World Applied Sciences Journal 32 (1): 66-77, 2014
ISSN 1818-4952

DOI: 10.5829/idosi.wasj.2014.32.01.83167

Microbial Protein Contribution in Biological Control: Minireview

[1]Sanaa S. Kabeil, [2]Abdelaal Shamseldin, [3]Elsayed E. Hafez,
[4]Mahfouz M. Abd-Elgawad and [5]A.M Aboulenein

[1]Department of Protein Research, Genetic Engineering and Biotechnology Research Institute, City of Scientific Research and Technology Applications, New Borg El-Arab, Alexandria, Egypt
[2]Department of Environmental Biotechnology at Genetic Engineering and Biotechnology, Research Institute, City of Scientific Research and Technology Applications New Borg El-Arab, Alexandria, Egypt
[3]Department of Plant Protection and Biomolecular Diagnosis, Arid Land Cultivation Research Institute at City of Scientific Research and Technological Applications New Borg El-Arab, Alexandria, Egypt
[4]Department of Plant Pathology, National Research Center, Giza, Egypt
[5]Department of Biochemistry, Faculty of Agriculture, Cairo University, Giza, Egypt

Abstract: The use of biological control is a fascinating phenomenon that help small farmers and large producers in the agricultural sector to prevent or reduce the plant infections with pests, fungi, insects, beetles, butterfly and viruses, therefore it is consequently necessary to renewal and updating the novel in methodology and knowledge that reach and help the scientist to improve this safe, applicable and inexpensive technology to be available in the hand of users, in order to increase the production of foods and to reduce the losses of crop yield due to plant pathology. Our review article discuses the contribution of different microorganisms such as bacteria, fungi and viruses as biological control agent, the mechanism of defense against diseases and the mode of action of some specific bacterocins such as Bt generated from *Bacillius thuregenesis* protein and Nisin for protecting plants from infection diseases.

Key words: Biological Control · Plant Pathogens · Bacteriocins · Bacteria Virus · Nematode As Biological Control

INTRODUCTION

Proteins are organic compounds consist of amino acids arranged in a linear chain and folded into a globular or fibrous form. The amino acids are joined together by the peptide bonds between the carboxyl of amino acid and the amino groups of adjacent amino acid residues forming poly polymer. The sequence of amino acids in a protein is defined by the sequence of a gene that is encoded in the genetic code [1]. In general, the genetic code specifies 20 standard amino acids; however, in certain organisms the genetic code can include seleno cysteine. Shortly after or even during synthesis, the residues in a protein are often chemically modified by post-translational modification, which alters the physical and the chemical protein structure as well as folding, stability, activity and ultimately, the function of the proteins. Proteins can also expressed together to achieve a particular function and they often associate to form stable complexes [2, 3]. Proteins are essential parts of organisms and participate in virtually every process within cells and many proteins are enzymes that catalyze biochemical reactions and are vital to metabolism.

Therefore, the aim of the present paper is to give an overview of the contribution of specific proteins and their functions to increase the ability of plant against pathogens and magnify their useful uses in biological control.

Proteins and Biological Control: Biological control of pests in agriculture is a method of controlling pests (including insects, mites, weeds and plant diseases) that

Corresponding Author: Sanaa S. Kabeil, Department of Protein Research, Genetic Engineering and Biotechnology Research Institute, City of Scientific Research and Technology Applications; New Borg El-Arab, Alexandria, Egypt. Tel: +2034593423; Fax: +2034593420.

relies on predation, parasitism, herbivory, or other natural mechanisms [4]. It can be an important component of integrated pest management (IPM) programs. Biological control is defined as the reduction of pest populations by natural enemies and typically involves an active human role. Natural enemies of insect pests, also known as biological control agents and include predators, parasitoids and pathogens. Biological control agents of plant diseases are most often referred to as antagonists. Biological control agents of weeds include herbivores and plant pathogens. Predators, such as birds, lady beetles and lacewings, are mainly free-living species that consume a large number of preys during their whole lifetime. Parasitoids are species that's immature develops on paper or within a single insect host, ultimately killing the host. Many species of wasps and some flies are parasitoids. Pathogens are disease-causing microorganisms including bacteria, fungi and viruses. They kill or debilitate their own host and are relatively specific. There are three basic types of biological control strategies; conservation, classical biological control and augmentation. Since all biological control agents include protein as an essential component in their body, some examples of biological control will be mentioned [5].

Biological Control of Insects: Bacteria as Biological Control: Over 90 species of naturally occurring, insect-specific (entomopathogenic) bacteria have been isolated from insects, plants and the soil, but only a few have been studied intensively. Much attention has been given to *Bacillus thuringiensis*, a species that has been developed as a microbial insecticide. *Bacillus thuringiensis* (Bt) occurs naturally in the soil and on plants. Different varieties of this bacterium produce a crystal protein that is toxic to specific groups of insects. Bt has been available in North America as a commercial microbial insecticide since the 1960s and is handling in the market under different trade names. These products have an excellent safety record and can be used on crops until close to the day of harvest [6].

Pests Attacked: Numerous moth, butterfly larvae, some beetle and fly larvae are susceptible to infection. Formulations of Bt variety kurstaki are available for the control of many caterpillar pests. Bt variety from Israel is marketed for use against black flies and mosquitoes, fungus gnats, although unless they used on a community-wide basis, it is probably more effective to eliminate standing water and control weeds at the edges of ponds. Bt variety aizawai is used to control wax moth larvae in bee hives and various caterpillars as well as for controling of diamondback moth caterpillar which has developed resistance to Bt variety kurstaki in some areas. Some available Bt varieties and target pests that have commercially scale are: *Bacillus thuringiensis* var. tenebrionis-Colorado potato beetle and elm leaf beetle larvae, var. kurstaki-caterpillars, var. israelensis-mosquito, black fly and fungus.

Mode of Action: The toxic crystal Bt protein in commercial formulations is effective only, when eaten by insects having a specific gut pH (usually alkaline) and the specific gut membrane structures is necessary to bind the toxin. [7]. Not only the insect must have the correct physiology and must be at a susceptible stage of development, but the bacterium must be eaten in sufficient quantity. When ingested by a susceptible insect, the protein toxin damages the gut lining, leading to gut paralysis. Affected insects stop feeding and die from the combined effects of starvation and tissue damage. Bt spores do not usually spread to other insects or cause disease outbreaks on their own as occurs with many pathogens [8].

