A B S T R A C T

PEPTIDES AND DERIVATIVES THEREOF FOR DETECTION AND CONTROL OF *SALMONELLA*

The present subject matter relates to isolated polypeptide having one of the sequences SEQ. ID. No 8, 9, 10, 11, 12, 13, 14 or fragments or derivatives thereof, having the ability to recognize and bind to a broad range of *Salmonella* strains.

Moreover, the present subject matter further relates to nucleic acid molecules having one of the SEQ. ID. No 1, 2, 3, 4, 5, 6, 7 encoding said recombinant polypeptide or fragments or derivatives thereof, vectors comprising said nucleic acid molecules and host cells comprising either said nucleic acid molecules or said vectors.

D E S C R I P T I O N

PEPTIDES AND DERIVATIVES THEREOF FOR DETECTION AND CONTROL OF *SALMONELLA*

FIELD OF THE INVENTION

The present subject matter relates to isolated polypeptides having the sequences SEQ. ID. No 8, 9, 10, 11, 12, 13, 14 or fragments or derivatives thereof, having the ability to recognize and bind to a broad range of *Salmonella* strains. Moreover, the present subject matter further relates to nucleic acid molecules SEQ. ID. No 1, 2, 3, 4, 5, 6, 7 encoding said recombinant polypeptide or fragments or derivatives thereof, vectors comprising said nucleic acid molecules and host cells comprising either said nucleic acid molecules or said vectors.

In addition, the present subject matter relates to said polypeptide, fragments or derivatives thereof for use as a detection tool or a kit for the identification of *Salmonella* strains in foodstuff, food processing equipment, food processing plants, food processing surfaces, medical devices, surfaces in hospitals and surgeries, human stools, environmental samples or any other samples where *Salmonella* might be present.

The present subject matter also relates to said polypeptide, fragments or derivatives thereof for use as a medicament, in particular for the treatment or prevention of *Salmonella spp*. infections.

The present subject matter also relates to the use of said polypeptide, fragments or derivatives thereof for the

treatment or prevention of bacterial contamination, particularly of *Salmonella spp*. contamination, of foodstuff, of food processing equipment, of food processing plants, of surfaces coming into contact with foodstuff, of medical devices, of surfaces in hospitals and surgeries or other places where *Salmonella* might be present.
Furthermore, the present subject matter relates to a pharmaceutical composition comprising said polypeptide, fragments or derivatives thereof.

BACKGROUND OF THE INVENTION

Foodborne diseases are of major concern due to their worldwide impact. The Center for Disease Control and Prevention (CDC) estimates that 76 million cases of foodborne diseases occur every year in the United States, causing roughly 5000 deaths. From the reported cases, it has been observed that *Salmonella* is the most common and widely distributed causing agent constituting thus a major public health burden with significant impact in the society costs worldwide. Besides the effort that has been done to control this pathogen, including the general improvement of food safety knowledge and good practices, they are still a major cause of the increasing occurrence of foodborne diseases5.
Moreover, the production and distribution of foodstuff increased dramatically in volume and in number of consumers, increasing the risk of mass epidemics5 . Consequently, it is critical to identify and quantify the presence of such pathogen to monitor the safety of foodstuff and also to define strategies to reduce the number of outbreaks.

The golden standard for *Salmonella* detection is still the bacteriological culture (ISO 6579:2002) which is time-consuming, laborious, expensive and ineffective in detecting non-cultivable organisms8. *Salmonella* detection methods typically take 3-5 days to obtain a result and during this long period portions of the food may have been distributed, marketed, sold, and eaten before a problem is even detected. A number of methods aiming at reducing the time required for the identification of foodborne pathogens have been developed, which include the use of antibodies to detect microbial antigens such as the enzyme-linked immunosorbent assay (ELISA) and nucleic acid-based techniques such as the polymerase chain reaction (PCR), DNA microarrays/DNA chips, sequencing-based identification and DNA hybridization2,3,4.However, the sensitivity of immunological methods has been found to be low, with a variable specificity depending on the antibody used, commonly with the occurrence of cross-reactivity phenomena. Often, these methods do not allow the discrimination between dead and living microorganisms. Also, the feasibility of the method depends on the origin of samples1.Despite the high sensitivity of the nucleic acid-based techniques and their increased value in the detection of fastidious microorganisms, they usually require extensive sample preparation, often including a DNA extraction step. These procedures are laborious, expensive and time consuming and may not be enough to remove all the contaminants, commonly found in the samples, that may inhibit (or influence) the reactions needed for the microorganisms detection. Moreover, the nucleic acid-based techniques such as the PCR and microarrays demand a high initial investment in equipment and expertise personnel1.

Bacteriophages (phages) are naturally evolved entities that due to their intrinsic characteristics present high potential in the control and detection of bacteria, namely foodborne pathogens. Phages are viruses that only infect bacteria and are obligate intracellular parasites lacking their own metabolism. They are extremely specific, usually infecting a single species or even strain and consequently they have been found to present potential in the rapid detection of bacteria6.

Phage PVPSE1 belongs to the family Myoviridae and is characterized by an icosahedral head of 84 nm in apical diameter and a contractile tail of 120 x 18 nm with short tail fibers. This phage has the ability to bind to a very broad range of *Salmonella* strains which makes it an interesting phage to be used in the detection of *Salmonella*9. In addition the lytic spectrum of this phage is broader than that described by Felix O1. We observed that PVPSE1 is able to infect *Salmonella* several mutants defective in core polysaccharide assembly suggesting that the receptor for this phage is the conserved LPS inner core region and explains its broad lytic spectrum. The use of tail proteins (e.g tail fibers and base plate) as recognition elements has been suggested by others, however, the present invention respects to a group of tail proteins that can cover a very broad range of *Salmonella* strains.

SUMMARY OF THE INVENTION

An embodiment of the present invention relates to new polypeptides isolated from phage PVPSE1 able to specifically bind to different *Salmonella* species and useful in the detection and control of *Salmonella* spp.,

namely an isolated polypeptide having the sequence of one of SEQ ID No 8, 9, 10, 11, 12, 13, or 14, or fragments or derivatives thereof, or combinations thereof, for recognizing or binding to *Salmonella*.

Namely:

SEQ ID No. 8 – Protein sequence of wt PVPSE1gp40 (604 amino acids)

MYPIPCLFLTLSGGGTEPLPPGSVKKVAFTRGLVGGATKRSMAILLIDGRLYTQGDNAW
SECANGNISPFKDHWHLAANGVADVFGGGRAFVVKYNNGGWQYCGDTSQFTGSGSIYSS
WTSFPSSITGTVSLANLQSVSCALGNTLWQMVDGRLYGSGSNTNGCLGSGNTTVISIPR
SISASSVRAYSLNACVTYLNNIGLPRVCGATHQIDGTSTTQTQNFIDVSFASVTETVYV
KEWLANETNSMAIASTGVDDTEHYLYTRGIGTAQYSKKEGIGPFETFRVIDGGQSHFLI
ADNKLYGLGDLSAQLGLGTPSTMVLEPTLVPVPTGRDWDLSKLTYIVDMKTDVLNQGNS
ISHWMVYDGNLYYAGNLYGFFGSTDSTGEFTNIPEASFGGTTADAITTGSIPYAIKGSR
SQLTWTVEPADAEIYDISFTSSAPNIATVDSNGIMTFLEEGGFDITMTAKTGSGADAKT
LTDTSGGYVSIFSVTTDSIPQKEVGDVFVFMDKNSPDYTPGPNVVGMEISPANVDTNFI
DGELTTTNPDVVMIDEGGFLSCIAVGDARCGVRLIYREGQVEAFDDSYVSVSDFTAPPD
PVDPGEPVVPSQPQ

SEQ ID No. 9 – Protein sequence of wt PVPSE1gp41 (602 amino acids)

MADMTQFEQAVDQVVEDSERLHKVVNGTASETVVTEDGSTIPTVRKALLDNLFFKTPPM
PWIAGTQTTVFNQLYAFNGTNGVQWWYAPTATASAPVVLPQNPANSVNWRLYNDAAAMA
SIYAPINSPILTGNPQAPTPAANSNSTTIATTAFVTTAIASALSSISGGSVTFANLSVT
GATTLNSLVVGGTIDLNGPVNADNSTGRFQNLILTKELSSLTFVFTDADNPTFFKTRLD
PYAIQTHSIQTDIIVNGTVAEDDTTMSLTGVGNNVFDYVYIRGNASKDPTAPRLKVSGT
TEVENLNITGNVTGITFSVNGLDISPNSVTTADGVTVGGDLQVSGVTNLGNATIGGLDI
TSDLTVNGNTTLEDFSAGNGNITGPFTVGGLTSLNGGFTTGTADGTIGGKLSVTGTSEF
IEDLSVLADVTVQDNLTVNGDVNLNNATGTTTVNNLVIQGTVTGLSVDLTGQNINVGSL
SSTGAVTANSLTVQDSAILTKASVEFLTLIAEDIDSSTAAWSPSGDSNIYNVTVDADLT
IGAWPEPTDAFSAVIYLTQDGTGGHTVTLDPNYLVLNSETINETAGSVTILQLTYNGVE
GGVIDTVIVRRP

