

DATA NOTE

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Complete genome sequence of *Pseudolactococcus raffinolactis* strain GCULR from a spotted seal (*Phoca largha*) in Korea

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Abstract

Objectives *Pseudolactococcus raffinolactis*—a lactic acid-producing bacterium recently reclassified from *Lactococcus*—demonstrates emerging potential for probiotics and food applications. We report the complete genome of strain GCULR isolated from a stranded spotted seal (*Phoca largha*) in Korea, to provide genomic data for understanding beneficial bacteria in marine mammals.

Data description *P. raffinolactis* strain GCULR was isolated from a stranded spotted seal in Korea, and its whole genome was sequenced using PacBio and Illumina X-10 platforms. Its complete genome comprises a single circular chromosome of 2,270,555 bp with 39.9% GC content and no plasmids. Annotation revealed 2,260 genes, including 2,145 protein-coding sequences, 56 transfer RNAs, 13 ribosomal RNAs, 3 non-coding RNAs, and 43 pseudogenes. Comparative analysis showed an average nucleotide identity of 99.3% with strain WiKim0068, confirming its close relationship with a food-derived isolate. Genome-based screening revealed the presence of several potential virulence- and antimicrobial resistance-associated genes and two intact prophage regions. AntiSMASH analysis predicted a conserved bacteriocin biosynthetic cluster, indicating its functional potential. These findings offer novel insights into the beneficial bacteria in endangered marine mammals and expand our understanding of the ecological and functional diversity of *P. raffinolactis*.

Keywords *Pseudolactococcus raffinolactis*, Lactic acid-producing bacteria, Marine mammal, Bacteriocin

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Objective

Several marine mammalian species have been classified as endangered or vulnerable, and understanding their health status is essential for establishing effective conservation strategies [1]. Microbial research on marine mammals has primarily focused on detecting pathogens and their biological and genetic characteristics, offering insights into host health, environmental exposure, and potential zoonotic risks [2, 3]. However, research on symbiotic or beneficial microbes in marine mammals is limited. *Pseudolactococcus* (*P.*) is a genus of gram-positive, non-motile, and catalase-negative cocci that was recently



reclassified from *Lactococcus* based on genome-based analyses and currently comprises 11 validly published species [4]. *P. raffinolactis* demonstrates promising probiotic and industrial potential. Strain WiKim0068 from kimchi exhibited vitamin B₃ biosynthesis capacity and lacked antibiotic resistance genes [5]. Strain 37 enhanced acid production during dairy fermentation when co-cultured with *Lactococcus lactis* [6], whereas APC 3967 produced bacteriocins active against *L. lactis* [7]. Most *P. raffinolactis* strains are isolated from dairy or fermented foods, and studies on their environmental distribution are limited.

In this study, we report the complete genome of *P. raffinolactis* strain GCULR, isolated from a spotted seal (*Phoca largha*) stranded in Korea in 2023. To our knowledge, this is the first report of potentially beneficial bacteria in Korean marine mammals. Sequencing the complete genome facilitates the understanding of *Pseudolactococcus* diversity across environments, enables comparative genomic studies, and expands the knowledge of beneficial bacteria in endangered marine mammals and the ecological diversity of *P. raffinolactis*.

Data description

Strain GCULR was isolated from sterile swab samples of the body fluid of a stranded spotted seal, which was deposited at the Cetacean Research Institute, National Institute of Fisheries Science, Korea (voucher no. CRI012528) [8], using tryptic soy agar at 37 °C for 24 h. The isolate was identified using 16 S ribosomal RNA (rRNA) sequencing. The 16 S rRNA gene was amplified using universal primers 27 F/1492R, sequenced with primers 785 F/907R (Macrogen Inc., Seoul, Korea), and compared with the EzBioCloud database (<https://www.ezbiocloud.net>). The GCULR strain exhibited >99.8% nucleotide sequence similarity with other *P. raffinolactis* strains in GenBank. Antimicrobial susceptibility was tested following the Clinical and Laboratory Standards Institute M45 guidelines [9] using E-test strips (Liofilchem, Roseto degli Abruzzi, Italy), and the isolate did not show any significant resistance to the tested antibiotics (Table 1, Data file 1) [10].

