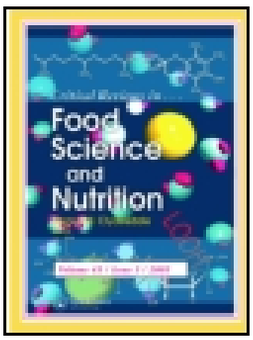

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REVIEW

Seeds, fermented foods, and agricultural by-products as sources of plant-derived antibacterial peptides

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ABSTRACT

The emergence of bacterial resistance against conventional antibiotics and the growing interest in developing alternative, natural antibacterial agents have prompted the search for plant-derived antibacterial peptides in recent decades. Different classes of endogenous antibacterial peptides have been identified from various plant species. Moreover, protein hydrolysates and hydrolysate-derived peptides with potent antibacterial effects have also been identified from numerous plant sources. Antibacterial peptides are often cationic and amphipathic, consisting of fewer than 100 amino acids. They are able to disrupt bacterial membrane integrity via pore formation and/or compromise bacterial metabolic processes. In this review, we summarize current knowledge on the characteristics and modes of action of antibacterial peptides, as well as salient points concerning the production of antibacterial protein hydrolysates from plant proteins. Examples of plant-derived antibacterial hydrolysates and peptides will be highlighted, with particular attention to less explored seeds, fermented plant foods and agricultural by-products. Promising future research directions with regards to the application of plant-derived antibacterial hydrolysates and peptides in food preservation, farm animal disease management, and nutraceutical/functional food development will be proposed.

KEYWORDS

Antimicrobial peptide; application; mode of action; protein hydrolysate

Introduction

Antibacterial peptides are a diverse group of biomolecules produced in various microorganisms, plants, and animals. In Antimicrobial Peptide Database (APD), a continuously updated database containing 2983 antimicrobial peptide sequences, 2505 records are antibacterial peptides (Wang et al. 2016a) (accessed on 20 June 2018). BIOPEP (Minkiewicz et al. 2008) and EROP-Moscow (Zamyatnin et al. 2006) are two other curated peptide databases which currently store 464 and 186 records of antibacterial peptides, respectively (accessed on 20 June 2018). Such databases, together with the accumulating body of knowledge in the literature, indicate strong interest in the scientific community in antibacterial peptides.

Plant antibacterial peptides reported in the literature can be broadly categorized into two groups: (i) endogenous peptides, which are already present in the plant matrixes, and (ii) peptides generated from the plant proteomes by means of processing (e.g., enzymatic hydrolysis and fermentation). Endogenous antibacterial peptides are indispensable, ubiquitous defense components that occur in many plant organs. Defensins, lipid transfer proteins, glycine-rich proteins, thionins (types I–V), cyclotides, snakins, and heveins are some types of endogenous plant antibacterial peptides that have been well-investigated (Patel and Akhtar 2017). Among plant sources, soybean has received abundant attention as a source of antibacterial and other bioactive peptides (Agyei 2015; De Mejia and De Lumen 2006; Maestri et al. 2016; Malaguti et al. 2014; Singh et al. 2014). However, there are other less explored plant seeds that are also valuable sources of potent antibacterial peptides, which deserve more attention from researchers.

Fermentation is a traditional food processing method that prolongs shelf life and improves the organoleptic properties of foods (Marco et al. 2017). It involves the application of living microorganisms, such as bacteria and yeasts, to achieve the enzymatic conversion of complex food components into simple compounds, such as peptides, simple sugars, free phenolic compounds, alcohols, and organic acids (Cho et al. 2011). Many of the fermented plant-based food products have been reviewed intensively by Tamang et al. (2016). Common microorganisms, such as yeasts and lactic acid bacteria, that are associated with the fermentation of different raw plant materials, including cereals, vegetables, legumes, and roots/tubers, were reported in the review. Many fermented food products, for example, fermented soybean (Cho et al. 2011; Gibbs et al. 2004; Hori et al. 2001; Iwai et al. 2002; Zhang et al. 2006), fermented red bean

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(Chang et al. 2012), fermented cereals (Coda et al. 2012), and fermented vegetables (Hu et al. 2013; Jiang et al. 2012), are the result of either single or multitude of food-microbe combinations. These products have attracted increasing attention from researchers in recent years. Some of them have been marketed as functional foods due to their health-promoting effects, such as antioxidant, antimicrobial, antihypertensive, hypolipidemic and anti-inflammatory activities (Pihlanto and Korhonen 2015; Udenigwe and Aluko 2012). Despite the increasing number of studies exploring antibacterial peptides derived from fermented plant foods, there is very little discussion on such a category of antibacterial peptides in recent reviews. On that note, a discussion on other types of bioactive peptides derived from fermented plant materials was presented in a recent review (Piovesana et al. 2018).

By-products or wastes that are continuously generated from the agri-food sector are considered a cheap source of biomass that is suitable for bioactive peptide production. Peptide production for applications either in the food or pharmaceutical industries can contribute to the valorization of such bio-materials, in addition to the reduction of waste generation and the cost of disposal (Piovesana et al. 2018). In contrast to the larger body of research on antibacterial peptide production using animal-based agricultural by-products (Bah et al. 2016; Zamora-Sillero et al. 2018), fewer studies have been carried out on plant-based agricultural by-products. A recent review discusses very briefly previous researches on antibacterial peptides derived from plant-food by-products (Guil-Guerrero et al. 2016). A more elaborate review of bioactive peptides derived from agro-industrial wastes (e.g., date seeds, cherry seeds, brewers' spent grain, cauliflower wastes, rice bran and tomato waste) focusses on antioxidant and anti-angiotensin converting enzyme activities (Piovesana et al. 2018). A growing number of studies highlighted the potential of plant-based agricultural by-products as sources of antibacterial hydrolysates and/or peptides. Thus a review of the current literature in this context is pertinent.

In the following sections of this review, structural characteristics and mechanisms of plant antibacterial peptides will be discussed. Selected examples of antibacterial protein hydrolysates/peptides derived from less explored seeds, fermented plant foods, and plant-based agricultural by-products will be presented. Figure 1 depicts the primary structures of selected examples of such antibacterial peptides. Future research directions in relation to applications in the food industry will also be proposed.

Characteristics and modes of action of plant antibacterial peptides

Endogenous antibacterial peptides are a key component of plant immune response. Plant antibacterial peptides of different classes, including defensins, albumins, cyclotides, and snakins, have been reported (de Souza Cândido et al. 2014). These peptides are frequently investigated in research pertaining to human diseases, including cancer treatment research (Leite et al. 2018). Furthermore, the protective effects conferred by defensins in crops against phytopathogens have also been explored (de Souza Cândido et al. 2014).

Plant nonspecific lipid transfer proteins (nsLTPs) possess antibacterial activity *in vitro* against a number of phytopathogens (Gonorazky et al. 2005; Lin et al. 2007). nsLTPs are small proteins of 6.5–10.5 kDa that are abundant in plants, accounting for up to 4% of total soluble protein. The tertiary structures of nsLTPs are characterized by an eight-cysteine residue conserved motif and an internal hydrophobic cavity that can bind various lipids and hydrophobic compounds. Overexpression of nsLTPs genes is known to enhance plant resistance against bacterial attacks (Liu et al. 2015).

The plant defensin family comprises antibacterial peptides that are similar in amino acid sequence to those of the animal defensin family. Plant defensins are basic, cysteine-rich peptides that are inhibitory against numerous Gram-positive and Gram-negative pathogens. These peptides are nontoxic to mammalian and plant cells (Yokoyama et al. 2008). Besides defensins, the glycine-rich proteins (GRPs) is another a group of ubiquitous antibacterial peptides in living organisms. For example, Pg-AMP1, a GRP that exhibited inhibitory activity against human pathogenic Gram-negative bacteria had been isolated from guava seeds (Pelegrini et al. 2008; Tavares et al. 2012). GRPs typically have a high glycine content (up to 70%) and comprise glycine-containing motifs consisting of repetitive amino acid residues (Czolpinska and Rurek 2018).