SEQ ID No. 10 – Protein sequence of wt PVPSE1gp46 (373 amino acids)

MAAPTVPIEIWAYGDIVLPNTHELNKARPIDDLWNKGWDLGEKPTVEEFNYVLNMLTAW
AKYITGEQIPGLDSRFLRVNQNLADLADKAAARTNLDVWSKTESDTRYVNISGDTMTGA
LSVPRLNLQPSESDYAYITTTNPAADTTFFDFVVGDNIGNAPGTSSIDSMRFRFVPSGG
SIFTMMELNAISGTAALCRVTGNIIASGSISGASVTATTANFTNTTVSGTLNAPTIQST
TIRTGTLTATGNVQGFNVVATSSLTTPYASVNGQCNVNSLVVNNNSATVGGRNVVRAIN
GATADGNGNVTLNLSGFVQQIRLGNRFATGVSESRFYAGHVMTGWAFGNKKELRGATYY
TAPLQYLINGQWVTVSNLD

SEQ ID No. 11 – Protein sequence of wt PVPSE1gp48 (497 amino acids)

MAAQYGLNDYGFAIPSLDDLIADTKQSLIRTFGENFNTQANTVVDKLTTILNEREYQLI
LLAAAVYSAQTLAGAEGIYLDELLGRRGIYRRGKTRGSGTIQMVVNNTVPYNMIYSSST
YSIDSGNFVLTQDTPVAGNILAQQILNQDWVLGNYTFQMINQNDGSTKSMNLTLSNKVP
NSPQLNAFMSSIKDFIVDNSTQLNEDRIFIDSAGGAMYIGYDANKKMIGLNSRVDFRSS
PVVGQRTITIEVIAAEAGAISREANTVTNITPTPSGFISMTNMTAFNDGSDVETDTDYK
VRASQSTAAGAAATRPAVISAVLNVEGVSKVRVFSNNTGETDQFGVPAYKFETVVYGGS
TEEISEALYNTIALSNATYGNVFYDVTTEDDQTERIYHSKAQARELAVRVRYKGKLLSV
TEQNTIKDALKAVVDPLNIADTLYNIQLVSAVGSSISPGRFTQLLVDVKNTDQPDSAYT
NSDVVAGMTEVFALDTDDITFQQII

SEQ ID No. 12 – Protein sequence of wt PVPSE1gp50 (929 amino acids)

MAQLRHNIDNVFQLIDGHIQFLERNKGETIDPTVQHYILNLQNVLANNRHFINWTAQEA
QPNGDATTEGQSVLILGCAYAYLATNDPKYLELAEDFWQAYIDWFFAGQPIPDPPAVYR
PNWIINGKEPRLAHYPLTDDGYPTHGGFKGSVMSWTNGKTLIPHGAPHWGEYLDKAWFA
FDGNLGWNSVNATVYAANADGTTNWDQYGSQWDVDWIIDRLGRKVDWDGNILEEGFPEA
EWGTVQLKDTTVTGNFKFNYATCNPVEHGGYLMDRNTMWHNRPVNVPIEMGFQDNASDA
ETWWCDANYVMYQITGERKYWLCWQSSLIVCDNYTDIDRFDKFFRKSTFAIIPFTDGIS
YDYSYPSTAVPEYSRDPEGYIGIRQAVSAQTTLEQQAIWFRVDGNSKLRVQFAGKDDLG
NGLLFRPELDLNKTKSETGRVTYRCGLPRGTDSIVSMDIPLSNFVRLTPPGGGNYIVAD
PRIVVDWGDNTVVDFEYQTGILGRINDQACTLITDADGGCTIGFWLTDSETADLSAFTY

RSYADNFNITITDENNWRWWAMIPATNGEWTTLQLTTTSFVLSSYQPDHPETDPRPNQI
DLHNLKQINLAPDTDPVDGVSARIDWYCVNAVPELYTSTGGDDYTMYFRITVSGDNPYS
AKLGDCTIIDYKTNSLHYTPGLIPFSNISDPNTALYDGWRGLPYPGYQYPTLYCFKGQN
IDWTRLNNTIDFLYDSQMWFYNSFNPVMPGPMAQAFVWDRWDAKKYGEPNTFTMKHWNE
KAWDGYEARAFFCVCRCVYELSQRGEAIPSKLFTVAKNWVDYLKWFQDNNEGRTPTIFN
PDGTVLAPEDDFTSHMSALFMGGCSMLGMAGMRDRIPNIDLVAERCFDLIQENYIILTP
NHPMNGCWSVAPRADTDNGMFFGFHAGELLRGFGLYAMYRNLTR

SEQ ID No. 13 – Protein sequence of wt PVPSE1gp51 (224 amino acids)

MADVSFPTVKVSDLPSAVTVSGGDYVVMDQADTTRKASLDTIMTRMGIMKVVFFSEGGF
LESKKDLAFFDTNGKYYTWNGVYPKTIPMSSSPSTTGGISENAWQEFGASGGGGSTGKV
VNLGNVQGTATCNLNRGDSFVANLTGGQCVVIITNPSTAQDVSQSFTLSLTQGTGANLV
SWPSNIKWNYGRVPVLSYKTGVRDIFQFVTYDGGNSWFGSLIMAGVE

SEQ ID No. 14 – Protein sequence of wt PVPSE1gp69 (918 amino acids)

MADYNDKVVNAEPILPEGTGGGTFVDDGQFHTEDTTTVTLLGNGNSTTPLKAAVVVDPA
SGNALVAGPDGLSVGVSPLSDNLLVLAGGKLYVAPPQIVVPISTKTGNVLVEVTDPGEE
GLFVPPGEKGDKGDKGDQGDPGVGIYIQGIIGDPSQLPPAATMQEGDTYVIGTHYWTVV
KGTWADLGDFAGPQGQDGIGLVIRGAFSDTSLLPTQGNTEGDTYIIQDQMWVWTGDADG
WQPVGQVGPAGPQGATGATGPQGPKGDKGDRGEKGDQGIQGIQGLTGPQGPKGDKGDKG
NDAAIVKLKGTKATVDELPEFGNAVADAWVVQTDNHVWVWTSDDTWEDIGPVQGPKGDQ
GDVGPQGPQGQKGDQGPEGPAGAEGPAGAEGPQGIQGPIGPKGDKGDTGLTGPVGPQGP
QGPKGDRGETGYGARVLGTKGAVSDLPATGTPGDAWIIVPNLYVWSQADAQWINVGPYV
GPKGDKGDTGATGDVGPKGDTGADGPQGPKGDQGDEGPQGPAGATGPQGPMGVSLTARG
TVANQAALPSGAATGDLYTTEDTGEAFAFDGTNWVNLGVMRGPQGDPGIQGDQGPMGAS
IVAKGVIATYEDLLDVQNPEQGWMYSVTSGGNKGYSYVYSGTAWEPMGDLTGPEGPQGP
QGPEGQMGAGVEILGKLDNTTELPSTGELGQGYLIEGDFWGWTGTTYENLGPIQGPQGD
VGPQGPQGPQGIQGIQGIKGDQGTLWLNFSRNPGPADGRIGDYFINKSTLEYFQKTSAT
AWASLGYMGGGNVYDTASTIPQARTNAGWVDVPVLEAPADTGYYVRVDNAWKKLDRYDL
LVTSTTGAMDVGVSQVFKVDGTANKTMSFTNLPANRAMTIVIVFSGSGASLTWPGNLAW
SNGTAVTLGTTRTVVTILWDGTNLTGTTSLTVD

Another preferred embodiment of the present invention provides new polypeptides that bind to *Salmonella* lipopolysaccharide(s) (LPS) comprising an isolated polynucleotide encoding the said polypeptide.

Another preferred embodiment of the present invention provides new polypeptides that bind to *Salmonella* lipopolysaccharide(s) (LPS) comprising the amino acid sequence encoded preferably by an isolated nucleotide sequences of SEQ ID No 1, 2, 3, 4, 5, 6, 7.

