



# Draft Genome Sequences of Three Virulent *Streptococcus thermophilus* Bacteriophages Isolated from the Dairy Environment in the Veneto Region of Italy

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**ABSTRACT** *Streptococcus thermophilus*, a very important dairy species, is constantly threatened by phage infection. We report the genome sequences of three *S. thermophilus* bacteriophages isolated from a dairy environment in the Veneto region of Italy. These sequences will be used for the development of new strategies to detect and control phages in dairy environments.

*Streptococcus thermophilus* is a low-GC Gram-positive bacterium considered the second most important dairy species (1) and is commonly used to produce cheese and yogurts (2, 3). Currently, its statuses of generally recognized as safe (GRAS) and of qualified presumption of safety (QPS) (4) make it reach a market value of \$40 billion (5, 6).

Ubiquitous in the dairy environment, bacterial viruses or bacteriophages are a constant threat to *S. thermophilus* starter cultures (7, 8). Overall, economic losses due to phage infection in dairy products are related to low fermentation activity and reduced product quality that may lead to total process failure (9, 10).

Here, we report the genome sequences of three *S. thermophilus* bacteriophages isolated from a dairy environment in Northeast Italy, vB\_SthS\_VA214, vB\_SthS\_VA460, and the partial genome sequence of vB\_SthS\_VA698 (VA214, VA460, and VA698, respectively).

Bacteriophages were concentrated and purified using polyethylene glycol 8,000, and their genomic DNA was extracted following the method described by Binetti et al. (11). Sequencing was performed with the Illumina MiSeq platform using paired-end (PE) reads (2 × 250 bp) and a Nextera library at the Ramaciotti Centre for Genomics (Sydney, Australia). After quality filtering and merging of the overlapping PE reads, a total of 56,194, 57,208, and 68,210 sequences were obtained. Raw reads were assembled *de novo* using CLC Genomic Workbench software (version 9.5). Coverage values obtained for VA214, VA460, and VA698 were approximately 367-, 308-, and 122-fold, respectively. Total genomes sizes of 38.2, 41.2, and 33.3 Kb were estimated for VA214, VA460, and VA698, respectively, with an average GC content of 38.6%.

The Rapid Annotations using Subsystems Technology (RAST) server (12) was used for gene finding and annotation. In total, 53, 56, and 38 coding sequences (CDS) were predicted for VA214, VA460, and VA698, respectively. For phage VA214 only, a gene cluster encoding seven tRNAs (Gly, Ala, Val, Lys, Leu, Thr, and Gly) and without introns or pseudogenes was identified using the tRNAscan-SE program (13).

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A BLASTn similarity search revealed that the genome of VA214 is related to *Streptococcus* phage P0091, whereas VA460 and VA698 are closely related to *Streptococcus* phage P9851 and *Streptococcus* phage P7132, respectively.

In this work, we present the complete genome sequences of three *S. thermophilus* bacteriophages, which will serve as the basis for developing new strategies useful to detect and control phages in dairy environments. In the future, we will also conduct studies of bacterial immunity against phages using *S. thermophilus* M17PTZA496 (14) and *S. thermophilus* TH1435/1436 (15).

**Accession number(s).** This whole-genome shotgun project has been deposited at DDBJ/ENA/GenBank under the accession numbers [MG708274](#), [MG708275](#), and [MG708273](#) for vB\_SthS\_VA214, vB\_SthS\_VA460, and vB\_SthS\_VA698, respectively. The versions described in this paper are the first versions, MG708274.1, MG708275.1, and MG708273.1, respectively.

