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Antimicrobial Peptides from the Plants

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Abstract: AMPs (antimicrobial peptides) are small, mostly basic peptides that range in size from 2-9 kDa, and they are an important component of the innate defense system of plants where they are effector molecules considered to be an important defense barrier to pathogens and pests. Nine families of antimicrobial peptides have been identified in plants, including thionins, defensins, lipid transfer proteins, hevein and knotting-like peptides, four cysteine-types, and the recently reported shepherdins, snakins and cyclotides. They are part of both permanent and inducible defense barriers of plants. Transgenic overexpression of the corresponding genes leads to enhanced tolerance to pathogens, and peptide-sensitive pathogen mutants have reduced virulence. In this review, the recent studies on peptides from plant sources, including peptides isolated from indigenous medicine and edible plants of Central-Asia, are briefly discussed with a focus on their origins, antioxidant, antitumor activities and the possible mechanisms of actions in order to provide a profile of important plant peptides.

Key words: AMPs, plant defense peptides, thionin, LTPs (lipid transfer proteins), Ib-AMPs (*Impatiens balsamina* antimicrobial peptides), disulfide bridge, pathogen.

1. Introduction

All living organism, ranging from microorganisms to plants and mammals, have evolved mechanisms to actively defend themselves against pathogen attack. The most sophisticated mechanisms deploy antibodies and killer cells to recognize and eliminate specific invaders, respectively. Peptides, a group of compounds consisting of two or more amino acids linked by peptide bond, are abundantly present in living organisms; they form an important and ancient mechanism of innate resistance providing rapid and metabolically inexpensive first line of defense against pathogens [1-3].

Thousands of peptides have been isolated from animals, plants and microorganisms with different biological activity, especially finding the new peptides classes of shepherdins [4], cyclotides [5] and snakins [6, 7], have make plant peptides antibiotics family more rich.

Pharmacological studies have proved that many peptides, including those isolated from plants, have a potential biological effect. These peptides have a number of advantages over other chemical agents including their low molecular weight, relatively simple structure, lower antigenicity and fewer adverse actions, easy absorption and a variety of routes of administration. Plants are a huge resource for organisms found in the earth. In recent years, one of the most active areas of research is the search for natural components of plants with potent biological activity and low toxicity. However, the study on the bioactivities of the peptides from plants, especially their antitumor, antioxidant and anti-hyperglycemia activities, has not been progressed as quickly as that of other compounds. Therefore, it is worthwhile emphasizing the importance and the potential future of

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research involving biologically active peptides from plant sources.

Antimicrobial peptides from animals may be linear or form complex globular structures in which antiparallel/3-sheets are stabilized by disulfide bonds, whereas in plants only disulfide-bonded peptides of the second type have been identified so far [8, 9]. Among plant antimicrobial peptides, thionins were the first whose activity against plant pathogens was demonstrated *in vitro* [8, 10]. Subsequently, several families of cysteine-rich peptides have been characterized, including defensins LTPs (lipid transfer proteins) [11, 12], hevein-type peptides [13], knottin-type peptides [14] and others. In this review, we summarize recent advances concerning the structural and functional properties of all these families of putative defense peptides from plants.

2. Classification and Structure of Plant Peptide Antibiotics

Plant peptides antibiotics all have common function of inhibiting or killing invading microorganisms, and their size range from approximately 10-90 amino acids. Despite that they are small low molecular weight proteins, they achieve antibiotics function using an extremely diverse range of structural and composition motifs. Structure diversity of antibiotics peptides are divided into five structure classes: those with α-helical structure, those with β-sheet structure, those with mixed helical/β-sheet structure, those with irregular structure, and those incorporating a macro cyclic structure. There are a significant diversity in both the size and charge of molecular within each of the classes and between the classes. The common feature of their three-dimensional structures is that they have a degree of amphipathic character in which there is separate localization of hydrophobic regions and positively charges regions [15].

According to the amino sequences and secondary-dimensional structure, there are nine classes of antimicrobial peptides (Table 1) which have been identified, among them eight classes belonging to cysteine-rich peptides with 4 to 12 cysteine residues involved in disulfide bridges and one class of cysteine free peptides that is rich of His/Gly or Gly. The origin, number, molecular weight and structure character of the plant peptides antibiotics are summarized in the Table 1.

There is no direct relationship between numbers of the disulfides bridge with classes of peptides, even they have difference in the number of disulfides bridges in the same classes, there are three (Ac-AMPS), four (hevein) and five (Ee-CBP) disulfides bridge in classes of heveins, but they have the same disulfide bonds linkage pattern in the same classes of peptides [47, 48] (Fig. 1), thionins are peptides with six or eight-cysteines, localized in protein bodies and/or cell walls of different species [16, 49], while defensins have eight- or 10-cysteines and are localized in vacuoles or cell wall [50]. In both cases the mature peptides have a half of total amino acids of the eight-cysteine motif peptides domain. Thionin tertiary structure as described for α1-purothionin [51] result in a hydrophobic underside with a structure of a Greek gamma letter, where the vertical stem is a pair of α-helices and the horizontal arm is a strand and short antiparallel β-sheet stabilized by four disulfide bridges (Fig. 1) in the core of peptides [28]. Defensins have adopted a different conformation [27], with a triple stranded antiparallel β-sheet, and a single α-helix lying in parallel with the β-sheet stabilized by four [52] or five bridges, such as in PhD1-2 defensin where the two additional cysteine form a new cysteine bond [29, 30]. The examples of linear cysteine-free (rich in histidine and glycine) antimicrobial peptides are from roots of *Capsella burse pastoris*—shepherins I and II [16] and glycine rich peptides from seeds of wheat [25]. It is interesting that glycine rich AMPs peptides are quite common in insect, but research results of Park [16] and Egorov [25] indicated that glycine rich AMPs peptides may be a constitutive element of defense in the plants as well.

Table 1 The origin and structure character of the plant peptides antibiotics.

Type	Number	Origin	Number of amino acids	Structural property
Shepherins [16]	2	*Capsella bursa-pastoris*	28~38	Rich of His, Gly (cysteine free) His, Gly, linear or rule less loop
Cyclotides [17-20]	45	*Caryophyllaceae* and *Annonaceae*	28~37	Three disulfide bridges, CCK (cyclic cystine knot) bonded with the β-sheet
Snakins [21, 22]	2	*Solanum tuberosum*	63~66	Six disulfide bounds
Thionins [23-25]	6	*Brusels sprouts* (cabbage) and oats, seeds of the Santalaceaes plants, leaf of barley, *Phoradendron tomentosun*, etc.	45~47	Three or four disulfide bridges, r-shaped molecular, the vertical, stem consists of a pair of anti-parallel α-helicas, and the horizontal arm consist of a pair anti-parallel β-sheet
Defensins [26-33]	80	Barley, wheat and maize, sorghum, oil spinach, beet, mustard, chile, turnip greens, radish, silkworm, *Clitoria ternatea* L., potato, Yunnan Bean, sunflower, *Aesculus hippocastanum*, etc.	45~54	Four disulfide bridges, three-dimensional structurewith triple-stranded, anti parallel β-sheet and a single α-helix lying in parallel with the β-sheet
Lipid transfer proteins [34-40]	10	Barley, wheat and maize, radish, onion, *Arabidopsis thaliana*, rubber, rapeseeds, radish seed, embryos of grape vine, etc.	90~93	Three or four disulfide bridges, four helices linked by three loops, a C-terminal tail without regular structure, the fold is stabilized by four disulfide bonds and helices enclosed. Internal hydrophobic cavity in which can be inserted by the aliphatic chain
Heveins [41-43]	9	Amaranth, *Pharbitis nil* (Linn.) *Choisy*, *Saintpaulia ionatha, Euonymus* L., etc.	29~44	Three, four or five disulfide bridges
Knotting [14, 25]	3	*Mirabilis jalapa*, pokeweed, seeds of wheat, etc.	35~37	Three disulfide bridges, short triple-stranded β-sheet stabilized by three disulfide bonds, forming a typical knotting, which binds various macromolecular ligands
Ib-AMPs [44-46]	2	Seed of *Impatients balsamina*, maize, jewelweed, seed of wheat, etc.	20~33	Two disulfide bridges

