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(54) Title: A NOVEL ANTIVIRAL PEPTIDE AGAINST AVIAN INFLUENZA VIRUS H9N2

(57) Abstract: The present invention relates to recombinant phages carrying fusion peptides that bind to avian influenza virus (AIV). Such phages are useful as diagnostic reagents to replace anti-AIV antibodies because the phages are capable of competing with the latter antibodies for binding sites on the virus. Synthetic peptides with the sequence CNDFRSKTC, either in linear or cyclic conformations, or fusion phages bearing the above said peptides inhibited AIV propagation in embryonated egg as well as in MDCK cell lines. Therefore they may be used as'therapeutic agents to control, to treat and to eradicate bird flu caused by avian influenza virus.

A NOVEL ANTIVIRAL PEPTIDE AGAINST AVIAN INFLUENZA VIRUS H9N2

1.0 FIELD OF INVENTION

The present invention relates to a novel antiviral peptide and a fusion phage which acts against Avian Influenza Virus (AIV) subtype H9N2. More specifically, the fusion phage displays the sequence CNDFRSKTC on its surface protein P3 which binds to AIV H9N2 with an IC_{50} value of less 10^{13} pfu/100 µl. Synthetic peptides with amino acid sequence CNDFRSKTC, either in linear or cyclic conformations, inhibited the propagation of AIV H9N2 with an IC_{50} value less than about 100 µM.

2.0 BACKGROUND OF INVENTION

Avian influenza virus (AIV) belongs to the family of *Orthomyxoviridae* which contains two genera, influenza A & B and influenza C (Lamb and Krug, 1996). These viruses are the major cause of morbidity and mortality among poultries in the world. It is the causative agent of the most dangerous disease, called bird flu in common terms (Webster *et* al.,, 1992). Although these viruses do not infect humans, several instances of human infections and outbreaks have been reported (CDC, 2008; Normile, 2004; Parry, 2004). Influenza A viruses are enveloped with lipid bilayer and contain eight single-stranded, segmented, negative sense RNAs. There are two glycoproteins present on the surface of the virions namely, haemagglutinin (HA) and neurammidase (NA), and one ion channel protein (M2). The glycoproteins are the major antigenic determinants of influenza viruses. The HA protein initiates the first step in the viral infection, which involves the attachment of viruses to the host cell surface sialic acid receptors (Lamb and Krug, 1996). The NA protein participates in the release of mature virions from the host cells (Palese *ef al.,* 1974). Therefore, in order to study the virus - host interaction and also to identify molecules that inhibit this process a bacteriophage displaying a specific peptide sequence was selected by its affinity to avian influenza virus strain H9N2 using a phage display library.

The preferred primary strategy for the prevention of influenza virus infection is annual vaccination among susceptible population. But the antiviral drugs play an important role in a comprehensive approach to control the illness and transmission (Hayden, 2006). There are two classes of antiviral drugs that have been approved for the treatment and prophylaxis. They are adamantane derivatives (amantadine and rimantadine) and neuraminidase inhibitors (NAIs; zanarnivir and oseltarnivir) (Nicholson *et* al., 2003). These adamantane derivatives act by binding and blocking the function of influenza A virus M2 ion channel protein and thereby prevents the viral replication inside the host cell (Wang *et* al., 1993)). Due to single point mutations in M2 proteins, adamantane resistant strains have emerged (Hay *et* al., 1986). These resistant viruses are typically fully pathogenic and transmissible (Hayden, 2006). The NAN inhibit the enzymatic activity of the neuraminidase protein and prevents viral release from the infected host cell. But NAI resistant strains have also emerged due to the mutations in the active site of the NA (Nicholson *et* al., 2003). The increasing resistance by the influenza A viruses against the both types of drugs highlights our necessity to identify novel drugs.

Traditionally, compounds from natural products obtained from plants, marine organisms, fungi or other microorganisms are used to identify antimicrobial or antiviral agents. Recently, combinatorial peptide libraries like phage display library are increasingly being used to identify peptide compounds for the same purpose (Doorbar *et* al., 1994). The broad structural diversity of peptides displayed on bacteriophages has made the phage display library an important tool to study proteinprotein interactions, especially in the identification of specific ligands that interact with a particular target (Devlin *et* al.,1990). Compounds that interact with target molecules are selected from phage libraries and screening processes are then used to identify lead compounds that have functional effects on the target. These lead compounds are then optimized for their activity and then the candidate drugs enter into clinical trials.

We used a cyclic peptide phage display library to identify peptide molecule that interacts with the influenza A virus H9N2 and proved its

antiviral property in *vitro* and in *ovo*.

3.0 SUMMARY OF THE INVENTION

Accordingly it is an object of the present invention to provide for novel peptides and fusion phages that has antiviral activity against avian influenza virus A H9N2.

