REVIEW PAPER

Application of antimicrobial peptides in agriculture and food industry

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Abstract Antimicrobial peptides have captured the attention of researchers in recent years because of their efficiency in fighting against pathogens. These peptides are found in nature and have been isolated from a wide range of organisms. Furthermore, analogs or synthetic derivatives have successfully been developed on the basis of natural peptide patterns. Long use of pesticides and antibiotics has led to development of resistance among pathogens and other pests as well as increase of environmental and health risks. Antimicrobial peptides are under consideration as new substitutes for conventional pesticides and antibiotics. Many plants and animals have been manipulated with antimicrobial peptide-encoding genes and several pesticides and drugs have been produced based on these peptides. Such strategies and products may still have a long way to go before being confirmed by regulatory bodies and others need to surmount technical problems before being accepted as applicable ones. In spite of these facts, several cases of successful use of antimicrobial peptides in agriculture and food industry indicate a promising future for extensive application of these peptides. In this review, we consider the developing field of antimicrobial peptide applications in various agricultural activities.

Keywords Antimicrobial peptide · Agriculture · Food industry · Fish · Livestock · Plant

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Introduction

Antimicrobial peptides (AMP)s are usually composed of 12–50 amino acids. These peptides synthesized by micro-organisms as well as multicellular organisms from the plant and animal kingdoms, and they are part of innate host defense mechanisms (Brown and Hancock 2006). Peptide-based antimicrobial defense is an evolutionary ancient mechanism, with immediate and non-specific effects against most Gram-negative and Gram-positive bacteria, fungi, viruses and eukaryotic parasites (Wang et al. 2006; Wang and Ng 2005; Alexander 2005; Vizioli and Salzet 2002a). In addition to the wide range of AMPs that have been discovered in the past two decades, many new ones have also been synthesized (Powers and Hancock 2003; Lee et al. 2004).

By their mode of production, AMPs fall into two categories; non-ribosomally synthesized peptides and ribosomally synthesized (natural) peptides. Whereas the first group is mostly produced by bacteria, the other is produced by all organisms including bacteria (Hancock and Chapple 1999). Electrostatic charge, the most important characteristic by which the action of AMPs is explained, is a trait that clusters AMPs into two main groups. The larger group consists of positively charged peptides, whereas the other group includes non-cationic peptides and it is divided into subgroups such as anionic peptides, aromatic peptides and peptides derived from oxygen-binding proteins (Vizioli and Salzet 2002b). Non-cationic peptides in comparison with the first group are scarce and very often in scientific texts, the term "antimicrobial peptide" only refers to cationic AMPs.

The naturally derived AMPs are divided into peptides with β-sheet, α-helical, extended helical and loop structures and among them the first two groups are more abundant in nature (Powers and Hancock 2003).

Some AMPs have developed naturally with high specificity against fungi. Over 100 antifungal peptides have been isolated from a wide range of organisms (De Lucca and Walsh 2000). Besides, there are AMPs with low MIC values against Gram-positive and/or -negative bacteria, and even AMPs with considerable effect on viruses and tumors (Chernysh et al. 2002).

AMPs have been isolated from many organisms and act efficiently against pathogens without any damage to the host. The structural differences between host and target cell membranes has an essential role in the selective action of AMPs (Yount and Yeaman 2005). Typically, AMPs follow a common pattern of action. Sequence, size, degree of structure formation, cationicity, hydrophobicity and amphipathicity are structural parameters that have key roles in the interaction of AMPs with target cells (Giangaspero et al. 2001; Soltani et al. 2007). After hydrophobic interaction with the target membrane, the accumulation of peptide molecules continues to reach a threshold in which peptide molecules through the membrane deformation (e.g. by making pores or channels in it), derange normal biological activity of membrane (Yeaman and Yount 2003). Though membrane dysfunction is lethal, the antimicrobial effect of AMPs is not limited to membrane perturbation and they have other potential targets such as cell division processes, macromolecular synthesis, and the cell wall (Hancock 2005).

In addition to antimicrobial action, AMPs have other less known properties. There are evidences that indicate some AMPs have mitogenic characteristics or are involved in particular signaling pathways as signaling molecules. Some of them have proven role in promotion of adaptive immune response by direct or indirect effects on immune cells (Kamysz et al. 2003).

The food situation worldwide will become critical as the vulnerability of modern agriculture to diseases and pests increases. Annually, high amounts of pesticides are used to inhibit the loss of yield imposed by plant diseases and pest insects. However, chemical pesticides cause other problems due to their risks for the environment and consumers' health, mainly because of untimely or excessive use of them (Rekha et al. 2006). Misuse of pesticides in combination with monoculture of high yielding varieties imposes strong selection pressure on pathogens that in turn culminates in development of resistant pathogen strains and plant resistance breakage (Ma and Michailides 2005; Webb and Fellers 2004). On the other hand, extensive use of antibiotics in animal husbandry as therapeutics or growth promoters is widespread (Khachatourians 1998).Treatment of animals with antibiotics leads to antibiotic residue problems in veterinary products and possible transmission of antibiotic-resistant microbes to humans (Molina et al. 2003).