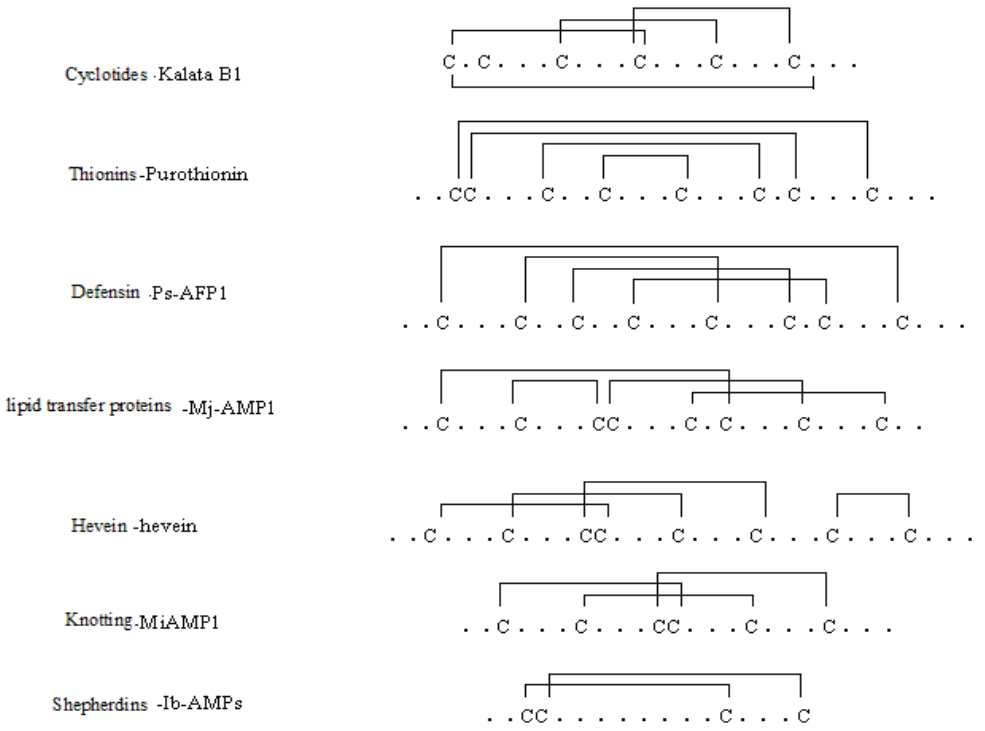

Fig. 1 Schematic representation of the disulfide bond patterns of different classes of peptides.
Cysteine positions are indicated by vertical lines on the schemes.

Shepherin I and shepherin II have 67.9% and 65.8 % (mol/mol) glycine, respectively, and 28.6% and 21.1% (mol/mol) histidine, respectively. Both shepherins have a Gly-Gly-His motif. Circular dichroism spectra of shepherin I and shepherin II showed that shepherin I and shepherin II in 50% trifluoroethanol have 66.7% and 75% random coils, respectively, without any alpha-helices [16].

3. Biological Character of the Plant Peptides Antibiotics

A lot of research results indicated that plant peptides antibiotics possess broad antimicrobial activity against Gram-positive and/or Gram negative bacteria, fungi, or enveloped viruses [53]. At present, most of the experiments for biological and function activities of plant peptides antibiotics were carried out *in vitro*, the inhibition growth of microbe and fungi are the most important biological character of the plant peptides antibiotics, but some of their new biological activities have been found recently. For example, cyclotides of Kalata B1 have activities of inhibition growth and development of larvae from the Lepidopteran species of *H. punctigera*. Circulins A and B have anti-HIV activity. Plant defensins, knotting and lipid transfer proteins have inhibition activity against α-amylase etc. [54]. Plant peptides as antibiotics were produced by plant species to prevent pathogen invasion of their tissue. Plant antibiotics has properties such as small (30-90 residues), positive charge, and a high portion of hydrophobic residues (about 30%) that allow them to fold into an amphiphilic structure with distinct pathches of hydrophobic and positive charged amino acids [55]. These structural features ensure effective interaction with plasma membranes of pathogenic microorganisms assumed to be their primary target. Cationic peptide is one of great interests in peptide antibotics group. They can kill bacteria quickly by the physical disruption of cell membrances because it has two distinguishing features [56]. Different amino acid sequences and adoption of diverse conformations were properties of plant AMPs which with a vast majority of them belong to cysteine-rich polypeptides and their structure are stabilized by intrachain disulfide bonds (2-5), providing the molecules with high structural stability [57]. Cationic peptides have a net positve charge of at least +2 (and usually 4, 5 or 6) by virtue of their possession of the aminoacides atginine and lysine. These amino acids are positively charged at neutral PH and are also folded in three dimensions so that they have both a hydrophobic face, comprising non-polar aminoacid side-chains, and a hydrophobic face of polar and positively charged residues, and these molecules are amphipathic.

As one of the unraveled mode of action of plant AMPs, the microbial plasma membrane is supposed to be the primary target for most of them. The cationic and amphiphilic nature of AMPs ensures their direct interation with anionic cell surfaces of microbial pathogens: lipopolysaccharides in Gram-negative and teichoic acids in Gram-positive bacteria. Subsequently, AMPs come in contact with membrane phospholipids. After the insertion into the membrane bilayer, they act either by disruption of the membrane integrity through its thinning, formation of pores and/or interference with the barrier funtion, or by affecting intracellular targets. Several models describe the interactions of AMPs with plasma membranes resulting in the formation of barrel-stave or toroidal channels, and the disruption of the membrane integrity by a “carpet” of AMPs on the membrane surface. The classes of AMPs include defensins, thionins, LTPs (lipid-transfer proteins), hevein- and knottin-like peptides, and macrocyclic peptides and their biological activity are presented in Table 2.

Recently, interest has emerged in the search for natural antioxidants used in the food industry or in medical materials as replacements for synthetic antioxidants, the use of which is limited by their carcinogenicity [58]. Peptides of various bioactivities have been isolated from seeds of several plants, such

Table 2 Biological activity of different classes of peptides.

Type	G^+	G^-	Fungi	Insects	Cell of animal and plants	Anti oxidant	Anti cancer (Caco-2)	Another	Inhibition
Shepherdins	–	+	+	–	–			–	–
Cyclotides	+	+	+	+	Anti-HIV			Uterus contraction	Na^+
Snakins	+	+	+	–	–			–	K^+, Ca^{2+}
Thionins	+	+	+	+	+			–	Ca^{2+}, Zn^{2+}, Fe^{2+}
Defensins	+	+	+	–	–	+	+	Inhibition activity of α-amylase	K^+, Mg^{2+}
LTP	+	+	+	–	–	+		Inhibition activity of α-amylase	
Heveins	+	–	+	–	–				K^+, Ca^{2+}
Knotting	+	–	+					Inhibition activity of α-amylase	K^+, Ca^{2+}
1b-AMPS	+	–	+						

+ = positive, – = negative; G^+ = gram positive bacteria; G^- = gram negative bacteria.

as antimicrobial peptides and antioxidative peptides [59, 60]. Many antioxidative peptides are released from animals by enzymatic hydrolysis. However, antioxidative peptides from plants are little studied, in particular, natural antioxidative peptides.

4. Proposed Mechanism of Action of Cationic Peptides

In spite of the fact that the mechanism of action is not satisfactory established for all cationic peptides, the structural model established by Shai-Matzusaki-Huang [61] provides a reasonable explanation for most antimicrobial activities of these compounds [62]. The model proposes that these linear amphipatic-helical peptides interact with bacterial membranes and increase their permeability, either by the effect of their positive charges with anionic lipids of the target membrane or by membrane destabilization through lipid displacements due to the drastic changes in the net charge of the composed system. A similar mechanism has been proposed for the cysteine-rich peptides such as defensins, which are suggested to form ion-permeable channels in the lipid bilayer. In contrast, some peptides penetrate into cells to exert their action over target molecules [63]. Several additional hypotheses have been proposed to explain the mechanisms by which peptides kill target cells; such hypotheses include induction of hydrolases which degrade the cell wall, disturb the membrane functions and damage crucial intracellular targets after internalization of the peptide [64].