It is another object of the present invention to provide for a pharmaceutical composition containing a recombinant phage or a synthetic peptide according to the invention.

It is yet another object of the present invention to provide a diagnostic reagent containing a recombinant phage or a synthetic peptide according to the invention.

These objectives are accomplished by,

An isolated and purified recombinant phage bearing a fusion peptide or synthetic peptides derived from the fusion phage that binds to avian influenza virus A H9N2 and inhibits the propagation of the virus.

To identify a novel antiviral molecule, a population of recombinant phages displaying random disulfide constrained heptapeptide sequences was screened against the virus. Then the peptide displayed on the surface of the fusion phage was synthesized chemically. The isolated peptide has the sequence of NDFRSKT with an IC50 value of less than 100 ~M The specificity of the recombinant phage against the influenza virus A H9N2 was proved by an antibody - phage competition assay, in which the phages were able to compete with polyclonal antibodies for binding site on the viral surface proteins.

In the present invention, synthetic peptides based upon one of the isolated sequences, either in linear or cyclic conformation, such as the synthetic peptide CNDFRSKTC, are able to inhibit the propagation of NDV, thereby preventing disease and spread of infection.

4.0 BRIEF DESCRIPTION OF THE FIGURES AND TABLE

Table I shows the heptapeptide sequences obtained from three rounds of biopanning against AIV H9N2.

Table 2 shows haernagglutination inhibition activities of synthetic peptides and fusion phages.

Figure I shows the results of the competition between recombinant phages bearing specific heptapeptide sequence and polyclonal antibodies for AIV H9N2.

Figure 2 shows the inhibition of AIV propagation with synthetic peptides in *ovo*.

Figure 3 shows the inhibition of AIV propagation with fusion phages *in ovo*.

Figure 4 shows the inhibition of AIV propagation with fusion phages *in vitro*.

Figure 5 shows the effect of fusion phage on viral replication *in vitro*.

5.0 DETAILED DESCRIPTION OF THE TABLES AND FIGURES

Table I shows the heptapeptide sequences obtained from four rounds of biopanning against AIV. After 4 rounds of selection and amplification 20, 35 and 35 individual clones from the 2^{nd}, 3^{rd} and 4^{th} rounds, respectively, were sequenced.

Table 2 shows the inhibitory ability of the cyclic and linear peptides against the haernaggluti nation activity of the avian influenza virus H9N2. Experiments were performed in triplicates. + in the presence of, - in the absence of.

Figure I shows the results of the competition between recombinant phages bearing specific heptapeptide sequence and polyclonal antibodies for AIV. The polyclonal antibodies (pAb) inhibited the association of the fusion phage with AIV, suggesting that the phages may share some common binding sites with the antibody. Experiments were done in triplicates and the error bars represent the standard deviation of the mean. Series I - Fusion phage; Series 2 - Fusion phage with polyclonal Antibodies.

Figure 2 shows the results of determination of the IC50 values of the synthetic peptides in ovo against AIV H9N2 propagation. Peptide concentration needed to inhibit 50% of the virus growth was determined using different concentrations of peptides. Experiments were done in triplicates and the error bars represent the standard deviation of the mean.

Figure 3 shows the results Of IC_{50} values of fusion phage FP-P1 against AIV H9N2 virus propagation *in ovo*. Experiments were done in triplicates and the error bars represent the standard deviation of the mean. Series I - Fusion phage FP-Pl; Series 2 - Wild phage as control.

Figure 4 shows the results of antiviral activity of the fusion phage FP-P1 *in vitro*. Experiments were done in triplicates and the error bars represent the standard deviation of the mean. Series I - FP-P 1; Series 2 - Wild Phage M 13 as control.

Figure 5 shows the results of the effect of the fusion phage FP-P1 on viral replication. MDCK cells were inoculated with untreated (0 value) or FP-P I treated virus, and viral titers were determined on supernatants 72 hpi. Series I - FP-Pl; Series 2 - Wild phage M 13 as control.

6.0 DETAILED DESCRIPTION OF THE INVENTION

The description described herein will be more fully understood with the following detailed description which is divided into different sections. Materials and methods employed in this invention are set forth in Examples. Examples I to 5 are the conventional materials and methods which are prerequisites of the invention.

Principles of phage display technology

In phage display technology, random peptides are usually displayed on the surface of bacteriophage molecules as fusion protein by inserting synthetic oligonucleotides in the gene encoding the coat proteins. A collection of these recombinant phages are known as phage display library, allows the screening of vast numbers of peptide sequences against a target by an in *vitro* selection procedure known as biopanning (Parmley *et al.*, 1988 and Smith *et al.*, 1993). In the present invention, the target used was whole AIV H9N2 virus particles and the library employed was the disulfide constrained library obtained from

New England Biolabs, Inc. In this particular library, the displayed peptide molecules are flanked by a cysteine residue to the gpIII protein of phage M13. The cysteine residues help the peptides to attain a fixed cyclic shape, in the absence of reducing agents. This disulfide constrained library useful for targets whose interacting components (native ligands) have discontinuous binding sites (conformational epitope) (Hoess *et al.,* 1994); in which amino acids are brought from different positions in a polypeptide to form an essential contact area. Besides, the disulfide constrained cyclic peptide library is more useful in selecting high affinity ligands rather than a linear peptide library (O'Neil *et aL,,* 1992; Gho *et al.,* 1997).

