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Special Issue

Advances in Food-Borne Pathogens and Food Safety

Edited by
Prof. Dr. Giuseppe Comi



<https://doi.org/10.3390/microorganisms11030611>



Article

Thermophilin 13: In Silico Analysis Provides New Insight in Genes Involved in Bacteriocin Production

Francesco Salini ^{1,2}, Lucilla Iacumin ^{1,*} , Giuseppe Comi ¹ and Leon M. T. Dicks ^{2,*} ¹ Department of Agriculture, Food, Environmental and Animal Science, University of Udine, 33100 Udine, Italy² Department of Microbiology, Stellenbosch University, Stellenbosch 7600, South Africa

* Correspondence: lucilla.iacumin@uniud.it (L.I.); lmttd@sun.ac.za (L.M.T.D.)

Abstract: Bacteriocins are a large family of ribosomally synthesised proteinaceous toxins that are produced by bacteria and archaea and have antimicrobial activity against closely related species to the producer strain. Antimicrobial proteinaceous compounds are associated with a wide range of applications, including as a pathogen inhibitor in food and medical use. Among the several lactic acid bacteria (LAB) commonly used in fresh and fermented food preservation, *Streptococcus thermophilus* is well known for its importance as a starter culture for yoghurt and cheese. Previous studies described the bacteriocin thermophilin 13 exclusively in *S. thermophilus* SFi13 and the genes encoding its production as an operon consisting of two genes (*thmA* and *thmB*). However, the majority of bacteriocins possess a complex production system, which involves several genes encoding dedicated proteins with relatively specific functions. Up to now, far too little attention has been paid to the genes involved in the synthesis, regulation and expression of thermophilin 13. The aim of the present study, using in silico gene mining, was to investigate the presence of a regulation system involved in thermophilin 13 production. Results revealed the dedicated putative bacteriocin gene cluster (PBGC), which shows high similarity with the class IIb bacteriocins genes. This newly revealed PBGC, which was also found within various strains of *Streptococcus thermophilus*, provides a new perspective and insights into understanding the mechanisms implicated in the production of thermophilin 13.

Keywords: thermophilin; *Streptococcus thermophilus*; gene organization; class IIb bacteriocin



Citation: Salini, F.; Iacumin, L.; Comi, G.; Dicks, L.M.T. Thermophilin 13: In Silico Analysis Provides New Insight in Genes Involved in Bacteriocin Production. *Microorganisms* **2023**, *11*, 611. <https://doi.org/10.3390/microorganisms11030611>

Academic Editor: Peter M. Muriana

Received: 9 January 2023

Revised: 23 February 2023

Accepted: 24 February 2023

Published: 28 February 2023



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1. Introduction

Streptococcus thermophilus is a nonpathogenic lactic acid bacterium commonly isolated from bovine mammary tissue and raw milk, producing lactic acid, exopolysaccharides (EPS) and several organoleptic compounds from the fermentation of lactose and galactose, and also is a well-known starter culture used in the production of yoghurt and cheese [1]. The species has GRAS (Generally Regarded As Safe) status from the FDA (Food and Drug Administration) and QPS (Qualified Presumption of Safety) status from the EFSA (European Food Safety Authority). Several strains of *S. thermophilus* produce bacteriocins, which are small, ribosomally synthesized peptides with narrow or broad spectrum antimicrobial activity [2]. Examples include thermophilin A (strain ST134) [3], thermophilin T (strain ACA-DC 0040) [4], thermophilin 110 (strain 580) [5] and thermophilin 1277 (strain SBT1277) [6]. Two other unnamed bacteriocins were reported in *S. thermophilus* strain 81 and *S. thermophilus* strain 580, but little is known about their peptide sequence and genes encoding them [7,8].

Furthermore, apart from the mentioned examples, *S. thermophilus* SFi13, an isolate belonging to the Nestle' strain collection, produces thermophilin 13. The inhibitory spectrum of thermophilin 13 includes *Clostridium botulinum*, *Listeria monocytogenes*, *Lactococcus lactis*, *Bacillus cereus*, *Bacillus subtilis* and *S. thermophilus* [9].

Expression of bacteriocin genes is usually subject to external induction factors (IFs) regulation. The gene encoding the pre-peptide is normally located in the same operon

as genes encoding the immunity protein, ABC transporter and accessory protein [10]. The accessory protein may also be involved in rendering immunity to the bacteriocin-producing cell [11]. The cleavage site that characterises peptides ThmA and ThmB is preceded by a double-glycine motif found in pre-peptides of class IIb bacteriocins [12,13]. However, thermophilin 13 has been described as an atypical bacteriocin in the sense that the activity of the antibacterial peptide ThmA is enhanced by the peptide ThmB, encoded by genes *thmA* and *thmB*, respectively, on a 960-bp operon (U93029.1) [9]. Thermophilin 13, in agreement with the classification proposed by Zouhir et al. (2010), shares common characteristics with class IIe bacteriocins by having a WX9GX3G motif ($1.02 \times 10^{-7} < p\text{-value} < 7.01 \times 10^{-6}$) in the enhancer peptide ThmB. However, the YGNGV-C motif is missing in both the peptides ThmA and ThmB. The YGNGV-C motif is typical of the anti-*Listeria*-active peptides [13]. Marciset et al. (1997) described thermophilin 13 as an ionophoric poration complex formed by the interaction between ThmA and ThmB [9]. Their results did not provide any information about the involvement of genes other than *thmA* and *thmB* forming the operon, which regulates the production of thermophilin 13. Among bacteriocin-producer strains, lactic acid bacteria play a key role in fresh and fermented food preservation. The present study is focused on *Streptococcus thermophilus* SFi13 strain, which is the only producer reported in the scientific literature of the bacteriocin thermophilin 13. Based on current knowledge, only two genes, *thmA* and *thmB*, are involved in the thermophilin 13 productions, and to our knowledge, no further studies have been pursued to investigate this bacteriocin's mode of action and gene organisation. Therefore, our study aimed to investigate the identity and organisation of all the genes encoding proteins involved in thermophilin 13 regulation, synthesis, transport and immunity, using in silico DNA comparisons.

2. Materials and Methods

2.1. Genome Sequences

The thermophilin 13 operon sequence (U93029.1) amounting to 960-bp and listed in the National Center for Biotechnology (NCBI, Bethesda, MD, USA; <https://www.ncbi.nlm.nih.gov/>; accessed on 2 October 2022) nucleotide database was used to conduct a similarity search using the NCBI Basic Local Alignment Search Tool (BLAST) [14]. Similarities to the thermophilin 13 operon were determined using NCBI Sequence Viewer [15]. The complete genome sequences of all bacterial strains showing the presence of 960-bp with an identity of 100% with the thermophilin 13 operon (U93029.1) sequence were downloaded from the NCBI database.

2.2. Identification and Analysis of the Thermophilin 13 Biosynthetic Gene Cluster (BGC)

To identify potential bacteriocins, biosynthetic gene clusters (BGC) of all genome sequences were analysed using the command-line antiSMASH version 5.0 [16] and BAGEL4 [17]. The ClusterFinder algorithm with additive cluster discovery was used. ClusterFinder source code is available from the GitHub repository (<https://github.com/petercim/ClusterFinder>; accessed on 2 October 2022).

Amino acid sequences with predicted ORFs (open reading frames) were compared against the non-redundant protein database using Blastp version 2.9.0+ (protein–protein BLAST) [18]. Using the Jukes–Cantor model, a Nearest-Neighbor-Interchange (NNI) tree with 1000 Bootstraps was constructed, including all bacteriocin gene sequences provided by antiSMASH and BAGEL4 hosted on the NCBI website. Analyses were conducted using the MEGA 11 software (Version 11.0.11) platform [19].