At present time, researchs on actions mechanism of plant peptides are concentrated on defensins and thionins. Plant defensins are small, basic peptides that have a characteristic three-dimensional folding pattern that is stabilized by eight disulfide-linked cysteines. They are termed plants defensins because they are structurally related to defensins found in other types of organism, including humans. To date, sequences of more than 80 different plant defensin genes from different plant species are available. In *Arabidopsis thaliana* at least 13 putative PDF (plants defensin) genes are present, encoding 11 different plants defensins fusions. Plant defensins inhibit the growth of a broad range of fungi but seem nontoxic to either mammalian or plant cells. Antifungal activity of defensins appears to require specific binding to membrane targets. Unlike insect and mammalian defensins, plant defensins neither form ion-permeable pores in artificial membranes nor change the electrical properties of artificial lipid bilayers [65]. Plant defensins induce an array of relatively rapid membrane responses, including Ca^{2+} uptake, K^+ efflux, and alkalinization of medium and membrane potential changes.

It has been proven that there are direct linkage

between the hyphal growth inhibition effect and the ion fluxes induced by the plant defensins [66]. In addition, specific, high-affinity binding sites for plant defensins on fungal cells and microsomal membranes have been identified based on studies with radio labeled plant defensins [67]. A mutant of the yeast, *Saccharomyces cerevisiae*, has been identified which, in contrast to the wild-type strain, is not sensitive to the plant defensins Dm-AMP1 (antimicrobial proteins from *Dahlia merchii* seeds). The capacity of this mutant, called DM1, to bind Dm-AMP1 to its plasma membrane is more than 10-fold less than that of the wild type, suggesting that binding of Dm-AMP1 to a specific binding site is a prerequisite to the antifungal activity of this plant defensins [68]. When combined, these observations suggest that the ion fluxes may result from: a) the interaction of the plant defensins with a binding site that transduce a signal to endogenous ion channels in the membrane. b) Binding-site-mediated insertion of the plant defensins into the membrane, with subsequent ion channel formation.

Multiple antimicrobial molecules through the animal and plant kingdom, belonging to different families, display similar modes of action against a wide range of bacteria and fungi. These molecules, including defensins, share common properties including broad-spectrum antimicrobial activity and cationic charge at physiological PH. There are currently two models describing the mode of antimicrobial activity of such cationic peptides. One model postulates the formation of multimeric pores within microbial membranes. After initial electrostatic binding of these positively charges peptides to negatively charged (phosphor) lipids on the target cell surface, they insert into the energized cell membrane and most likely form multimeric ion-permeable channels in a voltage-dependent manner [69-72]. The subsequent neutralization of the anionic lipid head groups disrupts the integrity of the lipid bilayers, causing transient gaps and allowing ions and larger molecules to cross the membrane [73, 74]. Recently, the theory that many cationic peptides exert their antimicrobial activity not only through cytoplasmic targets has gained support [75]. It was shown that apidaecins, short praline-arginine-rich insect peptides, enter bacterial cells through stereo specific interactions with the outer membrane and, once into the interior of cell, affect protein synthesis (castle). This and other evidence has led to the suggestion that membrane disruption by itself is not the primal cause of antimicrobial activity of possibly many cationic peptides, but rather inhibition of DNA, RNA or protein synthesis (FRIED). Thus the ability of cationic peptides in general to permeabilize cytoplasmic membranes might be a means to reach an intercellular target.

Shepherdins: Glycine histidine rich proteins shepherdins. An insect synthesizes a number of glycine/histidine rich antifungal proteins and polypeptides, including those from *Holotrichia diomphalia larvae* (holotrichin, 84 amino acids) [76], *Sarcophagi peregrine* (flesh fly, 67 amino acids) [77], and *Tenebrio molitor* (49 amino acids peptide tenecin) [78-80]. An alignment chart of these proteins shows that they are extremely rich in glycine and histidine, which comprise as much as 80% of the amino acids. Importantly, fungi inhibited *Candida albicans*, the most common human pathogen [81]. The mechanism of these proteins is not understood.

LTPs (Lipid-transfer Proteins): LTPs have the ability to transfer phospholipids between membranes. LTPs are small proteins (8-11 KDa) of ~90 amino acid stabilized by four disulfide bonds with a central tunnel-like hydrophobic cavity. They have been isolated from a number of sources, including mammals, plants, fungi, and bacteria [82-85], and may play several *in vivo* roles, including lipid exchange between cytoplasmic organelles and, importantly, defense against pathogens [86]. The number of alignments of LTPs note that although the proteins are from diverse sources, they have striking homologies

(between 37 to 90%). LTPs are active *in vitro* against a number of bacteria and fungi; however, the mechanism of action is not known. It may be that these proteins insert themselves into the fungal cell membrane, and the central hydrophobic cavity forms a pore, allowing efflux of intracellular ions and thus leading to fungal cell death. How this is related to their lipid transfer function is not clear.

Snakin peptides: Snakin peptides are basic and rich in cysteine residues, which may form six disulphide bridges to stabilize their structure. They cause a rapid aggregation of Gram-positive and Gram negative bacteria *in vitro*, the aggregation did not correlate with antimicrobial activity, but could play a role in the control of pathogen *in vivo* [87]. The mechanism of action of snakins remains unknown, but in contrast with other plant antibiotic peptides, they do not interact with artificial lipid membranes.

Hevein-like protein: It is a small chitin-binding protein that strongly resembles hevein from the rubber tree [88] and the hevein-like proteins from *Pharbitis nil* [89] and Arabidopsis with respect to its primary structure and physicochemical properties. It contains six to 10 Cys-residue that are all involved in disulfide bridges to stabilize the protein.

Knotting-like peptides and plant cyclotides: Knotting-like peptides are highly basic and consist of 35-37 amino acid residues, they contain three disulfide bridges that differ from other only by four amino acids. They exhibit a broad spectrum of antifungal activity to a variety of pathogenic fungi, and Gram-positive bacteria but were apparently nontoxic for Gram-negative bacteria and cultured human cells. Cyclotides comprise 29-31 amino acids, including six highly conserved Cys residues that form a cystine knot. The combination of a cystine knot embedded in a cyclic backbone, referred to as a cyclic cystine knot, produces a unique protein fold that is topologically complex and has exceptional chemical and biological stability. Antimicrobials activity of the cyclotides is salt dependent; this suggesting that the initial interaction between the cyclotides and the microbials surface is electrostatic, similar to that described for defensins. Jennings et al. [90] have reported that hemolytic activity of plant cyclotides is responsible for the insecticidal properties of cyclotides; they suggest possibility that insecticidal activity result from damage to membranes within the insect gut whatever the mode of action. Ib-AMPs is a basic, cysteine-rich peptide, and the antifungal activity of Ib-AMPs compares favorably with more active antifungal and antimicrobial peptides purified to date from plants. The mode of action of the Ib-AMPs is presently unknown. Tailor et al. [44] proved that Ib-AMPs even at very high rates (500 μg/mL) do not cause any visible lysis or membrane collapse on fungi; they suggest Ib-AMPS are not acting as ionospheres but rather inhibiting a distinct cellular process. The peptides are shown not to affect human cells and they are noncytotoxin to cultured insect and plants cells.

Sonkina et al. [91] has used model system named BLM (bilayer lipid membranes) for study interaction of bioactive peptides with membranes. The electrical characteristic has been studied under the modification with purified bioactive peptides from both seeds of *Daucus carota* and *Anethum graveolens*. The results show that samples from carrot seeds and *Anethum graveolens* that were assumed peptides increase conductivity of BLM with formation of single channel of two types. The first channel formation has been accompanied with noise current, consequently very swiftly open and shut of channel. It has been shown that the addition of peptides at concentration of 0.05 nM to the membrane washing solution did not possess membrane activate property. Peptides possessing membrane-active properties were observed, when applied them on membrane directly by concentration of 3.5 nM. Average amplitude of the first type channels has been 101.2 ± 1.53 pS (n = 5), and for the second type channels it has averaged 137.3 ± 5.89 pS (n = 5). The curve of current-voltage relationships is very symmetric but non-linearly. Observing on

conductivity and potential independence of channel situation concludes that bioactive peptides from seeds of *Daucus carota* and *Anethum graveolens* possess membrane activate properties.

