcus* genomes, together with genome-exclusion records and R scripts used to generate the phylogenomic, cgMLST, minimum-spanning-tree, ANI, AMR, and pan-genome figures. In addition to the de novo cgMLST scheme generated in this study, we considered the publicly available chewBBACA-compatible *L. garvieae* cgMLST schema deposited in GitHub and Zenodo as a reference resource for future standardised typing efforts (Ünal, 2026).

Declarations

Competing interests The authors declare no competing interests.

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