Genes encoding Bt have been transferred into other microorganisms to produce more active formulations, some of which are commercially available in the market. Additionally, researchers have genetically engineered varieties of several plant species to express the Bt toxin as part of the plant's normal development. This has led to the production of "insect-resistant" Bt-transformed lines of tobacco, cotton, corn, tomatoes, potatoes and others. The evaluation and development of management systems for these new plant lines is the subject of considerable research.

So, different Bt could be developed as bio pesticides.

Bio pesticides are materials that are the natural products of organisms that are shown to be toxic to nematodes and other pests. The frequent implication is that such materials are environmentally safe because they are produced naturally rather than synthetically. This assumption is unwarranted; however, as some naturally occurring toxins are known to be extremely carcinogenic. Researchers at the Mycogen Corporation isolated and patented a strain of BT that produces a toxin effective against certain plant-parasitic nematodes. Bt crystals are protein crystals formed during sporulation in some Bt strains. Bt produces proteins that aggregate to form a crystal. In insects, the crystal proteins bind specifically to certain receptors in the intestine.

Humans and other vertebrates do not have these receptors in their bodies and so are unaffected by the toxin. The Bt gene responsible for toxin production effective against certain insect pests has already been transgenically inserted into the genome of some plants. Introduction of the nematode-effective Bt gene into a plant genome would ostensibly render that cultivar resistant to nematode parasitism through antibiosis, while retaining its desirable agronomic or horticultural characteristics. Development of Bt-transgenic rootstocks would potentially confer nematode resistance with no effect on the characteristics of the scion. Bt toxins relatively easy to make and are safe to humans and vertebrate animals. Wei *et al.* [9] found that the Bt crystal protein Cry5B destroyed the intestine of *Caenorhabditis elegans*. They also found that Bt crystal proteins, Cry6A and Cry14A, reduced *C. elegans* fecundity. The toxic crystal molecules coded by the Bt gene are large that they range in molecular mass from 40 to >70 kDa [9]. Crystals would need to be ingested by the plant-feeding nematode and there has been some speculation that stylet-aperture exclusion would be a problem. However, *Meloidogyne incognita, Globodera rostochiensis* and *Rotylenchulus reniformis* are able to ingest green flourescent protein molecules of >28 kDa size from plant cells [10]. The size of the Bt crystal is 40-70 kDa and there is currently no evidence to suggest that crystals of that size would be excluded by the stylet aperture [8].

Viruses as Biological Control: Insect-specific viruses can be highly effective as natural bio product that can be used to control of several caterpillar pests. Different strains of naturally occurring nuclear polyhedrosis virus (NPV) and granulosis virus are present at low levels in many insect populations. Epizootics can occasionally devastate populations of some pests, especially when insect numbers are high. Insect viruses need to be eaten by an insect to cause infection but may also spread from insect to insect during mating or egg lying. In some cases, for example while searching for suitable hosts for egg laying, beneficial insects such as parasitoids may physically spread a virus through the pest population. No threat to humans or wildlife is posed by insect viruses. Virus diseases of caterpillar pests may cause indirect mortality of some beneficial larval parasitoids if the host insects die before the parasitoids have completed development. Predators and adult parasitoids are not directly affected. Viruses can overwinter in the environment or in overwintering insects to re-establish infection in subsequent seasons. NPV strains have only been mass produced in living insects, a costly procedure. Viral insecticide development is further hindered by the fact that the viruses are specific to one species or genus, ensuring a relatively small market [7].

Plant-Parasitic Nematodes and Biological Control: Because nematodes often occur in high numbers in soil, it is not surprising that a wide variety of soil organisms exploit nematodes as food, i.e., as sources of carbon, nitrogen and energy. Those organisms that seek out and consume nematodes are called predators. Predators of plant-parasitic nematodes include mites, collembola, flatworms, protozoa and other predacious nematodes. Some organisms may antagonize nematodes by producing nematoicidal or nemastatic compounds such as ammonia, certain fatty acids and avermectins. This mode of action is referred to as antibiosis and involves bacteria and fungi. One of these four mechanisms of antagonism (predation, parasitism, competition and antibiosis), parasitism has received the most research effort. Predators are difficult to handle and manipulate, while competition and antibiosis are less direct and more difficult to quantify than parasitism and predation. With the exception of one bacterium, *Pasteuria penetrans*, most effort has focused on fungal parasites of nematode eggs and females [11].

Biological Control Strategies

Classical Biological Control: Applying of organisms antagonistic to pests into an environment (one form of classical biological control methods) has been successful in entomology, but not in plant pathology or nematology. This approach involves the one-time or repetitive release of an antagonist in an area where it is not naturally present. Such approach has been successful against exotic insect pests and weeds that have escaped their natural enemies when introduced to a new area. Most nematode pests are not exotic (or not recently exotic) and it is considered difficult to introduce an organism into soil where all ecological niches are expected to be filled by the wide variety of organisms already present (the soil foodweb) [12].

Inundation of Biological Control: A number of researchers have applied large quantities of nematode antagonists to soil aiming to induce biological control. Although occasional success has been reported, this approach has generally failed. The reason for failure is unknown because the researchers typically did not

determine if the applied organisms became established. An exception is the application of rhizosphere bacteria through drip irrigation. Drip irrigation would seem to be a viable method of applying and maintaining large numbers of nematode antagonists (presumably antibiotic producers) in the rhizosphere [12].

Conservation Biological Control: The greatest success of utilizing biological control of phytonematodes has involved the conservation and enhancement of antagonists naturally present in soil. In England, the cereal cyst nematode, *Heterodera avenae*, is controlled successfully by growing monocultures of small grains which support high biomass of certain nematophagous fungi. Natural suppression of plant-parasitic nematodes has also been documented in peach orchards in the United States and vineyards in Australia. Methods for enhancing natural biological control include chitin amendments (to stimulate chitinolytic organisms that can degrade the eggshells of nematodes), collagen amendments (to stimulate collagenolytic organisms that can degrade the cuticle of vermiform nematodes) and other organic amendments (to increase the C: N ratio and, thus, stimulate the activity of nematode-trapping fungi) [12].