5. Prospective Application of Antifungal Proteins

Several applications of natural occurring antifungal proteins have been discussed during the last two decades. They seem to be an attractive alternative for chemical food additives, and may also be a new source of clinically useful therapeutics. Food preservation by use of antimicrobial proteins is not a totally new concept. The use of the antimicrobial protein nisin, which is produced by several *Lactococcus lactis* strains, has been approved by eight European countries.

The protein inhibits the growth of a wide range of Gram positive bacteria [92], and its mode of action is comparable to magainin [93]. It has been shown that an additional plasma membrane-based factor, named Lipid II, is needed for pore formation [94], which might also be a reason for the lack of antifungal activity of nisin. Another antifungal treatment which involves the use of antifungal proteins is the biological control by antagonistic organisms, such as *Trichoderma* spp., for crop protection [95]. However, one of the most promising tools for crop protection is the use of transgenic plants. Heterologous expression of RIPs (Ribosome-inactivating proteins), glucanases and chitinases in wheat as well as in tobacco resulted in increased protection of the plants against soil borne fungal pathogens [96, 97]. The transgenic expression of plant defensins led to a protection of vegetative tissue even under field conditions. For example the expression of the alfalfa antifungal protein, a plant defensin from the seeds of *Medicago sativa*, in transgenic potato plants resulted in a robust resistance of these plants to *Verticillium dahliae*, an important fungal plant pathogen [98].

Some plant defensins have been shown to interact with fungal-specific receptor-like structures. Since this interaction is very specific, plant defensins are discussed as being an attractive source for therapeutics [99]. These examples clearly show that food protection could be achieved by the use of antimicrobial and antifungal proteins. However, despite the ubiquitous occurrence of antifungal proteins, none of them is currently utilized, either in food preservation or in treatment of clinically relevant pathogenic fungi, although some proteins are evaluated for pharmaceutical use. For example, heliomycin is currently examined in preclinical tests for antifungal treatment [100]. A main hurdle that has hindered the development of antimicrobial and antifungal proteins as therapeutic agents is the fact that many naturally occurring proteins with antifungal activity *in vitro* (e.g., magainins) are only effective *in vivo* at very high doses, often close to the toxic doses of the peptide [101, 102]. One reason for this discrepancy between *in vitro* and *in vivo* activity might be the fact that the action of many antifungal proteins, especially membrane-acting proteins, has been shown to be cation sensitive. Sensitivity to high-ionic-strength conditions or a reduced activity under physiological conditions may be a crucial point in the application of antifungal proteins, since many food products have salt concentrations which also would lead to a decreased antifungal activity or even inactivation of the protein. However, plant defensins often retain their antifungal activity, even under high-ionic strength conditions [103], which would make them ideal candidates for an antifungal treatment of food products. A prerequisite for any application of antifungal proteins is the lack of effects on the host cells. A common method to demonstrate safety and selectivity is a hemolytic assay. The absence of cytolytic activity to red blood cells is generally accepted as proof that the protein can be regarded as safe [104]. Many antifungal proteins have been shown to act synergistically with other antifungal as well as antimicrobial proteins. Synergistic effects

can alter the activity or even the species specificity of a protein. For example, the antifungal protein cecropin B alone has no effect on *Escherichia coli*, but in combination with lysozyme it has been shown to efficiently kill the bacterium [75]. Although synergistic effects could be beneficial for many applications, they also may lead to negative effects, since synergistic interaction with human antifungal and antibacterial proteins might alter the antifungal or antibacterial spectrum.

Another aspect which has to be taken into account for prospective application of antifungal proteins is resistance. The extensive use of classical antibiotics has led to a huge increase of resistant bacteria and fungi. Since the mode of action of antifungal proteins is much more complex, development of resistance against these proteins is probably harder to archive. Nevertheless, it has been shown that fungi are able to adapt to the presence of cell wall-degrading enzymes [55, 105]. Macroconidia of *Fusarium solani* which were exposed to sublethal concentrations of cell wall-degrading enzymes became resistant to much higher concentrations that are lethal to noninduced fungi [106]. Evidence is accumulating that a weakened cell wall activates chitin synthesis and glucan synthase 2 [107]. Furthermore, the expression of the cell wall protein Cwp1p in *Saccharomyces cerevisiae* which limits the yeast cell wall permeability for nisin is positively influenced [108]. Resistance to membrane-acting proteins has not been reported.

Antifungal proteins may be powerful tools in food protection as well as in clinical treatment of pathogens. However, several aspects have to be thoroughly examined prior to a possible application. Activity under physiological conditions, resistance, selectivity and synergistic effects are only a few aspects which have to be clarified prior to application of antifungal proteins. In fact, there are other safety considerations such as immunogenicity or cross-reactions with other host receptors such as neuropeptide and peptide hormone receptors. Therefore, knowledge of the exact mode of action of antifungal proteins is a prerequisite for their application.

Pharmacological studies have proved that many peptides, including those isolated from plants, have a potential antitumor effect. These peptides have a number of advantages over other chemical agents including their low molecular weight, relatively simple structure, lower antigenicity and fewer adverse actions, easy absorption, and a variety of routes of administration. Plants are a huge resource for organisms found in the earth. Interest in the search for natural anti-oxidants for use in the food industry or medical materials as replacements for synthetic anti-oxidants and antibacterial peptides, the use of which is limited by their carcinogenicity, has recently risen [109]. Yili et al. [110-113] have isolated seven antioxidants peptides from seeds of *Apium graveolens* L. (celery) and chickpea, including two peptides isolated from seeds of *Apium graveolens* L. and five peptides molecular weights of 1.148, 4.68, 5.41, 5.48 and 9.086 kDa with antioxidant activity were isolated from the chickpea seeds and sprout for the first time. These systemic study of natural peptides from plants indicated that not only basic peptides, but also neutral and acidic peptides possess anti oxidant activity and this result provide the proof in medicinal or food addictive use possibility of natural peptides.

6. Conclusions

As judged from the evidence reviewed here, the role of antimicrobial peptides in the “innate immunity” system of plants seems to be well established. These peptides are part of developmentally regulated, preexisting defense barriers, and/or may be accumulated as a result of the induction of the corresponding genes upon infection. Two lines of evidence are particularly relevant in the demonstration of an important role of antimicrobial peptides in plant defense: over expression of some peptides enhance plant tolerance to pathogens and peptide-sensitive mutants of the pathogens show

significantly decreased virulence toward plant tissues in which these peptides are present. Furthermore, the latter type of evidence indicates that both plant and animal pathogens deal in a similar way with host defenses, as the equivalent mutants of animal pathogens also show decreased virulence and the possibility that the pathogen defense system against antimicrobial peptides may show specificity toward the peptide type being suggested and might be highly relevant in plant-pathogen interactions.

Summarizing the above review, despite that more than one hundred peptides have been isolated from the diverse plants, the mechanism of action of these peptides are as varied as their sources. This is particularly exciting since mode of the action of these peptides is vastly different from the currently used therapeutics, resistance to which is becoming a clinical problem. However, the mode of action of many peptides remains unknown and is the subject of active research. Equally important, bioactive proteins and peptide are being tested for use as pharmaceutical agents to treatment of human and animal diseases. There are a number of bioactive proteins and peptides in various stages of preclinical development, and the result of these experiment and the subsequent human clinical trials are eagerly anticipated.

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References

[1] Bell, A. A. 1981. "Biochemical Mechanisms of Disease Resistance." *Ann. Rev. Plant Physiol.* 32: 21-8.

[2] Collinge, D. B., and Slusarenko, A. J. 1987. "Plant Gene Expression in Response to Pathogens." *Plant Mol. Biol.* 9: 389-410.

[3] Luckner, M. 1984. *Peptides: Secondary Metabolism in Microorganisms, Plants and Animals*. Berlin: Springer Berlin Heidelberg, 466-80.

[4] Albersheim, P., and Valent, B. S. 1978. "Host-Pathogen Interactions in Plants. Plants, When Exposed to Oligosaccharides of Fungal Orign Defend Themselves by Accumulating Antibiotics." *The Journal of Cell Biology* 78(3): 627-43.

