



# Complete Genome Sequence of *Streptococcus thermophilus* Strain B59671, Which Naturally Produces the Broad-Spectrum Bacteriocin Thermophilin 110

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**ABSTRACT** *Streptococcus thermophilus* strain B59671 is a Gram-positive lactic acid bacterium that naturally produces a broad-spectrum bacteriocin, thermophilin 110, and is capable of producing gamma-aminobutyric acid (GABA). The complete genome sequence for this strain contains 1,821,173 nucleotides, 1,936 predicted genes, and an average G+C content of 39.1%.

*Streptococcus thermophilus* is a nonpathogenic lactic acid bacterium commonly isolated from bovine mammary tissue and raw milk, and it is used as a starter culture for the production of yogurt and cheese. Some *S. thermophilus* strains have been investigated for their production of broad-spectrum antimicrobial peptides called bacteriocins. Several strains naturally produce bacteriocins (1–7), while others require a synthetic 30-mer quorum-sensing peptide, BlpC30, to induce production (8–10). The chromosomal locus encoding BlpC30-induced bacteriocins was designated the bacteriocin-like peptide (*blp*) gene cluster when characterized in strains LMD-9, LMG18311, and CNRZ1066 (11). The *blp* gene cluster varied substantially within these strains, and only LMD-9 was induced to express an active bacteriocin (8, 12).

Here, we report the complete genome sequence of *S. thermophilus* strain B59671, which naturally produces a broad-spectrum bacteriocin, thermophilin 110, encoded within the *blp* gene cluster (13, 14). Thermophilin 110 was reported to inhibit the growth of *Listeria monocytogenes* (13), *Streptococcus mutans*, *Streptococcus pyogenes*, and *Propionibacterium acnes* (15). In addition, similar to *S. thermophilus* strains Y2 (16) and APC151 (17), strain B59671 expresses glutamate decarboxylase and is capable of converting glutamate to gamma-aminobutyric acid (GABA) (18), a potent neurotransmitter reported to have analgesic, antihypertensive, and antidiabetic activities (19).

Following growth in tryptone-yeast extract-lactose medium, cells were pretreated with mutanolysin, and genomic DNA was isolated using the DNeasy blood and tissue kit (Qiagen), according to the manufacturer's protocol. Genome sequencing was performed by the Genomics Core Facility, Clinical and Translational Research Institute, Drexel College of Medicine (Philadelphia, PA) with a Pacific Biosciences RSII system (PacBio, Menlo Park, CA). Single-molecule real-time (SMRT) sequencing was performed with P6/C4 PacBio chemistry. *De novo* assembly was achieved with HGAP assembler version 2.3, yielding a single contig supported by a mean coverage of 542-fold (20). The genome was circularized by permutation to begin at the *dnaA* gene, and terminal duplications were removed using Circlator version 1.0.2. The genome was resequenced with RS\_Modification\_and\_Motif\_Analysis version 2.3, and Prokka version 1.11 (21) and Taxator version 1.2 (22) were used for analysis. The fully assembled genome consisted of a single chromosome of 1,821,173 bp.

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Genome annotation was obtained by the NCBI Prokaryotic Genome Annotation Pipeline (Bethesda, MD) and revealed a total of 1,936 genes, 67 tRNAs, and 6 rRNAs (16S and 23S). In addition, there were 196 pseudogenes identified, which agreed with previous reports showing that approximately 10% of the genes in *S. thermophilus* are nonfunctional (11). The Clone Manager software (Sci-Ed Software, NC) was used to identify homologs of *blpA* and *blpX*, which flank the *blp* gene cluster (11). In B59671, this locus contained 10,067 bp, which was 2,501 and 2,172 bp less than the corresponding loci in strains LMD-9 and LMG18311, respectively (11). The nucleotide sequence from B59671 matched the sequences in LMD-9 and LMG18311 by 76 and 79%, respectively. Further studies will aim to fully characterize the *blp* gene cluster in B59671.

**Accession number(s).** The complete genome sequence for *S. thermophilus* B59671 has been deposited in GenBank under the accession number [CP022547](https://doi.org/10.1093/genome/10.1038/nbt1034).