Antibacterial peptides normally consist of fewer than 100 amino acids in length. Amino acid composition, amphiphilicity, cationicity, and molecular size are factors which influence the ability of the peptides to bind to and insert into the bacterial membranes, besides selectivity between prokaryotic and eukaryotic cells. Generally, higher hydrophobicity in antibacterial peptides leads to enhanced membrane disturbance and compromised cellular selectivity. By contrast, higher charge density enhances electrostatic interaction between peptides and bacterial membrane, thus lowering toxicity to mammal cells (Sun et al. 2014). Besides, the secondary structure of a peptide also influences its antibacterial activity. Antibacterial peptides are randomly distributed in water, but they form α-helices upon interacting with bacterial membrane (Beevers and Dixon 2010). Following environmental stimulations, changes in peptide secondary structure can cause more intense membrane leakage in bacteria targeted by the peptide (Strömstedt et al. 2009).

The mechanisms of action of antibacterial peptides involve the attachment of peptide to the bacterial membrane, followed by transmembrane pore formation, which may lead to membrane disintegration. Antibacterial peptides exhibit an amphipathic conformation, a high ratio of hydrophobic amino acids, and a net positive charge, which allow the peptides to bind easily to the negatively-charged bacterial membranes (Bechinger and Gorr 2017). In general, the peptides may firstly bind to the wall teichoic acids (anionic glycopolymers) in Gram-positive bacteria, or to lipopolysaccharides in Gram-negative bacteria (Vorland 1999), followed by interaction with the negatively-charged bacterial

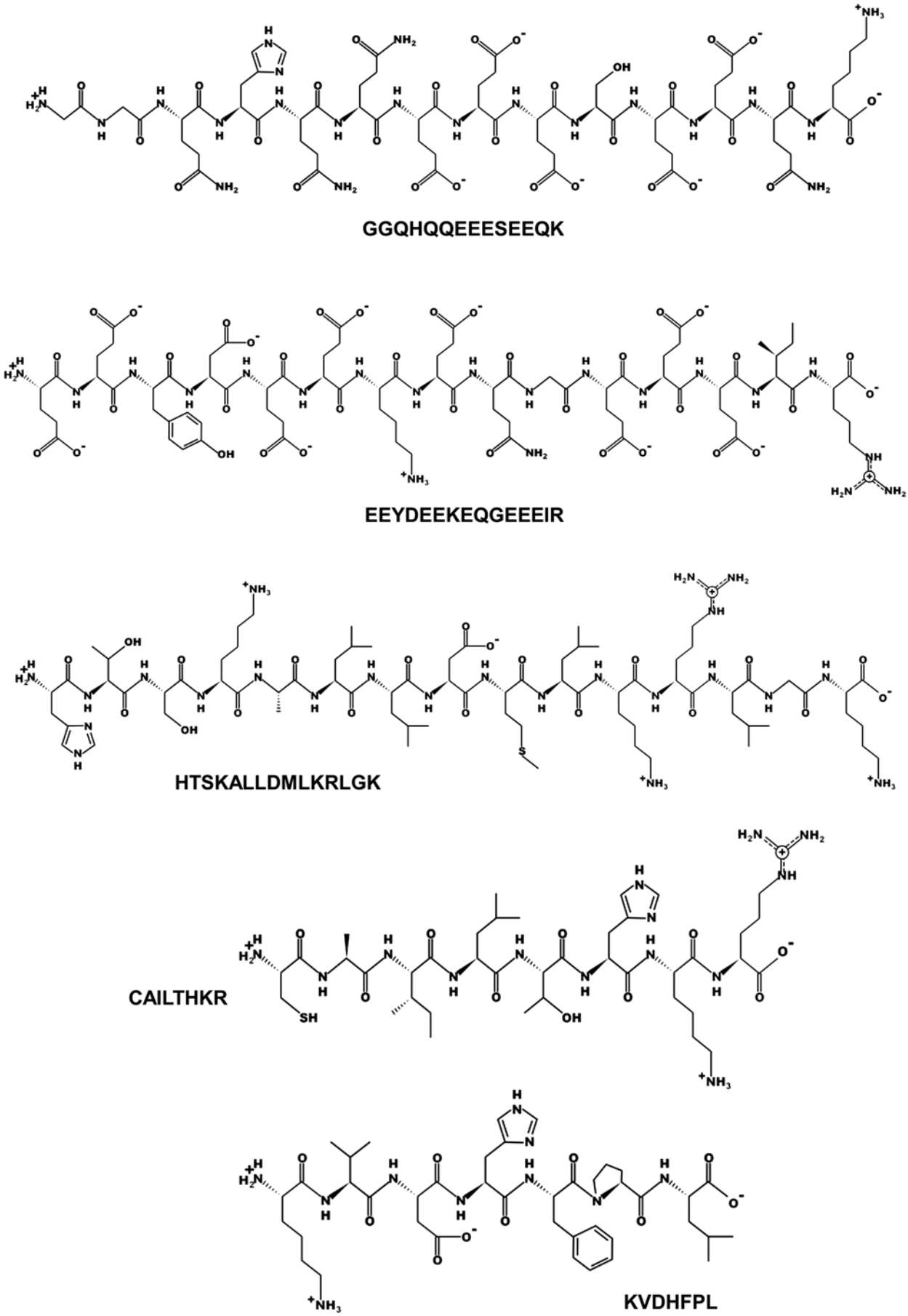

Figure 1. Selected examples of antibacterial peptides identified from *Vicia faba* seeds (GGQHQQEEESEEQK; EEYDEEKEQGEEEIR) (Karkouch et al. 2017), fermented soybean meal (HTSKALLDMLKRLGK) (Cheng et al. 2017), *Jatropha curcas* meal (CAILTHKR) (Xiao and Zhang 2012), and rice brans (KVDHFPL) (Pu and Tang 2017). The primary structures were drawn by using the Pepdraw.com server.

membranes. The interaction of peptides with target cell membranes begins with forming a beta-sheet-like structure on the cationic N-terminal site of the peptide and then bind to the cell surface. The hydrophobic C-terminal site of the peptide will then penetrate into the hydrophobic core of the cell membranes and bind to the mannose phosphotransferase permease (Nissen-Meyer et al. 2009). Binding of the peptides on bacterial membrane causes an electrostatic disruption to the cells, thus interfering with their normal physiological activities, such as biofilm formation and regulation of cell division. In addition, the cationic peptide can alter the membrane permeability through inducing dissipation of the electrochemical proton gradient, thereby initiating conformational alterations of protein, causing membrane depolarization and leading to cell lysis (Brogden 2005; Fitzgerald and Murray 2006; Lohner and Blondelle 2005).