Namely:

SEQ ID No. 1 – Nucleotide sequence of wt PVPSE1gp40 (1815bp)

atgtatcctattccatgtctcttcttgacattatctggaggaggaacagaaccactacc
accaggtagtgttaagaaggtggcctttacccgtggtctggttggtggtgctaccaaaa
ggtcaatggcgatcctcctgattgatgggagactctatacccaaggggataatgcgtgg
tctgaatgcgcaaacgggaacataagcccgtttaaggatcattggcacttggcggctaa
tggtgtagctgatgtttttggtggtggtagagcctttgttgtcaaatacaacaatggtg
gctggcagtattgtggggacaccagtcaattcactggtagtgggtctatctattcttct
tggacaagcttcccttcatcaatcacaggcacagtatccctcgcaaatttacaaagtgt
ttcttgtgctctcggtaacacactctggcagatggtagatggaagactttatggaagcg
ggtcaaacacaaatggatgccttggctctggtaatactaccgtaatatcgatcccaaga
agtattagtgcatcctctgtgagagcttacagcttaaatgcttgcgtgacatacctgaa
caatattggacttccccgtgtttgtggggccacccatcagatagatggtacttcaacaa
cacaaacacaaaactttatcgatgttagttttgcctctgtgactgaaaccgtctatgtt
aaagagtggttagcgaacgaaacaaactcaatggctatcgcctctacaggtgttgatga
tacagagcattatttatatacgaggggtatcgggacagcccaatactctaagaaagagg
gtattggtcctttcgagacattcagggttatcgacggtggtcagtctcatttcctgatt
gctgataacaagctttacggtcttggtgacctaagtgctcaacttggccttggcacacc
atcaacaatggtgttagaaccgacccttgttccagttcctacaggaagggattgggatc
tttcgaagctgacatacattgtagacatgaagacagatgtccttaaccaggggaactct
atcagccattggatggtgtatgacgggaacctttactacgctggtaacctttatggttt
ctttgggtcaaccgatagcactggtgagttcacaaacatcccagaagcatcctttgggg

ggacaactgcggatgctatcaccacagggtctatcccatacgctatcaaagggtcgaga
agtcaacttacgtggactgttgagccagcagatgctgagatttatgatatttcgttcac
atcgagtgctcctaatatcgcaactgtagactcaaatggtatcatgaccttccttgaag
agggtggttttgacatcacaatgactgctaaaacagggtctggcgcggatgctaaaaca
ctcacagatacttctggcggttatgtttccatcttctctgtgactaccgattccatccc
acaaaaggaagttggtgatgtgttcgtgttcatggataaaaacagtcctgactatacac
caggtccgaacgttgtcggaatggaaatttctccagccaatgttgatactaacttcata
gacggagaattaacaactacaaatccggatgtggtgatgattgatgagggtggattcct
ttcctgtattgcagttggtgatgcccgttgtggtgttcgtcttatttacagggagggtc
aggttgaggcatttgatgattcgtatgtcagtgtttcagacttcacagccccaccagat
cctgtggaccccggcgaaccagttgtaccttctcaaccgcaataa

SEQ ID No. 2 – Nucleotide sequence of wt PVPSE1gp41 (1809bp)

gtggcagacatgactcaatttgaacaggctgtcgatcaggttgttgaagactctgaacg
cctccacaaggttgtcaacggaacggcatccgaaactgttgttactgaagatggtagca
ccattccaacggttcgtaaagctcttcttgacaacctgttctttaaaacaccgcctatg
ccgtggatcgcgggtactcagaccacagtattcaaccagctttatgcttttaatggcac
aaatggggttcaatggtggtatgcaccaaccgcaacagcaagcgcccctgtcgttttac
cacaaaacccagctaacagtgttaactggagattgtacaacgacgcagcggcgatggca
tctatctatgcaccaatcaacagcccgatcctgacaggaaacccacaggctccaacccc
tgcggctaacagcaacagtacaacaattgccacaactgcctttgttacaaccgctattg
caagcgccttgtcaagcatctcaggtggtagtgttacctttgcaaacctgtctgtaaca
ggtgctacaacactcaatagtttggtggttggtgggacaatcgaccttaatggccctgt
aaacgcagataactcaacaggtcgtttccagaacctgatcctgacaaaagagttgtcaa
gtcttaccttcgtatttacagacgcagataatccgacattctttaagacgcgccttgac
ccatatgcgatccaaacccacagcatccagacggacattattgtcaacggaacagtggc
tgaagatgatacaaccatgtctctgacaggtgtgggtaataacgtcttcgattatgtgt
atatccgtggtaacgcaagtaaagatcctacagcaccgagattaaaggtctcaggtaca
actgaggtagaaaacctgaatattacaggtaacgtaacaggtatcaccttcagtgtcaa
cggacttgatatttcaccaaactctgttacaaccgcagacggtgtaactgtaggtggtg
acttacaggtcagtggtgttaccaaccttggaaacgcaacaatcggtggccttgatatt

acaagtgatctgaccgtaaacggtaatacaacactcgaagatttctctgcgggtaacgg
aaacatcactggtccattcacagtgggtgggttaaccagtctcaatggtggatttacaa
ccggaacagcagatggtacaattggcggaaaactctctgttacaggaacgtctgagttt
attgaggatcttagcgtacttgccgatgttacagtccaggataacctgaccgtaaacgg
tgatgtcaaccttaacaacgcaacaggaacaacgacagttaacaaccttgttattcagg
gtacggttacaggtctttctgttgacttgacaggtcagaacatcaacgtaggatctctt
tcctctactggtgcagtaactgccaatagccttacggtacaggattcggcaatcttaac
caaggcaagtgttgaattcctgacacttattgctgaggatattgatagctctacagcgg
catggtccccaagtggagattccaacatctacaacgtaacagtggatgcggatttaaca
attggtgcttggcctgaaccgacagatgctttttctgctgtgatttacctgactcaaga
tggtactggcggtcacactgtgactttagatcctaactacttggttttaaacagcgaaa
caattaacgagacggctggttcggtgactattcttcaattaacctataatggtgttgaa
ggcggtgttatcgacactgtaattgttcgtcgtccgtaa

SEQ ID No. 3 – Nucleotide sequence of wt PVPSE1gp46 (1122bp)

atggcagcgccaacagtacctattgagatttgggcttatggagatattgttcttcctaa
cacccatgaattaaacaaagcacgtcctatcgatgacctgtggaacaaaggttgggacc
ttggtgaaaagccaacagtcgaagaatttaactacgttttaaatatgctgactgcatgg
gccaagtatatcactggtgagcagatccctggtctcgacagtcgtttcttacgtgtgaa
tcagaaccttgcagaccttgcagacaaggccgcagcaagaacaaacctcgatgtttgga
gtaaaacagagtctgataccagatatgtgaacatttcaggcgacaccatgactggtgca
ttatctgttccacgcctgaacctccaaccatccgaatcagattacgcatacatcacgac
caccaacccagcagcagacaccacattctttgattttgtagttggtgataatatcggta
atgccccaggtacgtcgagcatcgatagtatgcgtttccgcttcgtcccttctggtggc
tcaatcttcacaatgatggagttgaatgcgatcagtggcactgctgcactgtgtagggt
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caaacttcacaaacactaccgtaagcggtacgctgaatgctccaactattcagtccaca
acaatcaggacagggaccttaacggcaacaggaaacgttcaagggttcaacgttgtcgc
aacaagctctttaacaaccccatatgccagtgtaaacggacagtgtaacgttaactccc
ttgttgtcaacaacaactccgctacagttggtggacgaaacgttgtgagagcaatcaat
ggtgcaacggcagatggtaacggtaacgtcacactaaacctgtctggatttgttcagca

gatccgtttaggtaataggtttgccacaggtgtaagtgaatccagattttatgcaggtc
acgtcatgaccgggtgggctttcggtaacaagaaagaattgaggggcgcaacttactac
actgcaccactacagtatctcattaacggacaatgggtaactgtttccaacctggacta
a

SEQ ID No. 4 – Nucleotide sequence of wt PVPSE1gp48 (1494bp)

gtggctgctcaatatggattaaatgactacggctttgcgatcccatccctcgatgattt
aatcgcagataccaagcagtcgctgatccgaacttttggtgaaaacttcaacactcagg
ctaacactgttgttgacaaacttaccacaattttgaatgaaagagaatatcagttgatc
cttctggctgcggctgtgtattctgcacagacacttgcaggggcagaaggtatttatct
tgatgaactgcttggtcgtcgtggtatttatcgtcgtggtaaaaccagaggatcaggta
ctatccagatggttgttaacaacaccgtcccgtacaacatgatttacagttcttcaaca
tatagcattgacagtggtaacttcgtactcacgcaggacactcctgtcgcagggaatat
cctggcgcaacaaatccttaaccaagattgggttcttggtaactatacgttccagatga
ttaatcagaacgatggttcaacaaaatcaatgaacttgacgctaagtaataaggtgcct
aacagccctcagttaaatgcgttcatgtcatcgattaaggatttcattgttgacaactc
cacacagttaaacgaagacaggatcttcattgactctgcgggtggagcgatgtatatcg
ggtatgatgctaacaagaaaatgatcggacttaacagtcgtgttgatttccgttcatca
cccgtagtgggccagagaacaattaccattgaagtaattgctgctgaagcaggggctat
ttctcgtgaagcaaatactgtaacgaatatcacaccgacaccaagcggttttatcagca
tgaccaacatgaccgcctttaatgacggttccgatgttgagacagacactgattacaag
gttcgtgcttcacaaagcaccgcagcaggtgctgctgccactagacctgccgttatttc
tgccgttttaaacgtcgagggcgtcagcaaggttcgtgtattctcaaacaacacagggg
aaacagaccagtttggtgttccggcatataagtttgagactgtagtttatggtggttcc
acagaagaaatcagtgaagcactttataacacaatcgcattgtccaacgcaacctatgg
taatgtttttatgatgttaccacagaggatgatcagacggaacgtatctatcacagca
aagcacaggctcgtgagcttgcagtccgtgttcgttataaaggaaagctcctgtccgtg
acagagcaaaacactatcaaagatgcattgaaagccgttgttgatcctctcaacattgc
agatacactttacaacatccagttggtatccgcagttggttcctctatttcaccagggc
gcttcacgcaattgttggtggatgttaagaacactgaccaacctgacagtgcttatact
aacagcgatgttgttgcaggtatgacagaagtctttgctttagatacagacgacattac
attccagcaaatcatctaa

