Microbiology Research Journal International

Volume 34, Issue 12, Page 55-75, 2024; Article no.MRJI.126274
ISSN: 2456-7043, NLM ID: 101726596
(Past name: British Microbiology Research Journal, Past ISSN: 2231-0886, NLM ID: 101608140)

Functional Peptides: Novel Tools for Controlling Plant Diseases

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

Article Information

DOI: https://doi.org/10.9734/mrji/2024/v34i121511

Open Peer Review History:
This journal follows the Advanced Open Peer Review policy. Identity of the Reviewers, Editor(s) and additional Reviewers, peer review comments, different versions of the manuscript, comments of the editors, etc are available here: https://www.sdiarticle5.com/review-history/126274

Review Article

Received: 11/09/2024
Accepted: 14/11/2024
Published: 05/12/2024

ABSTRACT

Chemical pesticides, which are today subject to stringent regulations and limits, are the primary means of controlling plant diseases. In terms of plant health, functional peptides are intriguing substances. Many novel synthetic and natural compounds have been found and employed in plant protection in recent years. Functional peptides are a good option among them to combat phytopathogens (Amso & Hayouka, 2019). Functional peptides are synthetic analogues or derived from living organisms, they offer new methods of action against plant diseases, making them potential biopesticide candidates. Functional peptides have long been proposed as potential antifungal and antibacterial agricultural agents (Van der Biezen, 2001). Functional peptides that target bacterial and fungal diseases have similar killing mechanisms despite their diverse sources. As summed up by numerous earlier reviews (Zasloff, 2002; Brogden, 2005; Melo et al., 2009; Bocchinfuso et al., 2011; Akalın Siben, 2014), to destroy infections, the majority of peptides target and breach the cell membrane directly. Peptides may be made available to the industry and

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Cite as: *Dole, Ayushi, Manish Maharania, Rahul Sahu, and Nidhee Yadav. 2024. "Functional Peptides: Novel Tools for Controlling Plant Diseases". Microbiology Research Journal International 34 (12):55-75. https://doi.org/10.9734/mrji/2024/v34i121511.*

growers on a big scale through chemical synthesis, biotechnological platforms, and natural sources. It is anticipated that a number of functional peptides may soon be offered for sale as plant disease control agents, although further research is required to confirm these peptides' effectiveness in real-world settings.

Keywords: Functional peptides; plant diseases; chemical pesticides; plant protection.

1. INTRODUCTION

Pesticides are a vital tool for protecting plants and are essential to agriculture and food security. The production of fruits and vegetables would decline by 78%, vegetables by 54%, and cereals by 32% if pesticides weren't used (Tudi et al., 2021). Pesticides help to boost crop yields globally, but they need to be updated to satisfy environmental safety regulations and agricultural development demands. Eco-friendly pesticides that are effective against pests and poses little threat to nontarget organisms are of paramount importance in the age of ecological agriculture, which emphasizes sustainable development. "The primary tenet of crop protection remains chemical control. However, due to the requirement to produce safe food and the fact that many pesticides have nontarget environmental consequences, several countries have limited the number and types of pesticides that are permitted. For instance, the European Union has mandated a significant decrease in the active ingredients in pesticides in recent years, and governments all over the world have followed suit. This allowed for the retention of more selective compounds with lower intrinsic toxicity and less detrimental effects on the environment. Following the restrictions' introduction, a number of pesticides were outlawed, and the absence of substances that effectively combat certain economically significant plant diseases has made management of these illnesses challenging. Several diseases may now be inadequately or completely uncontrolled as a result of the lack of adequate new chemicals, biopesticides, or effective cultural and management techniques to offset the decrease in the usage of conventional pesticides" (Zhang et al., 2023). Since there has traditionally been less bactericides than fungicides, the issue is more challenging when it comes to infections caused by bacteria than fungal diseases. Furthermore, a "number of regions have seen the establishment of new and re-emerging bacterial diseases of economic significance, such as bacterial leaf blight of rice (*Xanthomonas oryzae* pv. *oryzae*), bacterial wilt of tomato and potato (*Ralstonia solanacearum*), bacterial wilt of banana (*Xanthomonas vasicola* pv. *musacearum*), bacterial canker of kiwifruit (*Pseudomonas syringae* pv. *actini-diae*), bacterial blight of cassava (*Xanthomonas axonopodis* pv. *manihotis*), and fire blight of apple and pear (*Erwinia amylovora*) that have emerged and re-emerging bacterial diseases of economic importance" (Sundin et al., 2016).

Despite efforts by researchers to identify and develop new plant-protection products, pesticide companies are less interested in offering novel pesticides to growers due to the low return on investment (market value) and the difficulties in obtaining registration approval due to strict regulatory requirements. Additionally, a number of innovative disease control strategies are still being developed (such as RNA interference and defense elicitors), have safety issues (such as novel nanoparticle formulations), or have not undergone enough field testing and validation.