Therefore, in agriculture, there is a common trend to find and exploit elements that present sustainable resistance to a broad range of pests and pathogens and are safe for the host organisms with no side effects for the environment, animals or the people that consume the products. Due to the above-mentioned concerns and expectations, AMPs are suitable candidates to be used in various fields of agriculture. They are ubiquitous in nature, they have high selectivity against target organisms and resistance against them is rarely observed (Zasloff 2002). Table 1 shows the variety of sources, target organisms and applications of AMPs in agriculture at a glance.

Usually, AMPs are not used in their original structure. Considerations such as increase in potency of anti-pathogen activity, reduction of their hemolytic effect or inhibition of their degradation by host protease, persuade scientists to use analog peptides or derivatives of the original AMPs that could be created by amino acid alterations (Conlon et al. 2007; Li et al. 2002). Some synthetic AMPs are chimeras of natural peptides (e.g. CEMA is a hybrid of cecropin A and melittin; Osusky et al. 2000) or they are bioactive part(s) of a large protein (e.g. lactoferricin; Chan and Li-Chan 2007). Furthermore, many synthetic peptides with no natural counterpart have been made according to structural features of AMPs, which modulate their interaction with microbes. These features would be used as starting points in the rational design of AMPs with improved profile (Marcos et al. 2008). For more detailed information about AMPs, refer to http://www.bbcm.univ. trieste.it/~tossi/pag1.htm or http://aps.unmc.edu/AP/main. php.

In this review, we indicate applications of AMPs in various fields of agriculture and food industry with particular attention to experiments and studies that are reported in the scientific papers. As a result, genetic manipulations of organisms with AMP-encoding genes, which are the most reported cases, constitute a considerable portion of the review. Several cases are merely promising ones, the extensive use of which depends on considerations, some of which are mentioned at the end.

Applications in plants

Control of plant diseases

Recombinant DNA techniques have facilitated new strategies based on transgenic plants with AMP-expressing genes (van der Biezen 2001). Since generation of transgenic crops is a laborious and expensive procedure, *in vitro* assays are conducted to estimate bioactivity of AMPs against bacterial and fungal plant pathogens so that scientists can select suitable cases more confidently. For example, following leaf

Table 1 The variety of sources, target organisms and applications of AMPs

AMP	Origin	Source	Application	References
Cecropin B	Animal	*Hyalophora cecropia* and *Bombyx mori* (*H. cecropia* is scientific name for Giant silkmoth and *B. mori* is scientific name for domesticated silkmoth)	Antibacterial effect in transgenic rice, antiviral effect against important viral pathogens of fish	Sharma et al. (2000) and Chiou et al. (2002)
Lactoferricin		Cow (*Bos taurus*)	Mastitis control in goat	Zhang et al. (2007)
alfAFP	Plant	Alfalfa (*Medicago sativa*)	Antifungal effect in transgenic potato	Gao et al. (2000)
Pn-AMP2		Morning glory (*Pharbitis nil*)	Antifungal effect in transgenic tobacco	Koo et al. (2002)
CEMA	Synthetic	Chimera (cecropin-melittin)	Antifungal effect in transgenic tobacco, antibacterial effect against bacterial pathogen in fish	Yevtushenko et al. (2005) and Jia et al. (2000)
MSI-99		Magainin analog	Antifungal and antibacterial effect in transgenic tobacco, tomato, grapevine, banana	Chakrabarti et al. (2003), Vidal et al. (2003) and Alan et al. (2004)
Polyoxins	Microbial	*Streptomyces cacaoi*	Fungicide, insecticide	Reuveni et al. (2000) and Arakawa (2003)
Nisin		*Lactococcus lactis*	Food preservative	Delves-Broughton (2005) and Pawar et al. (2000)

disk assays of three cationic lytic peptides against various microbial pathogens, MSI-99, a synthetic derivative of magainin II with increased positive charge, was suggested as the best candidate for generation of transgenic tomato lines with enhanced resistance to bacterial and fungal diseases (Alan and Earle 2002). Though primary screening of AMPs through *in vitro* assays is informative, researchers are more interested in evaluation of *in planta* performance of AMPs before their possible use in transformation of target plants. An acceptable way to test *in planta* activity of candidate AMPs could be transformation of a model plant (e.g. *Arabidopsis taliana*) with AMP-expressing genes. Logically the results of *in planta* experiments are determinants for further decision-making and could have compatible or unexpectedly different results in comparison with counterpart *in vitro* assays. For instance, while β-purothionin, cecropin B, and phor21 exhibited antifungal activity *in vitro*, their expression in *A. taliana*, under an endogenous promoter with moderate-level activity and extracellular secretion, indicated that *in planta*, only β-purothionin preserved its high antibacterial and antifungal activity. Cecropin B enhanced only antibacterial resistance and phor21 did not improve antimicrobial resistance (Oard and Enright 2006).

