US 20150265692A1

(19) United States

(12) Patent Application Publication

(10) Pub. No.: US 2015/0265692 A1

(43) Pub. Date: Sep. 24, 2015

Entenza et al.

(54) **IMMUNOGENIC COMPOSITION COMPRISING AN INACTIVATED RECOMBINANT NON-PATHOGENIC BACTERIUM**

(71) Applicant: **Universite de Lausanne**, Lausanne (CH)

(72) Inventors: **Jose Entenza**, Lausanne (CH); **Philippe Moreillon**, Lausanne (CH); **Tiago Rafael Veloso**, Lausanne (CH); **Yok-ai Que**, Lausanne (CH); **Mathilde Ythier**, Lausanne (CH)

(21) Appl. No.: **14/426,273**

(22) PCT Filed: **Sep. 4, 2013**

(86) PCT No.: **PCT/EP2013/068231**

§ 371 (c)(1),
(2) Date: **Mar. 5, 2015**

(30) **Foreign Application Priority Data**

Sep. 6, 2012 (EP) 12183285.1

Publication Classification

(51) **Int. Cl.**

A61K 39/085 (2006.01)
C07K 14/31 (2006.01)
C07K 16/12 (2006.01)

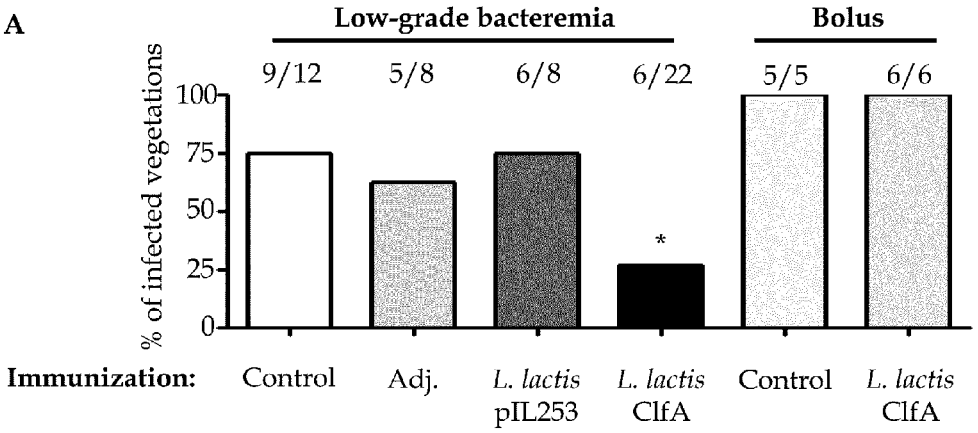
(52) **U.S. Cl.**

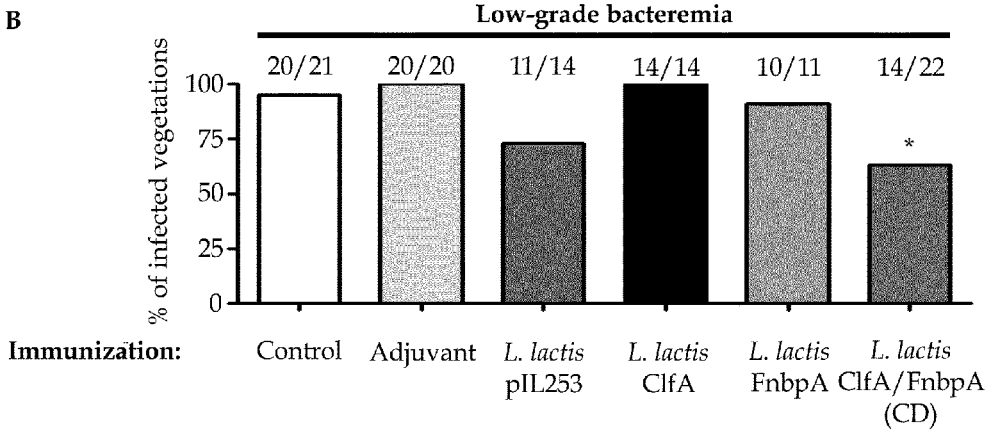
CPC ***A61K 39/085*** (2013.01); ***C07K 16/1271*** (2013.01); ***C07K 14/31*** (2013.01); *A61K 2039/521* (2013.01)

(57) **ABSTRACT**

The present invention relates to immunogenic compositions, vaccines and antibodies for the treatment and/or prevention of infections and diseases caused by *S. aureus* in a subject in need thereof.

Fig. 1


A
Low-grade bacteremia
Bolus
9/12
5/8
6/8
6/22
5/5
6/6
% of infected vegetations
100
75
50
25
0
*
Immunization:
Control
Adj.
L. lactis pIL253
L. lactis ClfA
Control
L. lactis ClfA


B
Low-grade bacteremia
20/21
20/20
11/14
14/14
10/11
14/22
% of infected vegetations
100
75
50
25
0
*
Immunization:
Control
Adjuvant
L. lactis pIL253
L. lactis ClfA
L. lactis FnbpA
L. lactis ClfA/FnbpA (CD)

IMMUNOGENIC COMPOSITION COMPRISING AN INACTIVATED RECOMBINANT NON-PATHOGENIC BACTERIUM

FIELD OF THE INVENTION

[0001] The present invention relates to immunogenic compositions, vaccines and antibodies for the treatment and/or prevention of infections and diseases caused by *S. aureus* in a subject in need thereof.

BACKGROUND OF THE INVENTION

[0002] *Staphylococcus aureus* is a major pathogen responsible for a variety of diseases, from benign skin infections, such as folliculitis and furunculosis, to life-threatening conditions, including erysipelas, deep-seated abscesses, osteomyelitis, pneumonia, sepsis, and infective endocarditis (IE). In addition to infections in which the organism is physically present at the infected site, *S. aureus* is also capable of producing "distant" diseases, which are mediated by the secretion of toxins. This success is ensured by the coordinated expression of numerous surface adhesins, which mediate host-tissue colonization, and secreted proteins and toxins, which promote invasion as well as strategies to escape the host immune system (Que et al., 2009).

[0003] For instance, *S. aureus* adhesins—which are collectively referred to as MSCRAMMs for Microbial Surface Components Reacting with Adherence Matrix Molecules—encompass at least 21 surface-anchored proteins including fibrinogen-binding proteins A and B (clumping factors A and B, or ClfA and ClfB), fibronectin-binding proteins A and B (FnPBA and FnBPB), collagen-binding protein (Cna) and protein A (Spa) to mention just a few (Patti et al., 1994).

[0004] Previous studies have shown that ClfA is essential for the development of IE. Moreover, after individual expression of ClfA in the non-pathogenic bacteria *Lactococcus lactis*, it has been shown that fibrinogen-binding was pivotal in promoting IE. In addition ClfA also interacts with the GPIIβIIIα receptor on the surface of platelets, a feature that plays an important indirect role in the ability of *S. aureus* to induce IE (Que et al., 2011).

[0005] Numerous attempts to develop vaccines against a variety of these structures, especially against the fibrinogen binding-domain of ClfA, have been attempted with various successes in animal models, but none of them have achieved sustainable efficacy in human clinical trials (Broughan et al., 2011).

[0006] In parallel, expressing antigens in non-pathogenic *L. lactis* has been attempted to trigger mucosal immunity. However, technical issues such as in vivo persistence of the bacterial strain as well as antigen release are as yet incompletely solved (Wells et al., 2008).

[0007] However, despite these studies and attempts, there is currently no efficacious immunogenic composition for treating and/or preventing *S. aureus* infections and diseases caused by *S. aureus*.

[0008] This object has been achieved by providing an immunogenic composition comprising an inactivated recombinant non-pathogenic bacterium, or a part thereof, expressing on its cell surface at least one folded sequence of a *S. aureus* adhesin, or a sequence having 80% or more sequence identity to said folded sequence of a *S. aureus* adhesin, wherein said at least one folded sequence of a *S. aureus* adhesion, or sequence having 80% or more sequence identity to said folded sequence of a *S. aureus* adhesin, is entirely accessible to trypsin digestion.

SUMMARY OF THE INVENTION

[0009] The invention provides an immunogenic composition comprising an inactivated recombinant non-pathogenic bacterium, or a part thereof, expressing on its cell surface at least one folded sequence of a *S. aureus* adhesin, or a sequence having 80% or more sequence identity to said folded sequence of a *S. aureus* adhesin, wherein said at least one folded sequence of a *S. aureus* adhesion, or sequence having 80% or more sequence identity to said folded sequence of a *S. aureus* adhesin, is entirely accessible to trypsin digestion.

[0010] Furthermore, the invention also provides a vaccine comprising an immunogenic composition of the invention in an immunologically acceptable carrier or diluent.

[0011] The invention further provides the use of the vaccine of the invention for the treatment and/or prevention of infections and diseases caused by *S. aureus* in a subject in need thereof.

[0012] Also provided is an isolated and/or purified antibody, antibody fragment or derivative thereof able to bind to the at least one folded sequence of a *S. aureus* adhesin expressed on the cell surface of an inactivated recombinant non-pathogenic bacterium.

BRIEF DESCRIPTION OF THE FIGURE

[0013] FIG. **1**. Prevention of *S. aureus* experimental endocarditis (i.e. infected vegetations in cardiac valves) in rats immunized with vaccine preparations. Rats were immunized with various vaccine preparations (see infra) for 6 weeks as described, before aortic vegetations were induced. 24 h later, they were challenged with identical inoculum sizes administered either by continuous infusion (0.0017 ml/min over 10 h), or by i.v. bolus (1 ml in 1 min). (A) Animals challenged with *S. aureus* Newman (10^4 CFU), and (B) with *S. aureus* P8 (10^6 CFU). * $P<0.05$ compared to the control group by χ^2 test. Control: group immunized with PBS; Adj.: group immunized with Freund's adjuvant group with the adjuvant emulsified at a 1:1 ratio in PBS; *L. lactis* plL253, *L. lactis* ClfA and *L. lactis* ClfA/FnBPA(CD): groups immunized with *L. lactis* plL253, ClfA or *L. lactis* ClfA/FnBPA (CD), respectively, emulsified at a 1:1 ratio in Freund's adjuvant.

DESCRIPTION OF THE INVENTION

[0014] 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. All publications, patent applications, patents, and other references mentioned herein are incorporated by reference in their entirety. The publications and applications discussed herein are provided solely for their disclosure prior to the filing date of the present application. Nothing herein is to be construed as an admission that the present invention is not entitled to antedate such publication by virtue of prior invention. In addition, the materials, methods, and examples are illustrative only and are not intended to be limiting.

[0015] Unless defined otherwise, all technical and scientific terms used herein have the same meaning as is commonly

understood by one of skill in art to which the subject matter herein belongs. As used herein, the following definitions are supplied in order to facilitate the understanding of the present invention.

[0016] The term "comprise" or "comprising" is generally used in the sense of include/including, that is to say permitting the presence of one or more features or components.

[0017] As used in the specification and claims, the singular form "a", "an" and "the" include plural references unless the context clearly dictates otherwise.

[0018] As used herein, "at least one" means "one or more."

[0019] As used herein, the terms "protein", "polypeptide", "polypeptidic", "peptide" and "peptidic" are used interchangeably herein to designate a series of amino acid residues connected to the other by peptide bonds between the alpha-amino and carboxy groups of adjacent residues.

[0020] As used herein the term "subject" is well-recognized in the art, and, is used herein to refer to a mammal, including dog, cat, rat, mouse, monkey, cow, horse, goat, sheep, pig, camel, and, most preferably, a human. The term does not denote a particular age or sex. Thus, adult and newborn subjects, whether male or female, are intended to be covered.

[0021] The present invention provides an immunogenic composition comprising an inactivated recombinant non-pathogenic bacterium, or a part thereof, expressing on its cell surface at least one folded sequence of a *S. aureus* adhesin, or a sequence having 80% or more sequence identity to said folded sequence of a *S. aureus* adhesin, wherein said at least one folded sequence of a *S. aureus* adhesion, or sequence having 80% or more sequence identity to said folded sequence of a *S. aureus* adhesin, is entirely accessible to trypsin digestion.

[0022] A "non-pathogenic bacterium" refers to a bacterium that does not cause a disease state when in contact with a subject. Examples of non-pathogenic bacteria are selected from the non-limiting group comprising the *Bacillus* genus, *Lactobacillus* genus, *Lactococcus* genus, *Sporolactobacillus* genus, *Bifidobacterium* genus, and the like bacteria. Preferably, the non-pathogenic bacterium is selected from the group comprising *L. lactis, L. acidophilus* and Lactobacilli. Particularly preferred is *L. lactis* and most particularly preferred is *L. lactis* subspecies *cremoris* 1363.

[0023] *Lactococcus lactis* is a nonpathogenic bacterium that is being developed, in its live form, as a vaccine delivery vehicle for immunization by mucosal routes.

[0024] However, the non-pathogenic bacterium of the invention is in an inactivated form. Inactivation is performed using methods and/or compounds known in the art, such as for example by H_2O_2, formaldehyde, heat or UV treatments.

[0025] The present invention also refers to a part of said inactivated recombinant non-pathogenic bacterium. Usually, this part consists in an isolated and/or purified cell wall of the inactivated recombinant non-pathogenic bacterium.

[0026] Generally, the non-pathogenic bacterium of the invention is a recombinant bacterium as it comprises at least one heterologous nucleic acid molecule encoding a folded sequence of a *S. aureus* adhesin.

[0027] Usually, the nucleic acid molecule encoding a folded sequence of a *S. aureus* adhesin is in the form of deoxyribonucleic acid (DNA). DNA which can be used herein is any polydeoxynuclotide sequence, including, e.g. double-stranded DNA, single-stranded DNA, double-stranded DNA wherein one or both strands are composed of two or more fragments, double-stranded DNA wherein one or both strands have an uninterrupted phosphodiester backbone, DNA containing one or more single-stranded portion(s) and one or more double-stranded portion(s), double-stranded DNA wherein the DNA strands are fully complementary, double-stranded DNA wherein the DNA strands are only partially complementary, circular DNA, covalently-closed DNA, linear DNA, covalently cross-linked DNA, cDNA, chemically-synthesized DNA, semi-synthetic DNA, biosynthetic DNA, naturally-isolated DNA, enzyme-digested DNA, sheared DNA, labeled DNA, such as radiolabeled DNA and fluorochrome-labeled DNA, DNA containing one or more non-naturally occurring species of nucleic acid, genomic or complementary DNA. Preferably, the DNA is a genomic DNA or a complementary DNA (cDNA).

[0028] DNA sequences that encode a folded sequence of a *S. aureus* adhesin can be synthesized by standard chemical techniques, for example, the phosphotriester method or via automated synthesis methods and PCR methods.

[0029] The DNA sequence encoding a folded sequence of a *S. aureus* adhesin according to the invention may also be produced by enzymatic techniques. Thus, restriction enzymes, which cleave nucleic acid molecules at predefined recognition sequences can be used to isolate nucleic acid sequences from larger nucleic acid molecules containing the nucleic acid sequence, such as DNA (or RNA) that codes for a peptide consisting a folded sequence of a *S. aureus* adhesin.

