

## **Antimicrobial proteins: key components of innate immunity**

### **Abstract**

Antimicrobial peptides (AMPs) are the small ubiquitous act as self-defence products which 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 are important mediators of an innate host defense system, with antimicrobial activities against a broad spectrum of microorganisms. 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.

**Key words:** Antimicrobial peptides (AMPs), thionins, defensins, Knottins, lipid transfer proteins

### **Introduction:**

A complex array of interactions between plants and microbes has evolved that reflects both the nutrient acquisition strategies of microbes and defense strategies of plants. Part of plant defense strategy includes an active offense against invading microbes using an array of antimicrobial gene products. More than 500 different antimicrobial proteins and peptides encoded within the genomes of many organisms, including plants, have been described (Kitajima and Sato 1999, Prasad et al., 2008, Tam et al., 2015; Tang et al., 2018). These molecules, which are either constitutive or inducible, are recognised as important components of innate defense mechanisms. Antimicrobial peptides (AMPs) are ubiquitous and found as

host defenses against pathogens and pests in diverse organisms ranging from microbes to animals (Egorov et al., 2005). AMPs exist in different molecular forms, although the majority of them are linear peptides from insects, animals, and plants.

Expression of genes encoding many antimicrobial proteins and peptides is pathogen inducible and is highly correlated with induced disease resistance phenomenon such as systemic acquired resistance (SAR) (Ryals et al., 1996; Maleck et al., 2000). Therefore, they generally are called defense genes and are also often referred to as 'disease resistance markers'. Many of these genes have been shown to not only alter the severity of disease symptoms when over-expressed in genetically engineered plants (Grison et al., 1996; Molina and Garcia-Olmedo 1997; Gao et al., 2000), but also offer the potential to enhance broad-spectrum disease resistance. Transgenic rice expressing gene for puroindoline (*pinA* and/or *pinB*) showed enhanced tolerance to rice blast and sheath blight (Krishnamurthy et al., 2001); and oat thionin in transgenic rice was effective in the control of bacterial diseases such as bacterial leaf blight and the disease caused by *Burk*. Plant defensin genes from *B. oleracea* and *B. campestris* have successfully conferred resistance to transgenic rice against blast and bacterial leaf blight (Kawata et al., 2003).

#### **Types of antimicrobial proteins:**

A common feature of the innate immune system of taxonomically diverse organisms such as mammals, insects, and plants is the ability to marshal the accumulation of antimicrobial proteins and peptides in response to an invasive challenge by foreign organisms (Maleck et al., 2000; Schenk et al., 2000; Reymond, 2001). Members of the major groups of antimicrobial proteins have been classified mainly on the basis of their biochemical (net charge) and/or structural features (linear/circular/amino acid composition), looking for common patterns that might help to distinguish them (Tossi and Sandri, 2002; Zasloff, 2002).

The most important groups from the plants are:

### ***Cecropins:***

This is a family of 3-4 kDa linear amphipathic peptides described in the haemolymph of insects in the early 1980s (Boman 1998; Zheng and Zheng 2002). These molecules are devoid of cysteine residues and contain two distinctive helical segments: a strongly basic N-terminal domain and a long hydrophobic C-terminal helix, linked by a short hinge. Shortly thereafter, other linear amphipathic peptides such as the magainins isolated from *Xenopus* skin, were isolated from vertebrates and included in the same group (Simmaco et al. 1998). These were the first molecules used to evaluate their biomedical applications (Hancock, 2000).

### ***Thionins:***

These are antimicrobial, and generally basic, plant peptides with a molecular weight of 5 kDa, which contain 6 or 8 conserved cysteine residues. Their *in vitro* toxicity against plant pathogenic bacteria and fungi indicates a role in conferring resistance to the plants (Bohlmann, 1999). Ligatoxin B, a new basic thionin containing 46 amino acid residues has been recently isolated from the mistletoe *Phoradendron liga*. Similarities observed by structural comparison of the helix-turn-helix (HTH) motifs of the thionins and the HTH DNA-binding proteins, led the authors to propose that thionins might represent a new group of DNA-binding proteins. Analysis of mRNA levels specific for barley leaf thionins showed that there is a fast increase in the synthesis of these molecules following inoculation with *Erysiphe graminis* f. sp. *hordei* spores (Bohlmann et al., 1988). The genes encoding thionins have already been used to obtain transgenic plants resistant to phytopathogenic fungi. The expression of  $\alpha$ -hordothionin gene in tobacco, under control of CaMV35S promoter, increased resistance against *Pseudomonas syringae* (Carmona et al., 1993), viscotoxins expression in *Arabidopsis thaliana* conferred resistance against *Plasmiodiophora brassicae* (Holtorf et al., 1998), and expression of an oat thionin in transgenic rice seedlings led to

protection against the phytopathogenic bacteria, *Burkholderia plantarii* and *B. glumae* (Iwai et al., 2002).