Here, we bring more detailed explanation on disease control by means of AMP-expressing genes in transgenic economically important plants.

Tobacco

Published reports indicate that tobacco has been successfully transformed by AMP-expressing genes from a wide range of organisms and as a result resistance of the transgenic plants has increased considerably against several fungal and bacterial diseases.

Two 38-amino acid peptides, SB-37 and Shiva-1, were produced as substitution analogs of Cecropin B, a natural lytic peptide of *Hyalophora cecropia*. First generation of seedlings expressing Shiva-1, exhibited delayed wilt symptoms and reduced disease severity and mortality after infection with a highly virulent strain of *Pseudomonas solanacearum* compared to control and SB-37 plants (Jaynes et al. 2002). Expression of the mammalian antimicrobial peptide cecropin P1 in transgenic tobacco led to enhanced resistance to phytopathogenic bacteria *Pseudomonas syringae* pv. *tabaci*, *Pseudomonas marginata*, and *Erwinia carotovora* (Zakharchenko et al. 2005). Expression of pnAMP-h2 cDNA which express Pn-AMP2, a hevein-like peptide from the seed of *Pharbitis nil* with potent *in vitro* antifungal activity, in transgenic tobacco resulted in improved resistance against *Phytophthora parasitica*, the causal agent of black shank disease (Koo et al. 2002). Esculentin-1 is a 46-residue AMP present in skin secretions of *Rana esculenta*. As a possible approach to enhance plant resistance, an esculentin-1 encoding gene, with the substitution Met-28Leu, introduced to tobacco. Transgenic plants indicated resistance against bacterial and fungal phytopathogens and showed insecticidal properties as well (Ponti et al. 2003).

Expression of defense-related genes under regulation of a promoter which activates only in response to pathogen attack or lesions is an advantageous trait that is highly recommended in genetic engineering of plants for disease-resistance enhancement. To attain this goal a plant transformation vector was constructed with transcriptional

fusion between the pathogen responsive win3.12T promoter from poplar and the gene encoding the novel hybrid peptide, CEMA. Expression level of the peptide as regulated by the win3.12T promoter was sufficient to confer resistance against highly virulent fungus *Fusarium solani* in transgenic tobacco (Yevtushenko et al. 2005).

Another recommended strategy for genetic engineering of plants is genetic manipulation of the chloroplast genome instead of the nuclear genome, due to higher levels of expression and prevention of pollen-mediated escape of transgenes. Expression of MSI-99 via the chloroplast genome led to highly improved resistance in transgenic tobacco against the fungal pathogen, *Colletotrichum destructivum* and the bacterial pathogen *Pseudomonas syringae* pv. *tabaci*. Meanwhile, *in vitro* assays confirmed growth inhibition of *P. syringae* pv. *tabaci*, *Aspergillus flavus*, *Fusarium moniliforme*, and *Verticillium dahliae* (DeGray et al. 2001).

Antiviral activity of AMPs is a controversial issue that is worthy of attention due to successful reports of tobacco mosaic virus (TMV) suppression in experiments that involved AMPs. Tobacco leaves inoculated with analogs of melittin that have a similar sequence and structure to an essential domain of the TMV coat protein were found to possess highly specific antiviral activity against TMV. The data suggests that the peptides are implicated in antiviral activity as a result of the similarity to the viral coat protein sequence and the folding that is induced by virus RNA (Marcos et al. 1995). According to this report, searching for sequences with homology to viral proteins among AMPs would be useful, as the transgenic plant potentially could have enhanced resistance against viral and microbial pathogens. While the above-mentioned report attributed the antiviral activity of melittin analogs to the structural features of the peptides, a recent report claims that the antiviral activity of PV5, a synthetic variant of polyphemusin which is a cationic peptide from *Limulus polyphemus*, is possibly rooted in the involvement of the AMP in modulation of defense gene(s) expression or its interaction with other defense-signaling pathways. In addition to TMV resistance, expression of PV5 in transgenic tobacco led to enhanced resistance against bacterial and fungal phytopathogens (Bhargava et al. 2007).

Though tobacco is cultivated as a field crop, due to its tractability to genetic manipulation and well documented protocols and papers about its genetic transformation, it is widely used as a model crop for improvement of recombinant DNA techniques and commercial or experimental expression of proteins. In this context, some of the above-mentioned researches have been done mainly for investigation of *in planta* performance of AMPs, maybe for use in other crops. On the other hand, expression of AMPs in other plants is done mainly for control of diseases and pests of the same plant.

