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, ~~YDYSFY,~~ 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 tones of fuel annually. Without antifouling measures, that fuel consumption would increase by as much as 40%, equivalent to an extra 120 million tones of fuel annually. The

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, ~~YDYSFY,~~ 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 10 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:

group consisting of YDYNWY, YDYNLY, ~~YDYSFY,~~ 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, ~~YDYSFY,~~ 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
MWSVPFOYNLYSNWW	Physcomitrella patens
MFSVPWDYNLYKNWF	Danio rerio
MFSVPFDYNLYKNWL	Tetraodon nigroviridis

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

MSRLIIVFIVVTMICSATALPSKKIIDEDEEDEKRSADVAGAVIDGASLSFDILKTVLEAL GNVKRKIAVGVDNESGKTWTALNTYFRSGTSDIVLPHKVPHGKALLYNGQKDRGPVA TGAVGVLAYLMSDGNTLAVLFSVPYDYNWYSNWWNVRIYKGKRRADQRMYE 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

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, ~~YDYSFY,~~ 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. W0/20071147098, 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. 26:647650 (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

CLAIMS

1. A peptide selected from the group consisting of YDYNWY, YDYNLY, ~~YDYSFY,~~ 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 concrete, a masonry, a glass, a metal and a plastic, the method comprising contacting the single cell organism with a composition devoid of cytotoxic or cytostatic activity and comprising an isolated or synthetic peptide comprising a sequence selected from the group consisting of YDYNWY, YDYNLY, ~~YDYSFY,~~ 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 comprises up to 50 amino acids.

3. The method of claim 2, wherein the peptide is synthetic.

~~4. The method of claim 2, wherein the sequence YDYNWY is comprised in a protein of sequence LFSVPYDYNWYSNWW.~~

~~5~~4. The method of claim 2, wherein the sequence YDYNLY is comprised in a protein of sequence MFSVPYDYNLYSNWV or LFSVPYDYNLYSNWW.

~~6~~5. The method of claim 2, wherein the sequence FDYNFY is comprised in a protein of sequence MFSVPFDYNFYSNWW or LFSVPFDYNFYSNWW.

~~7~~6. The method of claim 2, wherein the sequence FDYNLY is comprised in a protein of sequence selected from the group consisting of LFSVPFDYNLYSNWW, LFSIPFDYNLYSNWW, MFSVPFDYNLYSNWW, MFSVPFDYNLYTNWW, MWSVPFDYNLYSNWW and MFSVPFDYNLYKNWL.

~~8~~7. The method of claim 2, wherein the sequence WDYNLY is comprised in a protein of sequence MFSVPWDYNLYKNWF.

~~9~~8. The method of claim 2, wherein the sequence FDYNWY is comprised in a protein of sequence MFSVPFDYNWYSNWW.

~~10~~9. The method of claim 2, wherein the sequence YDWNLY is comprised in a protein of sequence MASIPYDWNLYQSWA or MASIPYDWNLYSAWA.

~~11~~10. The method of claim 2, wherein the sequence YDWHLY is comprised in a protein of sequence MASIPYDWHLYNAWA.

~~12~~11. The method of claim 2, wherein the peptide is isolated from an aquatic organism.

~~13~~12. The method of claim ~~12~~ 11, wherein the aquatic organism is selected from the group consisting of a fish and a sessile organism.

~~14~~13. The method of claim ~~13~~ 12, wherein the sessile organism is an anemone selected from the group consisting of *Aiptesia pulchella* and *Anemonia viridans*.

~~15~~14. The method of claim 2, wherein the peptide is isolated from a moss.

~~16~~15. The method of claim ~~15~~ 14, wherein 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*.

~~17~~16. The method of claim 2, wherein the composition inhibits aggregation of cells.

~~18~~17. The method of claim 2, wherein the single cell organism is comprised in a biofilm.

~~19~~18. The method of claim 2, wherein the single cell organism is selected from the group consisting of a bacteria, a fungi, a protozoa and an archaea.

~~20~~19. The method of claim ~~19~~ 18, wherein the fungi comprises a yeast.

~~21~~20. The method of claim 2, wherein the composition is in a form selected from the group consisting of a spray, a gel and a paint.

~~22~~21. The method of claim 2, wherein the surface is comprised in a device that is susceptible to biofilm formation, and wherein the device is selected from the group

consisting of a vessel hull, an automobile surface, an air plane surface, a membrane, a filter, or an industrial equipment.

~~23~~22. The method of claim ~~22~~ 21, wherein the filter is a reverse osmosis filter.

~~24~~23. A medical device comprising a composition devoid of cytotoxic or cytostatic activity and comprising an isolated or synthetic peptide comprising a sequence selected from the group consisting of YDYNWY, YDYNLY, ~~YDYSFY,~~ 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 intracorporea1 device or an extracorporea1 device, and wherein the peptide comprises up to 50 amino acids.