Furthermore, several models were proposed to illustrate phospholipid membrane permeation by membrane-active peptides, which include the "barrel-stave pore", "carpet mechanism", "toroidal pore" and "disordered toroidal pore" models (Melo et al. 2009; Shai and Oren 2001). Antibacterial peptides, for example, a helix-helix structured two-peptide bacteriocin (Nissen-Meyer et al. 2009), can penetrate into the bacterium through the cell membrane to interfere with intracellular functions, such as synthesis of macromolecules, cytoplasmic membrane septum formation, cell-wall synthesis and/or enzymatic activity, thus leading to bactericidal action (Brogden 2005). Complex mechanisms in modulating host immunity, including recruiting or activating immunocytes, neutralizing bacterial products to suppress inflammation, and enhancing nucleic acid recognition to promote auto-inflammation, were demonstrated by Zhang and Gallo (2016). In light of their antibacterial activities, it has been suggested that bacteriocins may exert probiotic function in different ways within the gastrointestinal tract to promote health effects: (1) They may act as colonizing peptides to facilitate the competition of probiotics with the niched pathogens. (2) They may also act as killing peptides to eliminate pathogenic strains. (3) They may act as signaling peptides to other cells or the host immune system (Dobson et al. 2012). Overall, the killing properties of antibacterial peptides are mediated by not only bacterial membrane disintegration but also disruption of the function(s) of intracellular biopolymers (Otvos 2005). On the other hand, pathogenic bacteria can develop immune responses by reducing the net negative surface charge of their outer membrane, mediated by dephosphorylation of lipopolysaccharides. This may reduce their interaction with the cationic bacteriocins (Bechinger and Gorr 2017). Some pathogenic strains could synthesize immunity proteins that protect themselves from being eliminated by forming complexes with bacteriocins (Nissen-Meyer et al. 2009).

Antibacterial peptide production and purification

In plant bioactive peptide research, production of peptides by proteolysis of precursor proteins is a common strategy. The strategy may involve applying one or more proteases either simultaneously or sequentially to a protein sample. Enzymatic hydrolysis is preferred over microbial fermentation as the former is less time-consuming and can be more easily controlled. When the hydrolysis conditions are optimized, reproducibility in the chemical and functional properties of the peptide product can be expected (Daliri et al. 2017; Piovesana et al. 2018).

Different proteases exhibit dissimilar substrate specificities. Thus, when applied to a plant protein sample, different proteases can generate peptide fragments with different types and/or levels of bioactivity. In the initial stage of an investigation, it is thus necessary to screen a series of proteases to identify one that leads to optimum production of antibacterial peptides. An example of such a necessity is the study of Pu and Tang (2017) who compared the antibacterial activity of seven rice bran protein hydrolysates generated using papain, bromelain, pepsin, trypsin, neutrase, alcalase, and flavourzyme. Interestingly, hydrolysates generated using papain, trypsin, and neutrase enhanced the growth of *Listeria monocytogenes* cells, which the authors proposed to be due to the hydrolysates providing nutrition for bacterial growth. Thus the three hydrolysates were excluded when selecting suitable hydrolysates for subsequent search for antibacterial peptides (Pu and Tang 2017). On the other hand, during different time-points of proteolysis, peptides released from precursor proteins would likely vary qualitatively and quantitatively. Moreover, whether peptides released into a complex mixture would interact among themselves over time and the exact nature of the interactions is not easily predictable. Thus, it is not surprising that among the seven hydrolysates generated in the study of Pu and Tang (2017), there were no consistently increasing or decreasing trends in antibacterial activity across the 0.5–4 hours' hydrolysis period. In other words, whereas the optimum pH and temperatures for a protease often fall within narrow, well-established ranges, hydrolysis duration required for generating an active antibacterial hydrolysate often requires optimization. On this note, the need to screen for optimal protease type and hydrolysis duration is not unique to the generation of potent antibacterial hydrolysates, but also relevant to the search for other bioactive protein hydrolysates (Admassu et al. 2018; Chai et al. 2015; Quah et al. 2018).

Production of bioactive peptides by microbial fermentation generally involves culturing bacteria, yeasts, or filamentous fungi on protein substrates, which allows secreted microbial proteases to digest the precursor plant proteins into peptides. Peptide production by fermentation is not as reproducible as that accomplished via enzymatic hydrolysis. This can be attributed to the use of live microbial cells, which may not be easily manipulated to have reproducible or consistent levels of protease production or capacity. Nevertheless, microbial fermentation is considered a less costly strategy compared with enzymatic hydrolysis (Daliri et al. 2017).

In researches which aimed to identify antibacterial peptides from plant protein hydrolysates, a combination of chromatographic and non-chromatographic techniques are

frequently employed to purify and isolate the targeted peptides. Following sequence identification by either Edman degradation or mass spectrometry (MS), it is often appropriate to chemically synthesize the identified peptide sequences and validate their bioactivity as well as conducting additional characterizations. Techniques involved in the aforementioned workflow is often similar to those adopted for the purification and identification of other bioactive peptides from plant and non-plant protein hydrolysates. Thus, we refer the reader to recent comprehensive reviews which discuss these techniques (Chai et al. 2017; Lemes et al. 2016; Piovesana et al. 2018). For a systematic summary of the basic principles, advantages and limitations of such techniques, we refer the reader to de Castro and Sato (2015).

An interesting technique developed by Xiao and Zhang (2012) is *Escherichia coli* cell membrane affinity extraction coupled to offline liquid chromatography time-of-flight mass spectrometry (MS), which they used for identifying antibacterial peptides from jatropha meal proteins. In short, the study screened for antibacterial peptides in a protein hydrolysate based on their ability to bind to *Escherichia coli* cell membranes that were immobilized onto the activated silica resins. Then by comparing the high-performance liquid chromatography (HPLC) fingerprint of the cell membrane affinity extraction effluent and that of the hydrolysate, peaks corresponding to resin-bound peptides were identified. Among these peaks, the one exhibiting the strongest antibacterial activity was taken to peptide sequence determination by tandem MS analysis (Xiao and Zhang 2012). Antibacterial hydrolysate and peptides are often reported to be more potent against Gram-positive bacteria, with weaker or no effects against Gram-negative bacteria (Pei et al. 2018; Tan et al. 2011; Xiao et al. 2011). Notably, the study of Xiao and Zhang (2012) showed that by using a suitable technology, it is possible to obtain an antibacterial peptide that exhibits greater potency against *Escherichia coli*, a Gram-negative bacteria, than against some Gram-positive bacteria.

Antibacterial peptides from seed proteins

Eight defense polypeptides were isolated from latent seeds of barnyard grass (*Echinochloa crusgalli* L.) (Rogozhin et al. 2012) (Table 1). Among these, EcLTP, a nonspecific 9-kDa lipid-transfer protein (N-terminal sequence consisting of 30 residues: ^{1}AISCGQVSSAIGPCLSYARGQGSAPSAGCC^{30}; molecular mass 9148.2 Da), inhibited the colony growth of Gram-positive bacteria *Pseudomonas syringae*. The other defense polypeptides did not exhibit antibacterial activity (Rogozhin et al. 2012). A hairpin-like antibacterial peptide named EcAMP3 was also isolated from barnyard grass seeds (Ryazantsev et al. 2014). EcAMP3 can inhibit *Pseudomonas syringae*, *Erwinia carotovora* and *Clavibacter michiganensis*. It is believed that the special feature of this hairpin-like peptide provides some hydrophobic interactions with fungal membrane besides exhibiting antibacterial effect on certain bacteria (Ryazantsev et al. 2014).

According to Yokoyama et al. (2008), peptides Cy-AMP1, Cy-AMP2, and Cy-AMP3 from the Cycad (*Cycas revoluta*) seeds showed inhibitory effect against Gram-negative and Gram-positive bacteria. Cy-AMP1 and Cy-AMP2 contain eight cysteine residues, two continuous sequences of cysteine, and a chitin-binding domain. The study suggested that Cy-AMP1 and Cy-AMP2 belong to a new hevein type and/or knottin type of defensin. The unique structures of Cy-AMP1 and Cy-AMP2 may contribute towards the understanding of the structure-function relationships in the antibacterial peptide of plants.