SEQ ID No. 5 – Nucleotide sequence of wt PVPSE1gp50 (2790bp)

atggcacagttacgccacaacattgataatgtttttcaattgatcgatggtcacataca
gttccttgaaagaaacaagggagagaccattgacccgacagttcagcactacatcctga
acttacagaatgttttggctaacaaccgtcactttatcaactggacggctcaagaagcc
cagcctaacggtgatgcaactaccgagggtcagtctgtcttgatcctcggttgcgcata
tgcatatcttgcaaccaacgaccctaaatatctggaacttgcagaagatttttggcaag
catatatcgactggttctttgctggtcagccaatcccagatcctccggcagtgtatcgc
ccgaactggatcattaacgggaaagagccaaggcttgcacattacccactgacagacga
tggatacccaacccacggtgggtttaagggtagcgttatgtcatggacaaatggtaaga
cgttaattcctcatggtgctccacattggggagaataccttgacaaggcttggttcgcc
tttgatggtaatcttggatggaattccgttaacgccacagtatatgctgctaacgcaga
tggtacgacaaattgggatcagtatgggtctcagtgggatgtcgattggatcattgaca
gactcggcagaaaagttgattgggacggtaatattcttgaagagggtttcccagaggcg
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gaaaccttacaagataa

SEQ ID No. 6 – Nucleotide sequence of wt PVPSE1gp51 (675bp)
atggcagatgtttcttttccaacggtgaaggtctctgaccttccttccgccgtcaccgt
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cccttattatggcaggagttgagtaa

SEQ ID No. 7 – Nucleotide sequence of wt PVPSE1gp69 (2757bp)

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aaggcgatacctacatcatccaagaccagatgtgggtttggactggcgatgcagacgga
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aagggattcagggcttaacagggcctcaaggacctaaaggtgacaagggcgataaaggt
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tcaaacggcacagcggttactctgggaactacccgcacggttgttactatcctctggga
cggtacaaacctgacaggtacaacctctttgactgtcgattaa

The nucleotide sequences coding for the polypeptides can be genetically fused to genes coding for fluorescent, bioluminescent or other biomarker proteins, or to a gene coding for a biotinylatable peptide or alternatively the polypeptides can be chemically conjugated to the above mentioned peptides. These fused recombinant proteins are able to specifically bind to *Salmonella* strains, to exhibit a fluorescent color (for instance green when excited at 475nm (emission maximum of 505nm)) and/or covalently bind to streptavidin-coated surfaces (which can be surfaces of detection devices or of magnetic beads). The polypeptides or derivatives thereof may additionally comprise of an affinity tag, e.g. histidine-tags, Strep-tags, Avi-tags, Myc-tags, Gst-tags, JS-tags, cysteine-tags, 6 FLAG-tags or other tags known in the art, thioredoxin or maltose binding

proteins (MBP) or other tags able to facilitate or increase protein expression and/or purification. Another preferred embodiment of the present invention provides an isolated nucleic acid molecule encoding the polypeptides.

Another embodiment of the present invention relates to a vector (e.g. plasmids or phages, or alike) comprising a nucleic acid molecule according to the present invention. An even more preferred embodiment of the present invention relates to a host cell comprising a nucleic acid molecule according to the present invention or a vector according to the present invention.

Another preferred embodiment of the present invention relates to the use of the polypeptide(s), or derivative(s) or a fusion protein or multiple combinations thereof according to the present invention for use as human medical, agro-food, environmental and veterinary diagnostic tool and/or antimicrobial agent. In a more preferred embodiment, said polypeptide(s) or derivative(s) according to the present invention is to be used in the detection/identification of *Salmonella spp*. in foodstuff, food processing equipment, food processing plants, food processing surfaces, medical devices, surfaces in hospitals and surgeries, human stools, environmental samples or any other samples where *Salmonella* might be present and also with specific antimicrobial properties, when fused to antimicrobial compounds, in food or on cosmetics, as disinfecting agent or in the environmental field. Another preferred embodiment, said polypeptide(s), a fragment(s) or derivative(s) or a fusion protein according to the present invention is(are) used in the production of a medicament for treatment or prevention of *Salmonella spp*. infections. Another preferred embodiment relates to the use of said polypeptide(s), a fragment(s) or derivative(s) or a fusion

protein according to the present invention for the treatment or prevention of *Salmonella spp*. contamination of foodstuff, of food processing equipment, of food processing plants, of surfaces coming into contact with foodstuff, of medical devices, of surfaces in hospitals and surgeries or other places where *Salmonella* might be present.
An isolated nucleic acid molecule encoding the polypeptide according to any one of claims 1 to 7.

Other preferred embodiment relates to a detection tool for recognizing Salmonella spp. comprising one of the polypeptides, or derivatives thereof, or combinations disclosed in the present subject matter; and described but it is not limited in figures 1 and 2.Another preferred embodiment of the present subject matter relates to a kit comprising the polypeptides or derivatives thereof, or combinations disclosed in the present subject matter.

Another preferred embodiment of the present subject matter relates to composition comprising the polypeptides or derivatives thereof described in the present invention. The pharmaceutical composition includes, but is not limited to, the fusion of antimicrobial compounds or cell-wall degrading enzymes and/or penetrating enzymes with polypeptides or derivatives described in the present invention in order to direct the antimicrobial compounds or cell- wall degrading enzyme to specific bacteria, that is, enabling specificity to the said antimicrobial compound or cell-wall degrading enzymes.

DETAILED DESCRIPTION

The following figures provide preferred embodiments for illustrating the description and should not be seen as limiting the scope of invention.

Figure 1 - shows the schematic representation of two examples of detection tools based on the peptides and derivatives covered by the present invention where in:
(Bi) represents a biotin;
(S) represents a streptavidin,
(pep)represents peptide,
(sup) represents solid sport,
(B) represents a target bacteria,
(MN) represents a magnetic nanoparticle,
(R) represents a reporter strain,
(C) represents a control,
(Sa) represents a sample,
(BP) represents a biochip platform,
(SE) represents a sensor,
(H) represents an external magnetic field,
(M) represents a label magnetization,
(a) represents a peptide immobilized on biochip platform,
(b) represents a biotinylated peptide immobilized on streptavidin modified magnetic nanoparticles
(1) represents a derivative immobilized on the surface of a microtiter plate;
(2) represents a target bacterium bound to the functionalized solid support;
(3) represents a reporter strain bound to free binding sites and the signal is measured and compared to that of the control
(4) represents a capture of the target bacteria by the biotinylated peptide immobilized on the magnetic nanoparticles;

(5) represents the peptide and target bacteria which are complementary leading to a biomolecular reaction;
(6) represents an external magnetic field that is applied and the sensor detects the fringe field due to label magnetization.

Figure 2 - shows the schematic representation of a non-specific antimicrobial fused to the peptides and derivatives covered by the present invention.
where in:
(pep)represents peptide,
(B) represents a target bacterium,
(NB) represents a non-target bacterium,
(AMC) represents an antimicrobial compound,
(7) represents the use of non-specific antimicrobials in the control of *Salmonella* spp,
(8) represents the use of non-specific antimicrobials fused to a tail protein in the control of *Salmonella* spp.

Figure 3 - shows the SDS-PAGE analysis of the recombinant expression and purification of PVPSE1gp51. SDS-PAGE analysis shows the elution protein fractions (E1, E2, E3) relative to the protometrics reference marker (M), as well as the flow through (FT) and waste fractions (W). Exp and ExpC are the expression culture before and after concentration by centrifugation respectively. The thick bands around 24 kDa indicate the high purity of the recombinant protein.

Figure 4 - shows the fluorescence intensity measured at 520 nm in the wells coated with different concentrations of PVPSE1gp40 and PVPSE1gp51 in PB (phosphate buffer 0.1 M, pH

7.5; 3.1 g $NaH_2PO_4.H_2O$, 10.9 g Na_2HPO_4)or SM buffers (sodium magnesium buffer 100 mM NaCl, 8mM $MgSO_4$, 50 mM Tris-HCl pH 7.5).

where in:

(51SM) represents PVPSE1gp40 in SM buffer,

(51PB) represents PVPSE1 gp40 in PB buffer at 0.1M,

(40PB) represents PVPSE1gp40 in PB buffer at 0.1M.

