Matkawala et al. (2019) Can J Biotech 3(2): 158–168
https://doi.org/10.24870/cjb.2019-000130

Canadian Journal of Biotechnology

ISSN 2560-8304

MINI REVIEW PAPER **OPEN ACCESS**

Antimicrobial Peptides in Plants: Classes, Databases, and Importance

Fatema Matkawala[1,2], Anand Nighojkar[2], and Anil Kumar[1*]

[1]School of Biotechnology, Devi Ahilya University, Khandwa Road, Indore 452001, INDIA

[2]Maharaja Ranjit Singh College of Professional Sciences, Khandwa Road, Indore 452001, INDIA

Received: Jul 12, 2019; Revised: Aug 19, 2019; Accepted: Aug 26, 2019

Abstract

Plant antimicrobial peptides (AMPs) are diverse molecules crucial in host defense mechanisms. These natural compounds display broad-spectrum antimicrobial activities and also play a significant role as immune modulators and anti-infective agents. They are classified into different families like defensins, thionins, cyclotides, snakins, and several others, based on the variation in their structure, the composition of amino acids, number of disulfide bonds, and mechanism of action. The ascending number of drug-resistant plant and animal pathogens has pushed researchers to search for novel peptides, which can be utilized as alternatives to chemical antibiotics. In addition, the exhaustive genomic and proteomic data available on the cyberspace encourage the development of peptide libraries used for the prediction of unexplored peptides, thus saving time and cost for wet-lab experimentation. Understanding the insights of the structure and function of plant AMPs would offer excellent opportunities to expand their use as therapeutics in pharmaceutical and agricultural industries. This study reviewed the basis of plant AMPs, provided information on recent advancements in omic tools, and updated newly added peptides in the databases. The potential application of these peptides in human healthcare and agribusiness was also discussed.

Keywords: antimicrobial peptides, plant proteins, protein database, therapeutics, defensin

Introduction

Antimicrobial peptides (AMPs) belong to a diverse group of bioactive molecules ranging from 10 to 100 amino acid residues in length [1]. Originally, these peptides were known to possess antagonistic action against numerous pathogens, including bacteria, fungi, viruses, protozoa. Recent discoveries have expanded their functions as immune modulators, antihypertensive molecules, anticancer, and antitumor agents [2,3]. Nowadays, AMPs are considered a fundamental component of the innate immune system [4]. These bioactive peptides have been naturally and synthetically derived from various prokaryotic and eukaryotic systems. Natural AMPs have been isolated from plants, amphibians, insects, mammals, fungi, bacteria, etc. [5,6]. Recent evidence of increasing resistance of microbes against antibiotics has drawn attention towards the use of peptides as novel therapeutics for the treatment of various Gram-positive and Gram-negative infections [7]. The use of AMPs in medicines has emerged rapidly because of their broad-spectrum activity and safety. Additionally, pathogens are unlikely to develop resistance against these peptides as compared to antibiotics.

Plants produce defensive molecules in response to the invasion of pathogens. An array of peptides isolated from different parts of the plants, namely roots, seeds, flowers, stems, and leaves, has demonstrated antimicrobial activity against phytopathogens, as well as pathogenic bacteria causing infection in human beings [8]. Based on the amino acid sequence homology, the main families of plant AMPs comprise defensins, thionins, lipid-transfer proteins, cyclotides, snakins, and hevein-like proteins [9]. The key features defining plant AMPs are that they are enriched in cysteine and glycine and the presence of disulfide bridges [10]. The major challenges faced by these compounds are the development of intrinsic toxicity in plants, lesser stability, and cost of production, restricting their use for commercial applications. With the advancement in biotechnological techniques, these peptides can serve as an attractive tool in the agriculture field for increasing crop yields [11].

This article summarized different AMPs found in plants and updated the recent progressions by searching plant AMPs

*Corresponding author: Email: ak_sbt@yahoo.com

through various databases. The review also focused on the development of AMP-based therapeutics, which can replace conventional antibiotics in the future, and provided insight to seek innovative methodologies for hunting novel AMPs with potential applicability.

Major Classes of Plant AMPs

A variety of plant species is known to serve as a source of natural products capable of controlling infections caused by pathogenic microorganisms. One of the major sources of antimicrobial compounds secreted as a part of their defense mechanism is AMPs. The repertoire of AMPs synthesized by plants is extremely diverse, with some of the plant species bearing hundreds of different types of AMPs [10]. A cumulative data on some of the recently reported plant AMPs are summarized in Table 1.

Seyedjavadi et al. [12] recently isolated an AMP, MCh-AMP1 from *Matricaria chamomilla* L. (Table 1), which shared more than 20% similarity with peptides belonging to plantaricin family (peptides derived from *Lactobacillus plantarum*). Several plant peptides sharing structural and functional similarity to animal-derived peptides have also been reported earlier. The criteria for categorizing different families of plant AMPs depend on several factors, including sequence homology of primary structure, the pattern of the disulfide bond, the charge on molecules, structural scaffold, molecular weight, etc. [19]. Based on this information, plant AMPs can be classified as defensins, thionins, cyclotides, lipid-transfer proteins, snakins, heveins, etc. The representative example of the three-dimensional structures of commonly found AMPs is listed in Figure 1.

Defensins

Defensins are the most ancient and commonly found AMPs secreted in eukaryotes. The very first plant defensins were isolated from wheat and barley and were initially designated as γ thionions. Afterward, these γ thionions were renamed as defensins due to their structural resemblance to animal defensins [20]. These peptides are characterized as smaller peptides having 40 to 55 amino acid residues with a molecular weight of 5 kDa, enriched in cysteine and comprises of four to five disulfide bonds in their structure [21]. These peptides have been reported to exhibit inhibitory activity against numerous bacterial pathogens of animals and fungi. They are abundant in seeds but have also been reported in other tissues, namely leaves, pods, tubers, fruits, roots, bark, and floral tissues. The NMR spectroscopy has revealed their three-dimensional structure, which presented three-stranded, anti-parallel β-sheets and one α-helix following a βαββ pattern, with eight cysteine residues forming four disulfide bridges that stabilize

Table 1: Some of the recent plant antimicrobial peptides (AMPs) reported in databases.

