Review

Streptococcus thermophilus bacteriocin, from production to their application: An overview

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Streptococcus thermophilus have potent antimicrobial activities toward closely related bacteria and undesirable harmful microorganisms. Certain S. thermophilus strains are able to produce substances that compete and prevent pathogenic bacteria from adhering to the receptors on epithelial cells of intestinal surfaces. The potential probiotic effects of S. thermophilus are well known in the human ecosystem and their production of antimicrobial peptides can contribute to elucidate the precise mechanisms by which S. thermophilus can dominate the intestinal microbiota and achieve their probiotic function. This paper presents a review of the antimicrobial proteinaceous compounds produced by various streptococcus strains, the attempts made to purify them, their characterization and useful applications.

Key words: Streptococcus thermophilus, bacteriocin.

INTRODUCTION

Streptococcus thermophilus is commonly used as starter culture for the preparation of different milk products and also considered as most important starter for industry after Lactococcus lactis (Hols et al., 2005). Traditionally, it is used for making yoghurt and different varieties of cheeses, for example, hard-cooked cheeses (Emmental, Gruyère, Parmigiano and Grana-types, etc.), Mozzarella and Cheddar cheeses. Being thermophilic in nature, it plays a vital role for different production processes used for the preparation of dairy products. It may be used alone or in combination with other lactobacilli and mesophilic starter culture used for cheese making, whereas in case of yoghurt it is always used with Lactobacillus delbrueckii sp. bulgaricus due to their symbiotic relationship (Auclair and Accolas, 1983). In milk fermentation, the role of S. thermophilus is attributed to the rapid rate of lactic acid production from lactose that result in reduction of pH and production of secondary metabolites. Together, S. thermophilus, S. salivarius and S. vestibularis form the salivarius group of viridans streptococci (Facklam, 2002). S. thermophilus is the only streptococcal species that is present in dairy products therefore regarded as GRAS. Whereas, other viridans streptococci showed commensals relationship in the oral cavity, gastrointestinal and genital tracts of mammals. Similarly, S. salivarius and S. vestibularis have been reported to isolate from the human oral cavity and are responsible for human infections. Some cases of meningitis, endocarditis and bacteraemia are reported due to oral streptococci (Doyuk et al., 2002; Idigoras et al., 2001; Ruoff et al., 1989). The genomes of these three S. thermophilus strains were sequenced recently which showed the inactivation or absence of virulence related genes which is attributing of the pathogenic streptococci.

The interest in the research of antimicrobial peptides including bacteriocins and bacteriocin-like compounds is increasing day by day because of their antimicrobial potential that improves the safety of different food products (Yildirim et al., 1999). The combinations of

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different species including *S. thermophilus* have found to be positively effected on diarrhea in young children, enterocolitis in premature neonates and inflammatory gut disease (Bibiloni et al., 2005; Bin-Nun et al., 2005; Shamir et al., 2005). In a probiotic mixture, the presence of *S. thermophilus* improves the efficiency of Bifidobacteria which in turn prevent the rotaviral diarrhea (Bin-Nun et al., 2005; Saavedra et al., 1994). Some specific strains are reported to obstruct the linkages of oral species, including cariogenic bacteria, to dental plaque, and of *Candida* spp. to silicone voice prostheses, which increases their life time (Busscher et al., 1997; Cornelli et al., 2002). Similarly different studies reported the characterization of several bacteriocins produced by *S. thermophilus* having strong antimicrobial activity against *Pediococcus acidilactici*, *Clostridium tyrobutyricum*, *Clostridium sporogenes*, *Clostridium botulinum*, *Bacillus cereus* and *Listeria monocytogenes* (Aktypis et al., 1998; Gibreth and Somkuti, 2005; Marciset et al., 1997; Mathot et al., 2003).