Figure 5 - shows the fluorescence intensity measured at 520 nm in the wells coated with 200µg/ml PVPSE1gp40 and PVPSE1gp51. Negative control is BSA (5%) and positive controls are phage PVPSE1 (1×10^9pfu's/ml) and Salmonella Polyclonal Antibody.

where in:

(I) represents Samples,

(II) represents BSA 5%,

(III) represents polyclonal antibody anti-Salmonella at 200 µg/ml,

(IV) represents phage PVP-SE1 at 1.0x109,

(V) represents PVP-SE1 gp40 at 200 µg/ml in PB buffer at 0.1M,

(VI) represents PVP-SE1 gp51 at 200 µg/ml in SM buffer.

Figure 6 - shows spots of immobilized PVPSE1gp51 on gold substrates specifically recognizing *Salmonella* Enteritidis cells (A: PVPSE1gp51 at 200 µg/ml, B: PVPSE1gp51 at approximately 10 mg/ml, C: PVPSE1gp51 at 10 mg/ml) and a Negative control of *Salmonella* Enteritidis cells detection using a spot of unspecific protein (Bovine Serum Albumin-BSA) (Image D). The Positive Control (E) shows spots of immobilized PVPSE1 phage on gold substrate specifically recognizing *Salmonella* Enteritidis cells.

Description

If appearing herein, the following terms shall have the definitions set out below.

The term "protein" as used herein refers synonymously to the term "polypeptide".
The term "protein/ polypeptide" as used herein refers to a linear polymer of amino acid residues linked by peptide bonds in a specific sequence. The amino acid residues of a protein may be modified by e.g. covalent attachments of various groups such as carbohydrates and phosphate. Other substances may be more loosely associated with the polypeptide chains, such as heme or lipid, giving rise to the conjugated proteins which are also comprised by the term “protein” as 20 used herein.

The term "fusion protein" as used herein refers to an expression product resulting from the fusion of two nucleic acid sequences. Such a protein may be produced, e.g., in recombinant DNA expression systems. Moreover, the term “fusion protein” as used herein refers to a fusion of a first amino acid sequence as e.g. tail protein, with a second or further amino acid sequence. Preferably, the second or further amino acid sequence is foreign to and not substantially homologous with any domain of the first amino acid sequence.

The term “tag” as used herein refers to a peptide which can be useful to facilitate expression and/or affinity purification of a polypeptide, to immobilize a polypeptide to a surface or to serve as a marker or a label moiety for

detection of a polypeptide e.g. by antibody binding in different ELISA assay formats.

The term “peptide” as used herein refers to short polypeptides consisting of from about 2 to about 100 amino acid residues, more preferably from about 4 to about 50 amino acid residues, more preferably from about 5 to about 30 amino acid residues, wherein the amino group of one amino acid residue is linked to the carboxyl group of another amino acid residue by a peptide bond. A peptide may have a specific function. A peptide can be a naturally occurring peptide or a synthetically designed and produced peptide. The peptide can be, for example, derived or removed from a native protein by enzymatic or chemical cleavage, or can be prepared using conventional peptide synthesis techniques (e.g., solid phase synthesis) or molecular biology techniques (see Sambrook, J. et al., Molecular Cloning: A Laboratory Manual, Cold Spring Harbor Press, Cold Spring Harbor, N.Y. (1989)).

The term “tail proteins” as used herein refers to a protein which is able to bind or adsorb to bacterial cell surfaces. Generally, tail proteins are able to bind different components on the surface of bacteria.

The term “wild type” or “wt” as used herein refers to the amino acid sequences of the PVPSE1 predicted tail proteins as depicted in SEQ ID No.: 8 to 14. The nucleic acid sequence encoding the wild PVPSE1 tail proteins are depicted in SEQ ID No. 1 to 7.

The term “derivative” as used herein refers to "fusion protein", an expression product resulting from the fusion

of two nucleic acid sequences or shorter versions of sequences that encode the tail proteins. The derivatives according to the present invention are fusion proteins.

The present invention relates to seven tail proteins of phage PVPSE1. This phage presents a very broad lytic spectrum and thus its tail proteins are useful for the construction of a diagnostic tool for the fast detection of *Salmonella* spp. and its specific control. The genes encoding for the polypeptides (SEQ ID No.8 to 14) comprise preferably one of the sequences SEQ ID No.1, 2, 3, 4, 5, 6 7.

The first derivatives are according to the present invention, the polypeptides fused at the N or C terminal of polypeptides to a fluorescent, bioluminescent or other biomarker protein. These derivatives are able to exhibit a fluorescence color (for instance green, when excited at 475nm (emission maximum of 505nm)).

The second derivatives comprise the polypeptides (SEQ ID No. 8, 9, 10, 11, 12, 13, 14) fused to a biotinylated peptide at the N- or C-terminal end. These derivatives are able to simultaneously bind to the bacterium to be detected and to streptavidin-coated surfaces. The biotin-streptavidin bond, once formed, is very resistant to extremes of temperature, pH organic solvents, denaturants, detergents and proteolytic enzymes requiring thus very harsh conditions to break the biotin-streptavidin interaction, conditions that often denature the protein of interest being purified. These features led to the widely use of biotin-streptavidin system in molecular biology and bionanotechnology, namely in the purification and detection

of various biomolecules and to attach various biomolecules to one another or onto a solid support.
The third derivatives are fused with a non-specific peptide or polypeptide with antimicrobial activity at the N or C terminal.

The first and second derivatives can be used directly in the construction of detection tools according, but not limited, to the following examples:
The immobilization of the derivatives on solid supports with streptavidin (see figure 1) through the biotinylated end.

The method developed through this approach consists in adding the sample to the wells of a microtiter plate coated with the derivatives in order to enable the target bacteria to be trapped by the peptides. This step can be repeated to increase the number of bound bacteria and thus sensitivity. Afterwards, wells are washed with buffer to remove unbound bacteria and a suspension with reporter bacterial strains with affinity to the immobilized peptides will then be added. The reporter strain can be a *Salmonella* strain with affinity to all the peptides of the present invention which must have a reporter gene (e.g. GFP and beta-galactosidase (beta- Gal)but not limited to these). The reporter strains bind to the free available binding surface producing a signal. By comparing this signal with the signal generated from wells saturated with the reporter strains (control) it will be possible to determine the number of target bacteria present in the sample.

The immobilization of the derivatives on magnetic beads coated with streptavidin and to a platform also coated with streptavidin (see figure 1).
The method developed through this approach consists in mixing the functionalized beads with the sample. The target bacteria will bind to the beads. Subsequently, the targeted bacteria from the sample now bound to the beads will bind to the sensor platform via immobilized second derivatives. The detection tool consists of a magnetoresistive sensor that will be used to detect the fringe fields emanating from magnetic nanoparticles that will attach to the platform as described elsewhere [7].

The first derivatives will also be used by direct addition to the sample. The said first derivatives will be used to detect the targeted bacteria present in samples, possibly contaminated with *Salmonella spp.* When samples are contaminated with *Salmonella spp.*, the said first derivatives will bind specifically to the targeted cells. The number of *Salmonella* cells specifically bound to the said first derivatives will be determined by direct observation of the sample under epifluorescence microscopy or by the determination of the intensity of the signal with a maximum emission at 475 nm when excited at maximum at 505 nm on a spectrofluorimeter, in case GFP was fused.
The third derivative can be used in medicament and pharmaceutical formulations in the treatment or prevention of Gram-negative bacterial infections, in particular for *Salmonella spp.* in cosmetic substance or sanitizing agents by increasing the specificity of antimicrobial agents to *Salmonella*. By fusing the third derivative to antimicrobial compound or protein (either recombinantly or chemically)

the resulting product will be directly guided towards *Salmonella spp.*, and as such increase the efficiency and specificity of the antimicrobial compound or protein (Figure 2).

The following example explains the present invention but is not considered to be limiting to the disclosure or the appended claims.

Unless indicated differently, molecular biological standard methods were used, as e.g., described by Sambrook et al., 1989, Molecular 15 Cloning: A Laboratory Manual, 2nd edition, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York.