Name	Species	Class	Peptide Sequence	Activity	Reference
MCh-AMP1	*Matricaria chamomilla* L.	Plantaricin- like	LSVKAFTGIQLRGVCGIEVKARG	Antifungal	[12]
ZmD32	*Zea mays*	Defensin	RTCQSQSHRFRGPCLRRSNCANVC RTEGFPGGRCRGFRRRCFCTTHC	Anti-Gram-positive, Anti-Gram-negative, Antifungal, Antibiofilm	[13]
NCR335	*Medicago truncatula*	Defensin	RLNTTFRPLNFKMLRFWGQNRNIM KHRGQKVHFSLILSDCKTNKDCPK LRRANVRCRKSYCVPI	Anti-Gram-positive, Anti-Gram-negative	[14]
Plantaricin JLA-9	Chinese fermented vegetables, *Lactobacillus plantarum*	Plantaricin	FWQKMSFA	Anti-Gram-positive, Anti-Gram-negative	[15]
AtPDF2.3	*Arabidopsis thaliana*	Defensin	RTCESKSHRFKGPCVSTHNCANVC HNEGFGGGKCRGFRRRCYCTRHC	Antifungal, Channel inhibitors	[16]
PSI-1.2	*Capsicum annuum*	Thionin-like	KACPRNCDTDIAYMVCPSSGERIIR KVCTNCCAAQKGCKLFRSNGSIKC TGT	Antifungal, Enzyme inhibitor	[17]
SmAMP3	*Stellaria media* L.	Gly-rich	VGPGGECGGRFGGCAGGQCCSRF GFCGSGPKYCAH	Antifungal	[18]

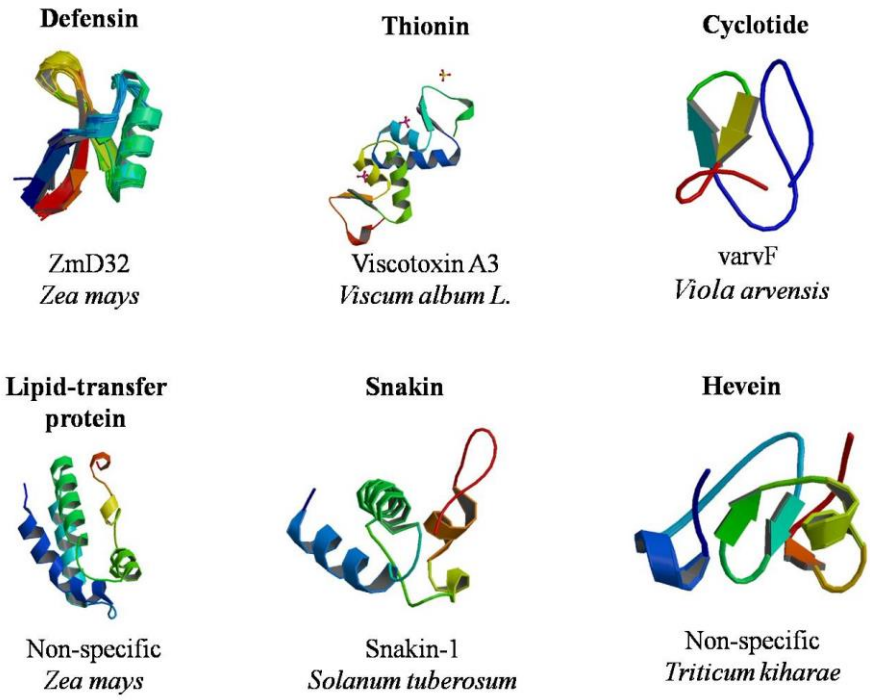

Figure 1: Three dimensional structures of some antimicrobial peptides (AMPs) belonging to different families, including defensin (PDB ID: 6DMZ), thionin (PDB ID: 1OKH), cyclotide (PDB ID: 3E4H), lipid-transfer protein (PDB ID: 1MZL), snakin (PDB ID: 5E5Q), hevein (PDB ID: 2LB7). All the structures were retrieved from the protein data bank (PDB).

the characteristic cysteine-stabilized alfa-beta (CS-αβ) motif [22]. Kerenga et al. [13] reported ZmD32 defensin isolated from *Zea mays* having three beta-strands and one helix in its structure. The highly conserved αβ conformation of defensin interacts with negatively charged membrane lipids, which increase cell permeability, thus causing cell death. Although defensins are well known for their antimicrobial action against numerous pathogens for a long time, they are now recognized for responding towards biotic stress and also play an important role in growth regulation and development. Recently, a novel plant defensin HXP124, having potent antifungal activity against dermatophytes and non-dermatophyte molds, was tested for a clinical trial in treating onychomycosis. The formulation is stable for 2 years and more efficient than the currently used antimycotic drugs [23].

Thionins

Thionins represent a family of smaller cysteine-enriched peptides ranging from 45 to 48 amino acids in length, having 5 kDa molecular weight, and are basic in nature. This family includes α1- and β- purothionins, α- and β-hordothionins, phoratoxin-A, *Pyrularia pubera* toxin, and viscotoxin A1, A3, and B2 [22]. The first AMP discovered in plants was purothionin isolated from wheat flour [24]. Besides, they have been identified in different organs, namely leaves, stems, roots, etc., of a wide range of monocotyledonous and dicotyledonous plant species. The α/β-thionins have shown a fold with two α-helices and a short antiparallel β-sheet, and its structure is stabilized by the presence of three or four conserved disulfide bonds [25]. These peptides possess antimicrobial activity against several Gram-positive and Gram-negative plant pathogenic bacteria and some phytopathogenic fungi. Thionins interact through charged residues, causing membrane disruption, or binds DNA through the structural helix-turn-helix motif, leading to cell death.

Cyclotides

Cyclotides are known to be important bactericidal peptides present in various plant families like *Rubiaceae*, *Violaceae*, *Apocynaceae*, *Cucurbitaceae*, and *Poaceae* [26]. These smaller (28 to 37 amino acids), low molecular weight (2.8–3.5 kDa) cyclic peptides have unique structural features, namely a head-to-tail cyclized backbone and a knotted arrangement of three disulfide bonds referred as a cyclic cystine knot (CCK) motif [25]. The presence of CCK framework in cyclotides confers thermal and chemical resistance and prevents them from proteolytic denaturation. Hence, these peptides can serve

as superior candidates in the development of peptide-based therapeutics in pharmaceuticals. Cyclotides are also known for their anti-tumor, anti-human immunodeficiency virus (HIV), nematicidal, hemolytic, and insecticidal activities. The antimicrobial action of cyclotides is associated with the electrostatic interaction between positively charged residues with the cell membrane causing disruption. Also, the presence of highly conserved Glu residue localized at loop 1 was found to play an important role in antimicrobial activities [27].