Putative bacteriocin genes within the respective genomes were annotated using the CLC Main Workbench—QIAGEN Bioinformatics software (CLC bio, Aarhus, Denmark). Sequence alignment was performed using Muscle WS [20] and displayed by Tree Of Life (iTOL) v4 online tool [21]. Comparative analyses of genomic datasets were performed using Operon-mapper [22] and loci of selected bacteriocin genes were visualised using cblaster (github.com/gamcil/clustermap.js; accessed on 16 November 2022) [23], Clinker

& Clustermap.js (github.com/gamcil/clinker; accessed on 16 November 2022) [24] and protein 3D prediction was obtained with ColabFold open-source software available at <https://github.com/sokrypton/ColabFold>; accessed on 15 February 2023 [25].

3. Results

Currently, no complete genome sequence of *S. thermophilus* SFi13 is available on the NCBI database. In a study by Comelli et al. (2002) and in the deposited patent USOO7491386B2, the authors described and evaluated bacterial strains with potential properties as oral probiotics, useful for the prevention of dental caries. According to the Nestlé Culture Collection (NCC), they also affirmed that strain *S. thermophilus* SFi13 was reclassified as *S. thermophilus* NCC 2008 [26,27].

All prior research on this bacteriocin only refers to the partial sequence with Accession Number U93029.1 (NCBI). Despite the reclassification of strain *S. thermophilus* SFi13 to *S. thermophilus* NCC 2008, the genome sequence is also unavailable on the NCBI database. The DNA sequences of operon U93029.1 contained genes *thmA* and *thmB*, putative promoter elements, ribosome binding sites, and a rho-independent terminator structure, as reported by Marciset et al. (1997) [9]. A similarity search using BLAST identified *S. thermophilus* B59671 (CP022547.1), *S. thermophilus* KLDS 3.1003 (CP016877.1), *S. thermophilus* STH_CIRM_1049 (LR822034.1), *S. thermophilus* STH_CIRM_1048 (LR822033.1), *S. thermophilus* CS9 (CP030927.1), *S. thermophilus* DMST-H2 (CP063275.1), TK-P3A (CP045596.1), ATCC 19258 (CP038020.1), *S. thermophilus* LMD-9 (CP086001.1), *S. thermophilus* NCTC12958 (LS483339), and *Streptococcus macedonicus* 19AS (PEBN00000000.1) shares identical DNA sequences to the thermophilin 13 operon (U93029.1) of *S. thermophilus* SFi13. All these strains have 100% similarity and 100% identity with the operon U93029.1. Even though some sequences had 79.22–84% of identity with operon U93029.1, their query cover ranged from 8–14%, and for this reason, they were automatically excluded from this study. General information and identification code of these 11 strains are listed in Table 1.

Table 1. List of strains and associated GenBank accessions code for genomes in which a 960-bp sequence with 100% identity to U93029.1 was found using BLASTn.

Strains	GenBank Code	Isolation/Source	Reference
<i>Streptococcus thermophilus</i> B59671	CP022547.1	Milk	[28]
<i>Streptococcus thermophilus</i> KLDS 3.1003	CP016877.1	Lactic starter (for yoghurt production)	[29]
<i>Streptococcus thermophilus</i> LMD-9	CP086001.1	Lactic starter (for yoghurt and mozzarella production)	[30]
<i>Streptococcus macedonicus</i> 19AS	PEBN00000000.1 ***	Cheese	[31]
<i>Streptococcus thermophilus</i> STH_CIRM_1049	LR822034.1	Lactic starter (for yoghurt production)	[32,33] **
<i>Streptococcus thermophilus</i> STH_CIRM_1048	LR822033.1	Lactic starter (for yoghurt production)	[32,33] **
<i>Streptococcus thermophilus</i> CS9	CP030927.1	Fermented milk	[34]
<i>Streptococcus thermophilus</i> DMST-H2	CP063275.1	Probiotic products	[35]
<i>Streptococcus thermophilus</i> TK-P3A	CP045596.1	Pasteurised milk	[36]
<i>Streptococcus thermophilus</i> ATCC 19258 *	CP038020.1	Milk	[37]
<i>Streptococcus thermophilus</i> NCTC 12958 *	LS483339.1	Milk	[38]

* Genome sequences of *S. thermophilus* NCTC 12958 and *S. thermophilus* ATCC 19258 are identical. In this study, both genomes were analysed independently. ** The identification name of the strains of *Streptococcus thermophilus* STH_CIRM_1048 and *Streptococcus thermophilus* STH_CIRM_1049 is related to the accession codes for genomes valid for the NCBI database; the same strains are reported as *Streptococcus thermophilus* CIRM-BIA1048 *Streptococcus thermophilus* CIRM-BIA1049 in citation [32,33]. *** GenBank code PEBN01000000.1 and PEBN01000052.1 both refer to *Streptococcus macedonicus* 19AS strain in NCBI database.

Data obtained using BAGEL4 and antiSMASH version 5.0 confirmed the distribution of thermophilin 13 BGC (biosynthetic gene cluster) in all 11 strains of *S. thermophilus*. All strains showed the same area of interest (AOI), with some variation in nucleotide sequences.

Strains *Streptococcus thermophilus* B59671 (CP022547.1) and *Streptococcus macedonicus* 19AS (PEBN00000000.1) were the most diverse based on AOI. Figure 1 shows a cladogram tree that is derived from the multiple sequence alignments of AOIs identified from the in silico study.

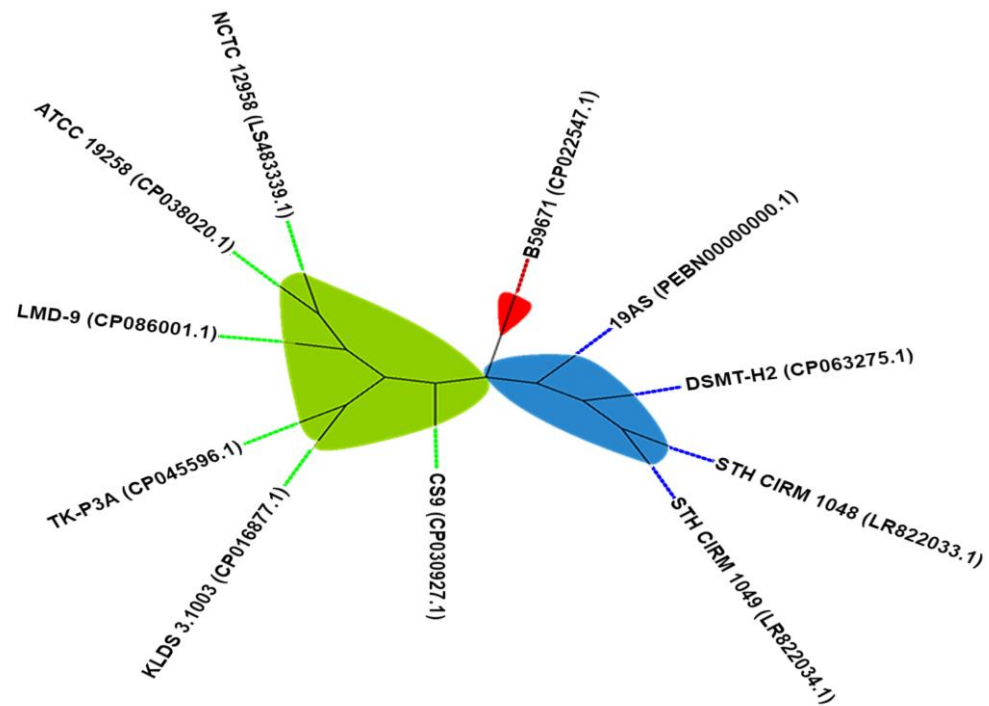


Figure 1. Cladogram tree showing the phylogenetic relatedness amongst gene loci, including thermophilin 13 (U93029.1). The tree was composed using the maximum likelihood method but visualised by removing branch length information. The three nodes are shown in different colours.

