

RAPID SPECIFIC PATHOGEN FREE ANIMAL

FIELD OF THE INVENTION

5 The present invention relates to methods of producing specific pathogen free and/or specific pathogen resistant animals.

BACKGROUND TO THE INVENTION

Specific pathogen free (SPF) non-human animals are essential for research purposes and to maintain standardised health and farming. Technological advances in the past decade have led to more sensitive research and commercialization outcomes that are recognizably affected by
10 the presence of unwanted microorganisms, especially viruses. Accordingly, there is a need to produce/ farm animals which are free from these unwanted microorganisms (i.e. SPF animals).

Diseases are the bane of farmers everywhere, costing farmers large amounts of money annually. When one animal is affected by a disease, most animals in the vicinity or within the same farm will also be infected causing an epizootic that may be both dangerous to the health
15 of the human being and/or costing the human being lots of money. Many problems related to diseases are preventable by exercising common sense and science-based animal-rearing strategies. One of these methods is by using SPF non-human animals. For example, Early Mortality Syndrome (EMS) also known as Acute Hepatopancreatic Necrosis Syndrome (AHPNS) in shrimp typically manifests in the first 10-40 days after stocking in ponds. It began in
20 China in 2009, spread to Vietnam in 2010, to Malaysia in 2011 and then to Thailand in 2012 with global losses exceeding USD1 billion annually. Before the recent EMS epizootic, the only other shrimp virus capable of causing losses exceeding USD1 billion annually was the White Spot Syndrome Virus (WSSV). The pathogen responsible for EMS has recently been identified as a strain of *Vibrio parahaemolyticus* bacteria that has been transferred a toxic gene via a
25 specific bacteriophage.

A reason why SPF animals have become extremely important for example in aquaculture is because it is now commonplace to farm alien species that are not endemic to a particular nation and therefore, to prevent introduction of new microorganisms to a place, use of SPF animals are required. One example is the use of the fast growing shrimp species *Penaeus vannamei*
30 which although originally of Latin American origin, is now farmed in almost every nation where shrimp farming is a major aquaculture activity, replacing the slower growing Tiger Shrimp *Penaeus monodon*. For *P.vannamei*, under commercial conditions in Asian earthen ponds, typical growth rates of 1.0–1.5 g/wk (with 80–90 percent survival) are common in the high-density pond system (60–150/m²) currently in use in Thailand and Indonesia. In contrast, the
35 growth (and survival) rate of *P. monodon* has been declining in recent years from 1.2 to 1 g/wk

(and 45 percent to 55 percent survival) over the last few years in Thailand. In order to increase the survival rate of at least the *P. monodon*, use of SPF versions will help.

Standard practices for the production of SPF animals for example in aquaculture use has been described as long back as 1994 and little has changed since in terms of the actual process. The main objective has been to provide disease-free fry, fingerlings and post larvae to aquaculture farms to reduce the risk of disease introduction causing widespread epizootics.

Genuine SPF shrimp, by present conventions, are those which are produced from bio secure facilities, have been repeatedly examined, tested and found free of specified pathogens using intensive surveillance protocols and molecular methods, and originate from brood stock developed with strict founder population development protocols. These founder populations are generated by extensive quarantine procedures that result in SPF F1 generations derived from wild parents. The history of domestication programmes in various countries, in that such stocks may have been deliberately in-bred and consists entirely of siblings. This means that future generations of animals based only on such lines will probably lead to inbreeding within a few generations. Such inbreeding has been noted in stocks of *P. stylirostris* bred in Tahiti for 22 generations. It has also been noted in captive stocks of *P. vannamei*, which were characterized by a diminished ability to tolerate Taura Syndrome virus (TSV) challenges compared to a more diverse, heterozygous wild control population.

Accordingly, although SPF animals have their advantages, producing them is a time-consuming process that may result in other problems such as inbreeding. Further the potential drawback of SPF animals is that they are only SPF for the specific diseases for which they have been checked. However, there is yet to be any SPF source of EMS-free brood stock shrimp or post larvae available globally.

There is thus a need in the art for not only a quicker way of obtaining a SPF animal but also a simpler method of producing Specific Pathogen Resistant (SPR) animals.

SPR describes a genetic trait of a shrimp that confers some resistance against one specific pathogen. SPR shrimp usually result from a specific breeding programme designed to increase resistance to a particular virus. SPF and SPR are independent characteristics. Not all SPR shrimp are SPF and vice versa. A selective breeding programme for *P. vannamei* was initiated in 1995 in the Oceanic Institute in Hawaii. Original work was based on a selection index weighted equally for growth and TSV resistance (the major disease problem in the Americas at that time). Confirmation that growth and survival (to TSV challenge) responded well to selection was obtained, but there appeared to be a negative genetic correlation between these traits. Further investigation revealed that the shrimp selected only for growth were 21 percent larger

than unselected shrimp (24 vs. 20 g) after one generation, with a realized heritability (h^2) of 1. Females were 12.7 percent larger than males at about 22 g, but it was not possible to select for a higher percentage of females. Meanwhile, shrimp selected on an index weighted 70 percent for TSV resistance and 30 percent for growth showed an 18 percent increase in survival to a TSV challenge (46 vs. 39 percent) after one generation, with a realized heritability (h^2) of 0.28. However, selected shrimp were 5 percent smaller than control shrimp, revealing a negative genetic correlation between mean family growth and mean family survival to a TSV challenge. This negative correlation between growth and disease resistance must therefore be taken into account when developing breeding plans for these shrimp. Taura Syndrome Virus or TSV can cause significant losses in farms stocked with *P. vannamei* and can be transmitted easily through insect or avian vectors between ponds. Because of this, the use of TSV-resistant (TSV-SPR) strains combined with biosecurity measures to reduce infections with other viruses such as WSSV, IHHNV and YHV could greatly assist the development of the new culture industry for *P. vannamei* in Asia. Such a protocol was adopted by the United States of America industry that, as a result, has seen a 50 percent growth rate per year over the last few years.

In view of the above, SPF and SPR are both essential and improved methods of producing and breeding them is needed.

SUMMARY OF THE INVENTION

The present invention is defined in the appended independent claims. Some optional features of the present invention are defined in the appended dependent claims.

According to one aspect of the present invention, there is provided a method of producing at least one specific pathogen free (SPF) non-human animal, the method comprising:

- (a) selecting a surviving animal in an environment comprising at least one pathogen that is capable of infecting and/or killing the animal;
 - (b) administration of a fusion protein to the surviving animal wherein the fusion protein comprises at least one polypeptide B which is a Type 1 Ribosome Inactivating Protein (RIP) or fragment thereof; and
 - (i) at least one polypeptide A which is an Antimicrobial peptide; and/or
 - (ii) at least one polypeptide C which is a Cationic Antimicrobial Peptide (CAP) or fragment thereof; and
 - (c) resulting surviving animal is the SPF non-human animal.
- Step (c) may be confirmed using conventionally accepted molecular methods that show the absence of the pathogen in question.

In another aspect of the present invention, there is provided a method of producing at least one specific pathogen resistant (SPR) non-human animal, the method comprising:

- (a) producing a specific pathogen free animal according to any method of the present invention; and
- 5 (b) selective breeding of a male and female SPF non-human animal to produce a SPR non-human animal offspring.

Step (b) may occur at the first, second, third, forth, fifth or tenth generation in the procedure of selective breeding. In particular, step (b) may eventually occur.

- 10 According to a further aspect of the present invention, there is provided a specific pathogen free or resistant non-human animal produced by any method of the present invention.

As will be apparent from the following description, preferred embodiments of the present invention allow for a fusion protein with an optimal effectiveness with a broad spectrum therapy
15 and/or allowing oral delivery of the protein as some of the several applications.

BRIEF DESCRIPTION OF THE FIGURES

Preferred embodiments of the fusion protein will now be described by way of example with
20 reference to the accompanying figures in which:

Figure 1 is a translation map of RetroMAD1 (SEQ ID NO:1 and SEQ ID NO:2).

Figure 2 has two photos of gels showing A) Time course expression and B) Solubility of
25 RetroMAD1 expression in E. Coli BL21(DE3) cells. Cells harbouring pRMD were harvested before induction (0h), and after induction for 1h, 2h and 3h represents the pellet phase, the hours with asterisk (*) represents the supernatant phase. Proteins were analysed on a 15% SDS-PAGE. M: PageRuler™ Protein Ladder Fermentas, U: uninduced, IND: induced and IB: purified inclusion bodies. Arrow indicates E. coli produced RetroMAD1 (41.2 kDa).

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Figure 3 is a photo of an agarose gel showing the PCR products in particular, the expected band of 441 bp confirming the absence of the virus in the RetroMAD1 treated prawns.

Figure 4 shows the experimental set-up of Example 3 to test the effects of RetroMAD1 on
35 WSSV.

Figure 5 are graphs showing the results that RetroMAD1 treated prawns survived for a longer period of time compared to the control (i.e. WSSV infected prawns).

Figure 6 are gel images of showing the stability of fusion proteins, RetroMAD1, RetroGAD1, Amatilin and Tamapal1: A1 and A2 are RetroMAD1 subjected to temperature fluctuations; B1 and B2 are RetroGAD1 subjected to temperatures; C1 and C2 are Amatilin subjected to temperature fluctuations; D1 and D2 are Tamapal1 subjected to temperature fluctuations.

5 Protein Ladder is the marker for protein size; Control is untreated drug; T1-4 are the different temperature fluctuations (as shown in Table 6) BME is 2× β -mercaptoethanol, the samples are loaded with (+) or without (-) BME.

Figure 7 is a graph showing the percentage of viral reduction caused by Amatilin, RetroGAD1
10 and Tamapal1 exposed to various temperature fluctuations in simultaneous treatment determined by PCR.

Figure 8A-D are graphs showing concentration of RetroMAD1 ($\mu\text{g/ml}$) (A), RetroGAD1 ($\mu\text{g/ml}$)
15 (B), Amatilin ($\mu\text{g/ml}$) (C), Tamapal1 ($\mu\text{g/ml}$) (D) leached out against Time (minutes)

Figure 9 is a graph showing concentration of RetroMAD1 in hepatopancreas, tail muscle, faeces and control against time in a short-term pharmacokinetics study

Figure 10 is a graph showing concentration of RetroMAD1 in hepatopancreas, tail muscle,
20 faeces and control against time in a long-term pharmacokinetics study

Figure 11A and B is an image of plates showing the anti-bacterial activity of amatilin against *V. cholera* (A) and *V. parahemolyticus* (B). (Plate: 1. 322.5 $\mu\text{g/ml}$, 2. 161.25 $\mu\text{g/ml}$, 3. 80.63 $\mu\text{g/ml}$,
25 4. 40.31 $\mu\text{g/ml}$, 5. 20.16 $\mu\text{g/ml}$, 6. 10.08 $\mu\text{g/ml}$, 7. 5.04 $\mu\text{g/ml}$, 8. 2.52 $\mu\text{g/ml}$, 9. Untreated)

Figure 12 is a graph showing the percentage of viral reduction caused by Amatilin, RetroGAD1, RetroMAD1 and Tamapal1 in simultaneous treatment at 72h determined by PCR.

Figure 13 A-C is a graph showing the percentage of viral reduction caused by drugs (A: Amatilin; B: RetroGAD1, C: Tamapal1) incubated at different temperatures for 1, 7 and 30 days in simultaneous treatment determined by PCR. (* Thermostability was not tested for 50 °C for 30 days incubation)

Figure 14 is a schematic diagram showing the Supercritical Fluid Drying (SCFD) Process
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Figure 15 is a Scanning Electron Microscope (SEM) image of RetroMAD1 crystals

Figure 16 is a graph showing the percentage of viral reduction caused by RetroMAD1 micronized powder in simultaneous treatment determined by PCR.

DETAILED DESCRIPTION OF THE INVENTION

For convenience, certain terms employed in the specification, examples and appended claims are collected here.

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The term "adjuvant", as used in the context of the invention refers to an immunological adjuvant. By this, an adjuvant is meant to be a compound that is able to enhance or facilitate the immune system's response to the ingredient in question, thereby inducing an immune response or series of immune responses in the subject. The adjuvant can facilitate the effect of the therapeutic composition by forming depots (prolonging the half-life of the ingredient), provide additional T-cell help and stimulate cytokine production. Facilitation of antigen survival and unspecific stimulation by adjuvants may, in some cases, be required if the antigenic molecule are only weakly antigenic or only exerts weak to moderate interactions with compounds, molecules, or cells of the immune system.

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The term "analogue" as used in the context of the invention refers to a peptide that may be modified by varying the amino acid sequence to comprise one or more naturally-occurring and/or non-naturally-occurring amino acids, provided that the peptide analogue is capable of reducing or preventing growth of a tumour or cancer. For example, the term "analogue" encompasses an inhibitory peptide comprising one or more conservative amino acid changes. The term "analogue" also encompasses a peptide comprising, for example, one or more D-amino acids. Such an analogue has the characteristic of, for example, protease resistance. Analogues also include peptidomimetics, e.g., in which one or more peptide bonds have been modified. Preferred analogues include an analogues of a peptide as described according to any embodiment here comprising one or more non-naturally-occurring amino acid analogues.

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The term "comprising" as used in the context of the invention refers to where the various components, ingredients, or steps, can be conjointly employed in practicing the present invention. Accordingly, the term "comprising" encompasses the more restrictive terms "consisting essentially of" and "consisting of." With the term "consisting essentially of" it is understood that the epitope/antigen of the present invention "substantially" comprises the indicated sequence as "essential" element. Additional sequences may be included at the 5' end and/or at the 3' end. Accordingly, a polypeptide "consisting essentially of" sequence X will be novel in view of a known polypeptide accidentally comprising the sequence X. With the term "consisting of" it is understood that the polypeptide, polynucleotide and/or antigen according to the invention corresponds to at least one of the indicated sequence (for example a specific sequence indicated with a SEQ ID Number or a homologous sequence or fragment thereof).

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The term "derivative" as used in the context of the invention includes e.g., a fragment or processed form of the stated peptide, a variant or mutant comprising one or more amino acid substitutions, deletions or additions relative to the stated peptide, a fusion protein comprising the stated peptide or a peptide comprising one or more additional non-peptide components relative to the stated peptide e.g., a chemical component, e.g., polyethylene glycol (PEG). The term "derivative" also encompasses polypeptides comprising the fusion protein according to the invention. For example, the polypeptide comprises a label, such as, for example, an epitope, e.g., a FLAG epitope or a V5 epitope or an HA epitope. For example, the epitope is a FLAG epitope. Such a tag is useful for, for example, purifying the polypeptide. A preferred derivative of an antitumour or anticancer fusion protein of the invention has enhanced stability. For example, a cleavage site of a protease active in a subject to which a fusion protein is to be administered is mutated and/or deleted to produce a stable derivative of an antitumour or anticancer fusion protein of the invention. The term "derivative" also encompasses a derivatized peptide, such as, for example, a peptide modified to contain one or more-chemical moieties other than an amino acid. The chemical moiety may be linked covalently to the peptide e.g., via an amino terminal amino acid residue, a carboxy terminal amino acid residue, or at an internal amino acid residue. Such modifications include the addition of a protective or capping group on a reactive moiety in the peptide, addition of a detectable label, and other changes that do not adversely destroy the activity of the peptide compound.

Accordingly, acceptable amino acid substitutions are generally therefore based on the relative similarity of the amino acid side-chain substituents, for example, their hydrophobicity, hydrophilicity, charge, size, and the like. Exemplary substitutions which take several of the foregoing characteristics into consideration are well known to those of skill in the art and include: arginine and lysine; glutamate and aspartate; serine and threonine; glutamine and asparagine; and valine, leucine and isoleucine. The isolated peptides of the present invention can be prepared in a number of suitable ways known in the art including typical chemical synthesis processes to prepare a sequence of polypeptides.

The term "fragment" as used in the context of the invention refers to an incomplete or isolated portion of the full sequence of the fusion protein according to any aspect of the present invention which comprises the active site(s) that confers the sequence with the characteristics and function of the protein. In particular, it may be shorter by at least one amino acid. For example a fragment of the fusion protein according to the present invention comprises the active site(s) that enable the protein to recognise an aberrant cell such as a tumour cell or cancer cell. The fragment may at least be 10 amino acids in length. For example, a non-limiting fragment of RIP may at least comprise the core or the bioactive site of the RIP which may be approximately 5kDa in size.

The term "fusion protein(s)" as used in the context of the invention refers to proteins created through the joining of two or more genes, which originally coded for separate proteins. Translation of this fusion gene results in a single polypeptide with functional properties derived from each of the original proteins. Recombinant fusion proteins are created artificially by recombinant DNA technology for use in biological research or therapeutics. For example, the fusion protein according to any aspect of the present invention may comprise a polypeptide B; and a polypeptide C which is a CAP. The fusion protein may have antiviral properties. The fusion protein according to any aspect of the present invention may further comprise a polypeptide A. Each individual part and/or the whole the fusion protein may have antiviral properties. For example, polypeptide A, B, and/or C may have anticancer properties. As a whole A-B-C may have antiviral properties. The structure of the fusion protein may be A-B-C, A-C-B, C-A-B, C-B-A, B-A-C, B-C-A, A-B-C-C, A-B, B-C, B-C-C, C-C-B-C-C, or C-B-C. In particular, the fusion protein may comprise dimers and/or tandem repeats. More in particular, the structure of the fusion protein according to any aspect of the present invention may be repeats of the structure mentioned above. For example, the structure may be A-A-B-C-C, C-C-B-C-C, A-A-B-A-A and the like. The polypeptide A, B or C in each fusion protein may be the same protein or may be a different protein when repeated. Polypeptide A may be theta defensin, an analogue, or a fragment thereof. A fusion protein according to the present invention may comprise the sequence of SEQ ID NO:1, a variant, derivative or fragment thereof. The term "RetroMAD1" is used in the present invention to refer to a fusion protein with the structure A-B-C and with amino acid sequence SEQ ID NO:1. In particular, in RetroMAD1 polypeptide A may be Retrocyclin 101, polypeptide B may be MAP30 and polypeptide C may be Dermaseptin 1. These peptides may be directly fused to one another or connected to one another by a linker peptide.

The term "linker peptide", as used in the context of the invention is used interchangeably with the term "linker" herein. A linker peptide is a peptide that covalently or non-covalently connects two or more molecules or peptides, thereby creating a larger complex consisting of all molecules or peptides including the linker peptide. A non-limiting example of a linker peptide may be SEQ ID NO:3 and/or SEQ ID NO:27

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The term "pathogen" as used in the context of the invention may refer to any disease-producing agent, especially a virus, bacterium, or other microorganism. A virus may be selected from the group consisting of cytomegalovirus (CMV), Epstein-Barr virus (EBV), varicella zoster virus (VZV), HSV-1, HSV-2, HSV-6, BK-virus, influenza viruses, respiratory syncytial virus (RSV); human immunodeficiency virus (HIV), hepatitis A, B or C (HBV), polio viruses, enteroviruses, human coxsackie viruses, rhinoviruses, echoviruses, equine encephalitis viruses, rubella viruses, dengue viruses, encephalitis viruses, yellow fever, coronaviruses, vesicular stomatitis viruses, rabies viruses, ebola viruses, parainfluenza viruses, mumps virus, measles virus, Hanta

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viruses, bunga viruses, phleboviruses and Nairo viruses, hemorrhagic fever viruses, reoviruses, orbiviruses and rotaviruses, parvoviruses, papilloma viruses, polyoma viruses, adenoviruses, herpes simplex virus (HSV) 1 and HSV-2, varicella zoster virus, variola viruses, vaccinia viruses, pox viruses, African swine fever virus, Iridovirus, Infectious Salmonid Anaemia (ISA),

5 White Spot Syndrome Virus (WSSV), Hepatopancreatic parvo-like virus (HPV), Monodon Baculo virus (MBV), Infectious Hypodermal and Hematopoietic Necrosis Virus (IHHNV), Yellow Head Virus (YHV), Taura syndrome virus (TSV), Gill-associated virus (GAV), Laem-Singh Virus (LSNV), Infectious Myonecrosis Virus (IMNV), Mourilyan virus (MoV), Koi herpesvirus 1 (KHV 1), KHV2, KHV3, viral nervous necrosis (VNN), infectious pancreatic necrosis virus (IPNV),

10 channel catfish virus (CCV), fish lymphocystis disease virus (FLDV), infectious hematopoietic necrosis virus (IHNV) and viral hemorrhagic septicemia virus (VHSV), AVG, AMAV, swine hepatitis E virus, Circoviruses, Herpesviruses, Porcine cytomegalovirus, pseudorabies virus, Feline Panleukopenia virus (FPV), Feline herpesvirus, Feline calicivirus, Feline Leukemia Virus (FeLV), Feline Immunodeficiency Virus (FIV), Rabies virus, canine parvovirus, canine

15 coronavirus, canine distemper virus, canine influenza, canine hepatitis virus, canine herpesvirus, a virus that causes pseudorabies, canine minute virus and the like.

In particular, the viruses may only be viruses that are capable of infecting a non-human animal. The virus may be selected from the group consisting of Avian influenza viruses, Lymphoid

20 Leukosis, Visceral Leukosis (Marek's Disease), Quail Bronchitis viruses, Newcastle disease viruses, infectious bronchitis viruses, infectious Bursal disease viruses, rhinoviruses, echoviruses, equine encephalitis viruses, coronaviruses, vesicular stomatitis viruses, rabies viruses, ebola viruses, parainfluenza viruses, Hanta viruses, bunga viruses, phleboviruses and Nairo viruses, hemorrhagic fever viruses, reoviruses, orbiviruses and rotaviruses, parvoviruses,

25 papilloma viruses, polyoma viruses, adenoviruses, Aquabirnaviruses, Betanoda viruses, Salmonid alphaviruses, Epizootic Hematopoietic necrosis viruses, Infectious salmon anemia viruses (ISAV), Nervous necrosis viruses, Abalone Viral ganglioneuritis, Abalone Herpes-like viruses, variola viruses, vaccinia viruses, pox viruses, African swine fever virus, Iridovirus, Infectious Salmonid Anaemia (ISA), White Spot Syndrome Virus (WSSV), Hepatopancreatic

30 parvo-like virus (HPV), Monodon Baculo virus (MBV), Infectious Hypodermal and Hematopoietic Necrosis Virus (IHHNV), Yellow Head Virus (YHV), Taura syndrome virus (TSV), Gill-associated virus (GAV), Laem-Singh Virus (LSNV), Infectious Myonecrosis Virus (IMNV), Mourilyan virus (MoV), Koi herpesvirus 1 (KHV 1), KHV2, KHV3, viral nervous necrosis (VNN), infectious pancreatic necrosis virus (IPNV), channel catfish virus (CCV), fish lymphocystis disease virus

35 (FLDV), infectious hematopoietic necrosis virus (IHNV) and viral hemorrhagic septicemia virus (VHSV), AMAV, swine hepatitis E virus, Circoviruses, Herpesviruses, Porcine cytomegalovirus, pseudorabies virus, Feline Panleukopenia virus (FPV), Feline herpesvirus, Feline calicivirus, Feline Leukemia Virus (FeLV), Feline Immunodeficiency Virus (FIV), Rabies virus, canine

parvovirus, canine coronavirus, canine distemper virus, canine influenza, canine hepatitis virus, canine herpesvirus, a virus that causes pseudorabies, canine minute virus and the like.

5 A virus may include a bacteriophage, also known as a phage that includes a group of viruses that infect specific bacteria, usually causing their disintegration or dissolution. A bacteriophage may be selected from a group consisting of Myoviridae, Siphoviridae, Podoviridae, Lipothrixviridae, Rudiviridae, Ampullaviridae, Bicaudaviridae, Clavaviridae, Corticoviridae, Cystoviridae, Fuselloviridae, Globuloviridae, Guttavirus, Inoviridae, Leviviridae, Microviridae, Plasmaviridae, Tectiviridae and the like. In particular, the phage may be Lambda phage (λ phage) – lysogen (λ phage), T2 phage, T4 phage, T7 phage, T12 phage, R17 phage, M13 phage, MS2 phage, G4 phage, P1 phage, Enterobacteria phage P2, P4 phage, Phi X 174 phage, N4 phage, Pseudomonas phage Φ 6, Φ 29 phage, 186 phage and the like.