Lipid-Transfer Proteins

Plant lipid-transfer proteins (LTPs) are ubiquitous lipid-binding proteins involved in diverse stress responses besides holding antimicrobial activity [28]. They are expressed at different levels depending on the organs like roots, leaves, stems, seeds, and flower buds. These peptides are cationic with 70 to 95 amino acids in length (7–9 kDa) and are subdivided into two distinct families of LTP1 and LTP2. NMR spectroscopy and X-ray crystallography revealed the three-dimensional structure of LTPs, which consist of a conserved pattern of eight cysteine residues forming four disulfide bonds. The tertiary folded protein is formed by four α-helices linked by flexible loops with a hydrophobic cavity that comprises the lipid-binding site [29].

Snakins

Snakin peptides were first isolated from potato tubers having 63 amino acid residues with 6.9 kDa weight. Since then, homologous peptides have been isolated from other plant species exhibiting identical antimicrobial activity against various bacterial and fungal pathogens of plants [10]. All snakins have 12 conserved cysteine residues forming six disulfide bridges. Some of the snakins have shown functional similarity with hemotoxic snake venom. However, the exact mechanism of action of snakins still has to be revealed [27].

Heveins

The name, hevein peptide, was derived from the plant species, *Hevea brasiliensis* (rubber tree), after its isolation from the latex of this plant by Archer [30]. Hevein forms a cysteine-enriched single-chain protein of 43 amino acids (4 kDa) that shares structural similarity with chitin-binding domains of lectins and enzymes. The hevein domain is composed of an antiparallel β-sheet and occasional short α-helices, stabilized by three to five disulfide bonds, and exhibit antifungal properties [27]. Although hevein-like peptides have shown sequence homology, they are cationic and possess higher antifungal potency than hevein. Hevein-like peptides have been isolated from different plant species like *Pharbitis nil* [31], *Amaranthus caudatus* [32], *Beta vulgaris* [33], etc.

Mechanism of Action

Several mechanisms have been projected, providing insights to understand the molecular mechanisms responsible for the antimicrobial activity of AMPs. The electrostatic interaction between cationic residues of AMPs with the negatively charged plasma membrane causing membrane disruption is universally accepted [4]. Hall et al. [34] reported that interactions of AMP with the membrane depends on several factors like size, residue composition, overall charge, secondary structure, hydrophobicity, and amphiphilic characters and is highly selective towards specific membrane type, which prevents mammalian cells from the action of AMPs. Linear AMPs, mostly α-helix, including magainin (skin) and cecropin (hemolymph), usually adopt a helical structure upon binding to the membranes of pathogens. This conformational change helps the peptide to bind between the hydrophilic head groups and the fatty acyl chains of the membrane phospholipids, followed by the formation of pores. The β-sheet peptides, including plant defensins, have comparatively ordered structures, and the extent of rigidity depends on the presence of intramolecular disulfide bonds [35]. The penetration of AMP inside the membrane of the pathogen has been depicted by four existing models. The toroidal-pore model states that the insertion of peptides forces the phospholipid to bend continuously from one leaflet to the other, resulting in a pore. The barrel-stave model proposes that peptides are perpendicularly incorporated in the membrane, creating a pore. In the carpet model, peptides accumulate on the surface, causing tension, which leads to membrane disruption. Finally, in the aggregate-channel model, peptides bind to the head groups of phospholipids in the lipid bilayer and are inserted into the membrane by randomly aggregating with lipids creating channels for leakage. Upon penetration inside a cell, AMP exhibits several functions like inhibition of synthesis of DNA, mRNA, and proteins, inhibition of cell wall synthesis, activation of enzymes, etc. [36].

Besides targeting a variety of metabolic processes inside the organism, AMPs also have the potential to modulate immune cells by stimulating cytokine production, activating natural killer cells, repressing inflammation, killing cancer cells, promoting wound healing, and up-regulating the synthesis of antibodies [37]. Several plant-derived peptides like Cn-AMP1 from *Cocos nucifera*, LTP from *Capsicum annuum*, Zip1 from *Zea mays* possessing immunomodulatory activities have been reported in the literature.

Databases are Repository to AMPs

The rising interest in AMPs has motivated researchers to build up databases, providing information on nomenclature, classification, prediction, design, and statistics of AMPs. Some of the recently used databases, which provide general information on AMPs from organisms, are listed in Table 2.

Table 2: Commonly used databases, providing information about AMPs.

Database	Description	Website	Reference
dbAMP	useful for identifying antimicrobial peptides using transcriptomic and proteomic data	http://140.138.77.240/~dbamp/index.php	[1]
APD3	contains a database of peptides, prediction tools, and classification schema	http://aps.unmc.edu/AP/main.php	[38]
DBAASP	specializes in modeling structures of AMPs to help predict therapeutic potential for these compounds	https://dbaasp.org/	[39]
$CAMP_{R3}$	focuses on sequence patterns and hidden Markov models (HMMs) for AMPs	http://www.camp.bicnirrh.res.in/	[40]
ADAM	establishes associations between AMP sequences and structures	http://bioinformatics.cs.ntou.edu.tw/ADAM	[41]
LAMP	integrated open-access database, linking AMPs	http://biotechlab.fudan.edu.cn/database/lamp/	[42]
YADAMP	facilitates access to critical information on AMPs against bacteria	https://omictools.com/yadamp-tool	[43]
AMPer	contains an annotated set of antimicrobial database	https://omictools.com/amper-tool	[44]

Other databases focus on specific species, namely PhytAMP (Plantae), BACTIBASE (bacteria), PenBase (shrimp), Peptaibols (fungi), etc. These databases retrieve peptide information from PubMed, PDB, Google, and Swiss-Prot. The criteria for listing a peptide as AMP depend on the following factors:

- It should belong to a natural source
- It should possess antimicrobial activity with minimum inhibitory concentration (MIC) <100 µM
- The complete or at least partial amino acid sequence of the peptide should be known
- The length of the peptide should be <100 amino acids

The latest updated version of the AMP database is the APD3 database, including a total of 2619 natural AMPs obtained from different organisms, a larger part of which is shared by animal host defense peptides followed by peptides isolated from plants (Figure 2). Although most of the AMP databases provide the maximum information about the sequence, structure, and function of these peptides, there exist certain limitations. None of these databases include all the AMPs described in the literature [45]. They fail to provide necessary information like MIC, sequence-structure relationship, determination of secondary structures, and folding in some peptides [41,43]. Therefore, intensive research on the construction of advanced databases is ongoing, which would offer more detailed insight into understanding AMPs.