[5] Vance, C. P., Krik, T. K., and Sherwood, R. T. 1980. "Lignification as a Mechanism of Disease Resistance." *Ann. Rev. Phytopathol.* 18: 259-88.

[6] Aist, J. R. 1976. "Papillae and Related Wound Plugs of Plant Cells." *Ann. Rev. Phytopathol.* 14: 145-63.

[7] Hammerschmidt, R., Nuckles, E. M., Kuć, J. 1982. "Association of Enhanced Peroxidase Activity with Induces Systemic Resistance of Cucumber to Colletotrichum Lagenarium." *Physiological Plant Pathology* 20: 73-82.

[8] Stec, B. 2006. "Plant Thionins—The Structural Perspective." *Celluar and Molecular Life Sciences* 63: 1370-85.

[9] Legrand, M., Kauffmann, S., Geoffroy, P., and Fritig, B. 1987. "Biological Function of Pathogenesis-Related Proteins: Four Tobacco Pathogenesis-Related Proteins Are Chitinases." *Proc. Natl. Acad. Sci. USA* 84 (19): 6750-54.

[10] Cassab, G. I. 1998. "Plant Cell Wall Proteins." *Annual Review of Plant Biol*ogy 49: 281-309.

[11] Kauffmann, S., Legrand, M., Geoffroy, P., and Fritig, B. 1987. "Biological Function of 'Pathogenesis-Related' Proteins: Four PR Proteins of Tobacco Have 1,3-β-Glucanase Activity." The *EMBO Journal* 6 (11): 3209-12.

[12] Schlumbaum, A., Mauch, F., Vögeli, U., and Boller, T. 1986. "Plant Chitinases are Potent Inhibitors of Fungal Growth." *Nature* 324 (27): 365-67.

[13] Kombrink, E., Schroder, M., and Hahlbrock, K. 1988. "Several "Pathogenesis-Related" Proteins in Potato Are 1,3-*β*-Glucanases and Chitinases." *Proc. Natl. Acad. Sci. USA* 85 (3): 782-86.

[14] Cammue, B. P. A., De Bolle, M. F. C., Terras, F. R. G., Proost, P., Van Damme, J., Rees, S. B., Vanderleyden, J., and Broekaert, W. F. 1992. "Isolation and Characterization of a Novel Class of Plant Antimicrobial Peptides from *Mirabilis Jalapa* L. Seeds." *The Journal Biological Chemistry* 267 (4): 2228-33.

[15] Hancock, R. E., and Lehrer, R. 1998. "Cationic Peptides: A New Source of Anitibiotics." *Trends in Biotechnology* 16 (2): 82-8.

[16] Park, C. J., Park, C. B., Hong, S. S., Lee, H. S., Lee, S. Y., and Kim, S. C. 2000. "Characterization and cDNA Clonging of Two Glycine- and Histidine-Rich Antimicrobial Peptides from the Roots of Shepherd's Purse, Capsella Bursa-Pastoris." *Plant Mol. Biol.* 44 (2): 187-97.

[17] Morita, H., Sato, Y., and Kobayashi, J. I. 1999. "Cyclosquamosins A-G, Cyclic Peptides from the Seeds of *Annona squamosa*." *Tetrahedron* 55: 7509-18.

[18] Morita, H., Yun, Y. S., Takeya, K., Itokawa, H., and Shirota, O. 1997. "Thionation of Segetalins A and B, Cyclic Peptides with Estrogen-Like Activity from Seeds of *Vaccaria segetalis*." *Bioorganic & Medicinal Chemistry* 5 (3): 631-36.

[19] Morita, H., Yun, Y. S., Takeya, k., Itokawa, H., and Yamada, K. 1995. "Segetalins B, C and D, Three New Cyclic Peptides from *Vaccaria segetalis*." *Tetrahedron* 51 (21): 6003-14.

[20] Wélé, A., Zhang, Y. J., Brouard, J. P., Pousset, J. L., and Bodo, B. 2005. "Two Cyclopeptides from the Seeds of *Annona cherimola*." *Phytochemistry* 66 (19): 2376-80.

[21] Segura, A., Moreno, M., Madueño, F., Molina, A., and García-Olmedo, F. 1999. "Snakin-1, A Peptide from Potato That Is Active against Plant Pathogens." *Mol. Plant Microbe. Interact.* 12 (1): 16–23.

[22] Berrocal-Lobo, M., Segura, A., Moreno, M., López, G., and Molina, A. 2002. "Snakin-2, An Antimicrobial Peptide from Potato Whose Gene is Locally Induced by Wounding and Responds to Pathogen Infection." *Plant Physiology* 128 (3): 951-61.

[23] Egorov, T. A., Odintsova, T. I., Pukhalsky, V. A., Grishin, E. V. 2005. "Diversity of Wheat Anti-microbial Peptides." *Peptides* 26 (11): 2064-73.

[24] Berrocal-Lobo, M., Molina, A., Rodríguez-Palenzuela, P., García-Olmedo, F., and Rivas, L. 2009. "*Leishmania donovani*: Thionins, Plant Antimicrobial Peptides with Leishmanicidal Activity." *Experimental Parasitology* 122: 247-9.

[25] Duvick, J. P., Rood, T., Rao, A. G., and Marshak, D. R. 1992. "Purification and Characterization of a Novel Antimicrobial Peptide from Maize (*Zea mays* L.) Kernels." *The Journal of Biological Chemistry* 267 (26): 18814-20.

[26] Wong, J. H., and Ng, T. B. 2003. "Gymnin, a Potent Defensin-Like Antifungal Peptide from the Yunnan Bean (*Gymnocladus chinensis* Baill)." *Peptides* 24 (7): 963-8.

[27] Ngai, P. H. K., and Ng, T. B. 2004. "Coccinin, an Antifungal Peptide with Antiproliferative and HIV-1 Reverse Transcriptase Inhibitory Activities from Large Scarlet Runner Beans." *Peptides* 25: 2063-68.

[28] Francois, I. E. J. A., Hemelrijck, W. V., Aerts, A. M., Wouters, P. F. J., Proost, P., Broekaert, W. F., and Cammue, B. P. A. 2004. "Processing in *Arabidopsis thaliana* of a Heterologous Polyprotein Resulting in Differential Targeting of the Individual Plant Defensins." *Plant Science* 166 (1):113-21.

[29] Thomma, B. P. H. J., and Cammue, B. P. A. 2002. "Plant Defensins." *Planta* 216: 193-202.

[30] Almeida, M. S., Cabral, K. M. S., Zingali, R. B., and Kurtenbach, E. 2002. "Characterization of Two Novel Defense Peptides from Pea (*Pisum sativum*) Seeds." *Archives of Biochemistry and Biophysics* 378 (2): 278-86.

[31] Thevissen, K., Warnecke, D. C., Francois, I. E. J. A, Leipelt, M., Heinz, E., Ott, C., Zähringer, U., Thomma, B. P. H. J, Ferket, K. K. A., and Cammue, B. P. A. 2004. "Defensins from Insects and Plants Interact with Fungal Glucosylceramides." *The Journal of Biological Chemistry* 279 (6): 3900-05.

[32] Park, H. C., Kang, Y. H., Chun, H. J., Koo, J. C., Cheong, Y. H., Kim, C. Y., Kim, M. C., Chung, W. S., Kim, J. C., Yoo, J. H., Koo, Y. D., Koo, S. C., Lim, C. O., Lee, S. Y., and Cho, M. J. 2002. "Characterization of a Stamen-Specific cDNA Encoding a Novel Plant Defensin in Chinese Cabbage." *Plant Molecular Biology* 50: 59-69.

[33] Yokoyama, S., Kato, K., Koba, A., Minami, Y., Watanabe, K., and Yagi, F. 2008. "Purification, Characterization, and Sequencing of Antimicrobial Peptides, Cy-AMP1, Cy-AMP2, and Cy-AMP3, from the Cycad (*Cycas revoluta*) Seeds." *Peptides* 29: 2110-17.

[34] Cammue, B. P. A, Thevissen, K., Hendriks, M., Eggermont, K., Goderis, I. J., Proost, P., Damme, J. V., Osborn, R. W., Guerbette, F., Kader, J. C., and Broekaert, W. F. 1995. "A Potent Antimicrobial Protein from Onion Seeds Showing Sequence Homology to Plant Lipid Transfer Proteins." *Plant Physiol.* 109: 445-55.