[0030] Encompassed by the present invention is also a nucleic acid in the form of a polyribonucleotide (RNA), including, e.g., single-stranded RNA, cRNA, double-stranded RNA, double-stranded RNA wherein one or both strands are composed of two or more fragments, double-stranded RNA wherein one or both strands have an uninterrupted phosphodiester backbone, RNA containing one or more single-stranded portion(s) and one or more double-stranded portion(s), double-stranded RNA wherein the RNA strands are fully complementary, double-stranded RNA wherein the RNA strands are only partially complementary, covalently crosslinked RNA, enzyme-digested RNA, sheared RNA, mRNA, chemically-synthesized RNA, semi-synthetic RNA, biosynthetic RNA, naturally-isolated RNA, labeled RNA, such as radiolabeled RNA and fluorochrome-labeled RNA, RNA containing one or more non-naturally-occurring species of nucleic acid.

[0031] The present invention also includes variants of the aforementioned sequences that are nucleotide sequences that vary from the reference sequence by conservative nucleotide substitutions, whereby one or more nucleotides are substituted by another with same characteristics.

[0032] The invention also encompasses allelic and polymorphic variants of the aforementioned sequences; that is, naturally-occurring alternative forms of the folded sequence of a *S. aureus* adhesin that also encode peptides that are identical, homologous or related to that encoded by the sequence of a *S. aureus* adhesin. Alternatively, non-naturally occurring variants may be produced by mutagenesis techniques or by direct synthesis.

[0033] Also encompassed in the present invention is a sequence having 80% or more sequence identity to said folded sequence of a *S. aureus* adhesin. The percentage identity of a polynucleotide or polypeptide sequence is determined by aligning polynucleotide and polypeptide sequences; identifying the number of identical nucleic or amino acids over the aligned portions; dividing the number of

identical nucleic or amino acids by the total number of nucleic or amino acids of the polynucleotide or polypeptide of the present invention; and then multiplying by 100 to determine the percentage identity. Preferably, the sequence has at least 80%, at least 81%, at least 82%, at least 83%, at least 84%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% sequence identity to said folded sequence of a *S. aureus* adhesin.

[0034] In case the sequence of a *S. aureus* adhesin is the clumping factor A (ClfA) then the acid nucleic molecule encoding the folded sequence of a *S. aureus* adhesin, namely the ClfA, is a genomic DNA as set forth in SEQ ID No 2.

TABLE 1

SEQ ID No 2

```
   1    ggtaccataa attacacatc tgcttttgaa aaaatatgat
       ttcaagctag gattacatta

  61    ggtagagttc atattaataa taaaaaatgt ttgcaatcaa
       atcgtacgtt gtcgtttgta

 121    attcttaaaa tagcaataaa taaaatgttt gttagtaaag
       tattattgtg gataataaaa

 181    tatcgataca aattaattgc tataatgcaa ttttagtgta
       taattccatt aacagagatt

 241    aaatatatct ttaaagggta tatagttaat ataaaatgac
       tttttaaaaa gagggaataa

 301    aatgaatatg aagaaaaaag aaaaacacgc aattcggaaa
       aaatcgattg gcgtggcttc

 361    agtgcttgta ggtacgttaa tcggttttgg actactcagc
       agtaaagaag cagatgcaag

 421    tgaaaatagt gttacgcaat ctgatagcgc aagtaacgaa
       agcaaaagta atgattcaag

 481    tagcgttagt gctgcaccta aaacagacga cacaaacgtg
       agtgatacta aaacatcgtc

 541    aaacactaat aatggcgaaa cgagtgtggc gcaaaatcca
       gcacaacagg aaacgacaca

 601    atcatcatca acaaatgcaa ctacggaaga aacgccggta
       actggtgaag ctactactac

 661    gacaacgaat caagctaata caccggcaac aactcaatca
       agcaatacaa atgcggagga

 721    attagtgaat caaacaagta atgaaacgac ttttaatgat
       actaatacag tatcatctgt

 781    aaattcacct caaaattcta caaatgcgga aaatgtttca
       acaacgcaag atacttcaac

 841    tgaagcaaca ccttcaaaca atgaatcagc tccacagagt
       acagatgcaa gtaataaaga

 901    tgtagttaat caagcggtta atacaagtgc gcctagaatg
       agagcattta gtttagcggc

 961    agtagctgca gatgcaccgg cagctggcac agatattacg
       aatcagttga cgaatgtgac

1021    agttggtatt gactctggta cgactgtgta tccgcaccaa
       gcaggttatg tcaaactgaa

1081    ttatggtttt tcagtgccta attctgctgt taaaggtgac
       acattcaaaa taactgtacc
```

TABLE 1-continued

```
1141    taaagaatta aacttaaatg gtgtaacttc aactgctaaa
       gtgccaccaa ttatggctgg

1201    agatcaagta ttggcaaatg gtgtaatcga tagtgatggt
       aatgttattt atacatttac

1261    agactatgta aatactaaag atgatgtaaa agcaactttg
       accatgcccg cttatattga

1321    ccctgaaaat gttaaaaaga caggtaatgt gacattggct
       actggcatag gtagtacaac

1381    agcaaacaaa acagtattag tagattatga aaaatatggt
       aagttttata acttatctat

1441    taaaggtaca attgaccaaa tcgataaaac aaataatacg
       tatcgtcaga caatttatgt

1501    caatccaagt ggagataacg ttattgcgcc ggttttaaca
       ggtaatttaa aaccaaatac

1561    ggatagtaat gcattaatag atcagcaaaa tacaagtatt
       aaagtatata aagtagataa

1621    tgcagctgat ttatctgaaa gttactttgt gaatccagaa
       aactttgagg atgtcactaa

1681    tagtgtgaat attacattcc caaatccaaa tcaatataaa
       gtagagttta atacgcctga

1741    tgatcaaatt acaacaccgt atatagtagt tgttaatggt
       catattgatc cgaatagcaa

1801    aggtgattta gctttacgtt caactttata tgggtataac
       tcgaatataa tttggcgctc

1861    tatgtcatgg gacaacgaag tagcatttaa taacggatca
       ggttctggtg acggtatcga

1921    taaaccagtt gttcctgaac aacctgatga gcctggtgaa
       attgaaccaa ttccagagga

1981    ttcagattct gacccaggtt cagattctgg cagcgattct
       aattcagata gcggttcaga

2041    ttcgggtagt gattctacat cagatagtgg ttcagattca
       gcgagtgatt cagattcagc

2101    aagtgattca gactcagcga gtgattcaga ttcagcaagc
       gattccgact cagcgagcga

2161    ttccgactca gacaatgact cggattcaga tagcgattct
       gactcagaca gtgactcaga

2221    ttccgacagt gactcagatt cagatagcga ttctgactca
       gacagtgact cagattcaga

2281    tagcgattca gattcagata gcgattcaga ttccgacagt
       gattccgact cagacagcga

2341    ttctgactcc gacagtgatt ccgactcaga cagcgattca
       gattccgaca gtgattccga

2401    ctcagatagc gattccgact cagatagcga ctcagattca
       gacagcgatt cagattcaga

2461    cagcgattca gattcagata gcgattcaga ttccgacagt
       gactcagatt ccgacagtga

2521    ctcggattca gatagcgatt cagattccga cagtgactca
       gattccgaca gtgactcaga

2581    ctcagacagt gattcggatt cagcgagtga ttcggattca
       gatagtgatt ccgactccga

2641    cagtgactcg gattcagata gcgactcaga ctcggatagc
       gactcggatt cagatagcga
```

TABLE 1-continued

```
2701    ttcggactca gatagcgatt cagaatcaga cagcgattca
        gaatcagaca gcgattcaga

2761    ttcagacagc gactcagaca gtgactcaga ttcagatagt
        gactcggatt cagcgagtga

2821    ttcagactca ggtagtgact ccgattcatc aagtgattcc
        gactcagaaa gtgattcaaa

2881    tagcgattcc gagtcaggtt ctaacaataa tgtagttccg
        cctaattcac ctaaaaatgg

2941    tactaatgct tctaataaaa atgaggctaa agatagtaaa
        gaaccattac cagatacagg

3001    ttctgaagat gaagcaaata cgtcactaat ttggggatta
        ttagcatcaa taggttcatt

3061    actacttttc agaagaaaaa aagaaaataa agataagaaa
        taagtaataa tgatattaaa

3121    ttaatcatat gattcatgaa gaagccacct taaaaggtgc
        ttcttttact tggattttcc

3181    aaatatattg tttgaatata attaataatt aattcatcaa
        cagttaatta ttttaaaaag

3241    gtagatgtta tataatttgg cttggcgaaa aaatagggtg
        taaggtaggt tgttaattag

3301    ggaaaattaa ggagaaaata cagttgaaaa ataaattgct
        agttttatca ttgggagcat

3361    tatgtgtatc acaaatttgg gaaagtaatc gtgcgagtgc
        agtggtttct ggggagaaga

3421    atccatatgt atctgagtcg ttgaaactga ctaataataa
        aaataaatct agaacagtag

3481    aagagtataa gaaaagctt
```

[0035] In case the sequence of a *S. aureus* adhesin is the fibronectin-binding protein A (FnPBA) then the acid nucleic molecule encoding the folded sequence of a *S. aureus* adhesin, namely the FnPBA, is preferably a genomic DNA as set forth in SEQ ID No 3.

TABLE 2

SEQ ID No 3

```
   1    ttatgctttg tgattctttt tatttctgcg taataatgct
        aaacctagaa tgctgaataa

  61    tccgccgaac aacatacctt tgtttgttga ttcttctcca
        cctgtttcag gtagttcaga

 121    tttcttagat tgtggttttt tagttggtgc cactgcttta
        acctttttcat tgatttcaat

 181    aacaggtgtt actactttac cttgttccac tggtttagaa
        ggctttttag gttcttcttt

 241    ggcaggtggt actggtttac caggttcagc tggtacctct
        ggtgttggcg gtgttggagt

 301    ttctggctca ctcggcactt ctggtgtcgg tggtgttggt
        gtttccggct cacttggtac

 361    ttctggtgtt ggtggcgttg gtgtttccgg ctcacttggt
        acttctggtg tcggtggcgt

 421    tggtggcacg attggaggtg ttgtatcttc ttcaatcgtt
        tgttgacctt cattttggcc
```

TABLE 2-continued

```
 481    gcttactttt ggaagtgtat cttcttcaaa gtcaacacta
        ttgtgtccac cgaattgata

 541    acttggttta tctttatttg tatcttcttc aataatttca
        gtgtgcttat tgaatccgtg

 601    aatatgtggc acactgtcga agtcgatatc aatgatgtta
        ccgccatgtt catacttagg

 661    tttgtctttt tctgtatctt cctcgaatga ctgattacct
        ttattttgac catgaatttg

 721    aggtacacta tcaaaatcga tatctacgat attgccacct
        tgttcatatt taggtttgtc

 781    ttcttctgtg tcttcctcga atgactggtt accgctattt
        tggccacctt cataacctaa

 841    ttcactctta atatcaacgt ggctattttc ttcgatttct
        tcaatcacgt cataattccc

 901    gtgaccattt tcagttccta aaccagaatg agaaatatga
        tgattgtttt tagtaatttc

 961    ctcgactggt ccttgtgctt gaccatgctc ttcaggtaat
        tcatccacta attcaatcag

1021    attactttca gttgtatatt ctttcgtatc ttcaactgtt
        gtatgatcgc tcactgcgcc

1081    agttacaata ccttttgtag actcttcgtc aaattcaact
        aagttagact cagtagtaac

1141    ctgaccacca cctgggtttg tatcttcttc atattcaaca
        acatcagcgt gatgttttga

1201    attttcatgt gtagattctt caaagtcaat tggatttgat
        tcctcagagg actcagtgta

1261    tcctccaacg tgacctgctt cgctatccac agcagtatgg
        taatcgatat caatagctga

1321    tgaatccgtt tcttctattg tttcaatgta tccatcaaca
        tatccacctc caccatctat

1381    agctgtgtgg taatcaatgt caagagttga tgaatcatat
        tcctcttcaa cagtagttac

1441    taaattctta tcatattgac ctgtaagagt ttctttaatt
        gtatcttctt tatattcaaa

1501    tttattattt tgaataatcg gaccattttt ctcatttccg
        ttcgctttat tactgtataa

1561    aactaaacca ttatcccaag ttaaggtata tcctctatca
        taataatact tataaagttg

1621    ctctggatgt cctaccattt gtgttctaaa atcaacttca
        tcagtaccat ttaaatactc

1681    tccatcatag tgaacaacat aagttttatc tagattttct
        atattcaatg aatagcttcc

1741    attattttgt aaattcaaat tcccactcat attacttgtg
        acttctttaa atttagaagt

1801    atctgtcgta tttgcatata cactcttcgc tatgtcttca
        ttattaccca agtattcaaa

1861    tatcctaact tttggttgat ttccattctg attactacct
        ttcattaaag ttccagtaac

1921    agtcacactt gtcgttttac cattattagg tttaataaat
        gcaacatgcg aaaatctatt
```

TABLE 2-continued

```
1981  attcgcttta ttaaatgtct caatcgatcc atttaaattg
      gcataataat tcccaatacc

2041  atctttatat ttaacatcta attcctttga agtttgttct
      tcatttagtg ttgaagttat

2101  agtttgattt ccattagttt gtacagtttt aggatcaata
      aataaattaa tttctagttc

2161  agccgttaca tcaaccttat cttcaatatc atttgtaaat
      gtatatctaa tctttccacc

2221  ttctaaaact tcacctgtcg ccattacgac tgaaccattt
      ttaatttctg gtacttttct

2281  agcagttgat acgccatgcg tatttacatt atttgataaa
      gtaaagtcaa agtagtcacc

2341  ttgatgtaaa ccattctcaa atttcaactt atattttagt
      accgctcgtt gtcctgcatg

2401  aggttctact ttatttgtat tgttatgccc ctcaatagaa
      ccaatttcta ctgtaacttt

2461  acttgttaca tctgtacccg tttccacttt cgcgttacta
      gcttccttag cttccgctac

2521  atctgctgat cttgtcacac gtggcttact ttctgatgcc
      gttcttggct gtgccacttc

2581  aacttgtgtt tctgcgactt gattttgtgt agccttttta
      ggtgttaaat ctacttgtct

2641  ttgatctccg ctattgtctt gagattgtgt tgtttcctta
      acttgaggtt tcgcttcttc
```

TABLE 2-continued

```
2701  cttaactacc tcttctttaa ctgtttctat atttgctggt
      tgtgcagttt gtggtgcttg

2761  tactgctttt ggtgcttctt cagttgttac ttgtgttgcg
      tttgacggtt gttctgttac

2821  tgttgcgtta tatgattgag tttcttctat atgattaacg
      ttagttgcag ttgtttgtgt

2881  ttcacttgtt ttattatcag tagctgaatt cccattttct
      tctactgtag ttgtcttttg

2941  ttctgatgct gcagcttctt tgtcttgtcc cat
```

[0036] Previously, it has been shown that *S. aureus* proteins expressed on the surface of Lactococci were entirely accessible to trypsin digestion for analysis by LC-MS (liquid chromatography coupled with mass spectrometry) (Ythier et al., 2012). In contrast, this was not the case in *S. aureus*, where trypsin had a limited access to the surface proteins, indicating that part of their structure was embedded in the wall and inaccessible to digestion, and thus to recognition by antibodies as well. The trypsin shaving protocol is a classical protocol described in Ythier et al. and in the Example parts.