Research Needs in Biological Control: Where biological control of nematode pests appears to be occurring, it is essential that the mechanism of antagonism be established for the system in question. This is relatively easy for parasites and predators, but quite difficult for antibiosis and competition. In addition, researchers must be able to quantify the antagonist and antagonist activity. Without this information, they cannot understand and remedy the inconsistency of results that is characteristic of biological control research [13]. As their proximal sources, these food chains are dependant on plants and their fixing of carbon dioxide and water into carbohydrate through the process of photosynthesis. Primary consumers, including plant-parasitic nematodes, are among the initial links in the chains. Other initial links are direct release of plant materials and products into the soil that fuel pathways of decomposition; the fuel includes plant litter, rhizodeposition and root sloughing and root exudates.Several types of fungi are important antagonists of nematodes. Among them are the trapping fungi that capture nematodes in various forms of traps. Additional research is needed to determine which organisms are most effective and also to suggest methods for enhancing their activity in the soil [13].

Phytopathogenic Bacteria and Fungi as Biological Control Agents: Some *Paecilomyces fluorescens* strains (CHA0 or Pf-5, for example) present biocontrol properties, protecting the roots of some plant species against parasitic fungi such as *Fusarium* or *Pythium*, as well as some phytophagous nematodes [14]. To be specific, certain *P. fluorescens* isolates produce the secondary metabolite 2,4-diacetylphloroglucinol (2,4-DAPG), the compound found to be responsible for antiphytopathegenic and biocontrol properties in these strains [15]. Another example is the fungus *Trichoderma harzianum*: a biological control agent that has antagonistic activity against phytopathogenic fungi. The mechanism of this activity is to inhibit spore germination and germ-tube elongation and to degrade the tips of fungal hyphae. Multiple applications of fungicides are relied upon by apple growers almost exclusively to control this disease during the growing season. Transgenic 'McIntosh' apple trees expressing either the endo- or exo chitinase gene or both genes have increased resistance to apple scab. These results suggest the potential broad usage of chitinase transgenes to control fungal diseases of plants [16].

Biological Control Using Bacteriocin: Many of early studies explored the possibility of controlling disease such as anthrax and diphtheria using nonpathogenic antagonistic microorganisms. The inhibitory bacterial products include a wide range of substances such as classical low molecular weight of antibiotics, lytic agent enzyme, bacteriocins and bacteriophages [17, 18].

Definition and Characteristics of Bacteriocins: Bacteriocins may be defined as non-replicating anti-bacterial substances of protein origin that have specific inhibitory effect against closely related microorganisms. Originally, the term bacteriocins was applied to antibiotic-like compound produced by *Escherichia coli* "the Colicin", which were specific in killing closely related strains and were protein in nature. The specificity and chemical composition of these compounds distinguished them from classical antibiotics such as streptomycin, a low molecular weight broad spectrum glycoside [19]. Today bacteriocins are defined primarily in terms of specificity of inhibition. In identifying bacteriocins, it is important to make sure that the antagonism really is specific and not due to non-specific compound such as hydrogen, peroxide or lactic acid. This can be achieved by testing the compounds against the producer organism, as with genuine bacteriocins,

where the producer is rarely to be sensitive to its own bacteriocin. Several bacteriocins differ from most others antibiotics because they have a narrow spectrum of actions and lethal only for bacteria which are closely related to strain that produced them [20]. The classical criteria for bacteriocin characterization can be summarized as following: (1) A narrow spectrum of activity centered a round the homologous species, (2) The presence of an essential biologically active protein moiety, (3) Bactericidal mode of action even after exposing to high temperature (heat stability), (4) Attachment to specific cell receptors, (5) Production of bacteriocin and host cell bacteriocin immunity is specified by plasmid and (6) Production by lethal biosynthesis.

On the other hand, recent researchers have been concluded that some of the typical features associated with bacteriocin of Gram-positive bacteria showed some discrepancies from above criteria, that it has a wider spectrum of activity against organisms of different species and genera (occur through different mechanisms).

So criteria (2) and (3) have been generally applicable to well characterized bacteriocin of Gram-positive as well as Gram-negative bacteria, but criteria (4) and (5) required more analysis with some exceptions, while criterion (6) has only been studied in relation to a limited number of bacteriocins and may be found to be associated only with the inducible bacteriocins. Finally the term bacteriocin can be applied to those bacterial substance that have been shown to meet at least criteria (2) and (3) listed above.

Nomenclature and Classification of Bacteriocin: The type of bacteriocin is based sometimes on generic or on the species designation of the strains [21]. For example, bacteriocin produced by *Corynebacterium diphtheria* known as corycin or diphthericin, while those of *Staphylococcus aureocin* known as either Staphylococcus or aureoucin [22]. Similarly bacteriocins of the genus *Clostridium* have been termed clostricin or clostridiocin. In many cases, authors have added a terminal "e" to the bacteriocin name (e.g. staphylococcine and corycine). Due to the production of different types of bacteriocins from diverse organisms belonging to a single species, therefore, additional designation appeared to be required for further differentiation. In this respect, it has been suggested that the name of bacteriocin included a trivial designation of the producing strain with consecutive letters of the alphabet [23, 24]. For example colicin EI-K30 is a colicin of EI type produced by *E.coli* strain K30 and streptococcin A-FF22 is a bacteriocin produced by group A of Streptococcus strain FF22 [24]. Usually, the production of different bacteriocin by strains of a particular species is first suspected from variation in their spectral activity. This criterion is most frequently used as the bases for the provisional subdivision of a group of related bacteriocins. On the other hand, bacteriocins can be categorized on the basis of differences in their activity [25], on their distinctive characteristics such as production kinetics, mode of action [22] and their Sensitivity to proteolytic enzymes and heat [26].

Physical Properties of Bacteriocin: Substances that have been designated as bacteriocin are ranging from simple low molecular-weight protein such as streptococcin A-FF22 with a molecular-weight of 8,000 Daltons [27] to a complex defective phage particle with a molecular-weight in a range of 10^6 Daltons. However, Bradley [27] divided the bacteriocins into two groups designated as low and high molecular-weight bacteriocins. Low molecular-weight bacteriocins are characterized as more susceptible to trypsin digestion, less sensitive to heat inactivation and were not phage related, while the high molecular-weight bacteriocins are phage related. Bacteriocins may be chemically divers, but they must contain an essential protein. To identify the essential component of bacteriocin, test of sensitivity to specific enzymes, proteinases, lipases, etc.; are necessary used [28]. According to chemical analysis, bacteriocins defined as simple protein structure, however most of them consist of complex of protein with lipids or carbohydrates such as some Staphylococcin and Clostridiocin [29, 25].