Bacteriocins are antimicrobial peptides excreted by certain bacteria having ability to inhibit the growth of other closely related bacteria (Hardy, 1975). Several bacteriocin-producing strains of *S. thermophilus* have been reported but to our knowledge, only eleven bacteriocins have been partially characterized: thermophilin 347 produced by a strain isolated from yogurt (Villani et al., 1995), thermophilin A (Ward and Somkuti 1995), thermophilin T produced by a strain isolated from “feta” cheese (Aktypis et al., 1998), thermophilin 13, which has been sequenced (Marciset et al., 1997) and a bacteriocin from *S. thermophilus* 81 with a broad inhibitory spectrum (also active against Gram-negative bacteria) (Ivanova et al., 1998). Bacteriocins are lethal to closely related bacteriocin-related species, food-borne pathogens and spoilage bacteria (Tagg et al., 1976; Klaenhammer, 1993). Among streptococcus species, *S. thermophilus* strains have been extensively utilized as probiotic cultures in dairy and numerous reports have proved its ability to produce bacteriocin (Bin-Nun et al., 2005), yet no review has been published summarizing its bacteriocin production. Therefore, the purpose of this paper is to have an overview and summarize the study of previous authors on bacteriocin producing *S. thermophilus* strains.

Since Marciset et al. (1997) reported that the strain *S. thermophilus*ACA-DC 0001 has a wide antimicrobial activity against Gram-positive and Gram-negative microorganisms; this means that some bacteriocin-producing strains could be used in thermophilic starters since not all bacteriocins are active against thermophilic *lactobacilli*. The temperature of incubation and heat treatment has influence on the biochemical activity of *S. thermophilus* in cow’s milk and this was investigated by Singh (1983). *S. thermophilus* has a proto-cooperation and shows antagonistic effect with *L. bulgaricus* including growth rate, acidification and aroma formation. However Simova et al. (2008) reported that when *S. thermophilus* is co-cultured with *L. bulgaricus*, it seems to enhance the bacteriocin production. Bacteriocins from both species contain a considerable application not only in bio-preservation but also in the extension of shelf-life. The most common peptide bacteriocins in *S. thermophilus* are lantibiotics. The first lantibiotic which was characterized is lantibiotic salivaricin A from *S. salivarius* (Ross et al., 1993).

**BACTERIOCIN PRODUCTION AND ITS GENERAL PROPERTIES**

The first attempt on production of bacteriocins by streptococci dates back to the 1960’s (Mindich, 1966). *S. thermophilus* identified as anaerobic, aerotolerant, catalase-negative and Gram-positive, growing as linear chains of ovoid cells and unable to grow at 10°C, at pH 9.6 or in 6.5% NaCl broth, belong to the genus *Streptococcus* (Moscetti et al., 1998; Sherman, 1937). Some strains of *S. thermophilus* produce a bacteriocin namely thermophilin which is active against numerous LAB and food spoilage bacteria such as *C. sporogenes* and is considered as a potential bio-preservation (Aktypis et al., 2007). Although numerous reports are available in literature on LAB bacteriocins but there are only a few ones regarding production of bacteriocins by *S. thermophilus* (Smaczny and Kramer, 1984; Citano et al., 1990; Marciset and Mollet, 1993). Thermophilin 13 consists of two substances while thermophilin A was apparently a glycosylated bacteriocin. It has been reported that thermophilin 81 and thermophilin ST-1 from *S. thermophilus*ACA-DC 0001 have antimicrobial activity against Gram-negative bacteria (Kabuki et al., 2006). Thermophilin 347 shows a bactericidal effect against *L. monocytogenes* as well as some other closely related lactic acid bacteria. The activity of thermophilin 347 is lost due to protease enzyme but it retains its activity after heating (Villani et al., 1995).

Recently, isolation and characterization of streptococcal bacteriocins have been done against pathogenic streptococci. Lantibiotics are the most prevalent peptide bacteriocins in streptococci and the majority belongs to the elongated cationic type A lantibiotics. Two-peptide lantibiotics have also been isolated from *streptococci*. The lantibiotic salivaricin A was the first *S. salivarius* lantibiotic to be characterized (Ross et al., 1993) and it strongly inhibited *S. pyogenes* strains. Its bacteriocin is isolated, identified and partially purified by ammonium sulfate precipitation, dialysis and gel filtration chromatography. The molecular weight of bacteriocin is found to be approximately 4.5 kDa through Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (Harun-ur-Rashid et al., 2007). The bacteriocin is purified from a culture of *S. thermophilus* 81 grown in MRS medium with pH 6.0 and the culture is obtained by
incubation followed by centrifugation. The proteins precipitated and the upper butanol fractions with antimicrobial activity are separated into a gel-filtration column (Ivanova et al., 1998). Table 1 represents a summary of the antimicrobial activity of various species purified from S. thermophilus bacteria that has been purified and named.