15 A bacteria may include *Aeromonas hydrophila*, *Aeromonas salmonicida*, *Aeromonas sobrio*, *Enterobacter aerogenes*, *Enterococcus faecalis*, *Escherichia coli*, *Flavobacterium meningosepticum*, *Helicobacter pylori*, *Klebsiella pneumonia*, *Listeria monocytogenes*, *Listonella anguillarum*, Methicillin-resistant *Staphylococcus aureus*, *Micrococcus luteus*, *Morganella morganii*, *Pasturella multocida*, *Pseudomonas aeruginosa*, *Salmonella typhimurium*, *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Staphylococcus haemolyticus*,
20 *Streptococcus agalactiae*, *Streptococcus equi*, *Streptococcus iniae*, *Streptococcus uberis*, *Vibrio alginolyticus*, *Vibrio anguillarum*, *Vibrio cholera*, *Vibrio damsela*, *Vibrio fluvialis*, *Vibrio furnissi*, *Vibrio harveyi*, *Vibrio hollisae*, *Vibrio metschnikovii*, *Vibrio mimicus*, *Vibrio parahaemolyticus*, *Vibrio proteolyticus*, *Vibrio vulnificus*, *Vibrio splendidus*, *Yersinia ruckeri* and the like.

25 The term "polypeptide" as used in the context of the invention may refer to a long, continuous, and unbranched peptide and may include cyclic polypeptides. Proteins consist of one or more polypeptides arranged in a biologically functional way and may often be bound to cofactors, or other proteins. In particular, the protein according to any aspect of the present invention may
30 be naturally occurring, de novo and/or synthetic.

The terms "subject", "patient" and "individual" are used interchangeably and are used in the context of the invention refers to either a human or a non-human animal. These terms include mammals, such as humans, primates, livestock animals (including bovines, porcines, etc.),
35 companion animals (e.g. canines, felines, etc) and rodents (e.g. mice and rats). In particular, the subject is an aquatic animal. The aquatic animal can be any animal, either vertebrate or invertebrate, which lives in the water for most or all of its life. The aquatic animal may be an arthropod for example a Horseshoe crab. In particular, the aquatic animal can be any crustacean which includes but is not limited to crabs, lobsters, crayfish, langoustine, shrimp,

and prawn. For example, a prawn can be decapod crustaceans. The term "prawn" can include cold water prawn, warm water prawn, caridean shrimp, whiteleg shrimp, Atlantic white shrimp, Indian prawn, banana prawn, tiger prawn and the like. In another example, the aquatic animal can be any fish, such as, for example, the Toad fish, zebra fish, Grouper or salmon; any crustacean such as, for example fiddler crab, or crayfish; or any cephalopod such as, for example, a squid. The aquatic animal can also be an amphibian such as, for example, a frog or salamander. The aquatic animal can be an animal adapted to fresh water, seawater, or brackish water. Both brackish water and seawater are saltwater. Brackish water has more salinity than fresh water, but less than seawater, such as the water in estuaries.

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The term "variant", as used in the context of the invention can alternatively or additionally be characterised by a certain degree of sequence identity to the parent polypeptide from which it is derived. More precisely, a variant in the context of the present invention exhibits at least 30% sequence identity, in particular at least 40%, 50%, 60%, 70%, 80% or 90% sequence identity.

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More in particular, a variant in the context of the present invention exhibits at least 95% sequence identity to its parent polypeptide. The variants of the present invention exhibit the indicated sequence identity, and preferably the sequence identity is over a continuous stretch of 100, 150, 200, 300, 315, 320, 330, 340, 344 or more amino acids. The similarity of nucleotide and amino acid sequences, i.e. the percentage of sequence identity, can be determined via

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sequence alignments. Such alignments can be carried out with several art-known algorithms, preferably with the mathematical algorithm of Karlin and Altschul (Karlin & Altschul (1993) Proc. Natl. Acad. Sci. USA 90: 5873-5877), with hmalign (HMMER package, <http://hmmer.wustl.edu/>) or with the CLUSTAL available e.g. on <http://www.ebi.ac.uk/Tools/clustalw/>. Preferred parameters used are the default parameters as

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they are set on <http://www.ebi.ac.uk/Tools/clustalw/> or <http://www.ebi.ac.uk/Tools/clustalw2/index.html>. The grade of sequence identity (sequence matching) may be calculated using e.g. BLAST, BLAT or BlastZ (or BlastX). Preferably, sequence matching analysis may be supplemented by established homology mapping techniques like Shuffle-LAGAN (Brudno M., Bioinformatics 2003b, 19 Suppl 1 :154-162) or

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Markov random fields. When percentages of sequence identity are referred to in the present application, these percentages are calculated in relation to the full length of the longer sequence, if not specifically indicated otherwise.

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The phrase "Specific pathogen free (SPF) animal" is a special stock of animals that are kept in specific pathogen free facilities under rigorous monitoring system, which are subjected to sensitive and accurate diagnostic methods. The traditional methods of producing SPF includes the animals being repeatedly bred under controlled conditions to maintain their freedom from specific pathogens and the SPF designation itself is tested on a regular basis over an extended period of time. The SPF animals may not innately be resistant to the specified pathogens or

infections, although they can possibly be developed as specific pathogen resistant (SPR) species. They are not produced to provide either superior genetic stock or improved culturing attributes such as faster growth. However, these characteristics can be incorporated into SPF stock to increase their commercial value. The SPF status of stock animals may be lost once the animals are removed from the designated facility even if the animals are not infected or develop any other disease symptoms. The SPF animals may be referred to as "high health" stock once they are transferred to other well-established unit with history of disease surveillance.

A person skilled in the art will appreciate that the present invention may be practiced without undue experimentation according to the method given herein. The methods, techniques and chemicals are as described in the references given or from protocols in standard biotechnology and molecular biology textbooks.

In one aspect of the present invention, there is provided a method of producing at least one specific pathogen free (SPF) non-human animal, the method comprising:

- (a) selecting a surviving animal in an environment comprising at least one pathogen that is capable of infecting and/or killing the animal;
- (b) administration of a fusion protein to the surviving animal wherein the fusion protein comprises at least one polypeptide B which is a Type 1 Ribosome Inactivating Protein (RIP) or fragment thereof; and
- (i) at least one polypeptide A which is an Antimicrobial peptide; and/or
- (ii) at least one polypeptide C which is a Cationic Antimicrobial Peptide (CAP) or fragment thereof; and
- (c) resulting surviving animal is the SPF non-human animal.

In particular, the specific pathogen free non-human animal may be considered an "instant specific pathogen free" or ISPF non-human animal that may be breeding stock indicating that a "viral clean-up" is possible.

The "surviving animal" in step (a) may be any animal that may be capable of enduring the environment with at least one pathogen thus staying alive in the presence of the pathogen. The environment may be considered "challenging" allowing selective breeding to take place thus the surviving animal may be considered a suitable candidate for SPF and/or SPR.

The method may further comprise a step of confirming that the surviving animal from step (a) expresses at least one marker of a pathogen resistant gene before the administration of the fusion protein of step (b). These markers may be well known in the art to be specific the particular animal. In particular, these markers may be known in the art to be expressed in a particular animal that is resistant to at least one pathogen. For example, if the animal is a

prawn, the marker may be selected from the group consisting of pmAV, c-type lectin, haemocyanin, beta-integrin, syntenin, alpha-2-macroglobulin, LPS-binding protein, beta-glucan binding protein, catalase gene, Ras-related nuclear protein, caspase-3 like gene, calreticulin, Rab GTPase gene, Mg-SOD gene and the like. Similarly, each species of animal may have
5 markers that are specific to that animal.

For example, the development of WSSV-resistant (WSSV-SPR) lines of *P. vannamei* is recognized a possibility and because WSSV remains the biggest disease problem in Asian shrimp culture, this would provide a much-needed impetus for the Asian shrimp culture industry as a whole. The recent applications of quantitative genetics to shrimp breeding, including the
10 identification of various molecular markers (particularly microsatellites) associated with disease resistance and growth, offer a method through which the selection of fast-growing, disease resistant strains might soon become much more efficient. It may also shed some light on invertebrate antiviral immunity, about which currently nothing is known. Such disease related markers have already been identified for IHHNV in *P. stylirostris* (Hizer S.E. et. al., 2002). The
15 genes that are up-regulated in shrimp during a WSSV infection has been reviewed (Liu H. et. al., 2009) and these may now be used as disease resistance markers for selective breeding.

The surviving animal in step (a) may be at least one animal that has been selectively bred for growth prior to carrying out the method according to any aspect of the present invention. In one
20 example, the animals bred in the environment comprising at least one pathogen that is capable of infecting and/or killing the animal have been pre-selected for growth and/or any other advantageous trait and may grow at a faster rate than the wild type of the animal. This may also the SPF animal using any method of the present invention may be achieved earlier than the methods known in the art.

25 There may be a negative correlation between growth and disease resistance (Argue B. et. al., 2002) and hence, it may be best to begin from a population that had been pre-selected for growth.

30 The presence of the SPF non-human animal in step (c) may be confirmed using any method known in the art. In particular, the presence of the SPF non-human animal may be confirmed by determining the presence or absence of the virus in the animal. The method of determining may be any method known in the art that is capable of identifying the presence of any genetic material of the virus in the animal. For example, the method of determining the presence of at
35 least one SPF non-human animal may be selected from the group consisting of PCR, ELISA, RT-PCR, LAMP and the like.

Thus, for example, any shrimp candidate or any aquatic animal, may be fed any fusion protein according to any aspect of the present invention along with its feed until it may become PCR negative for the specific viruses to be checked. If this animal were from a known SPF-line that had been selected for growth, preferably over 3 or more generations, and if this animal were to be grown in pond conditions where viruses were common, the 'survivors' of any resulting epizootic if any, would have a high probability of carrying resistance genes that may be screened using PCR, RT-PCR or microarrays and these could be used to selectively breed a SPR line over time. Microarrays may be used to study shrimp immune responses under various conditions which is a convenient and rapid method to screen survivor populations for resistance genes.

The SPF animal may be free from at least one pathogen selected from the group consisting of cytomegalovirus (CMV), Epstein-Barr virus (EBV), varicella zoster virus (VZV), HSV-1, HSV-2, HSV-6, BK-virus, influenza viruses, respiratory syncytial virus (RSV); human immunodeficiency virus (HIV), hepatitis A, B or C (HBV), polio viruses, enteroviruses, human coxsackie viruses, rhinoviruses, echoviruses, equine encephalitis viruses, rubella viruses, dengue viruses, encephalitis viruses, yellow fever, coronaviruses, vesicular stomatitis viruses, rabies viruses, ebola viruses, parainfluenza viruses, mumps virus, measles virus, respiratory syncytial virus, Hantaan viruses, bunya viruses, phleboviruses and Nairo viruses, hemorrhagic fever viruses, reoviruses, orbiviruses and rotaviruses, parvoviruses, papilloma viruses, polyoma viruses, adenoviruses, herpes simplex virus (HSV) 1 and HSV-2, varicella zoster virus, variola viruses, vaccinia viruses, pox viruses, African swine fever virus, WSSV, HPV, MBV, IHNV, YHV, TSV, GAV, LSNV, IMNV, MoV, KHV1, KHV2, KHV3, VNN, pancreatic necrosis virus (IPNV), channel catfish virus (CCV), fish lymphocystis disease virus (FLDV), hematopoietic necrosis virus (IHNV) and viral hemorrhagic septicemia virus (VHSV), AVG, AMAV, swine hepatitis E virus, Circoviruses, Herpesviruses, Porcine cytomegalovirus, pseudorabies virus, Feline Panleukopenia virus (FPV), Feline herpesvirus, Feline calicivirus, Feline Leukemia Virus (FeLV), Feline Immunodeficiency Virus (FIV), Rabies virus, canine parvovirus, canine coronavirus, canine distemper virus, canine influenza, canine hepatitis virus, canine herpesvirus, a virus that causes pseudorabies, and canine minute virus.

The fusion protein according to any aspect of the present invention may be an antiviral compound capable of a broad spectrum of applications and that may be economically produced without any limitation of raw material supply unlike certain antiviral compounds known in the art.

In order to achieve broad-spectrum activity, the fusion peptide according to any aspect of the present invention may be able to interfere with viral growth or proliferation in a number of different pathways. The fusion protein may thus have a multifunctional ability. An entire new class of antiviral drugs may thus be produced from the fusion protein according to any aspect of

the present invention. The number of combinations and permutations that may be obtained from expressed polypeptides A, B, and C as fusion antiviral proteins potentially numbers in the tens of thousands.

In particular, the fusion protein may comprise at least one formula selected from the group consisting of formulas I-XIX:

	Formula I:	A-B-C,
	Formula II:	A-B-C-C,
	Formula III:	A-B,
10	Formula IV:	A-C-B,
	Formula V:	C-A-B,
	Formula VI:	C-B-A,
	Formula VII:	C-B,
	Formula VIII:	B-A-C,
15	Formula IX:	B-A-C-C,
	Formula X:	B-C-A,
	Formula XI:	B-C,
	Formula XII:	B-A,
	Formula XIII:	C-C-B-C-C,
20	Formula XIV:	C-B-C,

Polypeptide A may be an antimicrobial peptide. In particular, polypeptide A may be a viral entry inhibitory protein. More in particular, polypeptide A may be a defensin, an analogue, or a fragment thereof. Even more in particular, the defensin may be an alpha, a beta, theta defensin, and a member of the Big defensins protein family, an analogue, or a fragment thereof. Polypeptide B may be Type 1 RIP, or a fragment thereof, polypeptide C may be Cationic Antimicrobial Peptide (CAP) or a fragment thereof; and - may be a direct linkage or a linker peptide.

In particular, the linker peptide may comprise a polypeptide sequence: [VPXVG]_n (SEQ ID NO:3) wherein X is an unknown or other amino acid and n is the number of repeats of SEQ ID NO:3 in each linker peptide. For example, n may be 1, 2, 3, 4 or 5. More in particular, X in SEQ ID NO:3 is G and n is 2.

In another example, the linker peptide may be a glycine-serine linker. In particular, the glycine-serine linker may have a sequence of [G-G-G-S]_n (SEQ ID NO:27).

In particular, the fusion protein may comprise the formula I:

A-B-C-

wherein, polypeptide A is a defensin (α , β , θ) an analogue, or a fragment thereof. In particular, polypeptide A may be a theta defensin, an analogue, or a fragment thereof, polypeptide B may be Type 1 RIP, or a fragment thereof, and polypeptide C may be CAP, or a fragment thereof and "-" may be a direct linkage or a linker peptide.

5

More in particular, polypeptide A may be fused to polypeptide B via at least one first linker peptide of SEQ ID NO: 3. Even more in particular, polypeptide A may be fused to polypeptide B via a peptide of SEQ ID NO: 3, wherein X is G and n is 2. Polypeptide B may be directly linked to polypeptide C with no linker peptide in-between. Polypeptide C in formula I may comprise a second linker peptide on the free end not linked to B. The second linker peptide may comprise the formula SEQ ID NO: 3. Even more in particular, in the second linker peptide X is G and n is 2.

15

Polypeptide A may be a viral entry inhibitor protein. In particular, polypeptide A may be a defensin (α , β , θ) an analogue, or a fragment thereof. In particular, polypeptide A may be a theta defensin of a vertebrate or invertebrate origin. In particular, theta Defensin may be from a bacterium, fungus, mammal, amphibian or reptile. The mammal may be a non-human primate and/or the invertebrate may be a horseshoe crab and/or an insect. The theta Defensin may be selected from the group consisting of Rhesus minidefensin (RTD-1), RTD-2, RTD-3, Retrocyclin-1, Retrocyclin-2, Retrocyclin-3 from *Macaca mulatta* of SEQ ID Nos: 7-12 respectively and the like (Tang YQ, 1999; Leonava L, 2001; Wang W, 2004).

25

The theta Defensin may be synthetic and may be selected from a group of retrocyclin congeners RC100-RC108 and RC110-RC114 of SEQ ID NO:13-25 respectively (Cole et. al. 2002: PNAS, V99(4):1813-1818 ; Wang et. al. 2003: J.Immunol. 170:4708-4716). The sequences of Retrocyclin (RC) 100-108 and RC110-RC114 are shown in Table 1a below.

SEQ ID NO:	Sequences
7	GFCRCLCRRGVCRCICTR
8	RCLCRRGVCRCLCRRGVC
9	RCICTRGFCRCICTRGFC
10	GICRCICGRGICRCICGR
11	GICRCICGRGICRCICGR
12	RICRCICGRRICRCICGR
13	GICRCICGRGICRCICGR
14	GICRCICGKGICRCICGR
15	GICRCYCGRGICRCICGR
16	GICRCICGRGICRCYCGR
17	GYCRCICGRGICRCICGR
18	GICRCICGRGYCRCICGR

19	GICYCICGRGICRCICGR
20	GICICICGYGICRCICGR
21	GICICICGRGICYCICGR
22	GICICICGRGICYCICGR
23	RGCICRCIGRGCICRCIG
24	RGCICRCIGRGCICRCIG
25	GICRCICGRGICRCICGR
26	GICRCICGKGICRCYCGR

Table 1a. Polypeptide sequences of naturally occurring and synthetic theta Defensin proteins.

Polypeptide A may be a beta defensin. In particular, polypeptide A may be avian beta defensin (AVBD103).

5

Alpha defensins for human are HNP 1-4 and Human Defensin 5-6, and alpha defensins of mice, monkeys, rats, rabbits, guinea pigs, hamster, horse, elephant, baboon, hedgehog, horse, chimpanzee, orang utan, macaque and marmoset.

10 Beta defensins are DEFB 1, DEFB 4A, DEFB 4B, DEFB 103A, DEFB 103B, DEFB 104A, DEFB 104B, DEFB 105A, DEFB 105B, DEFB 106A, DEFB 106B, DEFB 107A, DEFB 107B, DEFB 108B, DEFB108 P1-4, DEFB 109 P1, DEFB 109 P1B, DEFB 109 P2-3, DEFB 110, DEFB 112-119, DEFB 121-136.

15 Big defensins is a diverse family of antimicrobial peptides. Members of the Big defensins protein family originate from (i) Amphioxus – *Branchiostoma florida* and *Branchiostoma belcheri*; (ii) Horseshoecrab – *Tachypleus tridentatus*; (iii) Mussel – *Mytilus galloprovincialis*; (iv) Clam – *Ruditapes philippinam*; and (v) Oyster – *Crassostrea gigas*.

20 Polypeptide B may be a Type 1 Ribosome Inactivating Protein selected from the group consisting of Ebulitins, Nigritins, Amarandins, Amaranthus antiviral/RIP, Amaranthin, Atriplex patens RIP, Beta vulgaris RIP, β -vulgin, Celosia cristata RIP, Chenopodium album RIP, CAP30B, Spinacea oleracea RIP, Quinqueginsin, Asparins, Agrostin, Dianthins, DAPs, Dianthus chinensis', Lychnin, Petroglauclin, Petrograndin, Saponaria ocymoides RIP, Vacuolas saporin, Saporins, Vaccaria hispanica RIP, Benincasins, Hispin, Byrocin's, Colocins, Cucumis figarei RIP, Melonin, C.moschata RIP, Cucurmosin, Moschatins, Pepocin, Gynostemmin, Gynostemma pentaphyllum RIP, Gypsophilin, Lagenin, Luffaculin, Luffangulin, Luffin, MORs, Momordin II, Momorcharin's, Momorcochin, Momorcochin-S, Sechiumin, Momorgrosvin, Trichoanguin, Kirilowin, α -trichosanthin, TAP-29, Trichokirin, Trichomislin, Trichosanthin, Karasurin, Trichomaglin, Trichobakin, Crotin, Euserratin Antiviral Protein GAP-31, Gelonin, Hura crepitans RIP, Curcin, Jathropa curcas RIP, Mapalmin, Manutins, α -pisavin, Charibdin,

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Hyacinthus orientalis RIP, Musarmin, Iris hollandica RIP, Cleroendrum aculeatum RIP, CIPs,),Crip-31, Bouganin, Bougainvillea spectabilis RIP, Bougainvillea x buttiana Antiviral protein 1 (BBAP1), Malic enzymes, MAP-S, pokeweed antiviral proteins (PAP), PD-SI, DP-S2, Dodecandrin, PIP, PIP2, Phytolacca octandra anti-viral proteins, Hordeum vulgare RIPs, 5 Hordeum vulgare sub sp. Vulgare Translational inhibitor II, Secale cereale RIP, Tritin, Zea diploperemis RIPs, Malus x domestica RIP, Momordica Anti-HIV Protein, Gelonium multiflorum, Mirabilis expansa 1, phage MU1, betavulgin (Bvg), curcin 2, saporin 6, Maize RIP (B-32), Tobacco RIP (TRIP), Beetins, Mirabilis antiviral protein (MAP), Trichosanthin (TCS), luffins, Momorcharins, Ocymoidin, Bryodin, Pepopsin, β -trichosanthin, Camphorin, YLP, Insularin, 10 Barley RIP, Tritins, Lamjarin, Volvariella volvacea RIP and the like of plant origin.

Polypeptide C may be selected from the group consisting of Cyclotides, Siamycins, NP-06, Gramicidin A, Circulins, Kalatas, Ginkbilobin, Alpha-Basrubin, Lunatusin, Sesquin, Tricyclon A, Cycloviolacins, Polyphemusins, hfl-B5, Protegrins (Pig Cathelicidin), Rat Defensins, Human β - 15 defensins, Temporins, Caerins, Ranatuerins, Reptile Defensin, Piscidin's, Lactoferricin B, Rabbit Neutrophils, Rabbit α -Defensin, Retrocyclins, Human α -Defensins, Human β -defensin III (HBD3), Rhesus minidefensin (RTD-1, θ -defensin), rhesus θ -defensins, Human neutrophil peptides, Cecropin As, Melittin, EP5-1, Magainin 2s, hybrid (CE-MA), hepcidin TH1-5, Epinecidin-1, Indolicidin, Cathelicidin-4, LL-37 Cathelicidin, Dermaseptins, Maximins, Brevinins, 20 Ranatuerins, Esculentins, Maculatin 1.3, Maximin H5 and Piscidins, Mundtacin KS Enterocin CRL-35, Lunatusin, FK-13 (GI-20 is a derivative), Tachyplesins, Alpha-MSH, Antiviral protein Y3, Palustrin-3AR, Ponericin L2, Spinigerin, Melectin, Clavanin B, Cow cathelicidin's, Guinea pig cathelicidin CAP11, Sakacin 5X, Plectasin, Fungal Defensin, GLK-19, lactoferrin (Lf) peptide 2, Alloferon 1, Uperin 3.6, Dahlein 5.6, Ascaphin-8, Human Histatin 5, Guineapig neutrophils, 25 Mytilins, EP5-1,Hexapeptide (synthetic) Corticostatin IV Rabbit Neutrophil 2, Aureins, Latarcin, Plectasin, Cycloviolins, Varv Peptide E, Palicourein, VHL-1, Gaegurin 5, Gaegurin 6 and the like (US 8,076,284 B2; Kim, S. et al, Peptides, 2003, 24, 945-953).

In particular, polypeptide C may be Gaegurin 5, Gaegurin 6, their analogues, derivatives or 30 fragments thereof, which may have pro-apoptotic properties that may act upon drug sensitive and multidrug resistant tumour cell lines.

A Cationic Antimicrobial Peptide (CAP) may be an anti-microbial CAP that may have anticancer and/or antiviral properties. CAPs may be a maximum of 100 amino acids in length. CAPs may 35 either be a naturally occurring CAP with sequence with reported anticancer properties or a synthetic CAP sequence with anticancer properties. CAPs may mostly be of animal origin. However, there may also be CAPs, which are from plants, which include but are not limited to cyclotides. For example, bacteria CAPs may include but are not limited to Siamycin, NP-06 and Gramicidin A. Plant CAPs may include Circulin A, B, Kalata B1 and B8; Plant CAPs which may

function as entry inhibitors may include Kalata B8, Ginkbilobin, Alpha-Basrubin, Lunatusin and Sesquin, Circulin A, C and D, Tricyclon A and Cycloviolacin H4. Animal CAPs may include Polyphemusin I and II, hfl-B5, Protegrin (Pig Cathelicidin), Rat Defensin NP1, NP2, NP3 and NP4, Human β -defensin I and II, Temporin A, Temporin-LTc, Temporin-Pta, Caerin 1.1, 5 Ranatuerin 6 and 9, Reptile Defensin and Piscidin 1 and 2, Lactoferricin B, Rabbit Neutrophil-1 Corticostatin III a, Rabbit Neutrophil-3A, Rabbit α -Defensin, Retrocyclin-1, Retrocyclin-2, Retrocyclin-3, Human α -Defensin HNP-1, 2, 3,4,5 & 6, Human β -defensin III (HBD3), Rhesus minidefensin (RTD-1, θ -defensin), RTD-2 rhesus θ -defensin, RTD-3 rhesus θ -defensin, Human neutrophil peptide-2, Human neutrophil peptide-3 and human neutrophil peptide-4, Cecropin A, 10 Melittin, EP5-1, Magainin 2, hepcidin TH1-5, and Epinecidin-1, Indolicidin, Cathelicidin-4, Human neutrophil peptide-1, LL-37 Cathelicidin, Dermaseptin-S1, S4 and S9, Maximin 1, 3, 4 and 5, Brevinin 1, Ranatuerin 2P, 6 and 9 Esculentin 2P, Esculentin-1 Arb, Caerin 1.1, 1.9 and 4.1, Brevinin-2-related, Maculatin 1.3, Maximin H5 and Piscidin 1 and 2. Other CAPs may include Mundtacin KS Enterocin CRL-35, Lunatusin, FK-13 (GI-20 is a derivative), Tachyplesin I, 15 Alpha-MSH, Antiviral protein Y3, Piscidin 3, Palustrin-3AR, Ponericin L2, Spinigerin, Melectin, Clavanin B, Cow cathelicidin BMAP-27, BMAP-28, Guinea pig cathelicidin CAP11, Sakacin 5X, Plectasin, Fungal Defensin, GLK-19, lactoferrin (Lf) peptide 2, Kalata B8, Tricyclon A, Alloferon 1, Uperin 3.6, Dahlein 5.6, Ascapin-8, Human Histatin 5, Guineapig neutrophil CAP2 & CAP1, Mytilin B & C, EP5-1, and Hexapeptide (synthetic) Corticostatin IV Rabbit Neutrophil 2.