Affinity selection of peptides that bind to AIV

The avian influenza virus contains two surface glycoproteins namely haernagglutinin (HA) and neuraminidase (NA) which is responsible for virus entry and exit into and from the host cell. We therefore decided to employ the disulfide constrained library against the whole virus particles to select for conformational ligands which bind the surface proteins. Figure I summarizes the major steps involved in the selection of these ligands. First, the whole virus particles were directly attached to the surface of a high binding microtiter plate well. The library was then added into the well to allow the recombinant phage particles to bind with the virus particles. The unbound phages were washed out and the bound phages were eluted at low pH. The eluted phages were then amplified in the bacterium, *Escherichia coli* and the amplified phages were used in second round of biopanning. This procedure was repeated for four times. After four successive rounds of affinity selection and amplification, a subset of the selected phages was grown up individually and tile identity of the peptides that bind to core particles were obtained by sequencing tile gpIII gene carrying the insertion.

In order to select high affinity binding phages, the stringency of selection was increased by (i) performing the biopanning at room temperature (28'C), (1i) shortening the time of binding to I h to select for ligands with rapid on rates (Ko,), (iii) washing the wells thoroughly (10 times) to remove low affinity binding phages, and (iv) repeating 4 rounds of biopanning to enrich high affinity binding clones. 47% of phages analysed from the fourth round

panning carried the fusion peptide sequence NDFRSKT, 10.5% containing QHSTKWF motif followed by LPYAAKH and ILGDKVG, 5% each and other unrelated sequences (Table 1). Streptavidin target was used for positive control which gave a consensus motif of HPQ sequence in all clones, which in good agreement with that reported by Devlin et al. (1990). No recognizable consensus sequence was observed with bovine serum albumin (BSA), which was used as negative control.

As used herein, the term "fusion peptide" refers to amino acid sequence genetically encoded by a bacteriophage and physically linked to a coat protein of the phage. The claimed fusion peptides contain amino acid sequences NDFRSKT, but it is not limited to: (i) amino acid sequence which is shorter or longer than the claimed amino acid sequences; (ii) variations in the amino acid sequences, particularly amino acid substitutions within the same category as described below; (iii) amino acid sequences sharing at least 60% homology with those of the claimed fusion peptides and; (iv) either linear or constrained conformation.

AIV possesses two surface glycoproteins, HA and NA, which protrude from the viral lipid bilayer membrane. These glycoproteins are essential for the entry and release of viruses into and outside of the host cells respectively. Since the phages bearing the sequences NDFRSKT bound to the virion in solution, these sequences may, to a certain extent, resemble a region on the host cell receptor that interacts with the intact virion.

Phages compete with antibody for binding sites on AIV

The surface glycoproteins HA and NA are two important proteins that elicit antibody production in the host. In a phage - antibody competition assay, the phages were able to bind to one of the mimotopes and therefore inhibit the binding of antibodies raised against the virion. Figure I shows that the recombinant phages displaying the peptide NDFRSKT, selected from biopanning experiment, were able to compete with the antibodies for the binding site on AIV. In the presence of the antibody, the number of phages bound to the virus coated wells reduced dramatically as a result of competition between these two molecules for the same binding site on the virus. Thus the recombinant phage molecules may serve as a potential diagnostic agent for the

AIV infection.

Antiviral activity of synthetic peptides and fusion phages in ovo

AIV can easily be propagated in the allantoic fluid of embryonated chicken eggs. Therefore, the virus and the propagation system provide a simple model to assess directly the antiviral activities of a particular compound or peptide in vivo. The amount of virus present in the allantoic fluids, before and after the antiviral compounds treatment, is determined by haernagglutination titer. Injection of the claimed peptide either in cyclic or linear form and the fusion phage displaying the peptide reduced the AIV titer in the allantoic fluid (Figures 2 and 3). The cyclic peptide showed higher IC_{50} value at a peptide concentration lower than 100 μM to the linear peptide. Furthermore, cyclization can avoid the digestion of the peptides by exopeptidases. Thus the synthetic peptides claimed herein can be used as therapeutic agents to treat, control and also to eradicate the disease.