"In the realm of crop protection, functional peptides have been the focus of intense investigation as in medicine or food industry (Rajasekaran et al., 2012). Peptides are considered polypeptides of up to 50–60 amino acids (upper size limit considered as big peptides or small proteins) but also comprise pseudopeptides containing peptide bonds, non-natural or modified amino acids. The majority of peptides are derived from living things, and they have an antagonistic or antibiosis effect on microorganisms. Many different kinds of species, including humans, plants, animals, and invertebrates, form functional peptides which can be antimicrobial to ward off infection. Their defense systems work against pathogens in many ways" (Brogden, 2005). "They also form the first line of defense against stress in both plants and animals, as well as the immune system (Huan et al., 2020). AMPs have been reviewed in bacteria (Jack & Jung, 2000; Cooter et al., 2005; Raaijmakers et al., 2006), fungi (Degenkolb et al., 2003; Ng, 2004), insects (Hancock, 2001; Bulet et al., 2004), marine invertebrates (Tincu & Taylor, 2004), amphibians and mammals (Andreu & Rivas, 1998; Zasloff, 2002; Toke, 2005), plants (Garc´ıa-Olmedo et al.,

1998; Lay & Anderson, 2005).Based on their structural traits, the approximately 900 AMPs that have been described can be categorized into three groups: linear peptides that often take on helical shapes, cysteine-rich open-ended peptides with disulphide bridges, and cyclopeptides that form peptide rings. Peptides, which are mostly obtained from living organisms, are essential for lowering stress levels in both plants and animals. As the initial line of defense against bacteria, they function by either antagonistic or antibiosis. The present review summarizes the overview, steps involved in identifying and producing novel compounds and understanding their methods of action. It also discusses the existing knowledge of peptides that target plant pathogens and the crop diseases they cause, the production technologies that can be used, and the challenges and possibilities involved in developing novel biopesticides.

2. A COMPREHENSIVE OVERVIEW OF PEPTIDES

The earliest known peptide was secretin, which Bayliss and Starling discovered in animal gastrointestinal systems in 1902. (Tam et al., 2014). Later studies showed that oxytocin, which stimulates uterine contraction, and insulin (Muttenthaler et al., 2021), which lowers blood sugar, are examples of functional peptides. The foundation for automated synthesis was established in 1963 by solid phase peptide synthesis (SPPS), which was faster and easier to utilize than conventional liquid phase synthesis. For this innovation, Merrifield, the man behind SPPS, received the 1984 Chemistry Nobel Prize. Since then, there has been significant advancement in the field of peptide studies. Ten Nobel Prizes have been awarded to peptides, indicating their significant role in science and technology. The discovery of epidermal growth factor (EGF), a 53-amino acid peptide that stimulates the proliferation of skin and corneal cells, was made in 1986, and the person who made the discovery was granted the Nobel Prize in Physiology or Medicine. By controlling cellular transport and localization, signal peptides enable more efficient utilization of cells as "protein factories" for the manufacture of medications. For this discovery, they were awarded the 1999 Nobel Prize in Physiology or Medicine. The 2018 Chemistry Nobel Prize was given in recognition of the discovery of peptides manufactured by phages that can be used to treat autoimmune disorders. Peptides are particularly useful for protecting plants; this was highlighted by the 2020 Presidential Green Chemistry Challenge Award for the neuropeptide-based bio insecticide Spear®.

3. MICROORGANISM-DERIVED ANTIMICROBIAL PEPTIDES

Numerous antimicrobial peptides are produced by microorganisms, including secondary metabolites like peptaibols, cyclopeptides, and pseudopeptides that are produced by non-ribosomal synthesis as well as small bacteriocins and fungal defensins that are synthesized by ribosomal synthesis. The most often used classification approach takes into account the shapes that these molecules may develop in vivo, such as linear peptides with unusual bias and a-helix, b-sheet, b-hairpin, and looping topologies.

4. ANTIMICROBIAL PEPTIDES IN THE MANAGEMENT OF PLANT DISEASES

The majority of AMPs are cationic and enter the cytoplasmic membrane via binding to bacterial surfaces via receptor-mediated interaction. AMPs can interact with intracellular targets and halt the synthesis of proteins, nucleic acids, or enzymes by passing through cell membranes; certain AMPs damage membranes, while others do not. (Powers & Hancock, 2003; Brogden, 2005).

5. BACTERIOCINS AND FUNGAL DEFENSINS

Major bacterial groups secrete a form of protein and peptide called bacteriocins, which are capable of killing closely related species. Examples of bacteriocins that inhibit plant pathogenic bacteria have been reported from bacteria linked with plants, despite the fact that small bacteriocins have not been studied. (Ishimaru et al., 1988; Jabrane et al., 2002; Lavermicocca et al., 2002; Pham et al., 2004; Parret et al., 2005). Many filamentous fungi secrete AMPs, which are similar to plant and animal defensins. They are composed of 51–58 amino acid residues and have a compact structure of antiparallel strands bound together by disulphide bridges. AFP from *Aspergillus giganteus* (Lacadena et al., 1995), PAF from *Penicillium chrysogenum* and *Penicillium nalgiovense* (Kaiserer et al., 2003), and Anafp from *Aspergillus niger* (Lee et al., 1999) all have antifungal properties.