Mechanism of Bacteriocin Action: Much of information, explaining the mechanism of bacteriocin action, has been done on studies specific to colicin. The question is how many proteins such as colicin can kill bacteria? Biochemical studies on colicin revealed that it can be act in different mechanical ways, as they can kill cells by affecting the intra-cellular targets such as DNA or ribosomes [30, 19]. The second question is, how bacterial cells which possess impermeable cell wall to protein being affected by bacteriocin?. The common possible hypothesis explaining the mode of action of bacteriocin is the interaction of bacteriocin with sensitive cells occurs at different two stages. In the first stage the bacteriocin can be bind with the receptors of cell bacterial envelope. This binding does not lead to any permanent physiological change leading to cell death unless the cytoplasmic membrane is energized during this stage.

If the membrane produced enough energy, the cell death will be occurred in the second stage as a result of membrane irreversible physiological changes [20, 21].

Biochemical Effects of Bacteriocins: Several types of bacteriocin contact directly with the cytoplasmic membrane and they defined as bactericidal because they can disrupt and stop the transfer of energy through the membrane. The high-energy state of the membrane is therefore, essential for the action of these bacteriocins and appears to be their "biochemical target" [31]. The energy is needed for activating several amino acids and transports them in the cytoplasm for synthesing of proteins, some sugars and some ions that can be provided by electron transport, via the energized state of the membrane, without the necessity for ATP or other intermediate phosphorylated compounds. In addition, the active transport process is associated with several cations that have essential role as enzyme co-factors [32, 31]. Plate *et al.* [33] stated that when *E. coil* cells treated with colicin (E) or (K) this normally inhibits the active transport process in those treated cells and cation such as K^+ or Mg^{+2} (accumulated by active transport), are immediately released from the treated cells. The release of those cations may be enough to cause cell death. The evidence confirming this conclusion is that, when *E. coli* cells were not killed by treating them with colicin K in the presence of high concentration of K^+ and Mg^{+2} [33]. Further evidences were also observed using different Colicins or Colicin like-compounds. Colicin E3-CA38 and Cloicin DF13 that having a similar amino acid composition do not kill cells by disrupting the cytoplasmic membrane but by inhibiting protein synthesis [34], this effect actually happened due to in activatation of bacterial ribosome, where both bacteriocins found to have similar effect on ribosomes when mixed together *in vitro*. Nisin is also a bacteriocin produced by some strains of *Lactococcus lactis* subsp. lactis. It is the only bacteriocin from lactic acid bacteria produced on commercial scale [35]. It's role still unknown but may be it has effect on the synthesis of cytoplasmic membrane. However, Lazdunski [30] reported that nisin must intact cell surface of microorganism to be effective as biological agent. In conclusion, bacteriocins may comprise three distinct domains; (i) a domain involved in the recognition of a specific receptor, (ii) a domain involved in translocation and (iii) a domain acting as lethal substance [36].

The Role of Bacteriocin in Biocontrol: Biological control of plant disease by microbial agents has been extensively investigated during the last two eras [20]. The successful bacteria-biocontrol agent of pre-and post-harvest crops has been reported by El-Masry *et al.* [37] and Axelrood *et al.* [38]. Different evidences proved that bacteriocins can play a major role in affecting the composition of microbial communities in certain microhabitats such as plant infection courts [38]. The use of microorganisms as biological control agents for plant diseases is of interest, but sometimes *in vitro* antibiosis may not be effective for biocontrol in the field. In other cases, there is a correlation between assays *in vitro* and biocontrol *in vivo*. Generally, an effective biocontrol agent may not be discovered or constructed until the dynamics of five-ways interaction of antagonisms, pathogen, plant, epiphytic micro flora and environment are fully understood [39]. Within the pathogenic bacteria and related organisms, bacteriocins are produced by wide different taxonomic groups of microorganisms and show corresponding diversity in their nature and specificity. Studying the correlation between bacteriocin production and their applications for controlling diseases *in vitro* and *in vivo* is a useful criterion for the assessment of their affecting role on the molecules at the plant surface. Based on this basis, herbicolin production by *E. herbicola* appears to be having limited importance in competition with *Erwinia amylovora*, while bacteriocin production by *E. carotovora* and *Agrobacterium radiobacter* is more competitive than other biological agents. A bacteriocin produced by *Pseudomonas syringae* pv. *ciccaronei* can be used after purification at different concentrations in culture and in planta, in order to inhibited the multiplication of *P. syringae* subsp. savastanoi, the causal agent of olive knot disease and affect the epiphytic survival of the pathogen on the leaves and twigs of treated olive plants [40]. On the other hand, the use of antibiotic generated by *E. herbicola* Eh252 in biocontrol of *Erwinia amylovora* was approved as successful method, while *E. hebicola* Eh252 is a non-pathogenic epiphytic bacterium that reduces fire blight incidence when sprayed onto apple blossoms before inoculation with *E. amylovora* the causal agent of fire blight. *E. herbicola* Eh252 can produce antibiotic-like compound on minimal medium that inhibits the growth of *E. amylovora* [37,41]. Biological control of plant disease normally involves the external application of specific microorganisms (antagonists) to the infected plants or to the surrounding environment to limit the initiation and distribution of the disease. Such biological control agents can act at various points on the disease cycle such as reduce the survival of the pathogen in the external environment, rebuild-up of pathogen on the lost surface, limit the entry of pathogen into the host and transmission

of the pathogen between hosts [42]. However, biological control is mainly due to microbial antagonism, which can act as directly competition on space or antibiosis, or indirectly for induced host resistance [39, 41]. In most cases, to have an active biological control bacterial agent against plant diseases this product must has the ability to limit the growth and activity of phytopathogenic bacteria at the plant surface (aerial or subterranean) by acting as antagonistic to these pathogens [20, 39, 43, 44].

Detoxification and Degradation of Virulence Factors: The other mechanism of biological control is the detoxification of pathogen virulence factors. For example, certain biocontrol agents are able to detoxify albicidin toxin produced by *Xanthomonas albilineans* [45]. The detoxification mechanisms include production of a protein that reversibly binds with the toxin in both *Klebsiella oxytoca* [45] and *Alcaligenes denitrificans* as well as an irreversible detoxification of albicidin mediated by an esterase that occurs in *Pantoea dispersa.* Several different microorganisms, including strains of *B. cepacia* and *Ralstonia solanacearum*, can also hydrolyze fusaric acid, a phytotoxin produced by various *Fusarium* species [45]. More often though, pathogen toxins display a broad-spectrum activity and can suppress growth of microbial competitors, or detoxify antiobiotics produced by some biocontrol microorganisms, as a self-defense mechanism against biocontrol agents [46]. Lately, it has been discovered that certain PGPB quench pathogen quorum-sensing capacity by degrading autoinducer signals, thereby blocking expression of numerous virulence genes. Since most, if not all, bacterial plant pathogens rely upon autoinducer-mediated quorum-sensing to turn on gene cascades for their key virulence factors (e.g., cell-degrading enzymes and phytotoxins) [46], this approach holds tremendous potential for alleviating disease, even after the onset of infection, in a curative manner.