[0037] The trypsin digestion of adhesins such as, for example, ClfA expressed on the surface of lactococci and/or FnPBA expressed on the surface of Lactococci, releases a digestion profile which is different from the digestion profile of the same adhesins expressed on the surface of *S. aureus*.

[0038] For example, the digestion profile of ClfA expressed on the surface of lactococci reveals 10 peptides versus 9 for ClfA expressed on the surface of *S. aureus*. Among these 10 peptides, 3 peptides (SEQ IDs 5, 6, and 12) were specific of Lactococci.

TABLE 3

Peptides released after trysin digestion of ClfA expressed on

SEQ ID/Position (aa)	*S. aureus*	*L. lactis*
SEQ ID No 5 40-55		SENSVTQSDSASNESK
SEQ ID No 6 56-67		SNDSSSVSAAPK
SEQ ID No 7 200-212	DVVNQAVNTSAPR	DVVNQAVNTSAPR
SEQ ID No 8 259-271	LNYGFSVPNSAVK	LNYGFSVPNSAVK
SEQ ID No 9 282-293	ELNLNGVTSTAK	
SEQ ID No 10 331-345	ATLTMPAYIDPENVK	ATLTMPAYIDPENVK
SEQ ID No 11 347-363	TGNVTLATGIGSTTANK	TGNVTLATGIGSTTANK
SEQ ID No 12 375-381		FYNLSIK
SEQ ID No 13 396-434	QTIYVNPSGDNVIAPVLTGNLKPNTD K	QTIYVNPSGDNVIAPVLTGNLKPNTDSN ALIDQQNTSISNALIDQQNTSI K

TABLE 3-continued

Peptides released after trysin digestion of ClfA expressed on		
SEQ ID/Position (aa)	*S. aureus*	*L. lactis*
SEQ ID No 14 438-473	VDNAADLSESYFVNPENFEDVTNSVN ITFPNPNQYK	
SEQ ID No 15 474-500	VEFNTPDDQITTPYIVVVNGHIDPNSK	VEFNTPDDQITTPYIVVVNGHIDPNSK

[0039] As another example, the digestion profile of FnbpA expressed on the surface of Lactococci reveals 10 peptides versus 22 for FnbpA expressed on the surface of *S. aureus*. Among these peptides, 2 peptides (SEQ IDs 38 and 39) were specific of lactococci.

TABLE 4

Peptides released after trysin digestion of FnbpA expressed on		
Position (aa)	*S. aureus*	*L. lactis*
SEQ ID No 16 57-97	TSETQTTATNVNHIEETQSYNATVTEQPSNATQ VTTEEAPK	TSETQTTATNVNHIEETQSYNAT VTEQPSNATQVTTEEAPK
SEQ ID No 17 98-114	AVQAPQTAQPANIETVK	AVQAPQTAQPANIETVK
SEQ ID No 18 98-119	AVQAPQTAQPANIETVKEEVVK	
SEQ ID No 19 115-127	EEVVKEEAKPQVK	
SEQ ID No 20 148-165	KATQNQVAETQVEVAQPR	KATQNQVAETQVEVAQPR
SEQ ID No 21 149-165	ATQNQVAETQVEVAQPR	ATQNQVAETQVEVAQPR
SEQ ID No 22 201-216	VTVEIGSIEGHNNTNK	VTVEIGSIEGHNNTNK
SEQ ID No 23 233-260	FENGLHQGDYFDFTLSNNVNTHGVSTAR	
SEQ ID No 24 267-282	NGSWMATGEVLEGGK	
SEQ ID No 25 285-294	YTFTNDIEDK	
SEQ ID No 26 311-331	TVQTNGNQTITSTLNEEQTSK	TVQTNGNQTITSTLNEEQTSK
SEQ ID No 27 337-357	YKDGIGNYYANLNGSIETFNK	
SEQ ID No 28 339-357	DGIGNYYANLNGSIETFNK	
SEQ ID No 29 362-374	FSHVAFIKPNNGK	
SEQ ID No 30 375-386	TTSVTVTGTLMK	
SEQ ID No 31 399-411	IFEYLGNNEDIAK	IFEYLGNNEDIAK

TABLE 4-continued

Peptides released after trysin digestion of FnbpA expressed on

Position (aa)	*S. aureus*	*L. lactis*
SEQ ID No 32 423-451	FKEVTSNMSGNLNLQNNGSYSLNIENLDK	
SEQ ID. 33 452-471	TYWHYDGEYLNGTDEVDFR	
SEQ ID No 34 472-483	TQMVGHPEQLYK	
SEQ ID No 35 524-537	EDTIKETLTGQYDK	
SEQ ID No 36 622-655	HHADVVEYEEDTNPGGGQVTTESNLVEFDEESTK	
SEQ ID No 37 656-672	GIVTGAVSDHTTVEDTK	GIVTGAVSDHTTVEDTK
SEQ ID No 38 673-702		EYTTESNLIELVDELPEEHGQAQ GPVEEITK
SEQ ID No 39 764-786		YEQGGNIVDIDFDSVPQIHGQNK

[0040] Since all these domains are variously exposed on the *S. aureus* surface, the present invention shows that vaccination against only one binding-domain, which might become hidden in certain circumstances, is less effective than vaccination against the whole protein presented in a functional conformation on the surface of Lactococci.

[0041] Preferably, the sequence of the *S. aureus* adhesin of the invention is a folded sequence.

[0042] A "folded sequence" refers to a protein that is structured under physiologic conditions. Alternatively, the sequence of the invention is a sequence having 80% or more sequence identity to said folded sequence of a *S. aureus* adhesin. Preferably, the *S. aureus* adhesin is selected from the group comprising fibrinogen-binding protein A (clumping factor A; ClfA), fibrinogen-binding protein B (ClfB), fibronectin-binding protein

[0043] A (FnPBA) and fibronectin-binding protein B (FnBPB), collagen-binding protein (Cna) and protein A (Spa), Serine-aspartate repeat protein C, D and E (SdrC-E), Plasmin-sensitive protein (PIs), Factor affecting methicillin resistance in the presence of Triton X-100 (FmtB), surface protein A-K (SasA-K). A combination of one or more of the above listed *S. aureus* adhesin sequences is also envisioned.

[0044] Most preferably, the *S. aureus* adhesin is the fibrinogen-binding protein A (clumping factor A; ClfA). Clumping factor A (ClfA) is an MSCRAMM protein expressed by *S. aureus* that promotes binding of fibrinogen and fibrin to the bacterial cell surface. ClfA is the prototype of a recently identified multigene family of cell surface proteins characterized by a common domain composed of a unique serine-aspartate repeat. The gene encoding the fibrinogen-binding protein shows a 933-amino-acid polypeptide that contains structural features characteristic of many cell surface-associated proteins from gram-positive bacteria, including a typical cell wall attachment region comprising an LPXTG motif, a hydrophobic transmembrane sequence, and a positively charged C terminus. The fibrinogen-binding domain of ClfA has been localized to a 218-residue segment within region A.

[0045] More preferably, the sequence of the invention consists in a whole functional amino acid sequence of ClfA (amino acid 1 to 933, table 5).

TABLE 5

SEQ ID No 1

MNMKKKEKHAIRKKSIGVASVLVGTLIGFGLLSSKEADASENSVTQSDS

ASNESKSNDSSSVSAAPKTDDTNVSDTKTSSNTNNGETSVAQNPAQQET

TQSSSTNATTEETPVTGEATTTTTNQANTPATTQSSNTNAEELVNQTSN

ETTFNDTNTVSSVNSPQNSTNAENVSTTQDTSTEATPSNNESAPQSTDA

SNKDVVNQAVNTSAPRMRAFSLAAVAADAPAAGTDITNQLTNVTVGIDS

GTTVYPHQAGYVKLNYGFSVPNSAVKGDTFKITVPKELNLNGVTSTAKV

PPIMAGDQVLANGVIDSDGNVIYTFTDYVNTKDDVKATLTMPAYIDPEN

VKKTGNVTLATGIGSTTANKTVLVDYEKYGKFYNLSIKGTIDQIDKTNN

TYRQTIYVNPSGDNVIAPVLTGNLKPNTDSNALIDQQNTSIKVYKVDNA

ADLSESYFVNPENFEDVTNSVNITFPNPNQYKVEFNTPDDQITTPYIVV

VNGHIDPNSKGDLALRSTLYGYNSNIIWRSMSWDNEVAFNNGSGSGDGI

DKPVVPEQPDEPGEIEPIPEDSDSDPGSDSGSDSNSDSGSDSGSDSTSD

SGSDSASDSDSASDSDSASDSDSASDSDSASDSDSDNDSDSDSDSDSDS

DSDSDSDSDSDSDSDSDSDSDSDSDSDSDSDSDSDSDSDSDSDSDSD

SDSDSDSDSDSDSDSDSDSDSDSDSDSDSDSDSDSDSDSDSDSDS

DSDSDSDSDSDSDSDSDSDSDSDSDSDSDSDSASDSDSDSDSDSDSD

SDSDSDSDSDSDSDSDSDSDSDSESDSDSESDSDSDSDSDSDSDSDS

TABLE 5-continued

DSDSASDSDSGSDSDSSSDSDSESDSNSDSESGSNNNVVPPNSPKNGTN

ASNKNEAKDSKEPLPDTGSEDEANTSLIWGLLASIGSLLLFRRKKENKD

KK

[0046] More preferably also, the sequence of the invention consists in a whole functional amino acid sequence of FnPBA (amino acid 1 to 990, table 6).

TABLE 6

SEQ ID No 4

MGQDKEAAASEQKTTTVEENGNSATDNKTSETQTTATNVNHIEETQSY

NATVTEQPSNATQVTTEEAPKAVQAPQTAQPANIETVKEEVVKEEAKP

QVKETTQSQDNSGDQRQVDLTPKKATQNQVAETQVEVAQPRTASESKP

RVTRSADVAEAKEASNAKVETGTDVISKVTVEIGSIEGHNNTNKVEPH

AGQRAVLKYKLKFENGLHQGDYFDFTLSNNVNTHGVSTARKVPEIKNG

SVVMATGEVLEGGKIRYTFTNDIEDKVDVTAELEINLFIDPKTVQTNG

NQTITSTLNEEQTSKELDVKYKDGIGNYYANLNGSIETFNKANNRFSH

VAFIKPNNGKTTSVTVTGTLMKGSNQNGNQPKVRIFEYLGNNEDIAKS

VYANTTDTSKFKEVTSNMSGNLNLQNNGSYSLNIENLDKTYVVHYDGE

YLNGTDEVDFRTQMVGHPEQLYKYYYDRGYTLTWDNGLVLYSNKANGN

EKNGPIIQNNKFEYKEDTIKETLTGQYDKNLVTTVEEEYDSSTLDIDY

HTAIDGGGGYVDGYIETIEETDSSAIDIDYHTAVDSEAGHVGGYTESS

EESNPIDFEESTHENSKHHADVVEYEEDTNPGGGQVTTESNLVEFDEE

STKGIVTGAVSDHTTVEDTKEYTTESNLIELVDELPEEHGQAQGPVEE

ITKNNHHISHSGLGTENGHGNYDVIEEIEENSHVDIKSELGYEGGQNS

GNQSFEEDTEEDKPKYEQGGNIVDIDFDSVPQIHGQNKGNQSFEEDTE

KDKPKYEHGGNIIDIDFDSVPHIHGFNKHTEIIEEDTNKDKPSYQFGG

HNSVDFEEDTLPKVSGQNEGQQTIEEDTTPPIVPPTPPTPEVPSEPET

PTPPTPEVPSEPETPTPPTPEVPSEPETPTPPTPEVPAEPGKPVPPAK

EEPKKPSKPVEQGKVVTPVIEINEKVKAVAPTKKPQSKKSELPETGGE

ESTNKGMLFGGLFSILGLALLRRNKKNHKA

[0047] Surprisingly, the Applicants of the present invention have successfully prevented *S. aureus* experimental endocarditis in rats vaccinated with UV-killed *L. lactis* expressing heterologously the staphylococcal adhesin ClfA, or ClfA/FnBPA (CD). The success of vaccination is due to the method of antigen delivery, i.e. a whole functional *S. aureus* surface adhesin on a genuine bacterial surface, rather than only restricted peptides of the protein. This mode of delivery largely increases the repertoire of anti-staphylococcal antibodies generated by the host, and thus increases the efficacy of protection.

[0048] The cell wall anchored fibrinogen-binding protein ClfA has been the major target of vaccine candidates, due to its ability to binds to the γ-chain of the fibrinogen. Indeed, previous studies have shown that ClfA is essential for the development of IE due to the pivotal role of fibrinogen-binding (Moreillon et al., 1995; Yok-ai Que et al., 2005). However, numerous attempts to develop vaccines against a variety of *S. aureus* virulence factors have been attempted with various successes in animal models, but have as yet not achieved sustainable efficacy in human clinical trials.

[0049] The reasons for these failures are not entirely clarified, but there are at least two parameters of the *S. aureus* camouflage system that might have been underestimated until now. First, the fact that the bacterium may vary the way it exposes its antigenic structure on the surface, and second the fact that different strains may undergo antigenic variations. As a result, vaccination against one particular structure that is valid against one strain may not be efficacious against another strain. Therefore, a blocking or opsonizing antibody that is active in certain circumstances may become inactive in other settings.

[0050] Staphylococcal adhesins of the LPXTG-protein family are equipped with a spacer domain between the cell wall anchor and the outermost binding domain. This spacer is important to expose the binding domain on top of the plethora of other constituents of the staphylococcal envelope, in order to bind to the target host tissue. Thus, depending on the length of this spacer (which may vary between strains) and the size of other surface components (for instance polysaccharides and protein A), the binding domain of the adhesin may become embedded in other surface structures and hidden to the immune system (Scarpa et al., 2010). Indeed, it was shown that *S. aureus* exposed differently the fibrinogen-binding protein domain "A" of ClfA (Mcdevitt et al., 1994), as well as the surface capsule, at various stages of in vivo infection (Risley et al., 2007).

[0051] Another example comes from the length of anti-phagocytic protein A, which binds antibodies by their Fc fragment. It was recently shown that a longer spacer sequence allowed protein A to better prevent binding of antibodies to various antigenic structures presented on the staphylococcal surface (Scarpa et al., 2010). Moreover, the polymorphism of the protein A gene may affect the efficacy of vaccines in a strain-dependent manner. Indeed, the length of the spacer is determined by series of repeats that are known to vary between different staphylococcal strains, and thus are currently used as phylogenic markers (Kuhn et al., 2007).

[0052] Eventually, *S. aureus* produces a plethora of toxins and superantigens that may interfere with the immune response, and thus help the organism to circumvent existing host immune strategies. Vaccination against such structures were also recently attempted (Broughan et al., 2011).

