(54) Title: BACTERIAL VACCINE COMPONENTS FROM STAPHYLOCOCCUS AUREUS AND USES THEREOF

(57) Abrégé/Abstract:
Agents, compositions, methods and kits useful for the treatment and diagnosis of Staphylococcal intramammary infection are disclosed. The agents, compositions, methods and kits are derived from genes expressed during Staphylococcal intramammary
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(57) Abrégé(suite)/Abstract(continued):
infection, and more particularly genes SACOL0029, SACOL0264, SACOL0442, SACOL0718, SACOL0720, SACOL1353, SACOL1416, SACOL1611, SACOL1944, SACOL2144, SACOL2365 or SACOL2599, based on the gene nomenclature from the Staphylococcus aureus COL (SACOL) genome.
BACTERIAL VACCINE COMPONENTS FROM STAPHYLOCOCCUS AUREUS AND USES THEREOF

Abstract: Agents, compositions, methods and kits useful for the treatment and diagnosis of Staphylococcal intramammary infection are disclosed. The agents, compositions, methods and kits are derived from genes expressed during Staphylococcal intramammary infection, and more particularly genes SACOL0029, SACOL0264, SACOL0442, SACOL0718, SACOL0720, SACOL1353, SACOL1416, SACOL161, SACOL1944, SACOL2144, SACOL2365 or SACOL2599, based on the gene nomenclature from the Staphylococcus aureus COL (SACOL) genome.


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TITLE OF THE INVENTION

BACTERIAL VACCINE COMPONENTS FROM STAPHYLOCOCCUS AUREUS AND USES THEREOF

CROSS REFERENCE TO RELATED APPLICATIONS

[0001] This application is a PCT application no PCT/CA2011/* filed on March 17, 2011 and published in English under PCT Article 21(2), which itself claims benefit of U.S. provisional application serial No. 61/314,670, filed on March 17, 2010. All documents above are incorporated herein in their entirety by reference.

STATEMENT REGARDING FEDERALLY SPONSORED RESEARCH OR DEVELOPMENT

[0002] N.A.

FIELD OF THE INVENTION

[0003] The present invention relates to novel vaccine targets and components. More specifically, the present invention is concerned with novel antigens which represent vaccine components, processes of manufacturing same, methods using same, and methods of preventing and treating microbial infections involving the administration of same.

REFERENCE TO SEQUENCE LISTING

[0004] Pursuant to 37 C.F.R. 1.821(c), a sequence listing is submitted herewith as an ASCII compliant text file named Sequence listing_ST25, created on March 17, 2011 and having a size of 153 kilobytes. The content of the aforementioned file is hereby incorporated by reference in its entirety.

BACKGROUND OF THE INVENTION

[0005] Bovine mastitis is the most frequent and costly disease for dairy producers and Staphylococcus aureus is considered to be the transmittable bacterium that is the most often responsible for the development of the disease (Sears et al., 2003). Staphylococcal intramammary infections (IMI), which may lead to mastitis, are difficult to treat and frequent relapses are common (Sandholm et al., 1990). Bacterial susceptibility to antibiotics in vitro is a poor predictor of therapeutic efficacy in chronically infected cows (Owens et al., 1997). Although infections that follow treatment of mastitis can be due to newly acquired strains, they are often the result of the persistence of the original infective organism (Sandholm et al., 1990; Myllys et al., 1997). Existing therapies thus often fail to eliminate the infection and it would be highly desirable to find novel approaches to prevent or treat staphylococcal IMI.

[0006] A lack of vaccine efficacy and protective ability has been noted for commercially available S. aureus vaccines (Middleton, 2008). A number of additional Staphylococci vaccines and vaccine components have been
described and proposed. The use of milk or low-iron media as surrogate systems for exploring S. aureus genes that are expressed during IMI do not fully replicate the actual mammalian host environment that may vary in nutrient composition, in interactions with host cells and in immune response components, to name just a few differences. Hence, the S. aureus components currently proposed as vaccine are not necessarily the components that are expressed during IMI at multiple points in time, by multiple strains (including chronic strains) and in multiple hosts. Thus it would be highly desirable to identify S. aureus genes that are expressed during IMI at multiple points in time, by multiple strains, and in multiple hosts, so that a selection of genes and gene-encoded products (e.g., proteins) can be used either alone or in combination for protection against IMI and mastitis.

[0007] The present invention seeks to meet these and other needs.

[0008] The present description refers to a number of documents, the content of which is herein incorporated by reference in their entirety.

