Uluslararası İleri Doğa Bilimleri ve Mühendislik Araştırmaları Dergisi
Sayı 9, S. 150-161, 9, 2025

Araştırma Makalesi

IJANSER
https://as-proceeding.com/index.php/ijanser
ISSN:2980-0811

International Journal of Advanced Natural Sciences and Engineering Researches
Volume 9, pp. 150-161, 9, 2025

Research Article

Genomic Characterization and Antimicrobial Resistance Profiling of Dairy-Derived *Lactococcus garvieae* Strain MH3

Harun ÖNLÜ [1,2*] and Özlem OSMANAGAOGLU [3]

[1] *Department of Molecular Biology and Genetics, Faculty of Science and Literature, Muş Alparslan Uni-versity, 49250, Muş, Türkiye*
[2] *Department of Food Processing, Vocational School of Technical Sciences, Muş Alparslan University, 49250, Muş, Türkiye*

[3] *Department of Biology, Faculty of Science, Ankara University, 06100, Ankara, Türkiye*

[*] *(h.onlu@alparslan.edu.tr) Email of the corresponding author*

(Received: 11 September 2025, Accepted: 21 September2025)

(2nd International Conference on Pioneer and Academic Research ICPAR 2025, September 15-16, 2025)

ATIF/REFERENCE: Önlü, H. & Osmanagaoglu, Ö. (2025). Genomic Characterization and Antimicrobial Resistance Profiling of Dairy-Derived Lactococcus garvieae Strain MH3, *International Journal of Advanced Natural Sciences and Engineering Researches*, 9(9), 150-161.

Abstract –*Lactococcus garvieae* is a Gram-positive bacterium garnering increasing attention for its dual significance as both an opportunistic pathogen and a potential industrial microorganism. In this study, we present the whole-genome sequencing and antibiotic resistance gene profiling of *L. garvieae* strain MH3, isolated from traditional cow's milk cheese in Türkiye. The assembled genome consists of 73 contigs, totaling 2.21 Mb, with a GC content of 37%, 2,244 coding sequences, 7 rRNAs, 51 tRNAs, and 1 tmRNA. Genome annotation revealed multiple antimicrobial resistance (AMR) determinants, including resistance to oxacillin, erythromycin, kanamycin, gentamicin, daptomycin, colistin, chloramphenicol, and ampicillin. The presence of numerous AMR genes highlights the potential public health risk posed by this strain as a foodborne pathogen Although previous studies have highlighted the considerable industrial and probiotic potential of *L. garvieae* strains, our results suggest that the use of *L. garvieae* MH3 in such applications should be approached with caution, necessitating comprehensive safety evaluations Overall, this study underscores the essential role of genomic analyses in evaluating the safety and functional properties of newly emerging microbial strains.

Keywords – Lactococcus Garvieae MH3, Antibiotic Resistance Genes, Industrial Microorganism, Whole-Genome Sequencing, Pathogen Microorganism.

I. INTRODUCTION

Lactococcus garvieae is a nonmotile, gram-positive bacterium that belongs to the *Streptococcaceae* family [1], [2]. It usually inhabits aqua environments and is also known for being both a symbiont and a pathogen of various host species [3], [4]. In aquaculture, *L. garvieae* is considered one of the most critical lactococcosis-related acute fatal fish diseases, especially in so-called cold-water fish of commercial importance, such as rainbow fish, which cause significant economic losses [1], [5]. In addition to being isolated from fish, *L. garvieae* has also been isolated from several animals and, occasionally, from humans,

in which this bacterium causes unusual infections, mostly among immunocompromised hosts [6]. In general, because of its sensitivity to antibiotics [7], growing importance in various ecosystems, and potential for cross-species transmission for AMR genes, it is an essential subject for study in veterinary and human medicine [8]. Several studies have reported its occurrence not only in fish, but also in humans and other animals, indicating the possibility of host-to-host transmission across species boundaries [7], [9]. Furthermore, *L. garvieae* has been shown to harbor antimicrobial resistance genes, raising concerns about the transfer of AMR determinants through the food chain and between different hosts [10].

L. garvieae is considered a potential probiotic because of its ability to positively influence the microbiota of aquatic and terrestrial animals [11]. It can enhance immune responses and gut health and protect against harmful bacteria upon the introduction of probiotics into fish and livestock, which is beneficial for the aquaculture and agriculture sectors [12]-[14]. Many studies have demonstrated that *L. garvieae* improves growth performance, feed efficiency, and resistance [12], [15], [16]. Nevertheless, its inclusion in probiotic preparations for other animals, such as poultry and cattle, shows promising improvements in overall health and reduces the need for antibiotics. However, its dual role as both a helpful microbe and a potential pathogen is to select strains for care and monitoring to ensure the safety of probiotics.

Genomics and bioinformatics-enabled new tools have made a much more complete characterization of pathogens possible [17], [18]. These tools not only expand our understanding of the genetic and functional properties of pathogens but also provide new insights into the potential usage of resources [19]. Thanks to these developed tools, genomic studies have become a focal point in the scientific world, offering various application opportunities in the fields of biotechnology and healthcare, particularly in terms of metabolic potential, as well as industrial and clinical properties [20]. Moreover, with genomics and bioinformatics-based analysis, further insight into carbohydrate-active enzymes, CRISPR–Cas genes, antimicrobial resistance genes, virulence-associated genes, and bacteriocin-encoding genes of *L. garvieae* will be obtained [21].