Antiviral activity of fusion phages in vitro

MDCK cell lines are effective medium for the propagation of avian influenza viruses in *vitro*. The in *vitro* antiviral property of the fusion phages against the virus multiplication were checked in these cell lines. The inhibition of viral replication was indirectly checked by the cell viability, before and after the treatment with the fusion phage molecules. The cell viability increases nearly 2 fold after the infection with phage treated viruses to the untreated viruses (Figure 4). The reduction in viral titer was further confirmed by haemagglutination test of the supernatants (Figure 5). This experiment proved the antiviral property of the fusion phage molecules in *vitro*.

EXAMPLES

Example 1:

Propagation and purification of AIV: The propagation and purification methods were adapted and modified from Blaskovic and Styk (1967) and Yusoff *et al.* (1996), respectively. AIV subtype H9N2 was injected

into a 9 days old specific pathogen free embryonated chicken eggs. After 3 days of incubation at 37°C, the allantoic fluid was harvested and clarified by centrifugation (30 min, 35,000 x g, 4°C. The virus was purified from the clarified supernatants by 30%-60% sucrose gradient ultracentrifugation (3.5 h, 285,000 x g, 4°C and was used for the studies below.

Example 2:

Biopanning of purified AIV: The protein concentration of virus was measured using the Bradford assay (1976). AIV (15 µg/ml) was coated onto a microtiter plate well [$Na_2CO_3/NaHCO_3$ buffer (0.1 M, pH 9.6)]. Streptavidin and BSA were used as positive and negative controls respectively. The experiment was carried out at room temperature (about 25°C). Phages from a disulfide constrained *7-mer* phage display library (New England Biolabs, USA) were diluted to I X 10^{11} pfu in TBS [50 mM Tris-HCI (pH 7.5), 150 mM NaCl; 110 µl] and added into each coated well. The mixtures were incubated for 30 min. The wells were washed with TBST JBS + 0.1% v/v Tween-20). Bound phages were eluted with glycine-HCI [0. 1 M, (pH 2.2); 100 µl] by rocking gently for 7-8 min and neutralized with Tris-HCI [I M, (pH 9); 115 µl. The output pfu was determined by titrating the eluate (10 µl) while the remainder eluate was amplified in E. coli stain ER2738 (F' Iacl^q *Δ(IacZ)M]5proA$^+$B$^+$ zzf::TnIo(Tet^R)/fhuA2supE thi Δ(lac-proAB) Δ(hsdMS-mcrB)5* (r_K-m_K-McrBC-)} at 37°C, shaking for 5 hr. The panning process was repeated for a further 3 rounds.

Example 3:

Phage titration: Phage titration method was adapted from Sambrook *et al.* (1989).

Example 4:

Sequencing of the selected phages: Single plaques from all 4 rounds of panning were picked from (Luria Broth) LB plates (used in output titration) and grown in LB media. Single stranded DNA of the phages was extracted as described by Sambrook *et al*, (1989). Sequencing of inserts was done using the ABI automated sequencer by First Base Laboratories Sdn Bhd,

Kuala lumpur.

Example 5:

Antibody competition assay: Wells were coated with AIV (15 μg/ml) and polyclonal antibodies (1:500 dilution; 100 μl) raised against AIV were added with a series of different phage concentrations (10^9 - 10^{13} pfu; 100 μl). The mixtures were incubated for I hour and washed 6 X with Tris-buffered saline - Tween (TBST). Bound phages were eluted and titered as described above.

Example 6:

Determination Of IC_{50} of peptides and fusion phages in the inhibition of AIV propagation in *ovo*: Nine-day-old embryonated chicken eggs were inoculated with 100 μl of AIV (8 HA units) and an equal volume of different concentrations of peptide (0. 1 μM - I mM) or fusion phages (10^8 - 10^{13} pfu). The eggs were incubated at 37°C. Three eggs were used for each peptide concentration. For the positive control, only AIV was inoculated and for the negative control, the virus was substituted by peptides or phages. Peptides (100 μl) were injected into appropriate eggs daily for another 3 days. A day after the 4^{th} injection, the eggs were chilled overnight and the allantoic fluid was harvested. The haemagglutination activities of the allantoic fluid samples were then determined according to Grace *et al*, (1973).

Example 7:

Determination Of IC_{50} of fusion phages in the inhibition of AIV propagation in *vitro*: MDCK cells were inoculated with medium alone or phage treated (10^8 - 10^{13} pfu / 100 μl) or non-treated virus (multiplicity of infection [MoI] 0.05) for I hr at 37°C. Following adsorption, monolayers were washed and incubated in EMEM containing 5% fetal bovine serum (FBS). Cytopathic effect was monitored by light microscopy and quantitated by XTT Cell Viability Assay Kit (Biotium, USA). To assess viral replication, MDCK cells

were inoculated with phage-treated or untreated virus at an MOI of 0.05, supernatants were collected at 72 hpi, and viral titers were determined by HA titer. The IC_{50} value was estimated by interpolation of the dose-response curve.