Table 1. Antimicrobial cyclic-peptides

Type	Compound	Composition	Producer microorganism
Simple	Gramicidins	C10	*Bacillus brevis*
	Calophycin	C10	*Calothrix fusca*
	Laxaphycins	C11	*Anabaena laxa*
Tailed	Bacitracins	T5-C7	*Bacillus licheniformis*
Simple lipidic	Xanthostatin	R-C6	*Streptomyces spiroverticillatus*
	Echinocandins	R-C6.	*Aspergillus spp.*
	Cryptocandins	R-C6	*Cryptosporiopsis quercina*
	Fusaricidins	R-C6	*Paenibacillus polymixa*
Tailed lipidic	Viscosins	R-T2-C7	*Pseudomonas fluorescens*
	Polymixins	R-T3-C7	*Paenibacillus polymixa*
	Agrastatins	R-T2-C8	*Bacillus subtilis*
	Amphisins	R-T2-C9	*Pseudomonas fluorescens*
	Putisolvins	R-T8-C4	*Pseudomonas putida*

C, peptide ring size; T, peptide tail size; R, linked fatty acid

Table 2. Classification of some antimicrobial peptides

AMPs from Animals				
Peptide	**Source**	**Function**	**Species effectiveness**	**Refs.**
Abaecin	*Apis mellifera*	Antibacterial	*Agrobacterium tumefaciens* *Erwinia salicis* *Pseudomonas syringae* *Xanthomonas campestris*	Casteels et al. (1990)
Apidaecins	*Apis melifera*	Antibacterial	*A. tumefaciens* *E. salicis* *P. syringae* *Rhizobium meliloti*	Casteels et al. (1989); Casteels et al. (1994)
Cecropin B	*Hyalophora cecropia*	Antibacterial, Antifungal	*P. syringae pv. Tomato* *P. syringae pv. Syringae* *P. syringae pv. Tabaci* *X. campestris pv. Vesicatoria* *Clavibacter michiganensis subsp. Michiganensis* *Erwinia carotovora subsp. Carotovora* *E. carotovora subsp. Chrysanthemi* *A. tumefaciens* *Penicillium digitatum* *Phytophthora infestans*	Alan & Earle (2002)
Dermaseptin	*Rhacophorus*	Antibacterial	*Xylella fastidiosa*	Kuzina et al. (2006)
Drosomycin	*Drosophila melanogaster*	Antifungal	*Botrytis cinerea* *Fusarium culmorum* *Fusarium oxysporum* *Nectria haematococca* *Alternaria brassicola* *Alternaria longipes* *Trichoderma viride* *Ascochyta pisi*	Fehlbaum et al. (1994)
Indolicidin	*Bovine*	Antibacterial	*X. fastidiosa*	Kuzina et al. (2006)

AMPs from Animals				
Peptide	**Source**	**Function**	**Species effectiveness**	**Refs.**
LfcinB	*Bovine*	Antifungal	*P. digitatum* *Penicillium italicum* *Penicillium expansum* *Penicillium sp.* *Alternaria sp.* *Aspergillus nidulans* *B. cinerea* *F. oxysporum*	Munoz & Marcos (2006)
Magainin II	*Xenopus laevis*	Antibacterial, Antifungal	*P. syringae pv. Tomato* *P. syringae pv. Syringae* *P. syringae pv. Tabaci* *X. campestris pv. Vesicatoria* *C. michiganensis subsp. Michiganensis* *P. digitatum* *X. fastidiosa*	Alan & Earle (2002)
Penetratin	*Drosophilid*	Antibacterial	*Bacillus megaterium*	Palm et al. (2006)
PGQ	*X. laevis*	Antibacterial	*X. fastidiosa*	Kuzina et al. (2006)
pVEC	*Mammalian*	Antibacterial	*B. megaterium*	Palm et al. (2006)
Spodopsin Ia	*Spodoptera litura*	Antibacterial	*B. megaterium*	Choi et al. (1997)
AMPs from Plants				
α1-purothionin	*Triticum aestivum*	Antibacterial	*Xanthomonas* *Erwinia*	Caleya et al. (1972)
BLAD	*Lupinus albus*	Antifungal	*B. cinerea* *Erysiphales*	Pinheiro et al. (2018)
Ca-AFP	*Capsicum annuum*	Antifungal	*F. oxysporum* *Phytophthora capsici*	Capella et al. (2001)
Ca-LTP1	*C. annuum L.*	Antifungal	*F. oxysporum* *Colletotrichum lindemuthianum*	Cruz et al. (2010)
J1	*C. annuum*	Antifungal	*Colletotrichum gloeosporioide*	Diz et al. (2006);