Thermophilin 13 produced from S. thermophilus 13 consists of two antimicrobial peptides ThmA and ThmB. ThmA alone has antibacterial activity against S. thermophilus, C. botulinum, L. monocytogenes, and B. cereus, because its antimicrobial activity is enhanced 40-folds when an equal amount of ThmB is present. ThmB, by itself is not bacteriocidal and an excess of this peptide inhibits the activity of ThmA. Thermophilin 13, neither ThmA nor ThmB contains the YGNGV-C consensus sequence of Listeria-active peptides and post-translational modifications comparable to that in the lantibiotics are also absent. Mass spectrometry revealed the apparent oxidation of methionines in ThmA, which resulted in a peptide that could not be enhanced any longer by ThmB, whereas the intrinsic bacteriocidal activity was normal. Thermophilin 13 dispersed the membrane potential and the pH gradient in liposomes (Marciset et al., 1997).

Thermophilin 347 produced from S. thermophilus 347 is heat stable protein and shows a bacteriocidal effect against L. monocytogenes and several closely related lactic acid bacteria. The inhibitory substance exhibited a bacteriocidal effect against sensitive indicators and was shown to be proteinaceous by protease inactivation (a-Chymotrypsin, Trypsin, Pronase E, Papain, Protease K and Ficin) and precipitation with ammonium sulfate. It can be classified as a bacteriocin on the basis of the criteria of various authors (Tagg et al., 1976; Klaenhammer, 1988) and was designated as thermophilin 347. SDS-PAGE of partially purified thermophilin 347 was used to detect bacteriocin activity corresponding to an apparent molecular mass between 2.5 and 6.2 KDa (Villani et al., 1994).

Similarly, thermophilin A is a bacteriocin like substance present in the culture supernatant of S. thermophilus ST134. Apparently, it is a heat stable and glycosylated bacteriocin with a bacteriocidal mode of action against sensitive cells. It is purified to homogeneity by ammonium sulfate precipitation and ion exchange Chromatography followed by ultrafiltration (Ward and Somkuti, 1995; Whitford et al., 2001).

It has been reported that thermophilin 81, a new bacteriocin, produced by S. thermophilus 81 is heat labile but its activity is not altered by pH variation from 3 to 10. It has broad inhibitory spectrum and does not resemble any other S. thermophilus bacteriocin; the mode of action is bacteriostatic (retardation of bacterial growth) (Table 3). This bacteriocin as compared to 32 amino acids is efficient against several Bacillus species, L. monocytogenes, S. typhimurium, E. coli, Y. pseudotuberculosis and Y. enterocolitica. Six months of storage at 40°C did not influence its activity. The inactivation by detergents and the inability to resolve the protein in SDS–PAGE suggests a more complex structure (Ivanova et al., 1998).

Thermophilin ST-1 produced from S. thermophilus ACA-DC 0001 is a large heat-labile protein sensitive to the proteolytic enzymes pronase and trypsin at high acidic and alkaline conditions. It has antimicrobial activity against Gram-negative phytopathogen bacteria including L. innocua, Enterococcus faecalis, Staphylococcus aureus, Xanthomonas campestris, Pseudomonas syringae and Erwinia rubrifaciens. Production of thermophilin ST-1 starts during the early growth of the producer strain and reaches a maximum titre of 2560 AU·ml⁻¹ at the end of the exponential growth phase. Thermophilin ST-1 partially purified by ammonium sulfate precipitation, ion exchange and size-exclusion

