Chapter

Peptides and Microorganisms Isolated from Soybean Sources with Antimicrobial Activity

Rosalva Mora-Escobedo, María Del Carmen Robles-Ramírez, Alma Delia Román-Gutiérrez, Javier Castro-Rosas, Ciro Baruchs Muñoz-Llandes and Fabiola Araceli Guzmán-Ortiz

Abstract

Soybean has a high biological value because it is a potential nutraceutical that benefits human health. Isolated peptides of soybean have been associated with activities such as anticancer, antioxidants, antiobesity, antithrombotics, hypocholesterolemic, antidiabetic, immunomodulatory and antimicrobial, and this last activity is also obtained from microorganisms isolated from soybean subjected to processes such as fermentation, which can act directly against pathogens that are resistant to antibiotics or participate in the synthesis of new peptides with antimicrobial activity, so they could be used as preservatives in food as an alternative in the prevention of diseases. Strains of *Bacillus subtilis* isolated from soybean are mainly those that have the ability to inhibit the growth and proliferation of pathogens; some fungi such as *Rhizopus microspores* and *Aspergillus oryzae* have also had an inhibitory effect. This chapter describes the potential of microorganisms and peptides obtained from different sources of soybean against pathogenic microorganisms responsible for foodborne diseases.

Keywords: soybean, antimicrobial, peptides, microorganisms, fermentation

1. Introduction

Soy is a vegetable source of high protein content that simultaneously has several beneficial effects in human health. Its composition includes nutritional compounds of high biological value as lipids, vitamins, minerals, sugars, isoflavones, flavonoids, saponins, and peptides. Its nutritional content has proven its antihypertensive, anticholesterolemic, antioxidant, and anticancer activity [1]. Soy fermentation has mainly been conducted to preserve foods. However, it has been proven that this process is generated by certain microorganisms, expresses diverse nutritional compounds of certain biological value, and simultaneously lowers the concentration of antinutritional components as proteases, phytic acid, urease, and oxalic acids [2]. During this process, starter microorganisms turn complex organic compounds into simple compounds, improving their functionality and availability in the food matrix [3].

Different foods are derived from soy fermentation, in which lactic acid bacteria are used as starter microorganisms, mostly bacteria belonging to the genus *Bacillus*. These bacteria are responsible for the expression of certain antimicrobial components and provide the fermented products with certain organoleptic characteristics. Peptides, a product of protein hydrolysis, are one of the components expressed by starter microorganisms. They have been proven to exert a biological activity, largely due to the antagonistic and inhibitive effect of pathogenic microorganisms that cause gastrointestinal diseases and are transmitted by food. It has been demonstrated that short-chain peptides have a more efficient inhibitive action than long-chain ones [4].

On the other hand, it has been reported that some antimicrobial lipopeptides can interact with the cell's membrane, forming pores and leading to cell lysis [5].

This chapter consolidates relevant information on microorganisms and peptides isolated from soy-based foods that are known to have a microbial and/or antagonistic action in the presence of pathogenic bacteria. Reports are listed stating that, after fermentation, microorganism can express antimicrobial compounds that could be analyzed to be substituted by antibiotics.

2. Fermentation

Fermentation is a feasible way to produce peptides when compared against the use of enzymes in protein hydrolysis. Proteolytic enzymes produced by microorganisms involved in fermentation release peptides and free amino acids [6, 7]. For instance, the β-galactosidases in lactic acid bacteria in fermentation can hydrolyze oligosaccharides present in soy, reducing the unpleasant taste and flatulence [8–10]. Oligopeptides, dipeptides, and tripeptides are created as a result of protein hydrolysis by this type of proteolytic enzymes in soy milk fermentation [11]. The creation of peptides also depends on the soybean variety, the type of inoculation microorganism, and even the strain. Sanjukta et al. [7] found that the efficiency of hydrolyzed protein and free amino acids in fermented soybean with *Bacillus subtilis* MTCC5480 was higher than with *Bacillus subtilis* MTCC1747. The activity and generation of microorganisms during fermentation can vary depending on the substrate to produce a determined food. Among the possible sources of fermented-soybean products created from *Bacilli* are natto, kinema, and chungkookjang. Some other products as sufu, tempeh, douchi, miso, and combinations as doenjang are obtained from fungi. Other products derived from soybean as doubanjiang, meju, sokseongjang, cheonggukjang, kanjang, thua nao, hawaijar, and tungrymbai also create microorganisms with certain biological functions [6, 12, 13].

3. Fermented soybean products

3.1 Buckwheat sokseongjang

Buckwheat sokseongjang, a traditional Korean food, is an aged paste made from fermented soybeans. This fermentation takes place with *Bacillus subtilis* HJ18-4 as inoculum at 35°C for 36 h [14]. During the fermentation of buckwheat sokseongjang, there is a decrease in sugar content while the opposite occurs for protease and amylase activities [15]. Due to the type of microorganism, the soybean food shows a high antimicrobial spectrum. *Lactobacillus plantarum* JSA22 has also been isolated from buckwheat sokseongjang with probiotic properties that are able to inhibit infection by *S. typhimurium* in intestinal epithelial cells [16].

3.2 Cheonggukjang

This food is also produced from fermented soybeans and, as buckwheat sokseongjang, it is traditional in Korea [17]. Fermentation of cheonggukjang is completed at 40–43°C for 48–96 h [18], using natural microflora as *Bacillus subtilis* [19, 20]. During this process, the hydrolysis of compounds increases, leading to the conversion of glycosylated flavonoids into aglyconated forms. Additionally, several proteins are degraded into small peptides and amino acids [21, 22]. Cheonggukjang contains many enzymes, microorganisms, and bioactive compounds considered a source of proteins, hydrolyzed peptides, and lipids [19, 20, 23].

3.3 Miso

Miso is a soybean paste obtained by fermentation with lactic acid, yeast, and tane-koji (starter). It maintains some of the texture of the beans; however, the final product is a paste. *Aspergillus oryzae* strains are inoculated, allowing the fungus to grow and cover the beans; the process can take months and the same miso can be used as inoculum for new fermentations [24]. The presence of microorganisms as *Lactococcus* sp. GM005, which are capable of producing peptides with antimicrobial activity has been reported in miso fermentation [25].

3.4 Solid fermented soy foods

3.4.1 Douchi

Within the solid fermentation of soy products is douchi, a product of black soybeans that is traditional in China. Several types of douchi can be identified according to the type of microorganisms used in the fermentation (filamentous fungi or bacteria). The soybeans are washed and soaked for 3–4 h. Then, they are steamed for 50 min approximately and cooled at 30°C. The beans are inoculated with *Aspergillus aegyptiacus* [26], although *Mucor*, *Rhizopus*, and bacteria can also be used [27]. After inoculation, the beans are maintained at 30°C for 3–4 days to make koji. Afterwards, they are washed with water and mixed with 16% salt, water, ginger, and a mix of powdered spices. During maturation, the fungal enzymatic activity promotes an increase in amino nitrogen levels and organic acid as well as a reduction in the concentration of isoflavones [24, 26]. Some studies have reported that douchi inhibits the angiotensin-converting enzyme and α-glucosidase and has an antioxidant activity [28, 29].