EXAMPLE 1. Cloning, expression and purification of the tail proteins of the *Salmonella* Enteritis phage PVPSE1

Purified genomic DNA of phage PVPSE1 was used as a template for the amplification of the seven tail proteins in a standard PCR reaction with Phusion polymerase using the following PCR parameters: Start: 98°C, 2min; followed by 35 cycles of [98°C (20 sec) 50°C (30 sec) 72 °C (1 min)], followed by 72°C (7 min) & cooling to 4°C and storage.
Primers used during standard PCR amplification were as follows-:

- ORF 40 Fw 5' GGGCCGGATCCGACGACGACGACAAGATGTATCCTATTCCATGTCTCTTCTTG 3' - (SEQ ID No. 15)

- ORF 40 Rev 5' GGGCCGAGCTCTTATTGCGGTTGAGAAGGTACAACT 3' (SEQ ID No. 16)

- ORF 41 Fw 5' GGGCCGGATCCGACGACGACGACAAGGTGGCAGACATGACTCAATTTG 3' (SEQ ID No. 17)
- ORF 41 Rev 5' GGGCCGAGCTCTTACGGACGACGAACAATTACAG 3' (SEQ ID No. 18)
- ORF 46 Fw 5' GGGCCGGATCCGACGACGACGACAAGATGGCAGCGCCAACAGTAC 3' (SEQ ID No. 19)
- ORF 46 Rev 5' GGGCCGAGCTCTTAGTCCAGGTTGGAAACAGTTAC 3' (SEQ ID No. 20)
- ORF 48 Fw 5' GGGCCGGATCCGACGACGACGACAAGGTGGCTGCTCAATATGGATTAAA 3' (SEQ ID No. 21)
- ORF 48 Rev 5' GGGCCGAGCTCTTAGATGATTTGCTGGAATGTAATGT 3' (SEQ ID No. 22)
- ORF 50 Fw 5' GGGCCGGATCCGACGACGACGACAAGATGGCACAGTTACGCCACAA 3' (SEQ ID No. 23)
- ORF 50 Rev 5' GGGCCGAGCTCTTATCTTGTAAGGTTTCTGTACATAG 3' (SEQ ID No. 24)
- ORF 51 Fw 5' GGGCCGGATCCGACGACGACGACAAGATGGCAGATGTTTCTTTTCCAACG 3' (SEQ ID No. 25)
- ORF 51 Rev 5' GGGCCGAGCTCTTACTCAACTCCTGCCATAATAAGG 3' (SEQ ID No. 26)
- ORF 69 Fw 5' GGGCCGGATCCGACGACGACGACAAGATGGCAGATTACAATGATAAGGTCG 3' (SEQ ID No. 27)

- ORF 69 Rev 5' GGGCCGAGCTCTTAATCGACAGTCAAAGAGGTTGTAC 3' (SEQ ID No. 28)

The obtained PCR fragments were then ligated in the commercial available pET28a(+) expression vector (Novagen), which was previously provided with the gene, encoding a fluorescent, bioluminescent or other biomarker protein and/or a biotinylated peptide, following digestion with BamHI and SacI restriction enzymes of both vector and PCR fragment. Ligation was carried out by adding the vector, PCR fragment, T4 ligase (New England Biolabs) and respective buffer followed by incubation at 16°C for 2 hours.

The obtained DNA and amino acid sequences for the recombinant tail proteins are listed in SEQ ID No. 1 to 7 and SEQ ID No. 8 to 14, respectively. Recombinant expression of tail proteins was performed in exponentially growing E. coli BL21 (λDE3) pLysS cells after induction with 1 mM IPTG (isopropylthiogalactoside) at 37°C for a period of 4 to 18 hours. The tail proteins were then purified by Ni2+ affinity chromatography (Akta FPLC, GE Healthcare) using the C-terminal 6xHis-tag, encoded by the pET28a(+) expression vector. The Ni2+ affinity chromatography is performed in 4 subsequent steps, all at room temperature:

1. Equilibration of the Histrap HP 1 ml column (GE Healthcare) with 10 column volumes of Washing Buffer (60 mM imidazole, 0.5 mM NaCl and 20 mM NaH2P04-NaOH on pH 7.4) at a flow rate of 0.5 ml/min.
2. Loading of the total lysate (with the wanted tail protein) on the Histrap HP 1 ml column at a flow rate of 0.5 ml/min.

3. Washing of the column with 15 column volumes of Washing Buffer at a flow rate of 1 ml/min.
4. Elution of bound tail protein from the column with 10 column volumes of Elution Buffer (500 mM imidazole, 5 mM NaCl and 20 mM NaH2P04-NaOH on pH 7.4) at a flow rate of 0.5 ml/min.
The yield for the purification of recombinant tail protein per liter *E. coli* expression culture is determined by spectrophotometric measurement at 280 nm. Purified stock solution of recombinant tail protein in Elution Buffer (20 mM NaH2P04-NaOH pH7.4; 0.5 M NaCl; 500 mM imidazole) is determined visually on an SDS-PAGE gel (see Figure 3). The protein concentration is determined spectrophotometrically at a wavelength of 280 nm.

Example 2 Binding ability of the tail proteins of the *Salmonella* Enteritidis phage PVPSE1 by immunofluorescence

The tail proteins prepared as described in Example 1 (at concentrations of 100µg/ml, 200 µg/ml, 300 µg/ml, 400 µg/ml and 500 µg/ml in PB (phosphate buffer 0.1 M, pH 7.5; 3.1 g $NaH_2PO_4.H_2O$, 10.9 g Na_2HPO_4) or SM (sodium magnesium buffer 100 mM NaCl, 8mM $MgSO_4$, 50 mM Tris-HCl pH 7.5) and the positive/negative controls were immobilized onto a polystyrene microtiter plate by passive adsorption and incubated during 2 hours at room temperature, followed by a three times washing procedure with PB or SM. Positive controls consist of *Salmonella spp.* Polyclonal Antibody (200 µg/ml) and PVPSE1 (phage stock at a concentration $1.0x10^{10}$ pfu's/ml). Negative controls consisted of bovine serum albumin (BSA) 5% in PB and PB or SM.
After the immobilization of tail proteins, 1% of BSA in PB was added as a blocking agent to each well followed by a 40

min incubation at room temperature and a three times washing with PB.

One hundred milliliter of an exponential culture of *Salmonella enteritidis* S140013 grown in Luria Broth (37°C, 120 rpm) until an optical density at 600 nm of 0.4, which corresponds to $1.0x10^9$ cells/ml, was added to each well followed by a 1-hour incubation period at room temperature. After a washing step with PB to remove unbound bacteria, the wells were filled with 100 µl of *Salmonella spp*. Polyclonal Antibody, Biotin conjugated at 200 µg/ml (Thermo Scientific, ref PA1-73022) and incubated during 1 hour. After, the wells were washed with PB and filled with 100 µl Streptavidin Rhodamine conjugated (TRITC, Pierce, ref 21724) at 20 µg/ml and incubated 40 minutes at room temperature. After several washes with PB, a volume of 100 µl of PB was added to each well and the fluorescence was measured by a Synergy H1 microplate reader at 520 nm of excitation and at 570 nm of emission.

The results showed that the buffer and protein concentration influence the signal intensity and thus these two variables need to be optimized for each tail protein. For example, the gp40 presents a higher signal in PB at 200 µg/ml, while gp51 gives better results in SM at 200 µg/ml (Figure 4).

Comparison of the fluorescence intensity obtained with the peptides, the entire phage PVPSE1, *Salmonella* Polyclonal antibody and the negative control (Figure 5) showed that the tail proteins present the same signal intensity of a *Salmonella* Polyclonal antibody and of the entire phage particle showing the high binding efficiency of said proteins.

Example 3 Binding affinity of tail proteins immobilized on gold substrates

Au substrates (7x7 mm) were incubated for 2 hours in Microstrip® 3001(Fujifilm Electronic Materials, Belgium) at 65 °C for removal of the protective coating of photoresist polymer. The substrates were then washed three times with isopropanol and sterile distilled water and dried under N_2. After the cleaning step, the tail proteins at a concentration of 200 µg/ml, prepared in SM buffer, were immobilized by physical adsorption on the Au substrates in spots of 1 µl and incubated four hours at room temperature in a humidified atmosphere, in order to prevent evaporation. The unbound tail proteins were removed by washing three times with SM. The spots were then incubated with the blocking agent BSA 1% prepared in TE during 45 minutes and the excess of blocking solution was removed by washing the substrate with PB (0.1M, pH=7.5). All substrates were exposed to $2.8x10^9$ cells/ml during 1 hour and washed with PB (0.1M, pH=7.4) and distilled water. The bacterial coverage extent in spot and out spot was visualized by a Nikon SMZ 1500 microscope. Results are showed in figure 6 and were consistent with the results reported in Example 2, the tail protein concentration influences its binding ability, moreover the binding ability of the tail protein seems equal to that of the entire phage PVPSE1.

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The invention is of course not in any way restricted to the embodiments described and a person with ordinary skill in the art will foresee many possibilities to modifications thereof without departing from the basic idea of the invention as defined in the appended claims.

The following claims set out particular embodiments of the invention.

C L A I M S

1. An isolated polypeptide having the sequence of one of SEQ ID No 8, 9, 10, 11, 12, 13, or 14, or derivatives thereof, or combinations thereof, for recognizing or binding to *Salmonella*.

2. The polypeptide according to claim 1, wherein the polypeptide is fused with a biomarker protein at the N- or C-terminal end.

3. The polypeptides according to the previous claim wherein the said biomarker protein is a colored fluorescent protein or a bioluminescent protein, preferably GFP.

4. The polypeptides according to the previous claims, wherein the polypeptides are fused with a biotinylated peptide at the N- or C terminal end.

5. The polypeptides according to the previous claims, wherein the polypeptides are fused to a compound having an antimicrobial activity at the N- or C-terminal end.