Known bacteriocin loci were detected, e.g., the lantibiotic salivaricin 9 operon in the genome of *S. thermophilus* NCTC 12958 and *Streptococcus thermophilus* ATCC 19258 and thermophilin 110 operon in *S. thermophilus* B59671 [5]. The entire locus of Salivaricin 9 was fully characterized from *S. salivarius* strain JIM8780, and it was shown to consist of eight genes, having the following putative functions: sivK, sensor kinase; sivR, response regulator; sivA, Sal9 precursor peptide; sivM, lantibiotic modification enzyme; sivT, ABC transporter involved in the export of Sal9 and concomitant cleavage of its leader peptide; and sivFEG, encoding lantibiotic self-immunity [39]. The broad-spectrum bacteriocin thermophilin 110 is encoded within the blp gene cluster. Furthermore, thermophilin 110 was reported to inhibit the growth of *Listeria monocytogenes*, *Streptococcus mutans*, *Streptococcus pyogenes* and *Propionibacterium acnes*.

Manual curation and annotation were performed to compare the differences between the ORFs predicted by the bacteriocin mining tools. Comparisons of AOIs indicated that the gene loci in the thermophilin 13 operon are organised into eight genes/ORFs encoding proteins related to bacteriocin production, plus the two thermophilin 13 structural genes. These were consistent for all strains and include a response regulator (RR), sensor histidine protein kinase (HPK), quorum-sensing system pheromone BlpC, ABC-transporter, bacteriocin accessory protein, thiol–disulfide oxidoreductases, CAAX protease and genes *thmA* and *thmB*. Despite the similarity in translation, three operon patterns were observed using the Operon-mapper web server. These variations, including all strains analysed in the present study, are schematically visualised in Figure 2.

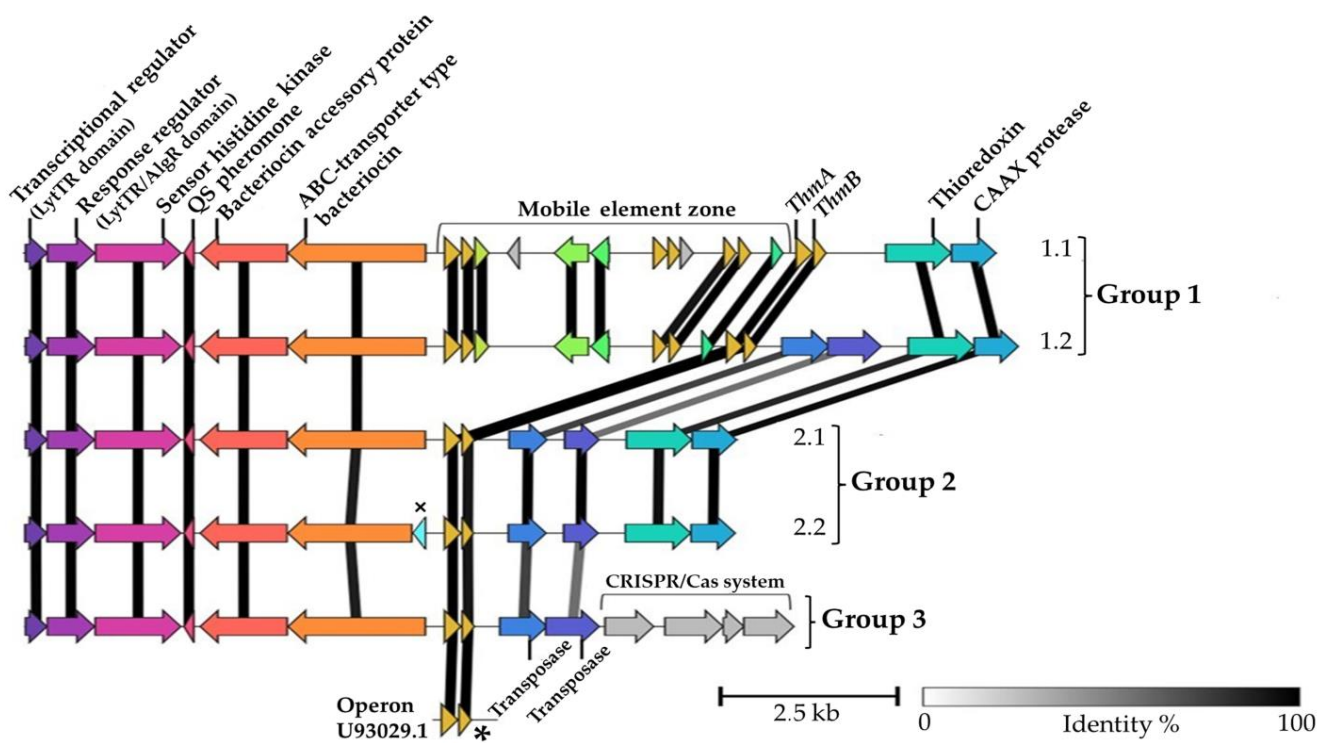


Figure 2. Comparison of the BGCs of thermophilin 13. Predicted ORFs are represented as arrows. Three main gene arrangements were detected. Subgroup 1.1 contains strain *S. macedonicus* 19AS and subgroup 1.2 strains *S. thermophilus* STH_CIRM_1049, *S. thermophilus* STH_CIRM_1048, *S. thermophilus* DMST-H2. Group 2 is divided into two subgroups, with strains *S. thermophilus* KLDS 3.1003, *S. thermophilus* TK-P3A, *S. thermophilus* CS9 in subgroup 2.1 and strains *S. thermophilus* LMD-9, *S. thermophilus* ATCC 19258 and *S. thermophilus* NCTC 12958 in subgroup 2.2. Group 3 is represented by strain *S. thermophilus* CS9. The symbol * indicates the third ORF (ORFC), encoded by the U93029.1 operon and symbol × indicate the C39 peptidase-like domains found only in subgroup 2.2. Colours are based on ORFs similarity found, including the mobile element zone, which were identified as small ORFs with apparent unrelated functions in the bacteriocins productions due to the prediction as hypothetical proteins.

A similar regulation and secretion system was observed for thermophilin 13 in groups 1, 2 and 3 (Figure 2). However, an additional nucleotide sequence (mobile element zone) was detected in group 1 (Figure 2). No variations in gene transcription upstream and downstream of this area were observed. In this regard, the insertion element, which is present in all sequenced BGCs of cluster 1, requires further investigation to assess possible interference with thermophilin 13 production due to the presence of transposases. A third ORF (ORFC), encoded by the U93029.1 operon, was reported by Marciset et al. (1997) [9] and was found in all BGCs groups shown in Figure 2. Structure models of the poration complex formed by Thermophilin 13 were described as the ThmA enhancing ThmB peptide with maximal explication in antimicrobial activity in equimolar concentration. However, the peptide ThmA alone resulted in antibacterial activity against *S. thermophilus*, *Clostridium botulinum*, *Listeria monocytogenes* and *Bacillus cereus*.