[0053] The importance of antigenic variation in *S. aureus* is less clear. Indeed, only two major capsular types (types 5 and 8) are implicated in infection in human, and surface proteins and toxins are relatively well conserved (Shinefield et al., 2002). However, many gram-positive pathogens, including group A streptococci and *Streptococcus pneumoniae*, undergo wide genetic variability at the level of the anti-phagocytic M protein and the polysaccharidic capsule, respectively. Taken together, the current consensus for anti-*S. aureus* vaccines is that they should comprise several antigens in order to be effective, and that single antigen-based vaccines are bound to fail (Broughan et al., 2011). However, the ideal antigen mixture to be used has yet to be elucidated.

[0054] Examples showed hereafter that *S. aureus* adhesins could be expressed functionally in L. *Lactis* in vitro, and could promote experimental endocarditis by the recombinant

Lactococci in vivo. When conjugated with the proteomic dissection of the surface proteome of *S. aureus*, it appeared that the *S. aureus* proteins expressed on the surface of Lactococci were entirely accessible to trypsin digestion for analysis by LC-MS (liquid chromatography coupled with mass spectrometry) (Ythier et al., 2012). In contrast, this was not the case in *S. aureus*, where trypsin had a limited access to the surface proteins, indicating that part of their structure was embedded in the wall and inaccessible to digestion, and thus to recognition by antibodies as well. Since all these domains were variously exposed on the *S. aureus* surface, it was conceivable that vaccination against only one binding-domain, which might become hidden in certain circumstances, might be less effective than vaccination against the whole protein presented in a functional conformation on the surface of lactococci.

[0055] As shown in the examples, immunizing series of rats with UV-killed lactococci expressing *S. aureus* ClfA, or ClfA/FnBPA (CD) protected animals from subsequent experimental endocarditis due to *S. aureus* induced by low-grade bacteremia ($P<0.05$ when compared to the several control groups). Remarkably, when the same vaccination schedule was tested in animals where the experimental endocarditis due to *S. aureus* was induced by high-grade bacteremia (traditional bolus infection) the infection rates increased (2/15 vs 6/6) and the protective effect was lost.

[0056] Attempts to vaccinate against severe infections in animal models are usually biased by the fact that most protocols administer very large bacterial inocula, in order to ensure that all untreated control animals will become infected. However, such large inocula—often in the range of 10 s of million bacteria injected intravenously—are incommensurably larger than inoculum sized expected during "natural" infection in human. Thus, it is possible that such large inocula may overwhelm the immune system, and falsely underestimate the efficacy of preventive or therapeutic strategies that would otherwise be efficacious. The Applicants recently showed that this was realistic in the model of experimental endocarditis, and that challenging animals with continuous low-grade bacteremia was as infectious that transient high-grade inoculation, but represented much more the reality of the disease in human (Veloso et al., 2011). Importantly, the good results of the vaccination strategy reported above were achieved with this very realistic model. Indeed, vaccination was less effective against the standard high-grade bacteremia model, an observation that could also explain failures with other types of potential anti-*S. aureus* vaccines tested in animals in the past, and abandoned.

[0057] The present invention also concerns a vaccine. Preferably, the vaccine comprises an immunogenic composition of the invention in an immunologically acceptable carrier or diluent. Preferably said vaccine is for treating and/or preventing infections and diseases caused by *S. aureus*. Examples of diseases caused by *S. aureus* are folliculitis, furunculosis, erysipelas, deep-seated abscesses, osteomyelitis, pneumonia, sepsis, and infective endocarditis (IE).

[0058] The vaccine of the invention may also contain several antibodies or antibody fragments, e.g. two or three antibodies or antibody fragments that recognize(s) at least one folded sequence of a *S. aureus* adhesin expressed on the cell surface of an inactivated recombinant non-pathogenic bacterium of the invention.

[0059] The immunologically acceptable carrier is usually selected from the group comprising polysaccharide materials forming hydrogels, bacterial ghosts and vesicular carriers. Preferably, the vesicular carriers are selected from the group comprising liposomes, niosomes, transfersomes, and ethosomes, and others known in the art.

[0060] Hydrogels envisioned as immunologically acceptable carrier as know in the art and are particularly adapted for mucosal, topical, oral or injectable delivery.

[0061] The vaccines of the invention may further comprise one or more adjuvant. Adjuvants can include, but are not limited to, MPL+TDM+CWS (SIGMA), MF59 (an oil-in-water emulsion that includes 5% squalene, 0.5% sorbitan monoleate and 0.5% sorbitan trioleate Chiron), Heat-labile toxin (HLT), CRMig (nontoxic genetic mutant of diphtheria toxin), Squalene (IDEC PHARMACEUTICALS CORP.), Ovalbumin (SIGMA), Quil A (SARGEANT, INC.), Aluminum phosphate gel (SUPERFOS BIOSECTOR), Cholera holotoxin (CT LIST BIOLOGICAL LAB.), Cholera toxin B subunit (CTB), Cholera toxin A subunit-Protein A D-fragment fusion protein, Muramyl dipeptide (MDP), Adjumera (polyphosphazene, VIRUS RESEARCH INSTITUTE), Montanide ISA 720, SPT (an emulsion of 5% squalene, 0.2% Tween 80, 1. 25% Pluronic L121 with phosphate-buffered saline ph 7. 4), Avridine (M6 PHARMACEUTICALS), Bay R1005 (BAYER), Calcitrol (SIGMA), Calcium phosphate gel (SARGEANT INC.), CRL 1005 (Block co-polymer P1205, VAXCEL CORP.), DHEA (MERCK), DMPC (GENZYME PHARMACEUTICALS and FINE CHEMICALS). DMPG (GENZYME PHARMACEUTICALS and FINE CHEMICALS), Gamma Inulin, Gerbu Adjuvant (CC BIOTECH CORP.), GM-CSF, (IMMUNE CORP.), GMDP (PEPTECH LIMITED), Imiquimod (3M PHARMACEUTICALS), ImmTher (ENDOREX CORPORATION), ISCOMTM (ISCOTEC AB), Iscoprep 7.0. 3 TM (ISCOTEC AB), Loxoribine, LT-Oral Adjuvant (*E. coli* labile enterotoxin, protoxin, BERNA PRODUCTS CORP.), MTP-PE (CIBA-GEIGY LTD), Murametide, (VACSYN S. A.), Murapalmitine (VACSYN S. A.), Pluronic L121 (IDEC PHARMACEUTICALS CORP.), PMMA (INSTITUT FUR PHARMAZEUTISCHE TECHNOLOGIE), SAF-1 (SYNTEX ADJUVANT FORMULATION CHIRON), Stearyl tyrosine (BIOCHEM THERAPEUTIC INC.), Theramidea (IMMUNO THERAPEUTICS INC.), Threonyl-MDP (CHIRON), FREUNDS adjuvant (complete or incomplete), aluminum hydroxide, dimethyldioctadecyl-ammonium bromide, Adjuvax (ALPHA-BETA TECHNOLOGY), Inject Alum (PIERCE), Monophosphoryl Lipid A (RIBI IMMUNOCHEM RESEARCH), MPL+TDM (RIBI IMMUNOCHEM RESEARCH), Titermax (CYTRX), QS21, t Ribi Adjuvant System, TiterMaxGold, QS21, Adjumer, Calcitrol, CTB, LT (*E. coli* toxin), LPS (lipopolysaccharide), Avridine, the CpG sequences (Singh et al., 1999 Singh, M. and Hagum, D., Nature Biotechnology 1999 17: 1075-81) toxins, toxoids, glycoproteins, lipids, glycolipids, bacterial cell walls, subunits (bacterial or viral), carbohydrate moieties (mono-, di, tri-, tetra-, oligo- and polysaccharide), or saponins. Combinations of various adjuvants may be used with the antigen to prepare the immunogen formulations. Adjuvants administered parentally or for the induction of mucosal immunity may also be used.

[0062] The present invention further contemplates isolated and/or purified antibody, antibody fragment or derivative thereof able to bind to the at least one folded sequence of a *S. aureus* adhesion of the invention, or to the sequence having 80% or more sequence identity to said folded sequence of a *S.*

aureus adhesion of the invention, expressed on the cell surface of an inactivated recombinant non-pathogenic bacterium.

[0063] As used herein, an "antibody" is a protein molecule that reacts with a specific antigenic determinant or epitope and belongs to one or five distinct classes based on structural properties: IgA, IgD, IgE, IgG and IgM. The antibody may be a polyclonal (e.g. a polyclonal serum) or a monoclonal antibody, including but not limited to fully assembled antibody, single chain antibody, antibody fragment, and chimeric antibody, humanized antibody as long as these molecules are still biologically active and still bind to one folded sequence of a *S. aureus* adhesin of the invention. Preferably the antibody is a monoclonal antibody. Preferably also the monoclonal antibody will be selected from the group comprising the IgGI, IgG2, IgG2a, IgG2b, IgG3 and IgG4 or a combination thereof. Most preferably, the monoclonal antibody is selected from the group comprising the IgGI, IgG2, IgG2a, and IgG2b, or a combination thereof.

[0064] A typical antibody is composed of two immunoglobulin (Ig) heavy chains and two Ig light chains. Several different types of heavy chain exist that define the class or isotype of an antibody. These heavy chain types vary between different animals. All heavy chains contain a series of immunoglobulin domains, usually with one variable (VH) domain that is important for binding antigen and several constant (CH) domains. Each light chain is composed of two tandem immunoglobulin domains: one constant (CL) domain and one variable domain (VL) that is important for antigen binding.

[0065] The term "isolated", when used as a modifier of an antibody of the invention means that the antibody is made by the hand of man or is separated, completely or at least in part, from their naturally occurring in vivo environment. Generally, isolated antibodies are substantially free of one or more materials with which they normally associate with in nature, for example, one or more protein. The term "isolated" does not exclude alternative physical forms of the antibodies, such as multimers/oligomers, modifications (e.g., phosphorylation, glycosylation, lipidation) or derivatized forms, or forms expressed in host cells produced by the hand of man

[0066] An "isolated" antibody can also be "substantially pure" or "purified" when free of most or all of the materials with which it typically associates with in nature. Thus, an isolated antibody that also is substantially pure or purified does not include polypeptides or polynucleotides present among millions of other sequences, such as antibodies of an antibody library or nucleic acids in a genomic or cDNA library.

[0067] Antibodies used in the present invention are not limited to whole antibody molecules and may be antibody fragments or derivatives as long as they are able to bind to the at least one folded sequence of a *S. aureus* adhesin expressed on the cell surface of an inactivated recombinant non-pathogenic bacterium and that they specifically recognize said folded sequence of a *S. aureus* adhesin.

[0068] Examples of isolated and/or purified antibody fragment or derivative thereof are selected amongst the group comprising a Fab-fragment, a F(ab2)'-fragment, a single-chain antibody, a chimeric antibody, a CDR-grafted antibody, a bivalent antibody-construct, a humanized antibody, a synthetic antibody, a chemically modified derivative thereof, a multispecific antibody, a diabody, a scFv-fragment; a dsFv-fragment, a labeled antibody, or another type of recombinant antibody. Specifically, an antibody fragment is synthesized by treating the antibody with an enzyme such as papain or pepsin, or genes encoding these antibody fragments are constructed, and expressed by appropriate host cells as known to the skilled artisan.

[0069] Yet another concern of the present invention is to provide an expression vector comprising at least one isolated and/or purified nucleic acid sequence encoding for at least one folded sequence of a *S. aureus* adhesin, or a sequence having 80% or more sequence identity to said folded sequence of a *S. aureus* adhesin. Preferably the nucleic acid molecule sequence encoding a peptide of the invention is DNA.

[0070] As used herein, "vector", "plasmid" and "expression vector" are used interchangeably, as the plasmid is the most commonly used vector form.

[0071] The vector may further comprise a promoter operably linked to the sequence encoding a folded sequence of a *S. aureus* adhesin. This means that the linked isolated and purified DNA sequence encoding the peptide of the present invention is under control of a suitable regulatory sequence which allows expression, i.e. transcription and translation of the inserted isolated and purified DNA sequence.

[0072] As used herein, the term "promoter" designates any additional regulatory sequences as known in the art e.g. a promoter and/or an enhancer, polyadenylation sites and splice junctions usually employed for the expression of the polypeptide or may include additionally one or more separate targeting sequences and may optionally encode a selectable marker. Promoters which can be used provided that such promoters are compatible with the host cell are e.g promoters obtained from the genomes of viruses such as polyoma virus, adenovirus (such as Adenovirus 2), papilloma virus (such as bovine papilloma virus), avian sarcoma virus, cytomegalovirus (such as murine or human cytomegalovirus immediate early promoter), a retrovirus, hepatitis-B virus, and Simian Virus 40 (such as SV 40 early and late promoters) or promoters obtained from heterologous mammalian promoters, such as the actin promoter or an immunoglobulin promoter or heat shock promoters.

[0073] Enhancers, which can be used, are e.g. enhancer sequences known from mammalian genes (globin, elastase, albumin, alpha-fetoprotein, and insulin) or enhancer from a eukaryotic cell virus. e.g. the SV40 enhancer, the cytomegalovirus early promoter enhancer, the polyoma, and adenovirus enhancers.

[0074] Useful expression vectors, for example, may consist of segments of chromosomal, non-chromosomal and synthetic DNA sequences. Suitable vectors include derivatives of SV40 and known bacterial plasmids, phage DNAs, yeast plasm ids such as the 2μ plasmid or derivatives thereof; vectors useful in eukaryotic cells, such as vectors useful in insect or mammalian cells; vectors derived from combinations of plasmids and phage DNAs, such as plasmids that have been modified to employ phage DNA or other expression control sequences; and the like. Most preferably the expression vector is a lactococcal plasmid. More preferably, the lactococcal plasmid is pOri23.

[0075] Also provided is a method for treating and/or preventing infections and diseases caused by *S. aureus*, in a subject in need thereof, comprising administering a pharmaceutically effective amount of an immunogenic composition according to the invention.

[0076] Usually, infections and diseases caused by *S. aureus* are selected among the non limiting group comprising IE,

intravascular and intravascular device infections, bloodstream infections, deep-seated abscesses, osteomyelitis, infection of prosthetic materials, and skin and soft tissue infections. Examples of diseases caused by *S. aureus* are folliculitis, furunculosis, erysipelas, deep-seated abscesses, osteomyelitis, pneumonia, sepsis, and infective endocarditis (IE).

[0077] Also envisioned is a method of inducing active immunity against a *S. aureus* infection in a subject in need thereof, comprising administering to said subject in need thereof i) an immunogenic composition comprising an inactivated recombinant non-pathogenic bacterium, or a part thereof, expressing on its cell surface, at least one folded sequence of a *S. aureus* adhesin or ii) a vaccine of the invention.

[0078] Also encompassed in the present invention is a method of inducing passive immunity against a *S. aureus* infection in a subject in need thereof, comprising administering to said subject in need thereof an isolated and/or purified antibody, antibody fragment or derivative thereof of the invention.