Potato

Potato is easy-to-grow and nutritious and is consumed daily by more than one billion people around the world. Recent advances in structural and functional genomics and the exclusive capacity for integration of foreign genes in genome of potato have made it an easy target for genetic modification (Mullins et al. 2006). There are many reports of genetic modification of potato by AMP-encoding genes. Alfalfa antifungal peptide (alfAFP) isolated from seeds of *Medicago sativa*, displays strong activity against the harmful fungal pathogen of potato, *V. dahliae* (Gao et al. 2000). MsrA3, an N-terminally modified analog of temporin A, as expressed in potato led to resistance against two prevalent potato diseases, late blight and pink rot that stem from *Phytophthora infestans* and *Phytophthora erythroseptica*, respectively. In this case, the activity of bacterial phytopathogen *E. carotovora* was also inhibited (Osusky et al. 2004). In a similar experiment, this time with low level expression of MsrA2, an analog of dermaseptin B1, transgenic potato showed high resistance to highly virulent fungal genera including *Alternaria*, *Cercospora*, *Fusarium*, *Phytophthora*, *Pythium*, *Rhizoctonia*, *Verticillium* and the bacterial phytopathogen *E. carotovora*. Late blight, pink rot and dry rot are important fungal diseases that were severely suppressed in this way (Osusky et al. 2005). Expression of a synthetic gene that encodes the N terminus-modified cecropin-melittin cationic peptide, the chimera MsrA1, resulted in resistance against several phytopathogens in the case of two potato varieties (Osusky et al. 2000). It is very significant that such broad-spectrum and powerful resistance to infective agents is attained by expression of relatively small peptides.

Tomato

Tomato is another member of the Solanaceae family which is vastly transformed by foreign genes. Since tomato is invaded by several fungal pathogens, many researchers have attempted to lessen the damage by transmission of AMP-encoding genes to tomato. Expression of MSI-99 in tomato led to symptom mitigation of bacterial speck disease, caused by *Pseudomonas syringae* pv. *Tomato* (Alan et al. 2004).Early blight, caused by *Alternaria solani*, is not only a prevalent, but also a highly serious fungal disease of tomato as it results in yield loss and reduction of fruit quality. Tomato lines which had been transformed by introduction of a gene from *Mirabilis jalapa*, encoding *Mj*-AMP1, showed enhanced resistance to early blight disease (Schaefer et al. 2005). Late blight was suppressed by expression of pep11 which is a very small AMP, through substitution of the gene that encodes this peptide instead of the systemin sequence in the prosystemin gene.

The problematic smallness of pep11 persuades scientists to exploit the proteolytic mechanism which is used by solanaceous plants to produce the very small, 18-amino acid-signaling peptide systemin from prosystemin (Jones et al. 2004).

Oilseed crops

Oilseed crops are valuable sources of both carbohydrate and protein. Therefore, making them resistant against pathogens is economically important. The so-called "white mold" or Sclerotinia disease that stems from a fungus with the same name *Sclerotinia sclerotiorum* is a significant cause of loss in oilseed crops such as sunflower, canola and soybean. Tachyplesin, an AMP isolated from the hemocytes of the *Tachypleus tridentatus* has been evaluated as a potential candidate for inhibition of this disease in sunflower (Lu 2003). Production of canola is affected by blackleg disease, which arises from a fungal pathogen *Leptosphaeria maculans*. Introduction of a gene encoding MiAMP1, a novel AMP isolated from the seeds of *Macadamia integrifolia*, to canola resulted in mitigation of the disease (Kazan et al. 2002). Also, expression of pea defensin led to limited resistance to blackleg in transgenic canola (Wang et al. 1999).

Rice

Rice is a major staple crop and serves as a model cereal crop plant for scientific studies (Peters 2006). Rice plants expressing the cecropin A gene of *Hyalophora cecropia* showed enhanced resistance to *Magnaporthe grisea*, the causal agent of rice blast disease. Two synthetic cecropin A genes, which were designed either to retain the cecropin A peptide in the endoplasmic reticulum (ER-CecA) or to secrete cecropin A to the extracellular space (Ap-CecA), were prepared. Though both genes expressed properly and products of both of them showed activity against *M. grisea in vitro*, due to negative effect of the latter gene version on fertility, ER-CecA was suggested as a potent candidate for protection of rice plants against the rice blast fungus *M. grisea* (Coca et al. 2006). This experiment is an example of how unintended effects of a gene expression could affect future decision-making. Another devastating rice disease is bacterial leaf blight caused by *Xanthomonas oryzae* pv. *Oryzae*. Transgenic expression of cecropin B, isolated from *Bombyx mori*, confined lesion development in the infected leaflets (Sharma et al. 2000).