[35] Wang, S. Y., Wu, J. H., Ng, T. B., Ye, X. Y., and Rao, P. F. 2004. "A Non-specific Lipid Transfer Protein with Antifungal and Antibacterial Activities from the Mung Bean." *Peptides* 25 (8): 1235-42.

[36] Castro, M. S., Gerhardt, I. R., Orrù, S., Pucci, P., and Jr, C. B. 2003. "Purification and Characterization of a Small (7.3 kDa) Putative Lipid Transfer Protein from Maize Seeds." *Journal of Chromatography B* 794: 109-14.

[37] Shao, F., Hu, Zh., Xiong, Y. M., Huang, Q. Z., Wang, C. G., and Wang, D. C. 1999. "A New Antifungal Peptide from the Seeds of *Phytolacca americana*: Characterization, Amino Acid Sequence and cDNA Cloning." *Biochimica et Biophysica Acta* 1430: 262-68.

[38] Lerche, M. H., Kragelund, B. B., Bech, L. M., and Poulsen, F. M. 1997. "Barley Lipid-Transfer Protein Complexed with Palmitoyl CoA: The Structure Reveals a Hydrophobic Binding Site That Can Expand to Fit both Large and Small Lipid-like Ligands." *Structure* 5 (2): 291-306.

[39] Diz, M. S. S., Carvallo, A. O., Rodrigues, R., Neves-Ferreira, A. G. C., Cunda, M. D., Alves, E. W., Okorokova-Façanha, A. L., Oliveira, M. A., Perales, J., Machado, O. L. T., and Gomes, V. M. 2006. "Antimicrobial Peptides from Chilli Pepper Seeds Causes Yeast Plasma Membrane Permeabilization and Inhibits

the Acidification of the Medium by Yeast Cells." *Biochimica et Biophysica Acta* 1760: 1323-32.

[40] Yili, A., Aisa, H. A., Imamu, X., Zhen, R. H., Zhang, Q., Maksimov, V. V., Veshkurova, O. N., and Salikhov, Sh. I. 2007. "Fungicidal Lipid-Transfer Peptide from *Daucus carota sativa* Seeds." *Chemistry of Natural Compounds* 43 (4): 450-53.

[41] Lipkin, A., Anisimova, V., Nikonorova, A., Babakov, A., Krause, E., Bienert, M., Grishin, E., and Egorov, T. 2005. "An Antimicrobial Peptide Ar-AMP from Amaranth (*Amaranthus retroflexus* L.) Seeds. " *Phytochemistry* 66 (20): 2426-31.

[42] Koo, J. C., Lee, S. Y., Chun, H. J., Cheong, Y. H., Choi, J. S., Kawabata, S. I., Miyagi, M., Tsunasawa, S., Ha, K. S., Bae, D. W., Han, C. D., Lee, B. L., and Cho, M. J. 1998. "Two Hevein Homologs Isolated From the Seed of *Pharbitis nil* L. Exhibit Potent Antifungal Activity." *Biochimica et Biophysica Acta* 1382: 80-90.

[43] Van Den Bergh, K. P. B., Proost, P., Damme, J. V., Coosemans, J., Van Damme, E. J. M., and Peumans, W. J. 2002. "Five Disulfide Bridges Stabilize a Hevein-Type Antimicrobial Peptide from the Bark of Spindle Tree (*Euonymus europaeus* L.). *Febs Letters* 530: 181-5.

[44] Tailor, R. H., Acland, D. P., Attenborough, S., Cammue, B. P., Evans, I. J., Osborn, R. W., Ray, J. A., Reeds, S. B., and Broekaert, W. F. 1997. "A Novel Family of Small Cysteine-Rich Antimicrobial Peptides from Seed of *Impatiens balsamina* is Derived from a Single Precursor Protein." *The Journal of Biological Chemistry* 272 (39): 24480-7.

[45] Thevissen, K., François, I. E. J. A., Sijtsma, L., Amerongen, A. V., Schaaper, W. M. M., Meloen, R., Posthuma-Trumpie, T., Broekaert, W. F., and Cammue, B. P. A. 2005. "Antifungal Activity of Synthetic Peptides Derived from *Impatiens balsamina* Antimicrobial Peptides Ib-AMP1 and Ib-AMP4." *Peptides* 26: 1113-19.

[46] Wang, P., Bang, J. K., Kim, H. J., Kim, J. K., Kim, Y., and Shin, S. Y. 2009. "Antimrobial Specificity and Mechanism of Action of Disulfide-Removed Linear Analogs of the Plant-Derived Cys-Rich Antimicrobial Peptide Ib-AMP1." *Peptides* 30: 2144-49.

[47] Tossi, A., and Sandri, L. 2002. "Molecular Diversity in Gene-Encoded, Cationic Antimicrobial Polypeptides." *Current Pharmaceutical Design* 8 (9): 743-61.

[48] Broekaert, W. F., Cammue, B. P. A., De Bolle, M. F. C., Thevissen, K., De Samblanx, G. W., Osborn, R. W., and Nielson, K. 1997. "Antimicrobial Peptides from Plants." *Critical Reviews in Plant Sciences* 16 (3): 297-323.

[49] Tam, J. P., Lu, Y. A., Yang, J. L., and Chiu, K. W. 1999. "An Unusual Structural Motif of Antimicrobial Peptides Containing End-to-End Macrocycle and Cystine-Knot Disulfides." *Proc. Natl. Acad. Sci. USA* 96 (16): 8913-18.

[50] Trabi, M., and Craik, D. J. 2002. "Circular Proteins-no End in Sight." *Trends in Biochemical Sciences* 27 (3): 132-8.

[51] Clore, G. M., Nilges, M., Sukumaran, D. K., Brünger, A. T., Karplus, M., and Gronenborn, A. M. 1986. "The Three-Demensional Structure of α1-Purothionin in Solution: Combined Use of Nuclear Magnetic Resonance, Distance Geometry and Restrained Molecular Dynamics." *The EMBO Journal* 5 (10): 2729-35.

[52] Pallaghy, P. K., Nielsen, K. J., Craik, D. J., and Norton, R. S. 1994. "A Common Structural Motif Incorporating a Cystine Knot and a Triple-Stranded Beta-Sheet in Toxic and Inhibitory Polypeptides." Protein Science 3 (10): 1833-39.

[53] Heidari, M., Hamir, A., Cutlip, R. C., and Brogden, K. A. 2002. "Antimicrobial Anionic Peptide Binds in Vivo to *Mannheimia* (*Pasteurella*) *haemolytica* Attached to Ovine Alveolar Epithelium." *International Journal of Antimicrobial Agents* 20 (1): 69-72.

[54] Derua, R., Gustafson, K. R., and Pannell, L. K. 1996. "Analysis of the Disulfide Linkage Pattern in Circulin A and B, HIV-Inhibitory Macrocyclic Peptides." *Biochem Biophys Res Commun* 228 (2): 632-38.

[55] Hancock, R. E. W., and Sahl, H. G. 2006. "Antimicrobial and Host-Defense Peptides as New Anti-infective Therapeutic Strategies." *Nature Biotechnology* 24 (12): 1551-57.

[56] Hancock, R. E. W. 1997. "Peptide Antibiotics." *The Lancet* 349 (9049): 418-22.

[57] Egorov, T. A., and Odintsova, T. I. 2012. "Defense Peptides of Plant Immunity." *Russian Journal of Bioorganic Chemistry* 38 (1): 1-9.

[58] Qian, Z. J., Jung, W. K., Byun, H. G., and Kim, S. K. 2008. "Protective Effect of an Antioxidative Peptide Purified from Gastrointestinal Digests of Oyster, Crassostrea Gigas against Free Radical Induced DNA Damage." *Bioresource Technology* 99 (9): 3365-71.

[59] Tassin, S., Broekaert, W. F., Marion, D., Acland, D. P., Ptak, M., Vovelle, F., and Sodano, P. 1998. "Solution Structure of Ace-AMP1, a Potent Antimicrobial Protein Extracted from Onion Seeds. Structural Analogies with Plant Nonspecific Lipid Transfer Proteins." *Biochemistry* 37 (11): 3623-37.