Biological Control of Some Plants and Weeds: The defense strategy of plants against stress factors involves a multitude of tools, including various types of stress proteins with putative protective functions [47]. A group of plant-coded proteins induced by different stress stimuli, named “pathogenesis related proteins” (PRs) is assigned an important role in plant defense against pathogenic constraints and in general adaptation to stressful environment. A large body of experimental data has been accumulated and changing views and concepts on this hot topic have been evolved [47]. A clear example is the fungus *Pleospora papaveracea* and it's Nep1, a phytotoxic protein from *Fusarium oxysporum*, which evaluated for its potential as a biocontrol agent on opium poppy (*Papaver somniferum*). Four treatments consisting of a control, *P. papaveracea* conidia, Nep1 (5 µg/ml) and *P. papaveracea* conidia plus Nep1 (5 µg/ml) were used in detached-leaf and whole-plant studies. Conidia of *P. papaveracea* remained viable for 38 days when stored at 20 or 4°C. Nep1 was stable in the presence of conidia for 38 days when stored at 4°C or for 28 days at 20°C. Other important examples are illustrated by two important groups, i.e. *Trichoderma* spp. and Mycorrhiza.

***Trichoderma* Spp. As Biocontrol Agent:** The mechanisms by which strains of *T. harzianum* function against phytopathogenic fungi are mycoparasitism, antibiosis, competition for nutrients or space, tolerance to stress through enhanced root and plant development, induced resistance through Prs and enzymes, solubilization and sequestration of inorganic nutrients, inactivation of the pathogen's enzymes or/and enzymatic hydrolysis. All mechanisms, except competition, can be used biocontrol nematoda [48]. The physical interaction between *Trichoderma* and the plant was observed by electron microscopy to be limited to the first few cell layers of plant epidermis and root outer cortex [49]. Elicitors from *Trichoderma* activate the expression of genes involved in the plant defense response system and promote the growth of the plant, root system and nutrient availability. This effect in turn augments the zone for colonization and the nutrients available for the biocontrol fungus, subsequently increasing the effect of antagonism to plant pathogens [50, 51]. Trichoderma strains must colonize plant roots prior to stimulation of plant growth and protection against infections. Colonization implies the ability to adhere and recognize plant roots, penetrate the plant and withstand toxic metabolites produced by the plants in response to invasion by a foreign organism, whether pathogen or not. There are no data in the literature concerning Trichoderma genes specifically expressed during the interaction between fungus and plant roots, but there are several reports on altered gene expression during mycorrhizal development [52]. Mycorrhizal fungi interaction is modulated by plant flavonoids and fungal auxins, followed by morphogenetic events that include aspersorium development [52]. In addition, genes that encode hydrophobins and other cell-wall structural proteins are specifically expressed, or their expression is up-regulated [52].

Mycorrhiza and Biocontrol: Plants associate with many soil microbial symbiont that improve their nutrition. The most widespread association is mycorrhizal symbiosis, involving soil fungi and plant roots [53, 54]. In a mycorrhiza, the plant receives water and mineral nutrients collected in soil by the fungal partner [50]. Some plants also fulfill their nitrogen requirements by contracting associations with N_2-fixing prokaryotes, such as legumes with Rhizobiaceae and several Rosids with Cyanobacteria and Actinomycetes [55,56]. In the competition model, the mycorrhizal fungus is depleting nutrients in the rhizosphere and plant tissues, as measured in some studies [57] thus limiting the settlement of pathogenic intruders. In plant physiological modification models, fungal symbiont elicit plant defenses that although allowing mycorrhizal colonization, prevent pathogenic aggressions (and perhaps limit excessive growth of symbionts). Biochemical molecular markers for plant defense (pathogenesis-related proteins with anti-microbial activities, phytoalexins and wall-reinforcing lignification.) are elicited in several model mycorrhizas [58,59]. Certain biological control agents have been demonstrated to suppress disease by parasitizing the plant pathogen. In most cases the biocontrol agent is a fungus that parasitizes on a plant pathogenic fungus. Lytic activity has been demonstrated to be involved in this phenomenon and to comprise degradation of the chitin and glucans in the fungal cell wall and osmotic disruption of the cellular membrane. Transformants of *Trichoderma harzianum* that over express a chitinase are more effective in inhibition of growth of *Rhizoctonia solani* [60]. More interestingly, transformants of *Trichoderma longibrachiatum* that over express the b-i, 4-endoglucanase gene egli were more effective in controlling effects of *P. ultimum* on cucumber plant emergence and health [61, 62] describe the genetic modification of *T. harzianum* strain P1, resulting in disruption of a single copy gene that encodes a 42-kDa endochitinase. Endochitinase activity is important in biocontrol of *B. cinerea* by *T. harzianum*, but for control of Pythium and Rhizoctonia other mechanisms appear to play a role. For bacteria the role of lytic activity in biological control of plant pathogens is less clear. Many chitin-degrading soil bacteria have the ability to inhibit fungal growth. However, in many cases, bacterial antagonism was not associated with chitinase production [63,64]. On the other hand, it has been suggested that lysis of fungal cell walls of *F. oxysporum* f. sp. cucurnerinum by *Paenibacillus* sp. 300 and *Streptomyces* sp. 385 is involved in biological control of fusarium wilt by these bacteria [65].