Trees

Exploitation of AMPs in plants is not limited to field crops. Trees are also transformed by AMP-encoding genes for resistance against devastating pathogens. Attacin E is an AMP originated from *Hyalophora cecropia*. Expression of attacin E in transgenic 'Royal Gala' apple resulted in significant resistance to *Erwinia amylovora*, the bacterial agent that causes fire blight disease (Norelli et al. 2000). Introduction of attacin E in transgenic pear led to similar results (Reynoird et al. 1999). Expression of magainin-type genes in transgenic grapevine led to strong resistance to *Agrobacterium vitis*, the bacterial agent of crown gall disease and mild resistance against *Uncinula necator*, the fungal agent of powdery mildew (Vidal et al. 2006).

Fusarium wilt that stems from *Fusarium oxysporum* f. sp. *Cubense* and yellow sigatoka which is generated by *Mycosphaerella musicola* are among diseases threaten banana culture. Expression of MSI-99 in transgenic banana plants, culminated in significant resistance to both of these diseases (Chakrabarti et al. 2003). Since world *Musa* production is currently about 97 million tones annually, of which bananas cultivated for the export trade accounts for 10%, protection of banana production from menacing diseases is vital for food security and economical prosperity, particularly in the humid tropics (Tripathi 2003).

Other than fruit trees, there are trees with valuable applications for which making them resistant against diseases by means of AMPs is an issue of interest. Hybrid poplars (*Populus* spp.) are fast-growing trees which are suitable for production of bioenergy, fiber and other bio-based products (Gallagher et al. 2006). The gene coding for D4E1, a synthetic antimicrobial peptide consisting of 17 amino acid residues, was introduced into poplar (*Populus tremula* L. × *Populus alba* L.) via *Agrobacterium*-mediated transformation. One transgenic poplar line, Tr23, bearing the highest transcript accumulation for the D4E1 gene, showed significant reduction in symptoms caused by *Xanthomonas campestris* pv. *populi*, the causal agent of bark necrosis in poplar, and *Agrobacterium tumefaciens* (Mentag et al. 2003). *Septoria musiva* is a bark canker agent which causes significant losses in poplar productivity in many ways. Ac-AMP1.2 is an analog of Ac-AMP1, a small chitin-binding protein isolated from the seed coat of *Amaranthus caudatus*. ESF12 is a synthetic peptide with 18 amino acids and mimics the amphipathic α-helix found in magainin. Plasmids pCA1 and pCWEA1, carrying antimicrobial peptide gene(s), Ac-AMP1.2 and ESF12, were used to transform hybrid poplar clones Ogy and NM6. When evaluated for resistance against *S. musiva* with an *in vitro* leaf disk assay, the transformed Ogy plants indicated significantly increased pathogen resistance as compared to the untransformed Ogy (Liang et al. 2002). American elm (*Ulmus americana* L.), the most popular landscape tree in the United States, is threatened by Dutch-elm disease arises from fungus *Ophiostoma novo-ulmi*. Expression of a transgene that encods ESF39A, a synthetic AMP, led to

less disease symptoms in *O. novo-ulmi* (Newhouse et al. 2007).

In the overall analysis, we should bear in mind that there is less knowledge about the genetics and physiology of trees in comparison with crops. There are many technical problems that reduce efficacy of efforts for genetic improvement of trees. While some of this deficiency is a result of novelty of the field, some specific features of trees such as long generation interval of most tree species, make many crop-breeding experiences and methods inefficient or impossible in the case of trees. The hardships are intensified when the desirable trait is controlled by a gene complex or when financial constraints make the breeder consider selection for more than one trait. These conditions make assessment trials, especially those with the aim of estimating the environmental impact of transformed trees very complicated. While the problems concerned with transformed non-fruit trees are mainly environmental, in fruit trees the safety of fruits for consumers is an additional matter of concern.

In this context transformation of trees with AMP-encoding genes is very advantageous, for only a single gene may make a great economical margin in the inhibition of deleterious disease(s). Furthermore, assessment trials are more efficient and informative when a single gene is involved. Once a promising transformant is isolated, it would be efficiently multipliable by means of vegetative propagation methods, which are normally well established for tree species.

Control of insect and nematode pests

The insecticidal aspects of some AMPs are useful traits that due to the mentioned advantages of AMPs should be considered for production of pest resistant plants and new pesticide chemicals. Pest control based on AMPs which act against bacterial endosymbionts could be a promising strategy that needs more attention. Mycetocyte symbionts are specialized cells found in some insect groups such as aphids. These cells contain endosymbiotic bacteria that provide essential amino acids and other chemicals to their host. The green-peach aphid, *Myzus persicae*, is a major agricultural pest with a wide range of host plants, causing damage by feeding or by virus transmission. The effect of indolicidin was tested on survival of *M. persicae* and its bacterial endosymbionts. Artificial diet bioassays indicated a significant lethal effect of indolicidin on *M. persicae* survival for annihilation of endosymbiotic bacteria by the peptide (Le-Feuvre et al. 2007). Derangement of the chitin synthesis process would be another appropriate control for insect pests. Polyoxins are a class of peptide nucleosides isolated from *Streptomyces cacaoi* var. *asoensis*. The original interest in polyoxins arises from their ability to inhibit the enzyme chitin synthase and therefore to prevent the biosynthesis of chitin. Much of the exterior and some of the internal parts of insects are constructed of chitin. Without these membranes, insects are highly vulnerable to infections (Arakawa 2003).