[60] Hsu, K. C. 2010. "Purification of Antioxidative Peptides Prepared from Enzymatic Hydrolysates of Tuna Dark Muscle by-Product." *Food Chemsitry* 122 (1): 42-8.

[61] Matsuzaki, K. 1999. "Why and How Peptide-Lipid Interactions Utilized for Self-defense? Magainins and Tachyplesins as Archetypes." *Biochimica et Biophysica Acta* 1462: 1-10.

[62] Zasloff, M. 2002. "Antimicrobial Peptides of Multicellular Organisms." *Nature* 415 (6870): 389-95.

[63] Kragol, G., Lovas, S., Varadi, G., Condie, B. A., Hoffmann, R., and Jr, L. O. 2001. "The Antibacterial Peptide Pyrrhocoricin Inhibits the ATPase Actions of DnaK and Prevents Chaperone-Assisted Protein Folding." *Biochemistry* 40 (10): 3016-26.

[64] Zasloff, M. 1987. "Magainins, a Class of Antimicrobial Peptides from *Xenopus* Skin: Isolation, Characterization of Two Active Forms, and Partial cDNA Sequence of a Precursor." *Proc. Natl. Acad. Sci. USA* 84 (15): 5449-53.

[65] Liu, Y. F., Luo, J. C., Xu, Ch. Y., Ren, F. Ch., Peng, Ch., Wu, G. Y., and Zhao, J. D. 2000. "Purification, Characterization, and Molecular Cloning of the Gene of a Seed-Specific Antimicrobial Protein from Pokeweed." *Plant Physiology* 122 (4): 1015-24.

[66] Thevissen, K., Ghazi, A., De Samblanx, G. W., Brownlee, C., Osborn, R. W., and Broekaert, W. F. 1996. "Fungal Membrane Responses Induced by Plant Defensins and Thionins." *The Journal of Biological Chemistry* 271 (25): 15018-25.

[67] Thevissen, K., Osborn, R. W., Acland, D. P., and Broekaert, W. F. 1998. "Specific, High Affinity Binding Sites for an Antifungal Plant Defensin on Neurospora Crassa Hyphae and Microsomal Membranes." *The Journal of Biological Chemistry* 272 (51): 32176-81.

[68] Thevissen, K., Cammue, B. P. A., Lemaire, K., Winderickx, J., Dickson, R. C., Lester, R. L., Ferket, K. K. A., Parret, A. H. A., and Broekaert, W. F. 2000. "A Gene Encoding a Sphingolipid Biosynthesis Enzyme Determines the Sensitivity of *Saccharomyces cerevisiae* to an Antifungal Plant Defensin from Dahlia (*Dahlia merckii*)." *Proc. Natl. Acad. Sci. USA* 97 (17): 9531-6.

[69] Carrasco, L., Vàzquez, D., Hernàndez-Lucas, C., Carbonero, P., and García-Olmedo, F. 1981. "Thionins: Plant Peptides that Modify Membrane Permeability in Cultured Mammalian Cells." *European Journal of Biochemistry* 116 (1): 185-9.

[70] Rosengren, K. J., Mcmanus, A. M., and Craik, D. J. 2002. "The Structural and Functional Diversity of Naturally Occurring Antimicrobial Peptides." *Current Medicinal Chemistry Anti-infective Agents* 1 (4): 319-41.

[71] Kagan, B. L., Selsted, M. E., Ganz, T., and Lehrer, R. I. 1990. "Antimicrobial Defensin Peptides form Voltage-Dependant Ion-Permeable Channels in Planar Lipid Bilayer Membranes." *Proc. Natl. Acad. Sci. USA* 87 (1): 210-14.

[72] Cociancich, S., Ghazi, A., Hetru, C., Hoffmann, J. A., and Letellier, L. 1993. "Insect Defensin, an Inducible Antibacterial Peptide, Forms Voltage-Dependent Channels in *Micrococcus luteus*." *Journal of Biological Chemistry* 268 (26): 19239-45.

[73] Bull, J., Mauch, F., Hertiq, C., Rebmann, G., and Dudler, R. 1992. "Sequence and Expression of a Wheat Gene that Encodes a Novel Protein Associated with Pathogen Defense." *Mol Plant Microbe Interact* 5 (6): 516-19.

[74] Caruso, C., Caporale, C., Chilosi, G., Vacca, F., Bertini, L., Maqro, P., Poerio, E., and Buonocore, V. 1996. "Structural and Antifungal Properties of a Pathogenesis-Related Protein from Wheat Kernel." *Journal of Protein Chemistry* 15 (1): 35-44.

[75] Wu, M., Maier, E., Benz, R., and Hancock, R. E. 1999. "Mechanism of Interaction of Different Classes of Cationic Antimicrobial Peptides with Planar Bilayers and with the Cytoplasmic Membrane of *Escherichia coli*." *Biochemistry* 38 (22): 7235-42.

[76] Lee, S. Y., Moon, H. J., Kurata, S., Natori, S., and Lee, B. L. 1995 "Purification and cDNA Cloning of an Antifungal Protein from the Hemolymph of *Holotrichia diomphalia* Larvae." *Biological & Pharmaceutical Bulletin* 18 (8): 1049-52.

[77] Iijima, R., Kurata, S., and Natori, S. 1993. "Purification, Characterization, and cDNA Cloning of an Antifungal Protein from the Hemolymph of *Sarcophaga peregrina* (Flesh Fly) Larvae." *The Journal of Biological Chemistry* 268 (16): 12055-61.

[78] Kim, D. H., Lee, Y. T., Lee, Y. J., Chung, J. H., Lee, B. L., Choi, B. S., and Lee, Y. 1998. "Bacterial Expression of Tenecin 3, an Insect Antifungal Protein Isolated from Tenebrio Molitor, and Its Efficient Purification." *Molecules and Cells* 8 (6): 786-89.

[79] Lee, K. M., Kim, D. H., Lee, Y. H., Choi, B. S., Chung, J. H., and Lee, B. L. 1999. "Antifungal Activities of Recombinant Antifungal Protein by Conjugation with Polyethylene Glycol." *Molecules and Cells* 9 (4): 410-16.

[80] Lee, Y. J., Chung, T. J., Park, Ch. W., Hahn, Y., Chung, J. H., Lee, B. L., Han, D. M., Jung, Y. H., Kim, S., and Lee, Y. 1996. "Structure and Expression of the Tenecin 3 gene in Tenebrio Molitor." *Biochem Biophys Res Commun* 218 (1): 6-11.

[81] Kim, D. H., Lee, D. G., Kim, K. L., and Lee, Y. 2001. "Internalization of Tenecin 3 by a Fungal Cellular Process is Essential for Its Fungicidal Effect on *Candida albicans*." *European Journal of Biochemistry* 268 (16): 4449-58.

[82] Coutos-Thevenot, P., Jouenne, T., Maes, O., Guerbette, F., Grosbois, M., Le Caer, J. P., Boulay, M., Beloire, A., Kader, J. C., and Guern, J. 1993. "Four 9-KDa Proteins Excreted by Somatic Embryos of Grapevine Are Isoforms of Lipid-Transfer Proteins." *European Journal of Biochemistry* 217 (3): 885-9.

[83] Molina, A., Segura, A., and García-Olmedo, F. 1993. "Lipid Transfer Proteins (nsLTPs) from Barley and Maize Leaves are Potent Inhibitors of Bacterial and Fungal Plant Pathogens." *FEBS Lett*ers 316 (2): 119-22.

[84] Molina, A., and García-Olmedo, F. 1993.

"Developmental and Pathogen-Induced Expression of Three Barley Genes Encoding Lipid Transfer Proteins." *The Plant Journal* 4 (6): 983-91.

[85] Segura, A., Moreno, M., and García-Olmedo, F. 1993. "Purification and Antipathogenic Activity of Lipid Transfer Proteins (LTPs) from the Leaves of *Arabidopsis* and Spinach." *FEBS Lett* 332 (3): 243-46.

[86] Bechinger, B. 1997. "Structure and Functions of Channel-Forming Peptides: Magainins, Cecropins, Melittin and Alamethicin." *J Membr Biol* 156 (3): 197-11.