Potentials of Antimicrobial Proteins and Genetic Engineering to Enhance Plant Resistance: Plants have both structural and biochemical defense strategies against pathogens. Plant pathogens, in turn, have counter strategies to ensure successful infection. Plant disease results when interaction between plants and pathogens leads to abnormal growth or crop yield. Plants grown for food, fiber, forage and ornamental purposes may be severely damaged and killed by diseases caused by pathogens. Chemical and biological treatments, cultural practices and resistant cultivars are used to control plant diseases and prevent severe crop losses. Unfortunately, these activities are not always successful. For instance, in one of the most widely cultivated fruit crops, apple, certain varieties are preferred by consumers and farmers for their fruit qualities and orchard characteristics. However, most of these 'accepted' apple varieties are susceptible to diseases and disease control is dependent on pesticides. Conventional plant breeding for single trait disease resistance in a perennial crop such as apple is hindered by self-incompatibility and heterozygosity. In other words, apples, as well as many other plants, do not breed true to variety. Even though they are disease resistant, progeny trees and plants often lack the table quality of the parents and are not accepted by consumers. Recent advances in genetic engineering offer alternative ways to transfer a resistance gene into popular commercial varieties without changing other favorable traits. One approach to improve plants' defense against a particular pathogen that has been made possible by genetic engineering is to use genes found in fungi, insects and animals. Antimicrobial proteins, peptides and lysozymes that naturally occur in insects [66], plants [67], animals [68] and humans [69, 70] are now a potential source of plant resistance. The production of active oxygen species like superoxide anions, hydroxy radicals and hydrogen peroxide, H_2O_2, have been observed in many plant-pathogen interactions and are known to play an important role in plant defense. Plants have been engineered to continuously produce active oxygen species. In transgenic potatoes containing a H_2O_2-generating glucose oxidase gene from the fungus *Aspergillus niger*, the resulting apoplastic accumulation of peroxide ions enhanced the plants resistance to *Phytophtora infestans*, late blight; *Verticillium dahliae*, Verticillium wilt; and *Alternaria solani*, early blight [71]. Lytic peptides are small proteins with an amphipathic a-helical structure which makes pores in membranes resulting in the lysis, for example, of the bacterial cell membrane [72]. Cecropins are antibacterial lytic peptides native to the hemolymph of *Hyalophora cecropia,* the

giant silk moth. Transgenic tobacco plants expressing cecropins have increased resistance to *Pseudomonas syringae* pv. *tabaci*, the cause of tobacco wildfire, a devastating disease that is difficult to control [73]. Bacterial blackleg of potato caused by *Erwinia carotovora* subsp. *atroseptica* can result in 30% yield reduction and 25% loss in storage even though chemical treatments and breeding for resistance are practiced. Synthetic lytic peptide analogs, Shiva-1 and SB-37, produced from transgenes in potato plants reduce bacterial infection caused by *E. carotovora* subsp. *atroseptica* in transgenic potato plants [74]. Attacins are another group of antibacterial proteins produced by *H. cecropia* pupae [75]. The mechanisms of antibacterial activity of this protein are to inhibit the synthesis of the outer membrane protein in gram negative bacteria [76].

CONCLUSION

Updating information and the knowledge of PRs use is important for estabilishing a good system for agricultural sustainable that will help to increase plant performances and production, disease resistance and general adaptation to stressful environment. This review article highlights the applicability of using PRs genes in gene-engineering technologies application for crop protection and improvement. However, fundamental aspects of PRs gene studies remain little understood, particularly the exact mechanisms of gene regulation; thus, the receptors, signal transducing cascades and molecular targets involved in PRs induction as a challenge for both fundamental and applied studies. Meanwhile, innovative technologies for screening plant resistance against pathogens are being tried using biochemical markers, especially enzymes, to expedite identification of plant resistance. On the other hand, Biological control of pests in agriculture is a method of controlling pests (including insects, mites, weeds and plant diseases) that relies on predation, parasitism, herbivory, or other natural mechanisms. It can be an important component of integrated pest management (IPM) programs. Various biocontrol methods and examples are presented herein to illustrate their potential in relation to proteins.

REFERENCES

1. Branden, C. and J. Tooze, 1999. Introduction to Protein Structure. New York: Garland Pub. ISBN 0-8153-2305.
2. Maton, A., J. Hopkins, C.W. McLaughlin, S. Johnson, M.Q. Warner, D. LaHart and J.D. Wright, 1993. Human Biology and Health. Englewood Cliffs, Michigan, USA: Prentice Hall. ISBN 0-13-981176-1. OCLC 32308337.
3. Ridley, M., 2006. Genome. New York, NY: Harper Perennial. ISBN 0-06-0194979
4. Flint, M. Louise and S.H. Dreistadt, 1998. Clark, Jack K., ed. Natural Enemies Handbook: The Illustrated Guide to Biological Pest Control. University of California Press. ISBN 9780520218017.
5. Douglas, A. D.A. Landis and D.B. Orr, 2012. Biological control: Approaches and Applications". University of Minnesota. Retrieved 3 September 2012.
6. Hoffmann, M.P. and A.C. Frodsham, 1993. Natural Enemies of Vegetable Insect Pests. Cooperative Extension, Cornell University, Ithaca, NY., pp: 63.
7. Bajwa, W.I. and M. Kogna, 2001. Bacillus thuringiensis based biological control of insect pests. Integrated Plant Protection Center (IPPC), Oregon State University, Corvallis.
8. Ali, A.S.R. and W.M. Mohd-Basri, 1997. A local Bacillus thuringiensis SRBT1 with potential for controlling *Metisa plana* (Wlk). Elaeis, 9: 34-45.
9. Wei, J.Z., K. Hale, L. Carta, E. Platzer, C. Wong, S.C. Fang and R.V. Aroian, 2003. Bacillus thuringiensis crystal proteins that target nematodes. Proceedings of the National Academy of Sciences of the United States of America, 100: 2760-2765.
10. Goverse, A., J. Biesheuvel, G.J. Wijers, F.J. Gommers, J. Bakker, A. Shots and J. helder, 1998. In planta monitoring of the activity of two constitutive promoters, CaMV 35S and TR2', in developing feeding cells induced by Globodera rostochiensis using green fluorescent protein in combination with confocal laser scanning microscopy. Physiology and Molecular Plant Pathology, 52: 275-284.
11. Agbenin, N.O., 2011. Biological control of plant parasitic nematodes: Prospects and challenges for the poor Africa farmer. Plant Protection Scieces, 47: 62-67.
12. Ferris, *et al*., 1992. Beyond pesticides-biological approaches to management in California.
13. http://plpnemweb.ucdavis.edu/nemaplex/Mangmnt/Biolmgmt.htm.
14. Haas, D. and C. Keel, 2003. Regulation of antibiotic production in root-colonizing Pseudomonas spp. and relevance for biological control of plant disease. Annual Reviews of Phytopathology, 41: 117-153.