3.4.2 Natto and kinema

Natto and kinema are soy products with similar elaboration processes. To make natto, soybeans are soaked in water at 21–23°C for 20 h and are boiled at 121°C for 40 min. They are then cooled at 50°C for inoculation with a pure culture of *Bacillus subtilis* natto spores that germinate at 50°C [30]. Similarly, to produce kinema, soybeans are soaked overnight at 25°C, cooked, and ground [31]. The fermentation of the product occurs naturally: during the process, bacterial spores able to survive cooking are generated; therefore, there is no need for starter microorganisms. Due to the type of microbiota generated during the fermentation of kinema (species of *Bacillus*, *Bacillus subtilis*, *Bacillus licheniformis*, *Bacillus cereus*, *Bacillus circulans*, *Bacillus thuringiensis*, and *Bacillus sphaericus* [32] some antimicrobial activity is likely to exist, mainly because of the presence of *Bacillus subtilis*, which has been reported to have an antimicrobial effect [33].

3.4.3 Tempeh

Tempeh is another solid fermented soy product. Its preparation starts with hull removal; then, the beans are soaked in water for 17 h, approximately. They are cooked in water for 30–40 min; the water is drained and the beans are cooled at room temperature for inoculation with *Rhizopus oligosporus* and incubated at 32°C for 26 h [34]. The fungal metabolism created during the fermentation causes changes in the composition of tempeh due to the increased protein degradation generating molecules of low molecular weight [35]. Some studies have reported that peptides, as bacteriocins, are generated from microorganisms isolated from tempeh and have an antibiotic effect [36].

4. Antimicrobial peptide action

The process of soy fermentation creates secondary metabolites, as antibiotics and peptides that have some biological activity beneficial to human health. Antimicrobial ability is one of the reported activities that secondary metabolites exhibit. It may be due to the peptides produced by protein hydrolysis present in the food or starter culture. It has been reported that *Bacillus subtilis* can produce a wide range of antimicrobial compounds with an antagonistic effect against bacteria and fungi [12, 14, 37, 38]. It is known that *Bacillus subtilis* is able to create bioactive peptides. Some pathogenic microorganisms susceptible to these peptides are: *Campylobacter* spp, *Clostridium botulinum*, *Listeria monocytogenes*, *Salmonella typhimurium*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Botrytis cinerea*, *Fusarium moniliforme*, *Micrococcus luteus*, and *S. typhimurium* [39]. The action mechanism of the antimicrobial activity can be different between peptides. Their hydrophobic characteristics allow them to interact with the lipid layer of the cell membrane. Those peptides in close contact with the bacterial cell must cross the capsular polysaccharide to interact with the outer membrane. Once the peptides have connected with the plasma membrane, they can interact with the lipid bilayer [40]. Through the interaction with the bacterial membrane, peptides cover the cell surface and lipids are aggregated. In consequence, peptides interact with the membrane and destabilize it, leading to pore formation and disruption

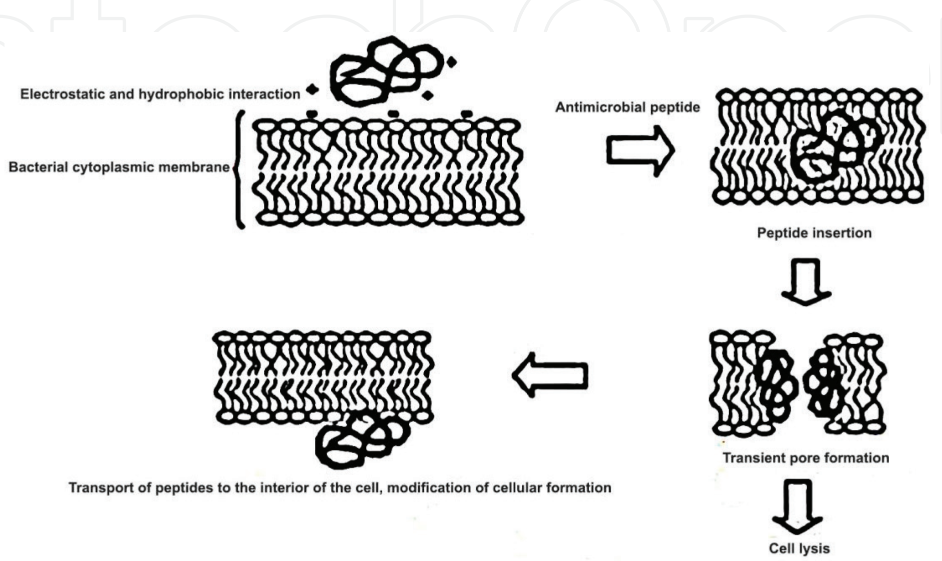

Figure 1.
Action mechanism of antimicrobial peptides (modified from Ruiz et al. [43]).

of the membrane, leading to cell lysis (**Figure 1**) [41–43]. In addition, peptides can produce indentations in the cell surface and induce micellization, disrupting the bacterial membrane [44]. On the other hand, an extensive presence of bubbles in bacterial cells promotes the release of intracellular material and, in consequence, antimicrobial peptides [45]. When peptides penetrate into the cells, they create clusters and aggregates, which promotes ion leakage from the cell [42]. The action mechanisms can depend on a number of physicochemical factors of the peptide's structure, charge, chain length, amino acid composition, and amino acid sequence.

5. Microorganisms

The fermentation quality of soy foods is determined by the type of bacteria generated. Lactic acid bacteria are especially interesting because of their function. They provide flavor and protect against contamination by pathogenic microorganisms [24]. These bacteria can produce bacteriocins in fermented-soybean products that contribute to control the growth of microorganisms as *Listeria* sp. and *Klebsiella* spp. Strain *Bacillus subtilis* is able to produce antibiotics and peptides as bacteriocins and lipopeptides with antimicrobial activity. This genus is a strong producer of hydrolytic enzymes that allow for the digestibility of fermented-soybean products [46]. The most common contamination in fermented products is caused by *Bacillus cereus*; this microorganism can produce enterotoxins on cooked soybeans, i.e., in kinema. However, only small amounts of enterotoxins were formed in the presence of a competitive dominance of *Bacillus subtilis* [47].

5.1 Lactic acid bacteria

Lactic acid bacteria are considered probiotic microorganisms thanks to the diverse beneficial effects they produce on human health. Still, they have also been proven to have antimicrobial activity against pathogenic bacteria [48]. Recent studies on probiotics have reported that fermented-soybean products have strong antibactericidal effects against pathogens transmitted by food [49]. These bacteria show inhibitive effects on many pathogenic organisms both *in vivo* and *in vitro*, including *Salmonella*, *Shigella*, *Clostridium*, *Bacillus cereus*, *Staphylococcus aureus*, *Candida albicans*, *Listeria monocytogenes*, *Escherichia coli*, and *Campylobacter jejuni* [50]. El-Sayed et al. [51] analyzed the antimicrobial activity of probiotic lactic acid bacteria during the fermentation process of soymilk. They found those microorganisms have an antagonistic effect against pathogenic agents as *E. coli* and *S. aureus*. Additionally, the fermentation process generates secondary metabolites as organic acids, hydrogen peroxide, and certain bacteriocins, which enhance the inhibitory capacity of pathogenic microorganisms. Bacteriocins are ribosomally synthesized antimicrobial peptides with inhibitory activity towards a wide range of pathogens [52]. Lactic acid bacteria isolated from *Tungtap*, a fermented species of Puntius, and *Tungrymbai*, a fermented-soy product, can produce bacteriocins. The bacterocins of *lactobacillus*, *Pediococcus* and *Enterococcus* possess better inhibitory properties against pathogens than antibiotics. Bacteriocins have an inhibitory effect against β-lactamase greater than antibiotics, which is desired because this enzyme provides resistance to bacteria to be inhibited [53]. *Streptococcus pyogenes*, *Enterococcus faecalis*, *Escherichia coli*, *Klebsiella pneumoniae*, and *Bacillus cereus* are the main microorganisms inhibited by this type of bacteriocins produced from lactic acid bacteria.