Tavares et al. (2012) reported that the glycine-rich recombinant Pg-AMP1 peptide from guava seeds exerted inhibitory activity against human pathogenic Gram-negative and Gram-positive bacteria. Specifically, the recombinant peptide showed bacteriostatic activity against *Staphylococcus aureus*, *Staphylococcus epidermides*, *Klebsiella pneumoniae* and *Pseudomonas aeruginosa*. Bactericidal activity was detected against *Escherichia coli*. Results of hemolysis assay suggest that the recombinant peptide had a greater affinity towards prokaryotic or bacterial membranes and less towards eukaryotic or human cell membranes. Hence, the recombinant Pg-AMP1 represents a potential biotechnological tool for control of infectious diseases in humans. The native Pg-AMP1 from guava seeds is not abundant enough to provide for biopharmaceutical production. Moreover, plant peptide expression is likely influenced by environment factors. This also justifies the use of the recombinant Pg-AMP1 peptide as a biotechnological alternative (Tavares et al. 2012).

Bioactive peptides released by enzymatic hydrolysis of dietary proteins are gaining interest among researchers. Recently, four peptides exhibiting antibiofilm activity against *Pseudomonas aeruginosa* was identified from the tryptic hydrolysate of *Vicia faba* seed proteins (Karkouch et al. 2017). Two peptides LSPGDVLVIPAGYPVAIK and EEYDEEKEQGEEEIR were found to inhibit biofilm formation without compromising the growth of planktonic bacteria; this suggests that the observed antibiofilm activity was independent of antibacterial activity. Furthermore, the antibiofilm activity of the two peptides may be attributable to the presence of hydrophobic and basic amino acid residues within the sequences. Among the four antibiofilm peptides identified in the study, GGQHQQEEESEEQK, which lacks hydrophobic residues, were shown to have the weakest activity. It was proposed that the peptide likely suppress biofilm formation by compromising the expression of biofilm-related genes (Karkouch et al. 2017).

A napin-like polypeptide isolated from the seeds of dwarf Chinese white cabbage (*Brassica chinensis* cv dwarf) was found to exhibit antibacterial activity against *Pseudomonas aeruginosa*, *Bacillus subtilis*, *Bacillus cereus*, and *Bacillus megaterium*. The polypeptide comprises a 4 kDa and a 7 kDa subunit, thus resembling *Brassica napus* napin in having two structurally dissimilar subunits (Ngai and Ng 2004).

Antibacterial peptides from fermented plant foods

Antibacterial peptides in fermented plant products vary from $<$5 kDa to $>$30 kDa (Klaenhammer 1993; Rai and Jeyaram 2017). Generally, they can be categorized into two major

Table 1. Examples of antibacterial peptide investigations on seed proteins.

Plant sample/source	Antibacterial peptide/ hydrolysate production	Peptide purification	Peptide identification	Peptide sequence identified	Activity	Reference
Seeds of barnyard grass (*Echinochloa crusgalli* L.)	Endogenous peptides extracted by using acetic acid, followed by acetone precipitation.	RP-HPLC	MALDI-TOF MS, Edman degradation	[1]AISCGQVSSAIGPCLSYARGQ-GSAPSAGCC[30]	Inhibitory against *Pseudomonas syringae* at IC_{50} 5 μM	Rogozhin et al. 2012
Seeds of barnyard grass (*Echinochloa crusgalli* L.)	Endogenous peptides extracted by using acetic acid, followed by acetone precipitation.	AF, SEC, RP-HPLC	Edman degradation	EcAMP3 (GADRCRERCERRHRGDWQ-GKQRCLMECRRREQEED)	Inhibitory against *Pseudomonas syringae*, *Erwinia carotovora* and *Clavibacter michiganensis* (MIC 2.1 – 9.8 μM)	Ryazantsev et al. 2014
Seeds of cycad (*Cycas revoluta*)	Endogenous peptides extracted by using sodium acetate buffer.	CM cellulofine column, HPIEC, RP-HPLC	MALDI-TOF MS, Edman degradation	Cy-AMP1 (44 amino acids), Cy-AMP2 (44 amino acids), Cy-AMP3 (90 amino acids)	Inhibitory against Gram-positive (*Clavibacterium michiganensis* and *Curtobacterium flaccumfaciens*) and Gram-negative (*Agrobacterium radiobacter*, *Agrobacterium rhizogenes*, and *Erwinia carobora*) bacteria (IC_{50} 6 – 260 μg/mL)	Yokoyama et al. 2008
Seeds of guava (*Psidium guajava*)	Antibacterial peptide Pg-AMP1 identified from guava seeds was expressed in *Escherichia coli*.	IMAC	No	No	Inhibitory against Gram-negative (*Escherichia coli*, *Klebsiella pneumonia* and *Pseudomonas aeruginosa*) and Gram-positive (*Staphylococcus aureus* and *Staphylococcus epidermides*) bacteria (MIC 50 – 100 μg/mL)	Tavares et al. 2012
Vicia faba seeds	Hydrolysis of proteins by using trypsin	IEC	MS/MS, de novo peptide sequencing	GGQHQQEEESEEQK, LSPGDVLVIPAGYPVAIK, VESEAGLTETWNPNHPELR, and EEYDEEKEQGEEEIR	Antibiofilm activity against *Pseudomonas aeruginosa* with $MBIC_{50}$ value ranged from 12 to 35 μM	Karkouch et al. 2017
Seeds of dwarf Chinese white cabbage	Endogenous peptides extracted by using Tris-HCl buffer.	IEC, AF, FPLC-IEC, FPLC-GF	Edman degradation	Heterodimeric 11 kDa napin-like polypeptide	Inhibitory activity against *Pseudomonas aeruginosa*, *Bacillus subtilis*, *Bacillus cereus*, and *Bacillus megaterium* (IC_{50} 66-236 μM)	Ngai and Ng 2004

AF, affinity chromatography; FPLC, fast protein liquid chromatography; GF, gel filtration; HPIEC, high performance ion-exchange chromatography; IEC, ion exchange chromatography; IMAC, immobilized metal affinity chromatography; MALDI, matrix-assisted laser desorption/ionization; MIC, minimal inhibitory concentration; $MBIC_{50}$, minimal biofilm inhibitory concentration leading to 50% decrease in adherent cells; MS/MS, tandem mass spectrometry; RP-HPLC, reversed-phase high-performance liquid chromatography; SEC, size exclusion chromatography.

groups: (1) bacteriocins that are ribosomally synthesized by the starter cultures, and (2) other peptides that are produced by bacterial hydrolysis of plant proteins during fermentation process (Gibbs et al. 2004; Hartmann and Meisel 2007; Khan et al. 2010; Peng et al. 2003; Wang et al. 2009).

Bacteriocins are abundant, diverse, low molecular weight, heat-stable antibacterial peptides synthesized by bacteria during fermentations to target other bacteria, whereas the producer has a specific immunity mechanism (Hegarty et al. 2016). The types of bacteriocins are diversified with respect to their size and structure, inhibitory mechanisms and spectrums, as well as their target receptors (Gillor et al. 2008). Bacteriocins can be classified into four classes: Class I bacteriocins, or lantibiotics, are heat-stable peptides (<5 kDa) and containing lanthionine and methyllanthionine; Class II bacteriocins are also heat-stable peptides (<10 kDa) synthesized by Gram-positive bacteria but do not contain lanthionine. They are subdivided into four classes: (i) class IIa, anti-*listerial* one-peptide, (ii) class IIb, two different peptides which both are important for antibacterial activity, (iii) class IIc, cyclic peptides, and (iv) class IId, linear non-pediocin-like one-peptide; Class III heat-labile proteins (>30 kDa); Class IV complex peptides conjugated with lipid or carbohydrate moieties (Lee and Kim 2011; Nissen-Meyer et al. 2009). Tremendous attention has been paid to Classes I and II bacteriocins by many researchers mostly due to their heat-stable property which is an essential characteristic for a bio-preservative ingredient (Deegan et al. 2006). An example of well-established bio-preservative peptide is nisin (E234 preservative, 34 amino acids), a 3.5 kDa bacteriocin consisting of uncommon amino acids lanthionine, methyllanthionine, didehydroalanine, and didehydroaminobutyric acid. Nisin is synthesized by *Lactococcus lactis* strains in milk fermentation, such as yogurt (Deegan et al. 2006). Other than nisin, many other bacteriocins were also successfully isolated from plant-based fermented foods, such as Kimchi, Chinese fermented vegetables and fermented soybean food (Table 2).