20

The Type 1 RIP may:

- (i) act as a pro-apoptotic polypeptide which up regulate pro-apoptotic genes that may include but not limited to caspase-12, Bax and the like, or down regulate anti-apoptotic gene including but not limited to Bcl-2 and the like in tumour or cancer cells (Fan, J-M., 25 et al, Mol Biotechnol, 2008, 39, 79-86);
- (ii) act as a DNA glycosylase / apurinic (AP) lyase capable of irreversibly relaxing tumour or cancer cell supercoiled DNA and catalyzing double-stranded breakage to form inactive products;
- (iii) act in alternative cytochrome pathways as well as Mn^{2+} and Zn^{2+} interactions with 30 negatively charged surfaces next to catalytic sites, facilitating DNA substrate binding instead of directly participating in catalysis (Wang et al, Cell, 1999, 99, 433-442);
- (iv) as an RNA N-Glycosidase which hydrolyses the N-C glycosidic bond of adenosine at position 4324 of the universally conserved sarcin/ricin domain(S/R domain) of the 28S-rRNA in the eukaryotic ribosome and render it incapable of carrying out protein 35 synthesis thus, functionally, blocking translation.

In particular, the Type 1 RIP may be selected from the group consisting of α -Ebulitin, β -Ebulitin, γ -Ebulitin, Nigritin f1, Nigritin f2, Amarandin-S, Amaranthus antiviral/RIP, Amarandin-1, Amarandin-2, Amaranthin, Atriplex patens RIP, Beta vulgaris RIP, β -vulgin, Celosia cristata RIP,

Chenopodium album RIP, CAP30B, Spinacea oleracea RIP, Quinqueginsin, Asparin 1, Asparin 2, Agrostin, Dianthin 29, DAP-30, DAP-32, Dianthin 30, Dianthus chinensis RIP1, Dianthus chinensis RIP2, Dianthus chinensis RIP3, Lychnin, Petroglauzin, Petrograndin, Saponaria ocymoides RIP, Vacuolas saporin, Saporin-1, Saporin-2, Saporin-3, Saporin-5, Saporin-6, Saporin-7, Saporin-9, Vaccaria hispanica RIP, Benincasin, α -benincasin, β -benincasin, Hispin, Byrodin I, Byrodin II, Colocin I, Colocin 2, Cucumis figarei RIP, Melonin, C.moschata RIP, Cucurmosin, Moschatin, Moschatin I, Moschatin II, Moschatin III, Moschatin IV, Moschatin V, Pepocin, Gynostemmin I, Gynostemmin II, Gynostemmin III, Gynostemmin IV, Gynostemmin V, Gynostemma pentaphyllum RIP, Gypsophilin, Lagenin, Luffaculin, Luffangulin, Luffin- α , Luffin-B, MOR-I, MOR-II, Momordin II, Alpha-momorcharin, β -momorcharin, γ δ -momorcharin, γ -momorcharin, Momorcochin, Momorcochin-S, Sechiumin, Momorgrosvin, Trichoanguin, α -kirilowin, β -kirilowin, α -trichosanthin, TAP-29, Trichokirin, Trichomislin, Trichosanthin, Karasurin-A, Karasurin-B, Trichomaglin, Trichobakin, Croton 2, Croton 3, Euserratin 1, Euserratin 2, Antiviral Protein GAP-31, Gelonin, Hura crepitans RIP, Curcin, Jathropa curcas RIP, Mapalmin, Manutin 1, Manutin 2, α -pisavin, Charibdin, Hyacinthus orientalis RIP, Musarmin 1, Musarmin 2, Musarmin 3, Musarmin 4, Iris hollandica RIP, Cleroendrum aculeatum RIP, CIP-29, CIP-34, Crip-31, Bougainin, Bougainvillea spectabilis RIP, Bougainvillea x buttiana Antiviral protein 1 (BBAP1), malic enzyme 1 (ME1), ME2, MAP-S, pokeweed antiviral protein (PAPa-1), PAPa-2, PAP- α , PAP-I, PAP-II, PAP-S, PD-SI, DP-S2, Dodecandrin, Anti-viral protein PAP, PIP, PIP2, Phytolacca octandra anti-viral protein, Phytolacca, octandra anti-viral protein II, Hordeum vulgare RIP-I, Hordeum vulgare RIP-II, Hordeum vulgare sub sp. Vulgare Translational inhibitor II, Secale cereale RIP, Tritin, Zea, diploperemis RIP-I, Zea diploperemis RIP-II, Malus x domestica RIP, Momordica Anti-HIV Protein (MAP30), Gelonium multiflorum (GAP31), pokeweed antiviral protein (PAP), Mirabilis expansa 1 (ME1), malic enzyme 2 (ME2), Bougainvillea x buttiana antiviral protein 1 (BBAP1), phage MU1, betavulgin (Bvg), curcin 2, saporin 6, Maize RIP (B-32), Tobacco RIP (TRIP), beetin (BE), BE27, Mirabilis antiviral protein (MAP), Trichosanthin (TCS), α -luffin, α -Momorcharin (α -MMC), β -MMC luffin, Ocymoidin, Bryodin, Pepopsin, β -trichosanthin, Camphorin, YLP, Insularin, Barley RIP, Tritins, Lamjarin, Volvariella volvacea RIP and the like of plant origin.

30

In particular, polypeptide A may be a Retrocyclin, polypeptide B may be MAP30 and polypeptide C may be a Dermaseptin. More in particular, polypeptide A may be Retrocyclin 101 (RC101) and polypeptide C may be Dermaseptin 1. A polypeptide comprising RC101, MAP30 and Dermaseptin 1 as polypeptide A, B and C respectively is termed RetroMAD1 in the present invention.

35

In particular, polypeptide A may comprise amino acid sequence with SEQ ID NO: 4, a fragment or variant thereof, polypeptide B may comprise amino acid sequence with SEQ ID NO:5, a

fragment or variant thereof, and polypeptide C may comprise amino acid sequence with SEQ ID NO:6, a fragment or variant thereof.

More in particular, the fusion protein according to any aspect of the present invention may comprise the amino acid sequence SEQ ID NO:1. The fusion protein or the basic unit of the fusion protein may have a molecular weight of about 30-50kDa. In particular, the molecular weight of the fusion protein may be 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 36.5, 37, 37.5, 37.8, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48 or 49 kDa. The fusion protein may comprise repeats of the basic unit. A skilled person would understand that the weight of the fusion protein would be dependent on the multiples of the basic unit present in the protein. The nucleic acid coding for the fusion protein of SEQ ID NO:1 may be found in SEQ ID NO:2. The sequences are provided in Table 1b below.

In particular, polypeptide B may be Type 1 RIP, or a fragment thereof, and polypeptide C may be Cationic Antimicrobial Peptide or a fragment thereof; and - may be a direct linkage or a linker peptide.

In one example, polypeptide A may be Avian β -Defensin 103 (AVBD103), polypeptide B may be MAP30 and polypeptide C may be Mytilin C10C.

In another example, the fusion protein may comprise the formula XIV:

C-B-C

SEQ ID NO.	Sequences
1	MKYLLPTAAAGLLLLAAQPAMAMGRICRCICGRGICRCICGVPGVGVPGVGGATGSDVNFDLSTATAKTY TKFIEDFRATLPFSHKVYDIPLLYSTISDSRRFILLDLTSYAYETISVAIDVTNVVYRTRDVSYYFFK ESPPEAYNILFKGTRKITLPYTGNYENLQTAHKIRENIDLGLPALSSAITTLFYNAQSAPSALLVLIQ TTAEAAARFKYIERHVAKYVATNFKPNLAIISLENQWSALSQKIFLAQNQGGKFRNPVDLIKPTGERFQVT NVSDSDVVGKNIKLLNSRASTADENFITMTLLGESVVEFPWALWKTMLKELGTMLHAGKAALGAAADT ISQGTQVPGVGVPGVGVKLAALAEHHHHHH
2	atgaaatacctgctgcccagccgctgctgctggtctgctgctcctcgtgcccagccggcgatggccatgg ggcgtatttgccgttgcatattgcggccgctggcatttgccgctgcacatctgtggcggtgctggtgt tccgggtgtgggtggtgcgaccggatccgatgtgaactttgatctgagcaccgcgaccgcgaaaacctat accaaattcatcgaaagattttcgtgcgaccctgccgttttagccataaaagtgtatgatccccgctgctgt atagcaccattagcgatagccgctgcttttattctgctggatctgaccagctatgcgtatgaaaccattag cgtggcgattgatgtgaccaacgtgtatgtggtggcgatcgtaccgctgatgtgagctactttttcaaa gaaagcccgccggaagcgtacaacattctgtttaaaggcaccgcgtaaaattaccctgccgtataccggca actatgaaaacctgcagaccgcggcgataaaattcgtgaaaacatcgatctgggcctgccggccctgag cagcgcgattaccacctgttttattataacgcgcagagcgcgcgagcgcgctgctggtgctgattcag accaccgcggaagcggcgcgcttttaaatatattgaacgccacgtggcgaaatatgtggcgaccaacttta aaccgaacctggccattattagcctggaaaaccagtggagcgcctgagcaaacaaatttttctggccca gaaccagggcgcaaatttcgtaatccggtggatctgattaaaccgaccggcgaaacgttttcaggtgacc aatgtggatagcgatgtggtgaaaggcaacattaaactgctgctgaacagccgtgagcagccgcatg aaaactttattaccaccatgacctgctggcgaaaagcgtggtggaattcccgctggcgctgtggaaaac catgctgaaagaactgggcaccatggcgctgcatgcgggtaaagcggcgctgggtgcggcagcggatacc attagccagggcaccaggttcggggcgctggcgcttcggggcgcttggtgaagcttgcgccgcactcgagc accaccaccaccactga
3	[VPXVG] _n
4	GRICRCICGRGICRCICG
5	GSDVNFDLSTATAKTYTKFIEDFRATLPFSHKVYDIPLLYSTISDSRRFILLDLTSYAYETISVAIDVTN VVYVYRTRDVSYYFFKESPPPEAYNILFKGTRKITLPYTGNYENLQTAHKIRENIDLGLPALSSAITTLF YYNAQSAPSALLVLIQTAEAAARFKYIERHVAKYVATNFKPNLAIISLENQWSALSQKIFLAQNQGGKFR

	NPVDLIKPTGERFQVTNVDSDVVKGNIKLLNSRASTADENFITTMTLLGESVVEFPW
6	ALWKTMLKELGTMALHAGKAALGAAADTISQGTQ

Table 1b. Sequences of polypeptides and polynucleotides of the present invention.

- 5 In another example, the fusion protein may be Amatilin, RetroGAD1, Tamapal1 and the like. DNA and polypeptide sequences of Amatilin, RetroGAD1, and Tamapal1 are presented in Tables 1d and 1e.

- 10 Investigations on thermal behaviour of drug samples are important for obtaining information for their processing in pharmaceutical industry, for predicting their shelf lives and also for suitable storage conditions. These drugs were shown to be thermally stable.

Example	Polypeptide A	Polypeptide B	Polypeptide C
Fusion peptide	Defensin	RIP	CAP
RetroMAD1	Retrocyclin 101	MAP30	Dermaseptin1
RetroGAD1	Retrocyclin 101	GAP31	Dermaseptin1
Tamapal1	Tachyplesin	MAP30	Alloferon1
Amatilin	AVBD103	MAP30	Mytilin C10C

Table 1c. The polypeptides used for each drug.

Fusion Protein	SEQ ID NO.	DNA Sequence
Amatilin	37	GGGCAGTGAGCGGAAGGCCCATGAGGCCAGTTAATTAAGAGGTACCGAATTCTCAT TCGGTTTGTGTAGATTGAGAAGAGGTTTCTGTGCTCACGGTAGATGTAGATTCCCA TCCATCCCAATCGGTAGATGTTCCAGATTTCGTTTCAGTGTTGTAGAAGAGTTTGGGT CCCAGGTGTTGGTGTTCAGGTGTTGGAGGTGCTACTGGTTCTGATGTTAACTTCG ACTTGTCCACTGCTACTGCTAAGACTTACACTAAGTTCATCGAGGACTTCAGAGCT ACTTTGCCATTCTCCACAAGGTTTACGACATCCCTTTGTTGTACTCCACTATCTC CGACTCCAGAAGATTCATCTTGTGTAAGTTGACTTCCTACGCTTACGAGACTATCT CCGTTGCTATCGACGTTACAAACGTTTACGTTGTTGCTTACAGAACTAGAGATGTT TCCTACTTCTTCAAAGAGTCCCCACCAGAGGCTTACAACATCTTGTTCAGGGTAC TAGAAAGATCACTTTGCCATACACTGGTAACTACGAGAACTTGCAGACTGCTGCTC ACAAGATCAGAGAGAACATCGACTTGGGTTTGCCAGCTTTGTCTCCGCTATCACT ACTTTGTTCTACTACAACGCTCAGTCCGCTCCATCCGCTTTGTTGGTFTTGATCCA GACTACTGCTGAGGCTGCTAGATTCAAGTACATCGAGAGACACGTTGCTAAGTACG TTGCTACAACTTCAAGCCAACTTGGCTATCATCTCCTTGGAGAACCAGTGGTCT GCTTTGTCCAAGCAGATCTTCTTGGCTCAAAACCAGGGTGGTAAGTTCAGAAACCC AGTCGACTTGATCAAGCCAACCGGTGAGAGATTCCAGGTTACTAATGTTGACTCCG ACGTTGTTAAGGGTAACATCAAGTTGTTGTTGAACTCCAGAGCTTCCACTGCTGAC GAGAACTTCATCACTACTATGACTTTGTTGGGTGAGTCCGTTGTTAACTCCTGTGC TTCCAGATGTAAGGGTCACTGTAGAGCTAGAAGATGTGGTTACTACGTTTCCGTTT TGTACAGAGGTAGATGTTACTGTAAATGTTTGAGATGTGTCCCCGGTGTGGAGTC CCTGGTGTGCGGTGCGGCCGCGAGCTCATGGCGCGCTAGGCCTTGACGGCCTTCCG CCAATTTCGC

RetroGAD1	38	CGAATTGGCGGAAGGCCGTCAAGGCCACGTGTCTTGTCCAGGTACCGAATTCGGAA TCTGTAGATGCATCTGCGGTAGAGGTATCTGCAGATGTATTTGTGGAAGAGTCCCA GGTGTGGTGTTCAGGTGTTGGAGGTGCTACTGGTTCTGGTTTGGACACTGTTTC ATTCTCCACTAAGGGTGCTACTTACATCACTTACGTTAACTTTTTGAACGAGTTGA GAGTTAAGTTGAAGCCAGAGGGTAACCCCACGGTATCCCTTTGTTGAGAAAGAAG TGTGACGACCCAGGTAAGTGTTCGTTTTGGTTGCTTTGTCCAACGACAACGGTCA GTTGGCTGAGATTGCTATCGACGTTACTTCCGTTTACGTTGTTGGTTACCAGGTTA GAAACAGATCCTACTTCTTCAAGGACGCTCCAGACGCTGCTTACGAAGGTTTGTTC AAGAACACTATCAAGACTAGATTGCACCTCGGTGGTTCCTACCCATCTTTGGAAGG TGAGAAGGCTTACAGAGAGACTACTGACTTGGGTATCGAGCCATTGAGAATCGGTA TCAAGAAGTTGGACGAGAACGCTATCGACAACATAAGCCAACTGAGATCGCTTCC TCCTTGTGGTTGTTATCCAGATGGTTTCCGAGGCTGCTAGATTCACTTTCATCGA GAACCAGATCAGAAACAACCTCCAGCAGAGAATCAGACCAGCTAACAACACTATTT CCTTGGAGAACAAGTGGGGTAAGTTGTCCTTCCAGATCAGAACATCCGGTGCTAAC GGTATGTTCTCTGAGGCTGTTGAGTTGGAGAGAGCTAACGGTAAGAAGTACTACGT TACTGCTGTTGACCAGGTTAAGCCAAAGATCGCTTGTGAGTTGTTGACAAGG ACCCAAAGGGTTTGTGGTCCAAGATCAAAGAGGCTGCTAAGGCTGCTGGTAAGGCT GCTTTGAATGCTGTTACTGGTTTGGTTAACCAGGGTGACCAACCATCTGTCCCTGG TGTTGGAGTCCCTGGTGTGCGGTGCGGCCGCGAGCTCTGGAGCACAAGACTGGCCCTC ATGGGCCTTCCGCTCACTGC
Tamapall	39	GGATCCGTTCCGGGTGTGGGTGTTCCGGGTGTTGGTAAATGGTGTTCGTGTTTGT TATCGCGGTATTTGTTATCGTCGTTGTGCTGTGCCAGGCGTTGGCGTTCAGGCGT GGGTGGTGCAACCGGTAGTGATGTTAATTTTGATCTGAGCACCAGCAACCGCAAAAA CCTATACCAAATTTATCGAAGATTTTCGTGCAACCCTGCCGTTTAGCCATAAAGTT TATGATATTCCGCTGCTGTATAGCACCATTAGCGATAGCCGTCGTTTATTCTGCT GAATCTGACCAGCTATGCCTATGAAACCATTAGCGTTGCAATTGATGTGACCAATG TTTATGTTGTTGCATATCGTACCCGTGATGTGAGCTATTTTTTCAAAGAAAGCCCT CCGGAAGCCTATAACATTCTGTTTAAAGGCACCCGCAAAATCACCCCTGCCGTATAC CGGTAATTATGAAAATCTGCAGACCGCAGCACATAAAATTCGCGAAAATATTGATC TGGGTCTGCCTGCACTGAGCAGCGCAATTACCACCCTGTTTTATTACAATGCACAG AGCGCACCGAGCGCACTGCTGGTCTGATTAGACACCGCAGAAGCAGCAGCCTT TAAATACATTGAACGTCATGTTGCCAAATACGTGGCCACCAACTTAAACCGAATC TGGCAATTATTAGCCTGGAAAATCAGTGGTCAGCACTGAGCAAACAAATTTTCTG GCACAGAATCAGGGTGGCAAATTTGTAATCCGGTTGATCTGATTAAACCG ACCGGTGAACGTTTTTACGTTACCAATGTTGATAGTGATGTGGTGAAAGGCAACAT TAACTGCTGCTGAATAGCCGTGCAAGCACCGCAGATGAAAACCTTTATTACCACCA TGACCCTGCTGGGTGAAAGCGTTGTTAATGTTCTGGTGTGGCGTGCCGTGGTGTT GGTCATGGTGTAGCGGTCATGGTCAGCATGGTGTTCATGGTTAAAAGCTT

Table 1d. DNA sequences of Amatinin, RetroGAD1 and Tamapall1

Fusion Protein	SEQ ID NO.	Protein Sequence
Amatinin	28	SFGLCRLRRGFCAHGRCFRPSIPIGRCSRFBVQCCRRVWVPGVGVPGVGGATGSDVNF DLSTATAKTYTKFIEDFRATLPFSHKVYDIPLLYSTISDSRRFILLNLTSYAYETIS VAIDVTNVYVVAYRTRDVSFFKESPPAYNILFKGTRKITLPYTGNYENLQTAHAK IRENIDLGLPALSSAITTLFYNAQSAPSALLVLIQTAEAAAREFKYIERHVAKYVAT NFKPNLAIISLENQWSALSQIFLAQNQGGKFRNPVDLIKPTGERFQVTNVDSDVVK GNIKLLNSRASTADENFITTMTLLGESVNVNSCASRCKGHCRARRRCGYVSVLYRGR CYCKCLRCVPGVGVPGVG
RetroGAD 1	36	GICRCIGRGICRCICGRVPGVGVPGVGGATGSGLDTVSFSTKGATYITYVNFNLNLR VKLKPEGNSHGIPLLRKKCDPGKCFVLVALSNDNGQLAEIAIDVTSVYVVGQVRN RSYFFKDAPDAAYEGLFKNTIKTRLHFGGSYPSLEGEKAYRETTDLGIEPLRIGIKK LDENAIIDNYKPTEIASSLLVVIQMVSEAAARFTFIENQIRNNFQQRIIRPANNTISLEN KWGKLSFQIRTSGANGMFSEAVELERANGKKYYVTAVDQVKPKIALLLKFVDKDPKGL WSKIKEAAKAAGKAALNAVTGLVNQGDQPSVPGVGVPGVG
Tamapall	34	VPGVGVPGVGKWCFRVCYRGICYRRRCRVPGVGVPGVGGATGSDVNFDLSTATAKTYT

		KFIEDFRATLPFSHKVYDIPLLYSTISDSRRFILLNLTSYAYETISVAIDVTNVYVV AYRTRDVS YFFKES PPEAYN ILFKGTRKITLPYTGNYENLQTAAHKIRENIDLGLPA LSSAITTLFY YNAQSAPS ALLVLIQT TAEAA RFKYIERHVAKYVATNF KPNLAIISL ENQWSALS KQIFLA QNQG GKF RNPVDLIKPTGERFQVTNVDSDVVKGNIKLLLSRA STADENFIT TMTLLGESV VNVPGVGVPGVGHGVSGHGHGQHG VHG
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Table 1e. Polypeptide sequences of Amatilin, RetroGAD1 and Tamapal1

Modifications and changes may be made in the structure of the peptides of the present invention and DNA segments, which encode them and still obtain a functional molecule that encodes a protein or peptide with desirable characteristics. The amino acids changes may be achieved by changing the codons of the DNA sequence. For example, certain amino acids may be substituted for other amino acids in a protein structure without appreciable loss of interactive binding capacity with structures such as, for example, tumour or cancer cell-binding regions of fusion proteins. Since it is the interactive capacity and nature of a protein that defines that protein's biological functional activity, certain amino acid sequence substitutions can be made in a protein sequence, and, of course, its underlying DNA coding sequence, and nevertheless obtain a protein with like properties. Various changes may be made in the peptide sequences of the disclosed compositions, or corresponding DNA sequences, which encode said proteins without appreciable loss of their biological utility or activity. Amino acid substitutions of the fusion protein according to the present invention may be possible without affecting the antitumour or anticancer effect of the isolated peptides of the invention, provided that the substitutions provide amino acids having sufficiently similar properties to the ones in the original sequences.

The fusion peptide according to any aspect of the present invention may be thermostable over a prolonged period of time. Thermostability is an industrially significant attribute as cold-chain transportation will greatly increase logistics and handling costs that will contribute to the overall total cost of the medication. Also, if the drug is to be carried about to be consumed before meals, patient compliance will suffer if the requirement of low temperature storage in an absolute necessity. Thus, the ability to remain stable for 7 days even at elevated temperatures will allow for a wider usage and application of the therapeutic protein.

The fusion protein may further comprise a pharmaceutically acceptable carrier, excipient, adjuvant, diluent and/or detergent. Such formulations therefore include, in addition to the fusion protein, a physiologically acceptable carrier or diluent, possibly in admixture with one or more other agents such as other antibodies or drugs, such as an antibiotic. Suitable carriers include, but are not limited to, physiological saline, phosphate buffered saline, phosphate buffered saline glucose and buffered saline. Alternatively, the fusion protein may be lyophilized (freeze dried) and reconstituted for use when needed by the addition of an aqueous buffered solution as described above. Routes of administration are routinely parenteral, including intravenous,

intramuscular, subcutaneous and intraperitoneal injection or oral delivery. The administration can be systemic and/or local. The medicament may be used for topical or parenteral administration, such as subcutaneous, intradermal, intraperitoneal, intravenous, intramuscular or oral administration. For this, the fusion protein may be dissolved or suspended in a pharmaceutically acceptable, preferably aqueous carrier. The medicament may contain excipients, such as buffers, binding agents, blasting agents, diluents, flavours, lubricants, etc.