5.2 *Bacillus subtilis* SN7

Bacillus subtilis strain SN7 is isolated from meju, a fermented-soy product from Korea. Strain SN7 has an antimicrobial activity against pathogens as *Bacillus cereus* and is able to produce bacteriocins. This strain poses no risk to human health and is therefore widely recommended. It has been used in the fermentation of cheonggukjang. *Bacillus subtilis* SN7 efficiently inhibits the growth of *Bacillus cereus* vegetative cells and the inactivated germination of *Bacillus cereus* spores [54]. This is because when 4.2–4.3 log spores/mL of *B. cereus* ATCC 14579 and 6.2–6.3 log colony forming units (CFU) /mL of *B. subtilis* SN7, were inoculated at 37° C for 24 h in tryptic soy broth, *B. cereus* is not detected at 12 h of culture. The antimicrobial effect of *Bacillus subtilis* SN7 is mainly due to its capacity in the production of bacteriocins, and these act directly on the cell wall of the bacteria causing death and lysis of the cell [55]. This suggests that *Bacillus subtilis* SN7 has a great potential as control agent of *Bacillus cereus* and starter culture in Cheonggukjang. It has a wide-spectrum antagonistic activity against *Bacillus cereus*, *Bacillus subtilis*, *Bacillus licheniformis*, *Staphylococcus aureus*, *E. coli* O157: H7, and *Micrococcus luteus*. However, after extended incubation periods, the antimicrobial activity can be inhibited since the antimicrobial compound is a protein and thus it is destroyed by proteolytic enzymes produced by the same *Bacillus subtilis* SN7. Most of the *Bacillus* strains produce proteolytic enzymes at the end of the growth for spore extrusion through autocellular lysis [56]. The antimicrobial capacity produced by bacteriocin of *Bacillus subtilis* SN7 it can be inactivated by proteolytic enzymes but not by α-amylase or lipase. Mejucin, the bacteriocin produced by *Bacillus subtilis* SN7, has a sequence LGPQLNKGCATCS IGAACLVDGPIPDEIAG. The pH conditions do not affect the antimicrobial activity since Mejucin tolerates pH 3–9 and is stable between 4 and 37°C [54]. Mejucin modifies the cell membrane in such a way that its regeneration is not possible, and it also causes the leakage of intracellular components of *Bacillus cereus* [13].

5.3 *Bacillus* sp. LM7

Bacillus sp. LM7 is a microorganism isolated from Chungkookjang. During the stationary growth phase of *Bacillus* sp. LM7 and soy fermentation, the microorganisms releases an antimicrobial substance called anti-LM7, which has been reported to be stable at pH 4–9 and 80°C for 30 min. The reported antimicrobial activity mainly affects gram-positive bacteria, including *Bacillus cereus* and *Listeria monocytogenes*, and inhibits certain types of fungal strains. The antimicrobial effect is associated to the generation of lipopeptides from two families, bacillomycin D and surfactin analogues [57], so anti-LM7 was considered a lipopeptide. Bacillomycin D is composed of L-Asn, D-Tyr, D-Asn, L-Pro, L-Glu, D-Ser, and L-Thr [58, 59]. Surfactin consists of a hydrophobic fatty acid and seven amino acids, including L-Asp, 2 L-Leu, L-Glu, L-Val, and 2 D-Leu. However, its efficiency is negatively affected by enzymes (trypsins, lipases, pepsins, or proteinase k). Chymotrypsin cleaves the amide bond on the carboxyl side of tyrosine, tryptophan, phenylalanine, and leucine. The protease hydrolyzes peptide amide bonds on the carboxyl side of glutamic and aspartic acids. Therefore, chymotrypsin and protease can inhibit the anti-LM7 activity by recognizing and hydrolyzing an amino acid in the peptide loop. Chymotrypsin can hydrolyze peptide bonds on the carboxyl side of Tyr, Trp, Phe and Leu and the protease Glu and ASP. Anti-LM7 isolated from chungkookjang has a potential use in environmental, pharmaceutical, and food-processing industries, and this can be used in dairy products and in the production of vegetables and meats as a preservative to increase the food's shelf life [57]. It could also be an antibiotic source, considering the conditions under which its activity can be affected.

5.4 *Bacillus subtilis* SC-8

Bacillus subtilis SC-8 is a gram-positive bacterium that has been isolated from fermented soy. The microorganism inhibits the growth of *Bacillus cereus* species as *Bacillus anthracis*, *Bacillus mycoides*, *Bacillus pseudomycoides*, *Bacillus thuringiensis*, and *Bacillus weihenstephanensis* [60, 61]. Genes were also found on chromosome SC-8 of *Bacillus subtilis* (BSSC8_21740 to BSSC8_21920), these genes can code new antimicrobial peptides against *B. cereus*. [62]. As *Bacillus* sp. LM7, *Bacillus subtilis* SC-8 can express surfactin, fengycin, and iturin. Fengycin is an antifungal that inhibits filamentous fungi, yeasts, and bacteria. Its structure is given by: Glu-D-Orn-D-Tyr-DaThr-L-Glu-D-Al/Val-L-Pro-L-Gln-L-Tyr-L-Ile. The iturin family includes bacillomycin, iturin, and mycosubtilin; they are cyclic lipoheptapeptides bound by a β-amino acid residue. The members of this family have a strong antibiotic property. Its structure is given by iturin A, FA-β-NH2-L-Asn-D-Tyr-D-Asn-LGln-L-Pro-D-Asn-L-Ser and iturin C, FA-β-NH2-L-Asp-D-Tyr-D-Asn-LGln-L-Pro-D-Asn-L-Ser [5].