Determining the genomic characteristics of *Lactococcus garvieae* MH3 is essential for evaluating its potential industrial applications in dairy products as well as assessing its pathogenicity risk. In this study, we performed whole-genome sequencing and analyzed the antimicrobial resistance genes of *L. garvieae* MH3, which was isolated from cow milk–derived cheese.

II. MATERIALS AND METHOD

Bacterial Strain and DNA Isolation

In our previous study, *Lactococcus garvieae* MH3 (NCBI Accession number: MW633194; SRA: PRJNA792923) was isolated from cheese produced from cow milk [22]. Cheese samples collected under sterile conditions were transported to the laboratory, where standard handling protocols were applied to isolate lactic acid bacteria. The food samples were serially diluted in physiological saline (PS) to a final dilution of 10^{-8}. Aliquots of 100 µL from each dilution were spread onto MRS agar plates using the spread plate method and incubated at 37 °C for 48 h. Pure isolates were transferred to Eppendorf tubes containing 50% sterile glycerol and stored at −80 °C for subsequent experiments.

MH3 was cultivated in MRS broth at 37°C for 24 h under aerobic conditions. Total DNA isolation was performed according to the protocol of the EURX Tissue and Bacterial DNA Purification Kit (EURX, Poland). For molecular identification, 16S rDNA gene was amplified using primer pair 27-F (5'-AGA GTT TGA TCC TGG CTC AG-3') and 1492-R (5'-CTA CGG CTA CCT TGT TAC GA-3') and amplified PCR fragments were cleaned using a PCR purification kit (Promega, Agarose Gel DNA Extraction Kit) and then sequenced by BM Software Consult. and Lab. Sis. Ltd. Şti. (Ankara, Türkiye). The sequence results were analyzed with the NCBI-BLAST program [23].

A genomic Illumina 150 bp paired-end library was derived from chromosomal DNA and sequenced by BM Software Consult. and Lab. Sis. Ltd. Şti. (Ankara, Türkiye) using Il-lumina NovaSeq 6000 sequencing

technology. The raw sequencing data were assessed for quality using FastQC v0.11.9. Quality-filtered reads were then assembled into scaffolds using Shovill v0.9.0 and SPAdes v3.13.1 with automatic coverage cutoff. Genome assembly quality was evaluated using QUAST v5.0.2. Genome annotation was performed with Prokka v1.14.6, and genomic features such as antimicrobial resistance and virulence genes were screened using ABRicate v1.0.1.

Identification of Antimicrobial Resistance–Associated Genes

The detection of antibiotic resistance genes in MH3 was performed using the CARD RGI tool integrated within the Proksee platform [24].

Disk Diffusion Susceptibility and MIC Test

The disc diffusion test was performed according to the Kirby–Bauer [25] disk diffusion susceptibility test protocol. Briefly, the MH3 strain was incubated in MRS broth at 37 ° C for 24 h, and the final concentration was adjusted to 0.5 MacFarland. MH3 was inoculated on the test plate, and then ten antibiogram discs (BioAnalyze, Türkiye; kanamycin, doxycycline, colistin, clindamycin, tetracycline, vancomycin, rifampin, gentamicin, penicillin G, ampicillin) were placed on the agar plate. The plates were incubated at 37 °C for 24 h, after which the inhibition zone diameters were measured. Experiments were performed in triplicate. *Staphylococcus aureus* ATCC 25923 and *Escherichia coli* ATCC 25922 were used as control strains

The Minimum Inhibitory Concentration (MIC) testing was performed according to CLSI guidelines [26], with minor modifications as described below: The MIC of the antibiotic was assessed using the agar dilution technique. This involved preparing serial two-fold dilutions of the antibiotic in 96-well plates filled with MRS broth medium, covering a broad range of concentrations specific to each antibiotic. Microorganisms (10^4 CFU/well) were inoculated, starting with an antibiotic concentration of 500 µg/ml in the first well, which was then halved in each subsequent well down to 0.48 µg/ml. MIC was identified as the lowest concentration of the antibiotic that completely prevented bacterial growth after 24 hours of incubation. The antibiotics tested included penicillin, vancomycin, tetracycline, erythromycin, streptomycin, kanamycin, chloramphenicol, and ampicillin (Sigma, St. Louis, USA).