AMPs from Animals				
Peptide	**Source**	**Function**	**Species effectiveness**	**Refs.**
			Colletotrichum musae *F. oxysporum*	Seo et al. (2014)
NaD1	*Nicotiana alata*	Antibacterial, Antifungal	*B. cinerea* *F. oxysporum* *F. oxysporum f. Sp. Vasinfectum* *Thielaviopsis basicola* *Verticillium dahlia* *Leptosphaeria maculans* *A. nidulans*	Kerenga et al. (2019); Van der Weerden et al. (2010); Van der Weerden et al. (2008)
Pa-AFP1	*Passiflora alata Curtis*	Antifungal	*C. gloeosporioide*	Ribeiro et al. (2011)
Pe-AFP1	*Passiflora edulis*	Antifungal	*Aspergillus fumigatus* *F. oxysporum*	Pelegrini et al. (2006)
Peptide-1	*Oryza sativa*	Antifungal	*Magnaporthe oryzae*	Sagehashi et al. (2017)
Pf2	*Passiflora edulis f. Flavicarpa*	Antifungal	*F. oxysporum* *C. musae* *C. lindemuthianum*	Agizzio et al. (2003)
PhD1	*Petunia hybrida*	Antifungal	*B. cinerea* *F. oxysporum*	Lay et al. (2003); Jenssen et al. (2006)
PhD2	*P. hybrida*	Antifungal	*B. cinerea*	Lay et al. (2003); Jenssen et al. (2006)
PvD1	*Phaseolus vulgaris*	Antifungal	*F. oxysporum* *Fusarium solani* *Fusarium laterithium*	Mello et al. (2011)
Snakin-1	*Solanum tuberosum*	Antibacterial, Antifungal	*B. cinerea* *F. solani* *F. culmorum* *F. oxysporum* *Plectosphaerella cucumerina* *Colletotrichum lagenarium*	Berrocal-Lobo et al. (2002), Segura et al. (1999)

AMPs from Animals				
Peptide	**Source**	**Function**	**Species effectiveness**	**Refs.**
			Colletotrichum graminicola *Bipolaris maydis* *Aspergillus flavus* *C. michiganensis* *Ralstonia solanacearum*	
Snakin-2	*S. tuberosum*	Antibacterial, Antifungal	*C. michiganensis* *R. solanacearum (rfa-)* *R. meliloti* *B. cinerea* *F. solani* *F. culmorum* *F. oxysporum f. Sp. Conglutinans* *F. oxysporum f. Sp. Lycopersici* *P. cucumerina* *C. graminicola* *C. lagenarium* *B. maydis* *A. flavus*	Berrocal-Lobo et al. (2002)
ZmPep1	*Z. mays*	Antifungal	*Pythiumspp.* *Fusarium*	Marx (2004), Huffaker et al. (2011)
AMPs from microorganism				
AFP	*Aspergillus giganteus*	Antifungal	*F. culmorum* *Fusarium equiseti* *Fusarium lini* *Fusarium moniliforme* *F. oxysporum* *Fusarium poae* *Fusarium proliferatum* *F. solani* *Fusarium sporotrichoides* *Fusarium vasinfectum* *Magnaporthe grisea*	Barna et al. (2008)

AMPs from Animals				
Peptide	**Source**	**Function**	**Species effectiveness**	**Refs.**
			P. infestans	
ANAFP	*A. niger*	Antifungal	*A. fumigatus* *A. flavus* *F. oxysporum* *F. solani*	Barna et al. (2008)
NAF	*Penicillium nalgiovense*	Antifungal	*A. flavus* *F. solani* *P. italicum*	Barna et al. (2008)
PAF	*Penicillium chrysogenum*	Antifungal	*A. fumigatus* *A. flavus* *A. niger* *B. cinerea* *Cochliobolus carbonum* *F. oxysporum* *Blumeria graminis f. Sp. Hordei* *Puccinia recondita f.sp. Tritici*	Barna et al. (2008); Kaiserer et al. (2003)

Table 3. Peptides used to regulate plant pathogens

Peptides	**Origin**	**Targeting pathogens**	**Methods of testing**	**Refs**
Thionin	*Arabidopsis thaliana*	*Ralstonia solanacearum, Fusarium oxysporum*	Transgenic expression	(Chan et al., 2005)
Snakin-1	Potato	*Clavibacter michiganensis, Botrytis cinerea*	Transgenic expression	(Segura et al., 1999)
alfAFP	Alfalfa	*Verticillium dahliae*	Transgenic expression	(Gao et al., 2000)
Melittin	*Apis mellifera*	*Xanthomonas oryzae*	In-vitro killing assay	(Shi et al., 2016)

6. SYNTHETIC AMPs

Synthetic molecules containing six to 47 amino acid residues have been developed or analogues from plants and animals have been used. Solid-phase techniques have been used to produce synthetic AMPs (Andreu et al., 1983). Combinatorial chemistry is a potent method for designing novel compounds that diverge from better leader compounds and concentrate their activity on specific target pathogens while reducing their toxicity to plants and animals and their susceptibility to protease digestion (Powell et al., 1995; Reed et al., 1997; Oh et al., 1999; Monroc et al., 2006a).

7. MULTIFUNCTIONAL PEPTIDES

Several examples of multifunctional peptides have been developed in various ways, such as by searching the genome, transcriptome, or proteome of a disease-resistant plant or by engineering from sequences of other peptides. Peptides with simultaneous mechanisms of action are interesting in plant protection because they counteract possible resistance in the pathogen and improve its activity. *Phakopsora pachyrhizi,* the causative agent of Asian soybean rust, is inhibited from germination and infections by the engineered peptide DS01-THA, a chimaera of dermaseptin and thanatin that adheres to the wax layer of soybean, barley and maize (Schwinges et al*.,* 2019). Among the various natural defence genes that may be in charge of HLB tolerance, the peptide MaSAMP was found in the huanglongbing (HLB)-tolerant *Microcitrus australasica* (Huang et al*.,* 2021, Wang 2021).The antimicrobial peptide MaSAMP, which is present in the plant's phloem, was one of the potential gene product regulators. Similar to BP178, the peptide's anticipated structure consists of two amphipathic a-helices joined by a hinge, with the helix-2 domain serving as the bactericidal motif. MaSAMP is bactericidal, inhibits Ca. *Liberibacter asiaticus* infections, and strengthens the citrus host's defenses.