~~25~~24. The medical device of claim ~~24~~ 23, wherein the peptide is synthetic.

~~26~~25. The medical device of claim ~~24~~ 23, wherein the device is an implantable medical device or a medical instrument.

~~27. The medical device of claim 24, wherein the sequence YDYNWY is comprised in a protein of sequence LFSVPYDYNWYSNWW.~~

~~28~~26. The medical device of claim ~~24~~ 23, wherein the sequence YDYNLY is comprised in a protein of sequence MFSVPYDYNLYSNWV or LFSVPYDYNLYSNWW.

~~29~~27. The medical device of claim ~~24~~ 23, wherein the sequence FDYNFY is comprised in a protein of sequence MFSVPFDYNFYSNWW or LFSVPFDYNFYSNWW.

~~30~~28. The medical device of claim ~~24~~ 23, wherein the sequence FDYNLY is comprised in a protein of sequence selected from the group consisting of LFSVPFDYNLYSNWW, LFSIPFDYNLYSNWW, MFSVPFDYNLYSNWW, MFSVPFDYNLYTNWW, MWSVPFDYNLYSNWW and MFSVPFDYNLYKNWL.

~~31~~29. The medical device of claim ~~24~~ 23, wherein the sequence WDYNLY is comprised in a protein of sequence MFSVPWDYNLYKNWF.

~~32~~30. The medical device of claim ~~24~~ 23, wherein the sequence FDYNWY is comprised in a protein of sequence MFSVPFDYNWYSNWW.

~~33~~31. The medical device of claim ~~24~~ 23, wherein the sequence YDWNLY is comprised in a protein of sequence MASIPYDWNLYQSWA or MASIPYDWNLYSAWA.

~~34~~32. The medical device of claim ~~24~~ 23, wherein the sequence YDWHLY is comprised in a protein of sequence MASIPYDWHLYNAWA.

~~35~~33. The medical device of claim ~~24~~ 23, wherein the peptide is isolated from an aquatic organism.

~~36~~34. The medical device of claim ~~24~~ 23, wherein the aquatic organism is selected from the group consisting of a fish and a sessile organism.

~~37~~35. The medical device of claim ~~36~~ 34, wherein the sessile organism is an anemone selected from the group consisting of *Aiptesia pulchella* and *Anemonia viridans*.

~~38~~36. The medical device of claim ~~24~~ 23, wherein the peptide is isolated from a moss.

~~39~~37. The medical device of claim ~~38~~ 36, wherein 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*.

~~40~~38. The medical device of claim ~~24~~ 23, wherein the composition inhibits aggregation of cells.

~~41~~39. A method of treating water to prevent or reduce biofilm formation or fouling of a filter, comprising treating the water with a composition devoid of cytotoxic or cytostatic activity and comprising an isolated or synthetic peptide comprising a sequence selected from the group consisting of YDYNWY, YDYNLY, ~~YDYSFY,~~ FDYNFY, FDYNLY, WDYNLY, FDYNWY, YDWNLY and YDWHLY, wherein the peptide comprises up to 50 amino acids.

~~42~~40. The method of claim ~~41~~ 39, wherein the peptide is synthetic.

~~43. The method of claim 41, wherein the sequence YDYNWY is comprised in a protein of sequence LFSVPYDYNWYSNWW.~~

~~44~~41. The method of claim ~~41~~ 39, wherein the sequence YDYNLY is comprised in a protein of sequence MFSVPYDYNLYSNWV or LFSVPYDYNLYSNWW.

~~45~~42. The method of claim ~~41~~ 39, wherein the sequence FDYNFY is comprised in a protein of sequence MFSVPFDYNFYSNWW or LFSVPFDYNFYSNWW.

~~46~~43. The method of claim ~~41~~ 39, wherein the sequence FDYNLY is comprised in a protein of sequence selected from the group consisting of LFSVPFDYNLYSNWW, LFSIPFDYNLYSNWW, MFSVPFDYNLYSNWW, MFSVPFDYNLYTNWW, MWSVPFDYNLYSNWW and MFSVPFDYNLYKNWL.

~~47~~44. The method of claim ~~41~~ 39, wherein the sequence WDYNLY is comprised in a protein of sequence MFSVPWDYNLYKNWF.

~~48~~45. The method of claim ~~41~~ 39, wherein the sequence FDYNWY is comprised in a protein of sequence MFSVPFDYNWYSNWW.

~~49~~46. The method of claim ~~41~~ 39, wherein the sequence YDWNLY is comprised in a protein of sequence MASIPYDWNLYQSWA or MASIPYDWNLYSAWA.

~~50~~47. The method of claim ~~41~~ 39, wherein the sequence YDWHLY is comprised in a protein of sequence MASIPYDWHLYNAWA.