Although the invention has been described above with respect to various presently preferred embodiments, it will be apparent to one of ordinary skill in the art that many variations and modifications may be made. Therefore, the invention is not to be understood as limited to the particular embodiments presented herein but, rather, is to be understood as embracing all such variations and modifications which fall within the scope of the claims appended hereto.

All references disclosed herein are incorporated by reference in their entirety.

Table 1

Rounds of panning	Heptapeptide sequences	Frequency of sequences (%)
2nd round	Unrelated sequences	100
3rd round	LPYAAKH/LPYGSKH	25
	ILGYKVG	17
	Unrelated sequences	58
4th round	**NDFRSKT**	47
	QHSTKWF	10.5
	LPYAAKH	5
	ILGDKVG	5
	Unrelated sequences	23
3rd round	**HPQ**FLSL	55
Streptavidin	GLYN**HPQ**	27
	Unrelated sequences	18

Table 2

Peptide concentration (mM; 50 µl) / Phage Particles	Agglutination of RBC (+ AIV 32 HAU; 50 µl)	Agglutination of RBC (- AIV 32 HAU; 50 µl)
Cyclic 1.0	No	No
Cyclic 0.1	No	No
Cyclic 0.01	Yes	No
Cyclic 0	Yes	No
Linear 1.0	No	No
Linear 0.1	No	No
Linear 0.01	Yes	No
Linear 0	Yes	No
Phage (10^{13} pfu / 100 µl)	No	No
Phage (10^{12} pfu / 100 µl)	Yes	No

WHAT IS CLAIMED IS:

1. An isolated and purified recombinant phage bearing a fusion peptide that binds to Avian influenza virus and inhibits the propagation of the virus.

2. A recombinant phage as claimed in claim I wherein the fusion peptide is amino acid of sequence NDFRSKT and other amino acid sequences related to them.

3. A synthetic peptide containing the amino acid sequence derived from the fusion peptide as claimed in claim I or 2.

4. The synthetic peptide of claim 3 which inhibits the propagation of AIV by binding to the virus.

5. The synthetic peptide of claim 3 wherein said peptide has the sequence CNDFRSKTC or sequences related to it.

6. The synthetic peptide of claim 3 wherein said peptide has an IC_{50} value less than 100 μm.

7. A pharmaceutical composition containing a recombinant phage as claimed in any one of claims I and 2.

8. A pharmaceutical composition containing a synthetic peptide as claimed in any one of claims 3 to 6.

9. A diagnostic reagent containing a recombinant phage as claimed in any one of claims I and 2.

10. A diagnostic reagent containing a synthetic peptide as claimed in any one claims 3 to 6.

11. A vaccine containing a recombinant phage as claimed in any one of claims I and 2.

12. A vaccine containing a synthetic peptide as claimed in any one of claims 3 to 6.

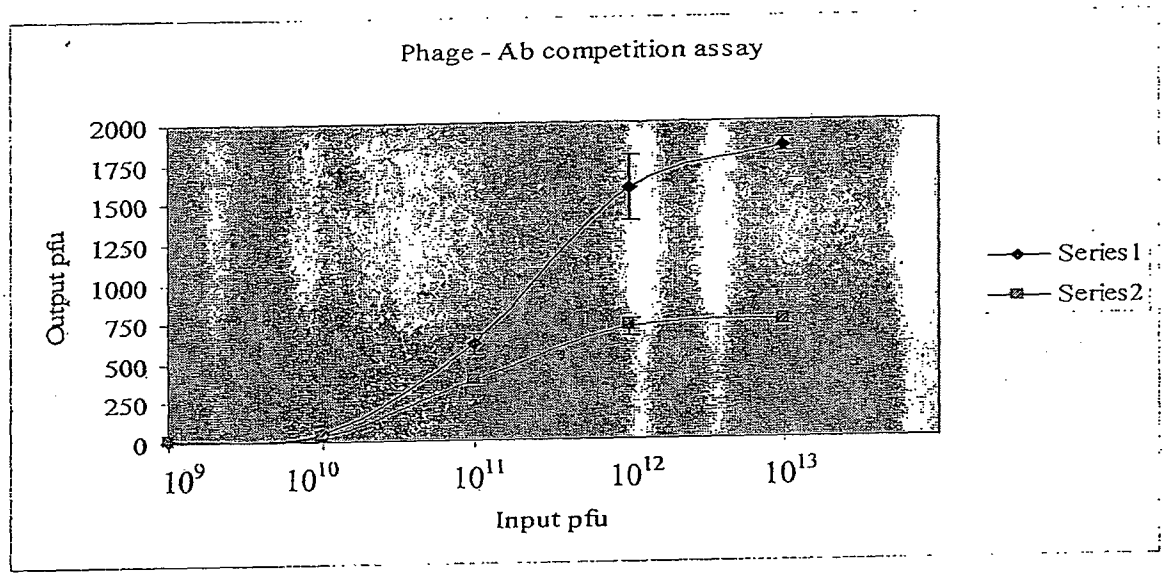
13. A synthetic peptide as claimed in any one of claims 3 to 6, which binds to the avian influenza virus H9N2 surface glycoproteins HA and NA.