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## REFERENCES

- Hols P, Hancy F, Fontaine L, Grossiord B, Prozzi D, Leblond-Bourget N, Decaris B, Bolotin A, Delorme C, Dusko Ehrlich S, Guédon E, Monnet V, Renault P, Kleerebezem M. 2005. New insights in the molecular biology and physiology of *Streptococcus thermophilus* revealed by comparative genomics. *FEMS Microbiol Rev* 29:435–463. <https://doi.org/10.1016/j.fm.2005.04.008>.
- Calasso M, Ercolini D, Mancini L, Stellato G, Minervini F, Di Cagno R, De Angelis M, Gobbetti M. 2016. Relationships among house, rind and core microbiotas during manufacture of traditional Italian cheeses at the same dairy plant. *Food Microbiol* 54:115–126. <https://doi.org/10.1016/j.fm.2015.10.008>.
- Marco ML, Heeney D, Binda S, Cifelli CJ, Cotter PD, Foligné B, Gänzle M, Kort R, Pasin G, Pihlanto A, Smid EJ, Hutkins R. 2017. Health benefits of fermented foods: microbiota and beyond. *Curr Opin Biotechnol* 44: 94–102. <https://doi.org/10.1016/j.copbio.2016.11.010>.
- Vendramin V, Treu L, Campanaro S, Lombardi A, Corich V, Giacomini A. 2017. Genome comparison and physiological characterization of eight *Streptococcus thermophilus* strains isolated from Italian dairy products. *Food Microbiol* 63:47–57. <https://doi.org/10.1016/j.fm.2016.11.002>.
- Bolotin A, Quinquis B, Renault P, Sorokin A, Ehrlich SD, Kulakauskas S, Lapidus A, Goltsman E, Mazur M, Pusch GD, Fonstein M, Overbeek R, Kyprides N, Purnelle B, Prozzi D, Ngui K, Masuy D, Hancy F, Burteau S, Boutry M, Delcour J, Goffeau A, Hols P. 2004. Complete sequence and comparative genome analysis of the dairy bacterium *Streptococcus thermophilus*. *Nat Biotechnol* 22:1554–1558. <https://doi.org/10.1038/nbt1034>.
- Hutkins RW. 2007. Microbiology and technology of fermented foods microbiology and technology of fermented foods John Wiley & Sons, Hoboken, NJ.
- Clokic MR, Millard AD, Letarov AV, Heaphy S. 2011. Phages in nature. *Bacteriophage* 1:31–45. <https://doi.org/10.4161/bact.1.1.14942>.
- Marcó MB, Moineau S, Quiberoni A. 2012. Bacteriophages and dairy fermentations. *Bacteriophage* 2:149–158. <https://doi.org/10.4161/bact.21868>.
- Bruttin A, Desiere F, D'amico N, Guérin JP, Sidoti J, Huni B, Lucchini S, Brüßow H. 1997. Molecular ecology of *Streptococcus thermophilus* bacteriophage infections in a cheese factory. *Appl Environ Microbiol* 63: 3144–3150.
- Moineau S, Lévesque C. 2004. Control of Bacteriophages in Industrial Fermentations, p. 285–296. *In* Bacteriophages CRC Press, Boca Raton, FL.
- Binetti AG, Del Rio B, Martin MC, Alvarez MA. 2005. Detection and characterization of *Streptococcus thermophilus* bacteriophages by use of the antireceptor gene sequence. *Appl Environ Microbiol* 71:6096–6103. <https://doi.org/10.1128/AEM.71.10.6096-6103.2005>.
- Overbeek R, Olson R, Pusch GD, Olsen GJ, Davis JJ, Disz T, Edwards RA, Gerdes S, Parrello B, Shukla M, Vonstein V, Wattam AR, Xia F, Stevens R. 2014. The SEED and the rapid annotation of microbial genomes using subsystems technology (RAST). *Nucleic Acids Res* 42:D206–D214. <https://doi.org/10.1093/nar/gkt1226>.
- Lowe TM, Eddy SR. 1996. TRNAscan-SE: a program for improved detection of transfer RNA genes in genomic sequence. *Nucleic Acids Res* 25:955–964.
- Treu L, Vendramin V, Bovo B, Campanaro S, Corich V, Giacomini A. 2014. Genome sequences of *Streptococcus thermophilus* strains MTH17CL396 and M17PTZA496 from Fontina, an Italian PDO cheese. *Genome Announc* 2:e00067-14. <https://doi.org/10.1128/genomeA.00067-14>.
- Treu L, Vendramin V, Bovo B, Campanaro S, Corich V, Giacomini A. 2014. Whole-genome sequences of *Streptococcus thermophilus* strains TH1435 and TH1436, isolated from raw goat milk. *Genome Announc* 2:e01129-13. <https://doi.org/10.1128/genomeA.01129-13>.