In particular, the fusion protein may be produced as a solid dose by means of Supercritical Fluid Drying (SCFD) used to dry and produce a micronized form of powdered free-flowing RetroMAD1. The powder may be for incorporation into tablets, capsules and animal feed pellets whether for terrestrial or aquatic application. This allows for high process yields and may enable further ease of oral drug delivery in tablet and/or capsule form.

In particular, the fusion protein may be administered orally. In particular, the presence of MAP30 surprisingly renders the fusion protein according to any aspect of the present invention stable for oral administration. In particular, the fusion protein may be administered with or before food. More in particular, when the fusion protein is administered before food, it may be done with a drink for example water. In the case of aquatic animals that do not 'drink', it may be effectively administered by top-coating the feed pellets with the fusion protein. Furthermore, they can be coated further with proteins to prevent leaching. A non-limiting example is the use of proteins from chicken eggs and the like to protect against leaching.

The fusion protein according to any aspect of the present invention may be capable of maintaining its form in the digestive tract without fragmentation or enzymatic digestion. In one example, the fusion protein may be in a liquid form. In particular, the fusion protein may be ingested, as a drink diluted with water, or the like, and the retention time in either stomach or duodenum is only a matter of minutes allowing the protein to reach its target point without being digested.

The dosage of the fusion protein according to the present invention to be administered to a non-human animal may vary with the precise nature of the condition being treated and the recipient of the treatment. The dose will generally be in the range of about 0.005 to about 1000 mg for an adult patient, usually administered daily for a period between 1 day to 2 years. In particular, the daily dose may be 0.5 to 100 mg per day. In particular the daily dose may be about 0.8, 1, 1.2, 1.5, 2, 2.5, 3.2, 4, 4.5, 5, 10, 15, 20, 30, 45, 50, 75, 80, 90, 95 mg per day. The dosage may be applied in such a manner that the ligand may be present in the medicament in concentrations that provide in vivo concentrations of said ligand in a patient to be treated of between 0.001 mg/kg/day and 5 mg/kg/day. In one embodiment, the medicament, the peptide or ligand according to the invention is present in an amount to achieve a concentration in vivo of 1 µg/ml

or above with a maximum concentration of 100 µg/ml. the dosage regime may be varied depending on the results on the patient.

The fusion protein may be pegylated to aid in the medicament being suitable for oral delivery. In particular, the fusion protein may be pegylated with any PEG known in the art. The PEG may be selected from the group consisting of but not limited to PEG200,300,400,500,600,700,800,900,1000,1100,1200,1300,1400,1500,1600,1700,1800,1900,2000,2100,2200,2300,2400,2500,2600,2700,2800,3000,3250,3350,3500,3750,4000,4250,4500,4750,5000,5500,6000,6500,7000,7500,8000 and the like.

SPF animal may be free from only the pathogens that they have been tested for. In shrimp for example, typically this may consist of the viral pathogens which are known to cause major losses to the shrimp culture industry, including WSSV, YHV, TSV, IHHNV, BPV, HPV 3 and the like. However, when new diseases may emerge from mutations of previously non-pathogenic organisms - i.e. the highly mutable RNA viruses the SPF animal may not survive. Hence, it remains a possibility that importation of SPF shrimp may not rule out simultaneous importation of pathogens. Also, if SPF shrimp are stocked into facilities with high viral loads, substantial mortality can result as they are not necessarily more resistant to these diseases than non-SPF shrimp, and in some cases, less so. They may thus be more suited to culture in biosecure systems, which may explain the reliance of the big, non-biosecure pond farms of Latin America on SPR (Specific Pathogen Resistant), rather than SPF shrimp. Accordingly, even though SPF animals have their advantages, they have their limitations and an SPR animal may be needed that may be capable of resistance to all pathogens.

In another aspect of the present invention, there is provided a method of producing at least one specific pathogen resistant (SPR) non-human animal, the method comprising:

(a) producing a specific pathogen free animal according to any method of the present invention; and

(b) selective breeding of a male and female SPF non-human animal to produce a SPR non-human animal offspring.

According to a further aspect of the present invention, there is provided a specific pathogen free or resistant non-human animal produced by any method of the present invention. The animal may be an aquatic animal. More in particular, the aquatic animal may be a prawn of any species or a fish. In one example, the animal may be a non-aquatic animal for example a bird like a chicken and the like. The animal may be of any age and include every stage of the life-cycle of the animal. In particular, the animal may include an egg, larvae and the like of the animal.

A person skilled in the art will appreciate that the present invention may be practised without undue experimentation according to the method given herein. The methods, techniques and chemicals are as described in the references given or from protocols in standard biotechnology and molecular biology text books.

5

The fusion protein and/or pharmaceutical composition according to any aspect of the present invention may result in no or substantially no toxic side effects when taken by the animal.

10

Having now generally described the invention, the same will be more readily understood through reference to the following examples which are provided by way of illustration, and are not intended to be limiting of the present invention.

EXAMPLES

15

Standard molecular biology techniques known in the art and not specifically described were generally followed as described in Sambrook and Green, Molecular Cloning: A Laboratory Manual, Cold Springs Harbor Laboratory (Fourth Edition), New York (2012).

EXAMPLE 1

Construction and Design of Expression Vector

20

The gene encoding RetroMAD1 A-B-C with SEQ ID NO:1 was synthesized and cloned into backbone of vector pGA4 at the KpnI/SacI site by contract service (GeneArt AG, Germany). The expected product size was 1140bp, which encoded a 379 amino acid and an expected size of 41.2 kDa. The polynucleotide sequence and the translated polypeptide sequence are shown in Figure 1 from PCT. The gene was sub-cloned into a pET expression vector (Novagen), pET-25

26(b) at the NcoI/HindIII sites. Kanamycin was used as a marker for selection and maintenance of culture purposes. This vector was inducible under the addition of isopropyl-beta-D-thiogalactopyranoside (IPTG). The plasmid, pRMD1 was then transformed into BL21(DE23) cells (Novagen) and plated on a selective media with Kanamycin.

30

Expression of RetroMAD1 from *E. coli*

35

One recombinant clone was grown in 10ml of LB Bertani (DIFCO) medium, supplemented with 30µg/ml kanamycin, at 37°C overnight. This culture was used to inoculate 100ml of LB Bertani supplemented with 30µg/ml kanamycin and grown at 37°C until the optical reading was 0.4-0.6 at 600nm. IPTG was added at 1.0 mM final concentration. The growth period continued for 3 hours. An SDS-PAGE analysis of the fraction of RetroMAD1 in cells extracted in electrophoresis loading buffer showed that a protein had a molecular mass of about 37.5 kDa, the expected molecular size of RetroMAD1 was produced in the induced cells only (Figure 2A). Further solubility analysis by SDS-PAGE revealed that RetroMAD1 was found in the pellet fraction and

not in the supernatant fraction of the E. coli indicating that the protein was expressed and produced as inclusion bodies as shown in Figure 2B.

Isolation and Purification of RetroMAD1

- 5 Cells from 100ml of induced culture were harvested by centrifugation for 10 min at 5000 x g at 15°C. The cells were suspended in a lysis buffer containing 20mM Tris-HCl (pH 7.5), 10mM EDTA and 1% Triton-X 100. Cells were disrupted by sonication. The insoluble fraction was isolated from the soluble fraction by centrifugation at 8,000 x g for 20 min. The supernatant was discarded and the pellet was further washed by repeating the same step. The pellet was further
10 washed twice with RO water by resuspension via sonication and separation by centrifugation.

Solubilization of RetroMAD1

- The insoluble material was dissolved and sonicated in 10ml of 5-8 Urea or 6M Guanidine Hydrochloride and supplemented with 2-5% of Sodium-lauryl sarcosine and 100mM β -
15 mercaptoethanol. The solubilisation was carried out overnight. The solubilised protein was separated from the bacterial cell wall by centrifugation at 8,000 x g for 20 minutes.

Refolding of RetroMAD1

- Renaturation of the protein was carried out by using dialysis. The protein (10ml) was dialysed in
20 a 15kDa molecular weight cut-off dialysis membrane (Spectra/Por Lab). The protein was dialysed in 5L of RO water with the pH of 11.0 adjusted by NaOH. Incubation was done at room temperature for 15-20 hours. The refolded protein was transferred to a 50 ml tube and centrifuged at 8,000 x g to separate any insoluble material. Renatured protein was stored at -
25 20°C until further use. The bioactivity of RetroMAD1 in the following examples is proof of successful refolding of the protein.

EXAMPLE 2

Elimination of Hepatopancreatic ParvoVirus (HPV) from shrimp

30 Shrimp culture and RetroMAD1 treatment

- Naturally infected HPV shrimp (150 pieces) was obtained from a local aquarium shop. Twenty pieces of randomly selected shrimp was selected for DNA extraction to confirm for HPV (Hepatopancreatic Parvo Virus) infection in the population. For the experiment, 56 shrimps were reared in two 20 liters tank (24 each) containing de-chlorinated fresh water equipped with
35 aeration. Water exchange was carried out at 20% every two days. Shrimps were acclimatized for one week before the experiment.

For the experiment both tanks were given 0.25mg of feed daily, divided into 3 meals. Treated tanks were given a dose of 25ug of RetroMAD1 absorbed into the commercial feed for each meal for four days while the control was given sterile water absorbed into the feed. After the end

of the experiment (day 4), 24 pieces of shrimp were still alive in the treated tank while 23 pieces were still alive. All shrimp were subjected to a whole-body DNA extraction.

DNA extraction

- 5 DNA was extracted from whole body using salting-procedure (Aljanabi, S.M. and L. Martinez, 1997. Universal and rapid salt-extraction of high quality genomic DNA for PCR-based techniques. Nucleic Acid Res., 25: 4692-4693). Primers used in this experiment was HPVF: 5'-ACA-CTC-AGC-CTC-TAC-CTT-GT 3' and HPVR: 5' -GCA- TTA-CAA-GAG-CCA-AGC-AG - 3'. Thirty-five cycles of amplification were performed at 30 s at 94°C, 30 s at 55°C, and 50 s at 10 72°C for both primer pairs. The expected PCR products were analyzed in a 2% agarose gel, with the expected band of 441 bp as shown in Figure 3.

Results

- PCR analysis showed that RetroMAD1 in the treated Paleonetes sp tank, 92% (22/24) were 15 HPV negative while 8% (2/24) were HPV positive. In the control non-treated group, 95% (22/23) were HPV positive while 5 percent (1/23) were HPV negative.

EXAMPLE 3

Effect of RetroMAD1 on WSSV-infected shrimp

20

Shrimp culture

- White Leg Shrimp *Penaeus vannamei* (36 pieces) at an average of 8.0 ± 0.5 grams were used in this experiment they were obtained from pond-reared from SPF (specific pathogen free) post-larvae obtained from commercial hatcheries. Treated sea water was obtained from the 25 hatchery. Cultures of healthy shrimp were performed in a recirculation system (equipped with filter and aeration) with a salinity of 28-32 ppt in a bio-secure laboratory at 28°C. They were acclimatized 1 week before the infection experiment. Two groups of 18 prawns were reared in a 90 liter tank with and individual filter (Figure 4).

WSSV Infection

- Prawns were orally challenged by feeding frozen flesh from WSSV-PCR positive prawns obtained from a recently WSSV-killed pond at approximately 5% of body weight on the first day. The next day, were given RetroMAD1 at a concentration of 0.1mg/g body weight by coating it into a commercial feed. They were given the medicated feed for all meals (4 times a day). 35 Observation was carried in term mortality after 24 hours of infection. At the end of the experiments, all live prawns were collected. These moribund and live prawns were subjected to PCR analysis.

DNA Extraction

DNA was extracted from the pleopod using salting-procedure (Aljanabi, S.M. and L. Martinez, 1997). Primers used in this experiment was WSVF: 5'-TAT-TGT-CTC-TCC-TGA-CGT-AC- 3' and WSVR: 5' -CAC-ATT-CTT-CAC-GAG-TCT-AC- 3'. Thirty-five cycles of amplification were performed at 30 s at 94°C, 30 s at 55°C, and 50 s at 72°C for both primer pairs. The expected PCR products were analyzed in a 2% agarose gel, with the expected band of 298 bp.

Result

In the control tank, mortalities began on day 3 post-challenge and by day 8, nearly all of the 18 prawns were dead. By day 9 post-challenge, 100% mortality was observed in the control tank showing that the WSSV-infected carcass used was very much capable of causing 100% mortality within 9 days post-oral infection. In the treated tank, no mortality was observed until day fourteen.

PCR analysis showed that all moribund prawns (18/18) from the control group had high level of WSSV. Interestingly, in the treated group 9/18 had low infection, 3/18 had very low infection while 6/18 undetected (Figure 5).

EXAMPLE 4

Time needed for sero-reversal to occur in MBV-infected shrimp

Monodon Baculovirus (MBV) is an OIE 'listed for notification' shrimp DNA virus that has historically contributed to significant commercial losses in shrimp farming. A total of 5 MBV highly-PCR positive 6g *Penaeus vannamei* were detected from a subsample of 20 shrimp obtained live from a commercial shrimp farm in Tawau, Malaysia and tested based on sacrificing one pleopod for DNA extraction. These were individually kept in separate 10L aerated plastic aquariums that had 30% daily water exchange at 30ppt salinity for a 1 week acclimation period. Ammonia and Nitrite were monitored to ensure adequate water quality. They were then fed a commercial pellet feed (Charoen Pokphand) once a day with 100mg each feeding. The intentionally low feeding rate was to ensure that all the feed would be consumed and not contribute to developing ammonia in the experimental tank. RetroMAD1 at 2mg/ml concentration was added at 150ml/kg to prepare the stock feed for the experiment by applying it on the surface of the feed followed by convection drying at 35°C in an oven. These were then stored at 4°C in a refrigerator for the duration of the experiment. After a week, another pleopod was surgically removed and tested with standard Polymerase Chain Reaction (PCR) against the highly conserved coat protein of the virus. The primers used were: MBV F: 5' TACCATAAGCTAGCATACGCC 3' and MBV R: 5' GGGGGCACAAGTCTCACAAG 3'. Nucleic acid isolation and the PCR protocol used were the same as Example 3 above. The size of the PCR product was 305 bp.

At the end of week 1, it was found that all the resultant pleopod samples were still PCR positive but at the end of week 2, all the samples from another surgically removed pleopod showed that they had all become PCR negative. It is therefore suggested that sero-reversal in MBV infected

shrimp from PCR positive to PCR negative takes approximately 2 weeks after feeding with RetroMAD1.

<i>P. vannamei</i>	PCR Result		
	Day 0	Day 7	Day 14
Animal 1	Positive	Positive	Negative
Animal 2	Positive	Positive	Negative
Animal 3	Positive	Positive	Negative
Animal 4	Positive	Positive	Negative

Table 2. Results of PCR for MBV post RetroMAD1 treatment

5 EXAMPLE 5

Stability of RetroMAD1 to trypsin at pH8

The ability of RetroMAD1 to withstand action of digestive enzymes acting at their pH optima is shown in Table 3 below.

50mM DTT was prepared and added into pre-dissolved RetroMAD1 protein (1:1) made according to Example 1 and mixed. This was heated at 95°C for 10 minutes and used to carry out enzyme assays with proteases such as Trypsin (pH8) (Lonza, Walkersville), α -Chymotrypsin (pH8) (Sigma-Aldrich) and Pepsin (pH2) (Sigma-Aldrich). After 10 minutes of heating at 95°C, the reaction was allowed to cool to room temperature (Approx. 10mins) and proteases added to a final ratio of 1:100 (w/w) (protease:protein). This was incubated at 37°C for 2 hours and protease activity terminated by incubating the mixture at 65°C for 15 minutes. SDS-PAGE was used to analyze the fragments.

Other fusion proteins provided in Table 4 were made according to the method of Example 1 and the results of their fragmentation provided in Table 3.

Drug	Size of drug	SEQ ID NO:	Structure of drug	No of bands after protease digestion		
				Pepsin	Trypsin	Chymotrypsin
AM	40 kDa	28	A-B-C (AVBD103-MAP30-MYTILINC10C)	No fragment	No fragment	No fragment
CT	36 kDa	29	A-A-B-C (CERCROPIN A-CERCROPIN D-TAP29-DAP30-LATARCIN 2A)	No fragment	No fragment	No fragment
AB	32 kDa	30	(RETROCYCLIN 101-MORMODICA ANTI-HIV PROTEIN 30)	No fragment	No fragment	No fragment
BA	32 kDa	31	(MORMODICA ANTI-HIV PROTEIN 30- RETROCYCLIN 101)	No fragment	No fragment	No fragment
BC	35 kDa	32	(MORMODICA ANTI-HIV PROTEIN 30- DERMASEPTIN 1)	No fragment	No fragment	No fragment
CB	35 kDa	33	DERMASEPTIN 1- MORMODICA ANTI-HIV PROTEIN 30	No fragment	No fragment	No fragment
Tamapal1	35.93 kDa	34	C-B-C TACHYPLESIN- MAP30-ALLOFERON1	No fragment	No fragment	No fragment
K5	36.55 kDa	35	C-B-D (GAEGURIN 5-MAP30-(KLAKLAK)2	No fragment	No fragment	No fragment
RetroM	41.2kDa	1	A-B-C	No	No	No

AD1			(RETROCYCLIN 101- MAP30- DERMASEPTIN 1)	fragment	fragment	fragment
RetroG AD1	35.29kDa	36	A-B-C (RETROCYCLIN 101- GAP31- DERMASEPTIN 1)	No fragment	No fragment	No fragment

Table 3. Results of fragmentation of fusion proteins according to the present invention

SEQ ID NO:	SEQUENCE
27	[G-G-G-S] _n
28	SFGLCRLRRGFCAGRCRFPSPPIPIGRCSRFRVQCCRRVWVPGVGVPGVGGATGSDVNFDLSTATAKTYTK FIEDFRATLPFSHKVYDIPLLYSTISDSRRFILLNLTSYAYETISVAIDVTNVYVAYRTRDVSFFKE SPPEAYNILFKGTRKITLPYTGNENLQTAHKIRENIDLGLPALSSAITTLFYNAQSAPSALLVLIQ TTAEAAARFKYIERHVAKYVATNFKPNLAIISLENQWSALSQKIFLAQNQGGKFRNPVDLIKPTGERFQV TNVDSDVVKGNIKLLNSRASTADENFITTMTLLGESVNVSCASRCKGHCRARRCGYYVSVLYRGRCYC KCLRCVPGVGVPGVG
29	LEKRKWKLFKKIEKVGQRVRDAVISAGPAVATVAQATALAKNVPGVGVPGVGGATGSDVSFRLSGATSK KKVYFISNLRKALPNEKKLYDIPVRSSSGSKATAYTLNLANPSASQYSSFLDQIRNNVRDTSIYGGT DVAVIGAPSTTDKFLRLNFQGPRTVSLGLRRENLYVVAYLAMDNANVNRAYYFKNQITSAELTALFPE VVVANQKQLEYGEDYQAIEKNAKITTDGQSRKELGLGINLLITMIDGVNKKVRVVKDEARFLIAIOMT AEAAARFYIQNLVTKNFPNKFDSSENKVIQFQVSWSKIATAIFGDCNGVFNKDYDFGFGKVRQAKDLQM GLLKYLGPRKSSSIEANSTDDTADVLVPGVGVPGVG KTCENLADTFRGPCFATSNC
30	MGRICRCICGRGICRCICGVPVGVPGVGGSDVNFDLSTATAKTYTKFIEDFRATLPFSHKVYDIPLLY STISDSRRFILLDLTSYAYETISVAIDVTNVYVAYRTRDVSFFKESPEAYNILFKGTRKITLPYTG NYENLQTAHKIRENIDLGLPALSSAITTLFYNAQSAPSALLVLIQTAEAAARFKYIERHVAKYVATN FKPNLAIISLENQWSALSQKIFLAQNQGGKFRNPVDLIKPTGERFQVTNVDSDVVKGNIKLLNSRAST ADENFITTMTLLGESVVEFPW
31	MGSDVNFDLSTATAKTYTKFIEDFRATLPFSHKVYDIPLLYSTISDSRRFILLDLTSYAYETISVAIDV TNVYVAYRTRDVSFFKESPEAYNILFKGTRKITLPYTGNENLQTAHKIRENIDLGLPALSSAIT TLFYNAQSAPSALLVLIQTAEAAARFKYIERHVAKYVATNFKPNLAIISLENQWSALSQKIFLAQNQG GKFRNPVDLIKPTGERFQVTNVDSDVVKGNIKLLNSRASTADENFITTMTLLGESVVEFPWVPGVGP VGGRICRCICGRGICRCICG
32	MGSDVNFDLSTATAKTYTKFIEDFRATLPFSHKVYDIPLLYSTISDSRRFILLDLTSYAYETISVAIDV TNVYVAYRTRDVSFFKESPEAYNILFKGTRKITLPYTGNENLQTAHKIRENIDLGLPALSSAIT TLFYNAQSAPSALLVLIQTAEAAARFKYIERHVAKYVATNFKPNLAIISLENQWSALSQKIFLAQNQG GKFRNPVDLIKPTGERFQVTNVDSDVVKGNIKLLNSRASTADENFITTMTLLGESVVEFPWVPGVGP VGALWKTMLKELGTMLHAGKAALGAAADTISQGTQ*
33	MALWKTMLKELGTMLHAGKAALGAAADTISQGTQVPGVGVPGVGGSDVNFDLSTATAKTYTKFIEDFR ATLPFSHKVYDIPLLYSTISDSRRFILLDLTSYAYETISVAIDVTNVYVAYRTRDVSFFKESPEAY NILFKGTRKITLPYTGNENLQTAHKIRENIDLGLPALSSAITTLFYNAQSAPSALLVLIQTAEAA RFKYIERHVAKYVATNFKPNLAIISLENQWSALSQKIFLAQNQGGKFRNPVDLIKPTGERFQVTNVDS DVVKGNIKLLNSRASTADENFITTMTLLGESVVEFPW*
34	VPGVGVPGVGKWCFRVCYRGICYRRCRVPGVGVPGVGGATGSDVNFDLSTATAKTYTKFIEDFRATLP SHKVYDIPLLYSTISDSRRFILLNLTSYAYETISVAIDVTNVYVAYRTRDVSFFKESPEAYNILFK GTRKITLPYTGNENLQTAHKIRENIDLGLPALSSAITTLFYNAQSAPSALLVLIQTAEAAARFKYI ERHVAKYVATNFKPNLAIISLENQWSALSQKIFLAQNQGGKFRNPVDLIKPTGERFQVTNVDSDVVKGN IKLLNSRASTADENFITTMTLLGESVNVPGVGVPGVGHGVS GHGQHG VHG
35	VPGVGVPGVGFLLLAGLAANFLPTIICFISYKCVPGVGVPGVGGATGSDVNFDLSTATAKTYTKFIED FRATLPFSHKVYDIPLLYSTISDSRRFILLNLTSYAYETISVAIDVTNVYVAYRTRDVSFFKESPE AYNILFKGTRKITLPYTGNENLQTAHKIRENIDLGLPALSSAITTLFYNAQSAPSALLVLIQTAE AARFKYIERHVAKYVATNFKPNLAIISLENQWSALSQKIFLAQNQGGKFRNPVDLIKPTGERFQVTNV SDVVKGNIKLLNSRASTADENFITTMTLLGESVNVPGVGVPGVVKLAKLAK KLA LAK

Table 4. Examples of fusion proteins according to the present invention

The G.I. tract of shrimps and prawns consists of the proventriculus or gastric mill, digestive gland, midgut and its diverticula, and the rectum. Two distinct cell types occur in the digestive gland, a secretory type, and a mucopolysaccharide-containing type, whose function is not clear. The digestive gland has no intrinsic muscles, and depends on extrinsic muscles, and possibly ingested water, for filling and emptying. The midgut or hepatopancreas extends to the sixth abdominal somite and faecal material is contained in a peritrophic membrane. Defecation was at a peak 5-8 hr after food ingestion, but continued up to 20 hr. The rectum appeared to have the additional function of pumping water into the gut via the anus. As the pH of seawater is around 8, it is natural for trypsin and chymotrypsin to be the main physiological digestive enzymes whose pH optima is pH 8 unlike pepsin whose optima is at pH 2. Thus, pepsin was absent from the digestive tract of shrimps. The ability of RetroMAD1 to survive digestion by trypsin and chymotrypsin allowed it to be an effective therapeutic protein that could be administered along with the feed pellets itself thereby enabling a mode of delivery that is practical for shrimp farmers to employ. The results are shown in Table 3 above.

Conjugating these peptides with MAP30, surprisingly render the fusion protein stable for oral administration as shown in its ability to survive protease digestion.