III. RESULTS

The MH3 16S rDNA sequence was determined by BM Labosis (Ankara, Turkey). Sequence analysis results were opened in FinchTV program and converted to FASTA for-mat. The sequences in FASTA format were compared with all sequences identified in the NCBI database using the BLAST program and the sequence was registered in the NCBI-genbank database and assigned the accession number (MW633194) [22]. To evaluate the whole genome sequencing data's quality, the FastQC software (version 0.11.9) was employed. Examination of the samples (HO_M_1.fq.gz and HO_M_2.fq.gz) showed a total of 3,410,632 reads, with none marked as low quality. Additionally, there was no evidence of adapter contamination or overrepresented sequences, indicating that the raw data was clean and dependable. Following this, the QUAST tool was utilized to assemble the genome and assess its overall quality. The analysis identified 73 contigs, with the longest being 304,000 base pairs. The N50 value was determined to be 194,442 bp, reflecting a well-assembled genome. An impressive 99.92% of the reads were successfully mapped to the genome, and 98.42% were correctly paired. The average coverage depth reached 461x, suggesting that nearly the entire genome was thoroughly sequenced. Furthermore, the analysis predicted the presence of 1,030 unique genes. Collectively, these findings confirm that the sequencing process was highly successful, yielding data of sufficient quality for downstream genomic analyses. Assembly of the *Lactococcus garvieae* MH3 genome resulted in 73 contigs, with a GC content of 37%, a total length of 2,213,675 base pairs, 2,244 coding sequences (CDSs), 7 rRNAs, 51 tRNAs, and 1 tmRNA region. The sequencing data have been deposited in the NCBI SRA database under accession code PRJNA792923. A

circular genome representation was generated using Proksee [24] (https://proksee.ca/projects/new) (Figure 1). Quality scoring followed the Sanger/Illumina 1.9 standard, yielding a total of 3,410,632 sequences, none of which were flagged as low quality.

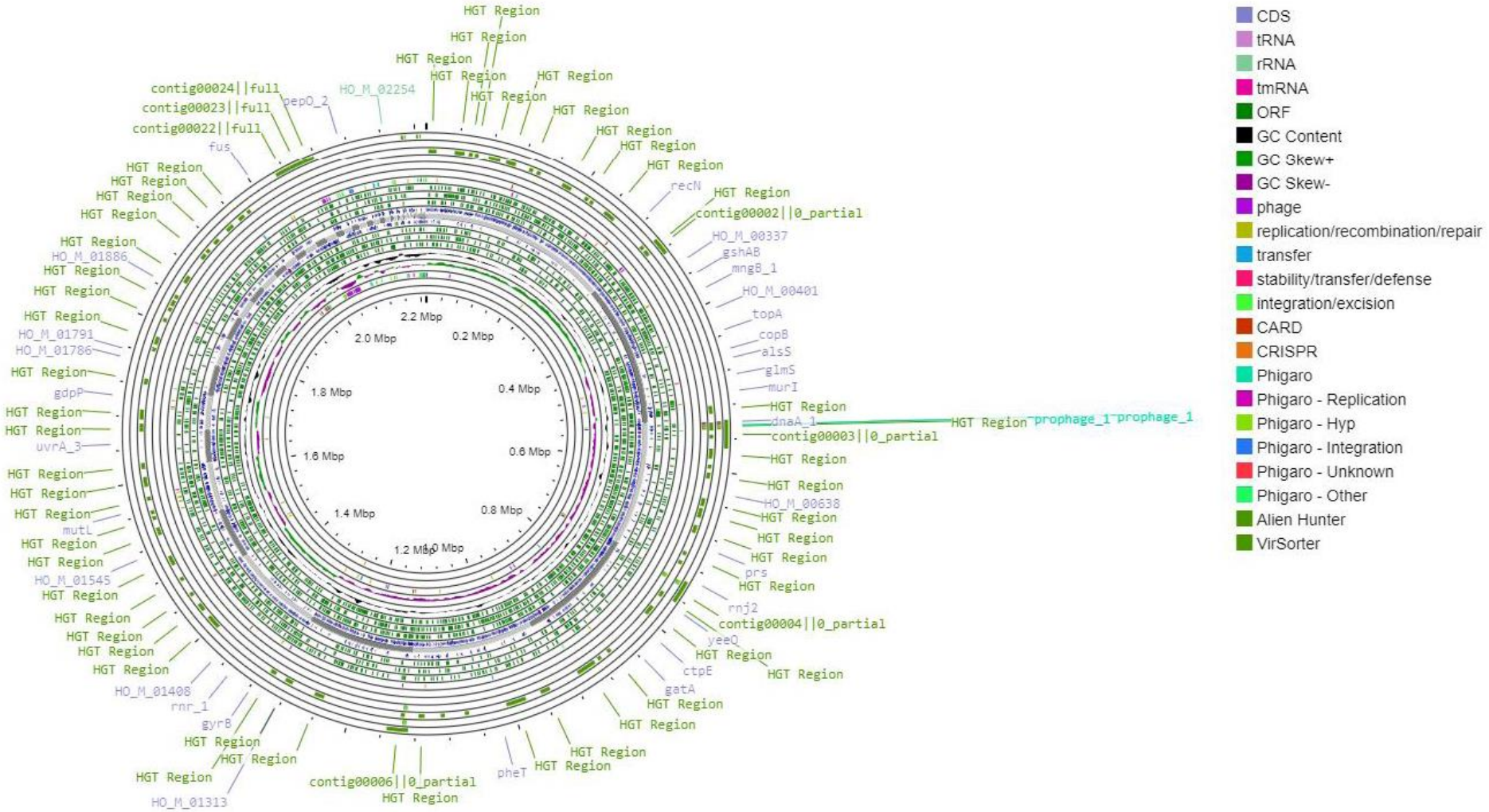

Fig. 1. Circular genome representation of *L. garvieae* MH3 (a circular map displays the distribution of the genome annotations of the MH3 **CDS**: coding DNA sequence**; CARD:** comprehensive antibiotic resistance database; **CRISPR**: CRISPR arrays and their associated Cas proteins; **Phigaro:** detect and annotate prophage regions; **Alien Hunter**: predict putative horizontal gene transfer (HGT) events; **VirSorter**: detect dsDNA and ssDNA virus genomes (phages)).