Role of Bioinformatics in the Development of AMPs

The enriched biodiversity of plants and widely available data on plant genomes unlock new opportunities to discover AMPs. Plant biodiversity remains largely unexplored for drug development and other potential applications [28]. Recent years have witnessed the advent in bioinformatics, allowing researchers to predict and screen AMPs from the vast genetic pool of plants. Various bioinformatics tools can be applied for searching AMPs using the genomic, transcriptional, proteomic, and metabolomic datasets of cultivated or wild plant species available in extensive databases. Currently, a total of 271 AMPs has been reported in PhytAMP database belonging to various plant families, such as *Amaranthaceae*, *Andropogoneae*, *Brassicaceae*, *Oryzeae*, *Santalaceae*, *Triticeae*, etc. The number of different classes of AMPs reported in some of the common plant species has been given in Table 3.

The growing volume of transcriptomic data available for several plant species augments the development of reliable algorithms for the prediction of potential AMPs and not just restricting the prediction of AMPs in model organisms. *In silico* modeling uses a variety of computational approaches, which can accelerate the process of antimicrobial drug

discovery and design [46]. Almaghrabi et al. [47] characterized thionin genes in *Arabidopsis* using *in silico* methods. Ramada et al. [48] developed a computer program named Kamal for the prediction of AMP sequences derived from internal sequences of plant proteins. In another report, Wang et al. [9] used a sequence alignment method for the prediction of AMPs. Additionally, the *in silico* approaches have also been successful in investigating the structure-function relationships of peptides used as antimicrobial agents. Porto and Franco [49] used *ab initio* method and comparative molecular modeling for the structure prediction of snakin-1. Melo et al. [50] predicted the structure and function of 2S albumin sequences characterizing its antimicrobial property. Recently, Shelenkov et al. [51] developed Cysmotif Searcher Pipeline to search and identify various AMPs in plant transcriptomes. Modern techniques, such as second-generation sequencing and peptidomic analysis, are commonly used for the analysis of AMP repertoire in insects [52,53] and other invertebrates. However, these methods fall short when applied to plants because of their extreme divergence. Moreover, bioinformatics tools are restricted to species whose "-Omic" data are available; otherwise, only heterology-based analysis is feasible [28]. More opportunities in the field need to be explored to design AMPs as there is only a little information available about the function and antimicrobial action of many peptides against target organisms.

Plant AMPs as Therapeutics

In the history of pharmacy, plant-derived products are known to play an important role in sustaining human health. The use of medicinal plants to relieve illness occupies an important niche in modern medicine [54]. The expanding biotechnological epoch urges researchers to explore novel antimicrobial drugs against dangerous pathogens infecting animals and plants. Also, the rising prevalence of multiple drug-resistant organisms is causing a clinical burden on researchers and pharmaceuticals. Therefore, the emergence of plant-based AMPs as novel potential antibiotic alternatives has drawn much attention towards their therapeutic use in human healthcare and agricultural industry.

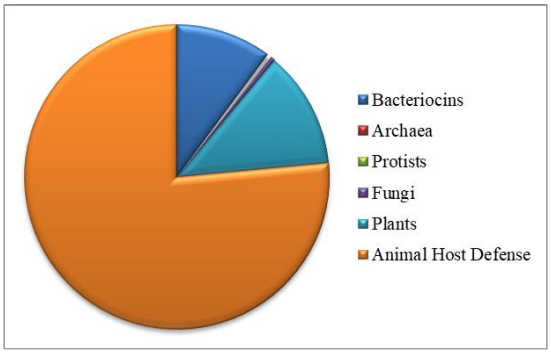

Figure 2: Pie chart representing the ratio of AMPs isolated from different organisms.

A considerable amount of losses in cultivated and stored crops occur due to the invasion of phytopathogens, affecting worldwide food distribution. To cope with this, crop protection relies mainly on chemical antimicrobials and pesticides, which increases production costs and causes environment pollution [22]. The topical application of AMPs is limited by higher production costs and decreased efficacy. One of the major prospective applications of AMPs is the use of genetic engineering to develop pathogen-resistant crops. Plant AMPs are encoded by small genes having conserved sequences, thus

Table 3: List of different families of AMPs reported on some of the common plant species as updated in the PhytAMP database.

Plant Species	Family of AMP					
	Defensin	Thionin	Lipid-Transfer	Snakin	Hevein	Other
Arabidopsis thaliana	14	6	3	4	-	-
Brassica napus	1	-	-	-	-	-
Zea mays	2	-	4	1	-	1
Oryza sativa	-	7	3	1	-	-
Capsicum annuum	2	-	-	-	-	-
Nicotiana tabacum	1	-	1	-	-	-
Allium cepa	-	-	1	-	-	-
Medicago sativa	1	-	-	-	-	-
Triticum aestivum	2	3	4	-	-	-
Beta vulgaris	2	-	2	-	1	-
Sorghum bicolour	-	-	2	-	-	-

they can be feasibly used in transgenesis to increase production and enhance the specific activity of selected peptides [10]. Studies have demonstrated that several transgenic plants expressing AMPs can confer different degrees of protection against diseases. Recently, Ghag et al. [55] cloned Sm-AMP-D, a defensin from chickweed, and expressed in transgenic banana plants, which showed high resistance to *Fusarium oxysporum*. Lacerda et al. [56] reported the expression of the defensin gene in transgenic *Pichia pastoris*, leading to enhanced resistance against obligate biotrophic fungi *Fusarium tucumaniae* and *Colletotrichum gossypii var*. Other scientists have also reported the expression of defensins in transgenic plants like tomato, tobacco, potato, and alfalfa, conferring resistance against fungal pathogens [57–59]. Similarly, thionins, cyclotides, protein transduction domains, and cell-penetrating peptides are other important peptides used for the development of transgenic plants. The success rate of transgenic technology using AMPs to improve disease resistance depends upon the recipient host as well as the source of the antimicrobial gene. Although the use of transgenic technology gives promising results, the overexpression of AMPs in plants by the use of constitutive promoters has an adverse effect on plant growth and development [60]. Unfortunately, commercial cultivars are unable to reach the market because of regulatory limitations and social concerns. Ongoing genetic improvement in plants may increase their pathogenic resistance and reduce crop losses in agriculture.