EXAMPLE 6

Trial using orally delivered RetroMAD1 and an immunostimulant Beta-Defense on Asian Seabass

Between 27th April 2012 to 23rd May 2012, a 4-week trial was carried out by Temanse Aquaculture S/B at its Sekayu Nursery centre in Malaysia where survivor Asian Sea Bass *Lates calcarifer* juveniles suffering from an unknown disease syndrome which was VNN and Iridovirus PCR -ve were treated with individual regimes of RetroMAD1 and Beta-Defense (a commercial immune-stimulant) and a combination regime, against an untreated control. From a shipment of 30,000 fingerlings from Singapore, 20,000 quickly died within a matter of days and 240 similar sized apparently healthy animals were selected from the 10,000 survivors and were placed in 4 aquariums measuring 40x60x100cm each with 60 fingerlings for 3 days acclimation. Some minor mortalities occurred during acclimation and the experiment began with 50 fishes per batch. All water quality parameters such as Dissolved Oxygen, pH, ammonia and nitrite are regularly measured to ensure these were within normal acceptable ranges. The data is presented in Table 5 below.

<i>Lates calcarifer</i> survivor juveniles	Survival Rate %					Weight gain and FCR		
Treatment	Day1	Day 6	Day 14	Day 22	Day 28	Initial Weight	Final Weight	FCR
Control - feed only	100	0	0	0	0	5.7	7.53	n.a.
Treatment - feed + BD	100	0	0	0	0	5.71	7.22	n.a.
Treatment - feed + RetroMAD1	100	100	100	48	48	5.65	15.8	1.8
Treatment - feed + BD + RetroMAD1	100	100	100	78	78	5.67	17.9	0.5

Carried out at Sekayu Nursery, Kuala Berang, Malaysia belonging to Temanse Aquaculture S/B

Table 5. Results of experiment

All control and Beta-Defense fishes died on day 6. No more mortalities occurred again until on day 22 and by day 28 at the end of the experiment, the RetroMAD1 treatment had 48% survival and the fingerlings had grown from 5.65g – 15.8g mean weight with an FCR of 1.8. The combination treatment of RetroMAD1 gave a very convincing 78% survival with growth from 5.67-17.9g mean weight with an FCR of 0.5. Beta-Defense was given at 45ml per 300g of pellet feed while RetroMAD1 was given at 0.1 ml diluted with 25 ml distilled water and added to the 300g of feed. In the combination treatment, both were given together to 300g of feed. Although the primary pathogen has not yet been determined, there was evidence of some secondary bacterial infection. We suspect however, that the primary pathogen is viral in nature as RetroMAD1 is a broad-spectrum antiviral oral-delivery protein drug. The efficacy shown with the 78% survival over 4 weeks also indicates that RetroMAD1 is efficacious in fishes also. Thus, there is every potential to use this method also in the production of SPF eggs and fingerlings in fish breeding.

EXAMPLE 7**The Stability of RetroMAD1, RetroGAD1, Amatilin and Tamapal1**

The polypeptides, RetroMAD1, RetroGAD1, Amatilin and Tamapal1 were capable of going through various thermocycler protocols that mimic post-extrusion processing temperatures in making extruded shrimp feed coated with RetroMAD1 and then coated again with a marine edible oil.

The fusion peptide solutions to be tested were loaded using a micropipette into 0.2ml PCR tubes that were then placed into a thermocycler (Labnet International, MultiGene Gradient) which was then programmed to run at various temperature regimes as mentioned in Table 6. Each regime was made up of a short high temperature phase of 15 minutes followed by a longer medium temperature phase of 45 minutes. These were to mimic the actual temperature conditions when an extruded feed in the form of a wafer shaped pellet left the extrusion barrel of a twin-screw extruder which in this case is a Clextral BC45. The wafer was then sprayed with sufficient squid oil post extrusion as to form an external lipid barrier. In these thermocycler trials, the harshest condition was a 15 minute 70°C exposure followed by a 45 minute 55°C exposure. Samples were then run on SDS-PAGE with the lanes as follows:- Lanes: M, marker; 1, negative control treated with 2× β-mercaptoethanol positive loading dye; 2, negative control treated with 2× β-mercaptoethanol negative loading dye; 3, Sample subjected to the temperature regime and treated with 2× β-mercaptoethanol positive loading dye; 4, sample subjected to the temperature regime and treated with 2× β-mercaptoethanol negative loading dye; 5, sample subjected to the temperature regime and treated with 2× β-mercaptoethanol positive loading

dye; 6, Sample subjected to the temperature regime and treated with 2× β -mercaptoethanol negative loading dye. Comparison of the gel bands against the control gave a physical evidence as to whether the protein was damaged by the heat treatment or not.

Parameters	Round 1/T1		Round 2/T2		Round 3/T3		Round 4/T4	
Temperature (°C)	60	50	55	45	50	40	70	55
Time (mins)	15	45	15	45	15	45	15	45

Table 6: Parameters of temperature fluctuations.

5

As can be seen in Figure 6, All four drugs, RetroMAD1 (A1 and A2), RetroGAD1 (B1 and B2), Amatillin (C1 and C2) and Tamapal1 (D1 and D2) are intact under all treatments despite the presence of BME. This indicates that all four drugs are stable and will not be affected by the change in temperature.

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EXAMPLE 8

The antiviral activity of peptides (subjected to various temperature fluctuations using thermocycler) against HSV-2

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Amatillin, RetroGAD1 and Tamapal1 as described in Example 7, were exposed to four sets of temperature fluctuations (T1, T2, T3 and T4) using thermocycler (Table 6 of Example 7). After exposure to various temperature fluctuations, the peptides were subjected to antiviral assay against HSV-2.

20

The cytotoxic activity of the peptides was quantified using MTS-based cell titer 96 non-radioactive cell proliferation assay. Briefly, monolayer cultures of Vero cells were exposed to increasing concentrations of all the three peptides for 24, 48 and 72h of incubation. After the incubation period, the maximal concentration of the extract that did not exert toxic effect which was regarded as the maximal non-toxic concentration (MNTD) was determined using MTS assay.

25

After exposure to various temperature fluctuations using thermocycler (Table 6), the antiviral activity of Amatillin, RetroGAD1 and Tamapal1 was evaluated by simultaneous treatment. For simultaneous treatment the mixture of the respective peptide and virus inoculated onto Vero cells in 24-well culture plates and incubated for 24, 48 and 72h at 37 °C under 5% CO₂ atmosphere. At the end of the time period the samples were harvested and viral DNA was extracted. The eluted DNA was then subjected to RT-PCR.

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The results obtained suggest that all the three peptides were thermal stable. Amatillin and Tamapal1 showed the strongest inhibitory activity against HSV-2 at all the four set of temperature fluctuations (Table 7 and Figure 7).

Peptides Set of temperature fluctuations	Amatilin	RetroGAD1	Tamapal1
T1	97.28	94.88	86.21
T2	94.05	96.95	90.36
T3	97.85	63.04	97.64
T4	86.00	75.91	93.65

Table 7: Percentage of viral reduction caused by Amatilin, RetroGAD1 and Tamapal1 exposed to various temperature fluctuations in simultaneous treatment determined by PCR.

EXAMPLE 9

Leaching rate of RetroMAD1, RetroGAD1, Amatilin and Tamapal1 from the wafer pellets produced in a pilot-scale manufacture

The leaching rate study for as the various fusion protein drugs as described in Example 7, was to study the time points when RetroMAD1, RetroGAD1, Amatilin and Tamapal1 were leached out from the wafers. Wafers containing the drugs were placed within in 30ppt sea salt water in 1:100 weight to volume ratio. Shrimp wafer pellets were formed by extrusion using a Clextral BC45 twin-screw extruder that was sprayed post extrusion with the fusion protein drugs to be tested followed by a spray coating in a vacuum chamber with squid oil to serve as an outer hydrophobic layer to 'lock-in' the test drug as well as to serve an a feeding attractant for the shrimp. Addition of RetroMAD1 was added at the rate of 300mg/kg of wafer pellets. At 0, 30, 60, 120 and 240 minutes, sea salt water was collected to determined the concentration of the fusion protein drugs that was leached out of the wafers into the sea salt water. Capture ELISA (Promega, Glomax Multidetection System) was used to determine the concentration of RetroMAD1, while Direct ELISA was used for RetroGAD1, Amatilin and Tamapal1. In Capture ELISA, a 96 U-bottom well plated was coated with 1:1000 of rabbit anti-RetroMAD1 antibody and was incubated at 4°C overnight. The plate was then washed with PBS-Tween20 six times before adding the samples collected at time point 0, 30, 60, 120 and 240 minutes and incubated at 37°C for an hour. Subsequently, 1:2500 human anti-RetroMAD1 antibodies were added to capture RetroMAD1 from the samples bound on the rabbit anti-RetroMAD1 antibody. While in direct ELISA, a 96 well U-bottomed plate was coated with the samples collected at time point 0, 30, 60, 120 and 240 minutes and incubated overnight at 4°C. The plate was then washed with PBS-Tween20 and added with 1:500 rabbit antibodies against RetroGAD1, Amatilin and Tamapal1 to capture the protein drug bound on the plate. Subsequently, 1:10000 anti-rabbit IgG were added to detect rabbit antibodies bind against the protein drugs. Absorbance was read at 490nm and 600nm. A standard curve of drug concentration against absorbance was plotted to determine the concentration of the drug in each sample.

Both RetroMAD1 and Tamapal1 began leaching out only after 120 minutes. Both Amatilin and RetroGAD1 did not show any signs of leaching even 240 minutes. This shows that since shrimp usually consume all their feed within 30-60 minutes, this method of oral administration of these fusion protein drugs is viable for the treatment of shrimp viruses. Furthermore, as shrimp

digestion is trypsin rather than chymotrypsin dependent, it does not matter that the drug is presented along with the feed.

Antibodies toward RetroMAD1, RetroGAD1, Amatilin and Tamapal1 were raised in 4 rabbits respectively. In each immunization, rabbits were immunized with RetroMAD1, RetroGAD1, Amatilin and Tamapal1 in single dose of 0.6ml per rabbit which is a dose of 0.2mg/kg body weight for RetroMAD1, 0.9ml per rabbit which is a dose of 0.25mg/kg body weight for RetroGAD1, 0.8ml per rabbit which is a dose of 0.25mg/kg body weight for Amatilin and 1ml per rabbit which is a dose of 0.25mg/kg body weight for Tamapal1.

Prior to immunization, on Day 0, blood was drawn from the rabbits. Pre-bleed blood collected on Day 0 was used as the base line in determining the antibody titer in rabbit. After pre-bleeding the rabbit, first immunization was given according to the dosage per body weight as mentioned above. Rabbits were bled before giving another immunization on Day7, Day 14, Day 28 and Day 35. Blood serum of rabbits collected on Day7, Day 14, Day 28 and Day 35 was used to determine antibody titer against RetroMAD1, RetroGAD1, Amatilin and Tamapal1. On Day 38, antibody towards RetroMAD1, RetroGAD1, Amatilin and Tamapal1 raised in rabbits were harvested. In harvesting the rabbit antibody, before bleeding, each rabbit was given anesthesia (Ketamine and Xylazine) intravenously; the sedative dose was calculated using the following formula

- Ketamine = (30 x body weight of the rabbit)/ (Concentration of Ketamine, 100mg/ml)
- Xylazine = (3 x body weight of the rabbit)/ (Concentration of Xylazine, 20mg/ml)

50ml of blood was collected from each rabbit. Blood was centrifuged at 4000rpm for 15 minutes; blood serum containing antibody towards RetroMAD1, RetroGAD1, Amatilin and Tamapal1 was collected and kept in -20°C for further use.

A direct ELISA was used to determine antibody titer in rabbit serum. A 96-well U-bottomed plate was coated with 1µg/ml of RetroMAD1, RetroGAD1, Amatilin and Tamapal1 in coating buffer (0.2 M sodium carbonate-bicarbonate, pH 9.6). The sample coated plate was incubated at 4°C overnight. Plates were washed six times with 0.05% Tween-20 in PBS 1x. 100µl of 1/10 rabbit serum was added to the well, a ½ serial dilution of the rabbit serum was made. Rabbit serum was diluted in 1/10, 1/20, 1/40, 1/80, 1/160, 1/320, 1/640, 1/1280, 1/2560, 1/5120 and 1/10240 to determine the antibody titer. After incubation at 37°C for 1 hour, plates were washed similarly and 100 µl/well of anti-rabbit IgG diluted 1:10000 in 5% BSA in PBS was added. After incubation at 37°C for 1 hour, plates were washed and 100 µl/well streptavidin- HRP diluted 1:20000 in 5% BSA in PBS was added. After incubation at 37°C for 1 hour in the dark, plates were washed and 100 µl/well of OPD added to each well. Plates were incubated in the dark for 30 min at room temperature and reaction stopped with 50 µl/well of 4N H₂SO₄. Optical densities (OD) were measured at 490 nm and 600 nm as background. The results are shown in Figure 8A-D and tables 8-11.

Time (minutes)	Concentration of RetroMAD1 (µg/ml)
30	0

60	0
120	0
240	1

Table 8: Concentration of RetroMAD1 leached out against time

Time (Minutes)	Concentration of RetroGAD1 (µg/ml)
0	0.00000
30	0.00000
60	0.00000
120	0.00000
240	0.00000
Control	0.00000

Table 9: Concentration of RetroGAD1 leached out at 0, 30, 60, 120 and 240 minutes

Time (Minutes)	Concentration of Amatilin (µg/ml)
0	0.00000
30	0.00000
60	0.00000
120	0.00000
240	0.00000
Control	0.00000

Table 10: Concentration of Amatilin leached out at 0, 30, 60, 120 and 240 minutes

Time (Minutes)	Concentration of Tamapal1 (µg/ml)
0	0.000000
30	0.000000
60	0.000000
120	0.000000
240	0.040167
Control	0.000000

Table 11: Concentration of Tamapal1 leached out at 0, 30, 60, 120 and 240 minutes

EXAMPLE 10**Short-term Pharmacokinetics of RetroMAD1 in shrimp using capture ELISA**

In the short-term feeding study, shrimps were fed with 0.06g of shrimp wafer pellets containing RetroMAD1 at an inclusion of 300mg/kg. This study is to determine the short term kinetics of RetroMAD1 in terms of absorption, retention and excretion. Shrimp wafer pellets were formed by extrusion using a Clextral BC45 twin-screw extruder that was sprayed post extrusion with the fusion protein drugs to be tested followed by a spray coating in a vacuum chamber with squid oil to serve as an outer hydrophobic layer to 'lock-in' the test drug as well as to serve as a feeding attractant for the shrimp. Addition of RetroMAD1 was added at the rate of 300mg/kg of wafer pellets.

Healthy specimens of the commonly cultured Pacific white shrimp *Penaeus vannamei* were selected from a shrimp farm in Tawau, Sabah, Malaysia and a single specimen ranging from 2.4-5.8g was placed in each transparent plastic aquarium tank of 10 litres total capacity containing 5 litres of seawater at 32 parts per thousand salinity. Specimens were acclimated for

a week prior to the experiment and 50% water was changed daily by siphoning. A single airstone was provided such that aeration was sufficiently provided such that the animal did not display any signs of being stressed. A plastic netting was provided on top to prevent the specimens from jumping out. For each sampling time point, tanks were present in triplicate as in Group 1,2 and 3. As there were 8 sampling time points, 24 tanks were prepared as shown in the Table 12.

Sampling Points	Number of Shrimp per tank		
Time (Hours)	Group 1	Group 2	Group 3
Control	1	1	1
0.5	1	1	1
1	1	1	1
1.5	1	1	1
2	1	1	1
3	1	1	1
5	1	1	1
8	1	1	1

Table 12: Experiment design for measuring short-term pharmacokinetics of RetroMAD1 in shrimp

At each sampling time point, the feces were collected by siphoning, the shrimp dissected removing the hepatopancreas well as the muscle of the last abdominal segment of the tail which was stored in PBS buffer and stored at -40°C . Note that the Control were fed normal shrimp pellets without RetroMAD1. The shrimp were unfed for the duration of the experiment after completely ingesting the test and control feeds. The weights of the feces, hepatopancreas and tail muscle (only the last abdominal segment) collected are presented in the table 13 below.

Time (Hour)	Tank	Weight (grams)			
		Whole Shrimp	Hepatopancreas	Tail Muscle	Feces
0 (Control)	1	3.238	0.120	0.108	0.000
	2	3.227	0.160	0.127	0.038
	3	3.554	0.190	0.231	0.043
0.5	1	3.600	0.250	1.960	0.045
	2	2.440	0.170	0.142	0.040
	3	3.720	0.187	0.136	0.020
1	1	2.785	0.152	0.100	0.065
	2	3.213	0.141	0.133	0.030
	3	4.236	0.211	0.208	0.015
1.5	1	2.130	0.126	0.095	0.070
	2	4.117	0.175	0.206	0.053
	3	1.612	0.100	0.083	0.086
2	1	5.500	0.236	0.222	0.041
	2	2.784	0.155	0.116	0.052
	3	2.993	0.182	0.160	0.056
3	1	3.538	0.190	0.142	0.024

	2	3.719	0.175	0.154	0.083
	3	3.995	0.199	0.147	0.054
5	1	3.508	0.154	0.188	0.060
	2	3.962	0.194	0.200	0.016
	3	2.443	0.155	0.104	0.050
8	1	4.995	0.245	0.245	0.035
	2	5.840	0.182	0.292	0.034
	3	3.460	0.148	0.146	0.038

Table 13: Weight of each shrimp, hepatopancreas, tail muscle (last segment only) and feces

Captured ELISA (Promega, Glomax Multidetector System) was used to determine concentration of RetroMAD1 in the samples. The tail muscle sampled was in the last abdominal segment after the anus to ensure any result did not come from the GI tract.

- 5 In captured ELISA, a 96 U-bottom well plated was coated with 1:1000 of rabbit anti-RetroMAD1 antibody (as mentioned in Example 9) and was incubated at 4°C overnight. Plate was then washed with PBS-Tween20 six times before adding the samples of hepatopancreas, tail muscle and feces and incubated at 37°C for an hour. Subsequently, 1:2500 human anti-RetroMAD1
- 10 RetroMAD1 antibody. Absorbance was read at 490nm and 600nm. A standard curve of concentration of RetroMAD1 (µg/ml) against absorbance as shown in Table 13 was plotted to determine the concentration of RetroMAD1 in each sample.

- Table 14 and Figure 9 show that hepatopancreal absorption of RetroMAD1 was detectable at
- 15 1.5 hours post-feeding and peaked at 5 hours post-feeding while RetroMAD1 was detectable in the tail muscle as early as 3 hours post-feeding.

Time (hours)	Treated with RetroMAD1			
	Hepatopancreas	Tail muscle	Feces	Control
0.5	0	0	0	0
1	0	0	0	0
1.5	0	0	0	0
2	1.133	0	0	0
3	1.983	0	0	0
5	3.867	0.9333	0	0
8	2.717	1.783	0	0

Table 14: Concentration of RetroMAD1 against time

EXAMPLE 11

20 Long-term Pharmacokinetics of RetroMAD1 in shrimp using capture ELISA

In the long-term feeding study, shrimps were fed with 0.2g of shrimp pellets containing RetroMAD1 at 300mg/kg inclusion rate. This study is to further determine the pharmacokinetics of RetroMAD1 in terms of absorption, retention and excretion over 7 days.

- 25 In this study, Healthy specimens of the commonly cultured Pacific white shrimp *Penaeus vannamei* were selected from a shrimp farm in Tawau, Sabah, Malaysia and 8 pcs of 10.0g +/-

0.5g specimen were placed in a single transparent plastic aquarium tank of 50 litres total capacity containing 40 litres of seawater at 32 parts per thousand salinity. Specimens were acclimated for a week prior to the experiment and 50% water was changed every alternate day by siphoning. A single airstone was provided such that aeration was sufficiently provided such that the animal did not display any signs of being stressed. A plastic netting was provided on top to prevent the specimens from jumping out. Feces were collected daily by siphoning and placed into a 1.5ml plastic tube that was capped and stored at -40°C until required.

For each sampling time point, tanks were present in triplicate as in Group 1,2 and 3. As there were 7 sampling time points as well as one control, 24 tanks were prepared each with 8 specimens as shown in Table 15:

Sampling Points	Number of Shrimps per tank		
Time (Days)	Group 1	Group 2	Group 3
Control	Control 1	Control 2	Control 3
1	8	8	8
2	8	8	8
3	8	8	8
4	8	8	8
5	8	8	8
6	8	8	8
7	8	8	8

Table 15 Experiment design for measuring long-term pharmacokinetics of RetroMAD1 in shrimp

Within the first day, there were 6 sampling points at 2, 4, 6, 8, 12 and 16 hours. As there were 8 animals, one was removed at each sampling point and the feces and dissected hepatopancreas as well as the tail muscle from the last abdominal segment pooled and kept in PBS at -40°C till required. The sampling point at 24 hours was taken at the start of Day 2 while the 36 hours sampling point taken mid-way through Day 2. Thereafter, there were sampling points daily.

Captured ELISA (Promega, Glomex Multidetec System) was used to determine concentration of RetroMAD1 in hepatopancreas, tail muscle and faeces, in the method previously described earlier.

Table 16 and Figure 10 showed that hepatopancreal RetroMAD1 peaked after 5 hours in agreement with the previous experiment (Example10). Residual RetroMAD1 could be detected up to 144 hours (day 6) even after faecal RetroMAD1 was no long detectable after 72 hours (day 3). Detectable RetroMAD1 in the tail muscle remained only for 8 hours showing that the drug residue is quickly broken down by active swimming shrimp.

This indicated a safe 'withdrawal period' of 14 days for head-on shrimp product (sold with the head containing the hepatopancreas) and 7 days for a headless shrimp product (where the head containing the hepatopancreas is removed at the processing factory prior to freezing).

Time (Hours)	Concentration of RetroMAD1 (µg/ml)			
	Hepatopancreas	Tail Muscle	Feces	Control
2	189.62	0	0	0
4	335.085	5.272	115.751	0
6	304.758	16.015	151.794	0

8	209.824	0	238.178	0
10	174.754	0	234.733	0
12	168.082	0	224.924	0
18	165.378	0	256.926	0
24	160.538	0	194.13	0
36	147.025	0	195.96	0
48	136.772	0	201.383	0
72	75.827	0	0	0
96	49.567	0	0	0
120	40.343	0	0	0
144	3.275	0	0	0
168	3.426	0	0	0

Table 16: Concentration of RetroMAD1 in hepatopancreas, tail muscle and feces at 2, 4, 6, 8, 10, 12, 18, 24, 36, 48, 72, 96, 120, 144 and 168 hours.

EXAMPLE 12

5 An orally administered WSSV challenge to post-larvae treated with various fusion proteins.

Protein concentrations were determined by Bradford Analysis (Quick Start Bradford Protein Assay, <http://www.bio-rad.com/webroot/web/pdf/lsr/literature/4110065A.pdf>).

10 The post-larvae were acclimatized for 1 week, continued with pre-infection period of 3 weeks and post-infection period of 1 week. The post-larvae were fed throughout the duration. Feeding was done 3 times a day mixed with the drugs at a ratio of 150ml/kg feed. RetroMAD1 and Tamapal1 were effective against the WSSV oral infection as shown in Tables 16-19. No mortality was observed until Day 30.

15 Feed was observed to be completely consumed justifying the increase in feeding rate over the period of the experiment. Tamapal1 differs from Tamapal1 (A) by use of a different refolding buffer.

Tail muscle tissue (20 mg) was used to determine the viral number retained in the shrimp. DNA extraction was carried out using a 'salting-out' method (Miller, S. A.; Dykes, D. D.; Polesky, H. F. (1988). "A simple salting out procedure for extracting DNA from human nucleated cells". *Nucleic acids research* **16** (3): 1215). Tissues were lysed in 600 µl of lysis buffer (25mM EDTA, 2% SDS) containing 1.5 µl of 20 mg/ml proteinase K. The lysate was incubated at 65°C for 45 minutes or until the solid tissue have been completely dissolved. The lysate was treated with 1.5 µl of 4 mg/ml RNase A for another 15 minutes followed by cooling to room temperature for 5 minutes. Protein precipitation solution (200 µl) was added and the lysate was vortexed for 25 seconds followed by incubation on ice for 5 minutes. The homogenate was centrifuged at 13,000 x g got 10 minutes and 600 µl of supernatant was transferred to a 1.5ml centrifuge containing 600 µl of isopropanol. The tube was inverted gently 40 times. DNA was precipitated

at 13,000 x g for 5 minutes, the supernatant was discarded. The DNA was further washed by adding 600 µl of 70% ethanol and centrifuged for another 3 minutes. Finally the supernatant was discarded and the tube was left to air-dry for 15 minutes. TE buffer was added at 100 µl and used for PCR.