Identification of Genes Related to Antimicrobial Resistance

The presence of antibiotic resistance genes (AMRs) was investigated using Proksee software. Analysis revealed multiple resistance gene regions within the genome of *Lactococcus garvieae* MH3 (Table 1). The findings indicate that MH3 exhibits resistance to a broad spectrum of antibiotics, including oxacillin, erythromycin, kanamycin A, gentamicin B, daptomycin, colistin A, colistin B, chloramphenicol, and ampicillin.

Table 1. Identification of Antimicrobial Resistance Genes in *Lactococcus garvieae* MH3.

Contig	Start/stop	Drug class	Resistance mechanism	AMR Gen Family	Antibiotic
2	593-1183	carbapenem; cephalosporin; penam	antibiotic inactivation	OXA beta-lactamase	cloxacillin; oxacillin; cefalotin
3	1195-1539	carbapenem	antibiotic inactivation	Subclass B1 *Vibrio cholerae* varG beta-lactamase	Meropenem
5	2038-2253	macrolide antibiotic	antibiotic inactivation	macrolide phosphotransferase (MPH)	Erythromycin
6	2479-3213	diaminopyrimidine antibiotic	antibiotic target replacement	trimethoprim resistant dihydrofolate reductase dfr	Trimethoprim
8	4036-4518	aminoglycoside antibiotic	antibiotic inactivation	AAC(2')	dibekacin; netilmicin; tobramycin; 6'-N-ethylnetilmicin; gentamicin
10	5406-7130	disinfecting agents and antiseptics	antibiotic efflux	resistance-nodulation-cell division (RND) antibiotic efflux pump	Triclosan
11	726-7853	streptogramin antibiotic; streptogramin A antibiotic	antibiotic inactivation	streptogramin vat acetyltransferase	virginiamycin M1; madumycin II; griseoviridin; dalfopristin
12	7890-8066	aminoglycoside antibiotic	antibiotic inactivation	APH(3')	neomycin; ribostamycin; kanamycin A; gentamicin B; paromomycin; lividomycin; gentamicin
14	11299-11952	tetracycline antibiotic	antibiotic efflux	ATP-binding cassette (ABC) antibiotic efflux pump	Tetracycline
17	13763-14347	cephalosporin; penam; peptide antibiotic	antibiotic efflux	ATP-binding cassette (ABC) antibiotic efflux pump	methicillin; daptomycin; cefotaxime; moenomycin A1
18	14465-17470	macrolide antibiotic; lincosamide antibiotic; streptogramin antibiotic	antibiotic target alteration	Erm 23S ribosomal RNA methyltransferase	Erythromycin
19	14957-16129	rifamycin antibioti	antibiotic inactivation	rifampin ADP-ribosyltransferase (Arr)	rifampin; rifaximin; rifabutin; rifapentine

20	16241-17161	fluoroquinolone antibiotic	antibiotic efflux	major facilitator superfamily (MFS) antibiotic efflux pump	ciprofloxacin; norfloxacin
23	19003-21087	cephalosporin; cephamycin; penam	antibiotic target alteration	Penicillin-binding protein mutations conferring resistance to beta-lactam antibiotics	Amoxicillin
28	23368-24156	carbapenem; cephalosporin; penam	antibiotic inactivation	OXA beta-lactamase	cloxacillin; oxacillin; cefalotin
29	24229-25512	phosphonic acid antibiotic	antibiotic target alteration	antibiotic-resistant murA transferase	Fosfomycin
30	25505- 25690	fluoroquinolone antibiotic	antibiotic target protection	quinolone resistance protein (qnr)	ciprofloxacin; levofloxacin; moxifloxacin; gatifloxacin; nalidixic acid; norfloxacin; sparfloxacin
31	25844-27127	macrolide antibiotic; monobactam; tetracycline antibiotic; aminocoumarin antibiotic	antibiotic efflux	resistance-nodulation-cell division (RND) antibiotic efflux pump	erythromycin; tetracycline; novobiocin; aztreonam; kitasamycin; rokitamycin
32	27291-27623	fluoroquinolone antibiotic; cephalosporin; glycylcycline; penam; tetracycline antibiotic; rifamycin antibiotic; phenicol antibiotic; disinfecting agents and antiseptics	antibiotic target alteration; antibiotic efflux	ATP-binding cassette (ABC) antibiotic efflux pump; major facilitator superfamily (MFS) antibiotic efflux pump; resistance-nodulation-cell division (RND) antibiotic efflux pump	tigecycline; ciprofloxacin; tetracycline; rifampin; chloramphenicol; ampicillin; nalidixic acid; norfloxacin; cefalotin; triclosan
33	27620-28297	aminoglycoside antibiotic	antibiotic inactivation	AAC(6')	dibekacin; sisomicin; netilmicin; tobramycin; 2'-N-ethylnetilmicin; 5-episisomicin; gentamicin
36	29859-30158	fluoroquinolone antibiotic; disinfecting agents and antiseptics	antibiotic efflux	multidrug and toxic compound extrusion (MATE) transporter	acriflavine; norfloxacin
39	31907-34141	disinfecting agents and antiseptics	antibiotic efflux	resistance-nodulation-cell division (RND) antibiotic efflux pump	Triclosan
40	34131-34643	peptide antibiotic	antibiotic target alteration;	pmr phosphoethanolamine transferase	colistin A; colistin B
42	35435-37150	cephalosporin; penam; penem	antibiotic inactivation	LAP beta-lactamase	cefepime; cefuroxime; amoxicillin; piperacillin; benzylpenicillin; cefalotin; ticarcillin
43	37175-37648	macrolide antibiotic; fluoroquinolone antibiotic; monobactam;	antibiotic efflux	resistance-nodulation-cell division (RND) antibiotic efflux pump	erythromycin; ciprofloxacin; tetracycline; ceftazidime;

