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[Continued on next page]

(54) Title: PEPTIDES AND COMPOSITIONS FOR PREVENTION OF CELL ADHESION AND METHODS OF USING SAME

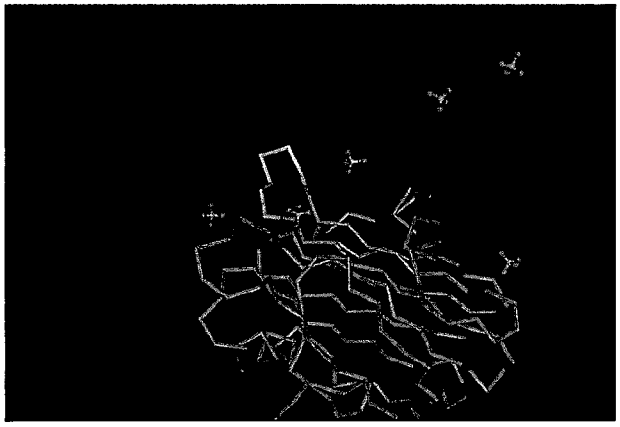

Figure 1: 1GWY Chain A, Crystal Structure Of The Water-Soluble State Of The Pore- Forming Cytolysin Sticholysin Ii

(57) Abstract: Compositions comprising an isolated peptide, which may for example optionally comprise a sequence selected from the group consisting of YDYNWY, YDYNLY, FDYNFY, FDYNLY, FDYNWY, YDWNLY, YDWHLY and WDYNLY, extracted from organisms such as aquatic organisms and mossor any other sequence described herein, and methods of using same, including for treatment of or prevention of formation of microbial biofilms and against adhesion of a cell to a surface.

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Title: PEPTIDES AND COMPOSITIONS FOR PREVENTION OF CELL ADHESION AND METHODS OF USING SAME

Inventors: Amir ZLOTKIN and Hen KESTENBOIM

FIELD OF THE INVENTION

The present invention relates to isolated natural peptides and their use in prevention of cell adhesion.

BACKGROUND OF THE INVENTION

Microorganisms can live and proliferate as individual cells swimming freely in the environment (as plankton), or they can grow as highly organized, multicellular communities encased in a self-produced polymeric matrix in close association with surfaces and interfaces. The latter microbial lifestyle is referred to as biofilms. Biofilm formation represents an ancient, protected mode of growth that allows microbial survival in hostile environments and allows microorganisms to disperse and colonize new niches [Hall-Stoodley et al., Nat Rev Microbiol. (2004) 2(2):95-108].

The composition of biofilms is complex and variable among different microbial species and even within the same species under different environmental conditions. Nonetheless, biofilm formation represents the normal lifestyle of microorganism in the environment and all microbes can make biofilms. Previous studies revealed that bacterial biofilm formation progresses through multiple developmental stages differing in protein profiles [Sauer et al., J Bacteriol. (2002) 184(4):1140-54], beginning with attachment to surface, followed by the immigration and division to form microcolonies and finally maturation involving expression of matrix polymers. Bacteria within each biofilm stage display phenotypes and possess properties that are markedly different from those of the same group growing planktonically [Sauer et al., J Bacteriol. (2004) 186(21):7312-26].

Biofilms are a major cause of systemic infections (e.g. nosocomial infections) in humans. In the body, biofilms can be associated with tissues (e.g., inner ears, teeth, gums, lungs, heart valves and the urogenital tract). An estimated 65 % of bacterial infections in humans are biofilm in nature. Additionally, after forming biofilms, microorganisms tend to change their characteristics, sometimes drastically, such that doses of antibiotics which normally kill the

organisms in suspended cultures are completely ineffective against the same microorganisms when the organisms are in attached or conglomerate biofilm form (U.S. Pat. No. 7189351).

One of the principal concerns with respect to products that are introduced into the body (e.g., contact lenses, central venous catheters, mechanical heart valves and pacemakers) or provide a pathway into the body is microbial infection and invariably biofilm formation. As these infections are difficult to treat with antibiotics, removal of the device is often necessitated, which is traumatic to the patient and increases the medical cost. Accordingly, for such medical apparatuses, the art has long sought means and methods of rendering those medical apparatuses and devices antimicrobial.

PCT Application No. WO 06/006172 discloses the use of anti-amyloid agents, such as aromatic compounds, for inhibiting formation or disintegrating a pre existing biofilm. . The application discloses that compounds preventing amyloid fibril formation in Alzheimers can act against fibril formation in biofilms, and concludes that amino acids having an aromatic arm are effective against biofilms. However, the analysis was limited to full length sequences.

SUMMARY OF THE INVENTION

The present invention provides broad spectrum natural factors that interfere with biofilm formation at its initial stages, in a wide range of microorganisms. From these natural factors, peptides with high conservation sequences were isolated, and showed high activity in prevention of microbial adherence in its synthetic conformation. The conserved sequence is found in several marine organisms, including various known species of sea anemone, several fish (including Danio rerio- zebra fish), and in moss Physcomitrella patens subsp. Patens..

All factors mentioned above show activity that is exclusively directed to the prevention of bacterial substrate adhesion and the derived biofilm formation. It is devoid of the commonly observed lethal bactericidal activity, revealed by the antibiotic peptides and secondary metabolites, which provides a strong selective pressure for rapid natural selection by the intensive microbial "biotic potential". On the other hand a wide range inhibition of bacterial colonization antagonizes a fundamental mechanism of bacterial survival. Therefore an adaptive modification of such mechanism has a low likelihood due to its vitality.

Sher et al. (Toxicon 45: 865-879, 2005) identified putative biologically active proteins and polypeptides expressed by hydrae which could be components of its allomonal system, using a bioinformatics approach. Hydrae were shown to express orthologs of cnidarian phospholipase A2 toxins and cytolysiens belonging to the actinoporin family, and to express proteins similar to

elapid-like phospholipases, cysteine-rich secretory proetins (CRISP), prokineticin-like polypeptides and toxic deoxyribonucleases.

The specific sequences responsible for cytotoxic activity in peptides isolated from natural sources have not hitherto been identified.

Unless otherwise defined, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this invention belongs. Although methods and materials similar or equivalent to those described herein can be used in the practice or testing of the present invention, suitable methods and materials are described below. In case of conflict, the patent specification, including definitions, will control. In addition, the materials, methods, and examples are illustrative only and not intended to be limiting.

As used herein, the terms "comprising" and "including" or grammatical variants thereof are to be taken as specifying the stated features, integers, steps or components but do not preclude the addition of one or more additional features, integers, steps, components or groups thereof. This term encompasses the terms "consisting of" and "consisting essentially of".

The phrase "consisting essentially of" or grammatical variants thereof when used herein are to be taken as specifying the stated features, integers, steps or components but do not preclude the addition of one or more additional features, integers, steps, components or groups thereof but only if the additional features, integers, steps, components or groups thereof do not materially alter the basic and novel characteristics of the claimed composition, device or method.

The term "method" refers to manners, means, techniques and procedures for accomplishing a given task including, but not limited to, those manners, means, techniques and procedures either known to, or readily developed from known manners, means, techniques and procedures by practitioners of the chemical, biological and biophysical arts.

As used herein the term "about" refers to ± 10 %.

Other features and advantages of the invention will be apparent from the following detailed description, and from the claims.

BRIEF DESCRIPTION OF THE DRAWINGS

The invention is herein described, by way of example only, with reference to the accompanying drawings. With specific reference now to the drawings in detail, it is stressed that the particulars shown are by way of example and for purposes of illustrative discussion of the preferred embodiments of the present invention only, and are presented in the cause of

providing what is believed to be the most useful and readily understood description of the principles and conceptual aspects of the invention. In this regard, no attempt is made to show structural details of the invention in more detail than is necessary for a fundamental understanding of the invention, the description taken with the drawings making apparent to those skilled in the art how the several forms of the invention may be embodied in practice.

In the drawings:

FIG. 1 shows the crystal structure of the 1GWY chain A of the water-soluble state of the pore-forming cytolysin sticholysin Ii, the active region marked in yellow;

FIG. 2 shows the crystal structure of the 1GWY chain B of the water-soluble state of the pore-forming cytolysin sticholysin Ii, the active region marked in yellow;

FIG. 3 shows the structure of the 1KD6 chain A of the eukaryotic pore-forming cytolysin equinatoxin Ii, the active region marked in yellow;

FIG. 4 shows the 3-dimensional construct of an equinatoxin mutant, the active region marked in yellow;

FIG. 5 is a bar chart showing the effects of different concentrations of synthetic proteins on growth of *Pseudomonas aeruginosa* ATCC 27853 over 24 hours; No bactericidal or bacteriostatic effect;

FIG. 6 is a bar chart showing the effects of different concentrations of synthetic proteins on biofilm formation by *Pseudomonas aeruginosa* ATCC 27853 over 24 hours;

FIG. 7 is a bar chart showing the effects of different concentrations of synthetic proteins on growth of a clinical isolate of *Acinetobacter Baumannii* over 24 hours; No bactericidal or bacteriostatic effect;

FIG. 8 is a bar chart showing the effects of different concentrations of synthetic proteins on biofilm formation by a clinical isolate of *Acinetobacter Baumannii* over 24 hours;

FIG. 9 is a bar chart showing the effects of selected tentacular fractions from *Actinia equina* on biofilm formation by *Acinetobacter baumannii*, with PBS as a positive control;

FIG. 10 is a bar chart showing the effects of fraction 13 on biofilm formation by various gram positive and gram negative bacteris;

FIG. 11 is a bar chart showing the effects of crude extracts from *Anemonia, Aiptasia* and *Physcomitrella* (Moss) on *Pseudomonas aeruginosa* in protein concentration of 50µg/m"l;

FIG. 12 is a bar chart showning the effects of five synthetic peptides and crude material from the moss of *Physcomitrella patens;*

FIG. 13 shows fractions obtained by separation of crude extract of *Aiptasia pulchella* on Sephadex G-10 column;

FIG. 14 shows peaks obtained by rechromatography of the high molecular weight fraction of FIG. 13;

FIG. 15 shows fractions obtained by reversed phase high performance liquid chromatography (RP-HPLC) separation with c-18 column, of the low molecular fraction of FIG. 14;

FIGS. 16A-B show the general structure of a cyclic lead with emulsifier arm of a peptide according to the principles of the present invention; and

FIG. 17 is a flow chart showing development of a cyclic peptide lead with emulsifying arm.

DESCRIPTION OF THE PREFERRED EMBODIMENTS

The present invention is of compositions comprising an isolated peptide which has one or more effects relating to prevention of bacterial substrate adhesion and the derived biofilm formation, and optionally also prevention of cell-cell adhesion. Other effects may also optionally be provided, additionally or alternatively. As a non-limiting example, the peptide may optionally and preferably comprise a sequence selected from the group consisting of YDYNWY, YDYNLY, FDYNFY, FDYNLY, FDYNWY, YDWNLY, YDWHLY and WDYNLY, extracted from organisms such as aquatic organisms and moss and methods of using same. Other sequences are described below.

One of the major concerns in medicine is microbial biofilm formation. In humans, biofilms are a cause of systemic infections (e.g., nosocomial infections) and are a major concern when introducing products into the body (e.g., contact lenses, central venous catheters, mechanical heart valves and pacemakers).

Biofilms are also a problem in many industries including the food, pharmaceutical, paint, water, shipping and engineering industries causing, amongst a wide range of negative effects, accelerated corrosion in industrial systems, oil souring and biofouling. For example, biofouling may be caused by the adhesion of organisms to any surface in a marine or freshwater environment, including cooling towers, water pipes and filters in cooling or desalinization installations, irrigation and power stations, and membranes, such as those used in wastewater and desalinization systems. Biofouling also occurs in aquaculture systems in fish farms.

Furthermore the commercial shipping fleets of the world consume approximately 300 million tonnes of fuel annually. Without antifouling measures, that fuel consumption would increase by as much as 40%, equivalent to an extra 120 million tonnes of fuel annually. The

economic cost of this was estimated as about $7.5 billion in 2000; a more recent estimate is $30 billion.

Biofilms are very difficult to eliminate since microbes growing within are highly organized and can withstand hostile environments, such as high temperatures and anti-microbial agents (e.g., antibiotics).

Marine and fresh water plants and organisms including soft bodied water invertebrates, fish and moss are surrounded by broad spectrum species of microbial organisms. Since such plant and organisms lack specific immunity, they produce several factors which can prevent microbial colonization on their body surface.

The most "sensitive" organisms are invertebrates belong to the phylum cnidaria that include the sea anemones, corals, jellyfish, hydroids, medusae, and sea fans. Such soft bodied organism, which lack physical protection such as scales or shells, use proteins and secondary metabolites to protect themselves from the microbial environment surrounding them.

It has been previously reported that marine organisms (e.g. sponges) produce secondary metabolites that exhibit antibacterial and antifungal activities [Amade et al., supra]. Moreover, sea anemones (e.g., Actinia equina) have been shown to produce toxic, pore forming peptides (i.e., equinatoxins), which lyse and kill eukaryotic cells similarly to other small antimicrobial peptides [Anderluh et al., supra].

Although it is known in the art that the full length sequences of various proteins are related to their cytolysic function, the specific peptides responsible for the cytolysic effect have not been previously identified.

The present inventors have demonstrated that several active fractions obtained from sea anemones using liquid chromatography separations show a high level of prevention of microbial adherence to abiotic surfaces. The sea anemone includes 46 families that can be found in water sources around the world. Most sea anemones are sessile, with a specialized foot used to anchor them in soft substrates, or attach themselves to rocks and corals. The anti-adhesive activity was demonstrated with several species of sea anemone belonging to different genera: *Actinia equine*, *Aiptasia* and *Anemonia*. The N terminus region of anemone cytotoxin has been shown to be involved in the cytotoxic effect [Ref: Kristan K, Podlesek Z, Hojnik V, Gutierrez-Aguirre I, Gunčar G, Turk D, Gonzalez-Manas JM, Lakey JH, Maček P, Anderluh G (2004): Pore formation by equinatoxin, an eukaryotic pore-forming toxin, requires a flexible N-terminal region and a stable beta sandwich. J Biol Chem. 279(45):46509-46517]. A protein having some resemblance to the C terminus region of anemone cytotoxin, which region is not involved in cytotoxicity, has also been identified in fish by the present inventors. This protein has a highly

conserved region, with unknown function, which is also a Trp-rich domain, and may be important for binding of the protein to lipid membrane. The present inventors have also found this region in the moss *Physcomitrella patens*.

The present inventors therefore hypothesized that this region provides a peptide which is highly effective in prevention in biofilm formation, while being devoid of cytotoxic activity. The present inventors have characterized and isolated a natural peptide comprising a sequence selected from the group consisting of YDYNWY, YDYNLY, FDYNFY, FDYNLY, WDYNLY, FDYNWY, YDWNLY and YDWHLY, having highly effective anti-biofilm properties.

According to some embodiments, the peptide comprises part of a sequence comprising up to about 30, up to about 40, or up to about 50 amino acids.

According to some embodiments, the peptide is selected from the group consisting of LFSVPYDYNWYSNWW, FSVPYDYNLYSNWW, MFSVPFDYNFYSNWW, MFSVPFDYNLYSNWW, MFSVPFDYNLYTNWW, MWSVPFDYNLYSNWW, MFSVPWDYNLYKNWF, MFSVPFDYNLYKNWL, MFSVPFFDYNWYSNWW, LFSVPFDYNLYSNWW, LFSVPYDYNWYSNWW, MASIPYDWNLYQSWA, MASIPYDWNLYSAWA, and MASIPYDWHLYNAWA. As is shown herein below and in the Examples section which follows, the present inventors have identified an active fraction extracted from *Aiptesia pulchella* anemone, using tandem mass spectroscopy (MS/MS) analysis.

The present inventors used the clustaLW program to identify biologically meaningful multiple sequence alignments of several anemone cytotoxin proteins and identify an anemone cytotoxin universal primer for use in a polymerase chain reaction (PCR). Amplification of a 250 bp region of cytotoxin proteins from two different anemones, aiptesia and anemonia viridansm, of sequence Eqt-F: GTR TCG ACA ACG AGT CRG G and Eqt-R252: TGA CAT YCC ACC AGT TGC TG, respectively, was achieved. Translation of these regions to peptides, and BlastX comparison to the genebank, showed that these regions are part of the conserved domain of anemone cytotoxin.As discussed in greater detail in the Examples section below, and shown in Figures 5 to 8, the present inventors compared the activities of a number of synthetic peptides from anemones and moss, and found that these peptides prevented the formation of biofilm [Figs 6 and 8 to 12], but did not kill or inhibit growth of bacteria [Figs 5 and 7].

The anti-adhesive effect was demonstrated on several bacterial species (Figure 10), which led the present inventors to conclude that the active materials are not species specific but active against a broad range of microbial species.

The conserved peptide region has been identified, for example, in the following natural proteins:

LFSVPYDYNWYSNWW	EqT-IV
FSVPYDYNLYSNWW	Actinoporin Or-A
MFSVPFDYNFYSNWW	HMg III from Heteractis magnifica
MFSVPFDYNLYSNWW	Avt-I RTX-A
MFSVPFDYNLYTNWW	Pstx20
MWSVPFDYNLYSNWW	Physcomitrella patens
MFSVPWDYNLYKNWF	Danio rerio
MFSVPFDYNLYKNWL	Tetraodon nigroviridis

Optionally and preferably, the peptide of the present invention comprises the sequence CMFSVPFDYNWYSNWWC. Optionally and preferably, the peptide of the present invention is comprised in a protein having from about 100 to about 300 amino acids.

Without wishing to be limited by a single hypothesis, based on the 3-dimensional structure of 2 anemone cytotoxin (equinatoxin and Sticholysin), as shown in Figures 1-4, the active region faces outwards.

Figures 1 and 2 shows the crystal structure of the 1GWY chains A and B, respectively, of cytolysin sticholysin Ii. Figure 3 shows the structure of the 1KD6 chain A of the eukaryotic pore-forming cytolysin equinatoxin Ii.