<table>
<thead>
<tr>
<th>Species</th>
<th>Peptide</th>
<th>Reference</th>
</tr>
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<tbody>
<tr>
<td>S. thermophilus</td>
<td>Thermophilin</td>
<td>Marciset et al. (1997)</td>
</tr>
<tr>
<td>S. thermophilus</td>
<td>Thermophilin</td>
<td>Villani et al. (1995)</td>
</tr>
<tr>
<td>S. thermophilus</td>
<td>Thermophilin</td>
<td>Ward and Somkuti (1995)</td>
</tr>
<tr>
<td>S. thermophilus</td>
<td>Thermophilin</td>
<td>Whitford et al. (2001)</td>
</tr>
<tr>
<td>S. thermophilus</td>
<td>Thermophilin</td>
<td>Ivanova et al. (1998)</td>
</tr>
<tr>
<td>S. thermophilus</td>
<td>Thermophilin</td>
<td>Aktypis et al. (1998)</td>
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<tr>
<td>S. thermophilus</td>
<td>Thermophilin</td>
<td>Mathot et al. (2003)</td>
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<td>S. thermophilus</td>
<td>Thermophilin</td>
<td>Gilbreth and Somkuti (2005)</td>
</tr>
<tr>
<td>S. thermophilus</td>
<td>Thermophilin</td>
<td>Kabuki et al. (2006)</td>
</tr>
<tr>
<td>S. thermophilus</td>
<td>Thermophilin</td>
<td>Laetitia and Hols (2008)</td>
</tr>
<tr>
<td>S. thermophilus</td>
<td>Bacteriocin</td>
<td>Khali (2009)</td>
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thermophilin ST-1 showed a single protein band with a molecular mass of 30 kg·mol⁻¹ by chromatography. SDS-PAGE electrophoresis of purified Thermophilin 1277 had an apparent molecular mass of 3.7 kDa. Bacteriocin production can be detected for 60 min but loses its activity against P. acidilactici F after 90 min or longer exposure. It is also susceptible to digestion by most proteases and α-amylase. It is isolated from culture supernatant after 16 h of growth and partially purified by chloroform extraction procedure. SDS-PAGE analysis revealed two components with estimated sizes between 4.0 and 4.5 kDa.

This bacteriocin may be especially useful in the food processing industries to control spoilage caused by pediococci (Gilbreth and Somkuti, 2005). Thermophilin 1277, which was produced by S. thermophilus SBT1277, showed stability with heat treatment but it is inactivated by protease K. It shows antimicrobial activity against several LAB and food spoilage bacteria including C. butylicum, C. sprogenes, B. cereus, and L. monocytogenes B. cereus. The partially purified Thermophilin 1277 had an apparent molecular mass of 3.7 kDa. Bacteriocin production can be detected in pH controlled ST broth at pH values of 5.5–6.5 (Kabuki et al., 2006). The blpSt cluster of S. thermophilus LMD-9 contains all the genetic information required for the production of bacteriocins. Thermophilin displays an inhibitory spectrum targeted toward related Gram-positive bacteria such as L. monocytogenes. Using deletion mutants, the contribution of the three putative bacteriocin-encoding operons; blpDS1-orf2, blpUST-orf3, and blpESt-blpFSt and of the blpGST gene, which encodes a putative modification protein to the inhibitory spectrum and immunity of strain LMD-9. blpSt locus encodes a