14. A method for the treatment of an individual (human being / animal or bird) infected with avian influenza virus comprising administering to said individual a therapeutically effective amount of a recombinant phage according to any one of claims I and 2.

15. The method according to claim 14, wherein the fusion peptide of the recombinant phage comprises the amino acid sequence selected from the group consisting of NDFRSKT or any other amino acid sequence derived therefrom.

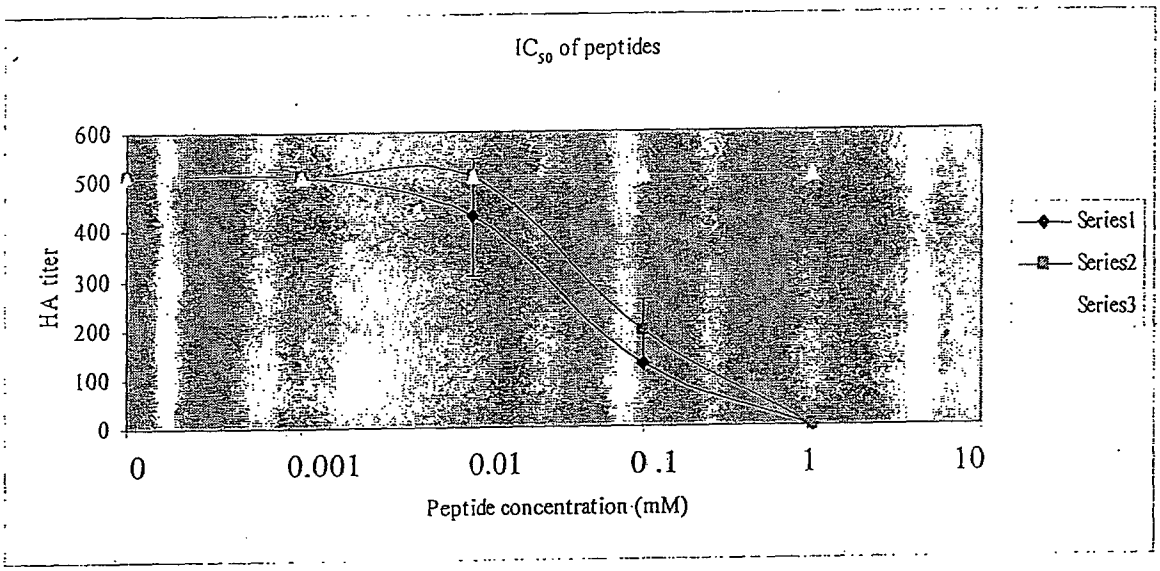
16. A method for the treatment of an individual (human being/animal or bird) infected with avian influenza virus comprising administering to said individual a therapeutically effective amount of a synthetic peptide according to any one of claims 3 to 6, and a pharmaceutically acceptable carrier.

17. The method according to claim 16, wherein the synthetic peptide comprises the amino acid sequence NDFRSKT or any other amino acid sequence derived therefrom.



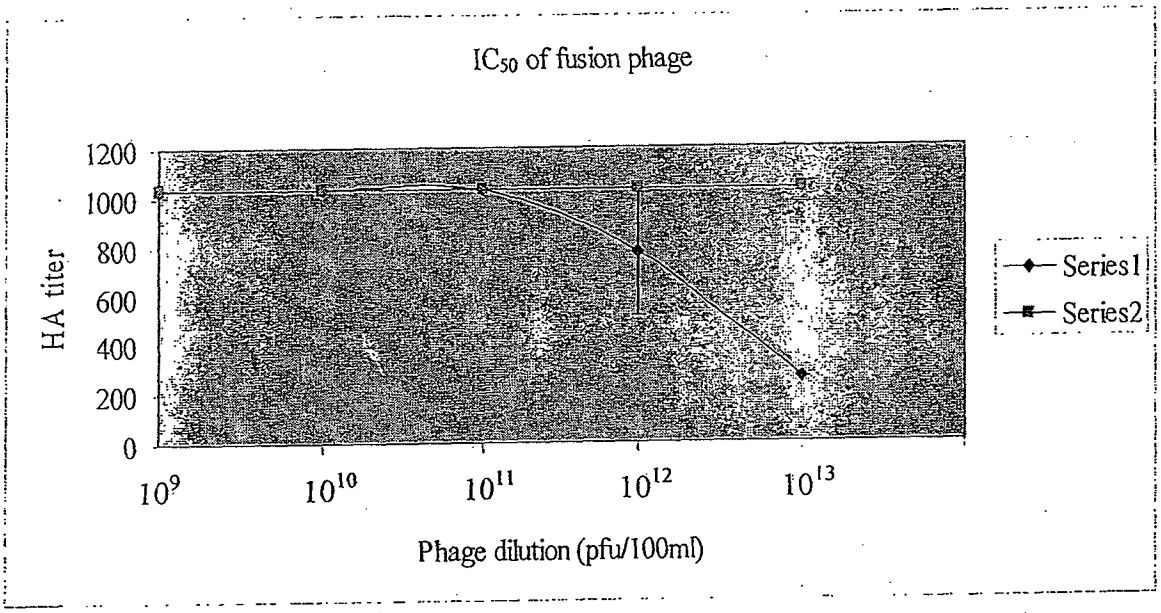
Series 1: FP-P1 phage
Series 2: FP-P1 phage with polyclonal antibodies