[87] Harris, P. W. R., Yang, S. H., Molina, A., López, G., Middleditch, M., and Brimble, M. A. 2014. "Plant Antimicrobial Peptides Snakin-1 and Snakin-2: Chemical Synthesis and Insights into the Disulfide Connectivity." *Chemistry-A European Journal* 20 (17): 5102-10.

[88] Gidrol, X., Chrestin, H., Tan, H. L., and Kush, A. 1994. "Hevein, a Lectin-like Protein from *Hevea brasiliensis* (Rubber Tree) Is Involved in the Coagulation of Latex." *The Journal of Biological Chemistry* 269 (12): 9278-83.

[89] Koo, J. C., Chun, H. J., Park, H. C., Kim, M. C., Koo, Y. D., Koo, S. C., Ok, H. M., Park, S. J., Lee, S. H., Yun, D. J., Lim, C. O., Bahk, J. D., Lee, S. Y., and Cho, M. J. 2002. "Over-Expression of a Seed Specific Hevein-Like Antimicrobial Peptide from *Pharbitis nil* Enhances Resistance to a Fungal Pathogen in Transgenic Tobacco Plants." *Plant Moleculary Biology* 50 (3): 441-52.

[90] Jennings, C., Wesr, J., Waine, C., Craik, D., and Anderson, M. 2001. "Biosynthesis and Insecticidal Properties of Plant Cyclotides: the Cyclic Knotted Proteins from *Oldenlandia affinis*." *Proc. Natl. Acad. Sci. USA* 98 (19): 10614-19.

[91] Sonkina, S. N., Yili, A., Maksimov, V. V., Veshkurova, O. N., Muratov, L. A., Sakahutdinov, B. A., and Aripov., T. F. 2008. "Investigation of the Action of Peptides from *Anethum graveolens* and *Daucus carota sativa* Seeds on Bilayer Lipid Membranes Conductivity." *Biological Journal of Uzbekistan* 3: 10-15. (in Russian)

[92] Delves-Broughton, J., Blackburn, P., Evans, R. J., and Hugenholtz, J. 1996. "Applications of the Bacteriocin Nisin." *Anthonie Van Leeuwenhoek* 69 (2): 193-202.

[93] Ludtke, S. J., He, K., Heller, W. T., Harroun, T. A., Yang, L., and Huang, H. W. 1996. "Membrane Pores Induced by Magainin."*Biochemistry* 35 (43): 13723-8.

[94] Brötz, H., Josten, M., Wiedemann, I., Schneider, U., Götz, F., Bierbaum, G., and Sahl, H. G. 1998. "Role of Lipid-Bound Peptidoglycan Precursors in the Formation of Pores by Nisin, Epidermin and other Lantibiotics." *Molecular Microbiology* 30 (2): 317-27.

[95] Papavizas, G. C. "Trichoderma and Gliocladium: Biology, Ecology and Potential for Biocontrol." *Ann. Rev. Phytopathol.* 23: 23-54.

[96] Jach, G., Görnhardt, B., Mundy, J., Logemann, J., Pinsdorf., E., Leah, R., Schell, J., and Mass, C. 1995. "Enhanced Quantitative Resistance against Fungal Disease by Combinatorial Expression of Different Barley Antifungal Proteins in Transgenic Tobacco." *The Plant Journal* 8 (1): 97-109.

[97] Oldach, K. H., Becker, D., and Lörz, H. 2001. "Heterologous Expression of Genes Mediating Enhanced Fungal Resistance in Transgenic Wheat." *Molecular Plant Microbe Interactions* 14 (7): 832-8.

[98] Gao, A. G., Hakimi, S. M., Mittanck, C. A., Wu, Y., Woerner, B. M., Stark, D. M., Shah, D. M., Liang, J., and Rommens, C. M. 2000. "Fungal Pathogen Protection in Potato by Expression of a Plant Defensin Peptide." *Nature Biotechnology* 18 (12): 1307-10.

[99] Thomma, B. P., Cammue, B. P., and Thevissen, K. 2003. "Mode of Action of Plant Defensins Suggests Therapeutic Potential." *Curr Drug Targets Infect Disord* 3 (1): 1-8.

[100] Banzet, N., Latorse, M. P., Bulet, P., François, E., Derpierre, C., and Dubald, M. 2002. "Expression of Insect Cytein-Rich Antifungal Peptides in Transgenic Tobacco Enhances Resistance to a Fungal Disease." *Plant Science* 162 (6): 995-1006.

[101] Darveau, R. P., Cunningham, M. D., Seachord, C. L., Cassiano-Clough, L., Cosand, W. L., Blake, J., and Watkins, C. S. 1991. "Beta-Lactam Antibiotics Potentiate Magainin 2 Antimicrobial Activity in Vitro and in Vivo." *Antimicrob Agents Ch* 35 (6): 1153-59.

[102] Guerbette, F., Grosbois, M., Jolliot-Croquin, A., Kader, J. C., and Zachowski, A. 1999. "Lipid-Transfer Proteins from Plants: Structure and Binding Properties." *Molecular and Cellular Biochemistry* 192 (1-2): 157-61.

[103] Terras, F. R. G., and Thevissen, K. 1999 "Permeabilization of Fungal Membranes by Plant Defensins Inhibits Fungal Growth." *Applied and Environmental Microbiology* 65 (12): 5451-58.

[104] Shai, Y. 1999. "Mechanism of the Binding, Insertion and Destabilization of Phospholipids Bilayer Membranes by α-Helical Antimicrobial and Cell Non-selective Membrane-Lytic Peptides." *Biochimica et Biophysica Acta Biomembranes* 1462 (1-2): 55-70.

[105] Vidal, S., Eriksson, A. R. B., Montesano, M., Denecke, J., and Palva, E. T. 1998. "Cell Wall-Degrading Enzymes from *Erwinia carotovora* Cooperate in the Salicylic Acid-independent Induction of a Plant Defense Response." *Mol. Plant Microbe In.* 11 (1): 23-32.

[106] Sela-Buurlage, M. B. 1996. "In Vitro Sensitivity and Tolerance of Fusarium Solani towards Chitinases and β-1, 3-Glucanases." Doctoral Thesis. Agricultural University of Wageningen.

[107] Broekaert, W. F., Terras, F. R., Cammue, B. P., and Osborn, R. W. 1995. "Plant Defensins: Novel Antimicrobial Peptides as Components of the Host

Defense System." *Plant Physiology* 108 (4): 1353-58.

[108] Ram, A. F., Van Den Ende, H., and Klis, F. M. 1998. "Green Fluorescent Protein-cell Wall Fusion Proteins Are Covalently Incorporated into the Cell Wall of *Saccharomyces cerevisiae*." *FEMS Microbiology* 162 (2): 249-55.

[109] Van't Hof, W., Veerman, E. C., Helmerhorst, E. J., and Amerongen, A. V. 2001. "Antimicrobial Peptides: Properties and Applicability." *Biological Chemistry* 382 (4): 597-619.

[110] Yili, A., Ma, Q. L., Gao, Y. H., Zhao, B., Jun, D., and Aisa, H. A. 2012. "Isolation of Two Antioxidant Peptides from Seeds of *Apium graveolens* Indigenous to China." *Chemistry of Natural Compounds* 48 (4):719-20.

[111] Ling, M. Q., Yili, A., Aisa, H. A., Bo, Z., Veshkurova, O. N., and Salikhov, Sh. I. 2011. "Isolation of a New Peptide from Seeds of *Apium graveolens* Indigenous to China." *Chemistry of Natural Compounds* 46 (6): 932-34.

[112] Yili, A., Ling, M. Q., Bo, Z., Asrorov, A. S., Oshchepkova, Yu. I., Salikhov, Sh. I., and Aisa, H. A. 2012. "New Peptide from Seeds of *Cicer arietinum*." *Chemistry of Natural Compounds* 47 (6): 959-62.

[113] Yili, A., Ma, Q. L., Lv, Q. Y., Gao, Y. H., Zhao, B., Veshkurova, O. N., Salikhov, Sh. I., and Aisa, H. A. 2012. "Antioxidant Peptides from *Cicer arietinum* of Xinjiang, China." *Chemistry of Natural Compounds* 48 (4): 643-5.