Many species of lactic acid bacteria in fermented vegetable products produce high levels of bacteriocins (Hu et al. 2013). Some bacteriocins exhibit significant antibacterial activity against pathogenic strains that have similar characteristics with the producer. Subtilosin A produced by *Bacillus subtilis* SC-8 isolated from Korean fermented soybean food, for example, demonstrated antibacterial activity against the *Bacillus cereus* group (Yeo et al. 2012). By contrast, some bacteriocins have a broad spectrum of activity. For example, plantaricin 163 produced by *Lactobacillus plantarum* 163 in Chinese fermented vegetables inhibited the growth of lactic acid bacteria, as well as Gram-positive and Gram-negative bacteria (Hu et al. 2013). Sakacin LSJ618, a bacteriocin produced by *Lactobacillus sakei* LSJ618 isolated from traditional Chinese fermented radish, exhibited antibacterial effects on Gram-positive and Gram-negative bacteria tested, but not against most of the lactic acid bacteria (Jiang et al. 2012) (Table 2).

Apart from bacteriocins, other antibacterial peptides produced through bacterial proteolysis of plant proteins have also been isolated and identified from fermented plant foods. A recent study on a Japanese fermented soybean, Natto, reported a novel antibacterial peptide, with 45 amino acid residues and rich in α-helix, which inhibited the growth of *Streptococcus pneumoniae* and *Bacillus subtilis* group strains (Kitagawa et al. 2017). Another similar study on antibacterial activity of the extract of an Indonesian fermented soybean, Tempe, demonstrated the adhesion interference on *Escherichia coli* K88 and growth inhibition on some Gram-positive bacteria (Kiers et al. 2002). Besides, it has been reported that fermentation of soybean meal using *Bacillus subtilis* E20 produced antibacterial peptides with potent antibacterial activity against *Vibrio alginolyticus* and *Vibrio parahaemolyticus* (Cheng et al. 2017). Other than soybean, some low-molecular-weight peptide fractions in flaxseed protein hydrolysate, which was produced by *Bacillus altitudinis* HK02, have also been reported to exhibit growth inhibition on *Pseudomonas aeruginosa* and *Escherichia coli* (Hwang et al. 2016).

Antibacterial peptides from agricultural by-products

Palm kernel cake, jatropha seed meal, rice brans and tomato seed meal are examples of agricultural by-products enriched in protein contents, which make them suitable raw materials for antibacterial peptide production (Table 3). Palm kernel cake (PKC), also known as palm kernel expeller, is a residue resulting from palm oil extraction. PKC is commonly used as livestock feeds (Alimon 2004). The work of Tan et al. (2011) showed that alcalase hydrolysis of PKE proteins enhanced the antibacterial activity of the resulting hydrolysates. The study screened the antibacterial activity of a series of alcalase hydrolysates with 50–100% degree of hydrolysis (DH). Alcalase hydrolysate with 70% DH exhibited the strongest antibacterial activity on spore-forming and non-spore-forming Gram-positive bacteria. By contrast, the alcalase hydrolysates exhibited limited or no antibacterial activity against Gram-negative bacteria (*Escherichia coli*, *Pseudomonas aeruginosa* and *Salmonella typhimurium*)(Tan et al. 2011). The lack of correlation between antibacterial activity and DH in the study (Tan et al. 2011) implies that at least for alcalase-mediated hydrolysate production, DH is not an efficient predictor of antibacterial potency. Nevertheless, the ability of the crude hydrolysate to target multiple spore-forming and non-spore-forming bacteria underscores its potential as cost-effective and versatile antibacterial ingredients for food preservation and pharmaceutical development (Tan et al. 2011).

A subsequent comparison between the 70% DH PKC hydrolysate and that of a gel filtration chromatography fraction purified from the hydrolysate revealed that the former was a stronger antibacterial agent (Tan et al. 2013a). The authors suggested that certain antibacterial components may have been lost during the chromatographic purification step (Tan et al. 2013a). Still, the possibility of synergism among peptides and non-peptides in the crude hydrolysate cannot be ruled out. Tan et al. (2013a) reported that the gel filtration chromatography fraction derived from the PKC hydrolysate was a mixture containing peptides, with high lauric acid derivatives. However, the sequences of the peptides were not identified. Interestingly, the aforementioned

Table 2. Examples of antibacterial peptide investigations on fermented plant foods

Plant-based fermented product	Antibacterial peptide/hydrolysate production	Peptide purification	Peptide identification	Peptide sequence identified	Activity	Reference
Kimchi	*Lactococcus lactis* BH5 produced Lacticin BH5 (3 to 3.5 kDa)	Salt precipitation, SDS-PAGE	No	No	Bactericidal activity against *Micrococcus flavus* ATCC 10240; broad spectrum of bactericidal activity against non-pathogenic and pathogenic microorganisms, including *Bacillus cereus* ATCC 11778, *Micrococcus flavus* ATCC 10240, and *Pseudomonas fluorescens*.	(Hur et al., 2000)
Kimchi	*Leuconostoc citreum* GJ7 produced kimchicin GJ7 (3.5 kDa)	DEAE-Sephacel column, tricine-SDS-PAGE	No	No	Antagonistic activity against a broad spectrum of micro-organisms, including *Lactobacillus acidophillus* KFRI 150, *Staphylococcus aureus* subsp. *aureus* ATCC 29213 and *Salmonella typhi* ATCC 19430.	(Chang et al., 2007)
Kimchi	*Pediococcus pentosaceus* K23-2 produced pediocin K23-2 (5 kDa)	Tricine-SDS-PAGE	No	No	Inhibitory to growth of both Gram-positive and Gram-negative bacteria, including *Enterococcus faecalis* KCTC 2011, *Listeria monocytogenes* KCTC 3569, KCTC 3710, and *Pediococcus dextrinicus* KCTC 3506.	(Shin et al., 2008)
Chinese fermented radish	*Lactobacillus sakei* LSJ618 produced sakacin LSJ618 (5.2 kDa)	Tricine-SDS-PAGE	No	No	Inhibitory to growth of food-spoiling bacteria and food-borne pathogens, including Gram-positive *Listeria monocytogenes*, *Staphylococcus aureus*, *Sarcina sp.*, *Micrococcus luteus*, and Gram-negative *Proteus sp.*, *Escherichia coli*, not against most of the lactic acid bacteria	(Jiang et al., 2012)
Chinese fermented vegetables	*Lactobacillus plantarum* 163 produced plantaricin 163 (3.55 kDa)	Salt precipitation, SEC, and RP-HPLC	MALDI-TOF-MS	VFHAYSARGNYY-GNCPANWPSC-RNNYKSAGGK	Inhibitory to growth of LAB and other tested Gram-positive and Gram-negative bacteria	(Hu et al., 2013)
Sichuan Pickle	*Pediococcus pentosaceus* 05-10 produced pediocin 05-10 (below 6.5 kDa)	Tricine-SDS-PAGE	No	No	Inhibitory to growth of *Listeria*, *Lactobacillus*, *Streptococcus*, *Enterococcus*, *Pediococcus* and *Leuconostoc*; Reduced the counts of *L. monocytogenes* 54002 in pork ham during storage at 4 °C for 10 days	(Huang et al., 2009)
Chinese fermented cabbage	*Lactobacillus sake* C2 produced sakacin C2 (5.5 kDa)	Tris-Tricine SDS–PAGE	No	No	Strongly inhibited the growth of *Staphylococcus aureus* ATCC 63589 and *Escherichia coli* ATCC 25922	(Gao et al., 2010)
Korean fermented soybean food	*Bacillus subtilis* SC-8 produced subtilosin A (BSSC_04620) (44 amino acids)	No	No	No	Inhibitory to growth of *B. cereus* group species, such as *B. cereus*, *B. anthracis*, *B. mycoides*, *B. pseudomycoides*, *B. thuringiensis*, and *B. weihenstephanensis*	(Yeo et al., 2012)
Fermented soybean meal	*Bacillus subtilis* E20 produced antibacterial peptide (5 kDa)	Ultrafiltration, SEC, and RP-HPLC	RP-nano-UPLC ESI-MS/MS	HTSKALLDMLKRLGK	Inhibitory to growth of *Vibrio alginolyticus* and *V. parahaemolyticus*	(Cheng et al., 2017)
Flaxseed protein	*Bacillus altitudinis* HK02 hydrolysed peptide (<1kDa)	Ultrafiltration	No	No	Inhibitory to growth of *Escherichia coli* BCRC 11634 and *Pseudomonas aeruginosa* BCRC 10944.	(Hwang et al., 2016)
Soybean tempe	*Rhizopus* spp. fermented extract	Centrifugation, filtration	No	No	Antagonistic effect against *Escherichia coli* K88 through inhibition of adhesion.	(Kiers et al., 2002)
Natto	*Bacillus subtilis* fermented peptide (45 amino acids)	Salt precipitation, Butyl-Sepharose High Performance column, Tris-Tricine SDS–PAGE, Chromatography on Wakosil-5C18HG column	Protein sequencer PPSQ-31B/33B	SMATPHVAGAAALILS-KHPTWTNAQVRD-RLESTATYLGNSFYYGK	Inhibitory to growth and causing lysis of *Streptococcus pneumonia* and *Bacillus subtilis* group (*B. subtitlis*, *B. pumilus*, *B. licheniformis*).	(Kitagawa et al., 2017)