In this regard, the presence of GxxxG-motifs or GxxxG-like motifs AxxxA and SxxxS motif, instead of the GxxxG-motif and a high helical content were related to the two-peptide bacteriocins into form membrane-penetrating helix-helix structures, explaining the increased helical content forming a dimer complex, in which an incremented antimicrobial action is attributable [40] This dual peptide interaction was described in several class IIb bacteriocins including thermophilin 13 as is reported by the authors Oppegård et al. (2008) and Nissen-Meyer et al. (2010) [41,42]. However, this aspect requires further investigation due to multiple GxxxG motifs, located in positions $^{21}\text{GxxxG}^{25}$, $^{32}\text{GxxxG}^{36}$, $^{40}\text{GxxxG}^{44}$,

$^{54}\text{GxxxG}^{58}$ for ThmA and $^5\text{GxxxG}^9$, $^{14}\text{GxxxG}^{18}$, $^{15}\text{GxxxG}^{19}$, $^{19}\text{GxxxG}^{23}$, $^{24}\text{GxxxG}^{28}$ for ThmB peptides, respectively, as is showed in Figure 3.

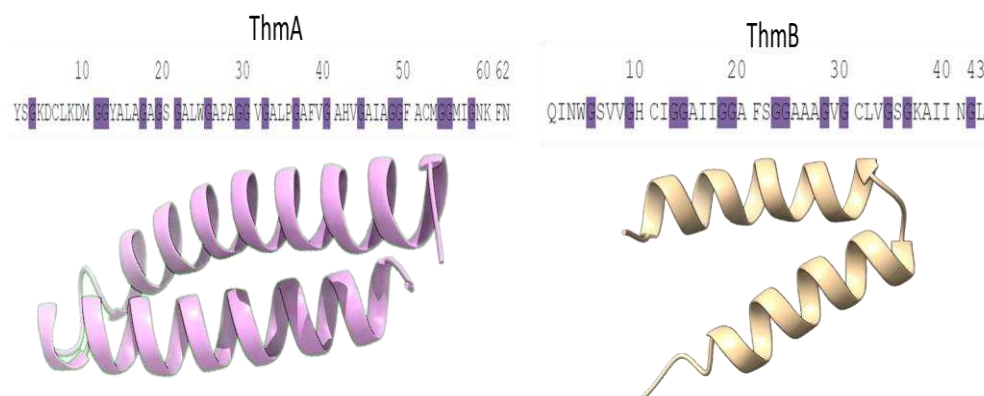


Figure 3. Amino acid sequences of the unmodified two-peptide subunit of thermophilin 13. The glycine residues in both peptides are matched in purple.

4. Discussion

Most bacteriocin operons include genes involved in the post-transcriptional modification and/or secretion of these peptides [12]. Based on that, the present study examined the thermophilin 13 operon (U93029.1) described by Marciset et al. (1997) [9], which appears lacking in bacteriocin-regulating genes involved in bacteriocin synthesis.

In silico analysis is an excellent predictor of “bacteriocin-associated driver genes” within genomes genes adding information on the mechanism related to the specific bacteriocin production. Starting from genomic or amino acid sequences, the main advantages of these methods are a significant reduction in time in comparison to the traditional screening method and, subsequently, the costs embroiled to the use of laboratory materials. Antimicrobial genome-mining tools have been closing the gap between a large number of predicted biosynthetic gene clusters (BGC) encoding bacteriocins, including ribosomally synthesized, post-translationally modified peptides (RiPPs) and also polyketide synthases (PKS) and non-ribosomal peptide synthetases (NRPS) [43]. However, the presence of bacteriocin genes in a strain is always directly related to an effective translation into biological antimicrobial activity [44].

This comprehensive in silico study reveals a complete thermophilin 13 gene cluster containing genes encoding a response regulator (RR), sensor histidine protein kinase (HPK), quorum-sensing system pheromone BlpC, ABC-transporter, bacteriocin accessory protein, thiol–disulfide oxidoreductases, CAAX protease and genes *thmA* and *thmB*.

Furthermore, we confirm the presence of ORFC in all strains; however, no correspondence related to this peptide has been associated with bacteriocin production in the databases.

Similarly to other bacteriocin gene clusters, response regulators grouped as LytR/AlgR family (RR) were predicted [45]. These regulators explicate their function in binding to promoters that initiate the transcription after phosphorylation of Asp residues promoting bacteriocin production and autoactivating their respective operons [46]. LytR Regulatory Systems represents the most abundant type of transcriptional regulator in the prokaryotic kingdom involved as either activators or repressors of single or operonic genes; of genes, including those involved in virulence, metabolism, quorum sensing motility and bacteriocins [45,47]. Furthermore, histidine kinases and response regulators mediate the actual response regarding bacteriocin production by a two-component signal-transducing system [48–51].

Peptide MTKHRTSLTAFTELSPSELHRISGGDWWDMKYFPSKQAIDSNKHKLG is present in all groups. By identifying the potential role in the bacteriocins biosynthetic gene cluster of this peptide, the prediction showed affinity to the quorum-sensing pheromone

BlpC (PF03047 HMM), which is also appointed as ComC/BlpC family leader-containing pheromone/bacteriocin. Interestingly, these peptides are different but are reported in several quorum-sensing regulated bacteriocins in *S. thermophilus*, stimulating the production of BLP (bacteriocin-like peptides) as a signal peptide for the activation of bacteriocin synthesis through a three-component regulatory system consisting of a peptide pheromone, a membrane-associated histidine protein kinase, and response regulators [52,53]. Plantaricins A, E/F and J/K by *L. plantarum* of C11, sakacin A of *L. sakei* Lb706EF and sakacin P of *L. sakei* LTH673102 are the best examples of bacteriocin of class II regulated by the three-component regulatory system, including inducing peptide (an indicator of the cell density), which is sensed by the corresponding (HPK), resulting in the activation of the RR [54].

A dedicated bacteriocin ABC-transporter, including a peptidase C39 motif, predicted to be a bacteriocin/lantibiotic transporter based on conserved domains (COG227400), and a bacteriocin accessory protein generally associated with transport, was observed [55,56]. ABC-transporter proteins related to the class II bacteriocin maturation and secretion carry a proteolytic peptidase C39 domain in their N-termini. The proteolytic peptidase C39 cleaves a double glycine (GG) motif-containing signal peptide from substrates before secretion, modulated in association with an ATP-binding cassette component located in the same protein [57,58]. Differences in ABC transporter sequences in Group 2 were detected in the C39 motif. Interestingly, an independent protein containing C39 peptidase domains, in terms of amino acid sequences, is present in subgroup 2.2. This protein conformation is termed C39 peptidase-like domains (CLD); additionally, their role is not yet completely understood, and they appear degenerated with nonproteolytic activity [59,60]. Most endopeptidases of family C39 are the less conservative component in the entire bifunctional transporter protein with a dedicated catalytic function for the secretion of the antimicrobial peptide of interest [61].

Thiol–disulfide oxidoreductases (TDORs) in Gram-positive bacteria play an essential role in forming disulfide bonds, allowing correct folding in class II bacteriocins through the R–S–S–R' bond of the CXXC catalytic site resulting in disulfide-bonded cysteines [62,63]. In this regard, only thmA has two cysteine residues in positions 6 and 53 of the aminoacidic backbones. The aminoacid methionine and single cysteines are also vulnerable to oxidation, but it has never been reported the disulfide bridge formation with this conformation in bacteriocins. However, in this protein, the LPxTG motif membrane-anchored transpeptidase, which cleaves proteins between the threonine (Thr) and the glycine (Gly), is conserved.