- 5 In this study, three sense primers (F1 [SEQ ID NO:40]: 5' ATGGATTGGAACCTAACA 3', F2 [SEQ ID NO:41]: 5' AATTCGTGGAGAGAGGTCC 3' and F3 [SEQ ID NO:42]: 5' ATCTCTACCGTCACACAGCC 3') and 1 antisense primer (R1 [SEQ ID NO:43]: 5' GAAGATTTTAATGTCCTTGCTCG 3') were designed from the nucleotide sequence of a 626 bp WSSV viral product (van Hulten M.C.W., Witteveldt J., Peters S., Kloosterboer N., Tarchini R., Fiers M., Sandbrink H., Klein Lankhorst R. and Vlak J.M. 2001. The white spot syndrome virus DNA genome sequence. Virol 67: 233–241).

- The F1 and R1 primers were used as external primers to generate a primary PCR product of 500 base pairs (bp) while F2 and F3 were used internal primers along with R1 to generate nested PCR product of 300 and 200 bp, respectively. The 30 µl PCR reaction contained 50 mM KCl, 10 mM Tris-HCl, pH 9.0, 1.5 mM MgCl₂, 0.2 mM each of deoxy (d) ATP, dCTP, dGTP and dTTP, 1 µM of R1, 0.3 µM of F1, 0.3 µM of F2 and 0.4 µM of F3, 1.25 U Taq polymerase. The PCR reaction initiated by heating the mixture 95 °C for 5 min followed by 30 cycles of 30 s at 95 °C, 30 s at 55 °C and 30 s at 72 °C with a final extension of 10 min at 72 °C. Following PCR, the amplified products were analyzed by electrophoresis in 2% agarose gel stained with ethidium bromide and visualized by ultraviolet transillumination.

Tanks	Mortality (times)						
	24 hours	48 hours	72 hours	96 hours	120 hours	144 hours	168 hours
Control (+) 1	0	6	9	10	10	10	10
Control (+) 2	0	2	10	10	10	10	10
Tamapal1 1	0	2	8	9	9	9	9
Tamapal1 2	0	0	0	0	0	0	0
Tamapal1 (A) 1	0	3	9	9	9	9	9
Tamapal1 (A) 2	0	5	8	10	10	10	10
RetroGAD1- 1	0	2	10	10	10	10	10
RetroGAD1- 2	0	1	8	10	10	10	10
Amatilin 1	0	4	10	10	10	10	10
Amatilin 2	0	4	10	10	10	10	10
RetroMAD1-1	0	0	0	0	0	0	0
RetroMAD1-2	0	0	0	0	0	0	0
Control (-) 1	0	0	0	0	0	0	0
Control (-) 2	0	0	0	0	0	0	0

Table 16: Post larvae mortality rate.

Tanks	Pcs									
	1	2	3	4	5	6	7	8	9	10
Control (+) 1	H	H	H	H	M	H	H	H	H	H
Control (+) 2	H	H	H	H	H	H	H	H	H	H
Tamapal1 1	H	H	H	H	H	H	H	H	H	
Tamapal1 2	-	-	-	-	-	-	-	-	-	-
Tamapal1 (A) 1	H	H	M	H	H	M	H	H	H	H
Tamapal1 (A) 2	H	H	H	H	H	M	L	L	H	H
RetroGAD1-1	H	H	M	H	H	H	H	H	H	H

RetroGAD1-2	H	H	H	H	H	H	H	H	H	H
Amatilin 1	H	H	H	M	H	H	H	H	H	H
Amatilin 2	H	H	H	M	H	H	M	H	M	H
RetroMAD1-1	-	-	-	-	-	-	-	-	-	-
RetroMAD1-2	-	-	-	-	-	-	-	-	-	-
Control (-) 1	-	-	-	-	-	-	-	-	-	-
Control (-) 2	-	-	-	-	-	-	-	-	-	-

Table 17: PCR result at the end of trial period. (Each set of drugs were tested in duplicates.)
H: High; M: Medium; L: Low; and -: no band observed.

	Concentration (mg/ml)
Tamapal1	0.468
Tamapal1 (A)	1.43
RetroGAD1	0.58
Amatilin	0.615
RetroMAD1	1.14

Table 18: Concentration of drugs used.

5

Pre & post infection	Feed/day/aquarium*	Amount of drug in µg ingested by a test animal assuming even feeding **				
		Tamapal1	Tamapal1 (A)	RetroGAD1	Amatilin	RetroMAD1
Day1-4	0.09g	0.63	1.9305	0.783	0.83025	1.539
Day5-9	0.12g	0.8424	2.574	1.044	1.107	2.052
Day10-16	0.18g	1.2636	3.861	1.566	1.6605	3.078
Day17-30	0.27g	1.8954	5.7915	2.349	2.4907	4.617

Table 19: Feeding rate from Day 1-30.

*10 pieces of post larvae per aquarium

**Even feeding assumes that all test animals ingested at the same rate.

10 EXAMPLE 13

Amatilin having antibacterial activity on *Vibrio cholera* and *Vibrio parahaemolyticus* from in vitro assay

The minimum inhibitory concentration (MIC) of Amatilin against *Vibrio cholera* and *Vibrio parahemolyticus* was performed according to the Clinical and Laboratory Standard Institute guidelines using the broth micro dilution method. Stock Amatilin at concentration of 1290 µg/ml was two-fold serially diluted in cationically adjusted Mueller Hinton broth (CAMHB) in 96 well round bottom plate to 50 µl. Bacterial cultures from -80°C glycerol stock were passaged twice on nutrient agar and resuspended in phosphate buffered saline (PBS) to OD₆₂₅ 0.08 – 0.1 which was equivalent to 1 – 2 x 10⁸ cfu/ml. The suspension was adjusted to 1 x 10⁶ cfu/ml and added in equal volume (50 µl) to the 96 well plate prepared with the serial dilutions of Amatilin. Final testing concentration of Amatilin ranged from 2.52 µg/ml to 322.5 µg/ml. Plates were incubated for 18-24 hours under 37°C. MIC was read as the lowest concentration of drug that completely inhibits the visible growth of the bacteria. Following that, aliquot of 10 µl from each well was serially ten-fold diluted in PBS and plated on Mueller Hinton agar for viable colony count. Wells with bacterial density of more than 1x 10¹⁰ cfu/ml was noted as "> 1 x 10¹⁰cfu/ml", implying no

45

Bacteria	Concentration (microgram/ml)	Cell count			
		Time (hr)	24hr		
			Cell counted ^a	Dilution factor	Actual cell no. (cfu/ml) ^b
<i>Vibrio cholerae</i>	Untreated	0	188	10 ³	1.88 x 10 ⁵
	Untreated	24	Overcrowded	10 ¹⁰	>1x10 ¹⁰
	322.5	24	0	10 ²	0
	161.25	24	51	10 ²	5.1x10 ³
	80.63	24	124	10 ⁹	>1x10 ¹⁰
	40.31	24	Overcrowded		
	20.16	24	Overcrowded		
	10.08	24	Overcrowded		
	5.04	24	Overcrowded		
	2.52	24	Overcrowded		
<i>Vibrio parahaemolyticus</i>	Untreated	0	26	10 ⁴	2.6 x 10 ⁵
	Untreated	24	Overcrowded	10 ¹⁰	>1x10 ¹⁰
	322.5		0	1	0
	161.25		0	1	0
	80.63		27	10 ⁸	2.7x10 ⁹
	40.31		Overcrowded	10 ¹⁰	>1x10 ¹⁰
	20.16		Overcrowded	10 ¹⁰	>1x10 ¹⁰
	10.08		Overcrowded	10 ¹⁰	>1x10 ¹⁰
	5.04		Overcrowded	10 ¹⁰	>1x10 ¹⁰
	2.52		Overcrowded	10 ¹⁰	>1x10 ¹⁰

^aPlates with more than 200 colonies were denoted as "overcrowded".

^bActual cell no. (cfu/ml) more than 1x10¹⁰ were denoted as ">1x10¹⁰".

inhibitory activity. Results as shown in Figures 11A and B and tables 20 and 21 were pooled from duplicate tests in three independent trials.

Bacteria	MIC (μg/ml)
<i>Vibrio cholerae</i>	161.25 (10.08)
<i>Vibrio parahaemolyticus</i>	161.25 (161.25)

Table 20: MIC of Amatilin

Table 21: Antibacterial effect of Amatilin on *V. cholerae* and *V. parahemolyticus*.

5

EXAMPLE 14

Antiviral activity of Amatilin, RetroGAD1, RetroMAD1 and Tamapal1 using HSV2

Cytotoxicity of tested peptides on Vero cells

The effect of Amatilin, RetroGAD1, RetroMAD1 and Tamapal1 on the growth of Vero cells was examined to rule out any direct cytotoxicity. Monolayer cultures of Vero cells were exposed to increasing concentrations of Amatilin, RetroGAD1, RetroMAD1 and Tamapal1. After 24, 48 and 72h of incubation, cell viability was determined using MTS assay. Results shown indicate the accepted maximal nontoxic concentrations of the four peptides on Vero cells. At the chosen Maximal non-toxic dose (MNTD) as shown in Table 22, the peptides did not impair the cell viability with respect to the untreated control group.

Peptide	MNTD (ug/ml)		
	24h	48h	72h
Amatilin	15	15	15
RetroGAD1	10	10	10
RetroMAD1	50	50	50
Tamapal1	15	15	15

Table 22: Maximal non-toxic dose of the peptides on Vero cells

10 The antiviral activity of peptides against HSV-2

The antiviral activity of Amatilin, RetroGAD1, RetroMAD1 and Tamapal1 was evaluated by simultaneous treatment. For simultaneous treatment the mixture of the respective peptide and virus was inoculated onto Vero cells and incubated for 24, 48 and 72h at 37 °C under 5% CO₂ atmosphere. At the end of the time period the samples were harvested and viral DNA was extracted. The eluted DNA was then subjected to RT-PCR.

The results obtained suggested that all the four peptides have strong inhibitory activity against HSV-2 via simultaneous treatment at the maximal non-toxic dose (MNTD) (Table 22). Amatilin showed 99.88, 99.99 and 99.98% of inhibition, respectively, after 24, 48 and 72h. RetroGAD1 exhibited 95.45, 91.71 and 89.95% inhibitory activity, respectively, at 24, 48 and 72h. RetroMAD1 showed 99.67, 99.96 and 99.87% of viral reduction, respectively, at 24, 48 and 72h. Tamapal1 showed 98.75, 98.00 and 98.98% inhibition, respectively, at 24, 48 and 72h (Table 23 and Figure 12).

Time Peptides	24h	48h	72h
Amatilin	99.88	99.99	99.98
RetroGAD1	95.45	91.71	89.95
RetroMAD1	99.67	99.96	99.87
Tamapal1	98.75	98.00	98.98

Table 23: Percentage of viral reduction caused by Amatilin, RetroGAD1, RetroMAD1 and Tamapal1 in simultaneous treatment at 72h determined by PCR.

Example 15**Effects of temperature on the stability of Amatilin, RetroGAD1 and Tamapal1 evaluated via antiviral activity**

- 5 Amatilin, RetroGAD1 and Tamapal1 were placed in -20, 4, 26 and 37 °C for up to 30 days. The peptides were also placed in 50 °C for up to 7 days. To further investigate the thermostability of the peptides, they were also exposed to four set of various temperature fluctuations. Subsequently, the peptides were analyzed for their antiviral activity against HSV-2 via simultaneous treatment.

10

The antiviral activity of peptides (subjected to various temperatures) against HSV-2

- The antiviral activity of Amatilin, RetroGAD1 and Tamapal1 after incubation at different temperatures (-20, 4, 26, 37 and 50 °C) for 1, 7 and 30 days was evaluated by simultaneous treatment. For simultaneous treatment the mixture of the respective peptide and virus
- 15 inoculated onto Vero cells and incubated for 24, 48 and 72h at 37 °C under 5% CO₂ atmosphere. At the end of the time period the samples were harvested and viral DNA was extracted. The eluted DNA was then subjected to RT-PCR.

- The results obtained suggest that all the three peptides were thermal stable. The peptides exposed to various temperatures for 1, 7 and 30 days showed strong inhibitory activity against
- 20 HSV-2 via simultaneous treatment at the maximal non-toxic dose (MNTD) (Table 20 in Example 14). Amatilin was stable at high temperatures, 26 and 37 °C for up to 30 days giving 99.95 and 91.78% of inhibition, respectively. Amatilin was also stable at 50 °C for up to 7 days with 94.75% inhibitory activity (Table 24 and Figure 13A). RetroGAD1 exhibited 99.01 and 78.52% inhibitory activity, respectively, after incubation at 26 and 37 °C for up to 30 days. The peptide
- 25 showed 95.03% of viral reduction after incubation at 50 °C for up to 7 days (Table 24 and Figure 13B). Tamapal1 was stable for up to 30 days at 26 and 37 °C giving 88.12 and 91.78% inhibitory activity, respectively. The peptide remained stable for 7 days at 50 °C with 99.42% of viral reduction (Table 24 and Figure 13C).

Peptides Temperature (°C)	Amatilin			RetroGAD1			Tamapal1		
	Day 1	Day 7	Day 30	Day 1	Day 7	Day 30	Day 1	Day 7	Day 30
-20	99.84	98.00	99.98	95.93	98.94	98.96	98.73	94.77	91.01
4	89.35	99.98	99.92	99.66	98.92	98.30	95.84	92.92	90.87
26	94.53	99.75	99.95	99.77	96.24	99.01	98.49	92.92	88.12
37	98.55	91.45	91.78	95.54	95.61	78.52	99.45	99.16	91.78
50	94.31	94.95	-	97.12	95.03	-	91.36	99.42	-

- 30 Table 24: Percentage of viral reduction caused by Amatilin, RetroGAD1 and Tamapal1 incubated at different temperatures for 1, 7 and 30 days in simultaneous treatment determined by PCR.

EXAMPLE 16

Method of micronizing RetroMAD1 into a free-flowing powder by Supercritical Fluid Drying (SCFD)

A 10L high pressure vessel of the configuration conventionally used for Supercritical Fluid Drying (SCFD) was used to dry and produce a micronized form of powdered free-flowing RetroMAD1 under the conditions of 120bar; 37°C; 300kg/hr CO₂ flow that gave 88-89% yield in 2 cases and a lower 58% yield in one case due to operator error. The resulting powder was observed to be slightly cubic when viewed under Scanning Electron Microscope (SEM) and about 1 micron in size on the average. This confirms that RetroMAD1 may be efficiently manufactured as a powder for incorporation into tablets, capsules and animal feed pellets whether for terrestrial or aquatic application. The schematics of the process is shown in Figure 14 and an SEM picture showing the morphology of the RetroMAD1 crystals is shown in Figure 15.

Bioactivity of RetroMAD1 SCFD micronized powder evaluated using HSV-2

The bioactivity of the micronized form of powdered free-flowing RetroMAD1 produced using SCFD was tested via antiviral assay against HSV-2. For the antiviral test, RetroMAD1 micronized powder was dissolved in two different solvents: (i) ultra pure water with 5.5 mM NaOH; and (ii) ultra pure water. Ultra pure water was produced using a Sastec ST-WP-UVF machine.

Cytotoxicity of RetroMAD1 micronized powder

Prior to screening RetroMAD1 micronized powder for its antiviral properties, it was subjected to cytotoxicity assay in order to identify the maximal concentration, which could be non-toxic to Vero cells. The cytotoxic activity of the peptides was quantified using MTS-based cell titer 96 non-radioactive cell proliferation assay. Briefly, monolayer cultures of Vero cells were exposed to increasing concentrations of the dissolved RetroMAD1 powder for 24, 48 and 72h of incubation. After the incubation period, the maximal concentration of the protein that did not exert toxic effect is regarded as the maximal non toxic concentration (MNTD) was determined using MTS assay.

Results as shown in Table 25 indicate that the accepted maximal nontoxic concentrations of RetroMAD1 micronized powder on Vero cells were less than 20 µg/ml. At the chosen MNTD, the peptides did not impair the cell viability with respect to the untreated control group.

Peptide	MNTD, ug/ml		
	24h	48h	72h
RetroMAD1 powder (in water + NaOH)	15	15	15
RetroMAD1 powder (in water)	5	5	5

Table 25. Maximal non-toxic dose of RetroMAD1 micronized powder on Vero cells

The antiviral activity of RetroMAD1 micronized powder against HSV-2

The antiviral activity of RetroMAD1 micronized powder was evaluated by simultaneous treatment. For simultaneous treatment, the mixture of RetroMAD1 and virus were inoculated onto Vero cells in 24-well culture plates and incubated for 24 and 48h at 37 °C under 5% CO₂ atmosphere. At the end of the time period the samples were harvested and viral DNA was extracted. The eluted DNA was then subjected to RT-PCR.

The results obtained suggested that RetroMAD1 in powder form exhibited strong inhibitory activity against HSV-2 via simultaneous treatment giving between 85% -100% of inhibition. RetroMAD1 powder was dissolved in ultrapure water with NaOH showed higher percentage of viral reduction compared to the powder dissolved in ultrapure water alone at the MNTD (Table 26 and Figure 16). SCFD was therefore a viable method of producing RetroMAD1 in a solid dose format good for incorporation into tablets, capsules, medicated chewing gum and aquatic feed pellets.

RetroMAD1	Time	
	24h	48h
RetroMAD1 (in water + NaOH) - 10µg/mL	98.33	93.06
RetroMAD1 (in water + NaOH) - 15µg/mL	100.00	100.00
RetroMAD1 (in water) - 5 µg/mL	87.13	85.43

Table 26 Percentage of viral reduction caused by RetroMAD1 micronized powder in simultaneous determined by PCR.

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 ttgccgttta gccataaagt gtatgatatc ccgctgctgt atagcaccat tagcgatagc 300

52

FP6545_ST25.txt

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cgtcgtttta ttctgctgga tctgaccagc tatgcgtagt aaaccattag cgtggcgatt    360
gatgtgacca acgtgtatgt ggtggcgtag cgtacccgtg atgtgagcta ctttttcaaa    420
jaaagcccg c ggaagcgta caacattctg tttaaaggca cccgtaaaat taccctgccg    480
tataccggca actatgaaaa cctgcagacc gcggcgcata aaattcgtga aaacatcgat    540
ctgggcctgc cggccctgag cagcgcgatt accaccctgt tttattataa cgcgagagc    600
jcgccgagcg cgctgctggt gctgattcag accaccgcgg aagcggcgcg ttttaaatat    660
attgaacgcc acgtggcgaa atatgtggcg accaacttta aaccgaacct ggccattatt    720
agcctggaaa accagtggag cgccctgagc aaacaaat tttctggcca gaaccagggc    780
jgcaaatttc gtaatccggt ggatctgatt aaaccgaccg gcgaacgttt tcaggtgacc    840
aatgtggata gcgatgtggt gaaaggcaac attaaactgc tgctgaacag ccgtgcgagc    900
accgcggatg aaaactttat taccaccatg accctgctgg gcgaaagcgt ggtggaattc    960
tcgtgggcgc tgtggaaaac catgctgaaa gaactgggca ccatggcgct gcatgcgggt   1020
aaagcggcg c tgggtgcggc agcggatacc attagccagg gcacccaggt tccgggcgtg   1080
jgcgttccgg gcgttggtaa gcttgcggcc gcactcgagc accaccacca ccaccactga   1140

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:210> 3
:211> 5
:212> PRT
:213> Artificial Sequence

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:220>
:223> A polypeptide of the present invention

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:220>
:221> misc_feature
:222> (3)..(3)
:223> Xaa can be any naturally occurring amino acid
:400> 3

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Val Pro Xaa Val Gly
      5

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:210> 4
:211> 18
:212> PRT
:213> Artificial Sequence

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:220>
:223> A polypeptide of the present invention

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:400> 4

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Gly Arg Ile Cys Arg Cys Ile Cys Gly Arg Gly Ile Cys Arg Cys Ile
      5              10              15

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ys Gly

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53

FP6545_ST25.txt

<210> 5

<211> 268

<212> PRT

<213> Artificial Sequence

<220>

<223> A polypeptide of the present invention

<400> 5

Gly Ser Asp Val Asn Phe Asp Leu Ser Thr Ala Thr Ala Lys Thr Tyr
1 5 10 15

Thr Lys Phe Ile Glu Asp Phe Arg Ala Thr Leu Pro Phe Ser His Lys
20 25 30

Val Tyr Asp Ile Pro Leu Leu Tyr Ser Thr Ile Ser Asp Ser Arg Arg
35 40 45

Phe Ile Leu Leu Asp Leu Thr Ser Tyr Ala Tyr Glu Thr Ile Ser Val
50 55 60

Ala Ile Asp Val Thr Asn Val Tyr Val Val Ala Tyr Arg Thr Arg Asp
65 70 75 80

Val Ser Tyr Phe Phe Lys Glu Ser Pro Pro Glu Ala Tyr Asn Ile Leu
85 90 95

Phe Lys Gly Thr Arg Lys Ile Thr Leu Pro Tyr Thr Gly Asn Tyr Glu
100 105 110

Asn Leu Gln Thr Ala Ala His Lys Ile Arg Glu Asn Ile Asp Leu Gly
115 120 125

Leu Pro Ala Leu Ser Ser Ala Ile Thr Thr Leu Phe Tyr Tyr Asn Ala
130 135 140

Gln Ser Ala Pro Ser Ala Leu Leu Val Leu Ile Gln Thr Thr Ala Glu
145 150 155 160

Ala Ala Arg Phe Lys Tyr Ile Glu Arg His Val Ala Lys Tyr Val Ala
165 170 175

Thr Asn Phe Lys Pro Asn Leu Ala Ile Ile Ser Leu Glu Asn Gln Trp
180 185 190

Ser Ala Leu Ser Lys Gln Ile Phe Leu Ala Gln Asn Gln Gly Gly Lys
195 200 205

Phe Arg Asn Pro Val Asp Leu Ile Lys Pro Thr Gly Glu Arg Phe Gln
210 215 220

Val Thr Asn Val Asp Ser Asp Val Val Lys Gly Asn Ile Lys Leu Leu
225 230 235 240

54

FP6545_ST25.txt

Leu Asn Ser Arg Ala Ser Thr Ala Asp Glu Asn Phe Ile Thr Thr Met
 245 250 255

Thr Leu Leu Gly Glu Ser Val Val Glu Phe Pro Trp
 260 265

<210> 6
 <211> 34
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> A polypeptide of the present invention

<400> 6

Ala Leu Trp Lys Thr Met Leu Lys Glu Leu Gly Thr Met Ala Leu His
 L 5 10 15

Ala Gly Lys Ala Ala Leu Gly Ala Ala Ala Asp Thr Ile Ser Gln Gly
 20 25 30

Thr Gln

<210> 7
 <211> 18
 <212> PRT
 <213> Artificial Sequence

<220>
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 Defensin proteins

<400> 7

Gly Phe Cys Arg Cys Leu Cys Arg Arg Gly Val Cys Arg Cys Ile Cys
 5 10 15

Thr Arg

<210> 8
 <211> 18
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> A polypeptide sequence of naturally occurring and synthetic theta
 Defensin proteins

<400> 8

Arg Cys Leu Cys Arg Arg Gly Val Cys Arg Cys Leu Cys Arg Arg Gly
 5 10 15

Val Cys

55

FP6545_ST25.txt

<210> 9
 <211> 18
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> A polypeptide sequence of naturally occurring and synthetic theta
 Defensin proteins

<400> 9

Arg Cys Ile Cys Thr Arg Gly Phe Cys Arg Cys Ile Cys Thr Arg Gly
 1 5 10 15

Phe Cys

<210> 10
 <211> 18
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> A polypeptide sequence of naturally occurring and synthetic theta
 Defensin proteins

<400> 10

Gly Ile Cys Arg Cys Ile Cys Gly Arg Gly Ile Cys Arg Cys Ile Cys
 1 5 10 15

Gly Arg

<210> 11
 <211> 18
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> A polypeptide sequence of naturally occurring and synthetic theta
 Defensin proteins

<400> 11

Gly Ile Cys Arg Cys Ile Cys Gly Arg Gly Ile Cys Arg Cys Ile Cys
 1 5 10 15

Gly Arg

<210> 12
 <211> 18
 <212> PRT
 <213> Artificial Sequence

<220>
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 Defensin proteins

56

FP6545_ST25.txt

<400> 12

Arg Ile Cys Arg Cys Ile Cys Gly Arg Arg Ile Cys Arg Cys Ile Cys
 5 10 15

Ily Arg

<210> 13

<211> 18

<212> PRT

<213> Artificial Sequence

<220>

<223> A polypeptide sequence of naturally occurring and synthetic theta
Defensin proteins