Another potential that should not be neglected is the antinematodal effect of AMPs. The peptide HP (2–20), derived from the N-terminal sequence of *Helicobacter pylori* ribosomal protein L1 (RPL1), has nematicidal activity against eggs and worms of *Caenorhabditis elegans*, a well known nematode, through structural disruption of the egg's shell and the cell membrane (Jang et al. 2004). PMAP-23, an AMP isolated from porcine myeloid showed similar effects against *C. elegans* (Park et al. 2004). Due to increasing damage that nematodes impose on plants and livestock, nematicidal feature of AMPs should be investigated seriously.

Control of post-harvest decay

Post-harvest decay that stems from pathogens causes significant losses via quality reduction or complete spoilage of agricultural products. Application of chemical fungicides to control post-harvest diseases is increasingly restricted, due to concerns about the safety of fungicides and development of pathogen resistance to many key fungicides (Janisiewicz and Korsten 2002). Application of AMPs would be a suitable alternative over conventional methods. Transformation of the potato by the MsrA3-encoding gene, in addition to disease control, resulted in prevention of storage losses, since transgenic tubers remained disease-free during storage for more than 2 years (Osusky et al. 2004). Synthetic hexapeptides, PAF19 and PAF26 inhibited growth of *Penicilium italicum* and *Penicilium digitatum*, the causal agents of blue and green rot which are two major post-harvest diseases of citrus (Lopez-Garcia et al. 2003). Gray mold, a prevalent post-harvest disease arising from *Botrytis cinerea*, is controlled in strawberry by CgPep33, an antifungal peptide isolated from enzymatic hydrolysates of *Crassostrea gigas* (Liu et al. 2007).

Application of microorganisms, especially yeasts that occur naturally on the surface of fruits or vegetables, usually has many advantages as compared to chemical compounds. There are many yeast-based biocontrol agents. The limitations of these biocontrol products can be resolved in many ways including making them more efficient by genetic manipulation (Janisiewicz and Korsten 2002). To make an improved biocontrol organism, the ability to produce a cecropin A-based peptide was introduced to yeast (*Saccharomyces cerevisiae*). Transformed yeast inhibited the growth of germinated *Colletotrichum coccodes*, a fungal pathogen which causes tomato decay (Jones and Prusky 2002). A recombinant version of the

peach defensin gene, rDFN1, was expressed in the yeast (*Pichia pastoris*).The transgenic yeast showed an improved ability to inhibit the spore germination of two major post-harvest fungal pathogens of apple, *Penicillium expansum* and *B. cinerea* (Wisniewski et al. 2005).

The vase life of flowers mainly depends on their ability to take up water after harvest. But above a certain threshold number, bacteria in vase water can block all xylem vessels. In cut roses this problem leads to reduction of vase life and ornamental value. Tachyplesin I and cecropin B have been suggested as environmentally friendly substitutes for hazardous chemicals that are used for controlling the bacterial population in the water (Florack et al. 1996).

Applications in aquaculture

Though the use of antibiotics and vaccination in intensive aquaculture reduce the damages of disease outbreaks, concerns about antibiotic resistance development and antibiotic residues in aquaculture products are increasing (Akinbowale et al. 2006). Use of AMPs is considered as a solution to some of these problems. In an experiment, Coho Salmon (*Oncorhynchus kisutch*) infected with *Vibrio anguillarum*, the causative agent of vibriosis, received daily doses of an amidated form of pleurocidin, a linear AMP found in the skin mucous secretions of *Pseudopleuronectes americanus*, and CEMA. The fish had significantly lower accumulated mortalities in comparison to control group (Jia et al. 2000). A synthetic, amidated form of epinecidin-1, an AMP isolated from leukocyte cDNA library of *Epinephelus coioides*, exhibited high antimicrobial activity against *Vibrio parahaemolyticus*, *Vibrio alginolyticus*, *Vibrio vulnificus*, *Pasturella multocida*, *Morganella morganii*, *Aeromonas sobrio*, *Aeromonas hydrophila*, *Flavobacterium meningosepticum* and *Escherichia coli*, *in vitro*. Most of these bacteria are known as very detrimental pathogens in aquaculture. Some fungi, such as *Candida albicans* and *Microsporosis sanis*, were also sensitive to this synthetic peptide (Yin et al. 2006). Antiviral effects of cecropin B and its synthetic analog, C17, against several important pathogens of fish such as infectious hematopoietic virus, viral hemorrhagic septicemia virus, snakehead rhabdovirus and infectious pancreatic necrosis virus were proved in another report (Chiou et al. 2002). Probably the peptide acts via inhibition of viral replication by viral envelope disruption and viral capsid disintegration. In this context, there is a great potential for the development of disease resistant transgenic fish using AMP-encoding genes. Expression of cecropins in transgenic *Oryzias latipas* resulted in significant resistance of the transgenic fish against two major pathogens, *V. anguillarum* and *Pseudomonas fluorescens* (Sarmasik et al. 2002).