15. Bangera, M.G. and L.S. Thomashow, 1999. Identification and characterization of a gene cluster for synthesis of the polyketide antibiotic 2,4-diacetylphloroglucinol from Pseudomonas fluorescens q2-87". Journal of Bacteriology, 181: 3155-3163.
16. Kisung, K., 2000. Using antimicrobial proteins to enhance plant resistance. APSnet Features. Online. doi: 10.1094/APSnetFeature-2000-0500C.
17. Stirling, G.R., 1991. Biological Control of Plant Parasitic Nematodes: Progress, Problems and Prospects. CAB International, UK., pp: 282.
18. Kabeil, S.S., S.M. Lashin, M.A. El-Saadani, M.M. Abd-Elgawad and A.M. Aboul-Einean, 2008. Potato brown rot disease in Egypt: current status and prospects. American-Eurasian Journal of Agriculture and Environmental Science, 4: 44-54.
19. Riley, M.A., 1998. Molecular mechanisms of bacteriocin evolution. Annual Review of Genetics, 32: 255-278.
20. Riely, M.A. and J.E. Wertz, 2002. Bacteriocins: evolution, ecology and application. Annual Review of Microbiology, 56: 117-137.
21. Hechard, Y. and H.G. Sahl, 2002. Mode of action of modified and unmodified bactericocins from Gram-positive bacteria. Biochimie, 84: 545-557.
22. Van Norman, G. and N. Groman, 1979. Method for quantitation sensitivity to Staphyococcal bacteriocin. Infection and Immunity, 26: 787-789.
23. Nomura, M., 1967. Colicins and related bacteriocins. Annual Review of Microbiology, 21: 257-284.
24. Gagliano, V.J. and R.D. Hinsdill, 1970. Characterization of a Staphylococcus aureus bacteriocin. Journal of Bacteriology, 104: 117-125.
25. Taqq, J.R., A.S. Dajani, L.W. Wannamaker and E.D. Gray, 1973. Group A streptococcal bacteriocin: production, purification and mode of action. The Journal of Experimental Medicine, 138: 1168-1183.
26. Yamamoto, Y., Y. Togawe, M. Shimosaka and M. Okazaki, 2003. Purification and characterization of a novel bacteriocin produced by Enterococcus faecalis strain RJ-11. Applied and Environmental Microbiology, 69: 5746-5753.
27. Bradley, D.E., 1967. Ultrastructure of bacteriophages and bacteriocins. Bacteriolological Review, 31: 230-314.
28. Tagg, J.R., A.S. Dajani and L.W. Wannamaker, 1976. Bacteriocin of Gram-positive bacteria. Bacteriological Review, 40: 722-756.
29. Ellison, J.S. and J.A. Kautter, 1970. Purification and some properties of two boticins. Journal of Bacteriology, 104: 19-26.
30. Hardy, K., 1984. Bacterial plasmids, Van Nostrand Reinhold. Co. Ltd; UK., pp: 104.
31. Lazdunski, C.J., 1995. Colicin import and pore formation: a system for studying protein transport across membranes. Molecular Microbiology, 16: 1059-1066.
32. Lazdunski, C.J., E. Bouveret, A. Rigal, L. Journet, R. Lloubes and H. Bendetti 1998. Colicin import into Escherichia coli cells. Journal of Bacteriology, 180: 4993-5002.
33. Plate, C.A., J.L. Suit, A.M. Jetten and S.E. Luria, 1974. Effects of colicira K on a mutant Escherichia coli deficient in Ca^{2+}, Mg -2+activated adenosine triphosphatase. Journal of Biology and Chemistry, 249: 6138-614.
34. Kopecky, A.L., D.P. Copeland and J.E. Lusk, 1975. Viability of Escherichia coli treated with colicin K. Proceeding of the National Academy of Science of the United States of America, 72: 4631-4634.
35. De Vuyst, L., 1994. Nisin production variability between natural Lactococcus lactis sub sp. *lactis* strains. Biotechnology Letters, 16: 287-292.
36. Bemdetti, H., M. Fremtte, D. Baty, M. Knibienhlen, F. Pattus and C. Lazdunki, 1991. Individual domains of colicin confer specificity in colicin uptake, in pore-properties and in immumity requirements. Journal of Molecular Biology, 217: 429-439.
37. El-Masry, M.H., T.A. Brown, H.A.S. Epton and D.C. Sigee, 1997. Transfer from Erwinia herbicola to Escherichia coli of a plasmid associated with biocontrol of fire Blight. Plant Pathology, 40: 865-870.
38. Axelrood, P.E., M. Rella and M.N. Schorth, 1988. Role of antibiosis in compelition of Erwinia strains in potato infection courts. Applied and Environmental Microbiology, 54: 3355-3359.
39. Sigee, D.C., 1993. Bacterial Plant Pathology: Cell and Molecular Aspects. Cambridge University Press, pp: 318.
40. Lavermicocca, P., S.L. Lonigro, F. Valerio, A. Evidente and A. Visconti, 2002. Reduction of olive knot disease by a Bacteriocin from Preudomonas syringae pv. ciccaronei. Applied and Environmental Microbiology, 68: 1403-1407.
41. Hattingh, M.J., S.V. Bear and E.W. Lawson, 1986. Scanning electronmicroscopy of apple blossom colonized by Erwinia amylovora and Erwinia herbicola. Phytopathology, 76: 900-904.