Interestingly, ThmA and ThmB peptides lack Thr residues; this is in accordance with Marciset et al. (1997), who observed that the oxidation of methionines to methoxides in position (Met¹⁰, Met⁵⁴ and/or Met⁵⁷) of ThmA seems the only possible explanation of the proposed poration complexes (AB)_n (i.e., Thermophilin 13) [9].

CAAX metalloprotease (bacteriocin-processing enzymes) detected in bacteriocin loci, including the Abi genes downstream of the bacteriocin structural genes, is likely involved in self-immunity. The role of these conserved motifs in the immunity function conferred a high degree of cross-resistance against each other's bacteriocins, suggesting the recognition of a common receptor. An example of this mechanism was found in *Lactobacillus sakei* 23K [64]. Furthermore, the bacteriocin-like gene sak23Kalpha showed antimicrobial activity when expressed in a heterologous host, and the associated Abi gene sak23Ki conferred immunity against the related bacteriocin [65,66]. Genes encoding the production, secretion, regulation, and immunity of thermophilin 13 are similar to gene sequences reported for class II bacteriocins from *S. thermophilus* strains LMG18311, CNRZ1066, and LMD-9 [67]. However, in *S. thermophilus* B59671, belonging to Group 3, TDOR and CAAX protease are replaced with a CRISPR/Cas system. Prior studies have noted the importance of quorum sensing induction peptides encoded by the different blp gene clusters found in *S. thermophilus* strains ST109, LMD-9, ST106, LMG18311, CNRZ1066, ND03, JIM8232, MN-ZLW002 and B59671 due to their homology to a bacteriocin-like peptide (blp) gene cluster in *S. pneumoniae* [28,51,68,69]. In relation to this aspect, the strains *S. thermophilus* B59671, ST106, ST109 and LMD-9 have been shown to produce a broad spec-

trum of bacteriocins encoded within a bacteriocin-like peptide (blp) gene cluster. However, the thermophilin 13 operon is also present in LMD-9 and B59671 strains but must not be confused with the bacteriocin-like peptide (blp) in *S. pneumoniae* gene clusters mentioned above. In this regard, strains LMD-9 and B59671 could be multiple-bacteriocins producer strains and should be highlighted for the necessary evaluation of the role of environmental factors and medium composition on bacteriocin production.

Bacteriocin production is an energy-utilising process involving a cascade of genetic mechanisms that varies greatly in how bacteriocin loci are organised. Among bacteriocin production mechanisms, in many strains, quorum-sensing (QS) circuits modulate various physiological responses, including the production of antimicrobial compounds [70,71]. However, in silico screens can be limited by their dependence on similarity to those previously described by Walsh et al. (2015) [72]. Further work is required to confirm that operon variation between strains influences the production of thermophilin 13. In summary, these results highlight that the production of peptides ThmA and ThmB is strongly related to its PBGC, which is not limited to only *thmA* and *thmB* genes. Therefore, it can be assumed that the QS system regulates the expression of thermophilin 13 bacteriocins in several *S. thermophilus* strains. It has to be considered that since 1997 no other investigations have been made on this bacteriocin. However, the evidence gathered in this study provides further insights into the mechanism of production and regulation of thermophilin 13; this has been observed and described to the scientific community after twenty-five years. All these reported strains are used in industrial applications, and their technological properties have already been proven, opening a new panorama of research that need further investigation. In light of the urgent need for new weapons to counteract pathogens without the use of antibiotics, the identification of the most suitable thermophilin 13 producer strain in terms of bacteriocin production and its applicability in food manufacturing is relevant.

5. Conclusions

The significance of antimicrobial peptides (AMPs) is growing for applicability in various fields, including as a bioprotector agent. There are still many challenges regarding bacteriocins looking for an answer, such as structural multiplicity, different modes of action, different classes, and the high cost of production. Furthermore, also for bacteriocins already applied as preservative agents, the major issues are connected to finding strategies for optimizing their maximum rate of production and developing more effective purification steps from the bacterial supernatant, which are currently long and complicated.

A large number of genomes available in public repositories offer novel approaches valuable in identifying novel bacteriocin genes and gene clusters [54]. Screening of putative bacteriocin gene clusters provides a deeper understanding of how these peptides are regulated. Genome mining indicates that operon thermophilin 13 is present in several strains grouped in three different clusters on the basis of the different genes organization of the eight genes involved in these bacteriocins' biosynthesis. As Marciset et al. (1997) suggested in their conclusion, thermophilin 13 showed peculiar and different characteristics in its mode of action that can share functional properties of lantibiotics. Our results also indicated that the thermophilin 13 two-component peptide system belongs to class IIb with its own related genes cluster, composed of a response regulator (RR), sensor histidine protein kinase (HPK), quorum-sensing system pheromone BpC, ABC-transporter, bacteriocin accessory protein, thiol–disulfide oxidoreductases, CAAX protease and genes *thmA* and *thmB*. However, the predictions obtained from the present research and the others in silico studies in general, must not be accepted as conclusive evidence for bacteriocin production, and we do not claim that all strains included in this study can produce thermophilin 13 in vitro and/or in vivo. Nevertheless, the information obtained in this study shed some light on the possible quorum sensing involvement in the mechanisms of regulation and secretion of thermophilin 13, which has already been reported in *Streptococcus thermophilus* strains having bacteriocin-like peptide (blp) gene cluster. This is a solid starting point for further investigation into a topic that has not been explored since 1997, which provides

opportunities to expand the knowledge of this antimicrobial peptide in order to target effective applications for food safety.

Author Contributions: Conceptualisation, F.S. and L.M.T.D.; methodology, F.S.; validation, L.M.T.D., L.I. and G.C.; formal analysis, F.S.; investigation, F.S.; resources, L.M.T.D., L.I. and G.C.; data curation, F.S.; writing—original draft preparation, F.S.; writing—review and editing, L.M.T.D., L.I. and G.C.; visualisation, F.S.; supervision, L.M.T.D., L.I. and G.C.; project administration, L.M.T.D. and L.I.; funding acquisition, L.M.T.D. and L.I. All authors have read and agreed to the published version of the manuscript.

Funding: This research received no external funding. Free research funds of L.I. covered expenses.