***Amino acid-enriched class:***

This is a distinctive class of antibacterial and antifungal cationic peptides, enriched in specific amino acids, with distinctive features depending on the organism from which they are isolated. These proline and glycine-rich peptides are mostly from insects and active against Gram-negative bacteria (Otvos, 2000); while cysteine-rich peptides, not related to defensins, represent the most diverse family among arthropods. On the other hand those enriched in histidine are particularly basic, mostly from mammals (Pollock et al. 1984). Among them, histatin recovered from saliva of humans and primates and primarily directed against fungal pathogens, outstands for its distinctive mechanism of action which does not involve channel formation in the fungal cytoplasmic membrane but rather translocates efficiently into the cell and targets the mitochondrion (Tsai and Bobek, 1998). Those enriched in histidine and glycine are quite large, also affecting fungal pathogens and a distinctive feature is that their residues are arranged in approximately regular but different structural repeats. Only two peptides enriched in tryptophan residues have been described, both derived from porcine cathelicidin precursors (Schibli et al. 2002). The outstanding feature though, is broad spectrum of activity including hundreds of Gram-positive and negative clinical isolates in addition to fungi and even the enveloped HIV virus (Gennaro and Zanetti, 2000).

***Histone derived compounds:***

This is a family of cationic helical peptides corresponding to cleaved forms of histones originally isolated from toad (butorin) (Park et al. 1996) and fish epithelia (parasin) (Park, 1998). These molecules are structurally similar to cecropins and quite active against bacteria and fungi. In the case of butorin II, at least, it was demonstrated that this molecule penetrates bacterial membranes and binds to nucleic acids thus, interfering with cell metabolism and

leading to rapid cell death (Park et al. 1998). AMPs are important factors in fish innate immunity (Zhou et al. 2002) and new contributions tend to demonstrate it. Recently, an active peptide was identified both in coho salmon mucus and blood, which display full identity with the N-terminus of trout H1 histone (Patrzykat et al. 2001). This is an indication that histone proteins may be a relatively ubiquitous component of host defenses (Hirsh, 1958). This assumption has been strengthened in recent years by the isolation of histone-like proteins in the cytoplasm of murine macrophages (Hiemstra et al. 1993) and the characterization of histone H2B fragments in human wound fluids (Frohm et al. 1996).

#### *Beta-hairpin:*

The third class of cationic peptides known includes a wide range of 2 to 8-kDa compounds containing beta-hairpin cross-linked by disulphide bridge(s). The smallest members of this class with one disulfide bridge, is represented by thanatin and brevinin. Those containing two disulfide bridges are represented by androctonin (Mandard et al. 1999) tachyplesin and protegrin I (Mandard et al. 2002). Members of the latter group are 2-kDa hairpin-structured peptides, isolated from both invertebrates and vertebrates and show preferential antibacterial and antifungal activities (Dimarcq et al. 1998).

#### *Lipid Transfer Proteins:*

The proteins are so named because of their ability to stimulate the transfer of a broad range of lipids through the membrane *in vitro* and their likely involvement in secretion or deposition of extracellular lipophilic materials such as cutin or wax. Defensive role for LTPs has been reviewed by Garcia-Olmedo et al. (1995). Transgenic tobacco and *Arabidopsis* plants constitutively expressing barley LTP2 protein were developed and were found to have enhanced tolerance to *Pseudomonas syringae* (Garcia-Olmedo et al., 1998).

The role of LTPs in plant protection has also been investigated by Ge et al. (2003). They studied the antifungal and antibacterial properties of LTP110, a lipid transfer protein from rice. The gene for this protein was cloned and the expressed protein was purified and tested *in vitro* against rice pathogens, *M. oryzae* and *X. oryzae*. LTP110 was able to inhibit the germination of *M. oryzae* spores, but could only slightly inhibit the growth of *Xanthomonas*.