<400> 13

Ily Ile Cys Arg Cys Ile Cys Gly Arg Gly Ile Cys Arg Cys Ile Cys
 5 10 15

Ily Arg

<210> 14

<211> 18

<212> PRT

<213> Artificial Sequence

<220>

<223> A polypeptide sequence of naturally occurring and synthetic theta
Defensin proteins

<400> 14

Ily Ile Cys Arg Cys Ile Cys Gly Lys Gly Ile Cys Arg Cys Ile Cys
 5 10 15

Ily Arg

<210> 15

<211> 18

<212> PRT

<213> Artificial Sequence

<220>

<223> A polypeptide sequence of naturally occurring and synthetic theta
Defensin proteins

<400> 15

Ily Ile Cys Arg Cys Tyr Cys Gly Arg Gly Ile Cys Arg Cys Ile Cys
 5 10 15

Ily Arg

<210> 16

57

FP6545_ST25.txt

<211> 18
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> A polypeptide sequence of naturally occurring and synthetic theta
 Defensin proteins

<400> 16

Ily Ile Cys Arg Cys Ile Cys Gly Arg Gly Ile Cys Arg Cys Tyr Cys
 L 5 10 15

Ily Arg

<210> 17
 <211> 18
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> A polypeptide sequence of naturally occurring and synthetic theta
 Defensin proteins

<400> 17

Ily Tyr Cys Arg Cys Ile Cys Gly Arg Gly Ile Cys Arg Cys Ile Cys
 5 10 15

Ily Arg

<210> 18
 <211> 18
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> A polypeptide sequence of naturally occurring and synthetic theta
 Defensin proteins

<400> 18

Ily Ile Cys Arg Cys Ile Cys Gly Arg Gly Tyr Cys Arg Cys Ile Cys
 5 10 15

Ily Arg

<210> 19
 <211> 18
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> A polypeptide sequence of naturally occurring and synthetic theta
 Defensin proteins

<400> 19

Ily Ile Cys Tyr Cys Ile Cys Gly Arg Gly Ile Cys Arg Cys Ile Cys

58

FP6545_ST25.txt

1

5

10

15

Gly Arg

<210> 20

<211> 18

<212> PRT

<213> Artificial Sequence

<220>

<223> A polypeptide sequence of naturally occurring and synthetic theta
Defensin proteins

<400> 20

Gly Ile Cys Ile Cys Ile Cys Gly Tyr Gly Ile Cys Arg Cys Ile Cys
1 5 10 15

Gly Arg

<210> 21

<211> 18

<212> PRT

<213> Artificial Sequence

<220>

<223> A polypeptide sequence of naturally occurring and synthetic theta
Defensin proteins

<400> 21

Gly Ile Cys Ile Cys Ile Cys Gly Arg Gly Ile Cys Tyr Cys Ile Cys
1 5 10 15

Gly Arg

<210> 22

<211> 18

<212> PRT

<213> Artificial Sequence

<220>

<223> A polypeptide sequence of naturally occurring and synthetic theta
Defensin proteins

<400> 22

Gly Ile Cys Ile Cys Ile Cys Gly Arg Gly Ile Cys Tyr Cys Ile Cys
1 5 10 15

Gly Arg

<210> 23

<211> 18

<212> PRT

<213> Artificial Sequence

59

FP6545_ST25.txt

:220>
 :223> A polypeptide sequence of naturally occurring and synthetic theta
 Defensin proteins

:400> 23

Arg Gly Cys Ile Cys Arg Cys Ile Gly Arg Gly Cys Ile Cys Arg Cys
 5 10 15

Ile Gly

:210> 24
 :211> 18
 :212> PRT
 :213> Artificial Sequence

:220>
 :223> A polypeptide sequence of naturally occurring and synthetic theta
 Defensin proteins

:400> 24

Arg Gly Cys Ile Cys Arg Cys Ile Gly Arg Gly Cys Ile Cys Arg Cys
 5 10 15

Ile Gly

:210> 25
 :211> 18
 :212> PRT
 :213> Artificial Sequence

:220>
 :223> A polypeptide sequence of naturally occurring and synthetic theta
 Defensin proteins

:400> 25

Ile Ile Cys Arg Cys Ile Cys Gly Arg Gly Ile Cys Arg Cys Ile Cys
 5 10 15

Ile Arg

:210> 26
 :211> 18
 :212> PRT
 :213> Artificial Sequence

:220>
 :223> A polypeptide sequence of naturally occurring and synthetic theta
 Defensin proteins

:400> 26

Ile Ile Cys Arg Cys Ile Cys Gly Lys Gly Ile Cys Arg Cys Tyr Cys
 5 10 15

60

FP6545_ST25.txt

Gly Arg

<210> 27
 <211> 4
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> An example of fusion proteins according to the present invention
 <400> 27

Gly Gly Gly Ser
L

<210> 28
 <211> 360
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> An example of fusion proteins according to the present invention
 <400> 28

Ser Phe Gly Leu Cys Arg Leu Arg Arg Gly Phe Cys Ala His Gly Arg
L 5 10 15Cys Arg Phe Pro Ser Ile Pro Ile Gly Arg Cys Ser Arg Phe Val Gln
20 25 30Cys Cys Arg Arg Val Trp Val Pro Gly Val Gly Val Pro Gly Val Gly
35 40 45Gly Ala Thr Gly Ser Asp Val Asn Phe Asp Leu Ser Thr Ala Thr Ala
50 55 60Lys Thr Tyr Thr Lys Phe Ile Glu Asp Phe Arg Ala Thr Leu Pro Phe
65 70 75 80Ser His Lys Val Tyr Asp Ile Pro Leu Leu Tyr Ser Thr Ile Ser Asp
85 90 95Ser Arg Arg Phe Ile Leu Leu Asn Leu Thr Ser Tyr Ala Tyr Glu Thr
100 105 110Ile Ser Val Ala Ile Asp Val Thr Asn Val Tyr Val Val Ala Tyr Arg
115 120 125Thr Arg Asp Val Ser Tyr Phe Phe Lys Glu Ser Pro Pro Glu Ala Tyr
130 135 140Asn Ile Leu Phe Lys Gly Thr Arg Lys Ile Thr Leu Pro Tyr Thr Gly
145 150 155 160

61

FP6545_ST25.txt

Asn Tyr Glu Asn Leu Gln Thr Ala Ala His Lys Ile Arg Glu Asn Ile
 165 170 175
 Asp Leu Gly Leu Pro Ala Leu Ser Ser Ala Ile Thr Thr Leu Phe Tyr
 180 185 190
 Tyr Asn Ala Gln Ser Ala Pro Ser Ala Leu Leu Val Leu Ile Gln Thr
 195 200 205
 Thr Ala Glu Ala Ala Arg Phe Lys Tyr Ile Glu Arg His Val Ala Lys
 210 215 220
 Tyr Val Ala Thr Asn Phe Lys Pro Asn Leu Ala Ile Ile Ser Leu Glu
 225 230 235 240
 Asn Gln Trp Ser Ala Leu Ser Lys Gln Ile Phe Leu Ala Gln Asn Gln
 245 250 255
 Gly Gly Lys Phe Arg Asn Pro Val Asp Leu Ile Lys Pro Thr Gly Glu
 260 265 270
 Arg Phe Gln Val Thr Asn Val Asp Ser Asp Val Val Lys Gly Asn Ile
 275 280 285
 Lys Leu Leu Leu Asn Ser Arg Ala Ser Thr Ala Asp Glu Asn Phe Ile
 290 295 300
 Thr Thr Met Thr Leu Leu Gly Glu Ser Val Val Asn Ser Cys Ala Ser
 305 310 315 320
 Arg Cys Lys Gly His Cys Arg Ala Arg Arg Cys Gly Tyr Tyr Val Ser
 325 330 335
 Val Leu Tyr Arg Gly Arg Cys Tyr Cys Lys Cys Leu Arg Cys Val Pro
 340 345 350
 Gly Val Gly Val Pro Gly Val Gly
 355 360

210> 29

211> 401

212> PRT

213> Artificial Sequence

220>

223> An example of fusion proteins according to the present invention

400> 29

eu Glu Lys Arg Lys Trp Lys Leu Phe Lys Lys Ile Glu Lys Val Gly
 5 10 15

In Arg Val Arg Asp Ala Val Ile Ser Ala Gly Pro Ala Val Ala Thr
 20 25 30

62

FP6545_ST25.txt

Val Ala Gln Ala Thr Ala Leu Ala Lys Asn Val Pro Gly Val Gly Val
 35 40 45
 Pro Gly Val Gly Gly Ala Thr Gly Ser Asp Val Ser Phe Arg Leu Ser
 50 55 60
 Gly Ala Thr Ser Lys Lys Lys Val Tyr Phe Ile Ser Asn Leu Arg Lys
 55 70 75 80
 Ala Leu Pro Asn Glu Lys Lys Leu Tyr Asp Ile Pro Leu Val Arg Ser
 85 90 95
 Ser Ser Gly Ser Lys Ala Thr Ala Tyr Thr Leu Asn Leu Ala Asn Pro
 100 105 110
 Ser Ala Ser Gln Tyr Ser Ser Phe Leu Asp Gln Ile Arg Asn Asn Val
 115 120 125
 Arg Asp Thr Ser Leu Ile Tyr Gly Gly Thr Asp Val Ala Val Ile Gly
 130 135 140
 Ala Pro Ser Thr Thr Asp Lys Phe Leu Arg Leu Asn Phe Gln Gly Pro
 145 150 155 160
 Arg Gly Thr Val Ser Leu Gly Leu Arg Arg Glu Asn Leu Tyr Val Val
 165 170 175
 Ala Tyr Leu Ala Met Asp Asn Ala Asn Val Asn Arg Ala Tyr Tyr Phe
 180 185 190
 Lys Asn Gln Ile Thr Ser Ala Glu Leu Thr Ala Leu Phe Pro Glu Val
 195 200 205
 Val Val Ala Asn Gln Lys Gln Leu Glu Tyr Gly Glu Asp Tyr Gln Ala
 210 215 220
 Ile Glu Lys Asn Ala Lys Ile Thr Thr Gly Asp Gln Ser Arg Lys Glu
 225 230 235 240
 Leu Gly Leu Gly Ile Asn Leu Leu Ile Thr Met Ile Asp Gly Val Asn
 245 250 255
 Lys Lys Val Arg Val Val Lys Asp Glu Ala Arg Phe Leu Leu Ile Ala
 260 265 270
 Ile Gln Met Thr Ala Glu Ala Ala Arg Phe Arg Tyr Ile Gln Asn Leu
 275 280 285
 Val Thr Lys Asn Phe Pro Asn Lys Phe Asp Ser Glu Asn Lys Val Ile
 290 295 300

63

FP6545_ST25.txt

Gln Phe Gln Val Ser Trp Ser Lys Ile Ser Thr Ala Ile Phe Gly Asp
305 310 315 320

Cys Lys Asn Gly Val Phe Asn Lys Asp Tyr Asp Phe Gly Phe Gly Lys
325 330 335

Val Arg Gln Ala Lys Asp Leu Gln Met Gly Leu Leu Lys Tyr Leu Gly
340 345 350

Arg Pro Lys Ser Ser Ser Ile Glu Ala Asn Ser Thr Asp Asp Thr Ala
355 360 365

Asp Val Leu Val Pro Gly Val Gly Val Pro Gly Val Gly Lys Thr Cys
370 375 380

Glu Asn Leu Ala Asp Thr Phe Arg Gly Pro Cys Phe Ala Thr Ser Asn
385 390 395 400

Cys

<210> 30
<211> 297
<212> PRT
<213> Artificial Sequence

<220>
<223> An example of fusion proteins according to the present invention
<400> 30

Met Gly Arg Ile Cys Arg Cys Ile Cys Gly Arg Gly Ile Cys Arg Cys
1 5 10 15

Ile Cys Gly Val Pro Gly Val Gly Val Pro Gly Val Gly Gly Ser Asp
20 25 30

Val Asn Phe Asp Leu Ser Thr Ala Thr Ala Lys Thr Tyr Thr Lys Phe
35 40 45

Ile Glu Asp Phe Arg Ala Thr Leu Pro Phe Ser His Lys Val Tyr Asp
50 55 60

Ile Pro Leu Leu Tyr Ser Thr Ile Ser Asp Ser Arg Arg Phe Ile Leu
65 70 75 80

Leu Asp Leu Thr Ser Tyr Ala Tyr Glu Thr Ile Ser Val Ala Ile Asp
85 90 95

Val Thr Asn Val Tyr Val Val Ala Tyr Arg Thr Arg Asp Val Ser Tyr
100 105 110

64

FP6545_ST25.txt

phe Phe Lys Glu Ser Pro Pro Glu Ala Tyr Asn Ile Leu Phe Lys Gly
 115 120 125
 thr Arg Lys Ile Thr Leu Pro Tyr Thr Gly Asn Tyr Glu Asn Leu Gln
 130 135 140
 thr Ala Ala His Lys Ile Arg Glu Asn Ile Asp Leu Gly Leu Pro Ala
 145 150 155 160
 Leu Ser Ser Ala Ile Thr Thr Leu Phe Tyr Tyr Asn Ala Gln Ser Ala
 165 170 175
 pro Ser Ala Leu Leu Val Leu Ile Gln Thr Thr Ala Glu Ala Ala Arg
 180 185 190
 phe Lys Tyr Ile Glu Arg His Val Ala Lys Tyr Val Ala Thr Asn Phe
 195 200 205
 Lys Pro Asn Leu Ala Ile Ile Ser Leu Glu Asn Gln Trp Ser Ala Leu
 210 215 220
 Ser Lys Gln Ile Phe Leu Ala Gln Asn Gln Gly Gly Lys Phe Arg Asn
 225 230 235 240
 pro Val Asp Leu Ile Lys Pro Thr Gly Glu Arg Phe Gln Val Thr Asn
 245 250 255
 Val Asp Ser Asp Val Val Lys Gly Asn Ile Lys Leu Leu Leu Asn Ser
 260 265 270
 Arg Ala Ser Thr Ala Asp Glu Asn Phe Ile Thr Thr Met Thr Leu Leu
 275 280 285
 Gly Glu Ser Val Val Glu Phe Pro Trp
 290 295

:210> 31
 :211> 297
 :212> PRT
 :213> Artificial Sequence
 :220>
 :223> An example of fusion proteins according to the present invention
 :400> 31

Met Gly Ser Asp Val Asn Phe Asp Leu Ser Thr Ala Thr Ala Lys Thr
 5 10 15
 Tyr Thr Lys Phe Ile Glu Asp Phe Arg Ala Thr Leu Pro Phe Ser His
 20 25 30
 Lys Val Tyr Asp Ile Pro Leu Leu Tyr Ser Thr Ile Ser Asp Ser Arg
 35 40 45