Plant AMPs have also gained attention in human health since they display antimicrobial activity not only against plant pathogens but also against human pathogens. Besides, these peptides have also proven to be effective analgesics, immunomodulators, and in the treatment of neurological disorders. Some studies have reported antimicrobial activities of AMPs isolated from fruits, which can be used in the treatment of infectious diseases caused by *Escherichia coli* and *Staphylococcus aureus* [61]. Some plant defensins, such as the phaseococcin, sesquin, and lunatusin, are reported to exhibit inhibition towards HIV as they restrain the activity of the viral reverse transcriptase [62]. Several AMPs with anti-infective activities have also been developed in order to investigate their antimicrobial mechanisms for therapeutic use [63]. Scientific reports describe different methods for producing AMPs, such as the isolation of the natural peptides from the part of the plant, where they are synthesized, but this method is limited because of the cost associated with the purification of these molecules in the active form. Also, the presence of these peptides in relatively low concentration limits their usage [27]. Another method makes use of chemical synthesis of peptides. The manipulation of chemical structure to create synthetic peptides represents a promising strategy for the development of AMPs, but constructing the correctly folded peptide showing functionality remains a major challenge for the researchers [54]. Recent years have witnessed the exploitation of the prokaryotic system (*E. coli*) for the expression of heterologous AMPs from plant sources. But there are several drawbacks associated with this technique like incorrect folding and interrupted post-translational modification in the prokaryotic system. The toxicity imposed by these peptides on host cells can be overcome by fusing peptides with different functional molecules for the development of a single compound exhibiting higher efficiency toward human pathogenic bacteria. Nowadays, the expression of these peptides in yeasts (*Pichia pastoris*) and plants (*Pisum sativum, Arabidopsis*) has been focused on improving their antimicrobial effectiveness [21].

Despite the ongoing efforts, there is no AMP agent currently approved by the Food and Drug Administration (FDA). Most AMPs undergoing clinical trials fall apart because of their susceptibility towards proteases, short half-life, cytotoxic effects imposed during systemic administration, and rapid clearance and degradation when administered orally [64]. Another major obstacle associated with these peptides is the cost of production, which is estimated to be roughly $50–$400 per 1 gram of amino acid when running commercial quantities. The stability of peptides can be improved by introducing unusual amino acids (mainly D-form amino acids) or modifying the terminal regions (acetylation or amidation) of peptides [65], but the synthesis of D-amino acids itself adds cost to the process. Alternatively, AMPs can be conjugated with various inorganic materials, such as silica, metal nanoparticles, carbon nanotubes, and polymers like poly(lactic-co-glycolic acid) (PLGA), polyethylene glycol (PEG), hydrogels, to enhance their stability and help in delivery into the host cells [64]. Conjugation of peptides with polymers increases viscosity in an aqueous medium, which in turn increases molecular weight. The use of efficient drug delivery systems, such as liposome encapsulation, can also prove to be effective for increasing stability and reducing the toxicity of these peptides, while the high production cost can be cut down by reducing the peptide size without losing activity [65]. Besides, *in silico* methods like sequence optimization of motifs, studying post-translational changes due to glycosylation can be used to produce tailored peptides with improved biological activity and production efficiency [66]. Advancement in drug delivery systems, use of nanotechnology for targeted therapy, exploration of expression systems can serve as promising alternatives for the development of AMPs that may substitute traditional antibiotics in the future.

Conclusion

The gigantic pool of plant AMPs having varied amino acid composition and diversified structures can exhibit potent broad-spectrum antimicrobial activities. The exhaustive genomic and proteomic data available for plant species has assisted in discovering AMPs encrypted in protein sequences followed by their expression and utility in constructing therapeutic drugs. Researchers have developed rapid screening

methods to build various databases that can provide updated information on these peptides. The increasing availability and use of innovative computer-assisted design strategies have considerable potential to boost the discovery of next-generation therapeutic peptides. Also, understanding the key element of their structure and function opens new opportunities for the treatment of infections caused by numerous animal and plant pathogens. Several AMPs are currently undergoing clinical trials validating the therapeutic benefits of these novel candidates, promising market authorization of new AMP-based drugs in the near future. Therefore, plant AMPs can serve as valuable natural alternatives to conventional antibiotics for curing human diseases and protect plants used in agriculture, thus increasing crop yields.

Acknowledgments

Authors acknowledge the facilities of the Department of Biotechnology, Ministry of Science and Technology, Government of India, New Delhi (DBT) provided under the Distributed Bioinformatics Sub Center and M.Sc. Biotechnology program in the School of Biotechnology, Devi Ahilya University, and used in this study.

Conflicts of Interest

The authors declare that they have no conflict of interest.

References

[1] Jhong, J.H., Chi, Y.H., Li, W.C., Lin, T.H., Huang, K.Y. and Lee, T.Y. (2019) dbAMP: an integrated resource for exploring antimicrobial peptides with functional activities and physicochemical properties on transcriptome and proteome data. *Nucleic Acids Res* 47: D285–D297. https://doi.org/10.1093/nar/gky1030

[2] Conlon, J.M., Mechkarska, M., Radosavljevic, G., Attoub, S., King, J.D., Lukic, M.L. and McClean, S. (2014) A family of antimicrobial and immunomodulatory peptides related to the frenatins from skin secretions of the Orinoco lime frog *Sphaenorhynchus lacteus* (Hylidae). *Peptides* 56: 132–140. https://doi.org/10.1016/j.peptides.2014.03.020

[3] Roudi, R., Syn, N.L. and Roudbary, M. (2017) Antimicrobial peptides as biologic and immunotherapeutic agents against cancer: a comprehensive overview. *Front Immunol* 8: 1320. https://doi.org/10.3389/fimmu.2017.01320

[4] Fjell, C.D., Hiss, J.A., Hancock, R.E. and Schneider, G. (2011) Designing antimicrobial peptides: form follows function. *Nat Rev Drug Discov* 11: 37–51. https://doi.org/10.1038/nrd3591

[5] Biswaro, L.S., da Costa Sousa, M.G., Rezende, T.M.B., Dias, S.C. and Franco, O.L. (2018) Antimicrobial peptides and nanotechnology, recent advances and challenges. *Front Microbiol* 9: 855. https://doi.org/10.3389/fmicb.2018.00855

[6] Lopez-Abarrategui, C., Alba, A., Silva, O.N., Reyes-Acosta, O., Vasconcelos, I.M., Oliveira, J.T., Migliolo, L., Costa, M.P., Costa, C.R., Silva, M.R., Garay, H.E., Dias, S.C., Franco, O.L. and Otero-Gonzalez, A.J. (2012) Functional characterization of a synthetic hydrophilic antifungal peptide derived from the marine snail *Cenchritis muricatus*. *Biochimie* 94: 968–974. https://doi.org/10.1016/j.biochi.2011.12.016