SDS-PAGE, sodium dodecyl sulphate-polyacrylamide gel electrophoresis; LAB, lactic acid bacteria; RP-HPLC, reversed-phase high performance liquid chromatography; ultrapure liquid chromatography; ESI, electrospray ionisation; MS/MS, tandem mass spectrometry; MALDI-TOF-MS, matrix-assisted laser desorption/ionisation time-of-flight mass spectrometry; SEC, size-exclusion chromatography.

Table 3. Examples of antibacterial peptide investigations on agricultural by-products.

Plant sample	Antibacterial peptide/ hydrolysate production	Peptide purification	Peptide identification	Peptide sequence identified	Activity	Reference
Palm kernel cake	Hydrolysis of proteins by using alcalase	No	No	No	Inhibitory against Gram-positive spore-forming bacteria (*Bacillus cereus*, *B. coagulans*, *B. megaterium*, *B. pumilus*, *B. stearothermophillus*, *B. subtilis*, *B. thuringiensis*, and *Clostridium perfringens*) and non-spore forming (*Lisinibacillus sphaericus* and *Listeria monocytogenes*)	Tan et al. 2011
Palm kernel cake	Hydrolysis of proteins by using alcalase	SEC	No	No	Crude hydrolysate and the 6th SEC fraction inhibitory against *Bacillus cereus*, *Bacillus subtilis*, *Bacillus thuringiensis*, *Lisinibacillus sphaericus*, *Clostridium perfringens*	Tan et al. 2013a
Palm kernel cake	Hydrolysis of proteins by using trypsin	SEC	No	No	SEC fraction was bacteriostatic, disrupting membrane integrity and interferes with RNA, DNA and also protein synthesis of *Bacillus cereus*.	Tan et al. 2013a
Palm kernel cake	Hydrolysis of proteins by using alcalase (PAH) and trypsin (PTH)	SEC	No	No	SEC fraction of PAH slightly more inhibitory than that of PTH against *Bacillus cereus*. Both bacteriostatic; not bactericidal. Both disrupting membrane integrity and inhibiting RNA synthesis and to a less extent, protein and DNA synthesis.	Tan et al. 2013b
Jatropha curcas meal	Hydrolysis of protein isolates by using protamex	*Escherichia coli* cell membrane affinity chromatography	MALDI-TOF MS	CAILTHKR	Inhibitory against Gram-negative bacteria (*Escherichia coli*, *Shigella dysenteriae*, *Pseudomonas aeruginosa*) and Gram-positive bacteria (*Staphylococcus aureus*, *Bacillus subtilis*, *Streptococcus pneumoniae*)	Xiao and Zhang 2012
Jatropha curcas meal	Endogenous peptides extracted by using phosphate-buffered saline.	Rat cell membrane affinity chromatography	MALDI-TOF MS	KVFLGLK	Inhibitory against Gram-negative bacteria (*Salmonella typhimurium*, *Shigella dysenteriae*, *Pseudomonas aeruginosa*) and Gram-positive bacteria (*Staphylococcus aureus*, *Bacillus subtilis*, *Streptococcus pneumoniae*)	Xiao et al., 2011
Rice brans	Hydrolysis of rice bran proteins by using bromelain	SEC, RP-HPLC	MALDI Q-TOF MS	KVDHFPL	Free and liposome-encapsulated peptide were bactericidal to *Listeria monocytogenes* planktonic and sessile (biofilm) cells	Pu and Tang 2017
Rice brans	Hydrolysis of rice bran proteins by using pepsin	RP-HPLC	MALDI-TOF MS	EKLLGKQDKGVIIRA and SSFSKGVQRAAF	Inhibitory against *Porphyromonas gingivalis* (IC_{50} 75.6 – 78.5 μM)	Taniguchi et al. 2017
Tomato seed meal	Submerged fermentation system by *Bacillus subtilis* A14h	No	No	No	Inhibitory activity against *Bacillus cereus* and *Escherichia coli*	Moayedi et al. 2016

SEC, size exclusion chromatography; LC, liquid chromatography; RP-HPLC, reversed-phase high-performance liquid chromatography; MALDI, matrix-assisted laser desorption/ionization; Q-TOF, quadrupole time-of-flight; MS, mass spectrometry.

fraction exhibited low hemolytic activity on human erythrocytes even when tested at 4-fold the MIC determined against *Bacillus cereus*. Moreover, the brighter coloration of the gel filtration chromatography fraction is advantageous as it would not influence the color of the final product upon incorporation into food (Tan et al. 2013a).

Jatropha kernel meal is a by-product of biodiesel production from jatropha seeds. In the past two decades, there has been growing interest among researchers to explore jatropha kernel meal as a source of bioactive proteins and peptides for nutritional and pharmaceutical applications (Devappa et al. 2010). Xiao and Zhang (2012) found that with the exception of flavourzyme, jatropha protein hydrolysates prepared using seven other proteases inhibited the growth of *Escherichia coli* to different extents. By contrast, Marrufo-Estrada et al. (2013) reported that alcalase and pepsin-pancreatin hydrolysates of jatropha seed proteins showed no inhibition on seven Gram-negative and Gram-positive bacterial pathogens, including *Escherichia coli*. Whether the discrepancy between the two studies was due to different types of antibacterial assays used is unclear. In any case, the identification of antibacterial peptides from jatropha meal proteins (Xiao and Zhang 2012; Xiao et al. 2011) substantiates the potential of jatropha kernel meal as a source of antibacterial hydrolysates and peptides.