Applications in animal husbandry

AMPs have numerous potentials to develop the quality of conventional animal husbandry and to resolve some of the existing problems which are consequences of the extensive use of antibiotics.

Use of chromatographically purified, chemical or recombinant synthetic AMPs in animal husbandry is limited, due to high costs or for technical reasons. Alternative cost-effective strategies would include immunomodulation and transgenic approaches (Zhang et al. 2000). Expression of a synthetic cecropin-class lytic peptide in transgenic mice has led to resistance to *Brucella abortus*, the causal agent of brucellosis (Reed et al. 1997). Successful experiences on model animals such as mouse may be generalized to important livestock. Infection of the mammary gland, in addition to animal distress, is a major economic burden of the dairy industry (Donovan et al. 2005). According to several reports, *Staphylococcus aureus*, the major contagious mastitis pathogen, is suppressed by expression of antibacterial peptides. Following a report of considerable inhibition of *S. aureus* by expression of a bioactive variant of lysostaphin, a potent bacteriolytic peptidoglycan, in mammary glands of mice (Kerr et al. 2001), the same lysostaphin gene was introduced into goat mammary glands by mean of an adenoviral vector (Fan et al. 2002). As a step toward prevention and cure of *S. aureus* mastitis, transgenic dairy cows that express lysostaphin in their mammary epithelium and secrete that into milk, were created (Donovan et al. 2005). In another effort to address the mastitis problem, a plasmid-mediated gene transfer technique was used to enable goat mammary cells to synthesize and secrete bovine lactoferricin and bovine tracheal antibacterial peptides. All of the milk samples collected from the mammary glands suppressed different bacterial pathogens (Zhang et al. 2007).

Applications in food industry

Adding preservative is a common way of preventing or slowing microbial growth, the major reason of spoilage and poisoning of food products. However, there is a shortage of efficient and safe preservatives as a result of appearance of resistant forms of food pathogens in response to massive use of preservatives. On the other hand, minimally processed natural foods are desirable for consumers. As naturally originated compounds, AMPs are advantageous options for use as new preservatives. Among AMPs, the bacteriocin group is the favorite (Rydlo et al. 2006). Since many bacteriocins are effective against Gram-positive bacteria (e.g. *Listeria monocytogenes*), which generate most of the food born illnesses, bacteriocins are in focus of

many studies. Lantibiotics as a group of bacteriocins are ribosomally synthesized and post-translationally modified AMPs (Sen et al. 1999). Nisin, a lantibiotic which is produced by certain strains of *Lactococcus lactis*, was approved as a food preservative by the Joint FAO/WHO Expert Committee on Food Additives in 1969. Nisin considerably inhibits activity of many food pathogens in a broad range of products from dairy products to sea foods (Delves-Broughton 2005). The drawback is that as with any other preservative, activity of nisin is affected by other factors such as temperature or additives such as sodium chloride. Therefore, doing experiments for assessment of nisin efficacy in interaction with other factors is prevalent (Pawar et al. 2000).

Dipping and spraying foods with bacteriocins possibly leads to reduction or loss of antimicrobial activity as a result of inactivation of the bacteriocins by food components or dilution below active concentration through migration into the food product (Appendini and Hotchkiss 2002). On the other hand, by gradual release of bacteriocins from a packaging film to the food surface, only the necessary amounts of bacteriocin would be used and the agent would not be a direct additive to the food product (Gennadios et al. 1997). The effectiveness of packaging films for levels of their bacteriocin content and their texture has been the subject of many experiments (Franklin et al. 2004).