EXAMPLES

Example 1

Materials and Methods

1.1 Plasmid Constructs and Bacterial Strains

[0079] The immunization protocol in this study was done using the recombinant strain of the non-pathogenic *L. lactis* subsp. *cremoris* 1363 expressing individual *S. aureus* ClfA, described elsewhere (Piroth et al., 2008; Que et al., 2000; Que et al., 2001) or *S. aureus* ClfA/FnBPA(CD). The *S. aureus* Newman ClfA gene was inserted in the lactococcal plasmid pOri23 together with an erythromycin resistance determinant as described by Que et al (Que et al., 2000). In the strain *L. lactis* ClfA/FnBPA(CD) *S. aureus* Newman ClfA gene was expressed as described above, and the CD domain of the *S. aureus* 8325 FnBPA gene was inserted in lactococcal plasmid pOri23 together with an kanamycin resistance determinant (Elonora Widmer MD/PhD thesis). The *L. lactis* plL253, containing the lactococcal plasmid pOri23 expressing only the erythromycin resistance determinant, and expressing no pathogenic factors, was used as the control mutant strain. All lactococci were grown at 30° C. without shaking in M17 medium (Oxoid) or on M17 agar plates supplemented with 0.5% glucose and 5 μg/m1 erythromycin (plus kanamycin when appropriate).

[0080] The well-described *S. aureus* strain Newman (i.e. methicillin-susceptible *S. aureus*) *S. aureus* strain P8 (i.e. methicillin-resistant *S. aureus*) (Entenza et al., 2001) was used in the animal model of endocarditis. The *S. aureus* bacterial isolates was grown at 37° C. in tryptic soy broth (Difco).

[0081] All the bacterial stocks were kept at −80° C. in liquid medium supplemented with 20% (vol/vol) of glycerol.

1.2 Immunization Protocol

[0082] (i) Animals. Four to six-week females (100 g of weight) Wistar Han rats were purchased from Charles River, France. The rats were supplied with water and food ad libitum, and randomly allocated to 6 treatment groups as follows: (a) Control, (b) Freund's adjuvant (Sigma), (c) *L. lactis* plL253 and (d) *L. lactis* ClfA, *L. lactis* FnBPA or *L. lactis* ClfA/FnBPA(CD). All animal experiments were carried out according to Swiss regulations (authorization 879.8).

(ii) Preparation of bacterial vaccine. The inactivated *L. lactis* vaccine was prepared as follows. *L. lactis* strains carrying either the empty plasmid pOri23, or the plasmid expressing ClfA or ClfA/FnBPA(CD) were cultured overnight at 30° C. without shaking in M17 medium (Oxoid), harvested by centrifugation, resuspended in sterilized PBS, and adjusted to 1×10^8 CFU/ml. The bacteria were then inactivated during 60 min under U.V. Then, the bacteria were emulsified at a 1:1 ratio in Freund's adjuvant. The first immunization was done with Freund's complete adjuvant, and the subsequent with Freund's incomplete adjuvant. The control group was immunized with PBS, and the Freund's adjuvant group with the adjuvant emulsified at a 1:1 ratio in PBS.

(iii) Vaccination schedule. The rats were immunized at 2-week intervals (days 0, 14 and 28). Three hundred microlitres of the preparations were injected intra-peritoneal to the respective groups. Blood samples were collected on days 7, 21 and 35; and the sera were harvested and stored at −80° C. to posterior in vitro analysis (Gong et al., 2010).

1.3. Animal Model of Endocarditis

[0083] Catheter-induced aortic vegetations were produced according to the method of Heraief et at (Héraïef et al., 1982) Insertion of an intravenous (i.v.) line in the jugular vein and connection to a programmable infusion pump (Pump 44; Harvard Apparatus, Inc., South Natick, Mass.) to deliver the inocula was performed as described (Fluckiger et al., 1994; Pea et al., 2011) on the day 40 of the vaccination schedule. Bacterial inocula were prepared from overnight cultures. Microorganisms were recovered by centrifugation, washed and adjusted to the desired inoculum size in saline. The inoculum size was confirmed by colony counts on blood agar plates. Animals were inoculated 24 h after catheterization, via the infusion pump, with 1 ml of 10^4 CFU(*S. aureus* Newman) or 106 CFU (*S. aureus* P8) progressively delivered at a pace of 0.0017 ml/min over 10 h in order to produce a low-grade of bacteremia (Veloso et al. 2011).

[0084] The traditional i.v. bolus inoculation (10^4 CFU/ml) provoking transient high-grade bacteremia was also performed. Rats were sacrificed 24 h after the end of inoculation. Quantitative valve cultures were performed as previously described (Fluckiger et al., 1994) This method permitted the detection of 2 log 10 CFU/g of vegetation.

1.4. Statistical Analysis

[0085] Statistical analyses were performed using GraphPad software (GraphPad Software, Inc., USA). The rates of valve infections of the various groups were compared by the x2 test. $P<0.05$ was considered to be statistically significant.

1.5. Trypsin Shaving Protocol (Ythier et al. 2012)

[0086] In brief, bacteria were grown in 300 ml liquid cultures in the different media described above. At various times of the logarithmic or stationary growth phases, samples (between 10 and 100 ml depending on the cell density) were removed, immediately chilled at 4° C., and harvested by centrifugation. Pellets were washed three times with ice-cold phosphate-buffered saline (PBS) and finally re-suspended in 1 ml of the same buffer. To allow semi-quantitative comparisons between the proteomes of different samples, cell con-

centrations were adjusted to 1×10^9 bacteria/ml in all samples. Cell counts were validated by optical microscopy (Neubauer cell) and viable colony counts on nutrient agar. There were <0.5 log 10 differences between the Neubauer cell and viable counts, indicating that the large majority of cells were alive. Samples were then shaved for 1 h with 1 μg/ml (final concentration) of trypsin (Promega, Madison, Wis.) at 37° C., after which they were chilled at 4° C. and bacterial cells removed by centrifugation for 10 min at 4000 rpm and 4° C. Supernatants containing trypsin-shaved peptides were filtered (0.22 μm) and freeze-dried until further use.

Example 2

Results

[0087] The efficacy of the immunization with *L. lactis* ClfA or *L. lactis* ClfA/FnBPA(CD) against the *S. aureus* induced experimental endocarditis due to low-grade bacteremia was compared to the different control groups. The results of the infectivity rate are shown in FIG. **1**.

[0088] In FIG. **1**A the proportion of infection in the group immunized with *L. lactis* ClfA (6/22; 27.2%) was significantly lower than in the control groups PBS (9/12; 75%), Adj. (5/8; 62.5%) and *L. lactis* pIL253 (6/8; 75%) (χ^2 test; P<0.05), in the case of low-grade bacteremia experimental endocarditis induced by *S. aureus* Newman. These results confirm the protective effect of the immunization using the non-pathogenic *L. lactis* expressing heterologously the staphylococcal adhesin ClfA. In contrast, when the same immunization schedule was used in animals exposed to high-grade bacteremia experimental endocarditis, the protective effect was not observed.

[0089] In FIG. **1**B the proportion of infection in the group immunized with *L. lactis* ClfA/FnBPA(CD) (14/22; 63.6%) was significantly lower (P<0.05) than in the control groups PBS (20/21; 95.2%), Adj. (20/20; 100%) but not for *L. lactis* pIL253 (11/15; 73.3%; χ^2 test; P=0.53). These results demonstrate a diminution in the infection after the effect of the immunization using the non-pathogenic *L. lactis* expressing heterologously the staphylococcal adhesins ClfA/FnBPA (CD).

REFERENCES

[0090] Broughan, J., Anderson, R., & Anderson, A. S. (2011). Strategies for and advances in the development of *Staphylococcus aureus* prophylactic vaccines. *Expert review of vaccines,* 10(5), 695-708.

[0091] Entenza, J. M., Que, Y. A., Vouillamoz, J., Glauser, M. P., & Moreillon, P. (2001). Efficacies of moxifloxacin, ciprofloxacin, and vancomycin against experimental endocarditis due to methicillin-resistant *Staphylococcus aureus* expressing various degrees of ciprofloxacin resistance. *Antimicrobial agents and chemotherapy,* 45(11), 3076-83.

[0092] Fluckiger, U., Moreillon, P., Blaser, L. J., Bickle, M., Glauser, M. P., & Francioli, P. (1994). Simulation of Amoxicillin Pharmacokinetics in Humans for the Prevention of Streptococcal Endocarditis in Rats. *Antimicrobial agents and chemotherapy,* 38(12), 2846-2849.

[0093] Gong, R., Hu, C., Xu, H., Guo, A., Chen, H., Zhang, G., & Shi, L. (2010). Evaluation of clumping factor A binding region A in a subunit vaccine against *Staphylococcus aureus*-induced mastitis in mice. *Clinical and vaccine immunology: CVI,* 17(11), 1746-52.

[0094] Héraïef E., Glauser, M. P., & Freedman, L. R. (1982). Natural history of aortic valve endocarditis in rats. *Infection and immunity,* 37(1), 127-31.

[0095] Kuhn, G., Francioli, P., & Blanc, D. S. (2007). Double-locus sequence typing using clfB and spa, a fast and simple method for epidemiological typing of methicillin-resistant *Staphylococcus aureus. Journal of clinical microbiology,* 45(1), 54-62.

[0096] Mcdevitt, D., Francois, P. F., Vaudaux, P., & Foster, T. J. (1994). Molecular characterization of the clumping factor (fibrinogen receptor) of *Staphylococcus aureus. Mol. Microbiol,* 11(2), 237-248.

[0097] Moreillon, P., Entenza, J. M., Francioli, P., McDevitt, D., Foster, T. J., & Vaudaux, P. (1995). Role of *Staphylococcus aureus* Coagulase and Clumping Factor in Pathogenesis of Experimental Endocarditis. *Infection and immunity,* 63(12), 4738-4743.

[0098] Patti, J. M., Allen, B. L., Mcgavin, M. J., & Hook, M. (1994). MSCRAMM-Mediated Adherence of Microorganisms to Host Tissues. *Ann. Rev. Microbiol.,* 48, 585-617.

[0099] Pea, F., Cojutti, P., Petrosillo, N., Furlanut, M., Entenza, J. M., Veloso, T. R., Vouillamoz, J., et al. (2011). Continuous infusion may improve the efficacy of vancomycin in treatment of experimental endocarditis due to heterogeneous vancomycin-intermediate *Staphylococcus aureus. Antimicrobial agents and chemotherapy,* 55(9), 4496;

[0100] Piroth, L., Que, Y., Widmer, E., Panchaud, A., & Moreillon, P. (2008). The Fibrinogen- and Fibronectin-Binding Domains of *Staphylococcus aureus* Fibronectin-Binding Protein A Synergistically Promote Endothelial Invasion and Experimental Endocarditis. *Infection and immunity,* 76(8), 3824-3831.

[0101] Que, Y, & Moreillon, P. (2011). Infective endocarditis. *The Lancet,* 363,139-149. Nature Publishing Group.

[0102] Que, Y. A., Haefliger, J. A., Francioli, P., & Moreillon, P. (2000). Expression of *Staphylococcus aureus* clumping factor A in *Lactococcus lactis* subsp. *cremoris* using a new shuttle vector. *Infection and immunity,* 68(6), 3516-22.

[0103] Que, Y. A., & Moreillon, P. (2009). *Principles and Practice in Infectious Diseases*. (G. Mandell, R. Douglas, & J. Bennett, Eds.) (p. Chapter 195). New York: Elsevier Inc.

[0104] Que, Yok-Ai, Franc, P., Haefliger, J., Vaudaux, P., & Moreillon, P. (2001). Reassessing the Role of *Staphylococcus aureus* Clumping Factor and Fibronectin-Binding Protein by Expression in *Lactococcus lactis. Infection and immunity,* 69(10), 6296-6302.

[0105] Que, Yok-Ai, Haefliger, J., Piroth, L., Widmer, E., Sinha, B., Herrmann, M., Francioli, P., et al. (2005). Fibrinogen and fibronectin binding cooperate for valve infection and invasion in *Staphylococcus aureus* experimental endocarditis. *JEM,* 201(610), 1627-1635.

[0106] Risley, A. L., Loughman, A., Cywes-Bentley, C., Foster, T. J., & Lee, J. C. (2007). Capsular polysaccharide masks clumping factor A-mediated adherence of *Staphylococcus aureus* to fibrinogen and platelets. *The Journal of infectious diseases,* 196(6), 919-27.

[0107] Scarpa, M., Piccinini, R., Brun, P., Grillo, A., Palù, G., Mengoli, C., Daprà, V., et al. (2010). Relationship

between virulence factor genes in bovine *Staphylococcus aureus* subclinical mastitis isolates and binding to anti-adhesin antibodies. *The Journal of dairy research,* 77(2), 159-67.

[0108] Shinefield, H., Black, S., Fattom, A., Horwith, G., Rasgon, S., Ordonez, J., Yeoh, H., et al. (2002). Use of a *Staphylococcus aureus* conjugate vaccine in patients receiving hemodialysis. *The New England journal of medicine,* 346(7), 491-6.

[0109] Veloso, T. R., Amiguet, M., Rousson, V., Giddey, M., Vouillamoz, J., Moreillon, P., & Entenza, J. M. (2011). Induction of experimental endocarditis by continuous low-grade bacteremia mimicking spontaneous bacteremia in humans. *Infection and immunity,* 79(5), 2006-11.

[0110] Wells, J. M., & Mercenier, A. (2008). Mucosal delivery of therapeutic and prophylactic molecules using lactic acid bacteria. *Nature reviews. Microbiology,* 6(5), 349-62.