Data Availability Statement: All Sequences are available in the NCBI database with related GenBank code reported in Table 1. The names of the repository/repositories and relative literature can be found in the article. Curated Putative Bacteriocin Gene Clusters (PBGs) described in this study are readily available upon reasonable request to the corresponding author.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Delorme, C.; Legravet, N.; Jamet, E.; Hoarau, C.; Alexandre, B.; El-Sharoud, W.M.; Darwish, M.S.; Renault, P. Study of *Streptococcus thermophilus* Population on a World-Wide and Historical Collection by a New MLST Scheme. *Int. J. Food Microbiol.* **2017**, *242*, 70–81. [[CrossRef](#)]
2. Rossi, F.; Marzotto, M.; Cremonese, S.; Rizzotti, L.; Torriani, S. Diversity of *Streptococcus thermophilus* in Bacteriocin Production; Inhibitory Spectrum and Occurrence of Thermophilin Genes. *Food Microbiol.* **2013**, *35*, 27–33. [[CrossRef](#)] [[PubMed](#)]
3. Ward, D.J.; Somkuti, G.A. Characterization of a bacteriocin produced by *Streptococcus thermophilus* ST134. *Appl. Microbiol. Biotechnol.* **1995**, *42*, 330–335. [[CrossRef](#)]
4. Anastasios, A.; George, K. Purification and Characterization of Thermophilin ST-1, a Novel Bacteriocin Produced by *Streptococcus thermophilus* ACA-DC 0001. *Le Lait* **2003**, *83*, 365–378. [[CrossRef](#)]
5. Gilbreth, S.E.; Somkuti, G.A. Thermophilin 110: A Bacteriocin of *Streptococcus thermophilus* ST110. *Curr. Microbiol.* **2005**, *51*, 175–182. [[CrossRef](#)] [[PubMed](#)]
6. Kabuki, T.; Uenishi, H.; Watanabe, M.; Seto, Y.; Nakajima, H. Characterization of a Bacteriocin, Thermophilin 1277, Produced by *Streptococcus thermophilus* SBT1277. *J. Appl. Microbiol.* **2007**, *102*, 971–980. [[CrossRef](#)]
7. Ivanova, I.; Miteva, V.; Stefanova, T.; Pantev, A.; Budakov, I.; Danova, S.; Moncheva, P.; Nikolova, I.; Dousset, X.; Boyaval, P. Characterization of a Bacteriocin Produced by *Streptococcus thermophilus* 81. *Int. J. Food Microbiol.* **1998**, *42*, 147–158. [[CrossRef](#)] [[PubMed](#)]
8. Mathot, A.G.; Beliard, E.; Thuault, D. *Streptococcus thermophilus* 580 Produces a Bacteriocin Potentially Suitable for Inhibition of *Clostridium Tyrobutyricum* in Hard Cheese. *J. Dairy Sci.* **2003**, *86*, 3068–3074. [[CrossRef](#)]
9. Marciset, O.; Jeronimus-Stratingh, M.C.; Mollet, B.; Poolman, B. Thermophilin 13, a nontypical antilisterial poration complex bacteriocin, that functions without a receptor. *J. Biol. Chem.* **1997**, *272*, 14277–14284. [[CrossRef](#)]
10. Oppgård, C.; Rogne, P.; Emanuelsen, L.; Kristiansen, P.E.; Fimland, G.; Nissen-Meyer, J. The Two-Peptide Class II Bacteriocins: Structure, Production, and Mode of Action. *J. Mol. Microbiol. Biotechnol.* **2007**, *13*, 210–219. [[CrossRef](#)]
11. Dimov, S.; Ivanova, P.; Harizanova, N. Genetics of Bacteriocins Biosynthesis by Lactic Acid Bacteria. *Biotechnol. Biotechnol. Equip.* **2005**, *19*, 4–10. [[CrossRef](#)]
12. Ditu, L.-M.; Chifiriuc, M.; Pelinescu, D.; Avram, I.; Pircalabioru, G.; Mihaescu, G. Class I and II Bacteriocins: Structure, Biosynthesis and Drug Delivery Systems for the Improvement of Their Antimicrobial Activity. *Curr. Proteom.* **2016**, *11*, 121–127. [[CrossRef](#)]
13. Zouhir, A.; Hammami, R.; Fliss, I.; Hamida, J. Ben A New Structure-Based Classification of Gram-Positive Bacteriocins. *Protein J.* **2010**, *29*, 432–439. [[CrossRef](#)] [[PubMed](#)]
14. Boratyn, G.M.; Camacho, C.; Cooper, P.S.; Coulouris, G.; Fong, A.; Ma, N.; Madden, T.L.; Matten, W.T.; McGinnis, S.D.; Merezuk, Y.; et al. BLAST: A More Efficient Report with Usability Improvements. *Nucleic Acids Res.* **2013**, *41*, W29–W33. [[CrossRef](#)]
15. Rangwala, S.H.; Kuznetsov, A.; Ananiev, V.; Asztalos, A.; Borodin, E.; Evgeniev, V.; Joukov, V.; Lotov, V.; Pannu, R.; Rudnev, D.; et al. Accessing NCBI Data Using the NCBI Sequence Viewer and Genome Data Viewer (GDV). *Genome Res.* **2021**, *31*, 159–169. [[CrossRef](#)]
16. Blin, K.; Shaw, S.; Steinke, K.; Villebro, R.; Ziemert, N.; Lee, S.Y.; Medema, M.H.; Weber, T. AntiSMASH 5.0: Updates to the Secondary Metabolite Genome Mining Pipeline. *Nucleic Acids Res.* **2019**, *47*, W81–W87. [[CrossRef](#)]
17. Van Heel, A.J.; De Jong, A.; Song, C.; Viel, J.H.; Kok, J.; Kuipers, O.P. BAGEL4: A User-Friendly Web Server to Thoroughly Mine RiPPs and Bacteriocins. *Nucleic Acids Res.* **2018**, *46*, W278–W281. [[CrossRef](#)]
18. Altschul, S.F.; Madden, T.L.; Schäffer, A.A.; Zhang, J.; Zhang, Z.; Miller, W.; Lipman, D.J. Gapped BLAST and PSI-BLAST: A New Generation of Protein Database Search Programs. *Nucleic Acids Res.* **1997**, *25*, 3389–3402. [[CrossRef](#)]