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## REFERENCES

- Villani F, Pepe O, Mauriello G, Salzano G, Moschetti G, Coppola S. 1995. Antilisterial activity of thermophilin 347, a bacteriocin produced by *Streptococcus thermophilus*. Int J Food Microbiol 25:179–190. [https://doi.org/10.1016/0168-1605\(94\)00153-W](https://doi.org/10.1016/0168-1605(94)00153-W).
- Ward DJ, Somkuti GA. 1995. Characterization of a bacteriocin produced by *Streptococcus thermophilus* ST134. Appl Microbiol Biotechnol 43:330–335. <https://doi.org/10.1007/BF00172834>.
- Marciset O, Jeronimus-Stratingh MC, Mollet B, Poolman B. 1997. Thermophilin 13, a nontypical antilisterial poration complex bacteriocin, that functions without a receptor. J Biol Chem 272:14277–14284. <https://doi.org/10.1074/jbc.272.22.14277>.
- Ivanova I, Miteva V, Stefanova Ts, Pantev A, Budakov I, Danova S, Moncheva P, Nikolova I, Dousset X, Boyaval P. 1998. Characterization of a bacteriocin produced by *Streptococcus thermophilus* 81. Int J Food Microbiol 42:147–158. [https://doi.org/10.1016/S0168-1605\(98\)00067-1](https://doi.org/10.1016/S0168-1605(98)00067-1).
- Mathot AG, Beliard E, Thuault D. 2003. *Streptococcus thermophilus* 580 produces a bacteriocin potentially suitable for inhibition of *Clostridium tyrobutyricum* in hard cheese. J Dairy Sci 86:3068–3074. [https://doi.org/10.3168/jds.S0022-0302\(03\)73906-X](https://doi.org/10.3168/jds.S0022-0302(03)73906-X).
- Kabuki T, Uenishi H, Watanabe M, Seto Y, Nakajima H. 2007. Characterization of a bacteriocin, Thermophilin 1277, produced by *Streptococcus thermophilus* SBT1277. J Appl Microbiol 102:971–980. <https://doi.org/10.1111/j.1365-2672.2006.03159.x>.
- Rossi F, Marzotto M, Cremonese S, Rizzotti L, Torriani S. 2013. Diversity of *Streptococcus thermophilus* in bacteriocin production; inhibitory spectrum and occurrence of thermophilin genes. Food Microbiol 35:27–33. <https://doi.org/10.1016/j.fm.2013.02.006>.
- Fontaine L, Boutry C, Guédon E, Guillot A, Ibrahim M, Grossiord B, Hols P. 2007. Quorum-sensing regulation of the production of Blp bacteriocins in *Streptococcus thermophilus*. J Bacteriol 189:7195–7205. <https://doi.org/10.1128/JB.00966-07>.
- Somkuti GA, Renyé JA, Jr. 2014. Effect of BlpC-based quorum-sensing induction peptide on bacteriocin production in *Streptococcus thermophilus*. J Food Res 4:88–96. <https://doi.org/10.5539/jfr.v4n1p88>.
- Renyé JA, Jr, Somkuti GA, Garabal JI, Steinberg DH. 2016. Bacteriocin production by *Streptococcus thermophilus* in complex growth media. Biotechnol Lett 38:1947–1954. <https://doi.org/10.1007/s10529-016-2184-2>.
- 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>.
- Fontaine L, Hols P. 2008. The inhibitory spectrum of thermophilin 9 from *Streptococcus thermophilus* LMD-9 depends on the production of multiple peptides and the activity of BlpG(St), a thiol-disulfide oxidase. Appl Environ Microbiol 74:1102–1110. <https://doi.org/10.1128/AEM.02030-07>.
- Gilbreth SE, Somkuti GA. 2005. Thermophilin 110: a bacteriocin of *Streptococcus thermophilus* ST110. Curr Microbiol 51:175–182. <https://doi.org/10.1007/s00284-005-4540-7>.
- Renyé JA, Jr, Somkuti GA. 2013. BlpC-regulated bacteriocin production in *Streptococcus thermophilus*. Biotechnol Lett 35:407–412. <https://doi.org/10.1007/s10529-012-1095-0>.
- Renyé JA, Jr, Somkuti GA. March 2017. Bacteriocin with novel activity. U.S. patent 9,598,471.
- Yang SY, Lü FX, Lu ZX, Bie XM, Jiao Y, Sun LJ, Yu B. 2008. Production of gamma-aminobutyric acid by *Streptococcus salivarius* subsp. *thermophilus* Y2 under submerged fermentation. Amino Acids 34:473–478. <https://doi.org/10.1007/s00726-007-0544-x>.
- Linares DM, O'Callaghan TF, O'Connor PM, Ross RP, Stanton C. 2016. *Streptococcus thermophilus* APC151 strain is suitable for the manufacture of naturally GABA-enriched bioactive yogurt. Front Microbiol 7:1876. <https://doi.org/10.3389/fmicb.2016.01876>.
- Somkuti GA, Renyé JA, Jr, Steinberg DH. 2012. Molecular analysis of the glutamate decarboxylase locus in *Streptococcus thermophilus* ST110. J Ind Microbiol Biotechnol 39:957–963. <https://doi.org/10.1007/s10295-012-1114-0>.
- Fayed AE. 2015. Review article: health benefits of some physiologically active ingredients and their suitability as yogurt fortifiers. J Food Sci Technol 52:2512–2521. <https://doi.org/10.1007/s13197-014-1393-8>.
- Chin CS, Alexander DH, Marks P, Klammer AA, Drake J, Heiner C, Clum A, Copeland A, Huddleston J, Eichler EE, Turner SW, Korlach J. 2013. Nonhybrid, finished microbial genome assemblies from long-read SMRT sequencing data. Nat Methods 10:563–569. <https://doi.org/10.1038/nmeth.2474>.
- Seemann T. 2014. Prokka: rapid prokaryotic genome annotation. Bioinformatics 30:2068–2069. <https://doi.org/10.1093/bioinformatics/btu153>.
- Dröge J, Gregor I, McHardy AC. 2015. Taxator-tk: precise taxonomic assignment of metagenomes by fast approximation of evolutionary neighborhoods. Bioinformatics 31:817–824. <https://doi.org/10.1093/bioinformatics/btu745>.