5.5 *Bacillus subtilis* NT-6

Bacillus subtilis NT-6 has been isolated from natto; it is able to release AMPNT-6, an amphipathic peptide with a hydrophobic fatty acid section and a hydrophilic peptide section. The molecular structures of these lipopeptides are cyclic because the C-terminal peptide residues connect with the β-hydroxy fatty acid through the hydroxyl group of the peptide residue directly with a β-amino acid [63]. Peptide AMPNT-6 has antimicrobial characteristics within a wide pH range (2–12). It is thermally stable, even at 100°C during 20 min, and has excellent solubility in both oil and water. It is efficient not only against gram-positive and gram-negative bacteria but also against fungi [64–66]. Xu et al. [67] reported a study on AMPNT-6 against *Vibrio parahaemolyticus*. The antimicrobial efficiency of the peptide depends on time and concentration. AMPNT-6 has a high inhibitory effect on *V. parahaemolyticus* at a minimal inhibitory concentration (MIC) (1.25 mg/mL). The action mechanism is the result of the destruction of the cell wall, forming pores in the cell membranes. Peptide AMPNT-6 has also been studied to determine its capacity to reduce adhesion and alter *Shewanella putrefaciens* preformed biofilms in two different contact surfaces (shell of shrimp, stainless steel blade). At minimal inhibitory concentration of 3 mg/mL, it was able to eliminate the formation of biofilms and prevent bacteria from forming them again in a model using a 96-well polystyrene microplate. It has also been reported that AMPNT-6, decreases the amount of extracellular polymeric substances secreted by bacteria, which is beneficial, since these substances are the main component of the biofilm responsible for adhesion [68, 69] and can strengthen the interactions between bacterial cells and participate in the formation of bacterial colonies on the contact surface [70]. At MIC of 2 mg/mL, it prevents bacteria from adhering to the surface of the microplate to form biofilms in a 3 h period while the elimination of formed biofilms takes place at 24 h [71]. *Staphylococcus aureus* and enterotoxin B, have also been controlled by the AMPNT-6 peptide, this may be due to the fact that they have been reported to contain three main lipopeptides such as surfactin, fengycin and iturin, capable of producing pores in the membranes that do not generate strains resistant to drugs [67, 72–74]. The antimicrobial activity of the AMPNT-6 peptide is not affected by the conditions used in meat products (temperature 25–37°C, sodium chloride concentration 7–8%, pH 7.4–8.4, and sodium metabisulfite concentration 0.2–0.4%); therefore, it can be viable for application in meat products [66].

5.6 *Bacillus subtilis* HJ18-4

Bacillus subtilis HJ18-4 is a microorganism that has been isolated from buckwheat sokseongjang. It has an antimicrobial activity against *Bacillus cereus* and other pathogens [75]. To demonstrate the antimicrobial effect of *B. subtilis* against *B. cereus*, these microorganisms have been inoculated in Luria-Bertani broth at 30° C for 24 h. *B. subtilis* has been inoculated at different concentrations (0.125, 0.25, 0.5 and 1%) and *B. cereus* at 0.5%. The highest inhibitory effect that *B. subtilis* showed against *B. cereus* was 0.5 and 1%, the survival of *B. cereus* decreases by 6.87–5.65 log CFU/mL, respectively [14]. In addition, gene expression has been used to prove the efficacy of the microorganism's antimicrobial capacity. Eom et al. [14] reported a decrease in the expression of genes related to toxin of *Bacillus cereus* as groEL, nheA, nheC, and entFM with *Bacillus subtilis* HJ18-4, proving its antimicrobial capacity.

5.7 *Bacillus subtilis* SCK-2

Among the bacteria isolated from the traditional Korean paste of fermented soy Kyeopjang, *Bacillus subtilis* SCK-2 shows antimicrobial activity against *Bacillus cereus* [76]. Peptide AMPC IC-1 has been identified from *Bacillus subtilis* SCK-2. It is a 33-residue thermostable peptide of 13 amino acids (Cys, Asn or Asp, Gln or Glu, Ser, Ala, Pro, Gly, Arg, Thr, Val, Ile, Leu and Lys) and its molecular weight is 3.4–3.6 kDa [77]. It has antimicrobial properties against species from the *Bacillus cereus* group. In addition, AMPC IC-1 inhibits the growth of *Bacillus cereus* KCTC 3624, KCTC 3674, and KCTC 3711 at a concentration of 50 μg/mL for 24 h [78]. However, it is not efficient for other pathogenic microorganisms as *Listeria monocytogenes*, *Salmonella enterica*, *Salmonella enteritidis*, *Staphylococcus aureus*, and *Escherichia coli* O157:H7 [77]. The antimicrobial activity of AMPC IC-1 is more stable under neutral and alkaline conditions [78]. The interaction between this peptide and proteases and proteinase K reduce antimicrobial activity [12]. The antimicrobial action mechanism takes place through the permeation of the cell membrane [76].

5.8 *Enterococcus faecium*

Enterococcus faecium is predominant in fermented foods, where it plays a key role thanks to its contribution to maturation and aroma development [79]. It is able to produce bacteriocins and has an inhibitory effect against pathogenic bacteria as *Listeria* sp., *Staphylococcus aureus*, *Vibrio cholerae*, *Clostridium* sp., *Bacillus* sp., and *Helicobacter pylori* [80–86]. Two bacteriocins (1 and 2) were obtained in tempeh from *E. faecium* LMG 19827 and *E. faecium* LMG 1982, respectively. Bacteriocins 1 and 2 have a molecular weight of 3.4 and 5.4 kDa. Their antimicrobial activity is mainly against *L. monocytogenes* and gram-positive indicators as *Enterococcus faecalis*, *E. faecium*, *Carnobacterium divergens*, *C. piscicola*, *Lactobacillus brevis*, *L. pentosus*, and *Paralactobacillus selangorensis*. The antimicrobial activity of bacteriocins is more stable after thermal treatment, except at alkaline pH values [36].

Enterococcus faecium has been isolated from chungkookjang. The strain is resistant against simulated gastrointestinal diseases. Strains S2C10 and S2C11 inhibit the viability of *Listeria monocytogenes* ATCC 19113. *L. plantarum* KCTC 1048, *Lactococcus lactis* KCTC 1913, *L. lactis* KCTC 3769, *Leuconostoc lactis* ATCC 19256, and *Pediococcus acidilactici* KCTC 1627. The ability is probably due to bacteriocin production. This antimicrobial effect is not altered at pH 2–8; however, at pH 10, the activity is reduced and often affected by enzymes as lipase [87].

5.9 *Lactococcus* sp. GM005

Another microorganism isolated from a fermented-soybean product (miso paste) is *Lactococcus* sp. GM005, which can produce 9.6-kDa bacteriocins against *L. sakei* (JCM1157 [25, 88]. This bacteriocin has been called GM005 because of the strain it is originated in. It contains a high proportion of hydrophobic amino acid residues and lanthionine [89]. Bacteriocin GM005 is sensitive to trypsin, has shown antibacterial activity against a producer of nisin that is immune to it, stable to heat and neutral pH [25]. Colony forming units (CFU) per mL of *L. sakei* JCM1157T have shown a decrease in their growth when treated with GM005 [25].

5.10 *Bacillus subtilis* E20

Cheng et al. [33] identified three peptides with antimicrobial activity. The peptides were isolated in a protein fraction from a solid-state fermentation of soy flour with *Bacillus subtilis* E20. These peptides showed an activity against *Vibrio alginolyticus* and *V. parahaemolyticus.* They have been identified as LSKKHEAALKAFTDAQKQ (2.01 kDa), LRFRAPAPVLRRIAKR (1.96 kDa), and HTSKALLDMLKRLGK (1.71 kDa). This last peptide has shown a more efficient antimicrobial activity. The minimum inhibitory concentration reported is 72 mM [90]. The growth inhibition of *Vibrio* can be by the interaction with the bacterial membranes that cause an increase in permeability [41, 91].