**Table 2. Spectrum of antimicrobial activity of the proteinaceous inhibitory compounds obtained from S. thermophilus.**

<table>
<thead>
<tr>
<th>Species</th>
<th>Inhibitory Spectrum</th>
<th>Reference</th>
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<tbody>
<tr>
<td>S. thermophilus Sfi 13</td>
<td>C. botulinum, L. monocytogenes, L. lactis, B. cereus, B. subtilis and S. thermophilus</td>
<td>Marciset et al. (1997)</td>
</tr>
<tr>
<td>S. thermophilus 347</td>
<td>L. monocytogenes, LAB</td>
<td>Villani et al. (1995)</td>
</tr>
<tr>
<td>S. thermophilus ST134</td>
<td>Against sensitive cells of producing culture</td>
<td>Ward and Somkuti (1995)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Whitford et al. (2001)</td>
</tr>
<tr>
<td>S. thermophilus 81</td>
<td>Bacillus species, L. monocytogenes, S. typhimurium, E. coli, Y. pseudotuberculosis and Y. enterocolitica</td>
<td>Ivanova et al. (1998)</td>
</tr>
<tr>
<td>S. thermophilus ACA-DC 0040</td>
<td>LAB, C. sporogenes, C. tyrobutyricum</td>
<td>Aktypis et al. (1998)</td>
</tr>
<tr>
<td>S. thermophilus 580</td>
<td>C. tyrobutyricum</td>
<td>Mathot et al. (2003)</td>
</tr>
<tr>
<td>S. thermophilus ST110</td>
<td>LAB, P. acidilactici</td>
<td>Gilbreth and Somkuti (2005)</td>
</tr>
<tr>
<td>S. thermophilus SBT1277</td>
<td>LAB, C. butylicum, C. sprogenes, B. cereus</td>
<td>Kabuki et al. (2006)</td>
</tr>
<tr>
<td>S. thermophilus LMD-9</td>
<td>Other S. thermophilus strains, L. monocytogenes</td>
<td>Lalettia and Hols (2008)</td>
</tr>
<tr>
<td>S. thermophilus CHCC 3534</td>
<td>LAB, S. typhimurium, S. aureus</td>
<td>Khali (2009)</td>
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multi-peptide bacteriocin system called thermophilin 9. The blpGST is specifically required for the anti-listeria activity of thermophilin 9 (Fontaine and Hols, 2008).

**PURIFICATION OF S. thermophilus BACTERIOCIN**

Thermophilin 13 was purified when trichloroacetic acid pellet of a 1 liter culture dissolved in 8 ml of 200 mM Tris-HCl, pH 8.0, 6 M urea and 2 M NaCl was used for purification on a 20 ml Source 15-Phe resin (15-Phe) packed into a HR16/10 column. The peaks were characterized by Electrospray Mass Spectroscopy and re-chromatographed separately after dilution (1:1) in 0.1% trifluoroacetic acid. Both peaks were collected from two independent runs, re-analyzed by electrospray-MS, and stored at -20°C in their elution solvent. By Mass Spectrometry Electrospray, mass spectra were recorded on an R 3010 quadrupole mass spectrometer equipped with electrospray ion source (Marciset et al., 1997). The molecular weight of the peptides was determined by the measurement of multiple charged ions (Covey et al., 1988).

The inhibitory substance thermophilin 347 was partially purified by ammonium sulfate precipitation (30, 40, 50, 55, 60 and 65% saturation) of cell-free supernatant fluid from a culture in MRS (de Man, Rogosa and Sharpe) broth of the producer strain. The best recovery (about 80% of the supernatant activity) and a maximal specific activity (128000 AU/mg proteins) were obtained by precipitation with ammonium sulfate at 55% saturation. This fraction (40 960 AU/ml) was used in all experiments (Villani et al., 1995). Direct detection of antimicrobial activity after electrophoresis on SDS-PAGE overlayed with *L. monocytogenes* Scott A indicated that thermophilin 347 had a molecular mass between 2.5 and 6.2 KDa. Nevertheless, SDS-PAGE did not reveal a specific stained band corresponding to the active thermophilin 347 had a molecular mass between 2.5 and 6.2 KDa. Nevertheless, SDS-PAGE did not reveal a specific stained band corresponding to the active fraction examined on an HPLC reversed-phase column. The peaks were characterized by Electrospray Mass Spectroscopy and re-chromatographed separately after dilution (1:1) in 0.1% trifluoroacetic acid. Both peaks were collected from two independent runs, re-analyzed by electrospray-MS, and stored at -20°C in their elution solvent. By Mass Spectrometry Electrospray, mass spectra were recorded on an R 3010 quadrupole mass spectrometer equipped with electrospray ion source (Marciset et al., 1997). The molecular weight of the peptides was determined by the measurement of multiple charged ions (Covey et al., 1988).