[7] Bamdad, F., Sun, X., Guan, L.L. and Chen, L. (2015) Preparation and characterization of antimicrobial cationized peptides from barley (*Hordeum vulgare L.*) proteins. *LWT - Food Sci Technol* 63: 29–36. https://doi.org/10.1016/j.lwt.2015.03.012

[8] Salas, C.E., Badillo-Corona, J.A., Ramírez-Sotelo, G. and Oliver-Salvador, C. (2015) Biologically active and antimicrobial peptides from plants. *Biomed Res Int* 2015*:* 102129. https://doi.org/10.1155/2015/102129

[9] Wang, P., Hu, L., Liu, G., Jiang, N., Chen, X., Xu, J., Zheng, W., Li, L., Tan, M., Chen, Z., Song, H., Cai, Y.D. and Chou, K.C. (2011) Prediction of antimicrobial peptides based on sequence alignment and feature selection methods. *PLoS ONE* 6: e18476. https://doi.org/10.1371/journal.pone.0018476

[10] Nawrot, R., Barylski, J., Nowicki, G., Broniarczyk, J., Buchwald, W. and Gozdzicka-Jozefiak, A. (2014) Plant antimicrobial peptides. *Folia Microbiol* (Praha) 59: 181–196. https://doi.org/10.1007/s12223-013-0280-4

[11] Pelegrini, P.B., Del Sarto, R.P., Silva, O.N., Franco, O.L. and Grossi-de-Sa, M.F. (2011) Antibacterial peptides from plants: What they are and how they probably work. *Biochem Res Int* 2011: 250349. http://dx.doi.org/10.1155/2011/250349

[12] Seyedjavadi, S.S., Khani, S., Zare-Zardini, H., Halabian, R., Goudarzi, M., Khatami, S., Imani Fooladi, A.A., Amani, J. and Razzaghi-Abyaneh, M. (2019) Isolation, functional characterization, and biological properties of MCh-AMP1, a novel antifungal peptide from *Matricaria chamomilla* L. *Chem Biol Drug Des* 93: 949–959. https://doi.org/10.1111/cbdd.13500

[13] Kerenga, B.K., McKenna, J.A., Harvey, P.J., Quimbar, P., Garcia-Ceron, D., Lay, F.T., Phan, T.K., Veneer, P.K., Vasa, S., Parisi, K., Shafee, T.M.A., van der Weerden, N.L., Hulett, M.D., Craik, D.J., Anderson, M.A. and Bleackley, M.R. (2019) Salt tolerant antifungal and antibacterial activities of the corn defensin ZmD32. *Front Microbiol* 10: 795. https://doi.org/10.3389/fmicb.2019.00795

[14] Farkas, A., Maroti, G., Kereszt, A. and Kondorosi, E. (2017) Comparative analysis of the bacterial membrane disruption effect of two natural plant antimicrobial peptides. *Front Microbiol* 8: 51.

https://doi.org/10.3389/fmicb.2017.00051

[15] Zhao, S., Han, J., Bie, X., Lu, Z., Zhang, C. and Lv, F. (2016) Purification and characterization of plantaricin JLA-9: A novel bacteriocin against *Bacillus* spp. produced by *Lactobacillus plantarum* JLA-9 from Suan-Tsai, a traditional chinese fermented cabbage. *J Agric Food Chem* 64: 2754–2764. https://doi.org/10.1021/acs.jafc.5b05717

[16] Vriens, K., Peigneur, S., De Coninck, B., Tytgat, J., Cammue, B.P. and Thevissen, K. (2016) The antifungal plant defensin AtPDF2.3 from *Arabidopsis thaliana* blocks potassium channels. *Sci Rep* 6: 32121. https://doi.org/10.1038/srep32121

[17] Vieira Bard, G.C., Nascimento, V.V., Ribeiro, S.F., Rodrigues, R., Perales, J., Teixeira-Ferreira, A., Carvalho, A.O., Fernandes, K.V. and Gomes, V.M. (2015) Characterization of peptides from *Capsicum annuum* hybrid seeds with inhibitory activity against α-amylase, serine proteinases and fungi. *Protein J* 34: 122–129. https://doi.org/10.1007/s10930-015-9604-3

[18] Rogozhin, E.A., Slezina, M.P., Slavokhotova, A.A., Istomina, E.A., Korostyleva, T.V., Smirnov, A.N., Grishin, E.V., Egorov, T.A. and Odintsova, T.I. (2015) A novel antifungal peptide from leaves of the weed *Stellaria media* L. *Biochimie* 116: 125–132. https://doi.org/10.1016/j.biochi.2015.07.014

[19] Samriti, Biswas, R. and Biswas, K. (2018) Plant antimicrobial peptides: a novel approach against drug resistant microorganisms. *Int J Pharm Sci Res* 9: 1–15. https://doi.org/10.13040/IJPSR.0975-8232.9(1).1-15

[20] Do, H.M., Lee, S.C., Jung, H.W., Kee, H.S. and Hwang, B.K. (2004) Differential expression and in situ localization of a pepper defensin (CADEF1) gene in response to pathogen infection, abiotic elicitors and environmental stresses in *Capsicum annuum*. *Plant Sci* 166: 1297–1305. https://doi.org/10.1016/j.plantsci.2004.01.008

[21] Carvalho Ade, O. and Gomes, V.M. (2009) Plant defensins- prospects for the biological functions and biotechnological properties. *Peptides* 30: 1007–1020. https://doi.org/10.1016/j.peptides.2009.01.018

[22] Lopez-García, B., San Segundo, S. and Coca, M. (2012) 'Antimicrobial peptides as a promising alternative for plant disease protection'. In Small Wonders: Peptides for Disease Control (Raja Sekaran K, Cary JW, Jaynes J, Montesinos E, Eds). American Chemical Society, Washington DC, USA, 263–294. https://doi.org/10.1021/bk-2012-1095.ch013

[23] Sher Khan, R, Iqbal, A., Malak, R., Shehryar, K., Attia, S., Ahmed, T., Ali Khan, M., Arif, M. and Mii, M. (2019) Plant defensins: types, mechanism of action and prospects of genetic engineering for enhanced disease resistance in plants. *3 Biotech* 9: 192. https://doi.org/10.1007/s13205-019-1725-5

[24] Hammami, R., Ben Hamida, J, Vergoten, G. and Fliss, I. (2009) PhytAMP: a database dedicated to antimicrobial plant peptides. *Nucleic Acids Res* 37: D963–D968. https://doi.org/10.1093/nar/gkn655

[25] Candido, E.S., Porto, W.F., Amaro, D.S., Viana, J.C., Dias, S.C. and Franco, O.L. (2011) Structural and functional insights into plant bactericidal peptides. In Science against microbial pathogens: communicating current research and technological advances (Mendez-Vilas A, Ed). Formatex Research Center, 951–960.