6. The polypeptide according to any of the preceding claims further comprising a tag.

7. The polypeptide according to the previous claim wherein the said tag is selected from histidine-tags, Strep-tags, Avi-tags, Myc-tags, Gst-tags, JS-tags, cysteine-tags, 6 FLAG-tags, and/or thioredoxin or maltose binding proteins.

8. The polypeptide according to any one of the preceding claims for use in medicine or as medicament.

9. The polypeptide according to the previous claim for use in treatment or prevention of *Salmonella spp.* infections.

10. The polypeptide according to any one of the claims 1 to 7 for use in the treatment or prevention of bacterial contamination, particularly of Gram-negative contamination, of foodstuff, of food processing equipment, of food processing plants, of surfaces coming into contact with foodstuff, of medical devices, surfaces in hospitals and surgeries, of environmental samples.

11. An isolated polynucleotide encoding the polypeptide according to any one of the claims 1-7.

12. An isolated nucleic acid molecule having the sequence of one of SEQ ID No 1, 2, 3, 4, 5, 6, 7.

13. An isolated nucleic acid molecule encoding the polypeptide according to any one of claims 1 to 7.

14. A vector comprising the nucleic acid molecule according to any one of the claims 11-13.

15. A host cell comprising the nucleic acid molecule according to claim 11-13 or the vector according to claim 14.

16. A detection tool for recognizing *Salmonella spp.* comprising one of the polypeptides, or derivatives

thereof, or combinations thereof according to claims 1 to 7

17. A kit comprising the polypeptides or derivatives thereof according to any one of the claims 1 to 7.

18. A pharmaceutical composition comprising the polypeptides or derivatives thereof described in any one of claims 1 to 7.