5.11 *Bacillus natto* TK-1

Lipopeptides can be obtained from *Bacillus natto* TK-1 in natto. The ability of *Bacillus natto* TK-1 to express components with antibacterial and antifungal activities has been proven [92, 93]. The effect is due to the production of lipopeptides as fengycin, which affects the surface of the cell membrane, inhibiting the development of bacteria as *E. coli*, *Salmonella typhimurium*, and *Staphylococcus aureus* and fungi as *Botrytis cinerea* and *Fusarium moniliforme* [91, 93–94]. However, the efficacy of inhibition against these microorganisms and other pathogens depends on the method of inoculation and the concentration of the antimicrobial [92].

6. Other peptides

Peptides isolated directly from soybeans have also proven to have antimicrobial activity. Peptides PGTAVFK and IKAFKEATKVDKVVVLWTA are protein sources from soy evaluated against *Listeria monocytogenes* and *Pseudomonas aeruginosa*. The long-chain peptide (IKAFKEATKVDKVVVLWTA) is more effective against both microorganisms at an inhibitory concentration of 37.2 μM. Still, peptide PGTAVFK does not significantly affect the development and proliferation of *P. aeruginosa* but inhibits *L. monocytogenes* at a concentration higher than 625 μM [95, 96]. PGTAVFK also has an antimicrobial activity against *E. coli* and *S. aureus* at a 31 μM concentration [97].

Peptides can also be produced by gastrointestinal digestion. Digestive enzymes hydrolyze proteins, producing peptides of different sizes and free amino acids. In *in vitro* systems, pepsin is used to act at stomach level and randomly hydrolyzes peptide bonds to produce relatively large peptides, a mix of pancreatic acid and pancreatins. The pancreatins are a mix of different peptidases as trypsin, α-chymotrypsin, elastase, and carboxypeptidases. All the enzymes used, with the exception of trypsin, hydrolyze peptide bonds, producing peptides that differ in amino acid sequence

[98]. Peptides (613 and 4932 Da) generated by acylated protein of soy seed and soymilk and subject to an *in vitro* digestion process have proven to have biological effects. Their size is slightly larger when compared to peptides produced by animal digestion. Hydrolysis can be limited due to the presence of some antinutritional factors as trypsin, lectins, and oligosaccharides inhibitors and structural barriers (cell wall), which are scarcely digestible and inhibit diffusion of digestive enzymes [24, 99].

The analysis of the data base and algorithms of peptide sequences predicted the generation of 11 peptides obtained from soy-seed digestion, 17 from soymilk protein, and 8 from antimicrobial soymilk protein precipitate. The generation of nine of those peptides is the consequence of glycine and β-conglycinin digestion. The sequences of the antimicrobial peptides reported were: IIIAQGKGALGV, SGGIKLPTDIISKISPLPVLKEI, SGGIKLPTDIISKISPLPV, and MIIIAQGKGALGV, IIVVQGKGAIG [97].

7. Conclusion

Soy is an important source of bioactive compounds and proteins. Some bioactive peptides obtained from soy and its fermented and non-fermented products show different biological actions as the antimicrobial activity. The fermentation process allows for the generation of microorganisms, mainly *Bacillus subtilis*, able to inhibit the growth of pathogenic bacteria. These microorganisms can participate in the production of new peptides with antagonistic activity towards pathogens, resulting in cellular lysis. Peptides can also be produced by fermentation, starter cultures, or hydrolysis of soy proteins. These products can be incorporated as additives in the development of functional foods and pharmaceutical products for preservation and prevention of health risks.

DOI: http://dx.doi.org/10.5772/intechopen.81243

Author details

Rosalva Mora-Escobedo[1], María Del Carmen Robles-Ramírez[1],
Alma Delia Román-Gutiérrez[2], Javier Castro-Rosas[2], Ciro Baruchs Muñoz-Llandes[2]
and Fabiola Araceli Guzmán-Ortiz[3]*

1 Departamento de Ingeniería Bioquímica, Escuela Nacional de Ciencias Biológicas, Instituto Politécnico Nacional, Unidad Adolfo López Mateos, Ciudad de México, Mexico

2 Área Académica de Química (AAQ), Universidad Autónoma del Estado de Hidalgo, Ciudad del Conocimiento, Mineral de la Reforma, Hidalgo, CP, México

3 CONACYT—Universidad Autónoma del Estado de Hidalgo, Ciudad del Conocimiento, Mineral de la Reforma, Hidalgo, CP, México

*Address all correspondence to: fabiguzman01@yahoo.com.mx, aroman@uaeh.edu.mx

References

[1] Kim SL, Berhow MA, Kim JT, Chi HY, Lee SJ, Chung IM. Evaluation of soyasaponin, isoflavone, protein, lipid, and free sugar accumulation in developing soybean seeds. Journal of Agricultural and Food Chemistry. 2006;**54**(26):10003-10010

[2] Egounlety M, Aworh OC. Effect of soaking, dehulling, cooking and fermentation with *Rhizopus oligosporus* on the oligosaccharides, trypsin inhibitor, phytic acid and tannins of soybean (*Glycine max* Merr.), cowpea (*Vigna unguiculata* L. Walp) and groundbean (*Macrotyloma geocarpa* Harms). Journal of Food Engineering. 2003;**56**(2-3):249-254

[3] Cho YS, Kim SK, Ahn CB, Je JY. Preparation, characterization, and antioxidant properties of gallic acid-grafted-chitosans. Carbohydrate Polymers. 2011;**83**(4):1617-1622

[4] Xiang N, Lyu Y, Zhu X, Bhunia AK, Narsimhan G. Effect of physicochemical properties of peptides from soy protein on their antimicrobial activity. Peptides. 2017;**94**:10-18

[5] Ariza Y. Determinación de metabolitos secundarios a partir de Bacillus subtilis efecto biocontrolador sobre Fusarium sp. Nova. 2012;**10**(18):135-250

[6] Rai AK, Jeyaram K. Health benefits of functional proteins in fermented foods. Health Benefits of Fermented Foods and Beverages. 2015;**44**:455-474

[7] Sanjukta S, Rai AK, Muhammed A, Jeyaram K, Talukdar NC. Enhancement of antioxidant properties of two soybean varieties of Sikkim Himalayan region by proteolytic Bacillus subtilis fermentation. Journal of Functional Foods. 2015;**14**:650-658

[8] Dhananjay S, Kulkarni S, Kapanoor KG, Naganagouda VK, Veerappa HM. Reduction of flatus-inducing factors in soymilk by immobilized-galactosidase. Biotechnology Applied Biochemistry. 2006;**45**:51-57

[9] Hati S, Vij S, Mandal S, Malik RK, Kumari V, Khetra Y. α-Galactosidase activity and oligosaccharides utilization by lactobacilli during fermentation of soy Milk. Journal of Food Processing and Preservation. 2014;**38**(3):1065-1071

[10] Scalabrini P, Rossi M, Spettoli P, Matteuzzi D. Characterization of bifidobacterium strains for use in soymilk fermentation. International Journal of Food Microbiology. 1998;**39**(3):213-219

[11] Agyei D, Danquah MK. Industrial-scale manufacturing of pharmaceutical-grade bioactive peptides. Biotechnology Advances. 2011;**29**(3):272-277

[12] Sanjukta S, Rai AK. Production of bioactive peptides during soybean fermentation and their potential health benefits. Trends in Food Science & Technology. 2016;**50**:1-10