		carbapenem; cephalosporin; cephamycin; penam; tetracycline antibiotic; peptide antibiotic; aminocoumarin antibiotic; diaminopyrimidine antibiotic; sulfonamide antibiotic; phenicol antibiotic; penem			ceftriaxone; meropenem; novobiocin; azithromycin; trimethoprim; sulfamethoxazole; chloramphenicol; aztreonam; colistin A; colistin B; ampicillin; nalidixic acid; panipenem; ticarcillin; trimethoprim-sulfamethoxazole
45	38207-40660	fluoroquinolone antibiotic; cephalosporin; cephamycin; penam	antibiotic efflux	resistance-nodulation-cell division (RND) antibiotic efflux pump	Ciprofloxacin
46	40783-41340	phenicol antibiotic	antibiotic efflux	resistance-nodulation-cell division (RND) antibiotic efflux pump	chloramphenicol; thiamphenicol
47	41588-43921	cephalosporin; cephamycin; penam	antibiotic inactivation	YRC Beta-lactamase	cefoxitin; ceftazidime; cefuroxime; amoxicillin; cefalotin
48	44159-44864	macrolide antibiotic	antibiotic target protection	Miscellaneous ABC-F subfamily ATP-binding cassette ribosomal protection proteins	Oleandomycin
49	44811-46001	fluoroquinolone antibiotic	antibiotic target protection	major facilitator superfamily (MFS) antibiotic efflux pump	ciprofloxacin; norfloxacin
50	45998- 46144	macrolide antibiotic	antibiotic inactivation	macrolide esterase	ciprofloxacin; norfloxacin
52	47817-51236	macrolide antibiotic; fluoroquinolone antibiotic; cephalosporin; fusidane antibiotic	antibiotic efflux	resistance-nodulation-cell division (RND) antibiotic efflux pump	Erythromycin
53	51282-51881	aminoglycoside antibiotic	antibiotic inactivation	AAC(3)	erythromycin; cefotaxime; fusidic acid
54	52061-52438	phosphonic acid antibiotic	antibiotic inactivation	fosfomycin thiol transferase	gentamicin A; gentamicin
55	52498-53028	peptide antibiotic	antibiotic target alteration	MCR phosphoethanolamine transferase	Fosfomycin
56	53190-53588	fluoroquinolone antibiotic; tetracycline antibiotic; disinfecting agents and antiseptics	antibiotic efflux	resistance-nodulation-cell division (RND) antibiotic efflux pump	colistin A; colistin B
57	53724-54497	glycopeptide antibiotic	antibiotic target alteration	vanH; glycopeptide resistance gene cluster	acriflavine; tetracycline; norfloxacin
58	54494-55039	carbapenem; cephalosporin; penam	antibiotic inactivation	SHV beta-lactamase	vancomycin; teicoplanin

Disc Diffusion and MIC Test

The disc diffusion assay was conducted following the Kirby–Bauer disc diffusion susceptibility testing protocol [25], and inhibition zone diameters were measured accordingly. The degree of inhibition was classified as resistant (R, zone diameter ≤ 14 mm), intermediate (I, zone diameter 15–19 mm), or susceptible (S, zone diameter > 20 mm). The analysis indicated that *Lactococcus garvieae* MH3 exhibited resistance to kanamycin, colistin, tetracycline, vancomycin, rifampin, and gentamicin (Table 2, Figure 2). Zone diameters for the control strains *Staphylococcus aureus* ATCC 25923 and *Escherichia coli* ATCC 25922 were measured and interpreted according to the breakpoints specified in the CLSI M100 guidelines [26]. Based on MIC testing, *L. garvieae* MH3 was resistant to all antibiotics at the tested concentrations, preventing determination of an exact MIC value (> 500 µg/ml)

Table 2. Antibiotic Susceptibility of *Lactococcus garvieae* MH3 Determined by Disc Diffusion Test (R=mm).