Figure 4 demonstrates the 3-dimensional construct of an equinatoxin mutant, having three cysteines introduced at positions 8, 18 and 69 (1TZQ Chain A). This mutant has been previously shown not to be hemolytically active (Kristan K, Podlesek Z, Hojnik V, Gutierrez-Aguirre I, Gunčar G, Turk D, Gonzalez-Manas JM, Lakey JH, Maček P, Anderluh G (2004): Pore formation by equinatoxin, an eukaryotic pore-forming toxin, requires a flexible N-terminal region and a stable beta sandwich. J Biol Chem. 279(45):46509-46517). The protein thus lost its cytotoxicity, but was still active against bacterial adherence.

The principles and operation of the present invention may be better understood with reference to the drawings and accompanying descriptions.

Before explaining at least one embodiment of the invention in detail, it is to be understood that the invention is not limited in its application to the details set forth in the following description or exemplified by the Examples. The invention is capable of other embodiments or of being practiced or carried out in various ways. Also, it is to be understood that the phraseology and terminology employed herein is for the purpose of description and should not be regarded as limiting.

According to one aspect of the present invention, there is provided a composition comprising an isolated natural peptide, the peptide comprising a sequence selected from the

group consisting of YDYNWY, YDYNLY, FDYNFY, FDYNLY, WDYNLY, FDYNWY, YDWNLY and YDWHLY.

According to an additional aspect of the present invention there is provided a method of preventing adhesion of a single cell organism to a surface, the method comprising contacting the cell with a composition of comprising an isolated natural peptide comprising a sequence selected from the group consisting of YDYNWY, YDYNLY, FDYNFY, FDYNLY, WDYNLY, FDYNWY, YDWNLY and YDWHLY, thereby preventing adhesion of a cell to a surface.

According to some embodiments of the present invention, there is preferably provided a domain which comprises at least one of the above peptides and which is effective against cell adhesion to a surface. More preferably, the domain is included as part of a protein. Optionally and most preferably, the domain exhibits anti-adhesive behavior, for example for the prevention of formation and/or treatment of a biofilm, but does not exhibit cytotoxic behavior.

A non-limiting selection of exemplary domains is provided in the table below.

Domain sequence	Species
LFSVPYDYNWYSNWW	EqT-IV
FSVPYDYNLYSNWW	Actinoporin Or-A
MFSVPFDYNFYSNWW	HMg III from Heteractis magnifica
MFSVPFDYNLYSNWW	Avt-I RTX-A
MFSVPFDYNLYTNWW	Pstx20
MWSVPFDYNLYSNWW	Physcomitrella patens
MFSVPWDYNLYKNWF	Danio rerio
MFSVPFDYNLYKNWL	Tetraodon nigroviridis

Further exemplary sequences are described herein, as being related to the following sequence:

MSRLIIVFIVVTMICSATALPSKKIIDEDEEDEKRSADVAGAVIDGASLSFDILKTV LEALGNVKRKIAVGVDNESGKTWTALNTYFRSGTSDIVLPHKVPHGKALLYNGQKDR GPVATGAVGVLAYLMSDGNTLAVLFSVPYDYNWYSNWWNVRIYKGKRRADQRMYE ELYYNLSPFRGDNGWHTRNLGYGLKSRGFMNSSGHAILEIHVSKA

This sequence has the GenBank accession identifier:

>gi|48428895|sp|P61914.1|ACTP2_ACTEQ Equinatoxin-2 precursor (Equinatoxin II) (EqT II) (EqTII) Actinia equine and is 214 aa in length. This sequence is also optionally an

exemplary sequence according to the present invention. Positions 38-213 of this sequence hit the annotated domain pfam06369, Anemone_cytotox, Sea anemone cytotoxic protein; therefore, this portion of the above sequence is also optionally an exemplary sequence according to the present invention.

In some embodiments, the present invention also includes any related sequence to the above sequence thereof. Such related sequences may optionally be found by running any type of sequence comparison software, including but not limited to BLASTP. Below are provided representative hits from selected taxa and their alignments to EqtII (the above sequence):

1. Sea Anemones –

1a. Stichodactyla helianthus

>gi|2815496|sp|P07845.2|ACTP2_STOHE Sticholysin-2 (Sticholysin II) (StnII) (Cytolysin St II) (Cytolysin III) (Cytotoxin)

ALAGTIIAGASLTFQVLDKVLEELGKVSRKIAVGIDNESGGTWTALNAYFRSGTTDVILP
EFVPNTKALLYSGRKDTGPVATGAVAAFAYYMSSGNTLGVMFSVPFDYNWYSNWWD
VKIYSGKRRADQGMYEDLYYGNPY
RGDNGWHEKNLGYGLRMKGIMTSAGEAKMQIKISR

Alignment:

>sp|P07845.2|ACTP2_STOHE Sticholysin-2 (Sticholysin II) (StnII) (Cytolysin St II) (Cytolysin III) (Cytotoxin)

Length=175

Score = 253 bits (646), Expect = 8e-66, Method: Composition-based stats.

Identities = 118/176 (67%), Positives = 144/176 (81%), Gaps = 1/176 (0%)

```
Query  38   DVAGAVIDGASLSFDILKTVLEALGNVKRKIAVGVDNESGKTWTALNTYFRSGTSDIVLP  97
            +AG +I GASL+F +L  VLE LG V RKIAVG+DNESG TWTALN YFRSGT+D++LP
Sbjct  1    ALAGTIIAGASLTFQVLDKVLEELGKVSRKIAVGIDNESGGTWTALNAYFRSGTTDVILP  60

Query  98   HKVPHGKALLYNGQKDRGPVATGAVGVLAYLMSDGNTLAVLFSVPYDYNWYSNWWNVRIY  157
              VP+ KALLY+G+KD GPVATGAV   AY MS GNTL V+FSVP+DYNWYSNWW+V+IY
Sbjct  61   EFVPNTKALLYSGRKDTGPVATGAVAAFAYYMSSGNTLGVMFSVPFDYNWYSNWWDVKIY  120

Query  158  KGKRRADQRMYEELYYNLSPFRGDNGWHTRNLGYGLKSRGFMNSSGHAILEIHVSK  213
             GKRRADQ MYE+LYY  +P+RGDNGWH +NLGYGL+ +G M S+G A ++I +S+
Sbjct  121  SGKRRADQGMYEDLYYG-NPYRGDNGWHEKNLGYGLRMKGIMTSAGEAKMQIKISR  175
```

2. Bony fish

2a. Danio rerio

>gi|125821212|ref|XP_001342650.1| PREDICTED: hypothetical protein [Danio rerio]

MTESAEAVAANVSSRRHATVEITNLTNNYCFLNPKVYLENGETSNPPQPTVRPLKTEVCTFSKSAAHATG
SVGVLTYDLFERRRNDYTETLAIMFSVPWDYNLYKNWFAVGIYPKGKECDQALYKEMYYQKNQHGFVREE
ANGSGINFEGKNLDIRATMCPMGRAIVKVEVWDKLLSPMAQMDC

Alignment:

>ref|XP_001342650.1| UniGene infoGene info PREDICTED: hypothetical protein [Danio rerio]

Length=184

GENE ID: 100002992 apnl | actinoporin-like protein [Danio rerio]

Score = 199 bits (505), Expect = 1e-49, Method: Composition-based stats.

Identities = 49/167 (29%), Positives = 73/167 (43%), Gaps = 12/167 (7%)

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Query  58   LEALGNVKRKIAVGVDNESG-KTWTALNTYFRSGTSDIVLPHKVPHGKALLYNGQKDRGP  116
            + A  + +R   V + N +    +     Y  +G +       V   K  +    K
Sbjct  8    VAANVSSRRHATVEITNLTNNYCFLNPKVYLENGETSNPPQPTVRPLKTEVCTFSKSAAH  67

Query  117  VATGAVGVLAYLMSD------GNTLAVLFSVPYDYNWYSNWWNVRIYKGKRRADQRMYEE  170
             ATG+VGVL Y + +        TLA++FSVP+DYN Y NW+ V IY    +  DQ +Y+E
Sbjct  68   -ATGSVGVLTYDLFERRRNDYTETLAIMFSVPWDYNLYKNWFAVGIYPKGKECDQALYKE  126

Query  171  LYYNLSPF----RGDNGWHTRNLGYGLKSRGFMNSSGHAILEIHVSK  213
             +YY  +         NG      G  L  R  M   G AI+++ V
Sbjct  127  MYYQKNQHGFVREEANGSGINFEGKNLDIRATMCPMGRAIVKVEVWD  173
```

2b. Tetraodon nigroviridis

>gi|47218822|emb|CAG02807.1| unnamed protein product [Tetraodon nigroviridis]

MESAEAVAADVSRSRSVTIEISNLTKNYCLINPRVYLESGETYNPPQPTVRPLMTEVCTFSKSSGIPTGS
VGVLTYELLERRSTMLPETLAIMFSVPYDYSFYNNWFAVGIYETGTKCNEGLYKQMYNEKKQAEHGFVRE
KANGSGINYVGGNLDIRATMNPLGKAIMKVEVWDAFFPFSE

Alignment:

>emb|CAG02807.1| unnamed protein product [Tetraodon nigroviridis]

Length=181

Score = 192 bits (489), Expect = 1e-47, Method: Composition-based stats.
Identities = 46/170 (27%), Positives = 76/170 (44%), Gaps = 14/170 (8%)

```
Query  58   LEALGNVKRKIAVGVDNES-GKTWTALNTYFRSGTSDIVLPHKVPHGKALLYNGQKDRGP  116
            + A  +  R + + + N +          Y  SG +       V      +    K  G
Sbjct  7    VAADVSRSRSVTIEISNLTKNYCLINPRVYLESGETYNPPQPTVRPLMTEVCTFSKSSG-  65

Query  117  VATGAVGVLAYLMSD------GNTLAVLFSVPYDYNWYSNWWNVRIYKGKRRADQRMYEE  170
            + TG+VGVL Y + +        TLA++FSVPYDY++Y+NW+ V IY+    + ++ +Y++
Sbjct  66   IPTGSVGVLTYELLERRSTMLPETLAIMFSVPYDYSFYNNWFAVGIYETGTKCNEGLYKQ  125

Query  171  LYYNLSPF------RGDNGWHTRNLGYGLKSRGFMNSSGHAILEIHVSKA  214
            +Y               NG     +G  L  R  MN  G AI+++ V  A
Sbjct  126  MYNEKKQAEHGFVREKANGSGINYVGGNLDIRATMNPLGKAIMKVEVWDA  175
```

3. Mosses

3a. Physcomitrella patens

>gi|168060237|ref|XP_001782104.1| predicted protein [Physcomitrella patens subsp. patens]

MVVHLIAMGLRYSETIMKTARMAEAIIPAAELSIKTLQNIVEGITGVDRKIAIGFKNLTDYTLENLGVYF
NSGSSDRSIAYKINAQEALLFSARKSDHTARGTVGTFSYYIQDEDKTVHVMWSVPFDYNLYSNWWNIAVV
DGRQPPDSNVHDNLYNGSGGMPYPNKFDQYINNEQKGFHLFGSMTNNGQATIEVELKKA

>ref|XP_001782104.1| Gene info predicted protein [Physcomitrella patens subsp. patens]
gb|EDQ53098.1| Gene info predicted protein [Physcomitrella patens subsp. patens]
Length=199

GENE ID: 5945292 PHYPADRAFT_61094 | hypothetical protein
[Physcomitrella patens subsp. patens]

Score = 230 bits (586), Expect = 7e-59, Method: Composition-based stats.
Identities = 63/183 (34%), Positives = 101/183 (55%), Gaps = 4/183 (2%)

```
Query  35   RSADVAGAVIDGASLSFDILKIVLEALGNVKRKIAVGVDNESGKTWTALNTYFRSGTSDI  94
            ++A +A A+I  A LS   L+ ++E +  V RKIA+G  N +  T   L  YF SG+SD
Sbjct  18   KTARMAEAIIPAAELSIKTLQNIVEGITGVDRKIAIGFKNLTDYTLENLGVYFNSGSSDR  77
```

```
Query  95   VLPHKVPHGKALLYNGQKDRGPVATGAVGVLAYLMSD-GNTLAVLFSVPYDYNWYSNWWN  153
            + +K+   +ALL++ +K     A G VG  +Y + D   T+ V++SVP+DYN YSNWWN
Sbjct  78   SIAYKINAQEALLFSARKSDH-TARGTVGTFSYYIQDEDKTVHVMWSVPFDYNLYSNWWN  136

Query  154  VRIYKGKRRADQRMYEELYYNL--SPFRGDNGWHTRNLGYGLKSRGFMNSSGHAILEIHV  211
            + +  G++  D  +++ LY      P+      +  N   G    G M ++G A +E+ +
Sbjct  137  IAVVDGRQPPDSNVHDNLYNGSCGMPYPNKPDQYINNEQKGFHLFGSMTNNGQATIEVEL  196

Query  212  SKA  214
             KA
Sbjct  197  KKA  199
```

4. Birds

4a. Gallus gallus

>gi|118129726|ref|XP_001231839.1| PREDICTED: hypothetical protein isoform 1 [Gallus gallus]

MPPKEKKENDKPCNDNCQPKPQCKGVESLMKNIDVCRSVGLEIINRTRTVTLTDFRSYCFSGKIVTTLPF
EIGPDSKGICIFAKTPYSLRGSVGTVVCKADTFFLAITFSNPYDYILYKIEFALEIFTEPNHLGNLGDVF
SKMMKSKPYCGSSLFQRAVLESEHETLEVSKGSIRVQAKMSNNRKAILKVQVEDMDPPPYSKGM

>ref|XP_001231839.1| UniGene infoGene info PREDICTED: hypothetical protein isoform 1 [Gallus gallus]

Length=204

GENE ID: 769729 LOC769729 | hypothetical protein LOC769729 [Gallus gallus]

Score = 150 bits (378), Expect = 9e-35, Method: Composition-based stats.

Identities = 33/172 (19%), Positives = 63/172 (36%), Gaps = 22/172 (12%)

```
Query  58   LEALGNVKRKIAVGVDNES-GKTWTALNTYFRSGTSDIVLPHKVPHGKALLYNGQKDRGP  116
            L    +V R + + + N +   T T   +Y  SG     LP ++      +    K
Sbjct  29   LMKNIDVCRSVGLEIINRTRTVTLTDFRSYCFSGKIVTTLPFEIGPDSKGICIFAKTP-Y  87

Query  117  VATGAVGVLAYLMSDGNTLAVLFSVPYDYNWYSNWWNVRIYKGKRRADQ-----RMYEEL  171
               G+VG +    +D   LA+ FS PYDY  Y   + + I+      +       ++ ++
Sbjct  88   SLRGSVGTVVCK-ADTFFLAITFSNPYDYILYKIEFALEIF---TEPNHLGNLGDVFSKM  143

Query  172  YYNLSPFRG----------DNGWHTRNLGYGLKSRGFMNSSGHAILEIHVSK  213
```

```
                P+ G                          ++ +  M+++  AIL++ V
Sbjct  144  MK-SKPYCGSSLFQRAVLESEHETLEVSKGSIRVQAKMSNNRKAILKVQVED  194
```

5. Platypus

5a. Ornithorhynchus anatinus

>gi|149491241|ref|XP_001516906.1| PREDICTED: hypothetical protein [Ornithorhynchus anatinus]

MAQTIEHLVHEVEAGRCVGIEITNTTNMTFRSPRTFCFSGHTLTPPTPIIHPNNAGFCIFVKRKFSLRGS
VGLLVYEIEDQTLAIMFSNPFDYNFFKVEFAVALSGYKEETQDLKAFFELLYHEKQKGWLKMAKEKLCEC
QCPVSLENNGIRVTATMSNNAKAIIKLSSPDAKPPEGDVADVQPTTVRRPNPPPFPSPRPRIGSDLTGDQ
LATLDFESGK

>ref|XP_001516906.1| Gene info PREDICTED: hypothetical protein [Ornithorhynchus anatinus]
Length=220

GENE ID: 100086848 LOC100086848 | hypothetical protein LOC100086848 [Ornithorhynchus anatinus]

Score = 168 bits (426), Expect = 2e-40, Method: Composition-based stats.
Identities = 36/167 (21%), Positives = 69/167 (41%), Gaps = 12/167 (7%)

```
Query  58   LEALGNVKRKIAVGVDNESGKTWTALNTYFRSGTSDIVLPHKVPHGKALLYNGQKDRGPV  117
            L       R + + + N +  T+ +  T+  SG +       +    A      K R
Sbjct  8    LVHEVEAGRCVGIEITNTTNMTFRSPRTFCFSGHTLTPPTPIIHPNNAGFCIFVK-RKFS  66

Query  118  ATGAVGVLAYLMSDGNTLAVLFSVPYDYNWYSNWWNVRI--YKGKRRADQRMYEELYYNL  175
               G+VG+L Y + D  TLA++FS P+DYN++   + V +  YK + +  +  +E LY+
Sbjct  67   LRGSVGLLVYEIED-QTLAIMFSNPFDYNFFKVEFAVALSGYKEETQDLKAFFELLYHEK  125

