Review Article

Functional Peptides: Novel Tools for Controlling Plant Diseases

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 and 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. For a long time, functional peptides have been suggested as possible anti-fungal and anti-bacteria reagents in agriculture (Van der Biezen, 2001). Functional peptides from different origins share similar killing mechanisms when targeting bacterial and fungal pathogens. As summarized by many previous reviews (Zasloff, 2002; Brogden, 2005; Melo et al., 2009; Bocchinfuso et al., 2011; Sibel Akalın, 2014), to destroy infections, the majority of peptides target and breach the cell membrane directly. Peptides may be made available to the industry and 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.

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). In the early 1990s, the European Union, the United States, and other nations implemented regulatory modifications to pesticide registration requirements, with the goal of halving the number of active ingredients currently in use. 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. Many different kinds of species, including humans, plants, animals, and invertebrates, form functional peptides which can be antimicrobial to ward off infection. Theirdefense systems work against pathogens in many ways (Brogden 2005). Peptides are considered polypeptides of up to

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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. 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). Reviews of AMPs have been carried out 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), and plants (Garc´ıa-Olmedo et al., 1998; Lay & Anderson, 2005). Approximately 900 AMPs have been reported; these can be divided into three types based on their structural characteristics: 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.

A Comprehensive Overview of Peptides

Secretin was the first peptide to be identified; it was found in the gastrointestinal tracts of animals by Bayliss and Starling in 1902 (Tam JKV et al. 2014). Subsequent research revealed that functional peptides include insulin, which decreases blood sugar, and oxytocin, which promotes uterine contraction. Solid phase peptide synthesis (SPPS), which was quicker and simpler to use than traditional liquid phase synthesis, laid the groundwork for automated synthesis in 1963. 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 53-amino acid peptide known as epidermal growth factor (EGF), which promotes skin and corneal cell proliferation, was discovered in 1986 and its discoverer was awarded the Nobel Prize in Physiology or Medicine. By controlling cellular transport and localization, signal peptides enable the more effective use 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®.

Microorganism-derived Antimicrobial Peptides

Microorganisms produce a wide range of antimicrobial peptides, including small bacteriocins and fungal defensins synthesised through ribosomal synthesis, as well as secondary metabolites

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produced by non-ribosomal synthesis such as peptaibols, cyclopeptides, and pseudopeptides.The most often used classification scheme involves considering the structures that these molecules can adopt in vivo, including a-helix, b-sheet, b-hairpin, and looping topologies, as well as linear peptides with unusual bias.

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

Antimicrobial Peptides in the Management of Plant Diseases

One of the things that fascinates AMPs as antimicrobial agents for managing plant diseases is their method of action on the target bacterium. Most AMPs are cationic and reach the cytoplasmic membrane because they bind to the surfaces of bacteria through receptor-mediated interaction. Certain AMPs cause membrane disruption, while others do not; they can pass through cell membranes to interact with intracellular targets and stop the creation of proteins, nucleic acids, or enzymes. (Powers & Hancock, 2003; Brogden, 2005).

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).

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Table 2. 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 a*l., 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)

Mechanism of action

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 others 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 colonisation structures.
- Alter the outer coat or behaviour of plant-pathogenic nematodes.
- Prevent the attachment or replication of viruses.

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 analysing defective mutants incapable of producing fengycin and bacillomycin D, as well as structural and functional characterisations 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).

Transgenic plants expressing AntimicrobialPeptides

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

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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*(Ohshima 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)

Large – scale Production of Functional Peptides

For in vitro screening, small amounts of peptides (milligrammes, for example) are needed; whereas, moderate-to-high quantities (grammes, 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 synthesised chemically, acquired directly from natural sources, or expressed heterologously in live biofactories.

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.(Sánchez A et al., 2017, Meneguetti BT 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 S et al., 2021, Klausmann P et al., 2021) or surfactin in B. subtilis 3NA, which produced yields that were exceptionally high at 26.5 g/L.(Cheng Q et al., 2018)

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 L et al., 2020 , Mueller LK et al., 2020 , Thayer A. et al., 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.

Biotechnological Production

The pharmaceutical industry has made extensive use of the relatively well-developed method of producing peptides by heterologous expression in biological systems (biofactories). This method yields linear peptides made of proteinogenic amino acids by ribosome synthesis.(Parachin NS, Mulder KC, Viana AA, Dias SC, Franco OL. 2012.) Though progress has been made in cloning biosynthetic gene clusters, biotechnological production of nonribosomally synthesised peptides (e.g., CLPs, peptaibols) is less advanced (165). One such instance is the cloned and inserted

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bacillomycin NRPS cluster from B. amyloliquefaciens FZB42 for heterologous expression in B. subtilis.(Liu Q, Shen Q, Bian X, Chen H, Fu J, et al. 2016)

Peptide-based Agrochemicals: Prospects

Optimising 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. Optimising 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.

Optimisation of structures

Natural peptides have poor stability and limited activity, hence several structural optimisation techniques, such as amino acid replacement, cyclisation tactics, mimic design, etc., have been developed to get around these problems.(Mora I, Cabrefiga J, Montesinos E. 2015.)(Badosa E, Moiset G, Montesinos L, Talleda M, Bardají E, et al. 2013.). 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 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.

Challenges

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. Therefore, improved stability, increased bioactivity, and reduced cost are necessary for peptide-based agrochemicals to be considered acceptable.

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 defence and antibacterial activity through many routes. It is possible to synthesise 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

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excellent defence 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 biofactories (plants, algae, and microbes).

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 optimise 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 synthesise functional peptides led by combinatorial chemical methods. Nevertheless, it has not yet been possible to utilise 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.

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