Genomic DNA was extracted using the QIAamp DNA Mini Kit (Qiagen, Hilden, Germany) and sequenced using the PacBio RSII System (Pacific Biosciences, Menlo Park, CA, USA) and Illumina HiSeq X-10 platform (Illumina, San Diego, CA, USA) [11]. The PacBio long reads (1,093,499,150 bp; 109,825 reads) and Illumina paired-end reads (1,712,874,598 bp; 11,348,536 reads) were assembled using the Microbial Genome Assembly application (<http://www.pacb.com/products-and-services/pacbio-systems/sequel/sequel-software/>) and error-corrected using Pilon v1.21 [12]. Genome annotation was performed using the National Center for Biotechnology

Information prokaryotic genome annotation pipeline (https://www.ncbi.nlm.nih.gov/genome/annotation_prok/). The GCULR genome comprised a single chromosome (2,270,555 bp, 39.9% G + C) with no plasmids (Table 2, Data file 2) [10] and contained 2,260 genes—2,145 protein-coding, 56 transfer RNAs, 13 rRNAs, 3 non-coding RNAs, and 43 pseudogenes.

Phylogenetic analysis of the 16 S rRNA gene against *Lactococcus*–*Pseudolactococcus* species confirmed clustering with the *P. raffinolactis* type strain JCM 5706^T—supporting the novel genus *Pseudolactococcus* [4] and confirming that the isolate can be classified as *P. raffinolactis* (Fig. 1, Data file 3) [10]. OrthoANI analysis (<https://www.ezbiocloud.net/tools/orthoani>) revealed a clear separation of *Pseudolactococcus* from *Lactococcus*, with GCULR exhibiting 99.3% average nucleotide identity with the *P. raffinolactis* strain WiKim0068 (Fig. 1, Data file 3) [10].

Screening for virulence- and antimicrobial resistance (AMR)-related genes and prophage regions [11] revealed several factors, including bacterial adherence, streptococcal enolase, manganese uptake, and protease (Table 3, Data file 4) [10]. These genes are commonly found in non-pathogenic and environmental bacteria [13, 14]. Two vancomycin resistance genes (*vanW* and *vanY*) were detected, but with low amino acid identity (28.6% and 28.2%, respectively) (Table 4, Data file 5) [10]. Two intact prophage regions were identified—30.6 kb (Region 1) and 42.5 kb (Region 2) (score >90) (Table 5, Data file 6) [10]. Most virulence- and AMR-related genes and prophage regions were nearly identical to those of WiKim0068.

Secondary metabolite prediction using antiSMASH 7.0 (<https://antismash.secondarymetabolites.org>) identified a biosynthetic gene cluster (BGC) (761,124–769,142 bp) encoding seven proteins, including Lactococcin 972 (Lcn 972) bacteriocin, with 99.8–100% amino acid identity and full query coverage to other *P. raffinolactis* strains (Table 6, Data file 7) [10]. This conserved BGC indicates antimicrobial compound production. Lactic acid bacteria-produced bacteriocins are considered safe by the US Food and Drug Administration [15]. Lcn 972, a Class IIc bacteriocin, binds to lipid II, thereby inhibiting septum formation and interrupting peptidoglycan synthesis in *L. lactis* [16]. The presence of the Lcn 972 gene indicates bacteriocin production potential in GCULR (Table 1).

Limitations

In summary, the complete genome of *P. raffinolactis* GCULR revealed distinct genetic features with potential applications in both probiotics and industrial fields. This study also highlights the genomic diversity of *P. raffinolactis* isolated from various environments, including marine mammals. However, several limitations should be considered. While *P. raffinolactis* strains isolated from