Query  176  --------SPFRGDNGWHTRNLGYGLKSRGFMNSSGHAILEIHVSKA  214
                          +          G++    M+++  AI+++    A
Sbjct  126  QKGWLKMAKEKLCECQCPVSLENNGIRVTATMSNNAKAIIKLSSPDA  172
```

As used herein, the term "isolated" refers to a composition that has been removed from its *in-vivo* location (e.g. aquatic organism or moss). Preferably the isolated compositions of the present invention are substantially free from other substances (e.g., other proteins that do not comprise anti-adhesive effects) that are present in their *in-vivo* location (i.e. purified or semi-purified).

As used herein the phrase "aquatic organism" refers to an organism living in a water environment (marine or freshwater) such as for example a fish or a sessile aquatic organism.

As used herein, the phrase "sessile aquatic organism" refers to an aquatic organism which is not freely moving for at least some a part of its life cycle. Aquatic sessile organisms are usually permanently attached to a solid substrate of some kind, such as to a rock or the hull of a ship due to physical anchorage to the substrate, or for any other reason (e.g. stone fish).

Exemplary sessile organisms include, but are not limited to, sessile cnidarians such as corals, sea anemones (e.g. Actinia equine and Aiptasia pulchella), sea pens, aquatic sessile larva (e.g., jellyfish larva), tube dwelling anemones and hydroids (e.g. Chlorohydra viridissima and Hydra vulgaris).

Exemplary fish that may be used according in embodiments of the present invention are preferably those dwelling in shallow waters or those that hide at the bottom layer of the ocean, sometimes in holes or caves. Such fish include eel and catfish.

As used herein the phrase "moss" refers to a non-vascular plant of the bryophyta division, including any of the classes takakiposida, sphyagnopsida, andreaeopsida, anderaeobryopsida, polytirchopsida, or bryopsisa.

The moss may comprise, for example, physcomitrella patens, Funaria hygrometrica; Eukaryota; Viridiplantae; Streptophyta; Embryophyta; Bryophyta;Moss Superclass V; Bryopsida; Funariidae; Funariales; Funariaceae; or Physcomitrella.

The compositions of the present invention may also be expressed in-vivo using genetic engineering techniques (e.g. using transgenic aquatic sessile organisms).

According to some embodiments of the present invention, the compositions of the present invention are devoid of cytotoxic or cytostatic activity, e.g. they are not bactericidal or bacteristatic.

According to some embodiments of the present invention, the compositions of the present invention are resistant to lyophilization – e.g. their activities are preserved following freeze drying.

As used herein the phrase "single cell organism" refers to a unicellular organism also termed a microorganism or a microbe. The single cell organism of the present invention can be

a eukaryotic single cell organism (e.g., protozoa or fungi for example yeast) or a prokaryotic single cell organism (e.g., bacteria or archaea). The single cell organisms of the present invention may be in any cellular environment, such as for example, in a biofilm, as isolated cells or as a cell suspension.

As used herein the term "biofilm" refers to an extracellular matrix in which microorganisms are dispersed and/or form colonies. The biofilm typically is made of polysaccharides and other macromolecules.

Exemplary bacterial cells, whose adhesion may be prevented according to the method of the present invention, include gram positive bacteria and gram negative bacteria.

The term "Gram-positive bacteria" as used herein refers to bacteria characterized by having as part of their cell wall structure peptidoglycan as well as polysaccharides and/or teichoic acids and are characterized by their blue-violet color reaction in the Gram-staining procedure. Representative Gram-positive bacteria include: Actinomyces spp., Bacillus anthracis, Bifidobacterium spp., Clostridium botulinum, Clostridium perfringens, Clostridium spp., Clostridium tetani, Corynebacterium diphtheriae, Corynebacterium jeikeium, Enterococcus faecalis, Enterococcus faecium, Erysipelothrix rhusiopathiae, Eubacterium spp., Gardnerella vaginalis, Gemella morbillorum, Leuconostoc spp., Mycobacterium abscessus, Mycobacterium avium complex, Mycobacterium chelonae, Mycobacterium fortuitum, Mycobacterium haemophilium, Mycobacterium kansasii, Mycobacterium leprae, Mycobacterium marinum, Mycobacterium scrofulaceum, Mycobacterium smegmatis, Mycobacterium terrae, Mycobacterium tuberculosis, Mycobacterium ulcerans, Nocardia spp., Peptococcus niger, Peptostreptococcus spp., Proprionibacterium spp., Sarcina lutea, Staphylococcus aureus, Staphylococcus auricularis, Staphylococcus capitis, Staphylococcus cohnii, Staphylococcus epidermidis, Staphylococcus haemolyticus, Staphylococcus hominis, Staphylococcus lugdanensis, Staphylococcus saccharolyticus, Staphylococcus saprophyticus, Staphylococcus schleiferi, Staphylococcus similans, Staphylococcus warneri, Staphylococcus xylosus, Streptococcus agalactiae (group B streptococcus), Streptococcus anginosus, Streptococcus bovis, Streptococcus canis, Streptococcus equi, Streptococcus milleri, Streptococcus mitior, Streptococcus mutans, Streptococcus pneumoniae, Streptococcus pyogenes (group A streptococcus), Streptococcus salivarius, Streptococcus sanguis.

The term "Gram-negative bacteria" as used herein refer to bacteria characterized by the presence of a double membrane surrounding each bacterial cell. Representative Gram-negative bacteria include Acinetobacter calcoaceticus, Acinetobacter baumannii, Actinobacillus actinomycetemcomitans, Aeromonas hydrophila, Alcaligenes xylosoxidans, Bacteroides,

Bacteroides fragilis, Bartonella bacilliformis, Bordetella spp., Borrelia burgdorferi, Branhamella catarrhalis, Brucella spp., Campylobacter spp., Chalmydia pneumoniae, Chlamydia psittaci, Chlamydia trachomatis, Chromobacterium violaceum, Citrobacter spp., Eikenella corrodens, Enterobacter aerogenes, Escherichia coli, Flavobacterium meningosepticum, Fusobacterium spp., Haemophilus influenzae, Haemophilus spp., Helicobacter pylori, Klebsiella pneumoniae, Klebsiella spp., Legionella spp., Leptospira spp., Moraxella catarrhalis, Morganella morganii, Mycoplasma pneumoniae, Neisseria gonorrhoeae, Neisseria meningitidis, Pasteurella multocida, Plesiomonas shigelloides, Prevotella spp., Proteus spp., Providencia rettgeri, Pseudomonas aeruginosa, Pseudomonas spp., Rickettsia prowazekii, Rickettsia rickettsii, Rochalimaea spp., Salmonella spp., Salmonella typhi, Serratia marcescens, Shigella spp., Shigella sonnei, Treponema carateum, Treponema pallidum, Treponema pallidum endemicum, Treponema pertenue, Veillonella spp., Vibrio cholerae, Vibrio vulnificus, Yersinia enterocolitica, Yersinia pestis.

The term "fungi" as used herein refers to the heterotrophic organisms characterized by the presence of a chitinous cell wall, and in the majority of species, filamentous growth as multicellular hyphae. Representative fungi whose adhesion may be prevented according to the method of the present invention include Candida albicans, Saccharomyces cerevisiae, Candida glabrata, Candida parapsilosis and Candida dubliniensis.

As used herein the phrase "preventing adhesion" refers to reducing or eliminating cell attachment to a surface (e.g. by reducing the rate of growth on a surface). Preferably, the compositions of the present invention prevent cell adhesion by as much as 10 %, more preferably by 20 %, more preferably by 30 %, more preferably by 40 %, more preferably by 50 %, more preferably by 60 %, more preferably by 70 %, more preferably by 80 %, more preferably by 90 % and most preferably by 100% as measured by a cell adhesion assay. Exemplary cell adhesion assays are described herein below and in the Examples section that follows. It will be appreciated that the compositions of the present invention may also be capable of preventing cell aggregation (i.e. cell aggregation not to a surface).

The present invention contemplates prevention of cellular adhesion to a wide variety of surfaces including fabrics, fibers, foams, films, concretes, masonries, glass, metals, plastics, polymers, and like.

According to one embodiment, the surface is comprised in a device that is susceptible to biofilm formation. Exemplary devices whose surfaces are contemplated by the present invention include, but are not limited to, vessel hulls, automobile surfaces, air plane surfaces, membranes, filters, and industrial equipment.

The surface may also be comprised in medical devices, instruments, and implants. Examples of such medical devices, instruments, and implants include any object that is capable of being implanted temporarily or permanently into a mammalian organism, such as a human. Representative medical devices, instruments, and implants that may be used according to the present invention include, for example, central venous catheters, urinary catheters, endotracheal tubes, mechanical heart valves, pacemakers, vascular grafts, stents and prosthetic joints. Methods of preventing cell attachment to medical devices and further examples thereof are described herein below.

According to another embodiment the surface is comprised in a biological tissue, such as for example, mammalian tissues e.g. the skin.

As mentioned, the method of the present invention is effected by contacting the cell with a composition from an organism capable of preventing adhesion of the cell to a surface.

As used herein the term "contacting" refers to the positioning of the compositions of the present invention such that they are in direct or indirect contact with the adhesive cells in such a way that the active agent comprised within is able to prevent adhesion of cells thereto. Thus, the present invention contemplates both applying the compositions of the present invention to a desirable surface and/or directly to the adhesive cells.

The contacting may be effected in vivo (i.e. within a mammalian body), ex vivo (i.e. in cells removed from the body) and/ or in vitro (i.e. outside a mammalian body).

Contacting the compositions with a surface can be effected using any method known in the art including spraying, spreading, wetting, immersing, dipping, painting, ultrasonic welding, welding, bonding or adhering. The compositions of the present invention may be attached as monolayers or multiple layers.

According to one embodiment, the compositions of the present invention may be comprised in a whole living organism. For example, the present invention contemplates adding live aquatic organisms to an underwater environment such that they are able to contact a surface and/or cells adhered thereto (e.g. underwater pipes, ship hull) preventing microorganism adhesion thereto. It will be appreciated that the active agent may be secreted from the aquatic organism. In this case, the aquatic organism does not have to be in direct contact with the surface or microorganism cells, but in sufficient proximity such that the active agent is able to diffuse to its site of action. Thus, the compositions of the present invention may be secreted into water and used in water purification treatments such as for example desalination of sea water or brackish water.

According to a further aspect of the present invention, there is provided a pharmaceutical composition comprising a pharmaceutically acceptable carrier or diluent and as an active ingredient a peptide isolated from an isolated natural peptide, said peptide comprising a sequence selected from the group consisting of YDYNWY, YDYNLY, FDYNFY, FDYNLY, WDYNLY, FDYNWY, YDWNLY and YDWHLY, or any other sequence as described herein.

According to other embodiments of the present invention, the above peptides may optionally be altered so as to form non-peptide analogs, including but not limited to replacing one or more bonds with less labile bonds, cyclization (described in greater detail below) and the like. Additionally or alternatively, a peptide may optionally be converted to a small molecule through computer modeling, as described for example in PCT Application No. WO/2007/147098, hereby incorporated by reference as if fully set forth herein.