Bacteriocins are not merely simple preservatives, but usually have important role in quality and flavor of the food product. This aspect is explained better with the following example. The most important step in the proteolytic degradation of casein to small peptides and free amino acids is the gradual lysis of the starter strain (e.g. *Lactococcus lactis*) during ripening and the subsequent release of intracellular proteinases and peptidases. These compounds contribute directly to the flavor characteristics of mature cheese and form the basis for further flavor reactions. Therefore, to obtain better flavor in shorter time, the rate of starter cell lysis must be increased (Lortal and Chapot-Chartier 2005). By using a commercial mesophilic LD-type starter made up of *Lactococcus lactis* subsp. *cremoris*, *Lactococcus lactis* subsp. *lactis*, *Lactococcus lactis* subsp. *lactis* var. *diacetylactis* and *Leuconostoc mesenteroides* subsp. *Cremoris*, Hispanico cheese can be formed. In this process, *Enterococcus faecalis* INIA 4 culture in milk is added as a bacteriocin-producing adjunct. Adding 1 g/kg bacteriocin-producing culture in milk culminated in a doubled level of activity of aminopeptidase in one-day-old cheese and an almost twofold increase in proteolysis and levels of free amino acids in comparison with control cheese of the same age. Bacteriocin-producing culture reduced the level of hydrophobic peptides in the resultant cheese, increased the concentrations of 3-methyl-1-butanal,

diacetyl and acetoin, and resulted in the best scores for flavor (Oumer et al. 2001).

Use of natural antimicrobials in food industry is not limited to bacteriocins. Because of the long history of milk use, milk bioactive agents are typically safe candidates for use in food industry. Lactoferrin, the natural iron-binding defense protein in milk, has many commercial applications including its usage as a food preservative (Weinberg 2003). Lactoferricin, a peptide derived from lactoferrin, shows high antimicrobial effects. In an experiment, treatment of ground beef with lactoferricin B reduced the population of *E. coli* (Venkitanarayanan et al. 1999).

AMPs: potentials, concerns and considerations

There are several potential applications of AMPs to develop new strategies or substitute the existing ones for more sustainable and efficient agricultural activities. Though not yet released as drugs, many AMPs have been assessed as potential therapeutics which could replace the abolished antibiotics (Gordon et al. 2005; Schröder and Harder 2006). These new antibiotics would revolutionize the health situation of human and domesticated animals.

Some of the AMP-based products have been introduced to markets and are used in a broad range of agricultural activities. Already we have mentioned vast use of AMPs in food industry. As effective inhibitors of chitin synthesis, polyoxin-based compounds are applied as commercial fungicides against fungi, of which chitin is an essential component of the cell wall structure. Polar, a polyoxin B compound, efficiently controlled powdery mildew in apple, grapevines and nectarine trees, in commercial orchards (Reuveni et al. 2000). Polyoxin D zinc salt is used in turf-covered grounds such as golf courses and parks as a fungicide against *Rhizoctonia solani*, which causes brown patch disease. Certainly, many other AMP based medicines and chemicals will be available in the future, after passing regulatory risk assessments and registration.

Plants, animals or microorganisms that are transformed by AMP-encoding genes, should be assessed before release. Biosafety evaluations are necessary as a matter of human health and environmental concerns. Environmental effects of transgenic plants with AMP transgenes have been the subject of some studies. For example, in an experiment bacterial and fungal communities associated with magainin-producing potato plants were compared with those communities from the non-transgenic potato cultivars (O'Callaghan et al. 2004). This kind of experiment, along with regular biosafety assessments should be done in a more organized manner so that for every given crop there would be an organized and reliable protocol for risk

assessment. This approach accelerates release of AMP-expressing transgenic plants convincingly. Due to the lack of biosafety guidelines, several hundreds of transgenic lines of *Musa* sp. with AMP-expressing transgenes could not be used in field trials in most tropical countries (Tripathi 2003).

Genetic engineering considerably broadens the scope of possibilities and makes it possible to obtain stable genotypes by transferring genes between distant species. However, it is recommended to use genes which are from the same species or its near relatives (Rommens 2004). This attitude should be considered in transformation of organisms with AMP transgenes because of the rich AMP arsenal of each organism. For instance, wheat is the source of many AMPs that could be used in future breeding programs of this important cereal or its near relatives (Egorov et al. 2005). Improvement of cereal varieties through introduction of AMP transgenes is worthy of more attention.

From a more innovative point of view, AMP-based strategies could be applied for management of soil microbial community for disease biocontrol or changing soil microbial ecosystems in favor of the cultivated crops. Since some plants are highly dependent on their microbial symbionts, AMPs have the potential to be used as herbicides through annihilation of the microbial symbionts of the weeds.

Though resistance to AMPs among microbes is less likely to happen, it is very unrealistic to think that such resistance is impossible to develop. In a selection experiment, propagation of *E.coli* and *Pseudomonas fluorescens* in medium supplemented with pexiganan, an analog of magainin, led to evolution of resistance to this AMP among the above-mentioned organisms after successive generations (Perron et al. 2006). Naturally, microbes become resistant to AMPs through constitutive (e.g. altered membrane energetics) or inducible (e.g. developing proteolytic activities) strategies (Yeaman and Yount 2003). Therefore, to prevent the problems that we encounter due to irregular use of conventional antibiotics, AMPs should be used properly and providently.

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