Figure 1



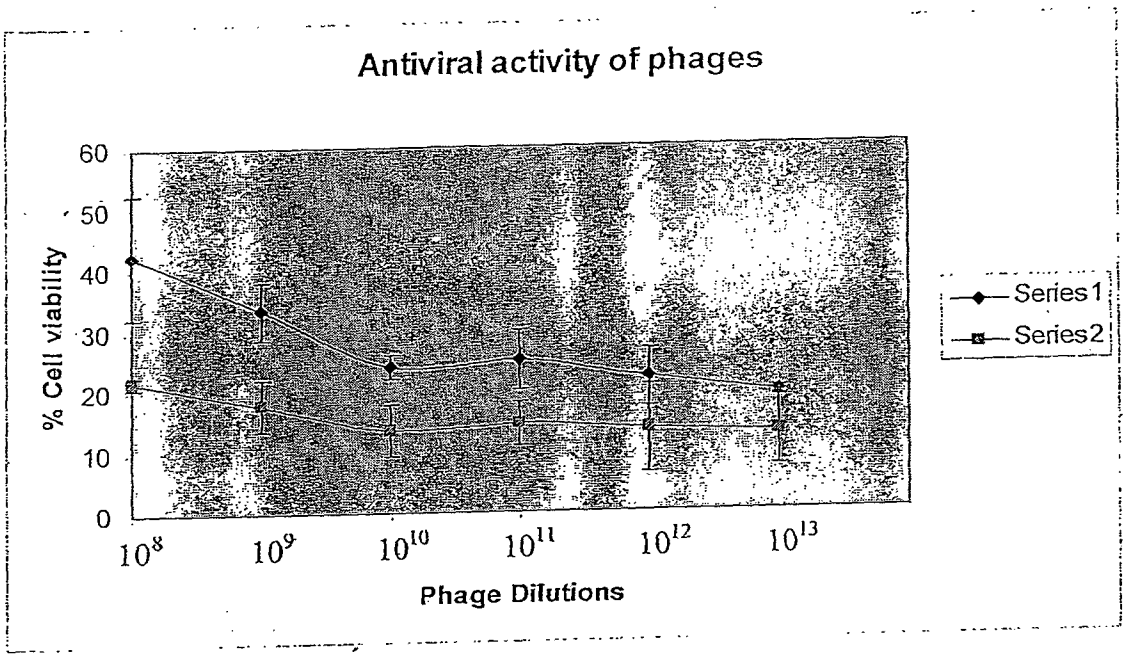
Series 1: Cyclic peptide [CNDFRSKTC]
Series 2: Linear peptide [NDFRSKT]
Series 3: Control [CSWGEYDMC]

Figure 2



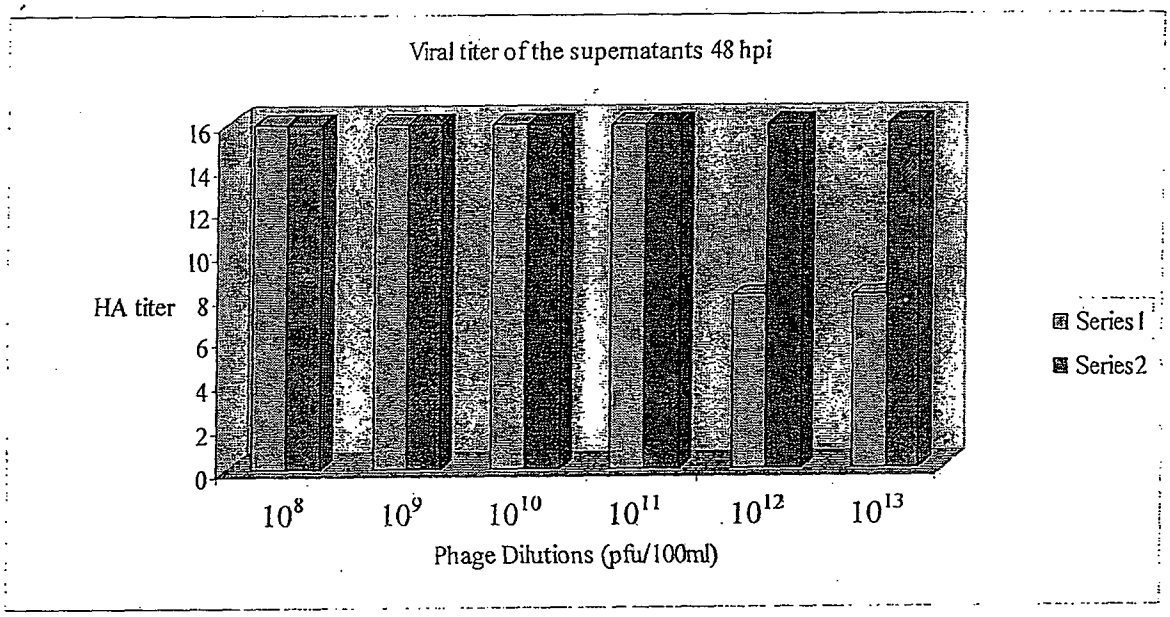
Series 1: Fusion phage (FP-P1)
Series 2: Control (Wild Phage M13)