A "peptidomimetic organic moiety" can optionally be substituted for amino acid residues in a peptide according to the present invention both as conservative and as non-conservative substitutions. These moieties are also termed "non-natural amino acids" and may optionally replace amino acid residues, amino acids or act as spacer groups within the peptides in lieu of deleted amino acids. The peptidomimetic organic moieties optionally and preferably have steric, electronic or configurational properties similar to the replaced amino acid and such peptidomimetics are used to replace amino acids in the essential positions, and are considered conservative substitutions. However such similarities are not necessarily required. The only restriction on the use of peptidomimetics is that the composition at least substantially retains its physiological activity as compared to the native peptide according to the present invention.

Peptidomimetics may optionally be used to inhibit degradation of the peptides by enzymatic or other degradative processes. The peptidomimetics can optionally and preferably be produced by organic synthetic techniques. Non-limiting examples of suitable peptidomimetics include D amino acids of the corresponding L amino acids, tetrazol (Zabrocki et al., J. Am. Chem. Soc. 110:5875 5880 (1988)); isosteres of amide bonds (Jones et al., Tetrahedron Lett. 29: 3853 3856 (1988)); LL 3 amino 2 propenidone 6 carboxylic acid (LL Acp) (Kemp et al., J. Org. Chem. 50:5834 5838 (1985)). Similar analogs are shown in Kemp et al., Tetrahedron Lett. 29:5081 5082 (1988) as well as Kemp et al., Tetrahedron Lett. 29:5057 5060 (1988), Kemp et al., Tetrahedron Lett. 29:4935 4938 (1988) and Kemp et al., J. Org. Chem. 54:109 115 (1987). Other suitable but exemplary peptidomimetics are shown in Nagai and Sato, Tetrahedron Lett. 26:647 650 (1985); Di Maio et al., J. Chem. Soc. Perkin Trans., 1687 (1985); Kahn et al., Tetrahedron Lett. 30:2317 (1989); Olson et al., J. Am. Chem. Soc. 112:323 333 (1990); Garvey et al., J. Org. Chem. 56:436 (1990). Further suitable exemplary

peptidomimetics include hydroxy 1,2,3,4 tetrahydroisoquinoline 3 carboxylate (Miyake et al., J. Takeda Res. Labs 43:53 76 (1989)); 1,2,3,4 tetrahydro- isoquinoline 3 carboxylate (Kazmierski et al., J. Am. Chem. Soc. 133:2275 2283 (1991)); histidine isoquinolone carboxylic acid (HIC) (Zechel et al., Int. J. Pep. Protein Res. 43 (1991)); (2S, 3S) methyl phenylalanine, (2S, 3R) methyl phenylalanine, (2R, 3S) methyl phenylalanine and (2R, 3R) methyl phenylalanine (Kazmierski and Hruby, Tetrahedron Lett. (1991.((

Exemplary, illustrative but non-limiting non-natural amino acids include beta-amino acids (beta3 and beta2), homo-amino acids, cyclic amino acids, aromatic amino acids, Pro and Pyr derivatives, 3-substituted Alanine derivatives, Glycine derivatives, ring-substituted Phe and Tyr Derivatives, linear core amino acids or diamino acids. They are available from a variety of suppliers, such as Sigma-Aldrich (USA) for example

In the present invention any part of a peptide may optionally be chemically modified, i.e. changed by addition of functional groups. The modification may optionally be performed during synthesis of the molecule if a chemical synthetic process is followed, for example by adding a chemically modified amino acid. However, chemical modification of an amino acid when it is already present in the molecule ("in situ" modification) is also possible .

The amino acid of any of the sequence regions of the molecule can optionally be modified according to any one of the following exemplary types of modification (in the peptide conceptually viewed as "chemically modified"). Non-limiting exemplary types of modification include carboxymethylation, acylation, phosphorylation, glycosylation or fatty acylation. Ether bonds can optionally be used to join the serine or threonine hydroxyl to the hydroxyl of a sugar. Amide bonds can optionally be used to join the glutamate or aspartate carboxyl groups to an amino group on a sugar (Garg and Jeanloz, Advances in Carbohydrate Chemistry and Biochemistry, Vol. 43, Academic Press (1985); Kunz, Ang. Chem. Int. Ed. English 26:294-308 (1987)). Acetal and ketal bonds can also optionally be formed between amino acids and carbohydrates. Fatty acid acyl derivatives can optionally be made, for example, by acylation of a free amino group (e.g., lysine) (Toth et al., Peptides: Chemistry, Structure and Biology, Rivier and Marshal, eds., ESCOM Publ., Leiden, 1078-1079 (1990)).

As used herein the term "chemical modification", when referring to a peptide according to the present invention, refers to a peptide where at least one of its amino acid residues is modified either by natural processes, such as processing or other post-translational modifications, or by chemical modification techniques which are well known in the art. Examples of the numerous known modifications typically include, but are not limited to: acetylation, acylation, amidation, ADP-ribosylation, glycosylation, GPI anchor formation,

covalent attachment of a lipid or lipid derivative, methylation, myristylation, pegylation, prenylation, phosphorylation, ubiquitination, or any similar process.

According to some embodiments of this aspect of the present invention, there is provided a method of preventing or treating a pathogen infection in a subject in need thereof, the method comprising administering to the subject a therapeutically effective amount of the pharmaceutical composition, thereby treating or preventing the pathogen infection.

According to alternative embodiments of this aspect of the present invention, there is provided a method of preventing attachment of exogenous bacteria to the gastrointestinal tract.

The mammalian gastrointestinal tract contains a wide variety of indigenous microflora, which provide resistance to colonization by enteric pathogen. In return for providing the host with enhanced defense against pathogens, the indigenous microflora gain access to a nutrient-enriched, stable environment, and thereby enter a symbiotic relation with the host's intestinal tract.

Symbiotic bacteria attach to the gastrointestinal epithelium in humans by high-affinity, receptor-mediated attachment. In contrast, exogenous bacteria attach to the epithelium by a low-affinity mechanism. Without wishing to be limited by a single hypothesis, the compositions of the present invention are expected to selectively prevent or decrease this low-affinity attachment, thereby preventing the initial step of biofilm formation.

The composition of the present invention is therefore useful for treatment or prevention of diseases of the gastrointestinal tract, such as, for example, Crohn's disease or ulcerative colitis, including, for example, collagneous colitis, lymphocytic colitis, ischaemic colitis, diversion colitis, infective colitis and Behcet's syndrome.

As used herein a "pharmaceutical composition" refers to a preparation of one or more of the active ingredients described herein with other chemical components such as physiologically suitable carriers and excipients. The purpose of a pharmaceutical composition is to facilitate administration of a compound to an organism.

As used herein the term "active ingredient" refers to the organism compositions (and agents purified therefrom) accountable for the intended biological effect.

Hereinafter, the phrases "physiologically acceptable carrier" and "pharmaceutically acceptable carrier," which may be used interchangeably, refer to a carrier or a diluent that does not cause significant irritation to an organism and does not abrogate the biological activity and properties of the administered compound. An adjuvant is included under these phrases.

Herein, the term "excipient" refers to an inert substance added to a pharmaceutical composition to further facilitate administration of an active ingredient. Examples, without

limitation, of excipients include calcium carbonate, calcium phosphate, various sugars and types of starch, cellulose derivatives, gelatin, vegetable oils, and polyethylene glycols.

Techniques for formulation and administration of drugs may be found in the latest edition of "Remington's Pharmaceutical Sciences," Mack Publishing Co., Easton, PA, which is herein fully incorporated by reference and are further described herein below.

As mentioned, the pharmaceutical compositions of the present invention may be administered to a subject in need thereof in order to prevent or treat a pathogen infection.

As used herein the term "subject in need thereof" refers to a mammal, preferably a human subject.

As used herein the term "treating" refers to curing, reversing, attenuating, alleviating, minimizing, suppressing or halting the deleterious effects of a pathogen infection.

As used herein the phrase "pathogen infection" refers to any medical condition which is caused by a pathogenic organism. Examples of pathogen infections include, but are not limited to, chronic infectious diseases, subacute infectious diseases, acute infectious diseases, viral diseases, bacterial diseases, protozoan diseases, parasitic diseases, fungal diseases, mycoplasma diseases, archaea diseases and prion diseases.

According to one embodiment, the pathogen infection is caused by an organism capable of growing in or on a biofilm.

Examples of pathogen infections caused by microbial biofilms include native valve endocarditis (NVE), otitis media (OM), chronic bacterial prostatitis, cystic fibrosis (CF) and periodontitis. Additional pathogen infections that are not specifically attributed to biofilms include, but are not limited to urinary infections, female genital tract infections and pneumonia. Infections due to implantation of medical devices include vascular catheter infections, arterial prosthetic infections, infections of prosthetic heart valves, prosthetic joint infections, infections of central nervous system shunts, orthopedic implant infections, pacemaker and defibrillator infections, hemodialysis and peritoneal dialysis infections, ocular infections, urinary tract infections, infections of the female genital tract, infections associated with endotracheal intubation and tracheostomy and dental infections.

As used herein the phrase "pathogenic organism" refers to any single cell organism which is capable of causing disease, especially a living microorganism such as a bacteria or fungi. Preferably the pathogenic organism is capable of growing in or on a biofilm. Many common pathogenic organisms exist in mammals (e.g. humans) as biofilms and cause disease. These include, but are not limited to, *Mannheimia haemolytica* and *Pasteurella multocida* (causing pneumonia), *Fusobacterium necrophorum* (causing liver abscess), *Staphylococcus*

aureus and *Pseudomonas aeruginosa* (causing wound infections), *Escherichia coli* and *Salmonella* spp (causing enteritis), *Staphylococcus aureus* and *Staphylococcus epidermidis* (causing OM), and *Streptococci* sp., *Staphylococci* sp., *Candida*, and *Aspergillus* sp. (causing NVE).

It will be appreciated that treatment of infectious diseases according to the present invention may be combined with other treatment methods known in the art (i.e., combination therapy). These include, but are not limited to, antimicrobial agents such as penicillins, cephalosporins, carbapenems, aminoglycosides, macrolides, lincomycins, tetracyclines, chloramphenicol, and griseofulvin.

Suitable routes of administration may, for example, include oral, rectal, transmucosal, especially transnasal, intestinal, or parenteral delivery, including intramuscular, subcutaneous, and intramedullary injections, as well as intrathecal, direct intraventricular, intravenous, inrtaperitoneal, intranasal, or intraocular injections.

Alternately, one may administer the pharmaceutical composition in a local rather than systemic manner, for example, via injection of the pharmaceutical composition directly into a tissue region of a patient.

Pharmaceutical compositions of the present invention may be manufactured by processes well known in the art, e.g., by means of conventional mixing, dissolving, granulating, dragee-making, levigating, emulsifying, encapsulating, entrapping, or lyophilizing processes.

Pharmaceutical compositions for use in accordance with the present invention thus may be formulated in conventional manner using one or more physiologically acceptable carriers comprising excipients and auxiliaries, which facilitate processing of the active ingredients into preparations that can be used pharmaceutically. Proper formulation is dependent upon the route of administration chosen.