[0111] Ythier, M., Resch, G., Waridel, P., Panchaud, A., Gfeller, A., Majcherczyk, P., Quadroni, M., et al. (2012). Proteomic and transcriptomic profiling of *Staphylococcus aureus* surface LPXTG-proteins: correlation with agr genotypes and adherence phenotypes. *Molecular & cellular proteomics: MCP.*

SEQUENCE LISTING

<160> NUMBER OF SEQ ID NOS: 39

<210> SEQ ID NO 1
<211> LENGTH: 933
<212> TYPE: PRT
<213> ORGANISM: Staphylococcus aureus

<400> SEQUENCE: 1

```
Met Asn Met Lys Lys Lys Glu Lys His Ala Ile Arg Lys Lys Ser Ile
1               5                   10                  15

Gly Val Ala Ser Val Leu Val Gly Thr Leu Ile Gly Phe Gly Leu Leu
            20                  25                  30

Ser Ser Lys Glu Ala Asp Ala Ser Glu Asn Ser Val Thr Gln Ser Asp
        35                  40                  45

Ser Ala Ser Asn Glu Ser Lys Ser Asn Asp Ser Ser Ser Val Ser Ala
    50                  55                  60

Ala Pro Lys Thr Asp Asp Thr Asn Val Ser Asp Thr Lys Thr Ser Ser
65                  70                  75                  80

Asn Thr Asn Asn Gly Glu Thr Ser Val Ala Gln Asn Pro Ala Gln Gln
                85                  90                  95

Glu Thr Thr Gln Ser Ser Ser Thr Asn Ala Thr Thr Glu Glu Thr Pro
            100                 105                 110

Val Thr Gly Glu Ala Thr Thr Thr Thr Thr Asn Gln Ala Asn Thr Pro
        115                 120                 125

Ala Thr Thr Gln Ser Ser Asn Thr Asn Ala Glu Glu Leu Val Asn Gln
    130                 135                 140

Thr Ser Asn Glu Thr Thr Phe Asn Asp Thr Asn Thr Val Ser Ser Val
145                 150                 155                 160

Asn Ser Pro Gln Asn Ser Thr Asn Ala Glu Asn Val Ser Thr Thr Gln
                165                 170                 175

Asp Thr Ser Thr Glu Ala Thr Pro Ser Asn Asn Glu Ser Ala Pro Gln
            180                 185                 190

Ser Thr Asp Ala Ser Asn Lys Asp Val Val Asn Gln Ala Val Asn Thr
        195                 200                 205

Ser Ala Pro Arg Met Arg Ala Phe Ser Leu Ala Ala Val Ala Ala Asp
    210                 215                 220

Ala Pro Ala Ala Gly Thr Asp Ile Thr Asn Gln Leu Thr Asn Val Thr
225                 230                 235                 240

Val Gly Ile Asp Ser Gly Thr Thr Val Tyr Pro His Gln Ala Gly Tyr
                245                 250                 255

Val Lys Leu Asn Tyr Gly Phe Ser Val Pro Asn Ser Ala Val Lys Gly
            260                 265                 270
```

-continued

```
Asp Thr Phe Lys Ile Thr Val Pro Lys Glu Leu Asn Leu Asn Gly Val
        275                 280                 285

Thr Ser Thr Ala Lys Val Pro Pro Ile Met Ala Gly Asp Gln Val Leu
    290                 295                 300

Ala Asn Gly Val Ile Asp Ser Asp Gly Asn Val Ile Tyr Thr Phe Thr
305                 310                 315                 320

Asp Tyr Val Asn Thr Lys Asp Asp Val Lys Ala Thr Leu Thr Met Pro
                325                 330                 335

Ala Tyr Ile Asp Pro Glu Asn Val Lys Lys Thr Gly Asn Val Thr Leu
            340                 345                 350

Ala Thr Gly Ile Gly Ser Thr Thr Ala Asn Lys Thr Val Leu Val Asp
        355                 360                 365

Tyr Glu Lys Tyr Gly Lys Phe Tyr Asn Leu Ser Ile Lys Gly Thr Ile
    370                 375                 380

Asp Gln Ile Asp Lys Thr Asn Asn Thr Tyr Arg Gln Thr Ile Tyr Val
385                 390                 395                 400

Asn Pro Ser Gly Asp Asn Val Ile Ala Pro Val Leu Thr Gly Asn Leu
                405                 410                 415

Lys Pro Asn Thr Asp Ser Asn Ala Leu Ile Asp Gln Gln Asn Thr Ser
            420                 425                 430

Ile Lys Val Tyr Lys Val Asp Asn Ala Ala Asp Leu Ser Glu Ser Tyr
        435                 440                 445

Phe Val Asn Pro Glu Asn Phe Glu Asp Val Thr Asn Ser Val Asn Ile
    450                 455                 460

Thr Phe Pro Asn Pro Asn Gln Tyr Lys Val Glu Phe Asn Thr Pro Asp
465                 470                 475                 480

Asp Gln Ile Thr Thr Pro Tyr Ile Val Val Val Asn Gly His Ile Asp
                485                 490                 495

Pro Asn Ser Lys Gly Asp Leu Ala Leu Arg Ser Thr Leu Tyr Gly Tyr
            500                 505                 510

Asn Ser Asn Ile Ile Trp Arg Ser Met Ser Trp Asp Asn Glu Val Ala
        515                 520                 525

Phe Asn Asn Gly Ser Gly Ser Gly Asp Gly Ile Asp Lys Pro Val Val
    530                 535                 540

Pro Glu Gln Pro Asp Glu Pro Gly Glu Ile Glu Pro Ile Pro Glu Asp
545                 550                 555                 560

Ser Asp Ser Asp Pro Gly Ser Asp Ser Gly Ser Asp Ser Asn Ser Asp
                565                 570                 575

Ser Gly Ser Asp Ser Gly Ser Asp Ser Thr Ser Asp Ser Gly Ser Asp
            580                 585                 590

Ser Ala Ser Asp Ser Asp Ser Ala Ser Asp Ser Asp Ser Ala Ser Asp
        595                 600                 605

Ser Asp Ser Ala Ser Asp Ser Asp Ser Ala Ser Asp Ser Asp Ser Asp
    610                 615                 620

Asn Asp Ser Asp Ser Asp Ser Asp Ser Asp Ser Asp Ser Asp Ser Asp
625                 630                 635                 640

Ser Asp Ser Asp Ser Asp Ser Asp Ser Asp Ser Asp Ser Asp Ser Asp
                645                 650                 655

Ser Asp Ser Asp Ser Asp Ser Asp Ser Asp Ser Asp Ser Asp Ser Asp
            660                 665                 670
```

-continued

```
Ser Asp Ser Asp Ser Asp Ser Asp Ser Asp Ser Asp Ser Asp Ser Asp
        675                 680                 685

Ser Asp Ser Asp Ser Asp Ser Asp Ser Asp Ser Asp Ser Asp Ser Asp
    690                 695                 700

Ser Asp Ser Asp Ser Asp Ser Asp Ser Asp Ser Asp Ser Asp Ser Asp
705                 710                 715                 720

Ser Asp Ser Asp Ser Asp Ser Asp Ser Asp Ser Asp Ser Asp Ser Asp
                725                 730                 735

Ser Asp Ser Asp Ser Asp Ser Asp Ser Asp Ser Asp Ser Asp Ser Asp
            740                 745                 750

Ser Asp Ser Asp Ser Asp Ser Asp Ser Asp Ser Asp Ser Asp Ser Ala
        755                 760                 765

Ser Asp Ser Asp Ser Asp Ser Asp Ser Asp Ser Asp Ser Asp Ser Asp
    770                 775                 780

Ser Asp Ser Asp Ser Asp Ser Asp Ser Asp Ser Asp Ser Asp Ser Asp
785                 790                 795                 800

Ser Asp Ser Asp Ser Asp Ser Glu Ser Asp Ser Asp Ser Glu Ser Asp
                805                 810                 815

Ser Asp Ser Asp Ser Asp Ser Asp Ser Asp Ser Asp Ser Asp Ser Asp
            820                 825                 830

Ser Asp Ser Asp Ser Ala Ser Asp Ser Asp Ser Gly Ser Asp Ser Asp
        835                 840                 845

Ser Ser Ser Asp Ser Asp Ser Glu Ser Asp Ser Asn Ser Asp Ser Glu
    850                 855                 860

Ser Gly Ser Asn Asn Asn Val Val Pro Pro Asn Ser Pro Lys Asn Gly
865                 870                 875                 880

Thr Asn Ala Ser Asn Lys Asn Glu Ala Lys Asp Ser Lys Glu Pro Leu
                885                 890                 895

Pro Asp Thr Gly Ser Glu Asp Glu Ala Asn Thr Ser Leu Ile Trp Gly
            900                 905                 910

Leu Leu Ala Ser Ile Gly Ser Leu Leu Leu Phe Arg Arg Lys Lys Glu
        915                 920                 925

Asn Lys Asp Lys Lys
    930


<210> SEQ ID NO 2
<211> LENGTH: 3499
<212> TYPE: DNA
<213> ORGANISM: Staphylococcus aureus

<400> SEQUENCE: 2

ggtaccataa attacacatc tgcttttgaa aaaatatgat ttcaagctag gattacatta     60

ggtagagttc atattaataa taaaaaatgt ttgcaatcaa atcgtacgtt gtcgtttgta    120

attcttaaaa tagcaataaa taaaatgttt gttagtaaag tattattgtg gataataaaa    180

tatcgataca aattaattgc tataatgcaa ttttagtgta taattccatt aacagagatt    240

aaatatatct ttaaagggta tatagttaat ataaaatgac tttttaaaaa gagggaataa    300

aatgaatatg aagaaaaaag aaaaacacgc aattcggaaa aaatcgattg gcgtggcttc    360

agtgcttgta ggtacgttaa tcggttttgg actactcagc agtaaagaag cagatgcaag    420

tgaaaatagt gttacgcaat ctgatagcgc aagtaacgaa agcaaaagta atgattcaag    480

tagcgttagt gctgcaccta aaacagacga cacaaacgtg agtgatacta aaacatcgtc    540
```

-continued

aaacactaat aatggcgaaa cgagtgtggc gcaaaatcca gcacaacagg aaacgacaca 600

atcatcatca acaaatgcaa ctacggaaga aacgccggta actggtgaag ctactactac 660

gacaacgaat caagctaata caccggcaac aactcaatca agcaatacaa atgcggagga 720

attagtgaat caaacaagta atgaaacgac ttttaatgat actaatacag tatcatctgt 780

aaattcacct caaaattcta caaatgcgga aaatgtttca acaacgcaag atacttcaac 840

tgaagcaaca ccttcaaaca atgaatcagc tccacagagt acagatgcaa gtaataaaga 900

tgtagttaat caagcggtta atacaagtgc gcctagaatg agagcattta gtttagcggc 960

agtagctgca gatgcaccgg cagctggcac agatattacg aatcagttga cgaatgtgac 1020

agttggtatt gactctggta cgactgtgta tccgcaccaa gcaggttatg tcaaactgaa 1080

ttatggtttt tcagtgccta attctgctgt taaaggtgac acattcaaaa taactgtacc 1140

taaagaatta aacttaaatg gtgtaacttc aactgctaaa gtgccaccaa ttatggctgg 1200

agatcaagta ttggcaaatg gtgtaatcga tagtgatggt aatgttattt atacatttac 1260

agactatgta aatactaaag atgatgtaaa agcaactttg accatgcccg cttatattga 1320

ccctgaaaat gttaaaaaga caggtaatgt gacattggct actggcatag gtagtacaac 1380

agcaaacaaa acagtattag tagattatga aaaatatggt aagttttata acttatctat 1440

taaaggtaca attgaccaaa tcgataaaac aaataatacg tatcgtcaga caatttatgt 1500

caatccaagt ggagataacg ttattgcgcc ggttttaaca ggtaatttaa aaccaaatac 1560

ggatagtaat gcattaatag atcagcaaaa tacaagtatt aaagtatata aagtagataa 1620

tgcagctgat ttatctgaaa gttactttgt gaatccagaa aactttgagg atgtcactaa 1680

tagtgtgaat attacattcc caaatccaaa tcaatataaa gtagagttta atacgcctga 1740

tgatcaaatt acaacaccgt atatagtagt tgttaatggt catattgatc cgaatagcaa 1800

aggtgattta gctttacgtt caactttata tgggtataac tcgaatataa tttggcgctc 1860

tatgtcatgg gacaacgaag tagcatttaa taacggatca ggttctggtg acggtatcga 1920

taaaccagtt gttcctgaac aacctgatga gcctggtgaa attgaaccaa ttccagagga 1980

ttcagattct gacccaggtt cagattctgg cagcgattct aattcagata gcggttcaga 2040

ttcgggtagt gattctacat cagatagtgg ttcagattca gcgagtgatt cagattcagc 2100

aagtgattca gactcagcga gtgattcaga ttcagcaagc gattccgact cagcgagcga 2160

ttccgactca gacaatgact cggattcaga tagcgattct gactcagaca gtgactcaga 2220

ttccgacagt gactcagatt cagatagcga ttctgactca gacagtgact cagattcaga 2280

tagcgattca gattcagata gcgattcaga ttccgacagt gattccgact cagacagcga 2340

ttctgactcc gacagtgatt ccgactcaga cagcgattca gattccgaca gtgattccga 2400

ctcagatagc gattccgact cagatagcga ctcagattca gacagcgatt cagattcaga 2460

cagcgattca gattcagata gcgattcaga ttccgacagt gactcagatt ccgacagtga 2520

ctcggattca gatagcgatt cagattccga cagtgactca gattccgaca gtgactcaga 2580

ctcagacagt gattcggatt cagcgagtga ttcggattca gatagtgatt ccgactccga 2640

cagtgactcg gattcagata gcgactcaga ctcggatagc gactcggatt cagatagcga 2700

ttcggactca gatagcgatt cagaatcaga cagcgattca gaatcagaca gcgattcaga 2760

ttcagacagc gactcagaca gtgactcaga ttcagatagt gactcggatt cagcgagtga 2820

-continued

ttcagactca ggtagtgact ccgattcatc aagtgattcc gactcagaaa gtgattcaaa 2880

tagcgattcc gagtcaggtt ctaacaataa tgtagttccg cctaattcac ctaaaaatgg 2940

tactaatgct tctaataaaa atgaggctaa agatagtaaa gaaccattac cagatacagg 3000

ttctgaagat gaagcaaata cgtcactaat ttggggatta ttagcatcaa taggttcatt 3060

actacttttc agaagaaaaa aagaaaataa agataagaaa taagtaataa tgatattaaa 3120

ttaatcatat gattcatgaa gaagccacct taaaaggtgc ttcttttact tggattttcc 3180

aaatatattg tttgaatata attaataatt aattcatcaa cagttaatta ttttaaaaag 3240

gtagatgtta tataatttgg cttggcgaaa aaatagggtg taaggtaggt tgttaattag 3300

ggaaaattaa ggagaaaata cagttgaaaa ataaattgct agttttatca ttgggagcat 3360

tatgtgtatc acaaatttgg gaaagtaatc gtgcgagtgc agtggtttct ggggagaaga 3420

atccatatgt atctgagtcg ttgaaactga ctaataataa aaataaatct agaacagtag 3480

aagagtataa gaaaagctt 3499

<210> SEQ ID NO 3
<211> LENGTH: 2973
<212> TYPE: DNA
<213> ORGANISM: Staphylococcus aureus