Figure 3



Series 1: Fusion Phage (FP-P1)
Series 2: Control (Wild phage M13)

Figure 4



Series 1: Fusion Phage (FP-P1)
Series 2: Control (Wild phage M13)

Figure 5

INTERNATIONAL SEARCH REPORT

International application No.
PCT/MY2009/000071

A. CLASSIFICATION OF SUBJECT MATTER

Int. Cl.

C07K 7/06 (2006.01)	***A61P 31/16*** (2006.01)	***C12N 15/62*** (2006.01)
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A61K 38/08 (2006.01)	***C12N 7/01*** (2006.01)	***G01N 33/53*** (2006.01)
A61K 39/145 (2006.01)	***C12N 15/44*** (2006.01)	

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
MEDLINE, HCA, BIOSIS, EPODOC, WPI: phage, bacteriophage, H9N2, bird, avian, flu, influenza, AIV, biopanning and like terms. In addition, GenomeQuest: NDFRSKT and CNDFRSKTC

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 2008/157419 A2 (THE GOVERNMENT OF THE UNITED STATES OF AMERICA) 24 December 2008 See whole document. Especially claim 48, p. 11-14, p. 24; ln. 26-31, p. 26; ln. 16-27, p.31; ln. 16-30, p. 16; ln. 12-14, p. 16; ln. 28-31, p. 32, and p. 43; ln. 32-33.	1, 3, 4, 7-12, 14 & 16
X	KUANG, Z. *et al.* 'Screening of peptides as broad-spectrum neuraminidase inhibitors against influenza viruses' Bing du Xue bao. 2007, vol. 23(3), pages 165-171 See English translation of abstract.	1, 3, 4 & 6
P, X	SONG, H. *et al.* 'Identification of peptide mimotopes of an abroad-spectrum neutralizing epitope of highly pathogenic avian influenza hemagglutinin' Bing du Xue bao. November 2008, vol. 24(6), pages 421-6 See English translation of abstract.	1, 3 & 4

[X] Further documents are listed in the continuation of Box C [X] See patent family annex

* Special categories of cited documents:

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Date of the actual completion of the international search	Date of mailing of the international search report
01 September 2009	23 SEP 2009
Name and mailing address of the ISA/AU AUSTRALIAN PATENT OFFICE PO BOX 200, WODEN ACT 2606, AUSTRALIA E-mail address: pct@ipaustralia.gov.au Facsimile No. +61 2 6283 7999	Authorized officer **JOSEPH NG** AUSTRALIAN PATENT OFFICE (ISO 9001 Quality Certified Service) Telephone No : +61 2 6225 6140

INTERNATIONAL SEARCH REPORT

International application No.
PCT/MY2009/000071

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
P, X	RAJIK, M. *et al.* 'Identification and characterisation of a novel anti-viral peptide against avian influenza virus H9N2' Virology Journal. 5 June 2009, vol. 6(74), pages 1-12 See whole document. In particular pages 2-10.	1-17
A	RAJIK, M. *et al.* 'A novel peptide inhibits the influenza virus replication by preventing the viral attachment to the host cells' International Journal of Biological Sciences. 8 August 2009, vol. 5(6), pages 543-548 See whole document	

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No.
PCT/MY2009/000071

This Annex lists the known "A" publication level patent family members relating to the patent documents cited in the above-mentioned international search report. The Australian Patent Office is in no way liable for these particulars which are merely given for the purpose of information.

Patent Document Cited in Search Report	Patent Family Member
WO 2008/157419	NONE

Due to data integration issues this family listing may not include 10 digit Australian applications filed since May 2001.

END OF ANNEX