

## Genome Sequence of *Lactococcus garvieae* IPLA 31405, a Bacteriocin-Producing, Tetracycline-Resistant Strain Isolated from a Raw-Milk Cheese

### Ana Belén Flórez,<sup>a</sup> Pilar Reimundo,<sup>b</sup> Susana Delgado,<sup>a</sup> Elena Fernández,<sup>a</sup> Ángel Alegría,<sup>a</sup> José A. Guijarro,<sup>b</sup> and Baltasar Mayo<sup>a</sup>

Departamento de Microbiología y Bioquímica de Productos Lácteos, Instituto de Productos Lácteos de Asturias (IPLA-CSIC), Villaviciosa, Asturias, Spain,<sup>a</sup> and Área de Microbiología, Departamento de Biología Funcional, Facultad de Medicina, IUBA, Universidad de Oviedo, Oviedo, Spain<sup>b</sup>

# This work describes the draft genome sequence of *Lactococcus garvieae* IPLA 31405, isolated from a traditional Spanish cheese. The genome contains a lactose-galactose operon, a bacteriocin locus, two integrated phages, a transposon harboring an active *tet*(M) gene, and two theta-type plasmid replicons. Genes encoding virulence factors were not recorded.

Lactocococcus garvieae has repeatedly been reported to be a majority component of the native microbiota of dairy products manufactured from raw milk (3, 5, 8, 10). This bacterium is the etiological agent of lactococcosis, affecting both marine and freshwater fish species (6). It also causes mastitis in cows (15) and has been involved in an increasing number of human infections (4). However, association between consumption of raw-milk cheese and human infections caused by *L. garvieae* has never been reported. Conversely, *L. garvieae* might contribute to the overall quality of dairy products, improving both safety and sensorial attributes (7, 9, 11).

Recently, the genomes of several *L. garvieae* strains have been released (1, 2, 12, 13, 14). Here we report the draft genome sequence of *L. garvieae* IPLA 31405, isolated from among the dominant microbiota of a traditional raw-milk cheese (3). IPLA 31405 grows well in lactose and produces a bacteriocin active against food-borne pathogens. It lacks hemolysin and gelatinase activities and does not produce biogenic amines (7). However, it showed resistance to tetracycline encoded by a *tet*(M) gene (7).

A genomic library of 0.5 kbp was constructed and subjected to paired-end sequencing, providing approximately 155-fold coverage, using a HiSeq 1000 System sequencer (Illumina). Quality-filtered reads were assembled into contigs using the software program Velvet (http://www.ebi.ac.uk/~zerbino/velvet/). Annotation was performed by merging the results obtained from RAST (http://rast .nmpdr.org/), BG (Era7, Granada, Spain), and BLAST analysis (http: //blast.ncbi.nlm.nih.gov). The KEGG Pathway (http://www.genome .jp/kegg/pathway.html), Uniprot (http://www.uniprot.org), and COG (http://www.ncbi.nlm.nih.gov/COG/) databases were consulted for descriptions of specific genes.

The draft genome sequence of IPLA 31405 includes 23 contigs from 598 to 1,017,382 bp (bp) and is composed of 2,052,308 bp with a GC content of 38.53%. It encodes 1,874 predicted coding sequences, which were classified into 23 classes and 308 subsystems by the RAST server. Single predicted copies of 16S, 23S, and 5S rRNA genes were found, as well as 46 genes for tRNAs. Two theta-type plasmid replicons and two integrated phages belonging to the P335 group of *Lactococcus lactis* phages were recorded. In addition, the *tet*(M) gene was harbored in a transposon highly similar to conjugative Tn6086 from *Enterococcus faecalis*.

IPLA 31045 may metabolize lactose by a lactose phosphotransferase operon (*lacXGEFDCBA*) identified in its genome. However, extracellular caseinolytic peptidases were not found. Production, resistance to, and secretion of a class IIb bacteriocin identical to garvieacin Q (16) are encoded by a four-gene operon. A gene cluster likely to be involved in the synthesis of cell wall exopoly-saccharides with a rhamnosyl backbone was detected, but typical capsule-encoding genes were not scored. Additionally, no evidence of virulence-related genes was obtained.

The genome sequence of strain IPLA 31405 provided further insights into the intraspecific variation of *L. garvieae*. Its comparison with those of other sequenced strains may supply information on the safety of the strains and on niche-specific genes.