Antibiotic	*L. garvieae* MH3	*S. aureus* ATCC 25923	*E. coli* ATCC 25922
Kanamycin	9 (R)	11 (R)	12 (R)
Doxycycline	23 (S)	25 (S)	13 (I)
Colistin	0 (R)	0 (R)	10 (R)
Clindamycin	15 (I)	22 (S)	11 (R)
Tetracycline	8 (R)	24 (S)	10 (R)
Vancomycin	7 (R)	13 (R)	0 (R)
Rifampin	12 (R)	26 (S)	0 (R)
Gentamicin	12 (R)	13 (I)	16 (S)
Penicillin G	18 (I)	38 (S)	0 (R)
Ampicillin	19 (I)	39 (S)	0 (R)

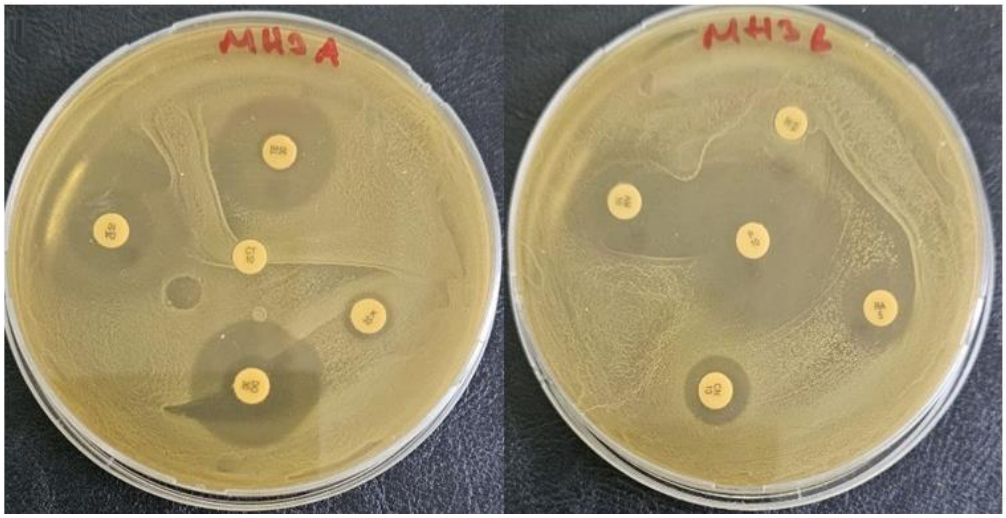

Fig 2. Antibiotic Susceptibility of *Lactococcus garvieae* MH3 Determined by Disc Diffusion Assay (TE: tetracycline, 30 µg; K: kanamycin, 30 µg; CT: colistin, 10 µg; VA: vancomycin, 10 µg; RA: rifampin, 5 µg; CN: gentamicin, 10 µg; P: penicillin G, 10 µg; AM: ampicillin, 30 µg; DA: clindamycin, 10 µg; DO: doxycycline, 30 µg).

IV. DISCUSSION

In our previous study, *Lactococcus garvieae* MH3 (NCBI Accession number: MW633194/SRA: PRJNA792923) was isolated from cow's milk cheese and demonstrated survival under acidic conditions (pH 2–3) and in the presence of bile salts (0.3–1%) for several hours, although viability gradually decreased over time [22]. Genome analysis revealed a G/C content of 37%, 73 contigs, 2,213,675 bp, 2,244 CDSs, 7 rRNAs, 51 tRNAs, and 1 tmRNA region. Comparative analyses indicated genomic variation among *L. garvieae* strains, suggesting possible gene loss or gain as part of niche adaptation. Similar diversity has also been observed in *Lactobacillus*, which displays an even broader range of genomic plasticity [27], [28]. According to Mahmoud et al. [29], the chromosome size of *L. garvieae* varies from 1.9 to 2.0 Mb, with a GC content of 38.2–38.9%, and the number of predicted protein-coding sequences ranges from 1922 to 1959. These chromosomes harbor essential genes involved in fundamental cellular functions—such as DNA replication, RNA metabolism, biosynthesis, and transport—as well as those associated with pathogenicity, including immune evasion, antiphagocytosis, secretion systems, and toxins [29].

Screening with Proksee-CARD RGI identified multiple antibiotic resistance (AMR) gene regions in the MH3 genome (Table 1), consistent with previous findings in other *L. garvieae* strains [3], [29]-[32]. Notably, MH3 exhibited resistance to oxacillin, erythromycin, kanamycin A, gentamicin B, daptomycin, colistin A/B, chloramphenicol, and ampicillin. These genomic predictions were confirmed by phenotypic disk diffusion assays (Table 2), which showed resistance to kanamycin, colistin, tetracycline, vancomycin, rifampin, and gentamicin (Figure 2). *L. garvieae* possesses intrinsic resistance to certain antibiotics, which may facilitate its adaptation within host microbiota. While some probiotic strains naturally tolerate specific antibiotics, the overall susceptibility profile of MH3 supports its potential safety for probiotic applications. Such intrinsic resistance could even help maintain probiotic populations during antibiotic treatment. Nonetheless, further investigation is required to determine how AMR genes are acquired and maintaine [33]. Additionally, the multidrug transporter *mdt*(A) gene previously described in other *L. garvieae* genomes was detected in MH3, with specific mutations linked to erythromycin sensitivity.