8. PLANT GROWTH-REGULATING PEPTIDES

Plant hormones that facilitate intercellular communication during development, such as auxin, cytokinin, and gibberellin, have an impact on plant growth and development. But according to recent research, peptide signal molecules are also crucial for a variety of plant development processes and environmental reactions, including meristematic stem cell differentiation, tissue and organ formation, fruit maturation, abscission, and biotic and abiotic stress adaptation (Chen et al., 2020). These peptides' precursors undergo processing in plants to become mature peptides, which subsequently interact with plant receptors and trigger downstream signal pathways to produce growth responses. PGRPs have a variety of roles in the growth and development of plants.

For instance, TIBO Crop Science discovered the functional peptide PY91 in 2021, which hinders crop growth. Meristem size is regulated by the CLAVATA3 peptide (Lay et al*.,* 2005). Cruciferous pollen's self-incompatibility is recognized by the SCR peptide (Fletcher et al*.,* 1999). A family of peptides known as RALFs is involved in the proliferation of plant cells (Okuda et al*.,* 2009).

Peptides of four different kinds have been employed as commercial plant growth regulators. The KEYLAN range of natural products, which includes KEYLAN Ca, KEYLAN Combi, KEYLAN Fe, KEYLAN Max, KEYLAN Mn, and KEYLAN Zn, has been developed by Italy Hello Nature (https://www.hello-nature.com/us/). These goods function as bio stimulants and offer micronutrients in a bio chelated form. KEYLANS are employed in hydroponic farming or soil fertilization to prevent and treat malnutrition. These products can be used across a wide range of soil pH levels, have good stability and water solubility, and can be safely combined with other calcium foliar fertilizers, growth regulators, adjuvants, insecticides, fungicides, and biocontrol protectants.

The active component of the commercial product Tandem, created by Italy Hello Nature, is the plant-derived peptide LRPP (https://www.hello-natur e.com/us/), which is also a bio stimulant. It is a potent bio stimulant that increases resilience to environmental stressors such poor soil, drought, and extremes in temperature. In order to establish a more intimate and advantageous interaction with seeds, this product is utilized at the sowing stage.

The active component of PHC-91398, which was created by PHC

(https://www.planthealthcare.com/), is the peptide 91,938. As a growth regulator, it promotes growth, metabolism, and natural plant defenses, protecting against nematodes and bacterial and fungal infections. Foliar spraying and seed treatment are suggested applications.

It has been demonstrated that Hicure® (https://www.syngenta.com/en), a natural bio stimulant with exceptional efficacy and adaptability that contains readily absorbed peptides and amino acids, improves plant quality and increases resilience to environmental stress. To get the greatest results, this product is used as a traditional spray or maceration solution prior to important developmental stages, pot changes and transplanting, environmental stress, or transportation. Hicure® works with the majority of fertilizer and plant protection products and doesn't require specialized equipment.

9. MECHANISM OF ACTION

Internal cell functions are affected by a class of antimicrobial peptides (Le et al., 2017) that can enter the target cell, sometimes rupturing the membrane, and disrupt the synthesis of proteins or nucleic acids, cell division, or proteinases. This is true for the antifungal PAF26 (Munoz et al., 2013), cathelicidins (which block translation, replication, and ion channels), and magainins (McMillan & Coombs., 2020), which impact DNA synthesis and metabolic activities in bacteria and fungus. However, some cell-penetrating peptides (CPPs) have been used to deliver cargo molecules to the cytoplasm of eukaryotic cells (plant, fungal, and human), such as BP100 to BY2 tobacco cells (Eggenberger et al., 2011) and BP16 to human tumor cells (Soler et al., 2014), or enhance the uptake of RNAi in plant cell transformation (Numata et al., 2014). These CPPs do not interfere with intracellular processes or break down cell membranes. A 10-nucleotide oligomer that targets the regulator gene acpP involved in the fatty acid biosynthesis in E. amylovora is one example of how other CPP, such as peptide nucleic acid (PNA) conjugates, enter plant pathogen cells and target specific genes (Patel et al., 2017).

Plant pathogens are impacted by functional peptides through a number of ways.

- They disorganize cell membranes and promote cell lysis by causing holes in cell membranes.
- Interfere with the production of nucleic acids, cell division, and cell-penetrating peptides, among each other's internal biological functions. This is the case with magainins, which have an impact on the synthesis of DNA and the metabolic activities of fungi and bacteria.
- Interact with extracellular structures like chitin in fungus and lipopolysaccharides, fimbriae, or flagella in bacteria.
- Prevent the production of biofilms and other bacterial colonization structures.
- Alter the outer coat or behaviour of plant-pathogenic nematodes.
- Prevent the attachment or replication of viruses.