42. Trigalet, A. and D. Trigalet-Demery, 1990. Use of avirulent mutants of Pseudomonas solanacearum for the biological control of bacterial wilt of tomato plants. Physiology and Molecular Plant Pathology, 36: 27-38.
43. Beer, S.V., 1981. Biogical control of fire flight. Acta Horticulture, 117: 123-127.
44. Epton, H.A.S., M. Wilson, S.L. Nicholson and D.C. Sigee, 1994. Biological control of Erwinia amylovora with Erwinia herbicola, pp335-352. In "Ecology of Plant Pathogens", Blakeman, J.P.& Williamson, B.J. (eds). Wallingford UK: C.A.B. International.
45. Compant, S., B. Duffy, J. Nowak, C. Clément and E. Barka, 2005. Use of plant growth-promoting bacteria for biocontrol of plant diseases: Principles, mechanisms of action and future prospects. Applied and Environmental Microbiology, 71: 4951-4959.
46. Leong, J., 1986. Siderophores: Their Biochemistry and Possible Role in the Biocontrol of Plant Pathogens. Annual Review of Phytology, 24: 187-209.
47. Edreva, A., 2005. Pathogenesis-related proteins: research progress in the last 15 years. General Applied Plant Physiology, 31: 105-124.
48. Abd-Elgawad, M.M.M. and S.S.A. Kabeil, 2010. Management of the Root knot nematode, Meloidogyne incognita on Tomato in Egypt. Journal of American Science, 6: 256-262.
49. Yedidia, I., N. Benhamou and I. Chet, 1999. Induction of defence responses in cucumber plants (Cucumis sativus L.) by the biocontrol agent Trichoderma harzianum. Applied and Environmental Microbiology, 65: 1061-1070.
50. Yedidia, I., M. Shoresh, K. Kerem, N. Benhamon, Y. Kapulnik and I. Chet, 2003. Concomitant induction of systemic resistance to Pseudomonas syringae pv. lachrymans in cucumber by Trichoderma asperellum (T-203) and the accumulation of phytoalexins. Applied and Environmental Microbiology, 69: 7343-7353.
51. Harman, G.E., C.R. Howell, A. Viterbo, I. Chet and M. Lorito, 2004. Trichoderma species-opportunistic, avirulent plant symbionts. Nature Review Microbiology, 2: 43-56.
52. Franken, P., G. Khun and V. Gianinazzi-Pearson, 2002. Development and molecular biology of arbuscular myeorrhizal fungi. In: Osiewacz HD (ed) Molecular biology of fungal development. Marcel Dekker, New York, pp: 325-348.
53. Smith, S.E. and D.J. Read, 1997. Mycorrhizal symbiosis, Academic Press, New York.
54. Brundrett, M.C., 2002. Co-evolution of roots and mycorrhizas of land plants. New Phytologist, 154: 275-304.
55. Werner, D., 1992. Symbiosis of plants and microbes. Chapman and Hall, London.
56. Brundrett, M.C., 2004. Diversity and classification of mycorrhizal associations. Biological Review, 79: 473-495.
57. Larsen, J. and L. Bodker, 2001. Interactions between pea root-inhabiting fungi examined using signature fatty acids, New Phytology, 149: 487-493.
58. Cordier, C., M.J. Pzier, J.M. Barea, S. Gianninazzi and V. Gianninazzi-Pearson, 1998. Cell defense responses associated with localized and systemic resistance to Phytophthora parasitica induced in tomato by an arbuscular mycorrhizal fungus, Molecular Plant-Microbe Interaction, 11: 1017-1028.
59. Weiss, M., J. Schmidt, D. Neuman, V. Wray, R. Chris and D. Strack, 1999. Phenylpropanoids in mycorrhizas of the Pinaceae. Planta, 208: 491-502.
60. Limon, M.C., J.A. Pintor-Toro and T. Benitez, 1999. Increased antifungal activity of Trichoderma harzianum transformants that overexpress a 33-kDa chitinase. Phytopathology, 89: 254-261.
61. Migheli, Q., L. Gonzalez-Candelas, L. Dealessi, A. Camponogara and D. Ramon-Vidal, 1998. Transformants of Trichoderma longibrachiatum overexpressing the b-i, 4-endoglucanase gene egli show enhanced biocontrol of Pythium ultimum on cucumber. Phytopathology, 88: 673-677.
62. Woo, S.L., B. Donzelli, F. Scala, R. Mach, G.E. Harman, C.P. K ubicek, G. Del Sorbo and M. Lorito, 1999. Disruption of *ech42* (endochitinase-encoding) gene affects biocontrol activity in Trichoderma harzianum strain P1. Molecular Plant Microbe Interaction, 12: 419-429.
63. De Beer, W., J.A. Klein Gunnewiek, P. Lafeber, J.D. Janse, B.E. Spit and J.W. Woldendorp, 1998. Anti-fungal properties of chitinolytic dune soil bacteria. Soil Biology and Bioçhemistry, 30: 193-203.
64. Friberg, S., 2001. Distribution and diversity of arbuscular mycorrhizal fungi in traditional agriculture on the Niger inland Delta, Mali West Africa. CBM: Skriftserie, 3: 53-80.
65. Singh, G., E.P. Kapoor, S.K. Pandey, U.K. Singh and R.K. Singh, 1998. Antibacterial investigation on volatile oils of some higher plants. Indian Drugs, 35: 591-598.

66. Jaynes, J.M., K.G. Xanthopoulos, L. Destéfano-Beltrán and J.H. Dodds, 1987. Increasing bacterial disease resistance in plants utilizing antibacterial genes from insects. Bioassays, 6: 263-270.
67. Broekaert, W.F., B.P.A. Cammue, M.F.C. De Bolle, K. Thevissen, G.W. De Samblanx and R.W. Osborn, 1997. Antimicrobial peptides from plants. Critical Reviews in Plant Sciences, 16: 297-323.
68. Vunnam, S., P. Juvvadi and R.B. Merrifield, 1997. Synthesis and antibacterial action of cecropin and proline-arginine-rich peptides from pig intestine. Journal of Peptide Research, 49: 59-66.
69. Mitra, A. and Z. Zhang, 1994. Expression of a human lactoferrin cDNA in tobacco cells produces antibacterial protein(s). Plant Physiology, 106: 977-981.
70. Nakajima, H., T. Muranaka, F. Ishige, K. Akutsu and K. Oeda, 1997. Fungal and bacterial disease resistance in transgenic plants expressing human lysozyme. Plant Cell Report, 16: 674-679.
71. Whipps, J.M., 2001. Microbial interactions and biocontrol in the rhizosphere. Journal of Expermental Botany, 52: 487-511.
72. Boman, H.G., 1991. Antibacterial peptides: Key components needed in immunity. Cell, 65: 205-207.
73. Huang, Y. and J.H. Mcbeath, 1997. Plant promoters controlled expression of cecropin B gene in transgenic potatoes renders disease resistance to soft rot bacteria. Phytopathology 87: S45. (Abstr.)
74. Arce, P., M. Moreno, M. Gutierrez, M. Gebauer, P. Dell'Orto, H. Torres, I. Acuna, P. Oliger, A. Venegas, X. Jordana, J. Kalazich and L. Holuigue, 1999. Enhanced resistance to bacterial infection by Erwinia carotovora subsp. *atroseptica* in transgenic potato plants expressing the attacin or the cecropin SB-37 genes. American Journal of Potato Research, 76: 169-177.
75. Hultmark, D., A. Engström, K. Andersson, H. Steiner, H. Bennich and H.G. Boman, 1983. Insect immunity Attacins, a family of antibacterial proteins from Hyalophora cecropia. EMBO Journal, 2: 571-576.
76. Carlsson, A., T. Nyström, H. de Cock and H. Bennich, 1998. Attacin-an insect immune protein-binds LPS and triggers the specific inhibition of bacterial outer-membrane protein synthesis. Microbiology, 144: 2179-2189.