The purification of thermophilin 110 was obtained by chloroform extraction-based purification. The solvent extraction method resulted in a 40% recovery of the initial total activity and a 522-fold increase in the specific activity of thermophilin 110. SDS-PAGE analysis on the Nu-PAGE Novex gels indicated the presence of two components in thermophilin 110 obtained by chloroform precipitation. The estimated size of each of the components was between 4 and 5 kDa. The putative peptide bands also picked up the glycoprotein-specific lantibiotic heat-stable peptide. Purification was done by Tricine SDS-polyacrylamide gel electrophoresis containing molecular size marker and the active fraction from Superose-12 gel-chromatography. Agarose gel electrophoresis of plasmid DNA was isolated from E. coli (Aktypis et al., 1998).

Thermophilin ST-1 from *S. thermophilus* ACA-DC 0001 was recovered following 55% saturation of the culture broth with ammonium sulfate, with a simultaneous increase in its specific activity at 10 970 (Fraction II). Upon an ion-exchange chromatography on sepharose QHP, the bacteriocin was not bound and eluted during washing of the column with buffer A in a volume of 50 ml. The specific activity at this stage was increased almost two-fold and the recovery was only 10% (Fraction III). Following the concentration and buffer exchange of bacteriocin with the ultrafiltration procedure, a high specific activity of 29 257 was achieved, but the yield was reduced to 4%. Then fraction IV was passed through a mono-S cation-exchange column, where a strong binding of activity was observed on the column. After elution of thermophilin ST-1, the specific activity was increased by four hundred-fold followed by a low recovery of 0.8%. Upon size-exclusion chromatography, the majority of the bacteriocin activity was eluted in 2 fractions of 1 mL each, with a titer of 1280 AU ml⁻¹. Finally, after this purification step, the specific activity increased five-hundred-fold with an overall recovery of bacteriocin at 0.1% and the elution volume (Ve) of thermophilin ST-1 activity from the superose12 column corresponded to a molecular mass of ca.30 kg·mol⁻¹ (Aktypis and Kalantzopoulos, 2003).

When contradictory observations made for *S. thermophilus* 580, it was observed that its bacteriocin activity measured in neutralized cell free supernatants (NCFS) which was obtained after neutralization to pH 6.5 and filter sterilization (Sartorius, 0.2 μm). Samples were mixed in sodium citrate (vol/vol, 1/1) at a final concentration of 0.05 M. After 1 h at 37°C, the solution was adjusted to pH 6.5 and centrifuged (2 min, 2700 g). After filter sterilization, the NCFS was assayed for bacteriocin activity. No effect of the treatment with sodium citrate on the amount of bacteriocin occurred. For determination of the molecular weight of the bacteriocin produced by ST 580, crude bacteriocin extract was subjected to ultrafiltration by centrifugation through membranes (centrisart, Sartorius) of different threshold: 5, 10, 20, and 100 kDa (Mathot et al. 2003).

The purification of thermophilin 110 was obtained by chloroform extraction-based purification. The solvent extraction method resulted in a 40% recovery of the initial total activity and a 522-fold increase in the specific activity of thermophilin 110. SDS-PAGE analysis on the Nu-PAGE Novex gels indicated the presence of two components in thermophilin 110 obtained by chloroform precipitation. The estimated size of each of the components was between 4 and 5 kDa. The putative peptide bands also picked up the glycoprotein-specific
stain, which confirmed the presence of a glycosidic moiety. MALDI-TOF-MS thermophilin 110 further purified by Sephadex G-25 chromatography identified three putative peptide components based on the presence of peaks with m/z values (m/z value is the mass/charge ratio, where z is usually 1). Although the estimated molecular size of each peptide detected by MALDI-TOF-MS fell within the estimated size range of the biologically active peptides identifiable by SDS-PAGE analysis (ca. 5,000 Da) and overlay agar diffusion assay, a direct relationship among these peptides could not be established (Gilbreth and Somkuti, 2005).