10. AMPs IN BIOCONTROL AGENTS

The ability of certain microorganisms to prevent bacterial and fungal plant diseases has been linked to the generation of AMPs. Nevertheless, the papers only show compelling evidence linking them to the genetically engineered biocontrol mechanism in a small number of cases. By analyzing defective mutants incapable of producing fengycin and bacillomycin D, as well as structural and functional characterizations of gene clusters involved in their production, cyclic lipopeptides have been implicated in providing *Bacillus amyloliquefaciens* FZB42 with the ability to control *F. oxysporum* (Koumoutsi et al., 2004). The antimicrobial metabolites known as lipopeptides are made up of fengycin, iturin, lichenycin, and surfactin. These compounds can damage the fungal membrane and compromise cellular integrity (Romero et al., 2007). *Bacillus subtilis* 155 produces cyclic lipopeptides and fengycin, which can harm rice membranes and prevent *M. grisea* hyphal development (Zhang & Sun, 2018).

11. TRANSGENIC PLANTS EXPRESSING ANTIMICROBIAL PEPTIDES

Gene constructs containing AMP-coding sequences have been expressed in model or agricultural plants, offering varying levels of defense against plant diseases. Several plants express the genes encoding animal defensin. Rice-expressed cecropins A and B provide defense against *Magneporthe grisea* (Coca et al., 2004) and *Xanthomonas oryzae* (Sharma et al., 2000), Magainin, which is found in tobacco, offers defense against a variety of bacteria and fungus (De Gray et al., 2001), and potato-expressed tachyplesin from crab proved helpful

against *E. carotovora* infections (Allefs et al., 1996). Tobacco-expressed insect defensins, heliomicin and drosomycin, provide defence against *B. cinerea*. (Banzet et al., 2002), and the fruit fly sarcotoxin found in tobacco offered protection against *E. carotovora ssp. carotovora* and *Pseudomonas syringae pv. Tabaci* (Ohshimax et al., 1999). Plants have also been shown to express plant defensins. Tobacco and tomato express the radish defensin Rs-AFP2, which provides defense against *Alternaria longipes* (Terras et al., 1995), Alf-AFP Potato-expressed lucerne defensin guards against *V. dahliae* (Gao et al., 2000), Tobacco-expressed SPI1 spruce defensin guards against *Heterobasidium annosum* (Elfstrand et al., 2001).

12. EXPLORING AND DEVELOPING PEPTIDES TO CONTROL PLANT DISEASES

Living organisms present excellent opportunities for the discovery of peptides, such as lactoferricin B, which is produced by acidic-pepsin hydrolysis of the lactoferrin found in cow's milk, or native chemicals, which are acquired by further hydrolysis from functional proteins (Tomita et al*.,* 1991). The foundation for creating analogues or newly created compounds that can be chemically synthesized to create peptide libraries is the understanding of the chemical structure, physico -chemical characteristics, and biological characteristics of natural peptides. The methods for producing analogues include using specific motifs (at the end, in chain, or repetitive sequences) such the ATCUN, Rana box, and LPS binding gamma-core motifs, as well as chemically altering existing compounds (e.g., halogenation, cyclisation, capping, conjugation) (Mueller et al., 2020 and Thayer, 2011). Tandem repeating sequences, cyclisation, or the addition of certain end sequences or amino acids (such D-amino acids) are examples of de novo peptide design. For the purpose of controlling plant diseases, a wide range of de novo designed cyclic peptides have been created. One such cyclic peptide, BPC194, which belongs to a cyclic decapeptide library, has strong antibacterial activity (Monroc et al*.,* 2006). Following this phase of producing peptide libraries, the compounds are put through an in vitro screening platform that evaluates their preliminary toxicity (haemolytic activity, phytotoxicity), stability under harsh physic-chemical conditions, susceptibility to protease hydrolysis, and antimicrobial activity (growth inhibition, killing assays). Microbial growth analyzers or viability methods (e.g., SYTOX green, resazurin, v-qPCR) can be used to examine fungicidal or bactericidal characteristics or to perform inhibition experiments that target plant-pathogenic bacteria or fungi (Baro et al*.,* 2020).

13. LARGE – SCALE PRODUCTION OF FUNCTIONAL PEPTIDES

For in vitro screening, small amounts of peptides (milligrams, for example) are needed; whereas, moderate-to-high quantities (grams, for example) are needed for plant assays or even field testing. Functional peptides' potential as plant-protection compounds mostly relay on their ability to be produced in large numbers using industrial platforms. Peptides can be synthesized chemically, acquired directly from natural sources, or expressed heterologous in live bio factories.

14. NATURAL SOURCES

Natural sources often include low amounts of peptides. Because it produces a significant number of by-products (such as blood, whey, etc.) that include peptides and proteins that can be processed either directly or by enzymatic digestion, the food sector can be a valuable source of peptides (Saucedo-Vázquez et al.,2022, Sanchez & Vázquez, 2017; Meneguetti et al., 2017). Peptides can be more prevalent in microbial fermentations; for example, nisin produced at 100–300 mg/L in fed-batch or batch fermentation reactors by enhanced strains of *Lactococcus lactis* (Klelissa et al., 2021; Klausmann et al., 2021) or surfactin in *B. subtilis* 3NA, which produced yields that were exceptionally high at 26.5 g/L (Cheng et al., 2018).