19. Tamura, K.; Stecher, G.; Kumar, S. MEGA11: Molecular Evolutionary Genetics Analysis Version 11. *Mol. Biol. Evol.* **2021**, *38*, 3022–3027. [[CrossRef](#)]
20. Edgar, R.C. MUSCLE: Multiple Sequence Alignment with High Accuracy and High Throughput. *Nucleic Acids Res.* **2004**, *32*, 1792–1797. [[CrossRef](#)]
21. Letunic, I.; Bork, P. Interactive Tree of Life (ITOL) v4: Recent Updates and New Developments. *Nucleic Acids Res.* **2019**, *47*, 256–259. [[CrossRef](#)] [[PubMed](#)]
22. Taboada, B.; Estrada, K.; Ciria, R.; Merino, E. Genome Analysis Operon-Mapper: A Web Server for Precise Operon Identification in Bacterial and Archaeal Genomes. *Bioinformatics* **2018**, *34*, 4118–4120. [[CrossRef](#)] [[PubMed](#)]
23. Gilchrist, C.L.M.; Booth, T.J.; van Wersch, B.; van Grieken, L.; Medema, M.H.; Chooi, Y.-H. Cblaster: A Remote Search Tool for Rapid Identification and Visualization of Homologous Gene Clusters. *Bioinform. Adv.* **2021**, *1*, vbab016. [[CrossRef](#)] [[PubMed](#)]
24. Gilchrist, C.L.M.; Chooi, Y.-H. Clinker & Clustermap.js: Automatic Generation of Gene Cluster Comparison Figures. *Bioinformatics* **2021**, *37*, 2473–2475. [[CrossRef](#)]
25. Mirdita, M.; Schütze, K.; Moriwaki, Y.; Heo, L.; Ovchinnikov, S.; Steinegger, M. ColabFold: Making Protein Folding Accessible to All. *Nat. Methods* **2022**, *19*, 679–682. [[CrossRef](#)] [[PubMed](#)]
26. Comelli, E.M.; Guggenheim, B.; Stingle, F.; Neeser, J.R. Selection of Dairy Bacterial Strains as Probiotics for Oral Health. *Eur. J. Oral Sci.* **2002**, *110*, 218–224. [[CrossRef](#)]
27. Comelli, E.; Guggenheim, B.; Neeser, J.; Stingle, F.; Cocconcelli, P.S. Treatment fo Actinomtces Naeslundii-Related Diseases with Exogenous Lactic Acid Bacteria Strains. U.S. Patent US 7491386 B2, 17 February 2009.
28. Renye, J.A.; Somkuti, G.A.; Steinberg, D.H. Thermophilin 109 Is a Naturally Produced Broad Spectrum Bacteriocin Encoded within the Blp Gene Cluster of *Streptococcus thermophilus*. *Biotechnol. Lett.* **2019**, *41*, 283–292. [[CrossRef](#)]
29. Alexandraki, V.; Kazou, M.; Blom, J.; Pot, B.; Papadimitriou, K.; Tsakalidou, E. Comparative Genomics of *Streptococcus thermophilus* Support Important Traits Concerning the Evolution, Biology and Technological Properties of the Species. *Front. Microbiol.* **2019**, *10*, 2916. [[CrossRef](#)]
30. Makarova, K.; Slesarev, A.; Wolf, Y.; Sorokin, A.; Mirkin, B.; Koonin, E.; Pavlov, A.; Pavlova, N.; Karamychev, V.; Polouchine, N.; et al. Comparative Genomics of the Lactic Acid Bacteria. *Proc. Natl. Acad. Sci. USA* **2006**, *103*, 15611–15616. [[CrossRef](#)]
31. Treu, L.; de Diego-Díaz, B.; Papadimitriou, K.; Tsakalidou, E.; Giacomini, A.; Corich, V. Whole-Genome Sequences of Three *Streptococcus macedonicus* Strains Isolated from Italian Cheeses in the Veneto Region. *Genome Announc.* **2017**, *5*, e01358-17. [[CrossRef](#)]
32. Roux, E.; Nicolas, A.; Valence, F.; Siekaniec, G.; Chuat, V.; Nicolas, J.; Le Loir, Y.; Guédon, E. The Genomic Basis of the *Streptococcus Thermophilus* Health-Promoting Properties. *BMC Genom.* **2022**, *23*, 210. [[CrossRef](#)] [[PubMed](#)]
33. CIRM-BIA: International Centre for Microbial Resources-Food-Associated Bacteria, STLO UMR 1253 INRAE-Agrocampus 65 rue de saint Briec, F-35042 Rennes Cedex, France. CIRM Associated Bacteria. Available online: <https://collection-cirmbia.fr/page/Cite%20CIRM-BIA> (accessed on 20 November 2022).
34. Hu, T.; Cui, Y.; Zhang, Y.; Qu, X.; Zhao, C. Genome Analysis and Physiological Characterization of Four *Streptococcus thermophilus* Strains Isolated From Chinese Traditional Fermented Milk. *Front. Microbiol.* **2020**, *11*, 184. [[CrossRef](#)] [[PubMed](#)]
35. Hu, J.-S.; Huang, Y.-Y.; Kuang, J.-H.; Yu, J.-J.; Zhou, Q.-Y.; Liu, D.-M. *Streptococcus thermophilus* DMST-H2 Promotes Recovery in Mice with Antibiotic-Associated Diarrhea. *Microorganisms* **2020**, *8*, 1650. [[CrossRef](#)] [[PubMed](#)]
36. Wang, Y.; Liang, Q.; Lu, B.; Shen, H.; Liu, S.; Shi, Y.; Leptihn, S.; Li, H.; Wei, J.; Liu, C.; et al. Whole-Genome Analysis of Probiotic Product Isolates Reveals the Presence of Genes Related to Antimicrobial Resistance, Virulence Factors, and Toxic Metabolites, Posing Potential Health Risks. *BMC Genom.* **2021**, *22*, 210. [[CrossRef](#)] [[PubMed](#)]
37. Cho, H.; Park, K.; Kim, K. Genome Analysis of *Streptococcus salivarius* Subsp. *Thermophilus* Type Strain ATCC 19258 and Its Comparison to Equivalent Strain NCTC 12958. *Arch. Microbiol.* **2021**, *203*, 1843–1849. [[CrossRef](#)] [[PubMed](#)]
38. Wescombe, P.A.; Upton, M.; Renault, P.; Wirawan, R.E.; Power, D.; Burton, J.P.; Chilcott, C.N.; Tagg, J.R. Salivaricin 9, a New Lantibiotic Produced by *Streptococcus salivarius*. *Microbiology* **2011**, *157*, 1290–1299. [[CrossRef](#)]
39. Tietz, J.I.; Schwalen, C.J.; Patel, P.S.; Maxson, T.; Blair, P.M.; Tai, H.-C.; Zakai, U.I.; Mitchell, D.A. A New Genome-Mining Tool Redefines the Lasso Peptide Biosynthetic Landscape. *Nat. Chem. Biol.* **2017**, *13*, 470–478. [[CrossRef](#)]
40. Ekblad, B.; Kristiansen, P.E. NMR Structures and Mutational Analysis of the Two Peptides Constituting the Bacteriocin Plantaricin S. *Sci. Rep.* **2019**, *9*, 1–10. [[CrossRef](#)]
41. Russell, A.H.; Truman, A.W. Genome Mining Strategies for Ribosomally Synthesised and Post-Translationally Modified Peptides. *Comput. Struct. Biotechnol. J.* **2020**, *18*, 1838–1851. [[CrossRef](#)]
42. Oppegård, C.; Schmidt, J.; Kristiansen, P.E.; Nissen-Meyer, J. Mutational Analysis of Putative Helix-Helix Interacting GxxxG-Motifs and Tryptophan Residues in the Two-Peptide Bacteriocin Lactococcin G. *Biochemistry* **2008**, *47*, 5242–5249. [[CrossRef](#)]
43. Nissen-Meyer, J.; Oppegård, C.; Rogne, P.; Haugen, H.S.; Kristiansen, P.E. Structure and Mode-of-Action of the Two-Peptide (Class-IIb) Bacteriocins. *Probiotics Antimicrob. Proteins* **2010**, *2*, 52–60. [[CrossRef](#)] [[PubMed](#)]
44. Nikolskaya, A.N.; Galperin, M.Y. A Novel Type of Conserved DNA-Binding Domain in the Transcriptional Regulators of the AlgR/AgrA/LytR Family. *Nucleic Acids Res.* **2002**, *30*, 2453–2459. [[CrossRef](#)] [[PubMed](#)]
45. Zou, Z.; Qin, H.; Brenner, A.E.; Raghavan, R.; Millar, J.A.; Gu, Q.; Xie, Z.; Kreth, J.; Merritt, J. LytTR Regulatory Systems: A Potential New Class of Prokaryotic Sensory System. *PLoS Genet.* **2018**, *14*, e1007709. [[CrossRef](#)] [[PubMed](#)]
46. Maddocks, S.E.; Oyston, P.C.F. Structure and Function of the LysR-Type Transcriptional Regulator (LTTR) Family Proteins. *Microbiology* **2008**, *154*, 3609–3623. [[CrossRef](#)]