[13] Lee SG, Chang HC. Purification and characterization of mejucin, a new bacteriocin produced by *Bacillus subtilis* SN7. LWT-Food Science and Technology. 2018;**87**:8-15

[14] Eom JS, Lee SY, Choi HS. Bacillus subtilis HJ18-4 from traditional fermented soybean food inhibits *Bacillus cereus* growth and toxin-related genes. Journal of Food Science. 2014;**79**(11): M2279-M2287

[15] Park NY, Lee SY, Kim JY, Choi HS. Quality characteristics of buckwheat Soksungjang manufactured by *Bacillus subtilis* HJ18-4. Korean Journal of Food Preservation. 2013;**20**(5):699-704

[16] Eom JS, Song J, Choi HS. Protective effects of a novel probiotic strain

of lactobacillus plantarum JSA22 from traditional fermented soybean food against infection by *Salmonella enterica* serovar Typhimurium. Journal Microbioly Biotechnoly. 2015;**25**(4):479-491

[17] Choi JH, Kim MJ, Cha YS. Cheonggukjang, a soybean paste fermented with B. licheniformis-67 prevents weight gain and improves glycemic control in high fat diet induced obese mice. Journal of Clinical Biochemistry and Nutrition. 2016;**59**(1):31-38

[18] Shin D, Jeong D. Korean traditional fermented soybean products: Jang. Journal of Ethnic Foods. 2015;**2**(1):2-7

[19] Lee JJ, Lee DS, Kim HB. Fermentation patterns of Chungkookjang and Kanjang by *Bacillus licheniformis* B1. The Korean Journal of Microbiology. 1999;**35**(4):296-301

[20] Su CL, Wu CJ, Chen FN, Wang BJ, Sheu SR, Won SJ. Supernatant of bacterial fermented soybean induces apoptosis of human hepatocellular carcinoma Hep 3B cells via activation of caspase 8 and mitochondria. Food and Chemical Toxicology. 2007;**45**(2):303-314

[21] Nakajima N, Nozaki N, Ishihara K, Ishikawa A, Tsuji H. Analysis of isoflavone content in tempeh, a fermented soybean, and preparation of a new isoflavone-enriched tempeh. Journal of Bioscience and Bioengineering. 2005;**100**(6):685-687

[22] Kwon DY, Jang JS, Lee JE, Kim YS, Shin DH, Park S. The isoflavonoid aglycone-rich fractions of Chungkookjang, fermented unsalted soybeans, enhance insulin signaling and peroxisome proliferator-activated receptor-γ activity in vitro. BioFactors. 2006;**26**(4):245-258

[23] Lee SJ, Rim HK, Jung JY, An HJ, Shin JS, Cho CW, et al. Immunostimulatory activity of polysaccharides from Cheonggukjang. Food and Chemical Toxicology. 2013;**59**:476-484

[24] Nout R. Quality, safety, biofunctionality and fermentation control in soya. In: Advances in Fermented Foods and Beverages. Woodhead Publishing Series in Food Science, Technology and Nutrition, Korea. 2015. pp. 409-434

[25] Onda T, Yanagida F, Tsuji M, Shinohara T, Yokotsuka K. Production and purification of a bacteriocin peptide produced by Lactococcus sp. strain GM005, isolated from miso-paste. International Journal of Food Microbiology. 2003;**87**(1-2):153-159

[26] Zhang JH, Tatsumi E, Fan JF, Li LT. Chemical components of Aspergillus-type Douchi, a Chinese traditional fermented soybean product, change during the fermentation process. International Journal of Food Science and Technology. 2007;**42**(3):263-268

[27] Chen T, Jiang S, Xiong S, Wang M, Zhu D, Wei H. Application of denaturing gradient gel electrophoresis to microbial diversity analysis in Chinese Douchi. Journal of the Science of Food and Agriculture. 2012;**92**(10):2171-2176

[28] Wang D, Wang LJ, Zhu FX, Zhu JY, Chen XD, Zou L, et al. In vitro and in vivo studies on the antioxidant activities of the aqueous extracts of Douchi (a traditional Chinese salt-fermented soybean food). Food Chemistry. 2008;**107**(4):1421-1428

[29] Iwaniak A, Minkiewicz P, Darewicz M. Food-originating ACE inhibitors, including antihypertensive peptides, as preventive food components in blood pressure reduction. Comprehensive Reviews in Food Science and Food Safety. 2014;**13**(2):114-134

[30] Wei Q, Chang SK. Characteristics of fermented natto products as

affected by soybean cultivars. Journal of Food Processing and Preservation. 2004;**28**(4):251-273

[31] Sarkar P, Tamang JP, Cook PE, Owens J. Kinema—A traditional soybean fermented food: Proximate composition and microflora. Food Microbiology. 1994;**11**(1):47-55

[32] Sarkar PK, Hasenack B, Nout MJR. Diversity and functionality of Bacillus and related genera isolated from spontaneously fermented soybeans (Indian Kinema) and locust beans (African Soumbala). International Journal of Food Microbiology. 2002;**77**(3):175-186

[33] Cheng AC, Lin HL, Shiu YL, Tyan YC, Liu CH. Isolation and characterization of antimicrobial peptides derived from *Bacillus subtilis* E20-fermented soybean meal and its use for preventing Vibrio infection in shrimp aquaculture. Fish & Shellfish Immunology. 2017;**67**:270-279

[34] Wei LS. Domestic soybean consumption in Asia. In: Soybean Processing for Food Uses. University of Illinois: Urbana-Champaign, INTSOY; 1991. pp. 162-183

[35] De Reu JC, Linssen VA, Rombouts FM, Nout MR. Consistency, polysaccharidase activities and non-starch polysaccharides content of soya beans during tempe fermentation. Journal of the Science of Food and Agriculture. 1997;**73**(3):357-363

[36] Moreno MRF, Leisner JJ, Tee LK, Ley C, Radu S, Rusul G, et al. Microbial analysis of Malaysian tempeh, and characterization of two bacteriocins produced by isolates of *Enterococcus faecium*. Journal of Applied Microbiology. 2002;**92**(1):147-157

[37] Kim HW, Kim KM, Ko EJ, Lee SK, He S, Song KB, et al. Development of antimicrobial edible film from defatted soybean meal fermented by *Bacillus subtilis*. Journal of Microbiology and Biotechnology. 2004;**14**(6):1303-1309

[38] Kim HW, Ko EJ, Ha SD, Song KB, Park SK, Chung DH, et al. Physical, mechanical, and antimicrobial properties of edible film produced from defatted soybean meal fermented by *Bacillus subtilis*. Journal of Microbiology and Biotechnology. 2005;**15**(4):815-822

[39] Baruzzi F, Quintieri L, Morea M, Caputo L. Antimicrobial compounds produced by *Bacillus* spp. and applications in food. Science against Microbial Pathogens: Communicating Current Research and Technological Advances. 2011;**2**:1102-1111

[40] Téllez GA, Castaño JC. Péptidos antimicrobianos. Infection. 2010;**14**(1):55-67

[41] Wimley WC. Describing the mechanism of antimicrobial peptide action with the interfacial activity model. ACS Chemical Biology. 2010;**5**(10):905-917