Xiao and Zhang (2012) employed the technique of *Escherichia coli* cell membrane affinity extraction for isolating antibacterial peptides from jatropha meal proteins. The investigation screened for antibacterial peptides based on their ability to bind to *Escherichia coli* cell membrane fragments immobilized onto silica resins. In the initial stage of the investigation, hydrolysis using pepsin, trypsin, protamex, neutrase, flavourzyme, papain, alcalase, and acid protease revealed no correlation between DH and antibacterial activity of the hydrolysates (Xiao and Zhang 2012). This suggests that besides the lack of correlation between DH and the antibacterial activity of alcalase hydrolysates (Tan et al. 2011), DH is apparently not an efficient predictor of antibacterial activity of hydrolysates generated using other proteases. Xiao and Zhang (2012) identified a cationic antibacterial peptide (CAILTHKR) from the protamex hydrolysate by tandem mass spectrometric analysis. Notably, unlike other antibacterial hydrolysates or peptides which target mainly Gram-positive bacteria, CAILTHKR was active against Gram-negative and Gram-positive bacteria. MIC values against the aforementioned bacteria ranged between 29 and 68 μg/mL (Xiao and Zhang 2012). In another study, Xiao et al. (2011) identified a cationic antibacterial peptide (KVFLGLK) from the endogenous peptidome of jatropha meal by using rat cell membrane affinity liquid chromatography. Similar to CAILTHKR (Xiao and Zhang 2012), KVFLGLK was active against Gram-negative bacteria and Gram-positive bacteria. MIC values against the bacteria ranged between 24 and 64 μg/mL (Xiao et al. 2011).

In general, the aforementioned results suggest that jatropha meal, *per se* or after protein hydrolysis, is a potential source of broad-spectrum antibacterial peptides. The studies also suggest that cell membrane affinity liquid chromatography is a promising technique for identifying peptides that target both Gram-negative and Gram-positive bacteria. Interestingly, CAILTHKR was the most inhibitory against the Gram-negative *Escherichia coli* (Xiao and Zhang 2012), whereas KVFLGLK was the most inhibitory against the Gram-positive *Bacillus subtilis* (Xiao et al. 2011). Such a difference may be in part because the techniques adopted in the two studies relied on the ability of peptides to bind to different cell membrane types. In any case, a more stringent comparison in this context is not possible because KVFLGLK was not tested against *Escherichia coli* (Xiao et al. 2011). Overall, cell membrane affinity chromatography would be a promising method for high-throughput screening of antibacterial peptides from not only jatropha meal but also likely other biosources (Xiao and Zhang 2012; Xiao et al. 2011).

Tomato pomace is the by-product remaining after the processing of tomatoes into food products. Tomato seeds, containing 22–34% proteins, are the main constituents of the pomace (Sogi et al. 2002). Moayedi et al. (2016) reported that using a submerged fermentation system mediated by *Bacillus subtilis* A14h, tomato seed meal proteins can be converted into antibacterial hydrolysates. In their study, different experimental parameters were evaluated and optimized by using Response Surface Methodology. Antibacterial activity of fermented tomato seed meal was associated with amino acids and peptides produced during fermentation. Nevertheless, the actual peptides responsible for the antibacterial effects were not identified. The authors found that the inhibition against *Bacillus cereus* was about 2-fold stronger than that against *Escherichia coli* (Moayedi et al. 2016), consistent with previous observation of greater sensitivity of Gram-positive bacteria towards antibacterial hydrolysate of palm kernel cake (Tan et al. 2011).

Rice bran is an abundant and low-cost agricultural by-product containing 10–15% protein contents (Fabian and Ju 2011). Pu and Tang (2017) identified an antibacterial peptide (KVDHFPL) from the bromelain hydrolysate of rice bran proteins. KVDHFPL exhibited bactericidal and antibiofilm effects against *Listeria monocytogenes* (Pu and Tang 2017). The pathogen is a common cause of food poisoning and foodborne infections (Buchanan et al. 2017; Gandhi and Chikindas 2007) and represents a risk for public health (Guilbaud et al. 2015). The prevalence of *Listeria monocytogenes* in food products and food processing environment is associated with its ability to form biofilms (Guilbaud et al. 2015). Pu and Tang (2017) found that both free and cationic liposome-encapsulated KVDHFPL were similarly bactericidal against planktonic listerial cells. However, encapsulated KVDHFPL showed a greater inhibition on the growth of sessile listerial cells (biofilm). Overall, the study highlights the potential application of liposome-encapsulated peptides for targeted delivery to pathogenic biofilm in the food industry (Pu and Tang 2017).

Two multifunctional cationic peptides that exhibited antibacterial, lipopolysaccharide-neutralizing and angiogenic activities were also identified from pepsin-hydrolyzed rice bran proteins (Taniguchi et al. 2017). The presence of lysine and arginine residues as well hydrophobic amino acids in

the peptides is believed to have contributed to their antibacterial effects, likely facilitating the binding of peptides to bacterial membranes and subsequently their insertion into and/or translocation across the bacterial membranes. The peptides exhibited low hemolytic activity, thus implying their low toxicity against mammalian cells (Taniguchi et al. 2017).

The identification of peptide sequences from plant food-derived antibacterial hydrolysates would contribute towards current knowledge of the structure–activity relationships of antibacterial peptides. Knowledge on structural determinants of antibacterial activity would benefit *in silico* prediction of potential antibacterial activity in new peptides. Moreover, the information can facilitate the development of strategies for enzyme-assisted production of antibacterial peptides from food and non-food proteins.

Among the aforementioned agro by-products, antibacterial peptides were identified from only jatropha meal (CAILTHKR and KVFLGLK)(Xiao and Zhang 2012; Xiao et al. 2011) and rice bran proteins (KVDHFPL, EKLLGKQDKGVIIRA and SSFSKGVQRAAF) (Pu and Tang 2017; Taniguchi et al. 2017). KVDHFPL is unusual as it has no net charges. The total net charges of CAILTHKR, KVFLGLK, EKLLGKQDKGVIIRA and SSFSKGVQRAAF are +3, +2, +2, and +2, respectively, consistent with the common observations of antibacterial peptides having net positive charges between +2 and +13 (Kumar et al. 2018). Net charges aside, the five aforementioned peptides do contain positive-charged amino acid residues (lysine (K), arginine (R), histidine (H)) often found in cationic antibacterial peptides (Kumar et al. 2018). On the other hand, the peptides also contain varying percentages of hydrophobic residues (43%, KVDHFPL; 50%, CAILTHKR; 57%, KVFLGLK; 40%, EKLLGKQDKGVIIRA; 33%, SSFSKGVQRAAF), which is typical of antibacterial peptides (Kumar et al. 2018). Thus, the discovery of these peptide sequences lends further support to current knowledge of typical molecular characteristics of antibacterial peptides.

Studies on the modes of action of antibacterial hydrolysates/peptides of agro by-products generally point to bacterial cell membrane disruption, and in some cases, a subsequent cell lysis. The modes of action of the gel filtration chromatography fractions derived from alcalase- and trypsin-hydrolyzed PKC were compared (Tan et al. 2012, 2013b). *Bacillus cereus*, a causal agent of food poisoning and serious opportunistic infections, was chosen as the bacterial model (Tran et al. 2011). Both alcalase hydrolysate (PAH) and tryptic hydrolysate (PTH) were found to be bacteriostatic, disrupt bacterial membrane integrity, and deplete intracellular ATP pools. Further indicating the ability of PAH and PTH to inhibit multiple cellular targets is their inhibitory effects on RNA synthesis, and to a less extent, protein and DNA synthesis (Tan et al. 2012, 2013b).