15. CHEMICAL SYNTHESIS

For the purpose of producing many peptides for medical use, large-scale chemical synthesis based on O-ring solid phase or liquid phase synthesis has been established (Andersson et al., 2020, Mueller et al., 2020; Thayer, 2011). Chemical synthesis works better in the pharmaceutical industry, where high-value goods are more dependable, than it does in agriculture, where plant protection calls for less costly products.

16. BIOTECHNOLOGICAL PRODUCTION

The pharmaceutical industry has made extensive use of the relatively well-developed method of

producing peptides by heterologous expression in biological systems (bio factories). This method yields linear peptides made of proteinogenic amino acids by ribosome synthesis (Parachin et al., 2012). Though progress has been made in cloning biosynthetic gene clusters, biotechnological production of nonribosomally synthesized peptides (e.g., CLPs, peptaibols) is less advanced (165). One such instance is the cloned and inserted bacillomycin NRPS cluster from B. amyloliquefaciens FZB42 for heterologous expression in *B. subtilis* (Liu et al., 2016).

17. PEPTIDE-BASED AGROCHEMICALS: PROSPECTS

Optimizing performance for formulation and structure: Enhancing the bioavailability and stability of naturally occurring peptides is crucial for the development of novel peptide-based medications and agrochemicals. Optimizing the structure and formulation of natural peptides can result in more palatable peptides or their mimics. Enhancing the delivery method can potentially produce peptide products with increased bioavailability.

Optimization of structures: Natural peptides have poor stability and limited activity, hence several structural optimization techniques, such as amino acid replacement, cyclisation tactics, mimic design, etc., have been developed to get around these problems (Mora et al., 2015; Badosa et al., 2013, Yao et al., 2018).). Genetic engineering can be used to alter naturally occurring peptides to create new peptides with desired characteristics. For instance, the natural spider venom peptide ω/κ-HXTX-Hv1a (Tan, H. J., & Tong, Y. L. (2022). was genetically engineered to include a glycine-serine dipeptide, leading to the development of the bioinsecticide Spear®. This product is regarded as a sustainable and efficient green tool for pest control in agriculture and public health since it has greater activity, lower risk, and more persistence than the natural product.

Formulation: The generation of distinct formulations, such as microemulsions, suspension agents, and capsule suspensions, can shield peptide molecules from environmental deterioration caused by elements including water, sunlight, temperature, and metabolic enzymes. This will also improve stability of functional peptides.

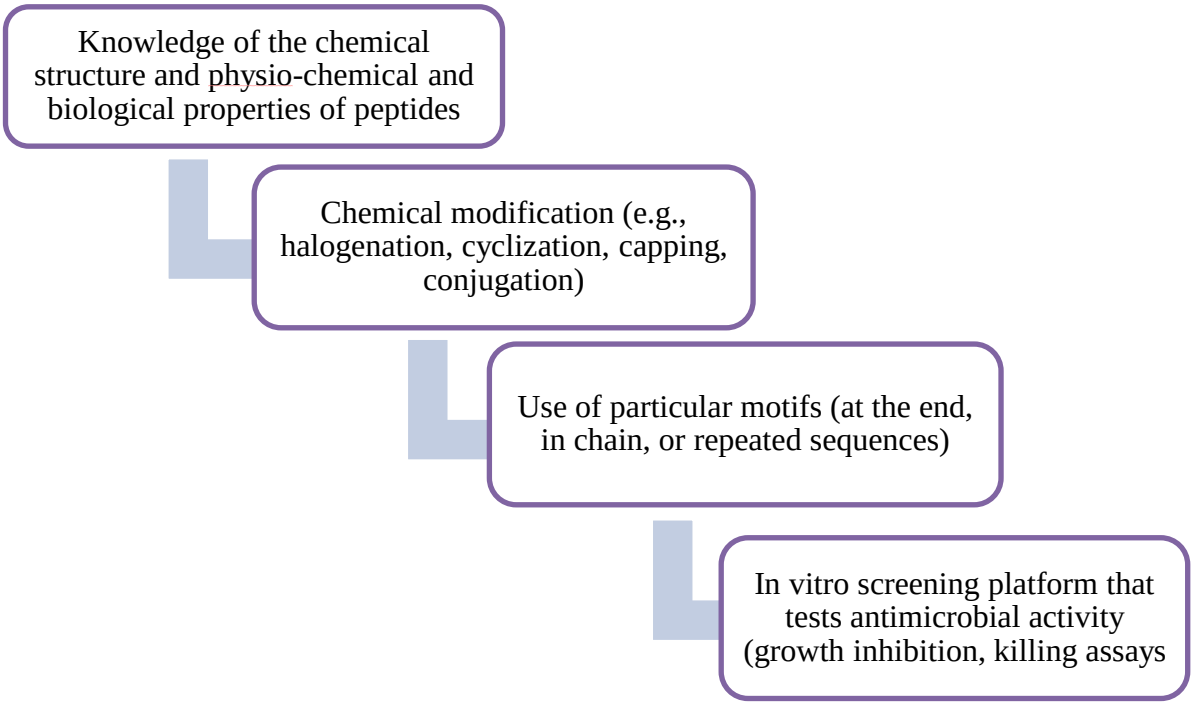

Fig. 1. Steps showing exploration and development of peptides to control plant diseases