V. CONCLUSION

In this study, we conducted a comprehensive genomic and phenotypic analysis of *Lactococcus garvieae* MH3, a strain isolated from cow's milk cheese. Genome sequencing revealed key features, including 73 contigs, a G/C content of 37%, and multiple genes associated with antibiotic resistance. The presence of resistance determinants was confirmed by phenotypic disk diffusion assays, which demonstrated resistance to several clinically relevant antibiotics. While *L. garvieae* exhibits traits that support its potential as a probiotic candidate, its capacity to harbor antimicrobial resistance (AMR) genes raises important concerns regarding biosafety and cross-species transmission. These findings highlight the dual nature of *L. garvieae* as both a promising industrial microorganism and a potential pathogen. Therefore, careful strain characterization and functional validation are essential before considering its application in food or probiotic formulations. Future studies should focus on in vivo assessments, expression profiling of resistance and virulence genes, and the development of genetically defined, non-pathogenic variants to ensure safe and effective utilization of this strain in biotechnological and clinical contexts.

ACKNOWLEDGMENT

This study was funded by the Muş Alparslan University-Scientific Research Coordination Unit under project number BAP-21-TBMY-4901-06.

REFERENCES

[1] Vendrell, D., Balcázar, J. L., Ruiz-Zarzuela, I., De Blas, I., Gironés, O., and Múzquiz, J. L. (2006). *Lactococcus garvieae* in fish: a review, Comparative immunology, microbiology and infectious diseases, 29 (4), 177-198. https://doi.org/10.1016/j.cimid.2006.06.003.

[2] Garvie, E. I., Farrow, J. A., and Phillips, B. A. (1981). A taxonomic study of some strains of streptococci which grow at 10° C but not at 45° C including *Streptococcus lactis* and *Streptococcus cremoris*, Zentralblatt für Bakteriologie Mikrobiologie und Hygiene: I. Abt. Originale C: Allgemeine, angewandte und ökologische Mikrobiologie, 2 (2), 151-165. https://doi.org/10.1016/S0721-9571(81)80037-3.

[3] Lin, Y. *et al.* (2023). Comparative genomic analyses of *Lactococcus garvieae* isolated from bovine mastitis in China, Microbiology Spectrum, 11 (3), e02995-22. https://doi.org/10.1128/spectrum.02995-22.

[4] Gibello, A., Galán-Sánchez, F., Blanco, M. M., Rodríguez-Iglesias, M., Domínguez, L., and Fernández-Garayzábal, J. F. (2016). The zoonotic potential of *Lactococcus garvieae*: An overview on microbiology, epidemiology, virulence factors and relationship with its presence in foods, Research in Veterinary Science, 109, 59-70. https://doi.org/10.1016/j.rvsc.2016.09.010.

[5] Haenen, O. L. M. *et al.* (2023). Bacterial diseases of tilapia, their zoonotic potential and risk of antimicrobial resistance, Reviews in Aquaculture, 15 (S1), 154-185. https://doi.org/10.1111/raq.12743.

[6] Francés-Cuesta, C., Ansari, I., Fernández-Garayzábal, J. F., Gibello, A., and González-Candelas, F. (2022). Comparative genomics and evolutionary analysis of *Lactococcus garvieae* isolated from human endocarditis, Microbial Genomics, 8 (2). https://doi.org/10.1099/mgen.0.000771.

[7] Meyburgh, C., Bragg, R., and Boucher, C. (2017). *Lactococcus garvieae*: an emerging bacterial pathogen of fish, Diseases of Aquatic Organisms, 123 (1), 67-79.

[8] Sohail, M. *et al.* (2023). The Threat of Transboundary Zoonosis, Zoonosis, Unique Scientific Publishers, Faisalabad, Pakistan, 4, 701-715.

[9] Wang, C. Y. *et al.* (2007). *Lactococcus garvieae* infections in humans: possible association with aquaculture outbreaks, Int J Clin Pract, 61 (1), 68-73. 10.1111/j.1742-1241.2006.00855.x.

[10] Aguado-Urda, M., Gibello, A., Blanco, M. M., López-Campos, G. H., Cutuli, M. T., and Fernández-Garayzábal, J. F. (2012). Characterization of Plasmids in a Human Clinical Strain of *Lactococcus garvieae*, PLOS ONE, 7 (6), e40119. 10.1371/journal.pone.0040119.

[11] Aguirre-Guzmán, G., Lara-Flores, M., Sánchez-Martínez, J. G., Campa-Córdova, A. I., and Luna-González, A. (2012). The use of probiotics in aquatic organisms: A review, African Journal of microbiology research, 6 (21), 4845-4857.

[12] Zhang, T., Xie, J., Zhang, M., Fu, N., and Zhang, Y. (2016). Effect of a potential probiotics *Lactococcus garvieae* B301 on the growth performance, immune parameters and caecum microflora of broiler chickens, Journal of Animal Physiology and Animal Nutrition, 100 (3), 413-421. https://doi.org/10.1111/jpn.12388.

[13] Wang, X.-Y. *et al.* (2024). Gut *Lactococcus garvieae* promotes protective immunity to foodborne *Clostridium perfringens* infection, Microbiology Spectrum, 12 (10), e04025-23. doi:10.1128/spectrum.04025-23.