[42] Li Y, Xiang Q, Zhang Q, Huang Y, Su Z. Overview on the recent study of antimicrobial peptides: Origins, functions, relative mechanisms and application. Peptides. 2012;**37**(2):207-215

[43] Ruiz JC, Segura-Campos MR, Betancur-Ancona D, Chel-Guerrero L. Proteínas y péptidos biológicamente activos con potencial nutracéutico. Omnia Science Monographs. 2013; 11-27

[44] Torrent M, Sánchez-Chardi A, Nogués MV, Boix E. Assessment of antimicrobial compounds by microscopy techniques. In: Microscopy: Science, Technology, Applications and Education. Formatex, Spain. 2010. pp. 1115-1126

[45] Tomasinsig L, Skerlavaj B, Scarsini M, Guida F, Piccinini R, Tossi A,

et al. Comparative activity and mechanism of action of three types of bovine antimicrobial peptides against pathogenic Prototheca spp. Journal of Peptide Science. 2012;**18**(2):105-113

[46] Wang CT, Ji BP, Li B, Nout MJR, Li PL, Ji H, et al. Purification and characterization of a fibrinolytic enzyme of *Bacillus subtilis* DC33, isolated from Chinese traditional Douchi. Journal of Industrial Microbiology and Biotechnology. 2006;**33**:750-758

[47] Nout MJR, Bakshi D, Sarkar PK. Microbiological safety of kinema, a fermented soya bean food. Food Control. 1998;**9**(6):357-362

[48] Donkor ON, Shah NP. Production of β-glucosidase and hydrolysis of isoflavone phytoestrogens by *Lactobacillus acidophilus*, *Bifidobacterium lactis*, and *Lactobacillus casei* in soymilk. Journal of Food Science. 2008;**73**(1):M15-M20

[49] Yesillik S, Yildirim N, Dikici A, Yildiz A. Antibacterial effects of some fermented commercial and homemade dairy products and 0.9% lactic acid against selected foodborne pathogens. Asian Journal of Animal Veterinary Advances. 2011;**6**(2):189-195

[50] Chou CC, Hou JW. Growth of bifidobacteria in soymilk and their survival in the fermented soymilk drink during storage. International Journal of Food Microbiology. 2000;**56**(2-3):113-121

[51] El-Sayed EM, El-Zeini HM, Hafez SA, Saleh FA. Antibacterial activity of probiotic yoghurt and soy-yoghurt against *Escherichia coli* and *Staphylococcus aureus*. Journal of Nutrition and Food Sciences. 2014;**4**(303):2

[52] De Vuyst L, Leroy F. Bacteriocins from lactic acid bacteria: Production, purification, and food applications. Journal of Molecular Microbiology and Biotechnology. 2007;**13**(4):194-199

[53] Biswas K, Upadhayay S, Rapsang GF, Joshi SR. Antibacterial and synergistic activity against β-lactamase-producing nosocomial Bacteria by Bacteriocin of LAB isolated from lesser known traditionally fermented products of India. HAYATI Journal of Biosciences. 2017;**24**(2):87-95

[54] Lee SG, Chang HC. Assessment of *Bacillus subtilis* SN7 as a starter culture for Cheonggukjang, a Korean traditional fermented soybean food, and its capability to control *Bacillus cereus* in Cheonggukjang. Food Control. 2017;**73**:946-953

[55] Cotter PD, Hill C, Ross RP. Bacteriocins: Developing innate immunity for food. Nature Reviews Microbiology. 2005;**3**(10):777-788

[56] Gálvez A, Abriouel H, López RL, Omar NB. Bacteriocin-based strategies for food biopreservation. International Journal of Food Microbiology. 2007;**120**(1-2):51-70

[57] Lee MH, Lee J, Nam YD, Lee JS, Seo MJ, Yi SH. Characterization of antimicrobial lipopeptides produced by *Bacillus* sp. LM7 isolated from chungkookjang, a Korean traditional fermented soybean food. International Journal of Food Microbiology. 2016;**221**:12-18

[58] Ongena M, Jacques P. Bacillus lipopeptides: Versatile weapons for plant disease biocontrol. Trends in Microbiology. 2008;**16**(3):115-125

[59] Shaligram NS, Singhal RS. Surfactin—A review on biosynthesis, fermentation, purification and applications. Food Technology and Biotechnology. 2010;**48**(2):119-134

[60] Lee NK, Yeo IC, Park JW, Kang BS, Hahm YT. Isolation and characterization

of a novel analyte from *Bacillus subtilis* SC-8 antagonistic to *Bacillus cereus*. Journal of Bioscience and Bioengineering. 2010;**110**(3):298-303

[61] Lee NK, Yeo IC, Park JW, Hahm YT. Growth inhibition and induction of stress protein, Gro EL, of *Bacillus cereus* exposed to antibacterial peptide isolated from *Bacillus subtilis* SC-8. Applied Biochemistry and Biotechnology. 2011;**165**(1):235-242

[62] Yeo IC, Lee NK, Hahm YT. Genome sequencing of *Bacillus subtilis* SC-8, antagonistic to the *Bacillus cereus* group, isolated from traditional Korean fermented-soybean food. Journal of Bacteriology. 2012;**194**(2):536-537

[63] Jasim B, Sreelakshmi KS, Mathew J, Radhakrishnan EK. Surfactin, iturin, and fengycin biosynthesis by *Endophytic bacillus* sp. from *Bacopa monnieri*. Microbial Ecology. 2016;**72**(1):106-119

[64] Banat IM, Franzetti A, Gandolfi I, Bestetti G, Martinotti MG, Fracchia L, et al. Microbial biosurfactants production, applications and future potential. Applied Microbiology and Biotechnology. 2010;**87**(2):427-444

[65] Sun L, Lu Z, Bie X, Lu F, Yang S. Isolation and characterization of a co-producer of fengycins and surfactins, endophytic *Bacillus amyloliquefaciens* ES-2, from *Scutellaria baicalensis* Georgi. World Journal of Microbiology and Biotechnology. 2006;**22**(12):1259-1266

[66] Zhang N, Pu Y, Sun L, Wang Y, Deng Q, Xu D, et al. Modeling the effects of different conditions on the inhibitory activity of antimicrobial lipopeptide (AMPNT-6) against *Staphylococcus aureus* growth and enterotoxin production in shrimp meat. Aquaculture International. 2017;**25**(1):57-70

[67] Xu D, Wang Y, Sun L, Liu H, Li J. Inhibitory activity of a novel antibacterial peptide AMPNT-6 from *Bacillus subtilis* against *Vibrio parahaemolyticus* in shrimp. Food Control. 2013;**30**(1):58-61

[68] Chen X, Stewart PS. Biofilm removal caused by chemical treatments. Water Research. 2000;**34**(17):4229-4233

[69] Croxatto A, Lauritz J, Chen C, Milton DL. Vibrio anguillarum colonization of rainbow trout integument requires a DNA locus involved in exopolysaccharide transport and biosynthesis. Environmental Microbiology. 2007;**9**(2):370-382

[70] Branda SS, Vik Å, Friedman L, Kolter R. Biofilms: The matrix revisited. Trends in Microbiology. 2005;**13**(1):20-26