Mechanistic studies on peptides of agro by-products were less in-depth. Ultrastructural analysis of Gram-positive *Staphylococcus aureus* upon exposure to CAILTHKR and KVFLGLK revealed strong membrane disruption and cell lysis (Xiao and Zhang 2012; Xiao et al. 2011). The two peptides were shown to target both Gram-positive and Gram-negative bacteria, thus an interesting question to address in future is whether the same mode of action is applicable to Gram-negative bacteria. Similarly, confocal scanning laser microscopy revealed that the antibiofilm effects of liposome-encapsulated KVDHFPL, purified from rice bran proteins, was associated with the loss of bacterial membrane integrity in the biofilm. The effectiveness of the encapsulated KVDHFPL was attributed to spontaneous adsorption of peptide-loaded liposomes to the biofilm, followed by peptide release (Pu and Tang 2017). Nevertheless, the actual mechanism of how the released antibacterial peptide disrupts the bacterial membrane remains unclear.

Applications and future perspectives

Food preservation, farm animal disease management, and nutraceutical/function food development are three areas of applications pertinent to plant food-derived antibacterial peptides, where the food industry is concerned. Among antibacterial peptides, nisin stands out as a bacteriocin approved as food preservative and awarded the Generally Recognised as Safe (GRAS) status by the U.S. Food and Drug Administration (FDA) since 1988 (Gharsallaoui et al. 2016b). Currently, nisin is commercially produced and used in the preservation of many food products. The application of nisin and other antibacterial peptides as preservatives for various food products has been discussed in a few reviews (Barbosa et al. 2017; Gharsallaoui et al. 2016b; Rai et al. 2016). Notwithstanding the success of nisin, its bactericidal effect in foods is compromised by the emergence of resistance in Gram-positive bacteria, particularly those repeatedly exposed to elevated nisin concentrations. Nisin resistance has been reported in various Gram-positive bacteria, including *Listeria monocytogenes*, *Bacillus cereus*, *Staphylococcus aureus* and *Clostridium botulinum* (Zhou et al. 2014). Besides bacterial resistance, activity loss due to interaction with fats and inactivation by proteolytic enzymes are other problems that weaken the efficacy of nisin when incorporated into food matrices (Gharsallaoui et al. 2016a). Thus there is need for discovery of new antibacterial peptides, which can be used to replace or complement nisin where necessary. Future research should search for antibacterial peptides which can ideally avoid some limitations of nisin application. Recently, lupin seed protein hydrolysate incorporated into minced beef was shown to exert antibacterial and antioxidative activities comparable to that of nisin during refrigerated storage (Osman et al. 2016). More such investigations comparing the antibacterial or bio-preservative efficacy between nisin and promising peptide candidates as well as potential synergism between them particularly in food model systems are needed in future.

Conventional antibiotics are often used for disease prevention and growth promotion in poultry and livestock production. Concerns over harmful antibiotic residues in the food animal products and emergence of antibiotic-resistant bacteria, however, are increasingly driving current research to search for alternative antibacterial agents for

use in food-producing animals (Van Boeckel et al. 2015). Owing to their low resistance induction ability and broad-spectrum antibacterial effects, antibacterial peptides are considered promising candidates as alternative antibiotics for the livestock industry (Li et al. 2018). Unlike conventional antibiotics, antibacterial peptides are less specific in their mechanisms; they act on not only multiple bacterial targets (e.g., bacterial membrane, cell wall synthesis, protein and DNA synthesis, and enzymatic activities), but also the host immune system. Such a multi-target mode of action is a major strength of antibacterial peptides which decreases bacterial survival, thus dampening resistance development in bacteria (Wang et al. 2016b). Bao et al. (2009) reported that defensin isolated from pig small intestine, when added to drinking water or feed, enhanced the growth of broilers, in addition to increasing intestinal ability to absorb nutrients and improving the intestine mucosal immunity. Antibacterial peptides are thus potentially useful as animal feed additives, which are also promising alternatives to antibiotic growth promoters (Bao et al. 2009). The ability of antibacterial peptides to modulate gut flora in swine and poultry by enhancing proliferation of beneficial microbes (e.g., *Lactobacillus* and *Bifidobacterium*) and suppressing harmful microbes (e.g., *Clostridium* and *Salmonella*) has also been reported (Wang et al. 2016b). Future research should investigate whether antibacterial peptides or hydrolysates derived from plant-based agricultural by-products can exert such beneficial effects on food-producing animals. This may provide a potential solution to the overuse of conventional antibiotics and concurrently contribute to valorization of agricultural by-products. Furthermore, it is also less likely to exacerbate the perceived competition between animals and humans for feed and food, compared with using other plant-derived antibacterial peptides or hydrolysates (e.g., from human-edible seeds).

Plant-derived antibacterial peptides or hydrolysates may be used as nutraceuticals in the form of supplements or as some functional food ingredients to promote human health. A multifunctional antibacterial peptide or hydrolysate which elicit multiple health benefits would be more valuable and versatile than a single-function peptide or hydrolysate (Daliri et al. 2017). Hence, future research in this context should target peptide candidates which not only protect against bacterial infections, but also exerting additional bioactivities, such as antioxidant, wound-healing and/or immunomodulatory activities. Such multifunctionality would also be more appealing to the consumers. Another consideration in the application of antibacterial peptides or hydrolysates as nutraceuticals or functional food is that when consumed, the peptide or hydrolysate should not compromise the beneficial intestinal microflora in the human body. As demonstrated in the *in vitro* study of Świątecka et al. (2010) using pea protein hydrolysate, common bacteria inhabiting the human small intestine responded differently to the same hydrolysate depending on whether they were planktonic or immobilized (biofilm-forming). Furthermore, among the immobilized bacteria, survival of *Escherichia coli* was compromised, whereas that of *Enterococcus faecalis* and *Lactobacillus acidophilus* was not (Świątecka et al. 2010). Qualitative and quantitative changes in gut microbiota composition may lead to diseases. Meanwhile, diets can affect human health by modulating the gut microflora (Nie et al. 2018). Thus, where the final goal is to develop nutraceuticals or functional food from an antibacterial peptide or hydrolysate, its effects on the gut microbiota should be evaluated.

Concluding remarks

Much work has been done to search for plant-derived antibacterial peptides or hydrolysates from plant sources, including plant-based fermented foods and agricultural by-products. It is conceivable that in the near future, the number of antibacterial peptides discovered from plant sources will continue to grow. To date, discovery of antibacterial peptides/hydrolysates and characterizations of their *in vitro* potency have progressed at a much faster pace than detailed research on their molecular characteristics, structure-function relationship, and inhibitory mechanisms. Similarly, other aspects of plant-derived antibacterial peptides or hydrolysates which are relevant to their food-related applications, such as stability under food processing conditions, *in vivo* potency, allergenicity, cytotoxicity, bioavailability, as well as effects on sensory and taste attributes of food products, require more attention in future. Large-scale production of functional peptides without losing the antibacterial properties would be a technological challenge. At present, much work has been done on plant food-derived antibacterial hydrolysates or peptides on the laboratory scale. The problem of scaling up antibacterial hydrolysate or peptide production to cope with market demands needs to be addressed in future. Where possible, close collaboration between academia and the food industry should be established to promote the development of antibacterial hydrolysates/peptides which are acceptable to the food industry with regards to efficacy, stability during food processing, and cost of production. Such partnerships with the industry, with their knowledge of consumer demand, will increase future success of translating laboratory findings on plant-derived antibacterial peptides or hydrolysates into marketable products.

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