Challenges: “The effectiveness of functional peptides as plant-protection products is hampered by a number of challenges. Plant pathogen populations' resistance to antimicrobial peptides is a significant problem. The peptide's interaction with the target plant-pathogen cell may be hampered by a number of mechanisms, such as adsorption by envelopes or external structures (biofilm barriers, exopolysaccharides, capsules), active removal from cells (e.g., efflux pumps, secretion of outer membrane vesicles), protease degradation, or enzymatic chemical modification” (Lima et al., 2021). A number of physicochemical factors, including as cations, pH, and phenolics, might decrease activity; these factors are especially significant for cationic amphipathic peptides.

“Peptides have been effectively employed in plant protection, however due to drawbacks such poor oral efficacy, limited systemic stability, and expensive production costs, they continue to confront a number of difficulties. Natural peptides often have low stability and low bioavailability because they are quickly broken down by the body's enzymes and impacted by external environmental factors like pH and light. Peptide insecticides that are too expensive will not be widely accepted in the commercial sector, in contrast to peptide-based medications. Undoubtedly, these challenges can be lessened by altering the peptide to include non-natural amino acids (like D-amino acids)” (Ng-Choi et al., 2014) or by using a suitable formulation (like nanoencapsulation), but these solutions always make the process of development and manufacturing more difficult.

Another significant obstacle is how to express or distribute the peptides into the plants. It appears that this method is more dependable than topical treatments given the large number of reports addressing the heterologous expression of peptides in plant crops; however, further research is needed to determine its effects on food safety and the environment, and its application to genetically modified self-protected plants may be restricted in some countries. High concentrations of peptides (e.g., kg/ha) are needed for the traditional spray or soil drench techniques of applying plant protection agents in agriculture. Endotherapy could be a viable option for trees, particularly when it comes to vascular system disorders like those brought on by *Xylella fastidiosa* (citrus variegated chlorosis, sudden death syndrome of olives, and leaf scorch of almonds) and *Candidatus Liberibacte rasiaticus* (Citrus HLB).

“One of the primary concerns is the cost of producing peptides for plant protection. Solid-phase chemical synthesis, which is frequently used for research or high-value products (pharmaceutical, for example), is too costly. Using mixtures of randomly synthesized peptides has been suggested as a way to lower the cost of chemical synthesis for agriculture” (Topman et al., 2018); however, this method produces combinations in which not all of the components may be active. A crude undecapeptide production in chemical synthesis currently costs several hundred dollars per gram, and the price goes up for larger peptides. Evaluation by the regulatory framework (such as the FDA in the US and the EFSA in the EU) is the final obstacle for peptides to be unique active compounds for creating plant-protection products. Given what we already know about peptides, it stands to reason that some of the more sophisticated ones will be able to satisfy the requirements for low-risk substances. Therefore, improved stability, increased bioactivity, and reduced cost are necessary for peptide-based agrochemicals to be considered acceptable.

18. SUMMARY

Potential biopesticides for use in next plant protection products are functional peptides. Peptides work against plant diseases and pathogens through a variety of methods of action, such as inducing plant defense and antibacterial activity through many routes. It is possible to synthesize functional peptides with many mechanisms of action at once, or to employ them as cell-penetrating peptides to help pathogens and plant cells reach their intracellular targets. Functional peptides produced by ribosomal synthesis are expressed heterologously in plants, providing excellent defense against pathogen infections. Large-scale peptides can be produced chemically, naturally (from food industry by-products, for example), or through microbial fermentations and heterologous expression in living bio factories (plants, algae, and microbes).

19. CONCLUDING REMARKS AND FUTURE PROSPECTS

Similar to the pharmaceutical industry, functional peptides have the potential to be very important

plant protection products in agriculture. Commercial development of functional peptides as biopesticides derived from various microbes secreting these chemicals has led to the successful usage of these compounds. Despite the development of many transgenic plants producing AMPs that offer varying degrees of disease resistance, commercial cultivars have not been released into the market due to social and legal constraints. Strong tools to optimize molecules generated from natural chemicals with enhanced activity against specific target pathogens, such as lower cytotoxicity and increased protease stability, are provided by synthetic procedures to synthesize functional peptides led by combinatorial chemical methods. Nevertheless, it has not yet been possible to utilize the large number of peptides as pesticide active components. Only a small number of functional peptides with potential applications are commercially available, and the bulk have only been investigated in vitro. Fewer molecules have been examined in plant pathosystems. There are various obstacles in the way of developing compounds that are ideal for use as pesticide ingredients in agriculture. These include the inherent toxicity and low stability of certain compounds, the necessity to create appropriate formulations, and the demand for low-cost plant protection solutions. Thus, future research priorities include creating chemicals that are less hazardous and more stable as well as lowering production costs through enhanced biotechnological processes and preparative synthesis that makes use of microbial systems or transgenic crops as plant factories.

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc.) and text-to-image generators have been used during the writing or editing of this manuscript.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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