For injection, the active ingredients of the pharmaceutical composition may be formulated in aqueous solutions, preferably in physiologically compatible buffers such as Hank's solution, Ringer's solution, or physiological salt buffer. For transmucosal administration, penetrants appropriate to the barrier to be permeated are used in the formulation. Such penetrants are generally known in the art.

For topical administration, the compositions of the present invention may be formulated as a gel, a cream, a wash, a rinse or a spray.

For oral administration, the pharmaceutical composition can be formulated readily by combining the active compounds with pharmaceutically acceptable carriers well known in the art. Such carriers enable the pharmaceutical composition to be formulated as tablets, pills, dragees,

capsules, liquids, gels, syrups, slurries, suspensions, and the like, for oral ingestion by a patient. Pharmacological preparations for oral use can be made using a solid excipient, optionally grinding the resulting mixture, and processing the mixture of granules, after adding suitable auxiliaries as desired, to obtain tablets or dragee cores. Suitable excipients are, in particular, fillers such as sugars, including lactose, sucrose, mannitol, or sorbitol; cellulose preparations such as, for example, maize starch, wheat starch, rice starch, potato starch, gelatin, gum tragacanth, methyl cellulose, hydroxypropylmethyl-cellulose, and sodium carbomethylcellulose; and/or physiologically acceptable polymers such as polyvinylpyrrolidone (PVP). If desired, disintegrating agents, such as cross-linked polyvinyl pyrrolidone, agar, or alginic acid or a salt thereof, such as sodium alginate, may be added.

Dragee cores are provided with suitable coatings. For this purpose, concentrated sugar solutions may be used which may optionally contain gum arabic, talc, polyvinyl pyrrolidone, carbopol gel, polyethylene glycol, titanium dioxide, lacquer solutions, and suitable organic solvents or solvent mixtures. Dyestuffs or pigments may be added to the tablets or dragee coatings for identification or to characterize different combinations of active compound doses.

Pharmaceutical compositions that can be used orally include push-fit capsules made of gelatin, as well as soft, sealed capsules made of gelatin and a plasticizer, such as glycerol or sorbitol. The push-fit capsules may contain the active ingredients in admixture with filler such as lactose, binders such as starches, lubricants such as talc or magnesium stearate, and, optionally, stabilizers. In soft capsules, the active ingredients may be dissolved or suspended in suitable liquids, such as fatty oils, liquid paraffin, or liquid polyethylene glycols. In addition, stabilizers may be added. All formulations for oral administration should be in dosages suitable for the chosen route of administration.

For buccal administration, the compositions may take the form of tablets or lozenges formulated in conventional manner.

For administration by nasal inhalation, the active ingredients for use according to the present invention are conveniently delivered in the form of an aerosol spray presentation from a pressurized pack or a nebulizer with the use of a suitable propellant, e.g., dichlorodifluoromethane, trichlorofluoromethane, dichloro-tetrafluoroethane, or carbon dioxide. In the case of a pressurized aerosol, the dosage may be determined by providing a valve to deliver a metered amount. Capsules and cartridges of, for example, gelatin for use in a dispenser may be formulated containing a powder mix of the compound and a suitable powder base, such as lactose or starch.

The pharmaceutical composition described herein may be formulated for parenteral administration, e.g., by bolus injection or continuous infusion. Formulations for injection may be presented in unit dosage form, e.g., in ampoules or in multidose containers with, optionally, an added preservative. The compositions may be suspensions, solutions, or emulsions in oily or aqueous vehicles, and may contain formulatory agents such as suspending, stabilizing, and/or dispersing agents.

Pharmaceutical compositions for parenteral administration include aqueous solutions of the active preparation in water-soluble form. Additionally, suspensions of the active ingredients may be prepared as appropriate oily or water-based injection suspensions. Suitable lipophilic solvents or vehicles include fatty oils such as sesame oil, or synthetic fatty acid esters such as ethyl oleate, triglycerides, or liposomes. Aqueous injection suspensions may contain substances that increase the viscosity of the suspension, such as sodium carboxymethyl cellulose, sorbitol, or dextran. Optionally, the suspension may also contain suitable stabilizers or agents that increase the solubility of the active ingredients, to allow for the preparation of highly concentrated solutions.

Alternatively, the active ingredient may be in powder form for constitution with a suitable vehicle, e.g., a sterile, pyrogen-free, water-based solution, before use.

The pharmaceutical composition of the present invention may also be formulated in rectal compositions such as suppositories or retention enemas, using, for example, conventional suppository bases such as cocoa butter or other glycerides.

Pharmaceutical compositions suitable for use in the context of the present invention include compositions wherein the active ingredients are contained in an amount effective to achieve the intended purpose. More specifically, a "therapeutically effective amount" means an amount of active ingredients (e.g., an aquatic organism composition or a moss composition) effective to prevent, alleviate, or ameliorate symptoms of a pathogenic infection (e.g., fever) or prolong the survival of the subject being treated.

Determination of a therapeutically effective amount is well within the capability of those skilled in the art, especially in light of the detailed disclosure provided herein.

For any preparation used in the methods of the invention, the dosage or the therapeutically effective amount can be estimated initially from in vitro and cell culture assays. For example, a dose can be formulated in animal models to achieve a desired concentration or titer. Such information can be used to more accurately determine useful doses in humans.

Toxicity and therapeutic efficacy of the active ingredients described herein can be determined by standard pharmaceutical procedures in vitro, in cell cultures or experimental

animals. The data obtained from these in vitro and cell culture assays and animal studies can be used in formulating a range of dosage for use in human. The dosage may vary depending upon the dosage form employed and the route of administration utilized. The exact formulation, route of administration, and dosage can be chosen by the individual physician in view of the patient's condition. (See, e.g., Fingl, E. et al. (1975), "The Pharmacological Basis of Therapeutics," Ch. 1, p.1.)

Dosage amount and administration intervals may be adjusted individually to provide sufficient plasma or brain levels of the active ingredient to induce or suppress the biological effect (i.e., minimally effective concentration, MEC). The MEC will vary for each preparation, but can be estimated from in vitro data. Dosages necessary to achieve the MEC will depend on individual characteristics and route of administration. Detection assays can be used to determine plasma concentrations.

Depending on the severity and responsiveness of the condition to be treated, dosing can be of a single or a plurality of administrations, with course of treatment lasting from several days to several weeks, or until cure is effected or diminution of the disease state is achieved.

The amount of a composition to be administered will, of course, be dependent on the subject being treated, the severity of the affliction, the manner of administration, the judgment of the prescribing physician, etc.

Compositions of the present invention may, if desired, be presented in a pack or dispenser device, such as an FDA-approved kit, which may contain one or more unit dosage forms containing the active ingredient. The pack may, for example, comprise metal or plastic foil, such as a blister pack. The pack or dispenser device may be accompanied by instructions for administration. The pack or dispenser device may also be accompanied by a notice in a form prescribed by a governmental agency regulating the manufacture, use, or sale of pharmaceuticals, which notice is reflective of approval by the agency of the form of the compositions for human or veterinary administration. Such notice, for example, may include labeling approved by the U.S. Food and Drug Administration for prescription drugs or of an approved product insert. Compositions comprising a preparation of the invention formulated in a pharmaceutically acceptable carrier may also be prepared, placed in an appropriate container, and labeled for treatment of an indicated condition, as further detailed above.

As mentioned, medical devices and implants are commonly infected with opportunistic bacteria and other infectious microorganisms (e.g., fungi) in some cases necessitating the removal of implantable devices. Such infections can also result in illness, long hospital stays, or

even death. The prevention of biofilm formation and infection of medical devices is therefore highly desirous.

Thus, the present invention also contemplates medical devices in which the above-described compositions are attached thereto.

As used herein the term "medical device" refers to any implant, instrument, apparatus, implement, machine, device or any other similar or related object (including any component or accessory), which is intended for use in the diagnosis, treatment, cure or prevention of disease or other conditions. Such medical device is intended for use in man or other animals and is anticipated to affect the structure or any function of the body. Such medical device does not achieve its primary intended purposes through chemical action and is not dependent upon being metabolized for the achievement of its primary intended purposes.

As used herein the term "implant" refers to any object intended for placement in a human body that is not a living tissue. The implant may be temporary or permanent. An implant can be an article comprising artificial components, such as catheters or pacemakers. Implants can also include naturally derived objects that have been processed so that their living tissues have been devitalized. As an example, bone grafts that have been processed so that their living cells are removed (acellularized), but so that their shape is retained to serve as a template for ingrowth of bone from a host. As another example, naturally occurring coral can be processed to yield hydroxyapatite preparations that can be applied to the body for certain orthopedic and dental therapies.

The present invention therefore envisions coating medical devices with the compositions of the present invention to prevent cell adherence thereto so as to reduce/eliminate any possible cell aggregation and biofilm formation known to occur following implantation. Device-related infections usually result from the introduction of microorganisms, primarily bacteria, during the device insertion or implantation procedure, or from attachment of blood-borne organisms to the newly inserted device and their subsequent propagation on its surface. Coating the medical device with the compositions of the present invention will therefore inhibit biofilm formation of one or more microbial species, will prevent medical device related infections, and consequently will reduce the need of antibiotic treatment or removal of the medical device from the subject.

Medical devices that may be coated according to the teachings of the present invention include, but not limiting to, artificial blood vessels, catheters and other devices for the removal or delivery of fluids to patients, artificial hearts, artificial kidneys, orthopedic pins, prosthetic joints, plates and implants; catheters and other tubes (including urological and biliary tubes, endotracheal tubes, peripherably insertable central venous catheters, dialysis catheters, long term