#### *Defensins:*

This is a highly complex group of 4-5 kDa open-ended cysteine-rich peptides arranged with different structural motifs. Defensins are a class of evolutionarily and structurally related small, highly basic, cysteine-rich peptides, displaying a broad-spectrum *in vitro* antifungal activity (Osborn et al., 1995; Thomma et al., 2002). Plant defensins are cysteine-rich, cationic peptides, which range in size from 45 to 54 amino acids and generally include eight cysteine residues involved in four disulphide bonds. They were first discovered in wheat and barley and it is now thought that most plants express defensins in a wide range of tissues. Their biological activities are targeted at many plant pests, including fungi, bacteria and insects. Raphanus sativus antifungal protein 1 (Rs-AFP1) and Dahlia merckii antimicrobial protein 1 (Dm-AMP 1) were widely used for genetic engineering in plant for enhancing diseases resistance (Jha et al., 2009a; Jha and Chattoo, 2009b). Enhanced resistance to rice blast and sheath blight was achieved in transgenic rice overexpressing Rs-AFP2 (Jha and Chattoo, 2009a) and Dm-AMP1 (Jha et al., 2009a). Expression of defensin gene from radish in transgenic wheat conferred increased resistance to *Fusarium graminearum* and *Rhizoctonia cerealis* (Li et al., 2011). They have a strong potential to be used for engineering disease resistance in crops because of their potent antifungal activity.

#### *Hevein-typ peptide:*

Hevein is a small cysteine- and glycine-rich chitin-binding peptide of 43 amino acid residues first discovered in the latex of rubber trees (Koo et al. 1998). Since then, a number of heaven-like antimicrobial peptides such as *Pn*-AMPs from morning glory (*Pharbitis nil*) (Koo et al. 1998), GAFP from Ginkgo biloba (Huang et al. 2000), Ee-CBP from the bark of spindle tree (*Euonymus europaeus* L.) (Karolien, 2002), and *Wj*-AMP-1 from the leaves of *Wasabia japonica* L. (Akinori et al. 2003) have been reported. Heaven-type peptides are all highly basic and have pI value above 10. They consist of three folded  $\beta$ -strands, the second and third strands being linked by an  $\alpha$ -helix. Heaven-type peptides have shown a wide range of *in vitro* antifungal activity. Their activities, which were found to be strongly dependent on the ionic composition of the growth medium, are essentially abolished in high salt media. However, it is not yet known how these chitin-binding proteins exhibit antifungal and antibacterial activities at the molecular level.

#### ***Knottin-type peptides:***

Knottins are a group of structurally related molecules with six disulfide-linked cysteines with a consensus pairing pattern (C1-C4, C2-C5, C3-C6). Knottins fold into a triple-stranded  $\beta$ -sheet and form a “knot-like” feature. Few knottin-like antimicrobial peptides have been reported so far. Examples include two highly homologous antimicrobial peptides *Mj*-AMP1 and *Mj*-AMP2 (36 and 37 residues, respectively) from *Mirabilis jalapa* L. seed (Cammue et al. 1992) and PAFP-s (38 residues) from the seeds of *Phytolacca American* (pokeweed) (Gao et al. 2001). These plant knottins show a broad antifungal activity with limited activity against bacteria.

*Mj*-AMPs have been identified in the seeds of *Mirabilis jalapa* (Cammue et al. 1995) and their structural and biological properties resemble those of defensins, a class of antimicrobial peptides found in diverse animal species, ranging from insects to mammals (Lehrer et al. 1991). *Mj*-AMPs are highly basic with homodimeric structures, have six cysteines that are

involved in disulfide bridges. They are synthesized as precursor proteins, which include an amino-terminal signal peptide for targeting of the preprotein into the apoplastic region. The *Mj*-AMPs exhibit a broad spectrum of antifungal activity since they are active against number of plant pathogens. Although the *Mj*-AMPs show sequence similarity to  $\mu$ -agatoxins, a class of insecticidal neurotoxic peptides isolated from the venom of spiders, they do not affect pulse transmission in insect nerves (Cammue et al. 1995). Despite the close similarity between *Mj*-AMP1 and *Mj*-AMP2 at the amino acid sequence level, the peptides differ markedly in their specific antifungal activity. On most fungi, *Mj*-AMP2 is at least 10-fold more active than *Mj*-AMP1 (Cammue et al. 1995). Constitutive expression of *Mj*-AMP2 in transgenic rice reduces the growth of *M. oryzae* by 63% with respect to untransformed control plant, and no effect on plant phenotype was observed. Transgene expression of *Mj*-AMP2 gene was not accompanied by an induction of pathogenesis-related (PR) gene expression indicating that the transgene product itself is directly active against the pathogen. The results presented in this study suggest that the *Mj*-AMP2 gene could be a useful candidate for protection of rice plants against the rice blast fungus *M. grisea* (Prasad et al., 2008).

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