In purification of Thermophilin 1277, the culture supernatants were obtained by centrifugation (8000 g for 10 min at 4°C). The supernatant applied to hydrophobic chromatography with OASIS-HLB column (500 mg resin; Waters, Milford, MA, USA) was equilibrated by miliQ and eluted in a stepwise manner by using water, 50% (v/v) methanol and 70% (v/v) methanol. Crude bacteriocin was eluted with 70% methanol and stored at -20°C. The molecular weight of the crude bacteriocin thermophilin 1277 was determined by Tricine SDS–PAGE and direct detection of antimicrobial activity by an in situ assay. From the results of Coomassie blue staining and an in situ assay of the gel, the molecular weight of bacteriocin was estimated to be 3.7 kDa (Kabuki et al. 2006).

Test strain of S. thermophilus CHCC 3534 when purified, was grown in M17 broth for 10 h at 37°C. Cells usually harvested by centrifugation and the bacteriocin were precipitated from the CFS with 45% saturated ammonium sulfate (Akyptis et al., 1998). The molecular weight of the bacteriocin was estimated according to the method of Sambrook and Russell (2001). The apparent molecular mass of the sample was calculated by comparison with the mobility of the standard markers.

**MODE OF ACTION OF S. thermophilus BACTERIOCIN**

To test for a bacteriocidal or bacteriolytic mode of action, it was investigated that thermophilin 13 did not need a specific component (proteinaceous or lipid) in the membrane for activity against Listeria species. It should be stressed that, although a receptor has not yet been identified for any lactic acid bacterial bacteriocin, the “non-lantibiotics” require an “additional factor” in the target membrane to exert pore-forming activity (Jack et al., 1995). In fact, only lantibiotics, and bacteriocins thought to contain lanthionines (for example, Plantaricin C), have so far been shown to exert pore-forming activity in COVs (Sahl et al., 1995; Gonzales et al., 1994). In contrast to the lantibiotics, Thermophilin 13 does not require a threshold membrane potential to disperse the pH gradient or a threshold pH gradient to dissipate the membrane potential (Marciset et al., 1997).

The effect of two concentrations of thermophilin 347 on the viability of non-growing cells of L. monocytogenes Scott A was checked out and found that this bacteriocin exhibited a bactericidal mode of action and acted rapidly. Cell death was observed within 5 min after treatment with 1280 and 640 AU/ml of thermophilin 347 (reduction in viable counts from 1.3 × 10⁸ to 4.0 × 10⁶ and from 1.3 × 10⁸ to 3.1 × 10⁵ CFU/ml respectively). Viable counts of cell suspensions without bacteriocin and with a-chymotrypsin treated bacteriocin were unchanged. Optical densities of the control and treated cell suspensions remained stable throughout the experiment. Thermophilin 347 was completely adsorbed on the sensitive strain L. monocytogenes Scott A at pH 7.0. Residual bacteriocin activity was also unchanged in cell suspensions of insensitive producer strain at pH ranging from 2.0 to 7.0 (Villani et al., 1995).

The mode of action of the bacteriocin of S. thermophilus 81 may be considered as bacteriostatic. To study the effect of the antibacterial compound on sensitive cells, 2 ml of neutralized culture supernatant was added to 10 ml growing cells of E. coli in the early exponential phase. The OD (optical density) was measured at appropriate intervals. This peptide of 32 amino acids is efficient against several Bacillus species, L. monocytogenes, S. typhimurium, E. coli, Y. pseudotuberculosis and Y. enterocolitica. The optical density of the test culture remained constant after the addition of the bacteriocin. Higher concentrations could be necessary in order to achieve a bactericidal effect (Ivanova et al., 1998).

The mode of action of Thermophilin 1277 against the sensitive strain L. helveticus SBT2171 was studied. The bactericidal activity of Thermophilin 1277 was found to be concentration dependant, and the bactericidal effect of Thermophilin 1277 was apparent within 30 min after affecting the indicator strains. It is generally accepted that bacteriocin-induced cell death occurs in a concentration and time dependent manner (Ennahar et al., 2000). Not only the inhibitor concentration, but also factors related to the target cell and the cell environment influence the effectiveness of a bacteriocin (Ennahar et al., 2000; McAuliffe et al., 2001).

