Complete Genome Sequence of the Dairy Isolate Streptococcus macedonicus ACA-DC 198

Konstantinos Papadimitriou,a Stéphanie Ferreira,b Nikolaos C. Papandreou,c Eleni Mavrogonatou,4 Philip Supply,4,a,f,g,h Bruno Pot,4,a,g,h and Effie Tsakalidoua

Laboratory of Dairy Research, Department of Food Science and Technology, Agricultural University of Athens, Athens, Greece; Genoscreen, Genomic Platform and R&D, Campus de l’Institut Pasteur, Lille, France; Department of Cell Biology and Biophysics, Faculty of Biology, University of Athens, Panepistimiopolis, Athens, Greece; Laboratory of Cell Proliferation and Ageing, Institute of Biology, National Center for Scientific Research Demokritos, Athens, Greece; Institut Pasteur de Lille, Center for Infection and Immunity of Lille, Lille, France; INSERM U1019, Lille, France; CNRS UMR8204, Lille, France; and Université Lille Nord de France, Lille, France.

The species Streptococcus macedonicus is associated with the food environment, especially with fermented dairy products. Here we present the complete 2.1-Mb genome sequence of strain ACA-DC 198, which was isolated from naturally fermented Greek kasseri cheese.

Streptococcus macedonicus is an intriguing streptococcal species, since its most frequent source of isolation to date is fermented foods, mainly of dairy origin (5). Within the genus Streptococcus, only Streptococcus thermophilus is considered nonpathogenic, due to its adaptation to the milk environment (3, 8). Even though S. macedonicus has been shown to possess important (bio)technological features similar to those of S. thermophilus (5), it belongs to the Streptococcus bovis/equisinus complex (13). It has been proposed that S. macedonicus is a subspecies of S. gallolyticus, along with S. gallolyticus subsp. galloyticus (formerly S. bovis biotype I) and Streptococcus pasteurianus (formerly S. bovis biotype II) (13). This classification scheme is not universally accepted (15); however, there is no doubt that S. macedonicus is phylogenetically related to streptococci associated with cases of endocarditis, colorectal cancer, bacteremia, and meningitis (1, 6). Accordingly, the pathogenicity status of S. macedonicus is ambivalent, raising concerns about the safety of its use as a starter or adjunct culture in food fermentations.

Sequencing of S. macedonicus ACA-DC 198 genome was performed using the 454 GS-FLX (Roche Diagnostics, Basel, Switzerland) and the HiSeq 2000 (Illumina, San Diego, CA) technologies at Genoscreen (Lille, France) and Fidelity Systems, Inc. (Gaithersburg, MD), respectively. Following an initial round of shotgun pyrosequencing, contigs were assembled using Newbler Assembler software (454 Life Sciences, Branford, CT) and further combined with 3-kb paired-end reads down to 7 scaffolds. An additional round of Illumina sequencing was necessary for complete gap closure and finishing. The hybrid assembly between 454 and HiSeq 2000 data (>200× coverage) after analysis with Velvet (16), Newbler, and Fidelity Systems’ in-house finishing software resulted in one continuous genomic scaffold of 2,130,034 bp and a plasmid of 12,728 bp. The genome assembly was validated against an Nhel optical map of the S. macedonicus genome that was produced at OpGen Technologies, Inc. (Madison, WI).

Sequences were annotated with the BaSys (14) and the RAST (2) pipelines and manually curated using Kodon (Applied Maths N.V., Sint-Martens-Latem, Belgium). Final corrections were made based on the quality assessment of the annotation using GenePRIMP (11). We found 2,192 protein-coding genes on the chromosome, 192 of which were identified as potential pseudogenes, indicating an ongoing genome decay process. This hypothesis is also supported by the fact that the S. macedonicus genome is approximately 220 kb smaller than the S. galloyticus genome (7, 9, 12), despite the high level of gene synteny between the two species. Such a reductive evolutionary process is common among lactic acid bacteria adapted to the food environment (10) and in the case of S. thermophilus was also accompanied by the loss of pathogenicity traits (3). Interestingly, S. macedonicus ACA-DC 198 does not carry the pil1 pilus locus, which is involved in infectious endocarditis caused by S. galloyticus (4). These findings illustrate the usefulness of and the need for comprehensive comparative genomic analysis of S. macedonicus against its related streptococcal pathogens in order to assess the safety of the species for its use in foods.

Nucleotide sequence accession numbers. The S. macedonicus ACA-DC 198 chromosome and plasmid pSMA198 sequences have been deposited in EMBL under accession numbers HE613569 and HE613570.

ACKNOWLEDGMENTS

The present work was cofinanced by the European Social Fund and the National resources EPEAEK and YPEPTH through the Thales project.

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Received 22 December 2011 Accepted 19 January 2012
Address correspondence to Konstantinos Papadimitriou, kpapadimitriou@aua.gr, or Effie Tsakalidou, et@aua.gr.
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