Phytochemistry 154 (2018) 94–105

Contents lists available at ScienceDirect

Phytochemistry

journal homepage: www.elsevier.com/locate/phytochem

Antimicrobial peptides from different plant sources: Isolation, characterisation, and purification

Swee-Seong Tang[a,*], Zakaria H. Prodhan[a,b], Sudhangshu K. Biswas[a], Cheng-Foh Le[c], Shamala D. Sekaran[d]

[a] Division of Microbiology, Institute of Biological Sciences, Faculty of Science, University of Malaya, 50603 Kuala Lumpur, Malaysia
[b] Department of Agronomy, Institute of Crop Science, College of Agriculture and Biotechnology, Zhejiang University, Hangzhou, China
[c] School of Biosciences, Faculty of Science, The University of Nottingham Malaysia Campus, Semenyih, Selangor, Malaysia
[d] Faculty of Medicine, MAHSA University, Saujana Putra Campus, 42610, Jenjarum, Selangor, Malaysia

ARTICLE INFO

Keywords:
Antimicrobial peptides
Therapeutic agent
Isolation
Purification
Plant source

ABSTRACT

Antimicrobial peptides (AMPs), the self-defence products of organisms, are extensively distributed in plants. They can be classified into several groups, including thionins, defensins, snakins, lipid transfer proteins, glycine-rich proteins, cyclotides and hevein-type proteins. AMPs can be extracted and isolated from different plants and plant organs such as stems, roots, seeds, flowers and leaves. They perform various physiological defensive mechanisms to eliminate viruses, bacteria, fungi and parasites, and so could be used as therapeutic and preservative agents. Research on AMPs has sought to obtain more detailed and reliable information regarding the selection of suitable plant sources and the use of appropriate isolation and purification techniques, as well as examining the mode of action of these peptides. Well-established AMP purification techniques currently used include salt precipitation methods, absorption-desorption, a combination of ion-exchange and reversed-phase C18 solid phase extraction, reversed-phase high-performance liquid chromatography (RP-HPLC), and the sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) method. Beyond these traditional methods, this review aims to highlight new and different approaches to the selection, characterisation, isolation, purification, mode of action and bioactivity assessment of a range of AMPs collected from plant sources. The information gathered will be helpful in the search for novel AMPs distributed in the plant kingdom, as well as providing future directions for the further investigation of AMPs for possible use on humans.

1. Introduction

Antimicrobial peptides (AMPs), also known as host defense peptides (HDPs), constitute part of the innate immune system found in almost all classes of life including microorganisms, arthropods, plants and animals (Bulet et al., 1999; Carvalho and Gomes, 2009; Zasloff, 2002). These AMPs are potent, broad-spectrum antibiotics against pathogenic bacteria (Gram-negative and Gram-positive), fungi, enveloped viruses and other parasites (Reddy et al., 2004).

Plant AMPs are an abundant group of proteinaceous compounds produced in plants (Jenssen et al., 2006). The first reported plant AMP was purothionin from wheat flour (*Triticum aestivum*) (De Caleya et al., 1972). Several groups of plant AMPs with antimicrobial activity have since been identified, characterised and purified, including defensins, snakins, puroindolines, glycine-rich proteins, cyclotides, hevein-type proteins, and lipid transfer proteins (Pelegrini and Franco, 2005; Witkowska et al., 2007). Such AMPs have been isolated from different plants and plant organs such as the stem, root, seed, flower and leaf. They exhibit potent microbicidal activities against viruses, bacteria, fungi, parasites and protozoa (Nawrot et al., 2014). Plant AMPs have become important candidates for developing potential new techniques for controlling crop losses as well as novel antibiotics for treating various infections in humans (Pelegrini et al., 2008).

The adverse effects of chemical pesticides, the frequent emergence of drug-resistant bacteria and the failure of some traditional antibiotics have all led to an urgent search for new antimicrobial agents (Nordström and Malmsten, 2017). Although more than 5000 AMPs have been identified from different sources so far (Hu et al., 2016; Zhao et al., 2013), only just over 2400 AMPs have been deposited in the AMP Database (http://aps.unmc.edu/AP/main.php), of which 343 are from

* Corresponding author.
E-mail addresses: sstang@um.edu.my, sstang2107@hotmail.com (S.-S. Tang), rajugenetics2003@gmail.com (Z.H. Prodhan), shu_genetics@yahoo.com (S.K. Biswas), chengfoh.le@nottingham.edu.my (C.-F. Le), shamaladevi@mahsa.edu.my (S.D. Sekaran).

https://doi.org/10.1016/j.phytochem.2018.07.002
Received 5 May 2018; Received in revised form 3 July 2018; Accepted 7 July 2018
Available online 17 July 2018
0031-9422/

Table 1
Main families of plant antimicrobial peptides with representative peptides and their sources.