tunneled central venous catheters, peripheral venous catheters, short term central venous catheters, arterial catheters, pulmonary catheters, Swan-Ganz catheters, urinary catheters, peritoneal catheters), urinary devices (including long term urinary devices, tissue bonding urinary devices, artificial urinary sphincters, urinary dilators), shunts (including ventricular or arterio-venous shunts); prostheses (including breast implants, penile prostheses, vascular grafting prostheses, aneurysm repair devices, mechanical heart valves, artificial joints, artificial larynxes, otological implants), anastomotic devices, vascular catheter ports, vascular stents, clamps, embolic devices, wound drain tubes, ocular lenses, dental implants, hydrocephalus shunts, pacemakers and implantable defibrillators, needleless connectors, voice prostheses and the like.

Another possible application of the compositions of the present invention is the coating of surfaces found in the medical and dental environment. Such surfaces include the inner and outer aspects of various instruments and devices, whether disposable or intended for repeated uses. Such surfaces include the entire spectrum of articles adapted for medical use, including without limitation, scalpels, needles, scissors and other devices used in invasive surgical, therapeutic or diagnostic procedures; blood filters. Other examples will be readily apparent to practitioners in these arts.

Surfaces found in the medical environment also include the inner and outer aspects of pieces of medical equipment, medical gear worn or carried by personnel in the health care setting. Such surfaces can include surfaces intended as biological barriers to infectious organisms in medical settings, such as gloves, aprons and faceshields. Commonly used materials for biological barriers are thermoplastic or polymeric materials such as polyethylene, dacron, nylon, polyesters, polytetrafluoroethylene, polyurethane, latex, silicone and vinyl. Other surfaces can include counter tops and fixtures in areas used for medical procedures or for preparing medical apparatus, tubes and canisters used in respiratory treatments, including the administration of oxygen, of solubilized drugs in nebulizers and of anesthetic agents. Other such surfaces can include handles and cables for medical or dental equipment not intended to be sterile. Additionally, such surfaces can include those non-sterile external surfaces of tubes and other apparatus found in areas where blood or body fluids or other hazardous biomaterials are commonly encountered.

The compositions of the present invention can be used on the surface of or within these medical devices to provide long term protection against microorganism colonization and reduce the incidence of device-related infections. These compositions can also be incorporated in combination with an anti-microbial agent (e.g., antibiotic agent) into coatings for medical

devices. Such a combination will sufficiently kill or inhibit the initial colonizing bacteria and prevent device-related infections as long as the substance is presented in an inhibitory concentration at the device-microbe interface.

The compositions of the present invention can be directly incorporated into the polymeric matrix of the medical device at the polymer synthesis stage or at the device manufacture stage. The compositions can also be covalently attached to the medical device polymer. These and many other methods of coating medical devices are evident to one of ordinary skill in the art.

Additional surfaces that can be treated according to the teachings of the present invention include the inner and outer aspects of those articles involved in water purification, water storage and water delivery, and those articles involved in food processing. Thus the present invention envisions coating a solid surface of a food or beverage container to extend the shelf life of its contents.

Surfaces related to health can also include the inner and outer aspects of those household articles involved in providing for nutrition, sanitation or disease prevention. Thus, the compositions of the present invention can be used for removal of disease-causing microorganisms from external surfaces. These can include, for example food processing equipment for home use, materials for infant care, tampons, soap, detergents, health and skincare products, household cleaners and toilet bowls.

The surface may be also be laboratory articles including, but not limited to, microscopic slide, a culturing hood, a Petri dish or any other suitable type of tissue culture vessel or container known in the art.

The inventors of this application also envision the use of the compositions of the present invention as anti-fouling agents.

As used herein the term "anti-fouling agents" refers to the compounds used to protect underwater surfaces from attaching single cell organisms. These single cell organisms include microorganism such as bacteria and fungi.

These underwater surfaces include any water immersed surface, including ships'/boats's hulls (i.e., the body or frame of a ship or boat), submergence vehicles, navigational aids, screens, nets, constructions, floating or emplaced offshore platforms (e.g., docks), buoys, signaling equipment and articles which come into contact with sea water or salty water. Other underwater surfaces include structures exposed to sea water including pilings, marine markers, undersea conveyances like cabling and pipes, fishing nets, bulkheads, cooling towers, and any device or structure that operates submerged.

The compositions of the present invention can be incorporated into marine coatings to limit undesirable marine fouling. Thus, the anti-fouling agents of the present invention can be formulated so as not to contain toxic materials (such as heavy metals), and still retain their efficacy. The anti-fouling paint of the present invention may further contain binders(s), pigment(s), solvent(s) and additive(s).

Examples of solvents that may be used include aromatic hydrocarbons such as xylene and toluene; aliphatic hydrocarbons such as hexane and heptane, esters such as ethyl acetate and butyl acetate; amides such as N-methylpyrrolidone and N,N-dimethylformamide; alcohols such as isopropyl alcohol and butyl alcohol; ethers such as dioxane, THF and diethyl ether; and ketones such as methyl ethyl ketone, methyl isobutyl ketone and methyl isoamyl ketone. The solvents may be used alone or in combination thereof.

Examples of binders that may be used include alkyd resin, acrylic or vinyl emulsions, polyurethane resins, epoxy resins, silicone based resins, acrylic resins, inorganic silicate based resins, vinyl resins, particularly a vinyl chloride/vinyl acetate copolymer, and rosin.

Examples of pigments that may be used include titanium dioxide, cuprous oxide, iron oxide, talc, aluminium flakes, mica flakes, ferric oxide, cuprous thiocyanate, zinc oxide, cupric acetate meta-arsenate, zinc chromate, zinc dimethyl dithiocarbamate, zinc ethylene bis(dithiocarbamate) and zinc diethyl dithiocarbamate.

Examples of additives that may be incorporated into the coating composition include dehumidifiers, wetting/dispersing agents, anti-settling agents, anti-skinning agents, drying/curing agents, anti-marring agents and additives ordinarily employed in coating compositions as stabilizers and anti-foaming agents. Additionally, any antibiotic which is relatively insoluble in seawater can be used with an anti-fouling marine paint.

Methods of preparing marine anti-fouling paints are explained in detail in U.S. Pat. No. 4,678,512; U.S. Pat. No. 4,286,988; U.S. Pat. No. 4,675,051; U.S. Pat. No. 4,865,909; and U.S. Pat. No. 5,143,545.

The compositions of the present invention may also be used for providing antibacterial properties in cosmetics, to prevent spoiling of the product.

The compositions may further be used to provide an antibacterial effect to the mouth, teeth and gums, such as by incorporation in a toothpaste, mouthwash, or chewing gum.Taken together the present teachings portray a wide range of novel anti-adhesive agents isolated from organisms such as aquatic organisms and moss. The broad spectrum of the anti adhesion effects of these agents (e.g. inhibiting adhesion of gram positive and gram negative bacteria) together with their ability to effect the initial, vulnerable stages of microbial biofilm formation, makes

these agents prime candidates as anti-biofilm agents. Moreover, the anti-adhesive agents described herein are clonable enabling modifications and mass production thereof. In addition their stability (i.e. resistance to environmental conditions) makes these agents suitable for a diverse array of applications.

Additional objects, advantages, and novel features of the present invention will become apparent to one ordinarily skilled in the art upon examination of the following examples, which are not intended to be limiting. Additionally, each of the various embodiments and aspects of the present invention as delineated hereinabove and as claimed in the claims section below finds experimental support in the following examples.

Generally, the nomenclature used herein and the laboratory procedures utilized in the present invention include molecular, biochemical, microbiological and recombinant DNA techniques. Such techniques are thoroughly explained in the literature. See, for example, "Molecular Cloning: A laboratory Manual" Sambrook et al., (1989); "Current Protocols in Molecular Biology" Volumes I-III Ausubel, R. M., ed. (1994); Ausubel et al., "Current Protocols in Molecular Biology", John Wiley and Sons, Baltimore, Maryland (1989); Perbal, "A Practical Guide to Molecular Cloning", John Wiley & Sons, New York (1988); Watson et al., "Recombinant DNA", Scientific American Books, New York; Birren et al. (eds) "Genome Analysis: A Laboratory Manual Series", Vols. 1-4, Cold Spring Harbor Laboratory Press, New York (1998); methodologies as set forth in U.S. Pat. Nos. 4,666,828; 4,683,202; 4,801,531; 5,192,659 and 5,272,057; "Cell Biology: A Laboratory Handbook", Volumes I-III Cellis, J. E., ed. (1994); "Current Protocols in Immunology" Volumes I-III Coligan J. E., ed. (1994); Stites et al. (eds), "Basic and Clinical Immunology" (8th Edition), Appleton & Lange, Norwalk, CT (1994); Mishell and Shiigi (eds), "Selected Methods in Cellular Immunology", W. H. Freeman and Co., New York (1980); available immunoassays are extensively described in the patent and scientific literature, see, for example, U.S. Pat. Nos. 3,791,932; 3,839,153; 3,850,752; 3,850,578; 3,853,987; 3,867,517; 3,879,262; 3,901,654; 3,935,074; 3,984,533; 3,996,345; 4,034,074; 4,098,876; 4,879,219; 5,011,771 and 5,281,521; "Oligonucleotide Synthesis" Gait, M. J., ed. (1984); "Nucleic Acid Hybridization" Hames, B. D., and Higgins S. J., eds. (1985); "Transcription and Translation" Hames, B. D., and Higgins S. J., Eds. (1984); "Animal Cell Culture" Freshney, R. I., ed. (1986); "Immobilized Cells and Enzymes" IRL Press, (1986); "A Practical Guide to Molecular Cloning" Perbal, B., (1984) and "Methods in Enzymology" Vol. 1-317, Academic Press; "PCR Protocols: A Guide To Methods And Applications", Academic Press, San Diego, CA (1990); Marshak et al., "Strategies for Protein Purification and Characterization - A Laboratory Course Manual" CSHL Press (1996); all of which are

incorporated by reference as if fully set forth herein. Other general references are provided throughout this document. The procedures therein are believed to be well known in the art and are provided for the convenience of the reader. All the information contained therein is incorporated herein by reference.

EXAMPLES

Reference is now made to the following examples, which together with the above description, illustrate the invention in a non limiting fashion.

Example 1: MS/MS analysis of an active fraction extracted from Aiptesia anemone

Crude extract of *Aiptasia pulchella* (whole organism) was separated on Sephadex G-10 column resulted in 2 fractions, both exhibiting anti-adherence/biofilm formation activity (Figure 13).

Rechromatography of the high molecular fraction from Sephadex G-10 on Sephadex G-75 resulted in two main peaks representing high and low molecular fractions (Figure 14).