[26] Mylne, J.S., Wang, C.K., van der Weerden, N.L. and Craik, D.J. (2010) Cyclotides are a component of the innate defense of *Oldenlandia affinis. Biopolymers* 94: 635–646. https://doi.org/10.1002/bip.21419

[27] De Souza Candido, E., e Silva Cardoso, M.H., Sousa, D.A., Viana, J.C., de Oliveira-Junior, N.G., Miranda, V. and Franco, O.L. (2014) The use of versatile plant antimicrobial peptides in agribusiness and human health. *Peptides* 55: 65–78. https://doi.org/10.1016/j.peptides.2014.02.003

[28] Pestana-Calsa, M.C. and Calsa Jr., T. (2011) In silico identification of plant-derived antimicrobial peptides. In Systems and Computational Biology – Molecular and Cellular Experimental Systems (Yang NS, Ed). IntechOpen. https://doi.org/10.5772/21172

[29] Carvalho Ade, O. and Gomes, V.M. (2007) Role of plant lipid transfer proteins in plant cell physiology-a concise review. *Peptides* 28: 1144–1153. https://doi.org/10.1016/j.peptides.2007.03.004

[30] Archer, B.L. (1960) The proteins of *Hevea brasiliensis* latex. 4. Isolation and characterization of crystalline hevein. *Biochem J* 75: 236–40. https://doi.org/10.1042/bj0750236

[31] Koo, J.C., Lee, S.Y., Chun, H.J., Cheong, Y.H., Choi, J.S., Kawabata, S., 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. *Biochim Biophys Acta* 1382: 80–90. https://doi.org/10.1016/s0167-4838(97)00148-9

[32] Broekaert, W.F., Marien, W., Terras, F.R., De Bolle, M.F., Proost, P., Van Damme, J., Dillen, L., Claeya, M., Rees, S.B., Vanderleyden, J. et al. (1992) Antimicrobial peptides from *Amaranthus caudatus* seeds with sequence homology to the cysteine/glycine-rich domain of chitin-binding proteins. *Biochemistry* 31: 4308–4314. https://doi.org/10.1021/bi00132a023

[33] Nielsen, K.K., Nielsen, J.E., Madrid, S.M. and Mikkelsen, J.D. (1997) Characterization of a new antifungal chitin-binding peptide from sugar beet leaves. *Plant Physiol* 113: 83–91. https://doi.org/10.1104/pp.113.1.83

[34] Hall, K., Mozsolits, H. and Aguilar, M.I. (2003) Surface plasmon resonance analysis of antimicrobial peptide-

membrane interactions: affinity & mechanism of action. *Lett Pept Sci* 10: 475–485. https://doi.org/10.1007/BF02442579

[35] Kang, S.J., Park, S.J., Mishig-Ochir, T. and Lee, B.J. (2014) Antimicrobial peptides: therapeutic potentials. *Expert Rev Anti Infect Ther* 12: 1477–1486. https://doi.org/10.1586/14787210.2014.976613

[36] Cruz, J., Ortiz, C., Guzman, F., Fernandez-Lafuente, R. and Torres, R. (2014) Antimicrobial peptides: promising compounds against pathogenic microorganisms. *Curr Med Chem* 21: 2299–2321. https://doi.org/10.2174/09298673216661402171101 55

[37] Maestri, E., Marmiroli, M. and Marmiroli, N. (2016) Bioactive peptides in plant-derived foodstuffs. *J Proteomics* 147: 140–155. https://doi.org/10.1016/j.jprot.2016.03.048

[38] Wang, G., Li, X. and Wang, Z. (2016) APD3: the antimicrobial peptide database as a tool for research and education. *Nucleic Acids Res* 44: D1087–D1093. https://doi.org/10.1093/nar/gkv1278

[39] Pirtskhalava, M., Gabrielian, A., Cruz, P., Griggs, H.L., Squires, R.B., Hurt, D.E., Grigolova, M., Chubinidze, M., Gogoladze, G., Vishnepolsky, B., Alekseyev, V., Rosenthal, A. and Tartakovsky, M. (2016) DBAASP v.2: an enhanced database of structure and antimicrobial/cytotoxic activity of natural and synthetic peptides. *Nucleic Acids Res* 44: D1104–D1112. https://doi.org/10.1093/nar/gkv1174

[40] Waghu, F.H., Gopi, L., Barai, R.S., Ramteke, P., Nizami, B. and Idicula-Thomas, S. (2014) CAMP: collection of sequences and structures of antimicrobial peptides. *Nucleic Acids Res* 42: D1154–D1158. https://doi.org/10.1093/nar/gkt1157

[41] Lee, H.T., Lee, C.C., Yang, J.R., Lai, J.Z.C. and Chang, K.Y. (2015) A large scale structural classification of antimicrobial peptides. *BioMed Res Int* 2015: 475062. https://doi.org/10.1155/2015/475062

[42] Zhao, X., Wu, H., Lu, H., Li, G. and Huang, Q. (2013) LAMP: A database linking antimicrobial peptides. *PLoS One* 8: e66557. https://doi.org/10.1371/journal.pone.0066557

[43] Piotto, S.P., Sessa, L., Concilio, S. and Iannelli, P. (2012) YADAMP: yet another database of antimicrobial peptides. *Int J Antimicrob Agents* 39: 346–351. https://doi.org/10.1016/j.ijantimicag.2011.12.003

[44] Fjell, C.D., Hancock, R.E. and Cherkasov, A. (2007) AMPer: a database and an automated discovery tool for antimicrobial peptides. *Bioinformatics* 23: 1148–1155. https://doi.org/10.1093/bioinformatics/btm068

[45] Thomas, S., Karnik, S., Barai, R.S., Jayaraman, V.K. and Idicula-Thomas, S. (2010) CAMP: a useful resource for research on antimicrobial peptides. *Nucleic Acids Res* 38: D774–D780. https://doi.org/10.1093/nar/gkp1021

[46] Hammami, R. and Fliss, I. (2010) Current trends in antimicrobial agent research: chemo- and bioinformatics approaches. *Drug Discov Today* 15: 540–546. https://doi.org/10.1016/j.drudis.2010.05.002