Family: Representative peptide	Plant organ	Plant	Reference
Cyclotides: Kalata B1 and B2	Leave & flowers	*Oldenlandia affinis*	(Craik, 2001; Nawrot et al., 2014; Selitrennikoff, 2001; Stec, 2006)
Impatiens: Ib-AMPs	Seeds	*Impatiens balsamina*	(Stepek et al., 2005; Tailor et al., 1997)
Knottin-peptides: PAFP-S	Seeds	*Phytolacca americana*, *Mirabilis jalapa*	(Marcus et al., 1999; Nawrot et al., 2014; Terras et al., 1995)
Lipid transfer proteins (LTPs): LTP1s and LTP2s	Seeds	*Zea mays*	(Nawrot et al., 2014; Pelegrini et al., 2007; Stec, 2006)
Defensins: Peptide PvD1 γ-hordothionin	Seeds	*Phaseolus vulgaris* *Hordeum vulgare*	(García-Olmedo et al., 1998; Mendez et al., 1990; Ramos et al., 2014)
Puroindolines: PINA and PINB	Endosperm	*Triticum aestivum*	(Liu et al., 2000; Nawrot et al., 2014; Tailor et al., 1997)
Snakins: StSN1 and StSN2	Tubers	*Solanum tuberosum*	(Fujimura et al., 2003; Nawrot et al., 2014; Terras et al., 1995)
Thionein: α-1-purothionin	Endosperm	*Triticum aestivum*	(De Caleya et al., 1972; Nawrot et al., 2014)
Vicilin-like AMPs: PMAPI	Seeds	*Macadamia integrifolia*	(Marcus et al., 1999)
Hevein-like AMPs: PMAPI	leaves	*Broussonetia papyrifera syn. Morus papyrifera* L.	(Zhao et al., 2011)
Others: Arietin	Seeds	*Cicer arietinum*	(Ramos et al., 2014; Tailor et al., 1997; Ye et al., 2002)
Ay-AMP	Seeds	*Amaranthus hypochondriacus*	(Rivillas-Acevedo and Soriano-García, 2007)
Cicerin	Seeds	*Cicer arietinum*	(Souza et al., 2011; Ye et al., 2002)
Shepherins	Roots	*Capsella bursa-pastoris*	(Marcus et al., 1999; Remuzgo et al., 2014)
Hispidulin	Seeds	*Benincasa hispida*	(Sharma et al., 2014)
Lunatusin	Seeds	*Phaseolus lunatus*	(Wong and Ng, 2005a)
Peptidesa	Seeds	*Brassica napus*	(del Mar Yust et al., 2004)
Vulgarinin	Seeds	*Phaseolus vulgaris*	(Wong and Ng, 2005b)

plants (Liu et al., 2017). Many of these identified AMPs have not been tested in clinical trials, and a number of those that have reached the clinical phase have failed due to unacceptable toxicity or lack of efficacy.

There is thus a need to search for new AMPs to widen the range of AMPs potentially available for therapeutic and similar uses. However, variations in screening, identification and purification methods, as well as the wide variety of different plant organs and species involved, have made the discovery process complicated and time-consuming. A comprehensive study involving the isolation, characterisation and purification of AMPs together with an analysis of their antimicrobial activity and cytotoxicity should help to identify novel AMPs with improved antimicrobial activity and reduced cytotoxicity, thereby accelerating the application of new AMPs for clinical and agricultural uses (Zhao et al., 2013).

This study summarises and updates current information on the isolation, identification and purification strategies for AMPs from plant sources. In addition to reviewing existing methods, this study also looks at a wide range of possible options for obtaining novel AMPs from the plant kingdom. Above all, this review aims to provide insights to help guide researchers interested in the isolation, identification, evaluation and how to test antimicrobial activity of novel AMPs from plant sources.

2. Isolation of antimicrobial peptides from plants

2.1. Selection of plant materials

The immense biodiversity in plant species means that there is enormous scope to explore new prospective AMPs for drug development in human welfare and other potential applications in agriculture. However, that very diversity also means that enormous efforts are required to select, identify and characterise plant materials in the search for AMPs. The availability of morphological, physiological and molecular data on plant species has made them suitable targets for research into the collection of AMPs. Data derived from molecular techniques designed to characterise and identify genes responsible for AMP synthesis and regulation, including the *in silico* analysis of AMPs along with the gene products responsible for inhibiting pathogens, is required to validate the functional aspects of AMPs (Clara Pestana-Calsa et al., 2010). The increasing number of plant species being deposited in public databases, together with the rapid growth in information available on proteins, genomes, transcripts and other molecular data, has made plants attractive as potential sources of AMPs.

Plant species that exhibit a strict ecological relationship with a pathogen and produce AMPs in a symbiotic context might be particularly suitable for use as sustainable biological controlling agents for pests and pathogens (Udwary et al., 2011). The first identified plant-derived AMP was purothionin, which was found to be active against *Xanthomonas phaseoli*, *Pseudomonas solanacearum*, *Erwinia amylovora*, *Corynebacterium flaccumfaciens*, *C. michiganense* and *C. fascians* (De Caleya et al., 1972). Several other plant AMPs have subsequently been discovered and characterised through a range of morphological, physiological and molecular analyses. The major AMPs collected from different plant species have been categorised into several groups, including defensins, cyclotides, lipid transfer proteins and thionins (Nawrot et al., 2014; Pelegrini et al., 2007; Stec, 2006; Udwary et al., 2011), as well as less common groups such as the impatiens, puroindolines, vicilin-like, glycine-rich, shepherins, snakins and heveins (Berrocal-Lobo et al., 2002; Fujimura et al., 2003; Liu et al., 2000; Marcus et al., 1999; Zottich et al., 2011).

AMPs have been isolated from whole plants, such as lunatusin collected from *Phaseolus lunatus* L. (the Chinese lima bean), as well as from seeds such as vulgarinin from *Phaseolus vulgaris* (haricot beans), hispidulin from a medicinal plant (*Benincasa hispida*), and cicerin and arietin from *Cicer arietinum* (chickpea) (Rivillas-Acevedo and Soriano-García, 2007; Wong and Ng, 2005a; b). Other AMPs have been obtained from the thick cell walls of *Spinacia oleracea* cv. Matador (spinach leaves) (Segura et al., 1998). The names of known AMPs, plant species, and the related references are summarised in Table 1 below.