[14] Ayyash, M. *et al.* (2020). Exopolysaccharide produced by the potential probiotic *Lactococcus garvieae* C47: Structural characteristics, rheological properties, bioactivities and impact on fermented camel milk, Food Chemistry, 333, 127418. https://doi.org/10.1016/j.foodchem.2020.127418.

[15] Le, L. T. H. L. *et al.* (2022). Dual functional roles of a novel bifunctional β-lactamase/esterase from *Lactococcus garvieae*, International Journal of Biological Macromolecules, 206, 203-212.

[16] Mi, H. *et al.* (2022). *Lactococcus garvieae* FUA009, a novel intestinal bacterium capable of producing the bioactive metabolite urolithin A from ellagic acid, Foods, 11 (17), 2621.

[17] Sciuto, S. *et al.* (2022). What can genetics do for the control of infectious diseases in aquaculture?, Animals, 12 (17), 2176. https://doi.org/10.3390/ani12172176.

[18] Fraser-Liggett, C. M. (2005). Insights on biology and evolution from microbial genome sequencing, Genome research, 15 (12), 1603-1610. http://www.genome.org/cgi/doi/10.1101/gr.3724205.

[19] Wu, C., Huang, J., and Zhou, R. (2017). Genomics of lactic acid bacteria: Current status and potential applications, Critical Reviews in Microbiology, 43 (4), 393-404. https://doi.org/10.1080/1040841X.2016.1179623.

[20] Stefanovic, E., Fitzgerald, G., and McAuliffe, O. (2017). Advances in the genomics and metabolomics of dairy lactobacilli: a review, Food microbiology, 61, 33-49. https://doi.org/10.1016/j.fm.2016.08.009.

[21] Li, W., Wu, Q., Kwok, L.-y., Zhang, H., Gan, R., and Sun, Z. (2024). Population and functional genomics of lactic acid bacteria, an important group of food microorganism: Current knowledge, challenges, and perspectives, Food Frontiers, 5 (1), 3-23. https://doi.org/10.1002/fft2.321.

[22] ONUR, M. and ONLU, H. (2021). Farklı Gıda Ürünlerinden İzole Edilen Laktik Asit Bakterilerinin Bazı Probiyotik Özelliklerinin Belirlenmesi, Avrupa Bilim ve Teknoloji Dergisi, (32), 562-572.

[23] Altschul, S. F. *et al.* (1997). Gapped BLAST and PSI-BLAST: a new generation of protein database search programs, Nucleic acids research, 25 (17), 3389-3402. https://doi.org/10.1093/nar/25.17.3389.

[24] Grant, J. R. *et al.* (2023). Proksee: in-depth characterization and visualization of bacterial genomes, Nucleic Acids Res, 51 (W1), W484-w492. 10.1093/nar/gkad326.

[25] Bauer, A., Kirby, W., Sherris, J. C., and Turck, M. (1966). Antibiotic susceptibility testing by a standardized single disk method, American journal of clinical pathology, 45 (4_ts), 493-496. https://doi.org/10.1093/ajcp/45.4_ts.493.

[26] CLSI, C. (2016). Performance standards for antimicrobial susceptibility testing, Clinical Lab Standards Institute, 35 (3), 16-38.

[27] Kant, R., Blom, J., Palva, A., Siezen, R. J., and de Vos, W. M. (2011). Comparative genomics of *Lactobacillus*, Microbial biotechnology, 4 (3), 323-332. https://doi.org/10.1111/j.1751-7915.2010.00215.x.

[28] Lynch, K. M., Lucid, A., Arendt, E. K., Sleator, R. D., Lucey, B., and Coffey, A. (2015). Genomics of *Weissella cibaria* with an examination of its metabolic traits, Microbiology, 161 (4), 914-930. https://doi.org/10.1099/mic.0.000053.

[29] Mahmoud, M. M. *et al.* (2023). Comparative genome analyses of three serotypes of *Lactococcus* bacteria isolated from diseased cultured striped jack (*Pseudocaranx dentex*), Journal of Fish Diseases, 46 (8), 829-839. https://doi.org/10.1111/jfd.13792.

[30] Chan, Y.-X. *et al.* (2024). Genomic investigation of *Lactococcus formosensis*, *Lactococcus garvieae*, and *Lactococcus petauri* reveals differences in species distribution by human and animal sources, Microbiology Spectrum, 12 (6), e00541-24. https://doi.org/10.1128/spectrum.00541-24.

[31] Altinok, I., Ozturk, R. C., and Ture, M. (2022). NGS analysis revealed that *Lactococcus garvieae* Lg-Per was *Lactococcus petauri* in Türkiye, Journal of Fish Diseases, 45 (12), 1839-1843. https://doi.org/10.1111/jfd.13708.

[32] Torres-Corral, Y. and Santos, Y. (2022). Predicting antimicrobial resistance of *Lactococcus garvieae*: PCR detection of resistance genes versus MALDI-TOF protein profiling, Aquaculture, 553, 738098. https://doi.org/10.1016/j.aquaculture.2022.738098.

[33] Thant, E. P. *et al.* (2024). Exploring *Weissella confusa* W1 and W2 Strains Isolated from Khao-Mahk as Probiotic Candidates: From Phenotypic Traits to Genomic Insights, Antibiotics, 13 (7), 604. https://doi.org/10.3390/antibiotics13070604.