ST-I has a bacteriocidal effect against sensitive organisms like L. lactis ssp. cremoris CNRZ 117. When the lower amount (40 AU·ml⁻¹ final inhibitory titre) of crude bacteriocin was added during exponential growth of this organism, growth stopped and the viability (cfu·ml⁻¹) was dropped by 2 log units within 24 h. At inhibitory titres of 80 and 160 AU·ml⁻¹, a drop in CFUm⁻¹ of 4 log units was observed during the first 2 h of incubation and growth was not resumed after prolonged incubation. Although the viability dropped dramatically upon the addition of crude bacteriocin, the optical density of the sensitive culture did not decrease, suggesting that lysis did not occur. Exposure of washed resting cells of L. lactis ssp. cremoris to 10 240 AU/ml⁻¹ crude bacteriocin resulted in the killing of over 92% (1 log reduction) of the cells within 5 min and 97% within 90 min (Aktypis and
Kalantzopoulos, 2003).

In studies designed to characterize its mode of antimicrobial activity, thermophilin 110 was observed to induce cell lysis in several pediococci. To explore the lysis phenomenon further, \textit{P. acidilactici} F was grown to an absorption of 0.5 at OD660, with the cells centrifuged and resuspended in 20 mM potassium phosphate buffer (pH 6.0). Partially purified thermophilin 110 was added to the cell suspension. Absorbance values were checked at 660 and 260 nm over a 2 h period to follow changes in cell density and to detect the release of intracellular material. As the cell density decreased, the OD260 readings increased indicating cell lysis. After about 2 h, the culture was completely lysed and the OD660 was close to zero while the OD260 increased to 0.8. Since cells apparently were lysing, experiments were carried out to measure the presence of the intracellular enzyme LDH in cell-free supernatant fluids. Samples were taken at various time points, the cells were removed, and the supernatant was stored at 4°C until they were assayed for the presence of LDH. As cell lysis progressed, the level of LDH in the supernatant increased, resulting in the oxidation of NADH and the decrease in OD340 values (Gilbreth and Somkuti, 2005).

**APPLICATION OF \textit{S. thermophilus} BACTERIOCIN**

The application of purified bacteriocin from thermophilic bacteria in wide range of food systems is not reported but its potential in such applications has not been disregarded. Many reports mention about the incorporation of thermophilus into different types of foods and beverages and their usefulness to human health. \textit{S. thermophilus} mostly used in fermented dairy products as a culture and potential bio-preservative. It is reported that \textit{S. thermophilus} TMC 1543 in yoghurt have hypocholesterolaemic and anti tumour effects and consumption of large quantities of cultured yoghurt lowers serum cholesterol levels in humans (Irkin and Eren, 2008). The ingestion of probiotics; \textit{L. delbrueckii} ssp. \textit{bulgaricus} and \textit{S. thermophilus} leads towards anti-carcinogenic effects through the detoxification of genotoxins in the gut of rats as well as these influence metabolic, immunological and protective functions in colon (Shah, 2007). Combinations of probiotic species containing \textit{S. thermophilus} are described as having positive effects on diarrhoea in young children, enterocolitis in premature neonates and inflammatory gut disease. \textit{S. thermophilus} improves the efficacy of Bifidobacteria in a probiotic mixture, preventing rotaviral diarrhoea (Bin-Nun et al., 2005; Saavedra et al., 1994).

Inhibitory ability of thermophilin ST-1 against Gram-negative phytopathogenic bacteria suggests that the producer strain could be used for the biological control of bacterial plant diseases, or for the control of post harvest plant diseases of fruit and vegetables under storage conditions. Similarly, a variety of lactic acid bacteria has also been reported to be antagonistic to test strains of the phytopathogens \textit{X. campestris}, \textit{Erwinia carotovora} and \textit{Pseudomonas syringae} (Visser et al., 1986).

The presence of enterococcal in pre-mature neonates and inflammatory gut diseases as well as diarrhoea in young children is a big problem. So, the combinations of probiotic species containing \textit{S. thermophilus} have been described as having positive effects on such diseases (Bibliioni et al., 2005; Bin-Nun et al., 2005; Shamir et al., 2005). Lysogenic character of \textit{S. thermophilus} is also important regarding bacteria viability during storage period of yoghurts.

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