[47] Almaghrabi B., Ali, M.A., Zahoor A., Shah, K.H. and Bohlmann., H. (2019) Arabidopsis thionin-like genes are involved in resistance against the beet-cyst nematode (*Heterodera schachtii*). *Plant Physiol Biochem* 140: 55–67. https://doi.org/10.1016/j.plaphy.2019.05.005

[48] Ramada, M.H.S., Brand, G.D., Abrao, F.Y., Oliveira, M., Cardozo Filho, J.L., Galbieri, R., Gramacho, K.P., Prates, M.V. and Bloch Jr., C. (2017) Encrypted antimicrobial peptides from plant proteins. *Sci Rep* 7: 13263. https://doi.org/10.1038/s41598-017-13685-6

[49] Porto, W.F. and Franco, O.L. (2013) Theoretical structural insights into the snakin/GASA family. *Peptides* 44: 163–167. https://doi.org/10.1016/j.peptides.2013.03.014

[50] Melo, F.R., Rigden, D.J., Franco O.L., Mello, L.V., Ary, M.B., Grossi de Sa, M.F. and Bloch Jr., C. (2002) Inhibition of trypsin by cowpea thionin: characterization, molecular modeling, and docking. *Proteins* 48: 311–319. https://doi.org/10.1002/prot.10142

[51] Shelenkov, A.A., Slavokhotova, A.A. and Odintsova, T.I. (2018) Cysmotif searcher pipeline for antimicrobial peptide identification in plant transcriptomes. *Biochemistry (Mosc)* 83: 1424–1432. https://doi.org/10.1134/S0006297918110135

[52] Wang, M. and Hu, X. (2017) Antimicrobial peptide repertoire of *Thitarodes armoricanus*, a host species of *Ophiocordyceps sinensis*, predicted based on de novo transcriptome sequencing and analysis. *Infect Genet Evol* 54: 238–244. https://doi.org/10.1016/j.meegid.2017.07.011

[53] Kim, I.W., Markkandan, K., Lee, J.H., Subramaniyam, S., Yoo, S., Park, J. and Hwang, J.S. (2016) Transcriptome profiling and in silico analysis of the antimicrobial peptides of the grasshopper *Oxya chinensis sinuosa*. *J Microbiol Biotechnol* 26: 1863–1870. https://doi.org/10.4014/jmb.1608.08029

[54] Candido Ede, S., Pinto, M.F., Pelegrini, P.B., Lima, T.B., Silva, O.N., Pogue, R., Grossi-de-Sá, M.F. and Franco, O.L. (2011) Plant storage proteins with antimicrobial activity: novel insights into plant defense mechanisms. *FASEB J* 25: 3290–3305. https://doi.org/10.1096/fj.11-184291

[55] Ghag, S.B., Shekhawat, U.K.S. and Ganapathi T.R. (2014) Transgenic banana plants expressing a Stellaria media defensin gene (*Sm-AMP-D1*) demonstrate improved resistance to *Fusarium oxysporum*. *Plant Cell Tissue Organ Cult* 119: 247–255. https://doi.org/10.1007/s11240-014-0529-x

[56] Lacerda, A.F., Del Sarto, R.P., Silva, M.S., de Vasconcelos, E.A.R., Coelho, R.R., Dos Santos, V.O.,

Godoy, C.V., Seixas, C.D.S., da Silva, M.C.M. and Grossi-de-Sa, M.F. (2016). The recombinant pea defensin Drr230a is active against impacting soybean and cotton pathogenic fungi from the genera *Fusarium*, *Colletotrichum* and *Phakopsora*. *3 Biotech* 6: 59. https://doi.org/10.1007/s13205-015-0320-7

[57] Schaefer, S.C., Gasic, K., Cammue, B., Broekaert, W., van Damme, E.J., Peumans, W.J. and Korban, S.S. (2005) Enhanced resistance to early blight in transgenic tomato lines expressing heterologous plant defense genes. *Planta* 222: 858–866. https://doi.org/10.1007/s00425-005-0026-x

[58] 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 Mol Biol* 50: 441–452. https://doi.org/10.1023/A:1019864222515

[59] 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. *Nat Biotechnol* 18: 1307–1310. https://doi.org/10.1038/82436

[60] Ali, S., Ganai, B.A., Kamili, A.N., Bhat, A.A., Mir, Z.A., Bhat, J.A., Tyagi, A., Islam, S.T., Mushtaq, M., Yadav, P., Rawat, S. and Grover, A. (2018) Pathogenesis-related proteins and peptides as promising tools for engineering plants with multiple stress tolerance. *Microbial Res* 212–213: 29–37. https://doi.org/10.1016/j.micres.2018.04.008

[61] Taveira, G.B., Mathias, L.S., da Motta, O.V., Machado, O.L., Rodrigues, R., Carvalho, A.O., Teixeira-Ferreira, A., Perales, J., Vasconcelos, I.M. and Gomes, V.M. (2014) Thionin-like peptides from *Capsicum annuum* fruits with high activity against human pathogenic bacteria and yeasts. *Biopolymers* 102: 30–39. https://doi.org/10.1002/bip.22351

[62] Da Rocha Pitta, M.G., da Rocha Pitta, M.G. and Galdino, S.L. (2010) Development of novel therapeutic drugs in humans from plant antimicrobial peptides. *Curr Protein Pept Sci* 11: 236–247. https://doi.org/10.2174/138920310791112066

[63] Dutta, P. and Das, S. (2016) Mammalian antimicrobial peptides: promising therapeutic targets against infection and chronic inflammation. *Curr Top Med Chem* 16: 99–129. https://doi.org/10.2174/1568026615666150703121819

[64] Kumar, P., Kizhakkedathu, J.N. and Straus, S.K. (2018) Antimicrobial peptides: diversity, mechanism of action and strategies to improve the activity and biocompatibility in vivo. *Biomolecules* 8: E4. https://doi.org/10.3390/biom8010004

[65] Seo, M.D., Won, H.S., Kim, J.H., Mishig-Ochir, T. and Lee, B.J. (2012) Antimicrobial peptides for therapeutic applications: A review. *Molecules* 17: 12276–12286. https://doi.org/10.3390/molecules171012276

[66] Da Cunha, N.B., Cobacho, N.B., Viana, J.F.C., Lima, L.A., Sampaio, K.B.O., Dohms, S.S.M., Ferreira, A.C.R., de la Fuente-Nunez, C., Costa, F.F., Franco, O.L. and Dias, S.C. (2017) The next generation of antimicrobial peptides (AMPs) as molecular therapeutic tools for the treatment of diseases with social and economic impacts. *Drug Discov Today* 22: 234–248. https://doi.org/10.1016/j.drudis.2016.10.017