Reversed phase high performance liquid chromatography (RP-HPLC) separation with c-18 column, of the low molecular fraction from the G-75 column, in linear gradients of acetonitrile (3 - 80 % from 5 to 75 minutes) in 0.1% TFA at a flow rate of 2 ml/min, resulted in several active fractions as anti adhesive compounds on *Pseudomonas aeruginosa* ATCC 27853. Fractions were collected every 2 minutes (Figure 15).

All active fractions where digested by trypsin, analyzed by LC-MS/MS on Qtof Premier (Waters) and on LTQ-Orbitrap (Thermo) and identified by Pep-Miner and Sequest software against the Eukaryotes part of the nr database. Active fraction eluted at 72.3% acetonitrile (marked in red arrow) was found to be similar to Equinatoxin 5 from *Actinia equine.*

Example 2: Identification of a conserved region of anemone cytotoxin

Purified template DNA was prepared from 25 mg of *Aiptasia pulchella* and *Anemonia viridans* using the wizard genomic DNA purification kit (Promega, USDA), according to the manufacturer's protocol for isolation of genomic DNA from animal tissu. PCR was carried out on 500 ng of purified template DNA from *Aiptasia pulchella* and *Anemonia viridans* using Reddy Mix PCR master mix (ABgene, UK), with the following protocol: 95°C-5min (95°C 30sec, 52°C 30sec and 72°C 1min) X35, 72°C for 10min.

Primers Eqt-F (GTR TCG ACA ACG AGT CRG G) and Eqt-R252 (TGA CAT YCC ACC AGT TGC TG) were added to the reaction mixture, to a final concentration of 0.5μM each.

Positive PCR reactions which gave DNA amplicon of size of ~250bp were sent for DNA sequencing.

A PCR amplicon from *Aiptasia pulchella* gave the following 265bp sequence:

GTGTCGCCAACGAGTCGGGATGCACTTGGGAAAAGCCAAATACATACTTCTTCTCTG
GTACTGAGGTATAAAGTGCCTCCCTCTAAAGCTTGAGAATAAAAAAGCACTTTTGTA
CGGCCCACGTAAGACAACAGGGCCTGTTGCCACGGGAGCTGTTGGAGTGCTCACTT
ACAAAATGTTGTGCACCAATGAGACGAACACTCTGGCTGTTCTTTTCAGTGTACCCT
TCGACTACAACTTGTACAGCAACTGGTGGAAATGTCAA

BLASTx comparison of the predicted amino acid sequence encoded by the above polynucleotide sequence to known protein sequences in the GeneBank provided the following results: Identities = 54/88 (61%), Positives = 62/88 (70%) To other anemone cytotoxins like: hemolytic toxin [Actineria villosa], PsTX-20A [Phyllodiscus semoni], cytolysin I precursor [Sagartia rosea] and equinatoxin IV precursor [Actinia equina]; (accession numbers: BAD74019.1, BAC45007.1, AAP04347.1 and AF057028_1).

The relevant peptide sequence [FSVPFDYNLYSNWW] appears in the Aiptasial sequence.

PCR amplicon from *Anemonia viridans* gave the following 254bp sequence:

TGTGTCGACAACGAGTCgGGCaagacgtGgaCCGCAntgaaCACATACTTCCGTTCTGGcAC
CTCTGATnTCrTCCTTCCCCATACAGTTCCACATGGTAAGGCACTGCTCTACAACGGT
CAGAAAGATCGTGGTCCAGTTGCGACTGGCGtgGTTGGAGTACTTGCTTATGcCATGA
GCgATGGAAACACCCtGGCCGTTTTgTTCAGCrTTCCCTaTGACTATAACCtGTACAGCA
ACTGGTGGAATGTCAA

BLASTn comparison to known nucleotide sequences in the GeneBank gave similarities of 97%, 96% and 95% to Equinatoxins 5 [accession number: AEU51900] , 4 [accession number: AF057028] and 2 [accession number: AEU41661], in correspondence.

Predicted amino acid sequence based on translation of the second positive ORF gave the following AA sequence:

CRQRVGMHLGKAKYILLLWY*GIKCLPLKLENKKALLYGPRKTTGPVATGAVGVLTYK
MLCTNETNTLAVLFSVPFDYNLYSNWWKCQ

Example 3: Comparison of activity of synthetic peptides

Peptides listed below were synthesized using solid-phase methods and purification to 90% scale was performed by Peptron Inc. (Taejeon, Korea).

The peptides were dissolved using 20µl dimethyl sulfoxide (DMSO) and diluted in double distilled-water to a concentration of 5mg/ml. Further dilutions were performed in phosphate buffered saline (PBS).

The activities of the following synthetic peptides were studied on a clinical isolate of *Acinetobacter Baumannii* and *Pseudomonas aeruginosa* ATCC 27853 at peptide concentrations ranging from 500- 0.5µg/ml. Peptides diluted to appropriate concentrations were incubated with the bacteria for 24-48 hours.

For bacterial adherence bioassys, biofilms were grown in 96-well round-bottom polystyrene plates. Briefly, 180 µl of overnight cultures were added to wells supplemented with 20µl of appropriate peptide diluted in PBS. After 24 h of incubation at 37°C, each well was washed with water and was stained with 250 µl of crystal violet solution. The dye was then removed by thorough washing with water. For quantification of attached cells, crystal violet was solubilized in 250 µl of 1% sodium dodecyl sulfate (SDS) and the absorbance was measured at 595nm.

CMFSVPFDYNWYSNWWC	AbacZ-17C
Ac-MFSVPFDYNWYSNWW-NH2	AbacZ-15
CFSVPFDYNWYSNWWC	AbacZ-16C
FDYNWY	AbacZ-6
CFDYNWYC	AbacZ-8C

Results are shown in Figures 1 to 12. As seen in Figures 1, 3, 5 and 7, the peptides did not kill or inhibit growth of bacteria. Figures 2, 4, 6, 8 and 10 to 12, demonstrate that the peptides prevented the formation of biofilms.

Example 4: Identification of preferred peptides

In order to identify the most active cyclic peptides according to the present invention, a manual Parallel Peptide synthesizer with peptide purifier is used to produce peptides which differ from each other in length and cyclization strategy. Each peptide is screened for anti-adhesive activity in microplate and flow cell assays, and selection of highly active peptides is performed. Computerized modeling of several versions of peptide is used to optimize selection of the active compounds.

For more sensitive screening the bioassay is scaled up using the BacTiter-Glo microbial cell viability Assay of Promega, USA. This method uses more sensitive spectrometric technique based on luminescence.

Various cyclization strategies are used to obtain optimized cyclic peptide from linear analog with satisfactory bioactivity. Figure 16A shows the generalized structure of the cyclic lead with emulsifier arm. The linear analog is a 14mer peptide with hydrophobic core (6aa) that is defined as a pharmacophore. Cyclic leads are prepared, preserving this pharmacophore and an emulsifying arm (hydrophobic moiety) such as polyethylene, polypropylene, Teflon etc. is then added to provide absorbing capabilities to a hydrophobic polymeric surface (Figure 16B).

Epitope mapping, escanning and Cycloscan methodologies are used for revealing shorter, more cost-effective, peptides possessing the desired biological activity.

The cyclic lead is prepared using 9H-fluoren-9-ylmethoxycarbonyl solid phase peptide synthesis (Fmoc SPPS).

According to a representative procedure, as shown in Figure 17, the first protected amino acid is condensed with chlorotitryl (Cl-Trt) resin using N,N-diisopropylethylamine (DIEA) in dichloromethane (DCM) or with Rink Amide using O-Benzotriazole-N,N,N',N'-tetramethyl-uronium-hexafluoro-phosphate (HBTU) as coupling reagent.

The next couplings are performed using a standard Fmoc protocol with 2-(1H-7-Azabenzotriazol-1-yl)--1,1,3,3-tetramethyl uronium hexafluorophosphate Methanaminium (HATU) or benzotriazol-1-yl-oxytripyrrolidinophosphonium hexafluorophosphate (PyBoP), DIEA in N-methyl-2-pyrrolidinone (NMP). The allyloxycarbonyl (alloc) groups are deprotected by Pd-triphenylphosphine (Tetrakis) using acetyl hydroxide/ N-methyl maleimide/diethene chloride (AcOH/NMM/DCE) cocktail.

The cyclization step is carried out as a standard coupling reaction (in the case of formation of an amide bond) or by bubbling oxygen (in the case of formation of a disulfide bridge). Other types of cyclizations as known in the art may also be performed.

The peptides are cleaved from the resin by treatment with trifluoroacetic acid:dichloromethane:tri-iso-propylsilane (TFA:DCM:TIS), 1:98:1 in the presence of 1,2-ethanedithiol for 30 min in case of the Cl-Trt resin, and in the presence of 95% TFA, TIS and H_2O in the case of Rink Amide.

The crude product in the solution (AcOH/H_2O 1:1) is purified by preparative HPLC or MPLC to afford pure cyclic peptide. The purity is determined by analytical HPLC. The structures are confirmed by LC-MS and AA analysis.

The next stage involves linkage of the hydrophobic arm for introducing absorbing properties to the cyclic peptide lead. This arm is linked using standard SPPS protocol.

Figure 17 shows a flowchart outlining the process for development of a cyclic peptide lead with an emulsifying arm.

Example 4: Treatment of water or fluid medium

The above peptides and/or compositions and/or organisms may optionally and preferably be used to treat water and/or a fluid medium, or a system or apparatus containing such, including but not limited to a reverse osmosis filter and/or filtration apparatus or system.

The effect of Actiniaria extracts on biofilm formation using polyamide coupons in a flow cell without filtration is tested as follows. The effect of biofilm growth is analyzed in a flowcell dedicated for confocal microscopy on a similar polyamide surface as an RO (reverse osmosis) active layer. A dual channel flowcell (FC 270, Biosurface Technologies, Montana, USA) is operated with both model strains and real microbial inoculum taken from reverse osmosis (RO) membrane coupon located in selected places on the Mediterranean sea supplemented with different concentrations (from nanograms to micrograms per ml) of the extracts. The flow regime in the flowcells is laminar and similar to a typical RO operational flow conditions. For the model strains, seawater synthetic media is determined (see (Fritzmann et al., 2007: Fritzmann, C., Lowenberg, J., Wintgens, T., and Melin, T. (2007) State-of-the-art of reverse osmosis desalination. Desalination 216: 1-76) and IDE reports http://www.ide-tech.tech.com/) and are used for cell attachment biofilm growth experiments. For the microbial consortium being isolated from the desalination plant in Palmachim, real seawater is used as a media for microbial attachment and biofilm growth experiments. Model strains to be used are Vibrio fisheri and Caulobacter crescentus. In the dual channel flowcell, one channel is supplemented with an extract and the other serves as a control with only the solvent being added to the media (for example, when the extract is dissolved in ethanol).

The flowcell biofilms are microscopically analyzed at different time points (up to 14 days of experiment) when viable cells, dead cells and Extra cellular polymeric substances (EPS) are stained with fluorescent probes (different flourescent labelled lectins are used for probing different polysacharide constitutents in the EPS) and visualized with laser scanning confocal microscopy (LSCM). Microscopic analysis is performed using image processing analysis softwares such as Imaris bitplane and COMSTAT (Heydorn et al., 2002: Ersboll, B., Kato, J., Hentzer, M., Parsek, M.R., Tolker-Nielsen, T. et al. (2002) Statistical analysis of Pseudomonas

aeruginosa biofilm development: impact of mutations in genes involved in twitching motility, cell-to-cell signaling, and stationary-phase sigma factor expression. Applied and Environmental Microbiology 68: 2008-2017).

Calcium, which has significant effect on the adhesiveness and the compactness of the biofilm is also monitored and visualized with LSCM using Calcium specific fluorochromes such as Fura-2 (Grynkiewicz et al., 1985: Grynkiewicz, G., Poenie, M., and Tsien, R.Y. (1985) A new generation of Ca2+ indicators with greatly improved fluorescence properties. Journal of Biological Chemistry 260: 3440-3450; Neu et al., 2002: Neu, T.R., Kuhlicke, U., and Lawrence, J.R. (2002) Assessment of Fluorochromes for Two-Photon Laser Scanning Microscopy of Biofilms. Applied and Environmental Microbiology 68: 901-909).

Antifouling properties are also examined by analyzing the adherence of different types of EPC/bacteria with QCM-D surface-modified crystals covalently bonded to different peptides.