[71] Deng Q, Pu Y, Sun L, Wang Y, Liu Y, Wang R, et al. Antimicrobial peptide AMPNT-6 from Bacillus subtilis inhibits biofilm formation by *Shewanella putrefaciens* and disrupts its preformed biofilms on both abiotic and shrimp shell surfaces. Food Research International. 2017;**102**:8-13

[72] Gabriel AA, Nakano H. Inactivation of *Salmonella*, *E. coli* and *Listeria monocytogenes* in phosphate-buffered saline and apple juice by ultraviolet and heat treatments. Food Control. 2009;**20**(4):443-446

[73] O'Connor NK, Hudson AS, Cobb SL, O'Neil D, Robertson J, Duncan V, et al. Novel fluorinated lipopeptides from Bacillus sp. CS93 via precursor-directed biosynthesis. Amino Acids. 2014;**46**(12):2745-2752

[74] Mandal SM, Sharma S, Pinnaka AK, Kumari A, Korpole S. Isolation and characterization of diverse antimicrobial lipopeptides produced by Citrobacter and Enterobacter. BMC Microbiology. 2013;**13**(1):152

[75] Lee SY, Kim JY, Baek SY, Yeo SH, Koo BS, Park HY, et al. Isolation

and characterization of oligotrophic strains with high enzyme activity from buckwheat sokseongjang. Korean Journal of Food Science and Technology. 2011;**43**(6):735-741

[76] Sumi CD, Yang BW, Yeo IC, Hahm YT. Antimicrobial peptides of the genus Bacillus: A new era for antibiotics. Canadian Journal of Microbiology. 2014;**61**(2):93-103

[77] Yeo IC, Lee NK, Cha CJ, Hahm YT. Narrow antagonistic activity of antimicrobial peptide from Bacillus subtilis SCK-2 against *Bacillus cereus*. Journal of Bioscience and Bioengineering. 2011;**112**(4):338-344

[78] Shafi J, Tian H, Ji M. Bacillus species as versatile weapons for plant pathogens: A review. Biotechnology & Biotechnological Equipment. 2017;**31**(3):446-459

[79] Cleveland J, Montville TJ, Nes IF, Chikindas ML. Bacteriocins: Safe, natural antimicrobials for food preservation. International Journal of Food Microbiology. 2001;**71**(1):1-20

[80] De Vuyst L, Moreno MF, Revets H. Screening for enterocins and detection of hemolysin and vancomycin resistance in enterococci of different origins. International Journal of Food Microbiology. 2003;**84**(3):299-318

[81] Gálvez A, Valdivia E, Abriouel H, Camafeita E, Mendez E, Martínez-Bueno M, et al. Isolation and characterization of enterocin EJ97, a bacteriocin produced by *Enterococcus faecalis* EJ97. Archives of Microbiology. 1998;**171**(1):59-65

[82] Herranz C, Casaus P, Mukhopadhyay S, Martınez JM, Rodrıguez JM, Nes IF, et al. *Enterococcus faecium* P21: A strain occurring naturally in dry-fermented sausages producing the class II bacteriocins enterocin A and enterocin B. Food Microbiology. 2001;**18**(2):115-131

[83] Hugas M, Garriga M, Aymerich MT. Functionalty of enterococci in meat products. International Journal of Food Microbiology. 2003;**88**(2-3):223-233

[84] Leroy F, Moreno MF, De Vuyst L. Enterococcus faecium RZS C5, an interesting bacteriocin producer to be used as a co-culture in food fermentation. International Journal of Food Microbiology. 2003;**88**(2-3):235-240

[85] Nam HR, Ha MS, Lee EJ, Lee YH. Effect of *Enterococcus faecalis* strain PL9003 on adherence and growth of *Helicobacter pylori*. Journal of Microbiology and Biotechnology. 2002;**12**(5):746-752

[86] Tsai CC, Huang LF, Lin CC, Tsen HY. Antagonistic activity against *Helicobacter pylori* infection in vitro by a strain of *Enterococcus faecium* TM39. International Journal of Food Microbiology. 2004;**96**(1):1-12

[87] Yoon MY, Kim YJ, Hwang HJ. Properties and safety aspects of *Enterococcus faecium* strains isolated from Chungkukjang, a fermented soy product. LWT-Food Science and Technology. 2008;**41**(5):925-933

[88] Onda T, Yanagida F, Tsuji M, Ogino S, Shinohara T. Isolation and characterization of the lactic acid bacterial strain GM005 producing a antibacterial substance from miso-paste product. Food Science and Technology Research. 1999;**5**(3):247-250

[89] Gross E, Morell JL. Structure of nisin. Journal of the American Chemical Society. 1971;**93**(18):4634-4635

[90] Shiu YL, Wong SL, Guei WC, Shin YC, Liu CH. Increase in the plant protein ratio in the diet of white shrimp,

Litopenaeus vannamei (Boone), using *Bacillus subtilis* E20-fermented soybean meal as a replacement. Aquaculture Research. 2015;**46**(2):382-394

[91] Cheng W, Chiu CS, Guu YK, Tsai ST, Liu CH. Expression of recombinant phytase of *Bacillus subtilis* E 20 in *Escherichia coli* HMS 174 and improving the growth performance of white shrimp, *Litopenaeus vannamei*, juveniles by using phytase-pretreated soybean meal-containing diet. Aquaculture Nutrition. 2013;**19**(2):117-127

[92] Cao XH, Liao ZY, Wang CL, Yang WY, Lu MF. Evaluation of a lipopeptide biosurfactant from *Bacillus natto* TK-1 as a potential source of anti-adhesive, antimicrobial and antitumor activities. Brazilian Journal of Microbiology. 2009;**40**(2):373-379

[93] Sheppard JD, Jumarie C, Cooper DG, Laprade R. Ionic channels induced by surfactin in planar lipid bilayer membranes. Biochimica et Biophysica Acta (BBA)—Biomembranes. 1991;**1064**(1):13-23

[94] Kim PI, Bai H, Bai D, Chae H, Chung S, Kim Y, et al. Purification and characterization of a lipopeptide produced by *Bacillus thuringiensis* CMB26. Journal of Applied Microbiology. 2004;**97**(5):942-949

[95] Dhayakaran RPA, Neethirajan S, Xue J, Shi J. Characterization of antimicrobial efficacy of soy isoflavones against pathogenic biofilms. LWT-Food Science and Technology. 2015;**63**(2):859-865

[96] Dhayakaran R, Neethirajan S, Weng X. Investigation of the antimicrobial activity of soy peptides by developing a high throughput drug screening assay. Biochemistry and Biophysics Reports. 2016;**6**:149-157

[97] McClean S, Beggs LB, Welch RW. Antimicrobial activity of antihypertensive food-derived peptides and selected alanine analogues. Food Chemistry. 2014;**146**:443-447

[98] Capriotti AL, Caruso G, Cavaliere C, Samperi R, Ventura S, Chiozzi RZ, et al. Identification of potential bioactive peptides generated by simulated gastrointestinal digestion of soybean seeds and soy milk proteins. Journal of Food Composition and Analysis. 2015;**44**:205-213

[99] Rashed NA, Mac Donald MH, Matthews BF. Protease inhibitor expression in soybean roots exhibiting susceptible and resistant interactions with soybean cyst nematode. Journal of Nematology. 2008;**40**(2):138