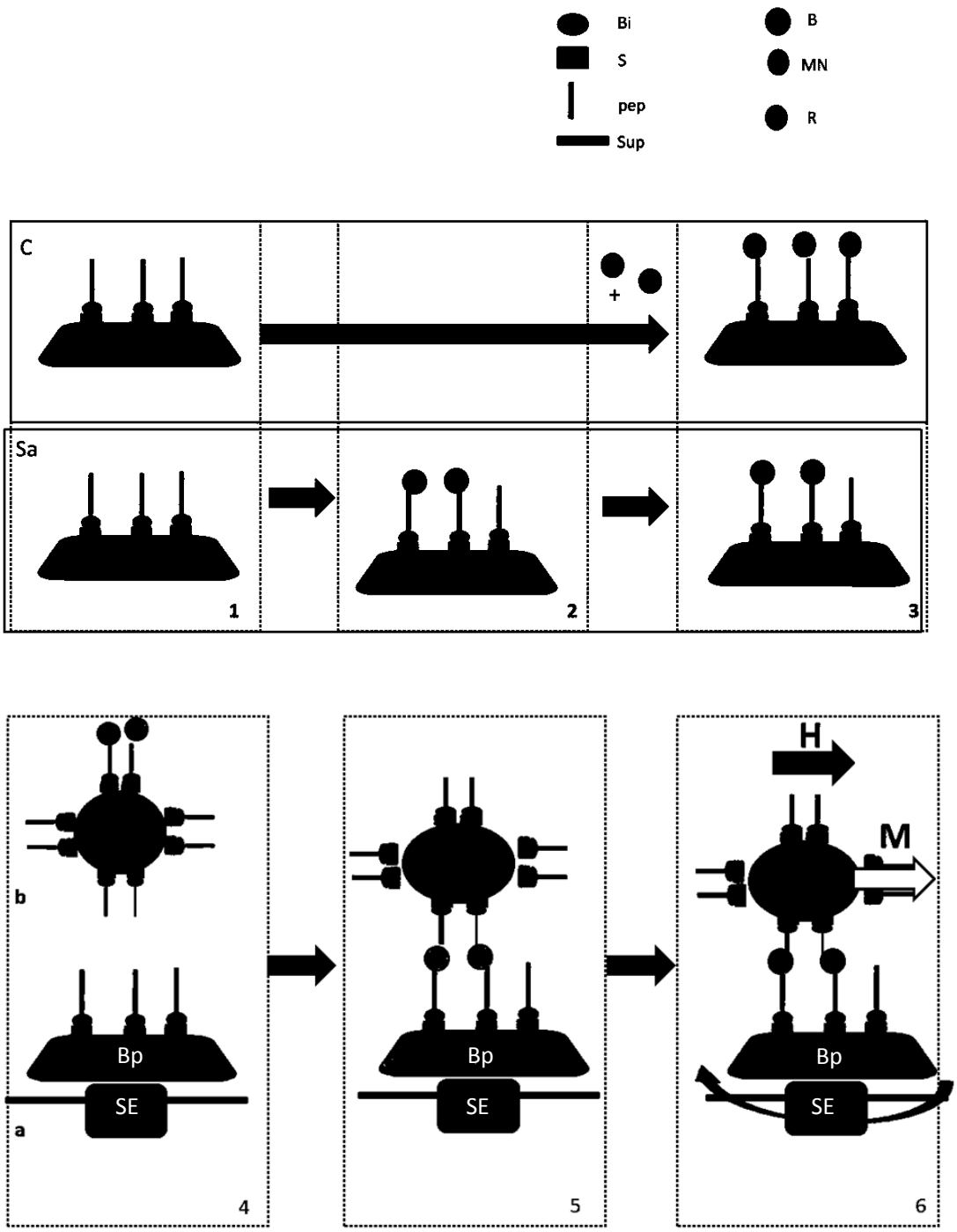

Figure 1

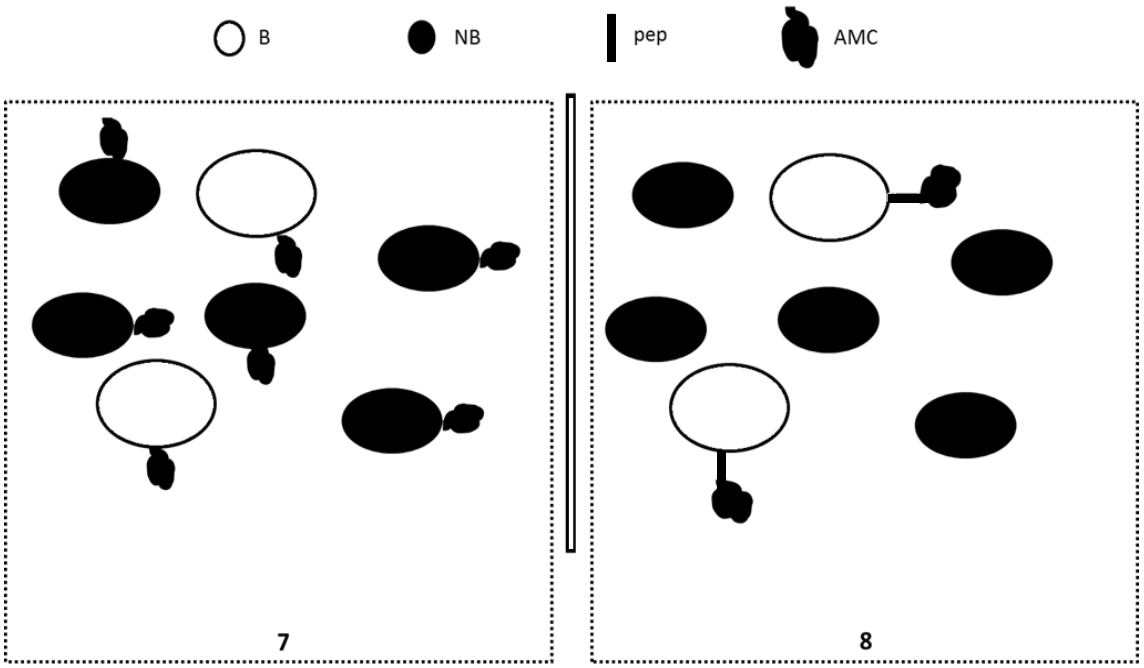

Figure 2

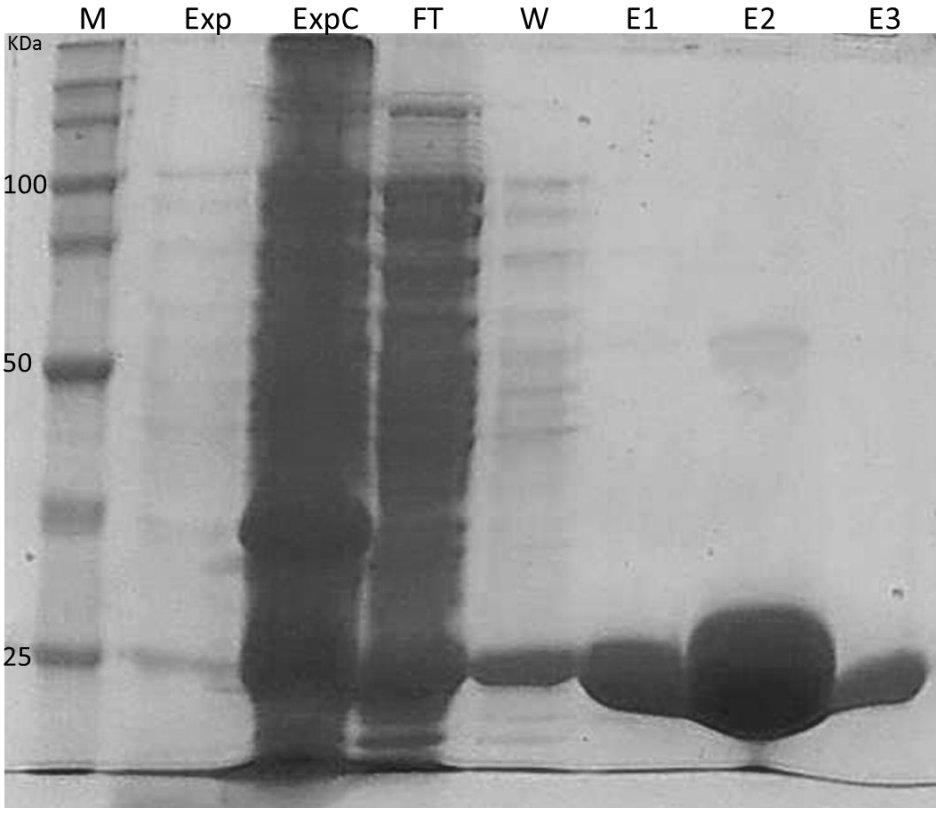

Figure 3

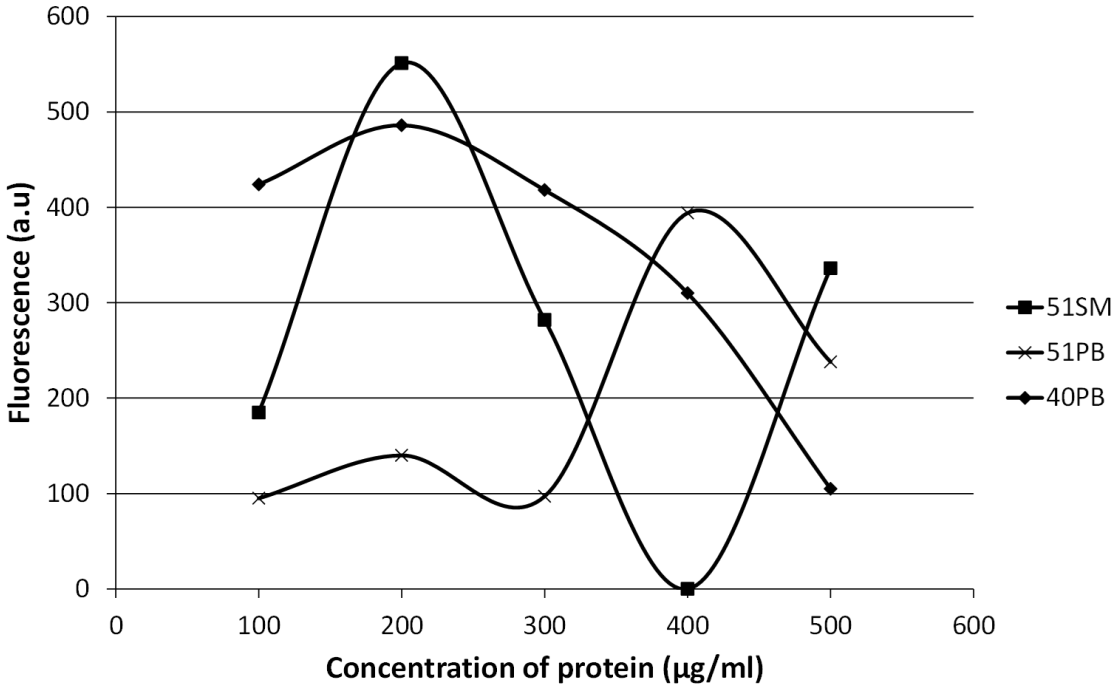

Figure 4

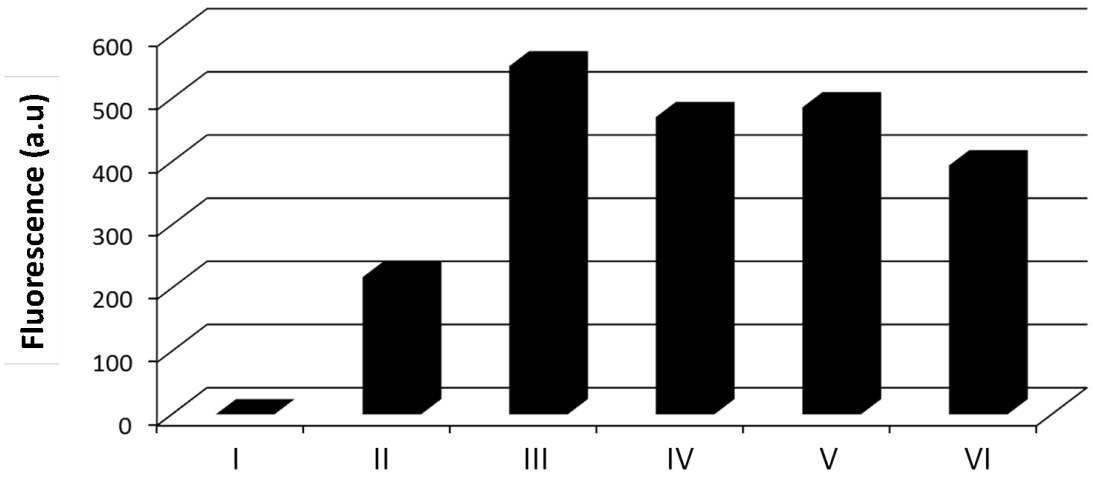

Figure 5

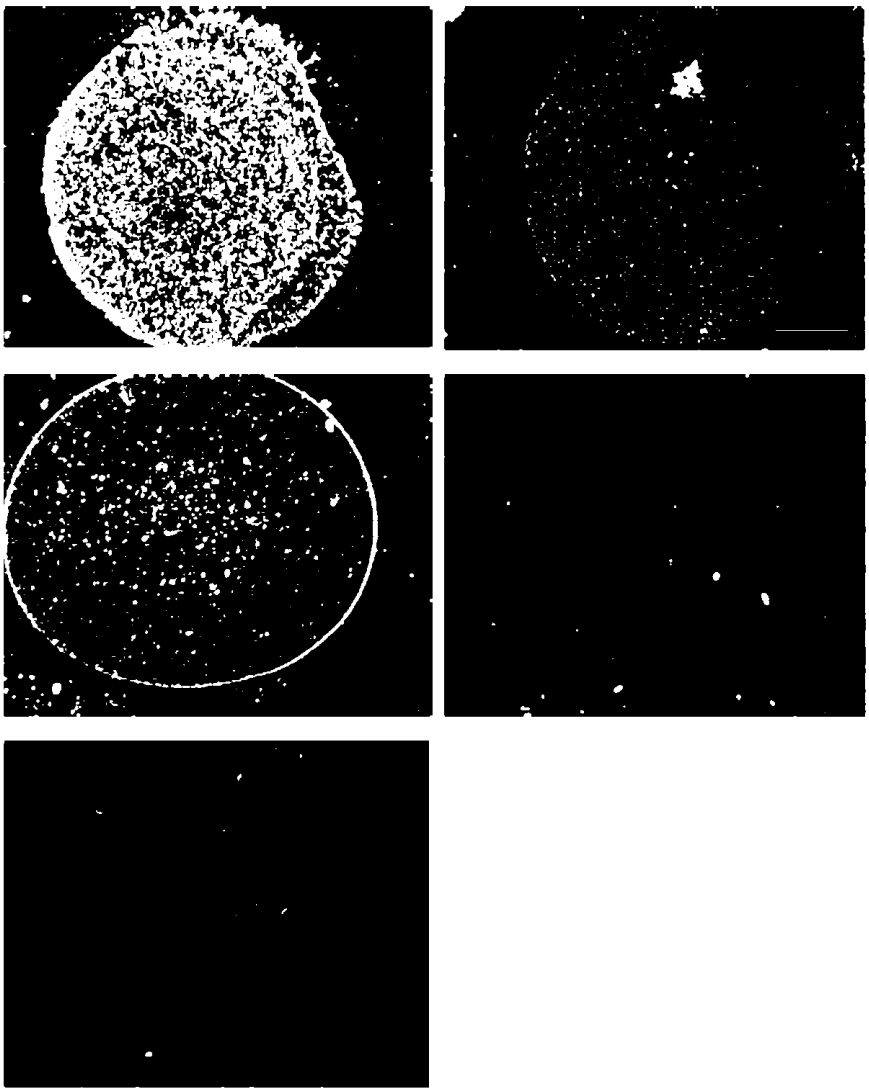

Figure 6