~~51~~48. The method of claim ~~41~~ 39, wherein the peptide is isolated from an aquatic organism.

~~52~~49. The method of claim ~~51~~ 48, wherein the aquatic organism is selected from the group consisting of a fish and a sessile organism.

~~53~~50. The method of claim ~~52~~ 49, wherein the sessile organism is an anemone selected from the group consisting of *Aiptesia pulchella* and *Anemonia viridans*.

~~54~~51. The method of claim ~~41~~ 39, wherein the peptide is isolated from a moss.

~~55~~52. The method of claim ~~54~~ 51, wherein 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*.

~~56~~53. The method of claim ~~41~~ 39, wherein the composition inhibits aggregation of cells.

~~57~~54. The method of claim ~~41~~ 39, wherein the treated water is applied to a reverse osmosis filter.

~~58~~55. The method of claim ~~41~~ 39, wherein the biofilm is formed by a single cell organism selected from the group consisting of a bacteria, a fungus, a protozoa and an archaea.

~~59~~56. The method of claim ~~58~~ 55, wherein the fungus comprises a yeast.

~~60~~57. The method of claim ~~41~~39, wherein the surface is comprised in a device that is susceptible to biofilm formation, and wherein the device is selected from the group consisting of a vessel hull, an automobile surface, an air plane surface, a membrane, a filter, or an industrial equipment.

~~61~~58. The method of claim ~~60~~ 57, wherein the filter is a reverse osmosis filter.

~~62~~59. A method of preventing or reducing biofilm formation in an external fluid which does not originate from an animal body, comprising treating the fluid with a composition devoid of cytotoxic or cytostatic activity and comprising an isolated or synthetic peptide comprising a sequence selected from the group consisting of YDYNWY, YDYNLY, ~~YDYSFY,~~ FDYNFY, FDYNLY, WDYNLY, FDYNWY, YDWNLY and YDWHLY, wherein the peptide comprises up to 50 amino acids.

~~63~~60. The method of claim ~~62~~ 59, wherein the peptide is synthetic.

~~64. The method of claim 62, wherein the sequence YDYNWY is comprised in a protein of sequence LFSVPYDYNWYSNWW.~~

~~65~~61. The method of claim ~~62~~ 59, wherein the sequence YDYNLY is comprised in a protein of sequence MFSVPYDYNLYSNWV or LFSVPYDYNLYSNWW.

~~66~~62. The method of claim ~~62~~ 59, wherein the sequence FDYNFY is comprised in a protein of sequence MFSVPFDYNFYSNWW or LFSVPFDYNFYSNWW.

~~67~~63. The method of claim ~~62~~ 59, wherein the sequence FDYNLY is comprised in a protein of sequence selected from the group consisting of LFSVPFDYNLYSNWW, LFSIPFDYNLYSNWW, MFSVPFDYNLYSNWW, MFSVPFDYNLYTNWW, MWSVPFDYNLYSNWW and MFSVPFDYNLYKNWL.

~~68~~64. The method of claim ~~62~~ 59, wherein the sequence WDYNLY is comprised in a protein of sequence MFSVPWDYNLYKNWF.

~~69~~65. The method of claim ~~62~~ 59, wherein the sequence FDYNWY is comprised in a protein of sequence MFSVPFDYNWYSNWW.

~~70~~66. The method of claim ~~62~~ 59, wherein the sequence YDWNLY is comprised in a protein of sequence MASIPYDWNLYQSWA or MASIPYDWNLYSAWA.

~~71~~67. The method of claim ~~62~~ 59, wherein the sequence YDWHLY is comprised in a protein of sequence MASIPYDWHLYNAWA.

~~72~~68. The method of claim ~~62~~ 59, wherein the peptide is isolated from an aquatic organism.

~~73~~69. The method of claim ~~72~~ 68, wherein the aquatic organism is selected from the group consisting of a fish and a sessile organism.

~~74~~70. The method of claim ~~72~~ 68, wherein the sessile organism is an anemone selected from the group consisting of *Aiptesia pulchella* and *Anemonia viridans*.

~~75~~71. The method of claim ~~62~~ 59, wherein the peptide is isolated from a moss.

~~76~~72. The method of claim ~~75~~ 71, wherein 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*.

~~77~~73. The method of claim ~~62~~ 59, wherein the composition inhibits aggregation of cells.

~~78~~74. The method of claims ~~62~~ 59, wherein the fluid is applied to a reverse osmosis filter.

~~79~~75. The method of claim ~~62~~ 59, wherein the biofilm is formed by a single cell organism selected from the group consisting of a bacteria, a fungus, a protozoa and an archaea.

~~80~~76. The method of claim ~~79~~ 75, wherein the fungus comprises a yeast.