QCM-D employs an ultra-sensitive mass sensor (silica-coated quartz crystal) housed inside a flow cell with a well-defined geometry and hydrodynamic characteristics, a design that allows real-time monitoring of mass adsorption with no required labeling. The piezoelectric quartz crystal oscillates laterally with an amplitude of 1-2 nm when a voltage is applied to the electrodes affixed to the quartz crystal. As deposition (adsorption) occurs on the crystal surface, it leads to a shift in the vibrational frequency of the crystal. In addition to monitoring the frequency shift to determine the adsorbed mass, thickness and structural conformation of the adsorbed layer can be extracted by simultaneous monitoring of the energy of dissipation, which is the sum of all energy losses within the system per oscillation cycle. Intriguingly, in addition to measuring the adsorption and adherence of the different types of EPS (i.e., changes in polysaccharides/protein contents) under different environmental conditions (i.e., changes in divalent cation concentrations), QCM-D can also reveal the visco-elastic properties, conformational changes, and thickness of the precipitated nano-layer.

Example 5: Effect of active peptides on reverse osmosis biofouling under desalination conditions

The effect of Actiniaria extracts on reverse osmosis biofouling under desalination conditions is determined.

Two RO (reverse osmosis) bench-scale units are operated for desalination of seawater, and biofouling experiments with both candidate model strains and microbial consortium isolated from the GES desalination plant (as mentioned above) are conducted both in a synthetic seawater media and a real seawater. Commercialized flat-sheet membranes SW-30 of Dow-

Filmtec are used for these biofouling experiments. Specific measures of process conditions are obtained: permeate flux, total organic carbon (TOC), oxygen concentrations in the permeate and in the brine solution, oxygen uptake rate, and the rejection of different ions and cations by the membrane. Different biofilm components are analyzed: Chemical analysis of the biofouling layer will include charcterization of proteins, carbohydrates, lipids, and DNA. Microscopic observation and analysis is performed as mentioned above.

While the invention has been described with respect to a limited number of embodiments, it will be appreciated that many variations, modifications and other applications of the invention may be made.

2009295575 15 Oct 2015

THE CLAIMS DEFINING THE INVENTION ARE AS FOLLOWS :

1. A peptide selected from the group consisting of YDYNWY, YDYNLY, FDYNFY, FDYNLY, WDYNLY, FDYNWY, YDWNLY and YDWHLY.

2. A method of preventing adhesion of a single cell organism to a surface selected from the group consisting of a fabric, a fiber, a foam, a film, a surface that comprises concrete, a surface that comprises masonry, a surface that comprises glass, a surface that comprises metal and a surface that comprises plastic, the method comprising the step of contacting the single cell organism with a composition which is devoid of cytotoxic or cytostatic activity and which comprises a peptide that comprises a sequence selected from the group consisting of YDYNWY, YDYNLY, FDYNFY, FDYNLY, WDYNLY, FDYNWY, YDWNLY and YDWHLY, wherein the composition prevents or reduces adhesion of the single cell organism to the surface, and wherein the peptide is cyclic and consists of a length of up to 50 amino acids.

3. A method as claimed in claim 2, in which the composition is in a form selected from the group consisting of a spray, a gel and a paint.

4. A medical device comprising a composition devoid of cytotoxic or cytostatic activity and comprising an isolated peptide comprising a sequence selected from the group consisting of YDYNWY, YDYNLY, FDYNFY, FDYNLY, WDYNLY, FDYNWY, YDWNLY and YDWHLY, wherein the composition is coated on a surface of the device or incorporated into a polymeric matrix of the medical device and the medical device is an intracorporeal device or an extracorporeal device, and wherein the peptide is cyclic and consists of a length of up to 50 amino acids.

5. A medical device as claimed in claim 4, in which the device is an implantable medical device or a medical instrument.

6. A method of treating water to prevent or reduce biofilm formation or fouling of a filter, the method comprising the step of treating the water with a composition which is devoid of cytotoxic or cytostatic activity and which comprises an isolated peptide comprising a sequence selected from the group consisting of YDYNWY, YDYNLY, FDYNFY, FDYNLY, WDYNLY, FDYNWY, YDWNLY and YDWHLY, and wherein the peptide is cyclic and consists of a length of up to 50 amino acids.

7. A method as claimed in claim 6, in which the treated water is applied to a reverse osmosis filter.

8. A method of preventing or reducing biofilm formation in an external fluid which does not originate from an animal body, the method comprising the step of treating the fluid with a composition which is devoid of cytotoxic or cytostatic activity and which comprises an isolated peptide comprising a sequence selected from the group consisting of YDYNWY, YDYNLY, FDYNFY, FDYNLY, WDYNLY, FDYNWY, YDWNLY and YDWHLY, and wherein the peptide is cyclic and consists of a length of up to 50 amino acids.

9. A

(a) method as claimed in any one of claims 2, 6, or 8, or

(b) medical device as claimed in claim 4,

in which the sequence YDYNWY is comprised in a protein which comprises the sequence LFSVPYDYNWYSNWW or LFSVPYDYNLYSNWW.

10. A

(a) method as claimed in any one of claims 2, 6, or 8, or

(b) medical device as claimed in claim 4,

in which the sequence YDYNLY is comprised in a protein which comprises the sequence MFSVPYDYNLYSNWV.

11. A

(a) method as claimed in any one of claims 2, 6, or 8, or

(b) medical device as claimed in claim 4,

in which the sequence FDYNFY is comprised in a protein which comprises the sequence MFSVPFDYNFYSNWW or LFSVPFDYNFYSNWW.

12. A

(a) method as claimed in any one of claims 2, 6, or 8, or

(b) medical device as claimed in claim 4,

in which the sequence FDYNLY is comprised in a protein which comprises a sequence selected from the group consisting of LFSVPFDYNLYSNWW, LFSIPFDYNLYSNWW, MFSVPFDYNLYSNWW, MFSVPFDYNLYTNWW, MWSVPFDYNLYSNWW and MFSVPFDYNLYKNWL.

13. A

(a) method as claimed in any one of claims 2, 6, or 8, or

(b) medical device as claimed in claim 4,

in which the sequence WDYNLY is comprised in a protein which comprises the sequence MFSVPFDYNLYKNWL or MFSVPFDYNLYKNWF.

14. A

(a) method as claimed in any one of claims 2, 6, or 8, or

(b) medical device as claimed in claim 4,

in which the sequence FDYNWY is comprised in a protein which comprises the sequence MFSVPFDYNWYSNWW.

15. A

(a) method as claimed in any one of claims 2, 6, or 8, or

(b) medical device as claimed in claim 4,

in which the sequence YDWNLY is comprised in a protein which comprises the sequence MASIPYDWNLYQSWA or MASIPYDWNLYSAWA.

16. A

(a) method as claimed in any one of claims 2, 6, or 8, or

(b) medical device as claimed in claim 4,

in which the sequence YDWHLY is comprised in a protein which comprises the sequence MASIPYDWHLYNAWA.

17. A

(a) method as claimed in any one of claims 2, 6, or 8, or

(b) medical device as claimed in claim 4,

in which the peptide is isolated from an aquatic organism.

18. A

(a) method as claimed in claim 17, or

(b) medical device as claimed in claim 17,

in which the aquatic organism is selected from the group consisting of a fish and a sessile organism.

19. A

(a) method as claimed in claim 18, or

(b) medical device as claimed in claim 18,

in which the sessile organism is an anemone selected from the group consisting of *Aiptesia pulchella* and *Anemonia viridans*.

20. A

(a) method as claimed in any one of claims 2, 6, or 8, or

(b) medical device as claimed in claim 4,

in which the peptide is isolated from a moss.

21. A

(a) method as claimed in claim 20, or

(b) medical device as claimed in claim 20,

in which the moss is selected from the group consisting of *Physcomitrella patens, Funaria hygrometrica; Eukaryota; Viridiplantae; Streptophyta; Embryophyta; Bryophyta; Moss Superclass V; Bryopsida; Funariidae; Funariales; Funariaceae;* and *Physcomitrella*.

22. A

(a) method as claimed in any one of claims 2, 6, or 8, or

(b) a medical device as claimed in claim 4,

in which the composition is resistant to lyophilization.

23. A

(a) method as claimed in any one of claims 2, 6, or 8, or

(b) a medical device as claimed in claim 4,

in which the composition inhibits aggregation of cells.

24. A method as claimed in claim 8, in which the fluid is applied to a reverse osmosis filter.

25. A method as claimed in any one of claims 2, 6, or 8, in which the biofilm is formed by a single cell organism selected from the group consisting of a bacterium, a fungus, a protozoa and an archaea.

26. A method as claimed in claim 25, in which the fungus is a yeast.

27. A peptide comprising a sequence selected from the group consisting of YDYNWY, YDYNLY, FDYNFY, FDYNLY, WDYNLY, FDYNWY, YDWNLY, and YDWHLY , and wherein the peptide is cyclic and consists of a length of up to 50 amino acids.

Dated: 15 October 2015

TEL HASHOMER MEDICAL RESEARCH, INFRASTRUCTURE AND SERVICES LTD.

By its Patent Attorneys

KNIGHTSBRIDGE PATENT ATTORNEYS

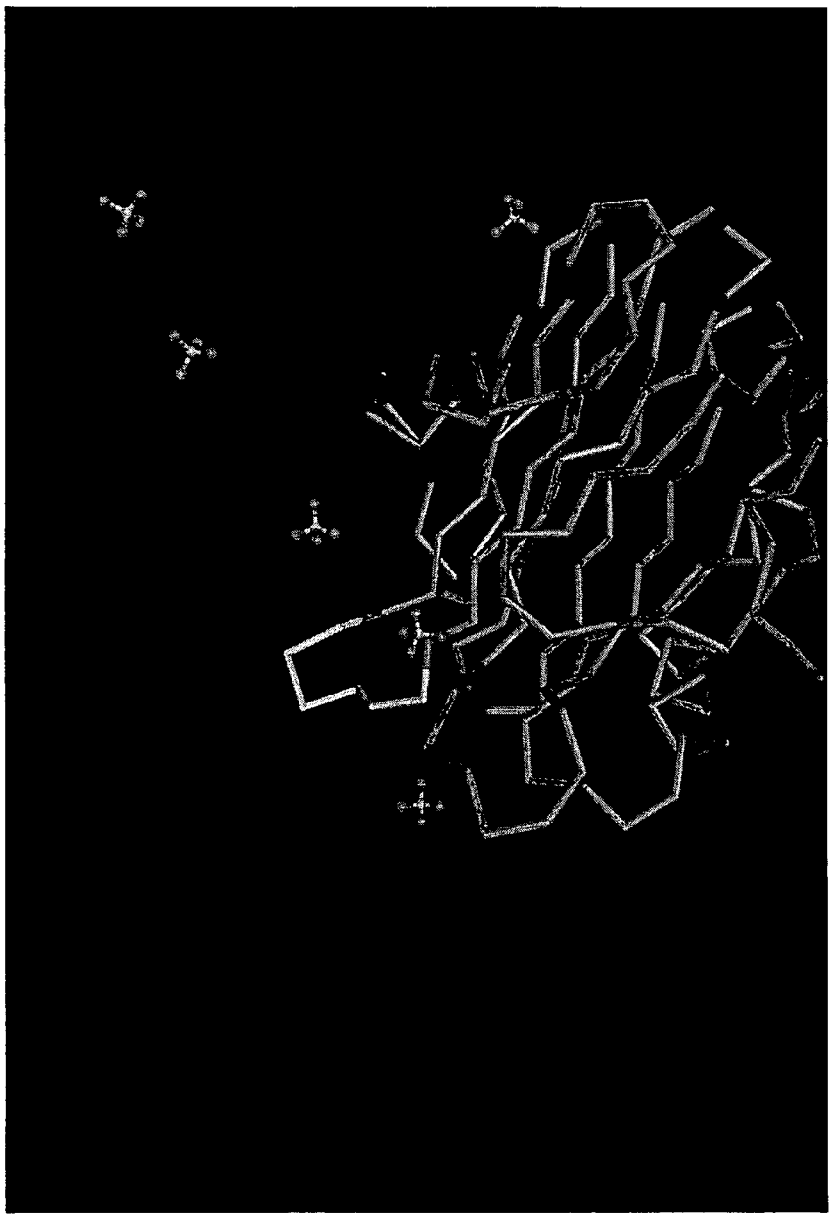


Figure 1: 1GWY Chain A, Crystal Structure Of The Water-Soluble State Of The Pore- Forming Cytolysin Sticholysin Ii

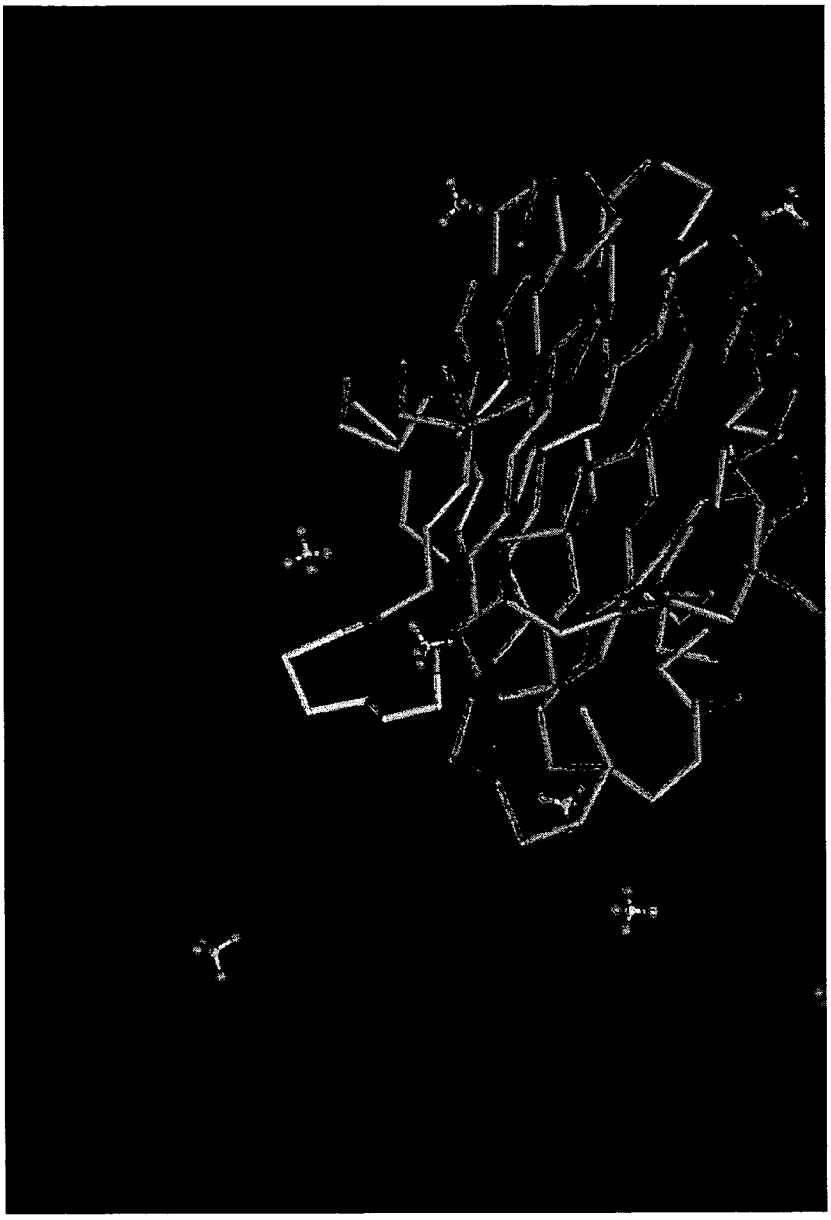


Figure 2: 1GWY Chain B, Crystal Structure Of The Water-Soluble State Of The Pore- Forming Cytolysin Sticholysin Ii

Figure 3: 1KD6 Chain A, Solution Structure Of The Eukaryotic Pore-Forming Cytolysin Equinatoxin Ii

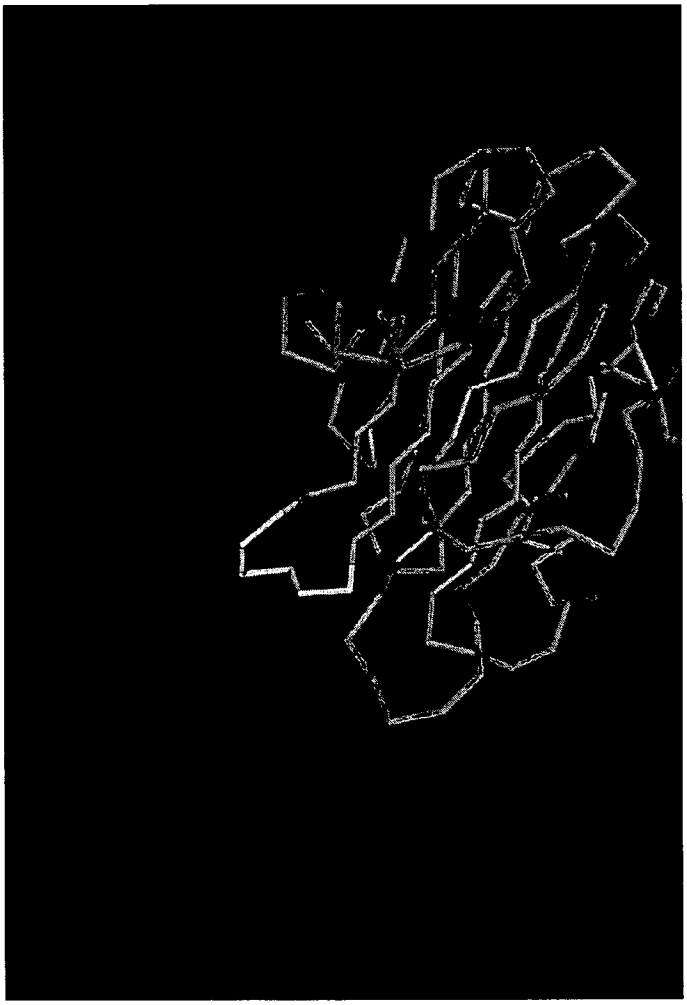


Figure 4: 1TZQ Chain A, Crystal Structure Of The Equinatoxin Ii 8-69 Double Cysteine Mutant

Figure 5

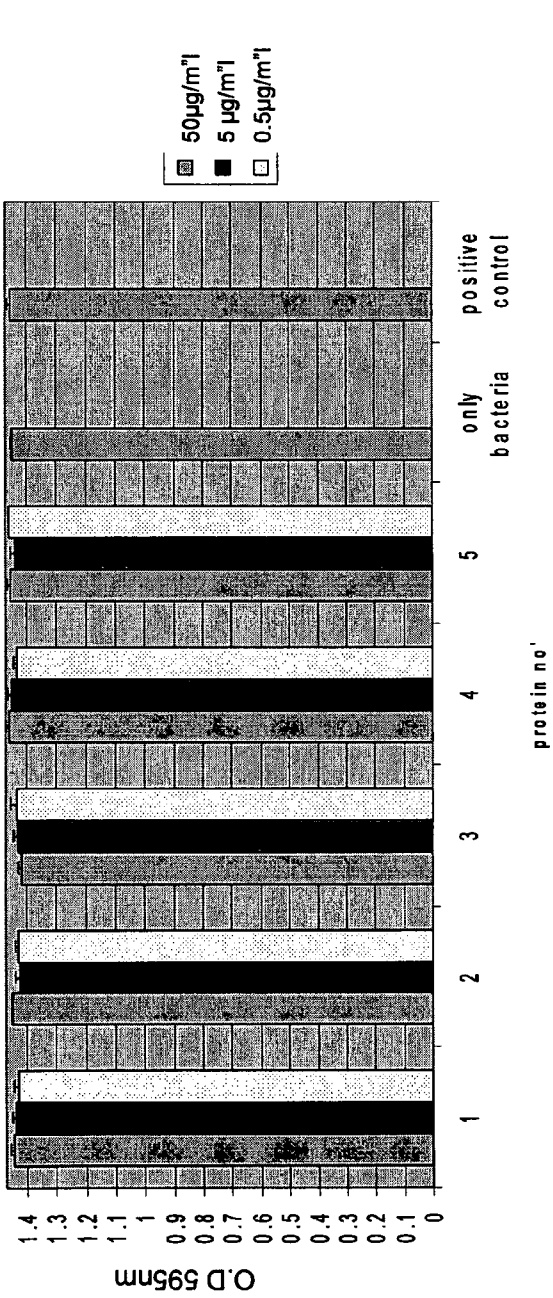

Pseudomonas aeruginosa ATCC 27853 growth 24hours
50µg/ml
5 µg/ml
0.5µg/ml
O.D 595nm
0
0.1
0.2
0.3
0.4
0.5
0.6
0.7
0.8
0.9
1
1.1
1.2
1.3
1.4
1
2
3
4
5
only bacteria
positive control
protein no'
1- AbacZ-17C
2- AbacZ-17
3- AbacZ-16C
4- AbacZ-6
5- AbacZ-8C

Figure 6

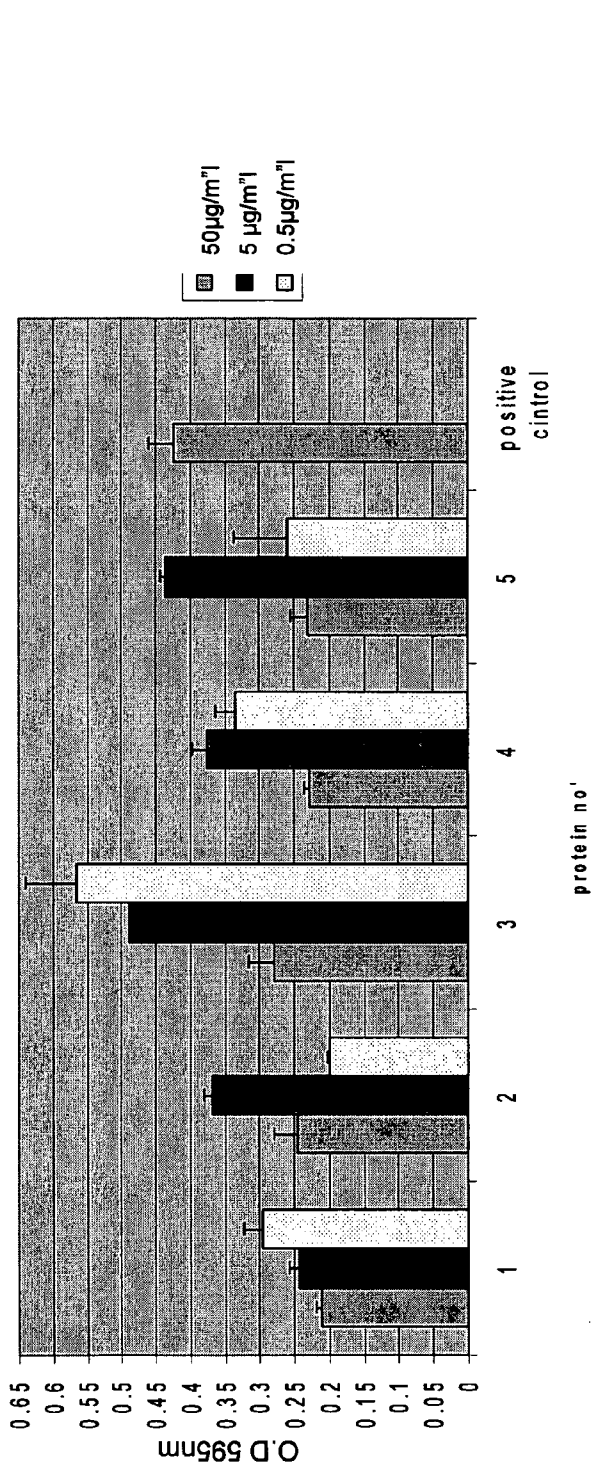

Prevention of Pseudomonas aeruginosa ATCC 27853 biofilm formation
50µg/ml
5 µg/ml
0.5µg/ml
O.D 595nm
0.65
0.6
0.55
0.5
0.45
0.4
0.35
0.3
0.25
0.2
0.15
0.1
0.05
0
1
2
3
4
5
positive cintrol
protein no'
1- AbacZ-17C
2- AbacZ-17
3- AbacZ-16C
4- AbacZ-6
5- AbacZ-8C

Figure 7

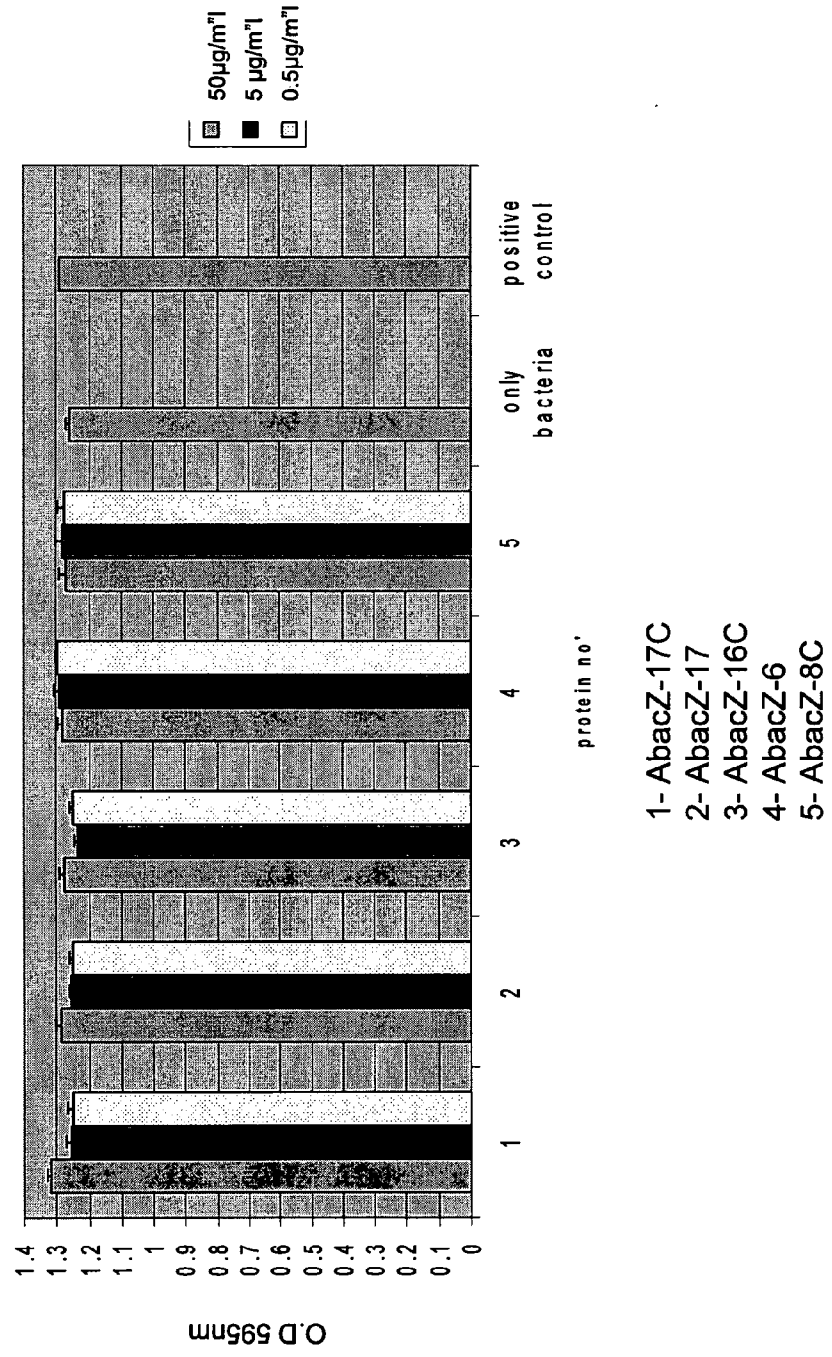
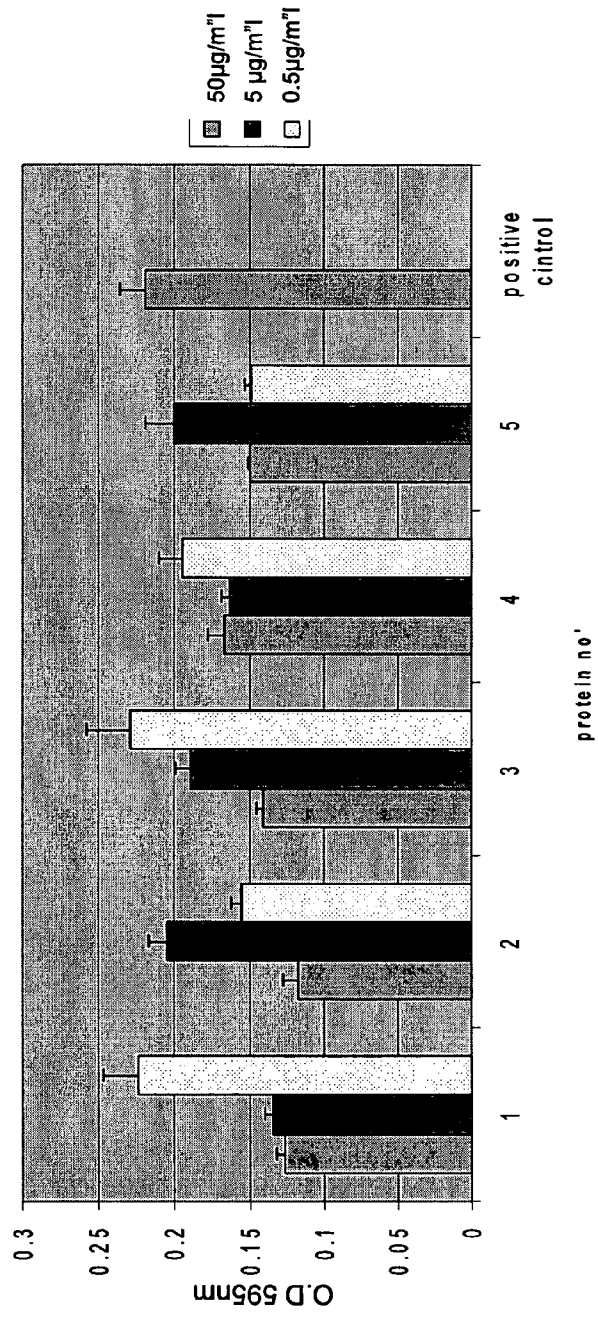

Acinetobacter Baumannii (clinical isolate) growth 24hours
50µg/ml
5 µg/ml
0.5µg/ml
O.D 595nm
1.4
1.3
1.2
1.1
1
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
1
2
3
4
5
only bacteria
positive control
protein no'
1- AbacZ-17C
2- AbacZ-17
3- AbacZ-16C
4- AbacZ-6
5- AbacZ-8C

Figure 8

Prevention of *Acinetobacter Baumannii* (clinical isolate) **biofilm formation**



1- AbacZ-17C
2- AbacZ-17
3- AbacZ-16C
4- AbacZ-6
5- AbacZ-8C

Figure 9
Prevention of *Actinetobacter baumannii* biofilm formation by *Actinia equina* fractions

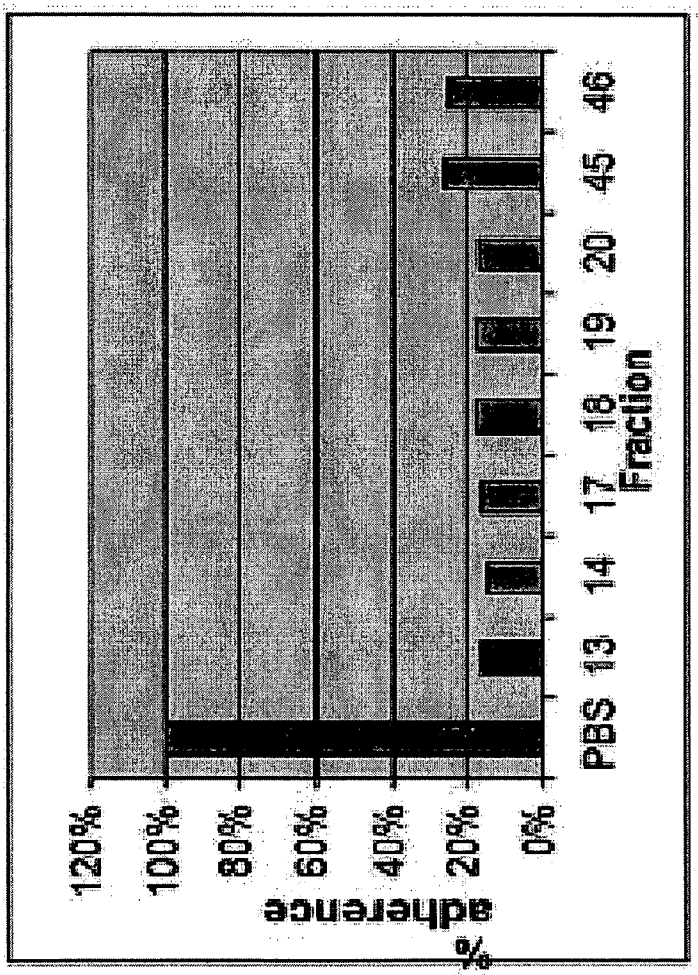
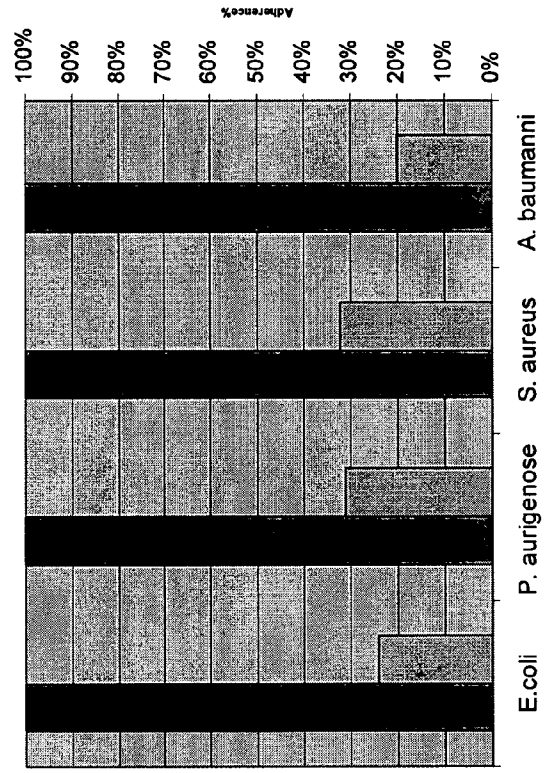


Figure 10
Prevention of biofilm formation by fraction 13



Prevention of *Pseudomonas aeruginosa* biofilm formation by *Anemonia, Aiptasia* and *Physcomitrella* (Moss)

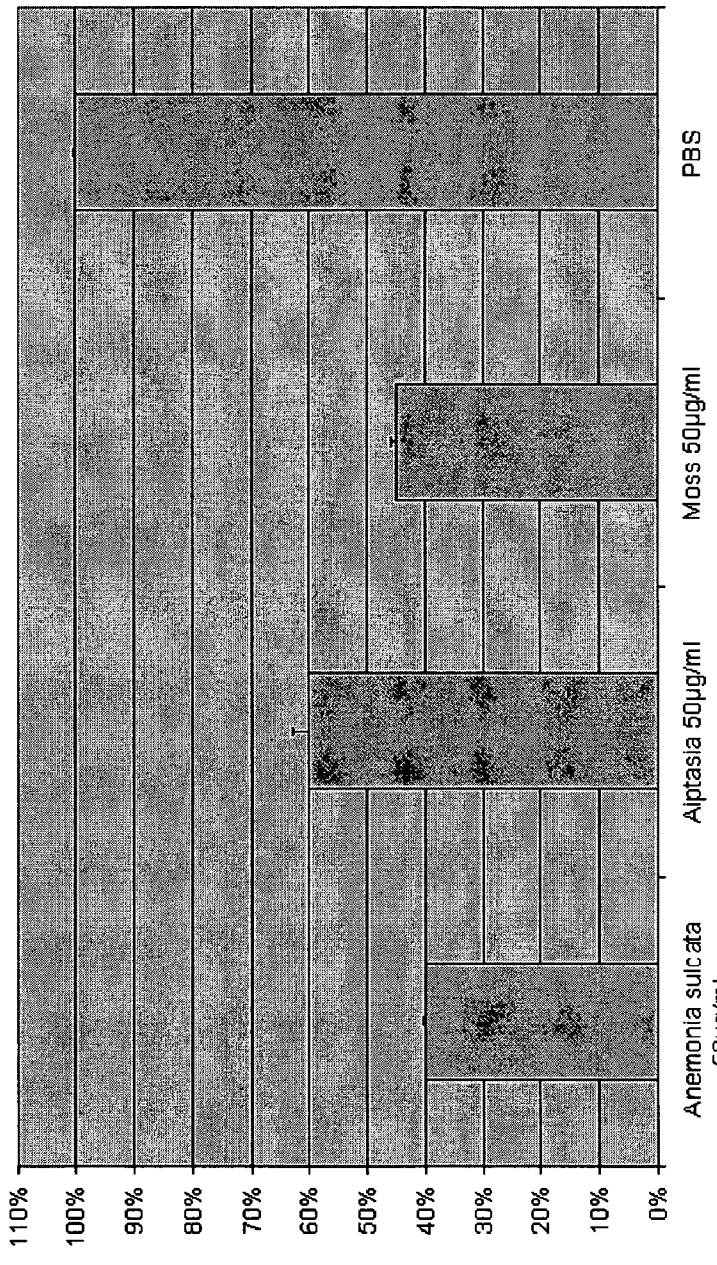


Figure 11

Figure 12

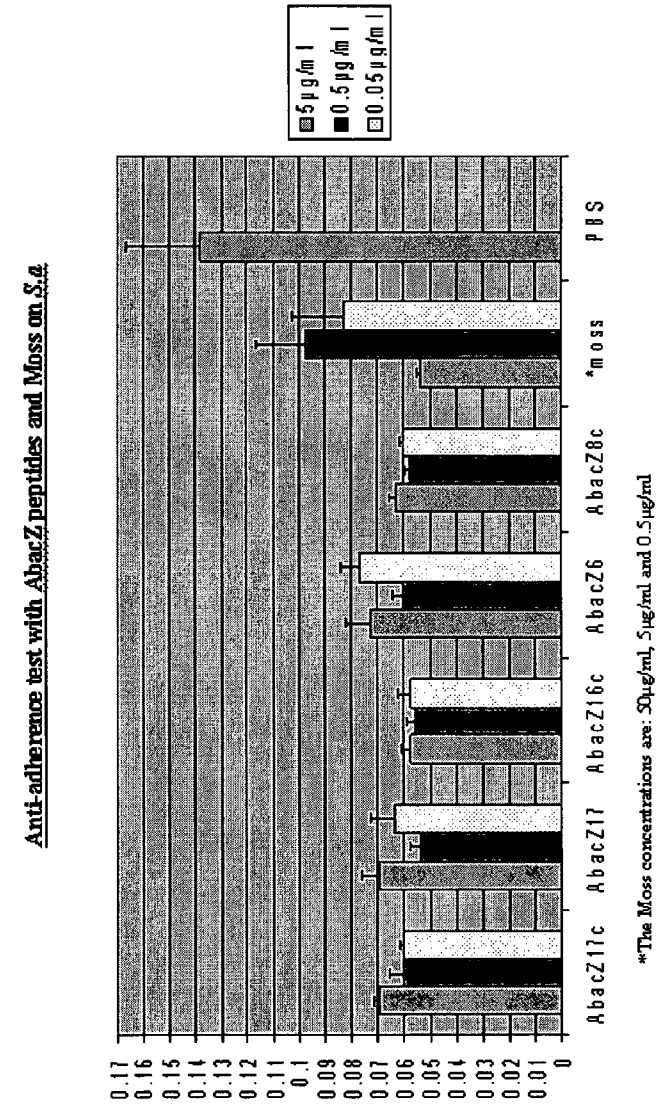

Anti-adherence test with AbacZ peptides and Moss on S.a
5µg/ml
0.5µg/ml
0.05µg/ml
0.17
0.16
0.15
0.14
0.13
0.12
0.11
0.1
0.09
0.08
0.07
0.06
0.05
0.04
0.03
0.02
0.01
0
AbacZ11c
AbacZ11
AbacZ16c
AbacZ6
AbacZ8c
*moss
PBS
*The Moss concentrations are: 50µg/ml, 5µg/ml and 0.5µg/ml

Figure 13

Fig. 8

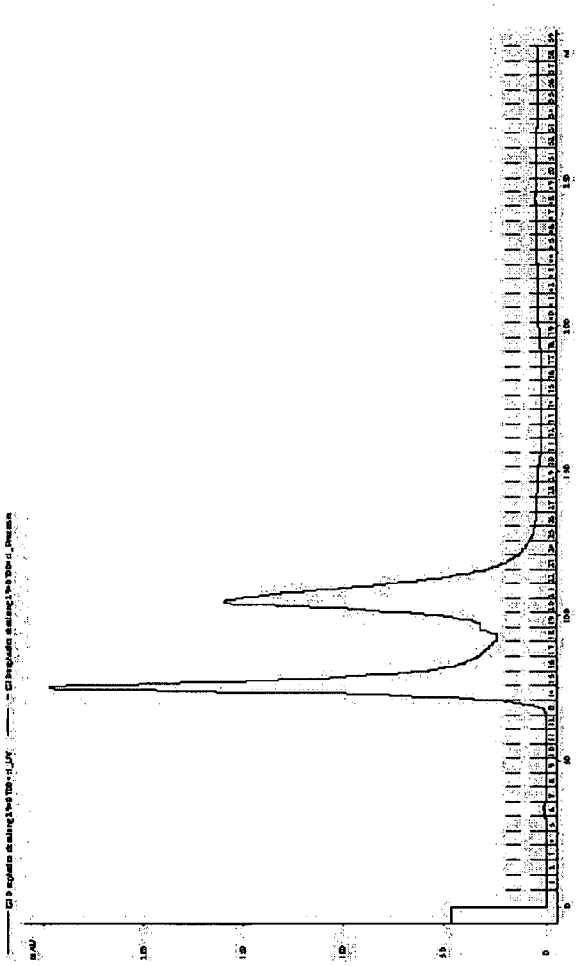


Figure 14

Fig. 9

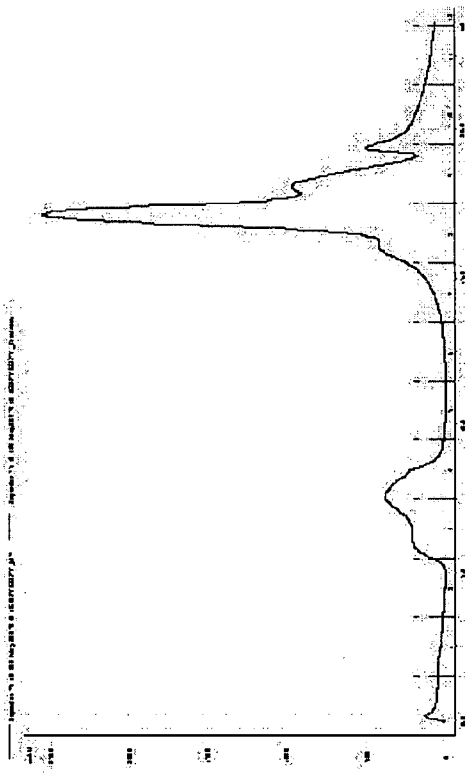


Figure 15

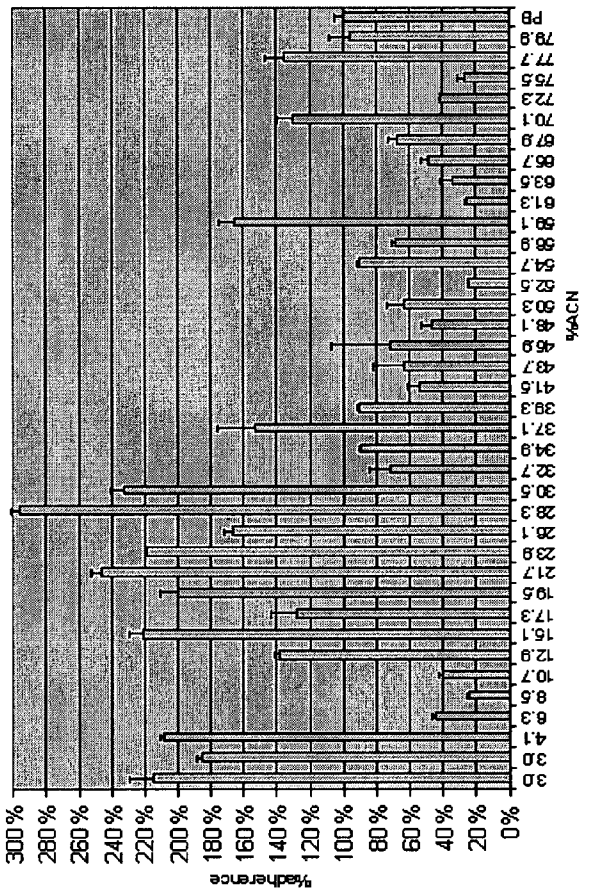
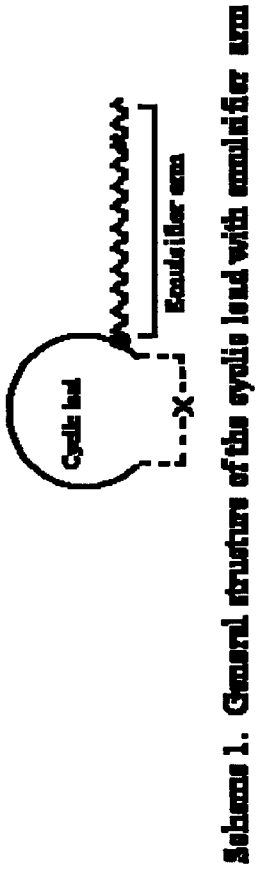

%adherence
300 %
280 %
260 %
240 %
220 %
200 %
180 %
160 %
140 %
120 %
100 %
80 %
60 %
40 %
20 %
0%
%ACN
3.0
3.0
4.1
6.3
8.5
10.7
12.9
15.1
17.3
19.5
21.7
23.9
26.1
28.3
30.5
32.7
34.9
37.1
39.3
41.5
43.7
45.9
48.1
50.3
52.5
54.7
56.9
59.1
61.3
63.5
65.7
67.9
70.1
72.3
75.5
77.7
79.9
PB

Figure 16A



Scheme 1. General structure of the cyclic load with equalizer arm

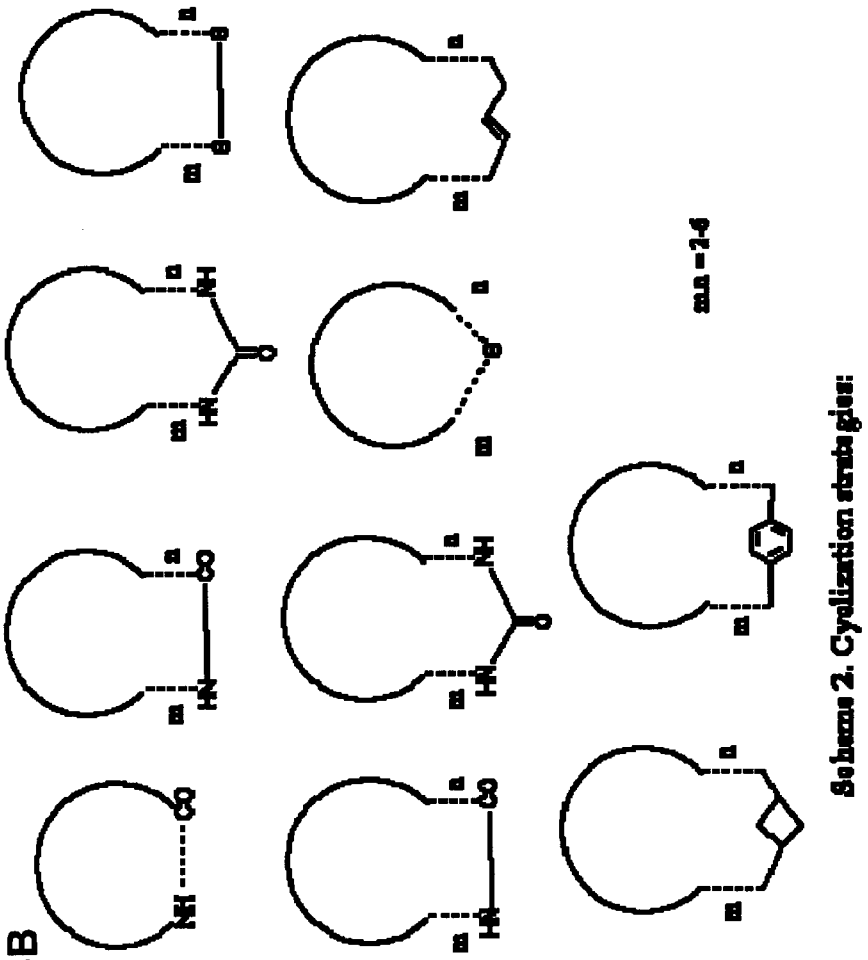


Figure 16B

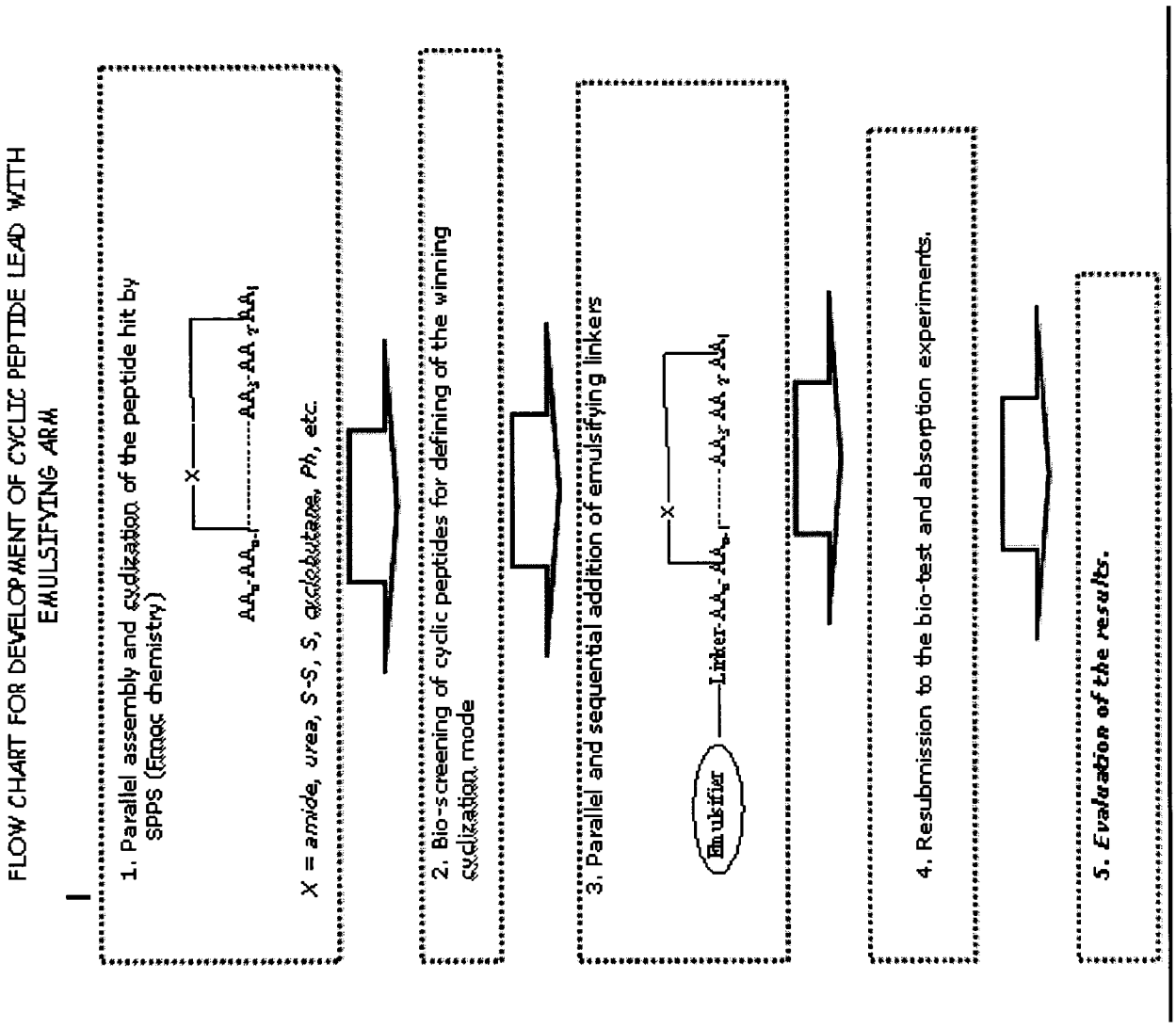

FLOW CHART FOR DEVELOPMENT OF CYCLIC PEPTIDE LEAD WITH EMULSIFYING ARM
1. Parallel assembly and cyclization of the peptide hit by SPPS (Fmoc chemistry)
X = amide, urea, S-S, S, cyclobutane, Ph, etc.
2. Bio-screening of cyclic peptides for defining of the winning cyclization mode
3. Parallel and sequential addition of emulsifying linkers
Emulsifier
Linker
4. Resubmission to the bio-test and absorption experiments.
5. Evaluation of the results.

Figure 17