Table 1 Overview of data files/data sets

Label	Name of data file/data set	File types (File extension)	Data repository and identifier (DOI or accession number)
Data file 1	Table 1. Results of the antibiotic susceptibility test for strain GCULR.	MS Excel file (.xlsx)	Figshare [10]: https://doi.org/10.6084/m9.figshare.30100798.v1
Data file 2	Table 2. General genomic features of the <i>P. raffinolactis</i> strain GCULR.	MS Excel file (.xlsx)	Figshare [10]: https://doi.org/10.6084/m9.figshare.30100798.v1
Data file 3	Figure 1. The phylogenetic and genomic identification of <i>P. raffinolactis</i> strain GCULR	Portable Document Format file (.pdf)	Figshare [10]: https://doi.org/10.6084/m9.figshare.30100798.v1
Data file 4	Table 3. Putative virulence factors of GCULR were identified using the Virulence Factor Database (VFDB).	MS Excel file (.xlsx)	Figshare [10]: https://doi.org/10.6084/m9.figshare.30100798.v1
Data file 5	Table 4. Profiles of antimicrobial resistance genes in the GCULR strain using the Comprehensive Antibiotic Resistance Database (CARD).	MS Excel file (.xlsx)	Figshare [10]: https://doi.org/10.6084/m9.figshare.30100798.v1
Data file 6	Table 5. The putative prophage regions of the strain GCULR were identified using Phage Search Tool with Enhanced Sequence Translation (PHASTEST).	MS Excel file (.xlsx)	Figshare [10]: https://doi.org/10.6084/m9.figshare.30100798.v1
Data file 7	Table 6. A putative biosynthetic gene cluster was identified in the genome of strain GCULR using antibiotics and Secondary Metabolite Analysis Shell (antiSMASH).	MS Excel file (.xlsx)	Figshare [10]: https://doi.org/10.6084/m9.figshare.30100798.v1
Data file 8	Paired-end Illumina reads	Fastq file (.fastq)	NCBI GenBank Database [17]: SRX30397417 http://identifiers.org/insdc.sra:SRX30397417
Data file 9	PacBio high-fidelity consensus reads	Fastq file (.fastq)	NCBI GenBank Database [18]: SRX30397418 http://identifiers.org/insdc.sra:SRX30397418
Data file 10	Complete genome of <i>P. raffinolactis</i> strain GCULR	Fasta file (.fasta)	NCBI GenBank Database [19]: CP178385 https://identifiers.org/insdc:CP178385

fermented foods are generally regarded as safe and beneficial, their ecological roles in marine mammals remain unclear due to the limited microbiological data available for marine mammals. Although strain GCULR was considered a potentially beneficial isolate in this study, further studies using samples from healthy populations are required to determine whether *P. raffinolactis* constitutes part of the commensal microbiota of the spotted seal (*P. largha*). The virulence-associated genes detected (adherence factors, enolase, manganese uptake, and protease) are commonly found in non-pathogenic lactic acid bacteria, where they function primarily in metabolism or host colonization. Notably, the putative vancomycin resistance genes (*vanW* and *vanY*) showed relatively low amino acid identity (<30%) to reference sequences and likely represent non-functional homologs, consistent with the observed vancomycin susceptibility in antimicrobial testing in this study. All functional predictions, including prophage regions and the conserved bacteriocin biosynthetic cluster, were derived solely from in silico analysis and thus require experimental validation through in vitro and in vivo assays. Future studies should focus on microbiological and functional characterization to elucidate the probiotic potential of *P. raffinolactis* from marine mammal environments.

Abbreviations

AMR Antimicrobial Resistance
antiSMASH Antibiotics & Secondary Metabolite Analysis Shell
BGC Biosynthetic gene cluster

rRNA Ribosomal RNA

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Author contributions

JHK and SYP conceived the project. TSC and KL collected the samples. TSC, KL, SL, and SC performed bacterial isolation, DNA extraction, and library preparation. TSC and YS performed bioinformatic analysis. TSC and KL performed the investigation. TSC and SL prepared the original draft, and all authors reviewed, edited, and approved the final manuscript.

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Data availability

P. raffinolactis GCULR strain was deposited in the Korean Collection for Type Cultures (KCTC 21253). Its complete genome sequence is available in GenBank (accession number CP178385), with corresponding BioSample (accession number SAMN46285626) and BioProject (accession number PRJNA1211812) entries.

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Competing interests

The authors declare no competing interests.

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