Nucleotide sequence accession numbers. This whole-genome shotgun project was deposited in DDBJ/EMBL/GenBank under accession number AKFO0000000 (BioProject PRJNA161657). The version described in this article is the first version, AKFO01000000.

### ACKNOWLEDGMENTS

This study was supported by projects of the Spanish Ministry of Economy and Competitiveness (AGL2007-61869 and AGL2011-24300).

V. Ladero, IPLA-CSIC, is gratefully acknowledged for assistance and helpful discussions during genome analysis.

### REFERENCES

- 1. Aguado-Urda M, et al. 2011. Genome sequence of *Lactococcus garvieae* 21881, isolated in a case of human septicemia. J. Bacteriol. 193:4033–4034.
- Aguado-Urda M, et al. 2011. Genome sequence of *Lactococcus garvieae* 8831, isolated from rainbow trout lactococcosis outbreaks in Spain. J. Bacteriol. 193:4263–4264.
- 3. Alegría A, et al. 2009. Diversity and evolution of microbial populations during manufacture and ripening of Casín, a traditional Spanish, starter-free cheese made from cow's milk. Int. J. Food Microbiol. 136:44–51.
- Chan JFW, et al. 2011. Primary infective spondylodiscitis caused by Lactococcus garvieae and a review of human L. garvieae infections. Infection 39:259–264.
- El-Baradei G, Delacroix-Buchet A, Ogier J-C. 2008. Bacterial diversity of traditional Zabady fermented milk. Int. J. Food Microbiol. 121:295–301.
- Eyngor M, et al. 2004. Clonality and diversity of the fish pathogen *Lactococcus garvieae* in Mediterranean countries. Appl. Environ. Microbiol. 70:5132–5137.

Received 4 June 2012 Accepted 29 June 2012

Address correspondence to Baltasar Mayo, baltasar.mayo@ipla.csic.es.

Copyright © 2012, American Society for Microbiology. All Rights Reserved. doi:10.1128/JB.00975-12

- 7. Fernández E, Alegría A, Delgado S, Mayo B. 2010. Phenotypic, genetic and technological characterization of *Lactococcus garvieae* strains isolated from a raw milk cheese. Int. Dairy J. 20:142–148.
- Fortina MG, et al. 2003. Genetic characterization of some lactic acid bacteria occurring in an artisanal protected denomination origin (PDO) Italian cheese, the Toma Piedemontese. Food Microbiol. 20:397–404.
- Fortina MG, et al. 2007. Phenotypic typing, technological properties and safety aspects of *Lactococcus garvieae* strains from dairy environments. J. Appl. Microbiol. 103:445–453.
- Foschino R, Picozzi C, Borghi M, Cerliani MC, Cresci E. 2006. Investigation on the microflora of Caprino Lombardo cheese from raw goat milk. Ital. J. Food Sci. 18.33–49.
- Foschino R, et al. 2008. Comparison of *Lactococcus garvieae* strains isolated in northern Italy from dairy products and fishes through molecular typing. J. Appl. Microbiol. 105:652–662.
- 12. Morita H, et al. 2011. Complete genome sequence and comparative

analysis of the fish pathogen *Lactococcus garvieae*. PLoS One 6:e23184. doi:10.1371/journal.pone.0023184.

- Reimundo P, et al. 2011. Genome sequence of *Lactococcus garvieae* UNIUD074, isolated in Italy from a lactococcosis outbreak. J. Bacteriol. 193:3684–3685.
- Ricci G, Ferrario C, Borgo F, Rollando A, Fortina MG. 2012. Genome sequences of *Lactococcus garvieae* TB25, isolated from Italian cheese, and *Lactococcus garvieae* LG9, isolated from Italian rainbow trout. J. Bacteriol. 194:1249–1250.
- Teixeira ML, et al. 1996. Phenotypic and genotypic characterization of atypical *Lactococcus garvieae* strains isolated from water buffalos with subclinical mastitis and confirmation of *L. garvieae* as a senior subjective synonym of *Enterococcus seriolicida*. Int. J. Syst. Bacteriol. 46:664–668.
- Tosukhowong A, et al. 2012. Garvieacin Q, a novel class II bacteriocin from *Lactococcus garvieae* BCC 43578. Appl. Environ. Microbiol. 78: 1619–1623.