47. Jia, F.F.; Pang, X.H.; Zhu, D.Q.; Zhu, Z.T.; Sun, S.R.; Meng, X.C. Role of the LuxS Gene in Bacteriocin Biosynthesis by *Lactobacillus Plantarum* KLDS1.0391: A Proteomic Analysis. *Sci. Rep.* **2017**, *7*, 13871. [[CrossRef](#)]
48. Hühne, K.; Axelsson, L.; Holck, A.; Kröckel, L. Analysis of the Sakacin P Gene Cluster from *Lactobacillus Sake* Lb674 and Its Expression in Sakacin-Negative Lb. *Sake Strains. Microbiology* **1996**, *142*, 1437–1448. [[CrossRef](#)]
49. Maldonado-Barragán, A.; West, S.A. The Cost and Benefit of Quorum Sensing-Controlled Bacteriocin Production in *Lactobacillus plantarum*. *J. Evol. Biol.* **2020**, *33*, 101–111. [[CrossRef](#)]
50. Renye, J.A.; Somkuti, G.A. BlpC-Regulated Bacteriocin Production in *Streptococcus thermophilus*. *Biotechnol. Lett.* **2013**, *35*, 407–412. [[CrossRef](#)]
51. Smits, S.H.J.; Schmitt, L.; Beis, K. Self-Immunity to Antibacterial Peptides by ABC Transporters. *FEBS Lett.* **2020**, *594*, 3920–3942. [[CrossRef](#)]
52. Venema, K.; Kok, J.; Marugg, J.D.; Toonen, M.Y.; Ledebroer, A.M.; Venema, G.; Chikindas, M.L. Functional Analysis of the Pediocin Operon of *Pediococcus Acidilactici* PAC1.0: PedB Is the Immunity Protein and PedD Is the Precursor Processing Enzyme. *Mol. Microbiol.* **1995**, *17*, 515–522. [[CrossRef](#)]
53. Zhang, T.; Zhang, Y.; Li, L.; Jiang, X.; Chen, Z.; Zhao, F.; Yi, Y. Biosynthesis and Production of Class II Bacteriocins of Food-Associated Lactic Acid Bacteria. *Fermentation* **2022**, *8*, 217. [[CrossRef](#)]
54. Franke, C.M.; Tiemersma, J.; Venema, G.; Kok, J. Membrane Topology of the Lactococcal Bacteriocin ATP-Binding Cassette Transporter Protein LcnC. Involvement of LcnC in Lactococcal A Maturation. *J. Biol. Chem.* **1999**, *274*, 8484–8490. [[CrossRef](#)] [[PubMed](#)]
55. Beis, K. Structural Basis for the Mechanism of ABC Transporters. *Biochem. Soc. Trans.* **2015**, *43*, 889–893. [[CrossRef](#)] [[PubMed](#)]
56. Kanonenberg, K.; Schwarz, C.K.W.; Schmitt, L. Type I Secretion Systems—A Story of Appendices. *Res. Microbiol.* **2013**, *164*, 596–604. [[CrossRef](#)]
57. Beis, K.; Rebuffat, S. Multifaceted ABC Transporters Associated to Microcin and Bacteriocin Export. *Res. Microbiol.* **2019**, *170*, 399–406. [[CrossRef](#)]
58. Wu, K.H.; Tai, P.C. Cys32 and His105 Ate the Critical Residues for the Calcium-Dependent Cysteine Proteolytic Activity of CvaB, an ATP-Binding Cassette Transporter. *J. Biol. Chem.* **2004**, *279*, 901–909. [[CrossRef](#)]
59. Mesa-Pereira, B.; O'Connor, P.M.; Rea, M.C.; Cotter, P.D.; Hill, C.; Ross, R.P. Controlled Functional Expression of the Bacteriocins Pediocin PA-1 and Bactofencin A in *Escherichia Coli*. *Sci. Rep.* **2017**, *7*, 1–11. [[CrossRef](#)]
60. García-Curiel, L.; del Rocío López-Cuellar, M.; Rodríguez-Hernández, A.I.; Chavarría-Hernández, N. Toward Understanding the Signals of Bacteriocin Production by *Streptococcus* Spp. and Their Importance in Current Applications. *World J. Microbiol. Biotechnol.* **2021**, *37*, 1–14. [[CrossRef](#)]
61. Duan, Y.; Niu, W.; Pang, L.; Bian, X.; Zhang, Y.; Zhong, G. Unusual Post-Translational Modifications in the Biosynthesis of Lasso Peptides. *Int. J. Mol. Sci.* **2022**, *23*, 7231. [[CrossRef](#)]
62. Davey, L.; Halperin, S.A.; Lee, S.F. Thiol-Disulfide Exchange in Gram-Positive Firmicutes. *Trends Microbiol.* **2016**, *24*, 902–915. [[CrossRef](#)]
63. Kjos, M.; Snipen, L.; Salehian, Z.; Nes, I.F.; Diep, D.B. The Abi Proteins and Their Involvement in Bacteriocin Self-Immunity. *J. Bacteriol.* **2010**, *192*, 2068–2076. [[CrossRef](#)] [[PubMed](#)]
64. Miller, E.L.; Abrudan, M.I.; Roberts, I.S.; Rozen, D.E. Diverse Ecological Strategies Are Encoded by *Streptococcus pneumoniae* Bacteriocin-Like Peptides. *Genome Biol. Evol.* **2016**, *8*, 1072–1090. [[CrossRef](#)] [[PubMed](#)]
65. Pei, J.; Mitchell, D.A.; Dixon, J.E.; Grishin, N.V. Expansion of Type II CAAX Proteases Reveals Evolutionary Origin of γ -Secretase Subunit APH-1. *J. Mol. Biol.* **2011**, *410*, 18–26. [[CrossRef](#)] [[PubMed](#)]
66. Fontaine, L.; Boutry, C.; Guédon, E.; Guillot, A.; Ibrahim, M.; Grossiord, B.; Hols, P. Quorum-Sensing Regulation of the Production of Blp Bacteriocins in *Streptococcus thermophilus*. *J. Bacteriol.* **2007**, *189*, 7195–7205. [[CrossRef](#)] [[PubMed](#)]
67. Hols, P.; Hancy, F.; Fontaine, L.; Grossiord, B.; Prozzi, D.; Leblond-Bourget, N.; Decaris, B.; Bolotin, A.; Delorme, C.; Duskoehrich, S. New Insights in the Molecular Biology and Physiology of *Streptococcus thermophilus* Revealed by Comparative Genomics. *FEMS Microbiol. Rev.* **2005**, *29*, 435–463. [[CrossRef](#)] [[PubMed](#)]
68. Renye, J.R.; Wales, N.; Somkuti, G.A. Bacteriocin with Novel Activity. US 2016/0368953 A1, 22 December 2016.
69. Blanchard, A.E.; Liao, C.; Lu, T. An Ecological Understanding of Quorum Sensing-Controlled Bacteriocin Synthesis. *Cell. Mol. Bioeng.* **2016**, *9*, 443–454. [[CrossRef](#)]
70. Meng, F.; Zhao, H.; Nie, T.; Lu, F.; Zhang, C.; Lu, Y.; Lu, Z. Acetate Activates *Lactobacillus* Bacteriocin Synthesis by Controlling Quorum Sensing. *Appl. Environ. Microbiol.* **2021**, *87*, e007202. [[CrossRef](#)]
71. Rohde, B.H.; Quadri, L.E.N. Functional Characterization of a Three-Component Regulatory System Involved in Quorum Sensing-Based Regulation of Peptide Antibiotic Production in *Carnobacterium maltaromaticum*. *BMC Microbiol.* **2006**, *6*, 93. [[CrossRef](#)]
72. Walsh, C.J.; Guinane, C.M.; Hill, C.; Ross, R.P.; O'Toole, P.W.; Cotter, P.D. In Silico Identification of Bacteriocin Gene Clusters in the Gastrointestinal Tract, Based on the Human Microbiome Project's Reference Genome Database. *BMC Microbiol.* **2015**, *15*, 183. [[CrossRef](#)]

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