65

FP6545_ST25.txt

```

Arg Phe Ile Leu Leu Asp Leu Thr Ser Tyr Ala Tyr Glu Thr Ile Ser
 50                               55                               60

Val Ala Ile Asp Val Thr Asn Val Tyr Val Val Ala Tyr Arg Thr Arg
35                               70                               75                               80

Asp Val Ser Tyr Phe Phe Lys Glu Ser Pro Pro Glu Ala Tyr Asn Ile
                        85                               90                               95

Leu Phe Lys Gly Thr Arg Lys Ile Thr Leu Pro Tyr Thr Gly Asn Tyr
                100                               105                               110

Glu Asn Leu Gln Thr Ala Ala His Lys Ile Arg Glu Asn Ile Asp Leu
        115                               120                               125

Gly Leu Pro Ala Leu Ser Ser Ala Ile Thr Thr Leu Phe Tyr Tyr Asn
        130                               135                               140

Ala Gln Ser Ala Pro Ser Ala Leu Leu Val Leu Ile Gln Thr Thr Ala
145                               150                               155                               160

Glu Ala Ala Arg Phe Lys Tyr Ile Glu Arg His Val Ala Lys Tyr Val
        165                               170                               175

Ala Thr Asn Phe Lys Pro Asn Leu Ala Ile Ile Ser Leu Glu Asn Gln
        180                               185                               190

Trp Ser Ala Leu Ser Lys Gln Ile Phe Leu Ala Gln Asn Gln Gly Gly
        195                               200                               205

Lys Phe Arg Asn Pro Val Asp Leu Ile Lys Pro Thr Gly Glu Arg Phe
        210                               215                               220

Gln Val Thr Asn Val Asp Ser Asp Val Val Lys Gly Asn Ile Lys Leu
125                               230                               235                               240

Leu Leu Asn Ser Arg Ala Ser Thr Ala Asp Glu Asn Phe Ile Thr Thr
        245                               250                               255

Ile Thr Leu Leu Gly Glu Ser Val Val Glu Phe Pro Trp Val Pro Gly
        260                               265                               270

Val Gly Val Pro Gly Val Gly Gly Arg Ile Cys Arg Cys Ile Cys Gly
        275                               280                               285

Arg Gly Ile Cys Arg Cys Ile Cys Gly
        290                               295

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210> 32
211> 313

FP6545_ST25.txt

In Val Thr Asn Val Asp Ser Asp Val Val Lys Gly Asn Ile Lys Leu
25 230 235 240

67

FP6545_ST25.txt

Leu Leu Asn Ser Arg Ala Ser Thr Ala Asp Glu Asn Phe Ile Thr Thr
 245 250 255

Met Thr Leu Leu Gly Glu Ser Val Val Glu Phe Pro Trp Val Pro Gly
 260 265 270

Val Gly Val Pro Gly Val Gly Ala Leu Trp Lys Thr Met Leu Lys Glu
 275 280 285

Leu Gly Thr Met Ala Leu His Ala Gly Lys Ala Ala Leu Gly Ala Ala
 290 295 300

Ala Asp Thr Ile Ser Gln Gly Thr Gln
 305 310

<210> 33
 <211> 313
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> An example of fusion proteins according to the present invention

<400> 33

Met Ala Leu Trp Lys Thr Met Leu Lys Glu Leu Gly Thr Met Ala Leu
 5 10 15

His Ala Gly Lys Ala Ala Leu Gly Ala Ala Ala Asp Thr Ile Ser Gln
 20 25 30

Gly Thr Gln Val Pro Gly Val Gly Val Pro Gly Val Gly Gly Ser Asp
 35 40 45

Val Asn Phe Asp Leu Ser Thr Ala Thr Ala Lys Thr Tyr Thr Lys Phe
 50 55 60

Ile Glu Asp Phe Arg Ala Thr Leu Pro Phe Ser His Lys Val Tyr Asp
 65 70 75 80

Ile Pro Leu Leu Tyr Ser Thr Ile Ser Asp Ser Arg Arg Phe Ile Leu
 85 90 95

Leu Asp Leu Thr Ser Tyr Ala Tyr Glu Thr Ile Ser Val Ala Ile Asp
 100 105 110

Val Thr Asn Val Tyr Val Val Ala Tyr Arg Thr Arg Asp Val Ser Tyr
 115 120 125

He Phe Lys Glu Ser Pro Pro Glu Ala Tyr Asn Ile Leu Phe Lys Gly
 130 135 140

Thr Arg Lys Ile Thr Leu Pro Tyr Thr Gly Asn Tyr Glu Asn Leu Gln
 145 150 155 160

68

FP6545_ST25.txt

Thr Ala Ala His Lys Ile Arg Glu Asn Ile Asp Leu Gly Leu Pro Ala
 165 170 175
 Leu Ser Ser Ala Ile Thr Thr Leu Phe Tyr Tyr Asn Ala Gln Ser Ala
 180 185 190
 Pro Ser Ala Leu Leu Val Leu Ile Gln Thr Thr Ala Glu Ala Ala Arg
 195 200 205
 Phe Lys Tyr Ile Glu Arg His Val Ala Lys Tyr Val Ala Thr Asn Phe
 210 215 220
 Lys Pro Asn Leu Ala Ile Ile Ser Leu Glu Asn Gln Trp Ser Ala Leu
 225 230 235 240
 Ser Lys Gln Ile Phe Leu Ala Gln Asn Gln Gly Gly Lys Phe Arg Asn
 245 250 255
 Pro Val Asp Leu Ile Lys Pro Thr Gly Glu Arg Phe Gln Val Thr Asn
 260 265 270
 Val Asp Ser Asp Val Val Lys Gly Asn Ile Lys Leu Leu Leu Asn Ser
 275 280 285
 Arg Ala Ser Thr Ala Asp Glu Asn Phe Ile Thr Thr Met Thr Leu Leu
 290 295 300
 Gly Glu Ser Val Val Glu Phe Pro Trp
 305 310

<210> 34
 <211> 328
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> An example of fusion proteins according to the present invention

<400> 34

/a| Pro Gly Val Gly Val Pro Gly Val Gly Lys Trp Cys Phe Arg Val
 L 5 10 15

Cys Tyr Arg Gly Ile Cys Tyr Arg Arg Cys Arg Val Pro Gly Val Gly
 20 25 30

/a| Pro Gly Val Gly Gly Ala Thr Gly Ser Asp Val Asn Phe Asp Leu
 35 40 45

Ser Thr Ala Thr Ala Lys Thr Tyr Thr Lys Phe Ile Glu Asp Phe Arg
 50 55 60

69

FP6545_ST25.txt

Ala Thr Leu Pro Phe Ser His Lys Val Tyr Asp Ile Pro Leu Leu Tyr
 55 70 75 80

Ser Thr Ile Ser Asp Ser Arg Arg Phe Ile Leu Leu Asn Leu Thr Ser
 85 90 95

Tyr Ala Tyr Glu Thr Ile Ser Val Ala Ile Asp Val Thr Asn Val Tyr
 100 105 110

Val Val Ala Tyr Arg Thr Arg Asp Val Ser Tyr Phe Phe Lys Glu Ser
 115 120 125

Pro Pro Glu Ala Tyr Asn Ile Leu Phe Lys Gly Thr Arg Lys Ile Thr
 130 135 140

Leu Pro Tyr Thr Gly Asn Tyr Glu Asn Leu Gln Thr Ala Ala His Lys
 145 150 155 160

Ile Arg Glu Asn Ile Asp Leu Gly Leu Pro Ala Leu Ser Ser Ala Ile
 165 170 175

Thr Thr Leu Phe Tyr Tyr Asn Ala Gln Ser Ala Pro Ser Ala Leu Leu
 180 185 190

Val Leu Ile Gln Thr Thr Ala Glu Ala Ala Arg Phe Lys Tyr Ile Glu
 195 200 205

Arg His Val Ala Lys Tyr Val Ala Thr Asn Phe Lys Pro Asn Leu Ala
 210 215 220

Ile Ile Ser Leu Glu Asn Gln Trp Ser Ala Leu Ser Lys Gln Ile Phe
 225 230 235 240

Leu Ala Gln Asn Gln Gly Gly Lys Phe Arg Asn Pro Val Asp Leu Ile
 245 250 255

Lys Pro Thr Gly Glu Arg Phe Gln Val Thr Asn Val Asp Ser Asp Val
 260 265 270

Val Lys Gly Asn Ile Lys Leu Leu Leu Asn Ser Arg Ala Ser Thr Ala
 275 280 285

Asp Glu Asn Phe Ile Thr Thr Met Thr Leu Leu Gly Glu Ser Val Val
 290 295 300

Asn Val Pro Gly Val Gly Val Pro Gly Val Gly His Gly Val Ser Gly
 305 310 315 320

His Gly Gln His Gly Val His Gly
 325

70

FP6545_ST25.txt

<210> 35

<211> 336

<212> PRT

<213> Artificial Sequence

<220>

<223> An example of fusion proteins according to the present invention

<400> 35

Val Pro Gly Val Gly Val Pro Gly Val Gly Phe Leu Pro Leu Leu Ala
 5 10 15

Gly Leu Ala Ala Asn Phe Leu Pro Thr Ile Ile Cys Phe Ile Ser Tyr
 20 25 30

Lys Cys Val Pro Gly Val Gly Val Pro Gly Val Gly Gly Ala Thr Gly
 35 40 45

Ser Asp Val Asn Phe Asp Leu Ser Thr Ala Thr Ala Lys Thr Tyr Thr
 50 55 60

Lys Phe Ile Glu Asp Phe Arg Ala Thr Leu Pro Phe Ser His Lys Val
 65 70 75 80

Tyr Asp Ile Pro Leu Leu Tyr Ser Thr Ile Ser Asp Ser Arg Arg Phe
 85 90 95

Ile Leu Leu Asn Leu Thr Ser Tyr Ala Tyr Glu Thr Ile Ser Val Ala
 100 105 110

Ile Asp Val Thr Asn Val Tyr Val Val Ala Tyr Arg Thr Arg Asp Val
 115 120 125

Ser Tyr Phe Phe Lys Glu Ser Pro Pro Glu Ala Tyr Asn Ile Leu Phe
 130 135 140

Lys Gly Thr Arg Lys Ile Thr Leu Pro Tyr Thr Gly Asn Tyr Glu Asn
 145 150 155 160

Leu Gln Thr Ala Ala His Lys Ile Arg Glu Asn Ile Asp Leu Gly Leu
 165 170 175

Pro Ala Leu Ser Ser Ala Ile Thr Thr Leu Phe Tyr Tyr Asn Ala Gln
 180 185 190

Ser Ala Pro Ser Ala Leu Leu Val Leu Ile Gln Thr Thr Ala Glu Ala
 195 200 205

Ile Arg Phe Lys Tyr Ile Glu Arg His Val Ala Lys Tyr Val Ala Thr
 210 215 220

Asn Phe Lys Pro Asn Leu Ala Ile Ile Ser Leu Glu Asn Gln Trp Ser
 225 230 235 240

71

FP6545_ST25.txt

Ala Leu Ser Lys Gln Ile Phe Leu Ala Gln Asn Gln Gly Gly Lys Phe
 245 250 255

Arg Asn Pro Val Asp Leu Ile Lys Pro Thr Gly Glu Arg Phe Gln Val
 260 265 270

Thr Asn Val Asp Ser Asp Val Val Lys Gly Asn Ile Lys Leu Leu Leu
 275 280 285

Asn Ser Arg Ala Ser Thr Ala Asp Glu Asn Phe Ile Thr Thr Met Thr
 290 295 300

Leu Leu Gly Glu Ser Val Val Asn Val Pro Gly Val Gly Val Pro Gly
 305 310 315 320

Val Gly Lys Leu Ala Lys Leu Ala Lys Lys Leu Ala Lys Leu Ala Lys
 325 330 335

210> 36
 211> 325
 212> PRT
 213> Artificial Sequence

220>
 223> Polypeptide sequence of RetroGAD1

400> 36

Ty Ile Cys Arg Cys Ile Gly Arg Gly Ile Cys Arg Cys Ile Cys Gly
 5 10 15

Arg Val Pro Gly Val Gly Val Pro Gly Val Gly Gly Ala Thr Gly Ser
 20 25 30

Ty Leu Asp Thr Val Ser Phe Ser Thr Lys Gly Ala Thr Tyr Ile Thr
 35 40 45

Yr Val Asn Phe Leu Asn Glu Leu Arg Val Lys Leu Lys Pro Glu Gly
 50 55 60

sn Ser His Gly Ile Pro Leu Leu Arg Lys Lys Cys Asp Asp Pro Gly
 5 70 75 80

ys Cys Phe Val Leu Val Ala Leu Ser Asn Asp Asn Gly Gln Leu Ala
 85 90 95

Iu Ile Ala Ile Asp Val Thr Ser Val Tyr Val Val Gly Tyr Gln Val
 100 105 110

rg Asn Arg Ser Tyr Phe Phe Lys Asp Ala Pro Asp Ala Ala Tyr Glu
 115 120 125

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Gly Leu Phe Lys Asn Thr Ile Lys Thr Arg Leu His Phe Gly Gly Ser
 130 135 140
 Tyr Pro Ser Leu Glu Gly Glu Lys Ala Tyr Arg Glu Thr Thr Asp Leu
 145 150 155 160
 Gly Ile Glu Pro Leu Arg Ile Gly Ile Lys Lys Leu Asp Glu Asn Ala
 165 170 175
 Ile Asp Asn Tyr Lys Pro Thr Glu Ile Ala Ser Ser Leu Leu Val Val
 180 185 190
 Ile Gln Met Val Ser Glu Ala Ala Arg Phe Thr Phe Ile Glu Asn Gln
 195 200 205
 Ile Arg Asn Asn Phe Gln Gln Arg Ile Arg Pro Ala Asn Asn Thr Ile
 210 215 220
 Ser Leu Glu Asn Lys Trp Gly Lys Leu Ser Phe Gln Ile Arg Thr Ser
 225 230 235 240
 Gly Ala Asn Gly Met Phe Ser Glu Ala Val Glu Leu Glu Arg Ala Asn
 245 250 255
 Gly Lys Lys Tyr Tyr Val Thr Ala Val Asp Gln Val Lys Pro Lys Ile
 260 265 270
 Ala Leu Leu Lys Phe Val Asp Lys Asp Pro Lys Gly Leu Trp Ser Lys
 275 280 285
 Ile Lys Glu Ala Ala Lys Ala Ala Gly Lys Ala Ala Leu Asn Ala Val
 290 295 300
 Thr Gly Leu Val Asn Gln Gly Asp Gln Pro Ser Val Pro Gly Val Gly
 305 310 315 320
 Val Pro Gly Val Gly
 325

210> 37
 211> 1185
 212> DNA
 213> Artificial Sequence

220>
 223> Coding sequence of Amatilin

400> 37
 ggcagtgcg cggaaggccc atgaggccag ttaattaaga ggtaccgaat tctcattcgg 60
 ttgtgtaga ttgagaagag gtttctgtgc tcacggtaga tgtagattcc catccatccc 120
 atcggtaga tggtccagat tcgttcagtg ttgtagaaga gtttgggtcc caggtgttgg 180
 gttccaggt gttggaggtg ctactggttc tgatgttaac ttcgacttgt cactgctac 240

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tgctaagact tacactaagt tcatcgagga cttcagagct actttgccat tctcccacaa	300
ggtttacgac atccctttgt tgtactccac tatctccgac tccagaagat tcatcttggt	360
gaacttgact tcctacgctt acgagactat ctccgttgct atcgacgtta caaacgttta	420
cgttgttgct tacagaacta gagatgtttc ctacttcttc aaagagtccc caccagaggc	480
ttacaacatc ttgttcaagg gtactagaaa gatcactttg ccatacactg gtaactacga	540
gaacttgag actgctgctc acaagatcag agagaacatc gacttgggtt tgccagcttt	600
gtcctccgct atcactactt tgttctacta caacgctcag tccgctccat ccgctttgtt	660
ggttttgatc cagactactg ctgaggctgc tagattcaag tacatcgaga gacacgttgc	720
taagtacgtt gctacaaact tcaagccaaa cttggctatc atctccttgg agaaccagt	780
gtctgctttg tccaagcaga tcttcttggc tcaaaaccag ggtggttaagt tcagaaaccc	840
agtcgacttg atcaagccaa ccggtgagag attccagggt actaatgttg actccgacgt	900
tgtaaggggt aacatcaagt tgttgttgaa ctccagagct tccactgctg acgagaactt	960
catcactact atgactttgt tgggtgagtc cggtgttaac tcctgtgctt ccagatgtaa	1020
gggtcactgt agagctagaa gatgtgggta ctacgtttcc gttctgtaca gaggtagatg	1080
ttactgtaaa tgtttgagat gtgtccccgg tgttgagtc cctggtgtcg gtgcggccgc	1140
gagctcatgg cgcgcttagg ccttgacggc cttccgccaa ttcgc	1185

<210> 38
 <211> 1084
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> Coding sequence for RetroGAD1

<400> 38	
cggaattggcg gaaggccgct aaggccacgt gtcttgicca ggtaccgaat tcggaatctg	60
tagatgcacg tgcggtagag gtatctgcag atgtatttgt ggaagagtcc cagggtgttg	120
tggtccagggt gttggagggtg ctactgggtc tgggttgagc actgtttcat tctccactaa	180
gggtgctact tacatcactt acgttaactt tttgaacgag ttgagagtta agttgaagcc	240
agagggtaac tcccacggta tccctttgtt gagaaagaag tgtgacgacc caggtaagt	300
tttcgttttg gttgctttgt ccaacgacaa cggctcagttg gctgagattg ctatcgacgt	360
tacttccggt tacgttggtg gttaccagggt tagaaacaga tcctacttct tcaaggacgc	420
tccagacgct gcttacgaag gtttggtcaa gaacactatc aagactagat tgcacttcgg	480
tggttcctac ccatcttttg aagggtgagaa ggcttacaga gagactactg acttgggtat	540
cgagccattg agaatcggtt tcaagaagtt ggacgagAAC gctatcgaca actacaagcc	600
actgagatc gcttctctct tgttggttgt tatccagatg gtttccgagg ctgctagatt	660
tactttcatc gagaaccaga tcagaaacaa cttccagcag agaatcagac cagctaacaa	720
tactatttcc ttggagaaca agtggggtaa gttgtccttc cagatcagaa catccggtgc	780

74

FP6545_ST25.txt

taacggtatg ttctctgagg ctgttgagtt ggagagagct aacggtaaga agtactacgt	840
tactgctgtt gaccagggtta agccaaagat cgctttgttg aagttcgttg acaaggaccc	900
aaagggtttg tgggtccaaga tcaaagaggc tgctaaggct gctggtaagg ctgctttgaa	960
tgctgttact ggtttggtta accagggtga ccaaccatct gtccctggtg ttggagtccc	1020
tgggtgctggt gcggccgcga gctctggagc acaagactgg cctcatgggc cttccgctca	1080
ctgc	1084

<210> 39
 <211> 998
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> Coding sequence of Tampil 1

<400> 39	
ggatccgttc cgggtgtggg tgttccgggt gttggtaaata ggtgtttcgt gtttgttatc	60
gcgggtatttg ttatcgtcgt tgcgtgtgc caggcgttgg cgttccaggc gtgggtggtg	120
caaccggtag tgatgttaata ttgatctga gcaccgcaac cgcaaaaacc tataccaaat	180
ttatcgaaga ttttcgtgca accctgccgt ttagccataa agtttatgat attccgctgc	240
tgtatagcac cattagcgat agccgtcgtt ttattctgct gaatctgacc agctatgcct	300
atgaaaccat tagcgttgca attgatgtga ccaatgttta tgttgttgca tatcgtaccc	360
gtgatgtgag ctattttttc aaagaaagcc ctccggaagc ctataacatt ctgtttaaag	420
gcacccgcaa aatcaccctg ccgtataaccg gtaattatga aaatctgcag accgcagcac	480
ataaaattcg cgaaaatatt gatctgggtc tgcctgcact gagcagcgca attaccaccc	540
tgttttatta caatgcacag agcgcaccga gcgcactgct ggttctgatt cagaccaccg	600
cagaagcagc acgcttttaa tacattgaac gtcattgtgc caaatacgtg gccaccaact	660
ttaaaccgaa tctggcaatt attagcctgg aaaatcagtg gtcagcactg agcaaacaaa	720
tttttctggc acagaatcag ggtggcaaat ttcgtaatcc ggttgatctg attaaaccga	780
ccggtgaacg ttttcagggt accaatgttg atagtgtgtt ggtgaaaggc aacattaaac	840
tgtgctgaa tagccgtgca agcaccgcag atgaaaactt tattaccacc atgaccctgc	900
tgggtgaaag cgttgttaat gttcctggtg ttggcgtgcc tgggtgttgt catggtgtta	960
gcggtcatgg tcagcatggt gttcatgggt aaaagctt	998

<210> 40
 <211> 20
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> Sense Primer F1 of Example 12

<400> 40
 itggatttgg caacctaaca

20

75

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<210> 41
<211> 19
<212> DNA
<213> Artificial Sequence

<220>
<223> Sense primer F2 of Example 12

<400> 41
aattcgtgga gagaggtcc 19

<210> 42
<211> 20
<212> DNA
<213> Artificial Sequence

<220>
<223> Sense primer F3 of Example 12

<400> 42
atctctaccg tcacacagcc 20

<210> 43
<211> 23
<212> DNA
<213> Artificial Sequence

<220>
<223> Antisense primer R1 of Example 12

<400> 43
gaagatttta atgtccttgc tcg 23

CLAIMS

1. A method of producing at least one specific pathogen free (SPF) non-human animal, the method comprising:
- 5 (a) selecting a surviving animal in an environment comprising at least one pathogen that is capable of infecting and/or killing the animal;
- (b) administration of a fusion protein to the surviving animal wherein the fusion protein comprises at least one polypeptide B which is a Type 1 Ribosome Inactivating Protein (RIP) or fragment thereof; and
- 10 (i) at least one polypeptide A which is an Antimicrobial peptide; and/or
- (ii) at least one polypeptide C which is a Cationic AntiMicrobial Peptide (CAP) or fragment thereof; and
- (c) resulting surviving animal is the SPF non-human animal.
- 15 2. The method according to claim 1, further comprising a step of confirming that the surviving animal from step (a) expresses at least one marker of a pathogen resistant gene before the administration of the fusion protein of step (b).
3. The method according to either claim 1 or 2, wherein the surviving animal is at least one
- 20 animal selectively bred for growth.
4. The method according to any one of the preceding claims, wherein the presence of the SPF non-human animal in step (c) is confirmed using at least one of the methods selected from the group consisting of PCR, ELISA, LAMP and RT-PCR.
- 25 5. The method according to any one of the preceding claims, wherein the non-human animal is at least one aquatic animal.
6. The method according to claim 5, wherein the aquatic animal is at least one crustacean.
- 30 7. The method according to claim 5, wherein the aquatic animal is at least one prawn of the genus *Macrobrachium* or shrimp of the genus *Penaeus* spp.
8. The method according to claim 2, wherein the marker is selected from the group consisting of pmAV, c-type lectin, haemocyanin, beta-integrin, syntenin, alpha-2-macroglobulin, LPS-binding protein, beta-glucan binding protein, catalase gene, Ras-related nuclear protein, caspase-3 like gene, calreticulin, Rab GTPase gene, and Mg-SOD gene.
- 35

9. The method according to any one of the preceding claims, wherein the administration is by oral delivery.
10. The method according to any one of the preceding claims, wherein the fusion protein is administered with food.
11. The method according to any one of the preceding claims, wherein the SPF is free from at least one pathogen selected from the group consisting of Avian influenza viruses, Lymphoid Leukosis, Visceral Leukosis (Marek's Disease), Quail Bronchitis viruses, Newcastle disease viruses, infectious bronchitis viruses, infectious Bursal disease viruses, rhinoviruses, echoviruses, equine encephalitis viruses, coronaviruses, vesicular stomatitis viruses, rabies viruses, ebola viruses, parainfluenza viruses, Hanta viruses, bunga viruses, phleboviruses and Nairo viruses, hemorrhagic fever viruses, reoviruses, orbiviruses and rotaviruses, parvoviruses, papilloma viruses, polyoma viruses, adenoviruses, Aquabirnaviruses, Betanoda viruses, Salmonid alphaviruses, Epizotic Hematopoietic necrosis viruses, Infectious salmon anemia viruses (ISAV), Nervous necrosis viruses, Abalone Viral ganglioneuritis, Abalone Herpes-like viruses, variola viruses, vaccinia viruses, pox viruses, African swine fever virus, Iridovirus, Infectious Salmonid Anaemia (ISA), White Spot Syndrome Virus (WSSV), Hepatopancreatic parvo-like virus (HPV), Monodon Baculo virus (MBV), Infectious Hypodermal and Hematopoietic Necrosis Virus (IHHNV), Yellow Head Virus (YHV), Taura syndrome virus (TSV), Gill-associated virus (GAV), Laem-Singh Virus (LSNV), Infectious Myonecrosis Virus (IMNV), Mourilyan virus (MoV), Koi herpesvirus 1 (KHV 1), KHV2, KHV3, viral nervous necrosis (VNN), infectious pancreatic necrosis virus (IPNV), channel catfish virus (CCV), fish lymphocystis disease virus (FLDV), infectious hematopoietic necrosis virus (IHN) and viral hemorrhagic septicemia virus (VHSV), AMAV, swine hepatitis E virus, Circoviruses, Herpesviruses, Porcine cytomegalovirus, pseudorabies virus, Feline Panleukopenia virus (FPV), Feline herpesvirus, Feline calicivirus, Feline Leukemia Virus (FeLV), Feline Immunodeficiency Virus (FIV), Rabies virus, canine parvovirus, canine coronavirus, canine distemper virus, canine influenza, canine hepatitis virus, canine herpesvirus, a virus that causes pseudorabies, canine minute virus and a bacteriophage.
12. The method according to claim 11, wherein the bacteriophage is selected from a group consisting of Myoviridae, Siphoviridae, Podoviridae, Lipothrixviridae, Rudoviridae, Ampullaviridae, Bicaudaviridae, Clavaviridae, Corticoviridae, Cystoviridae, Fuselloviridae, Globuloviridae, Guttavirus, Inoviridae, Leviviridae, Microviridae, Plasmaviridae, Tectiviridae and the like. In particular, the phage may be Lambda phage (λ phage) – lysogen (λ phage), T2 phage, T4 phage, T7 phage, T12 phage, R17 phage, M13 phage, MS2 phage, G4 phage, P1 phage, Enterobacteria phage P2, P4 phage, Phi X 174 phage, N4 phage, Pseudomonas phage Φ 6, Φ 29 phage, 186 phage and the like.

13. The method according to any one of the preceding claims, wherein the polypeptide A is a defensin and selected from the group consisting of alpha, beta, theta, a member of the big defensins protein family, an analogue, and a fragment thereof.
- 5 14. The method according to any one of the preceding claims, wherein the fusion protein comprises the structure A-B-C, A-C-B, C-A-B, C-B-A, B-A-C, B-C-A, A-B-C-C, A-B, B-A, B-C, C-B, C-B-C, or C-C-B-C-C.
- 10 15. The method according to any one of the preceding claims, wherein the fusion protein comprises polypeptides A, B and C.
16. The method according to any one of the preceding claims further comprising at least one linker peptide between each of the polypeptides A, B and/or C.
- 15 17. The method according to claim 16, wherein the linker peptide has SEQ ID NO: 3 or 35.
18. The method according to any one of the preceding claims, wherein polypeptide A is a theta defensin selected from the group consisting of Rhesus minidefensin (RTD-1), RTD-2, RTD-3, Retrocyclin-1, Retrocyclin-2, Retrocyclin-3, synthetic retrocyclin congener RC100, RC101, 20 RC102, RC103, RC104, RC105, RC106, RC107, RC108, RC110, RC111, RC112, RC113 and RC114.
19. The method according to any one of claims 1-17, wherein polypeptide A is an alpha-defensin selected from the group consisting of human neutrophil protein 1 (HNP-1), HNP-2, 25 HNP-3, HNP-4, Human defensin 5 and Human defensin 6, an analogue, or a fragment thereof.
20. The method according to any one of claims 1-17, wherein polypeptide A is a beta-defensin selected from the group consisting of DEFB 1, DEFB 4A, DEFB 4B, DEFB 103A, DEFB 103B, DEFB 104A, DEFB 104B, DEFB 105A, DEFB 105B, DEFB 106A, DEFB 106B, DEFB 107A, 30 DEFB 107B, DEFB 108B, DEFB108 P1-4, DEFB 109 P1, DEFB 109 P1B, DEFB 109 P2-3, DEFB 110, DEFB 112-119 and DEFB 121-136.
21. The method according to any one of the preceding claims, wherein the Type 1 RIP (polypeptide B) is selected from the group consisting of Ebulitins, Nigritins, Amarandins, 35 Amaranthus antiviral/RIP, Amaranthin, Atriplex patens RIP, Beta vulgaris RIP, β -vulgin, Celosia cristata RIP, Chenopodium album RIP, CAP30B, Spinacea oleracea RIP, Quinqueginsin, Asparins, Agrostin, Dianthins, DAPs, Dianthus chinensis', Lychnin, Petroglauclin, Petrograndin, Saponaria ocymoides RIP, Vacuolas saporin, Saporins, Vaccaria hispanica RIP, Benincasins, Hispin, Byrodins, Colocins, Cucumis figarei RIP, Melonin, C.moschata RIP, Cucurmosin,

- Moschatins, Pepocin, Gynostemmin, Gynostemma pentaphyllum RIP, Gypsophilin, Lagenin, Luffaculin, Luffangulin, Luffin, MORs, Momordin II, Momorcharins, Momorcochin, Momorcochin-S, Sechiumin, Momorgrosvin, Trichoanguin, Kirilowin, α -trichosanthin, TAP-29, Trichokirin, Trichomislin, Trichosanthin, Karasurin, Trichomaglin, Trichobakin, Crotin, Euserratin Antiviral
- 5 Protein GAP-31, Gelonin, Hura crepitans RIP, Curcin, Jathropa curcas RIP, Mapalmin, Manutins, α -pisavin, Charibdin, Hyacinthus orientalis RIP, Musarmin, Iris hollandica RIP, Cleroendrum aculeatum RIP, CIPs), Crip-31, Bouganin, Bougainvillea spectabilis RIP, Bougainvillea x buttiana Antiviral protein 1 (BBAP1), Malic enzymes, MAP-S, pokeweed antiviral proteins (PAP), PD-SI, DP-S2, Dodecandrin, PIP, PIP2, Phytolacca octandra anti-viral proteins,
- 10 Hordeum vulgare RIP's, Hordeum vulgare sub sp. Vulgare Translational inhibitor II, Secale cereale RIP, Tritin, Zea diploperemis RIPs, Malus x domestica RIP, Momordica Anti-HIV Protein, Gelonium multiflorum, Mirabilis expansa 1, phage MU1, betavulgin (Bvg), curcin 2, saporin 6, Maize RIP (B-32), Tobacco RIP (TRIP), Beetins, Mirabilis antiviral protein (MAP), Trichosanthin (TCS), luffins, Momorcharins, Ocymoidin, Bryodin, Pepopsin, β -trichosanthin,
- 15 Camphorin, YLP, Insularin, Barley RIP, Tritins, Lamjarin, and Volvariella volvacea RIP.

22. The method according to any one of the preceding claims, wherein the CAP (polypeptide C) is selected from the group consisting of Cyclotides, Siamycins, NP-06, Gramicidin A, Circulins, Kalatas, Ginkbilobin, Alpha-Basrubin, Lunatusin, Sesquin, Tricyclon A, Cycloviolacins,
- 20 Polyphemusins, hfl-B5, Protegrins (Pig Cathelicidin), Rat Defensins, Human β -defensins, Temporins, Caerins, Ranatuerins, Reptile Defensin, Piscidins, Lactoferricin B, Rabbit Neutrophils, Rabbit α -Defensin, Retrocyclins, Human α -Defensins, Human β -defensin III (HBD3), Rhesus minidefensin (RTD-1, θ -defensin), rhesus θ -defensins, Human neutrophil peptides, Cecropin As, Melittin, EP5-1, Magainin 2s, hybrid (CE-MA), hepcidin TH1-5,
- 25 Epinecidin-1, Indolicidin, Cathelicidin-4, LL-37 Cathelicidin, Dermaseptins, Maximins, Brevinins, Ranatuerins, Esculentins, Maculatin 1.3, Maximin H5 and Piscidins, Mundtacin KS Enterocin CRL-35, Lunatusin, FK-13 (GI-20 is a derivative), Tachyplesins, Alpha-MSH, Antiviral protein Y3, Palustrin-3AR, Ponericin L2, Spinigerin, Melectin, Clavanin B, Cow cathelicidins, Guinea pig cathelicidin CAP11, Sakacin 5X, Plectasin, Fungal Defensin, GLK-19, lactoferrin (Lf) peptide 2,
- 30 Alloferon 1, Uperin 3.6, Dahlein 5.6, Ascaphin-8, Human Histatin 5, Guinea pig neutrophils, Mytilins, EP5-1, Hexapeptide (synthetic) Corticostatin IV Rabbit Neutrophil 2, Aureins, Latacin, Plectasin, Cycloviolins, Varv Peptide E, Palicourein, VHL-1, and Buforins.

23. The method according to any one of the preceding claims, wherein the Type 1 RIP is
- 35 MAP30, the CAP is Dermaseptin 1 and the polypeptide A is Retrocyclin 101.

24. The method according to claim 23, wherein the fusion protein comprises the amino acid sequence SEQ ID NO:1.

25. The method according to any one of claims 1-22, wherein the Type 1 RIP is MAP30, the CAP is Alloferon1 and the polypeptide A is Tachyplesin.

5 26. The method according to claim 25, wherein the fusion protein comprises the amino acid sequence SEQ ID NO:34.

27. The method according to any one of claims 1-22, wherein the Type 1 RIP is MAP30, the CAP is Mytilin C10C and the polypeptide A is AVBD103.

10 28. The method according to claim 27, wherein the fusion protein comprises the amino acid sequence SEQ ID NO:28.

29. The method according to any one of claims 1-22, wherein the Type 1 RIP is GAP31, the CAP Dermaseptin1 and the polypeptide A Retrocyclin 101.

15 30. The method according to claim 29, wherein the fusion protein comprises the amino acid sequence SEQ ID NO:36.

20 31. A method of producing at least one specific pathogen resistant (SPR) non-human animal, the method comprising:

(a) producing a specific pathogen free animal according to any one of claims 1-30,

(b) selective breeding of a male and female SPF non-human animal to produce a SPR non-human animal offspring.

25

32. A specific pathogen free non-human animal according to any one of claims 1-30.

33. A specific pathogen resistant non-human animal according to claim 31.