<400> SEQUENCE: 3

ttatgctttg tgattctttt tatttctgcg taataatgct aaacctagaa tgctgaataa 60

tccgccgaac aacatacctt tgtttgttga ttcttctcca cctgtttcag gtagttcaga 120

tttcttagat tgtggttttt tagttggtgc cactgcttta acctttttcat tgatttcaat 180

aacaggtgtt actactttac cttgttccac tggtttagaa ggctttttag gttcttcttt 240

ggcaggtggt actggtttac caggttcagc tggtacctct ggtgttggcg gtgttggagt 300

ttctggctca ctcggcactt ctggtgtcgg tggtgttggt gtttccggct cacttggtac 360

ttctggtgtt ggtggcgttg gtgtttccgg ctcacttggt acttctggtg tcggtggcgt 420

tggtggcacg attggaggtg ttgtatcttc ttcaatcgtt tgttgacctt cattttggcc 480

gcttactttt ggaagtgtat cttcttcaaa gtcaacacta ttgtgtccac cgaattgata 540

acttggttta tctttatttg tatcttcttc aataatttca gtgtgcttat tgaatccgtg 600

aatatgtggc acactgtcga agtcgatatc aatgatgtta ccgccatgtt catacttagg 660

tttgtctttt tctgtatctt cctcgaatga ctgattacct ttattttgac catgaatttg 720

aggtacacta tcaaaatcga tatctacgat attgccacct tgttcatatt taggtttgtc 780

ttcttctgtg tcttcctcga atgactggtt accgctattt tggccacctt cataacctaa 840

ttcactctta atatcaacgt ggctattttc ttcgatttct tcaatcacgt cataattccc 900

gtgaccattt tcagttccta aaccagaatg agaaatatga tgattgtttt tagtaatttc 960

ctcgactggt ccttgtgctt gaccatgctc ttcaggtaat tcatccacta attcaatcag 1020

attactttca gttgtatatt ctttcgtatc ttcaactgtt gtatgatcgc tcactgcgcc 1080

agttacaata ccttttgtag actcttcgtc aaattcaact aagttagact cagtagtaac 1140

ctgaccacca cctgggtttg tatcttcttc atattcaaca acatcagcgt gatgttttga 1200

attttcatgt gtagattctt caaagtcaat tggatttgat tcctcagagg actcagtgta 1260

tcctccaacg tgacctgctt cgctatccac agcagtatgg taatcgatat caatagctga 1320

-continued

tgaatccgtt tcttctattg tttcaatgta tccatcaaca tatccacctc caccatctat 1380

agctgtgtgg taatcaatgt caagagttga tgaatcatat tcctcttcaa cagtagttac 1440

taaattctta tcatattgac ctgtaagagt ttctttaatt gtatcttctt tatattcaaa 1500

tttattattt tgaataatcg gaccattttt ctcatttccg ttcgctttat tactgtataa 1560

aactaaacca ttatcccaag ttaaggtata tcctctatca taataatact tataaagttg 1620

ctctggatgt cctaccattt gtgttctaaa atcaacttca tcagtaccat ttaaatactc 1680

tccatcatag tgaacaacat aagttttatc tagattttct atattcaatg aatagcttcc 1740

attattttgt aaattcaaat tcccactcat attacttgtg acttctttaa atttagaagt 1800

atctgtcgta tttgcatata cactcttcgc tatgtcttca ttattaccca agtattcaaa 1860

tatcctaact tttggttgat ttccattctg attactacct ttcattaaag ttccagtaac 1920

agtcacactt gtcgttttac cattattagg tttaataaat gcaacatgcg aaaatctatt 1980

attcgcttta ttaaatgtct caatcgatcc atttaaattg gcataataat tcccaatacc 2040

atctttatat ttaacatcta attcctttga agtttgttct tcatttagtg ttgaagttat 2100

agtttgattt ccattagttt gtacagtttt aggatcaata aataaattaa tttctagttc 2160

agccgttaca tcaaccttat cttcaatatc atttgtaaat gtatatctaa tctttccacc 2220

ttctaaaact tcacctgtcg ccattacgac tgaaccattt ttaatttctg gtacttttct 2280

agcagttgat acgccatgcg tatttacatt atttgataaa gtaaagtcaa agtagtcacc 2340

ttgatgtaaa ccattctcaa atttcaactt atattttagt accgctcgtt gtcctgcatg 2400

aggttctact ttatttgtat tgttatgccc ctcaatagaa ccaatttcta ctgtaacttt 2460

acttgttaca tctgtacccg tttccacttt cgcgttacta gcttccttag cttccgctac 2520

atctgctgat cttgtcacac gtggcttact ttctgatgcc gttcttggct gtgccacttc 2580

aacttgtgtt tctgcgactt gattttgtgt agccttttta ggtgttaaat ctacttgtct 2640

ttgatctccg ctattgtctt gagattgtgt tgtttcctta acttgaggtt tcgcttcttc 2700

cttaactacc tcttctttaa ctgtttctat atttgctggt tgtgcagttt gtggtgcttg 2760

tactgctttt ggtgcttctt cagttgttac ttgtgttgcg tttgacggtt gttctgttac 2820

tgttgcgtta tatgattgag tttcttctat atgattaacg ttagttgcag ttgtttgtgt 2880

ttcacttgtt ttattatcag tagctgaatt cccattttct tctactgtag ttgtcttttg 2940

ttctgatgct gcagcttctt tgtcttgtcc cat 2973

<210> SEQ ID NO 4
<211> LENGTH: 990
<212> TYPE: PRT
<213> ORGANISM: Staphylococcus aureus

<400> SEQUENCE: 4

Met Gly Gln Asp Lys Glu Ala Ala Ala Ser Glu Gln Lys Thr Thr Thr
1 5 10 15

Val Glu Glu Asn Gly Asn Ser Ala Thr Asp Asn Lys Thr Ser Glu Thr
20 25 30

Gln Thr Thr Ala Thr Asn Val Asn His Ile Glu Glu Thr Gln Ser Tyr
35 40 45

Asn Ala Thr Val Thr Glu Gln Pro Ser Asn Ala Thr Gln Val Thr Thr
50 55 60

-continued

```
Glu Glu Ala Pro Lys Ala Val Gln Ala Pro Gln Thr Ala Gln Pro Ala
65                  70                  75                  80

Asn Ile Glu Thr Val Lys Glu Glu Val Val Lys Glu Glu Ala Lys Pro
                85                  90                  95

Gln Val Lys Glu Thr Thr Gln Ser Gln Asp Asn Ser Gly Asp Gln Arg
            100                 105                 110

Gln Val Asp Leu Thr Pro Lys Lys Ala Thr Gln Asn Gln Val Ala Glu
        115                 120                 125

Thr Gln Val Glu Val Ala Gln Pro Arg Thr Ala Ser Glu Ser Lys Pro
    130                 135                 140

Arg Val Thr Arg Ser Ala Asp Val Ala Glu Ala Lys Glu Ala Ser Asn
145                 150                 155                 160

Ala Lys Val Glu Thr Gly Thr Asp Val Thr Ser Lys Val Thr Val Glu
                165                 170                 175

Ile Gly Ser Ile Glu Gly His Asn Asn Thr Asn Lys Val Glu Pro His
            180                 185                 190

Ala Gly Gln Arg Ala Val Leu Lys Tyr Lys Leu Lys Phe Glu Asn Gly
        195                 200                 205

Leu His Gln Gly Asp Tyr Phe Asp Phe Thr Leu Ser Asn Asn Val Asn
    210                 215                 220

Thr His Gly Val Ser Thr Ala Arg Lys Val Pro Glu Ile Lys Asn Gly
225                 230                 235                 240

Ser Val Val Met Ala Thr Gly Glu Val Leu Glu Gly Gly Lys Ile Arg
                245                 250                 255

Tyr Thr Phe Thr Asn Asp Ile Glu Asp Lys Val Asp Val Thr Ala Glu
            260                 265                 270

Leu Glu Ile Asn Leu Phe Ile Asp Pro Lys Thr Val Gln Thr Asn Gly
        275                 280                 285

Asn Gln Thr Ile Thr Ser Thr Leu Asn Glu Glu Gln Thr Ser Lys Glu
    290                 295                 300

Leu Asp Val Lys Tyr Lys Asp Gly Ile Gly Asn Tyr Tyr Ala Asn Leu
305                 310                 315                 320

Asn Gly Ser Ile Glu Thr Phe Asn Lys Ala Asn Asn Arg Phe Ser His
                325                 330                 335

Val Ala Phe Ile Lys Pro Asn Asn Gly Lys Thr Thr Ser Val Thr Val
            340                 345                 350

Thr Gly Thr Leu Met Lys Gly Ser Asn Gln Asn Gly Asn Gln Pro Lys
        355                 360                 365

Val Arg Ile Phe Glu Tyr Leu Gly Asn Asn Glu Asp Ile Ala Lys Ser
    370                 375                 380

Val Tyr Ala Asn Thr Thr Asp Thr Ser Lys Phe Lys Glu Val Thr Ser
385                 390                 395                 400

Asn Met Ser Gly Asn Leu Asn Leu Gln Asn Asn Gly Ser Tyr Ser Leu
                405                 410                 415

Asn Ile Glu Asn Leu Asp Lys Thr Tyr Val Val His Tyr Asp Gly Glu
            420                 425                 430

Tyr Leu Asn Gly Thr Asp Glu Val Asp Phe Arg Thr Gln Met Val Gly
        435                 440                 445

His Pro Glu Gln Leu Tyr Lys Tyr Tyr Tyr Asp Arg Gly Tyr Thr Leu
    450                 455                 460
```

-continued

```
Thr Trp Asp Asn Gly Leu Val Leu Tyr Ser Asn Lys Ala Asn Gly Asn
465                 470                 475                 480

Glu Lys Asn Gly Pro Ile Ile Gln Asn Asn Lys Phe Glu Tyr Lys Glu
                485                 490                 495

Asp Thr Ile Lys Glu Thr Leu Thr Gly Gln Tyr Asp Lys Asn Leu Val
            500                 505                 510

Thr Thr Val Glu Glu Glu Tyr Asp Ser Ser Thr Leu Asp Ile Asp Tyr
        515                 520                 525

His Thr Ala Ile Asp Gly Gly Gly Gly Tyr Val Asp Gly Tyr Ile Glu
    530                 535                 540

Thr Ile Glu Glu Thr Asp Ser Ser Ala Ile Asp Ile Asp Tyr His Thr
545                 550                 555                 560

Ala Val Asp Ser Glu Ala Gly His Val Gly Gly Tyr Thr Glu Ser Ser
                565                 570                 575

Glu Glu Ser Asn Pro Ile Asp Phe Glu Glu Ser Thr His Glu Asn Ser
            580                 585                 590

Lys His His Ala Asp Val Val Glu Tyr Glu Glu Asp Thr Asn Pro Gly
        595                 600                 605

Gly Gly Gln Val Thr Thr Glu Ser Asn Leu Val Glu Phe Asp Glu Glu
    610                 615                 620

Ser Thr Lys Gly Ile Val Thr Gly Ala Val Ser Asp His Thr Thr Val
625                 630                 635                 640

Glu Asp Thr Lys Glu Tyr Thr Thr Glu Ser Asn Leu Ile Glu Leu Val
                645                 650                 655

Asp Glu Leu Pro Glu Glu His Gly Gln Ala Gln Gly Pro Val Glu Glu
            660                 665                 670

Ile Thr Lys Asn Asn His His Ile Ser His Ser Gly Leu Gly Thr Glu
        675                 680                 685

Asn Gly His Gly Asn Tyr Asp Val Ile Glu Glu Ile Glu Glu Asn Ser
    690                 695                 700

His Val Asp Ile Lys Ser Glu Leu Gly Tyr Glu Gly Gly Gln Asn Ser
705                 710                 715                 720

Gly Asn Gln Ser Phe Glu Glu Asp Thr Glu Glu Asp Lys Pro Lys Tyr
                725                 730                 735

Glu Gln Gly Gly Asn Ile Val Asp Ile Asp Phe Asp Ser Val Pro Gln
            740                 745                 750

Ile His Gly Gln Asn Lys Gly Asn Gln Ser Phe Glu Glu Asp Thr Glu
        755                 760                 765

Lys Asp Lys Pro Lys Tyr Glu His Gly Gly Asn Ile Ile Asp Ile Asp
    770                 775                 780

Phe Asp Ser Val Pro His Ile His Gly Phe Asn Lys His Thr Glu Ile
785                 790                 795                 800

Ile Glu Glu Asp Thr Asn Lys Asp Lys Pro Ser Tyr Gln Phe Gly Gly
                805                 810                 815

His Asn Ser Val Asp Phe Glu Glu Asp Thr Leu Pro Lys Val Ser Gly
            820                 825                 830

Gln Asn Glu Gly Gln Gln Thr Ile Glu Glu Asp Thr Thr Pro Pro Ile
        835                 840                 845

Val Pro Pro Thr Pro Pro Thr Pro Glu Val Pro Ser Glu Pro Glu Thr
    850                 855                 860

Pro Thr Pro Pro Thr Pro Glu Val Pro Ser Glu Pro Glu Thr Pro Thr
```

-continued

```
865                 870                 875                 880

Pro Pro Thr Pro Glu Val Pro Ser Glu Pro Glu Thr Pro Thr Pro Pro
                885                 890                 895

Thr Pro Glu Val Pro Ala Glu Pro Gly Lys Pro Val Pro Pro Ala Lys
            900                 905                 910

Glu Glu Pro Lys Lys Pro Ser Lys Pro Val Glu Gln Gly Lys Val Val
        915                 920                 925

Thr Pro Val Ile Glu Ile Asn Glu Lys Val Lys Ala Val Ala Pro Thr
    930                 935                 940

Lys Lys Pro Gln Ser Lys Lys Ser Glu Leu Pro Glu Thr Gly Gly Glu
945                 950                 955                 960

Glu Ser Thr Asn Lys Gly Met Leu Phe Gly Gly Leu Phe Ser Ile Leu
                965                 970                 975

Gly Leu Ala Leu Leu Arg Arg Asn Lys Lys Asn His Lys Ala
            980                 985                 990


<210> SEQ ID NO 5
<211> LENGTH: 16
<212> TYPE: PRT
<213> ORGANISM: Lactobacillus lactis

<400> SEQUENCE: 5

Ser Glu Asn Ser Val Thr Gln Ser Asp Ser Ala Ser Asn Glu Ser Lys
1               5                   10                  15


<210> SEQ ID NO 6
<211> LENGTH: 12
<212> TYPE: PRT
<213> ORGANISM: Lactobacillus lactis

<400> SEQUENCE: 6

Ser Asn Asp Ser Ser Ser Val Ser Ala Ala Pro Lys
1               5                   10


<210> SEQ ID NO 7
<211> LENGTH: 13
<212> TYPE: PRT
<213> ORGANISM: Lactobacillus lactis

<400> SEQUENCE: 7

Asp Val Val Asn Gln Ala Val Asn Thr Ser Ala Pro Arg
1               5                   10


<210> SEQ ID NO 8
<211> LENGTH: 13
<212> TYPE: PRT
<213> ORGANISM: Lactobacillus lactis

<400> SEQUENCE: 8

Leu Asn Tyr Gly Phe Ser Val Pro Asn Ser Ala Val Lys
1               5                   10


<210> SEQ ID NO 9
<211> LENGTH: 12
<212> TYPE: PRT
<213> ORGANISM: Staphylococcus aureus

<400> SEQUENCE: 9

Glu Leu Asn Leu Asn Gly Val Thr Ser Thr Ala Lys
```

-continued

```
1               5                   10


<210> SEQ ID NO 10
<211> LENGTH: 15
<212> TYPE: PRT
<213> ORGANISM: Lactobacillus lactis

<400> SEQUENCE: 10

Ala Thr Leu Thr Met Pro Ala Tyr Ile Asp Pro Glu Asn Val Lys
1               5                   10                  15


<210> SEQ ID NO 11
<211> LENGTH: 17
<212> TYPE: PRT
<213> ORGANISM: Lactobacillus lactis

<400> SEQUENCE: 11

Thr Gly Asn Val Thr Leu Ala Thr Gly Ile Gly Ser Thr Thr Ala Asn
1               5                   10                  15

Lys


<210> SEQ ID NO 12
<211> LENGTH: 7
<212> TYPE: PRT
<213> ORGANISM: Lactobacillus lactis

<400> SEQUENCE: 12

Phe Tyr Asn Leu Ser Ile Lys
1               5


<210> SEQ ID NO 13
<211> LENGTH: 38
<212> TYPE: PRT
<213> ORGANISM: Lactobacillus lactis

<400> SEQUENCE: 13

Gln Thr Ile Tyr Val Asn Pro Ser Gly Asp Asn Val Ile Ala Pro Val
1               5                   10                  15

Leu Thr Gly Asn Leu Lys Pro Asn Thr Asp Ser Asn Ala Leu Ile Asp
            20                  25                  30

Gln Gln Asn Thr Ser Ile
        35


<210> SEQ ID NO 14
<211> LENGTH: 36
<212> TYPE: PRT
<213> ORGANISM: Staphylococcus aureus

<400> SEQUENCE: 14

Val Asp Asn Ala Ala Asp Leu Ser Glu Ser Tyr Phe Val Asn Pro Glu
1               5                   10                  15

Asn Phe Glu Asp Val Thr Asn Ser Val Asn Ile Thr Phe Pro Asn Pro
            20                  25                  30

Asn Gln Tyr Lys
        35


<210> SEQ ID NO 15
<211> LENGTH: 27
<212> TYPE: PRT
<213> ORGANISM: Lactobacillus lactis
```

-continued

<400> SEQUENCE: 15

Val Glu Phe Asn Thr Pro Asp Asp Gln Ile Thr Thr Pro Tyr Ile Val
1 5 10 15

Val Val Asn Gly His Ile Asp Pro Asn Ser Lys
20 25

<210> SEQ ID NO 16
<211> LENGTH: 41
<212> TYPE: PRT
<213> ORGANISM: Lactobacillus lactis

<400> SEQUENCE: 16

Thr Ser Glu Thr Gln Thr Thr Ala Thr Asn Val Asn His Ile Glu Glu
1 5 10 15

Thr Gln Ser Tyr Asn Ala Thr Val Thr Glu Gln Pro Ser Asn Ala Thr
20 25 30

Gln Val Thr Thr Glu Glu Ala Pro Lys
35 40

<210> SEQ ID NO 17
<211> LENGTH: 17
<212> TYPE: PRT
<213> ORGANISM: Lactobacillus lactis

<400> SEQUENCE: 17

Ala Val Gln Ala Pro Gln Thr Ala Gln Pro Ala Asn Ile Glu Thr Val
1 5 10 15

Lys

<210> SEQ ID NO 18
<211> LENGTH: 22
<212> TYPE: PRT
<213> ORGANISM: Staphylococcus aureus

<400> SEQUENCE: 18

Ala Val Gln Ala Pro Gln Thr Ala Gln Pro Ala Asn Ile Glu Thr Val
1 5 10 15

Lys Glu Glu Val Val Lys
20

<210> SEQ ID NO 19
<211> LENGTH: 13
<212> TYPE: PRT
<213> ORGANISM: Staphylococcus aureus

<400> SEQUENCE: 19

Glu Glu Val Val Lys Glu Glu Ala Lys Pro Gln Val Lys
1 5 10

<210> SEQ ID NO 20
<211> LENGTH: 18
<212> TYPE: PRT
<213> ORGANISM: Lactobacillus lactis

<400> SEQUENCE: 20

Lys Ala Thr Gln Asn Gln Val Ala Glu Thr Gln Val Glu Val Ala Gln
1 5 10 15

Pro Arg

-continued

<210> SEQ ID NO 21
<211> LENGTH: 17
<212> TYPE: PRT
<213> ORGANISM: Lactobacillus lactis

<400> SEQUENCE: 21

Ala Thr Gln Asn Gln Val Ala Glu Thr Gln Val Glu Val Ala Gln Pro
1 5 10 15

Arg

<210> SEQ ID NO 22
<211> LENGTH: 16
<212> TYPE: PRT
<213> ORGANISM: Lactobacillus lactis

<400> SEQUENCE: 22

Val Thr Val Glu Ile Gly Ser Ile Glu Gly His Asn Asn Thr Asn Lys
1 5 10 15

<210> SEQ ID NO 23
<211> LENGTH: 28
<212> TYPE: PRT
<213> ORGANISM: Staphylococcus aureus

<400> SEQUENCE: 23

Phe Glu Asn Gly Leu His Gln Gly Asp Tyr Phe Asp Phe Thr Leu Ser
1 5 10 15

Asn Asn Val Asn Thr His Gly Val Ser Thr Ala Arg
20 25

<210> SEQ ID NO 24
<211> LENGTH: 16
<212> TYPE: PRT
<213> ORGANISM: Staphylococcus aureus

<400> SEQUENCE: 24

Asn Gly Ser Val Val Met Ala Thr Gly Glu Val Leu Glu Gly Gly Lys
1 5 10 15

<210> SEQ ID NO 25
<211> LENGTH: 10
<212> TYPE: PRT
<213> ORGANISM: Staphylococcus aureus

<400> SEQUENCE: 25

Tyr Thr Phe Thr Asn Asp Ile Glu Asp Lys
1 5 10

<210> SEQ ID NO 26
<211> LENGTH: 21
<212> TYPE: PRT
<213> ORGANISM: Lactobacillus lactis

<400> SEQUENCE: 26

Thr Val Gln Thr Asn Gly Asn Gln Thr Ile Thr Ser Thr Leu Asn Glu
1 5 10 15

Glu Gln Thr Ser Lys
20

-continued

<210> SEQ ID NO 27
<211> LENGTH: 21
<212> TYPE: PRT
<213> ORGANISM: Staphylococcus aureus

<400> SEQUENCE: 27

Tyr Lys Asp Gly Ile Gly Asn Tyr Tyr Ala Asn Leu Asn Gly Ser Ile
1 5 10 15

Glu Thr Phe Asn Lys
20

<210> SEQ ID NO 28
<211> LENGTH: 19
<212> TYPE: PRT
<213> ORGANISM: Staphylococcus aureus

<400> SEQUENCE: 28

Asp Gly Ile Gly Asn Tyr Tyr Ala Asn Leu Asn Gly Ser Ile Glu Thr
1 5 10 15

Phe Asn Lys

<210> SEQ ID NO 29
<211> LENGTH: 13
<212> TYPE: PRT
<213> ORGANISM: Staphylococcus aureus

<400> SEQUENCE: 29

Phe Ser His Val Ala Phe Ile Lys Pro Asn Asn Gly Lys
1 5 10

<210> SEQ ID NO 30
<211> LENGTH: 12
<212> TYPE: PRT
<213> ORGANISM: Staphylococcus aureus

<400> SEQUENCE: 30

Thr Thr Ser Val Thr Val Thr Gly Thr Leu Met Lys
1 5 10

<210> SEQ ID NO 31
<211> LENGTH: 13
<212> TYPE: PRT
<213> ORGANISM: Lactobacillus lactis

<400> SEQUENCE: 31

Ile Phe Glu Tyr Leu Gly Asn Asn Glu Asp Ile Ala Lys
1 5 10

<210> SEQ ID NO 32
<211> LENGTH: 29
<212> TYPE: PRT
<213> ORGANISM: Staphylococcus aureus

<400> SEQUENCE: 32

Phe Lys Glu Val Thr Ser Asn Met Ser Gly Asn Leu Asn Leu Gln Asn
1 5 10 15

Asn Gly Ser Tyr Ser Leu Asn Ile Glu Asn Leu Asp Lys
20 25

<210> SEQ ID NO 33
<211> LENGTH: 20

-continued

<212> TYPE: PRT
<213> ORGANISM: Staphylococcus aureus

<400> SEQUENCE: 33

Thr Tyr Val Val His Tyr Asp Gly Glu Tyr Leu Asn Gly Thr Asp Glu
1 5 10 15

Val Asp Phe Arg
20

<210> SEQ ID NO 34
<211> LENGTH: 12
<212> TYPE: PRT
<213> ORGANISM: Staphylococcus aureus

<400> SEQUENCE: 34

Thr Gln Met Val Gly His Pro Glu Gln Leu Tyr Lys
1 5 10

<210> SEQ ID NO 35
<211> LENGTH: 14
<212> TYPE: PRT
<213> ORGANISM: Staphylococcus aureus

<400> SEQUENCE: 35

Glu Asp Thr Ile Lys Glu Thr Leu Thr Gly Gln Tyr Asp Lys
1 5 10

<210> SEQ ID NO 36
<211> LENGTH: 34
<212> TYPE: PRT
<213> ORGANISM: Staphylococcus aureus

<400> SEQUENCE: 36

His His Ala Asp Val Val Glu Tyr Glu Glu Asp Thr Asn Pro Gly Gly
1 5 10 15

Gly Gln Val Thr Thr Glu Ser Asn Leu Val Glu Phe Asp Glu Glu Ser
20 25 30

Thr Lys

<210> SEQ ID NO 37
<211> LENGTH: 17
<212> TYPE: PRT
<213> ORGANISM: Lactobacillus lactis

<400> SEQUENCE: 37

Gly Ile Val Thr Gly Ala Val Ser Asp His Thr Thr Val Glu Asp Thr
1 5 10 15

Lys

<210> SEQ ID NO 38
<211> LENGTH: 31
<212> TYPE: PRT
<213> ORGANISM: Lactobacillus lactis

<400> SEQUENCE: 38

Glu Tyr Thr Thr Glu Ser Asn Leu Ile Glu Leu Val Asp Glu Leu Pro
1 5 10 15

Glu Glu His Gly Gln Ala Gln Gly Pro Val Glu Glu Ile Thr Lys
20 25 30

-continued

<210> SEQ ID NO 39
<211> LENGTH: 23
<212> TYPE: PRT
<213> ORGANISM: Lactobacillus lactis

<400> SEQUENCE: 39

Tyr Glu Gln Gly Gly Asn Ile Val Asp Ile Asp Phe Asp Ser Val Pro
1 5 10 15

Gln Ile His Gly Gln Asn Lys
20

1. An immunogenic composition comprising an inactivated recombinant non-pathogenic bacterium, or a part thereof, expressing on its cell surface at least one folded sequence of a *S. aureus* adhesin, or a sequence having 80% or more sequence identity to said folded sequence of a *S. aureus* adhesin, wherein said at least one folded sequence of a *S. aureus* adhesin, or sequence having 80% or more sequence identity to said folded sequence of a *S. aureus* adhesin, is entirely accessible to trypsin digestion.

2. The immunogenic composition of claim **1**, wherein the trypsin digestion of said at least one folded sequence of a *S. aureus* adhesin, or of the sequence having 80% or more sequence identity to said folded sequence of a *S. aureus* adhesin, releases a digestion profile upon trypsin digestion which is different from a digestion profile upon trypsin digestion of said adhesin expressed on the surface of *S. aureus*.

3. The immunogenic composition of claim **1**, wherein the part of the inactivated recombinant non-pathogenic bacterium consists of an isolated and/or purified cell wall.

4. The immunogenic composition of claim **1**, wherein the at least one folded sequence of a *S. aureus* adhesin is selected from the group consisting of fibrinogen-binding protein A (clumping factor A ClfA), fibrinogen-binding protein B (ClfB), fibronectin-binding protein A (FnPBA) and fibronectin-binding protein B (FnBPB), collagen-binding protein (Cna) and protein A (Spa), Serine-aspartate repeat protein C, D and E (SdrC-E), Plasmin-sensitive protein (Pls), Factor affecting methicillin resistance in the presence of Triton X-100 (FmtB), and surface protein A-K (SasA-K), or a combination thereof.

5. The immunogenic composition of claim **4**, wherein the at least one folded sequence of a *S. aureus* adhesin consists of the fibrinogen-binding protein A (clumping factor A (ClfA)) and/or fibronectin-binding protein A (FnPBA).

6. The immunogenic composition of claim **1**, wherein the inactivated recombinant non-pathogenic bacterium is the *L. lactis* subspecies *cremoris* 1363.

7. The immunogenic composition of claim **4**, wherein the sequence of *S. aureus* fibrinogen-binding protein A (clumping factor A (ClfA)) is as set forth in SEQ ID No.1.

8. The immunogenic composition of claim **1**, wherein the *S. aureus* fibrinogen-binding protein A (clumping factor A (ClfA)) releases a digestion profile of 9 peptides upon trypsin digestion.

9. The immunogenic composition of claim **1**, wherein the *S. aureus* fibronectin-binding protein A (FnPBA) releases a digestion profile of 23 peptides upon trypsin digestion.

10. The immunogenic composition of claim **1**, wherein the inactivated recombinant non-pathogenic bacterium is UV inactivated.

11. A vaccine comprising an immunogenic composition of claim **1** in an immunologically acceptable carrier and/or diluent.

12. The vaccine of claim **11**, wherein the immunologically acceptable carrier is selected from the group consisting of polysaccharide materials forming hydrogels and vesicular carriers.

13. The vaccine of claim **12**, wherein the vesicular carriers are selected from the group consisting of bacterial ghosts, liposomes, niosomes, transfersomes, and ethosomes.

14. The vaccine of claim **11**, further comprising an adjuvant.

15. (canceled)

16. The method of claim **19**, wherein the infection or disease caused by *S. aureus* is selected from the group consisting of IE, intravascular and intravascular device infections, bloodstream infections, deep-seated abscesses, osteomyelitis, infection of prosthetic materials, and skin and soft tissue infections.

17. An isolated and/or purified antibody, antibody fragment or derivative thereof able to bind to the at least one folded sequence of a *S. aureus* adhesin, or to a sequence having 80% or more sequence identity to said folded sequence of a *S. aureus* adhesin, expressed on the cell surface of an inactivated recombinant non-pathogenic bacterium.

18. An expression vector comprising an isolated and/or purified nucleic acid sequence encoding for at least one folded sequence of a *S. aureus* adhesin, or a sequence having 80% or more sequence identity to said folded sequence of a *S. aureus* adhesin.

19. A method for treating and/or preventing an infection or disease caused by *S. aureus*, in a subject in need thereof, comprising administering a pharmaceutically effective amount of an immunogenic composition of claim **1**.

20. A method for inducing active immunity against an infection or disease caused by *S. aureus* in a subject in need thereof, comprising administering to said subject in need thereof i) an immunogenic composition of claim **1** or ii) a vaccine of claim **11**.

21. A method for inducing passive immunity against an infection or disease caused by *S. aureus* in a subject in need thereof, comprising administering to said subject in need thereof an isolated and/or purified antibody, antibody fragment or derivative thereof of claim **17**.

22. The method of claim **20**, wherein the infection or disease caused by *S. aureus* is selected from the group consisting

of IE, intravascular and intravascular device infections, bloodstream infections, deep-seated abscesses, osteomyelitis, infection of prosthetic materials, and skin and soft tissue infections.

23. The method of claim **21**, wherein the infection or disease caused by *S. aureus* is selected from the group consisting of IE, intravascular and intravascular device infections, bloodstream infections, deep-seated abscesses, osteomyelitis, infection of prosthetic materials, and skin and soft tissue infections.

* * * * *