SUMMARY OF THE INVENTION

[0009] In an aspect, the present invention provides a method for preventing and/or treating Staphylococcal intramammary infection (IMI) in a mammal, said method comprising administering to said mammal an effective amount of at least one agent, wherein said agent is: (a) a polypeptide encoded by a gene, wherein said gene is SACOL0029, SACOL0264, SACOL0442, SACOL0718, SACOL0720, SACOL1353, SACOL1416, SACOL1611, SACOL1944, SACOL2144, SACOL2365 or SACOL2599, based on the gene nomenclature from the Staphylococcus aureus COL (SACOL) genome set forth in NCBI Reference Sequence NC_002951.2; (b) a polypeptide encoded by a gene from a same operon as one of the genes of (a); (c) an immunogenic fragment of (a) or (b); (d) an immunogenic variant of any one of (a) to (c); (e) a nucleic acid encoding the polypeptide of any one of (a) to (d); or (f) any combination of (a) to (e).

[0010] In another aspect, the present invention provides a use of an agent, wherein said agent is: (a) a polypeptide encoded by a gene, wherein said gene is SACOL0029, SACOL0264, SACOL0442, SACOL0718, SACOL0720, SACOL1353, SACOL1416, SACOL1611, SACOL1944, SACOL2144, SACOL2365 or SACOL2599 based on the gene nomenclature from the Staphylococcus aureus COL (SACOL) genome set forth in NCBI Reference Sequence NC_002951.2; (b) a polypeptide encoded by a gene from a same operon as one of the genes of (a); (c) an immunogenic fragment of (a) or (b); (d) an immunogenic variant of any one of (a) to (c); (e) a nucleic acid encoding the polypeptide of any one of (a) to (d); or (f) any combination of (a) to (e), for preventing and/or treating Staphylococcal intramammary infection (IMI) in a mammal.

[0011] In another aspect, the present invention provides a use of an agent, wherein said agent is: (a) a polypeptide encoded by a gene, wherein said gene is SACOL0029, SACOL0264, SACOL0442, SACOL0718,
SACOL0720, SACOL1535, SACOL1416, SACOL1611, SACOL1944, SACOL2144, SACOL2365 or SACOL2599 based on the gene nomenclature from the Staphylococcus aureus COL (SACOL) genome set forth in NCBI Reference Sequence NC_002951.2; (b) a polypeptide encoded by a gene from a same operon as one of the genes of (a); (c) an immunogenic fragment of (a) or (b); (d) an immunogenic variant of any one of (a) to (c); (e) a nucleic acid encoding the polypeptide of any one of (a) to (d); or (f) any combination of (a) to (e), for the preparation of a medicament for preventing and/or treating Staphylococcal intramammary infection (IMI) in a mammal.

[0012] In another aspect, the present invention provides a pharmaceutical composition for preventing and/or treating Staphylococcal intramammary infection (IMI) in a mammal, said composition comprising: (a) at least one agent, wherein said agent is (i) a polypeptide encoded by a gene, wherein said gene is SACOL0029, SACOL0264, SACOL0442, SACOL0718, SACOL0720, SACOL1353, SACOL1416, SACOL1611, SACOL1944, SACOL2144, SACOL2365 or SACOL2599 based on the gene nomenclature from the Staphylococcus aureus COL (SACOL) genome set forth in NCBI Reference Sequence NC_002951.2; (ii) a polypeptide encoded by a gene from a same operon as one of the genes of (i); (iii) an immunogenic fragment of (i) or (ii); (iv) an immunogenic variant of any one of (i) to (iii); (v) a nucleic acid encoding the polypeptide of any one of (i) to (iv); or (vi) any combination of (i) to (v). It may optionally comprise (b) a pharmaceutically acceptable excipient.

[0013] In another aspect, the present invention provides a pharmaceutical composition comprising: (a) at least one agent, wherein said agent is: (i) a polypeptide encoded by a gene, wherein said gene is SACOL0029, SACOL0264, SACOL0442, SACOL0718, SACOL0720, SACOL1353, SACOL1416, SACOL1611, SACOL1944, SACOL2144, SACOL2365 or SACOL2599, based on the gene nomenclature from the Staphylococcus aureus COL (SACOL) genome set forth in NCBI Reference Sequence NC_002951.2; (ii) a polypeptide encoded by a gene from a same operon as one of the genes of (a); (iii) an immunogenic fragment of (i) or (ii); (iv) an immunogenic variant of any one of (i) to (iii); (v) a nucleic acid encoding the polypeptide of any one of (i) to (iv); or (vi) any combination of (i) to (v); and (b) a pharmaceutically acceptable excipient.

[0014] In another aspect, the present invention provides a kit for the prevention and/or treatment of Staphylococcal IMI, comprising (a) at least one agent, wherein said agent is: (i) a polypeptide encoded by a gene, wherein said gene is SACOL0029, SACOL0264, SACOL0442, SACOL0718, SACOL0720, SACOL1353, SACOL1416, SACOL1611, SACOL1944, SACOL2144, SACOL2365 or SACOL2599, based on the gene nomenclature from the Staphylococcus aureus COL (SACOL) genome set forth in NCBI Reference Sequence NC_002951.2; (ii) a polypeptide encoded by a gene from a same operon as one of the genes of (a); (iii) an immunogenic fragment of (i) or (ii); (iv) an immunogenic variant of any one of (i) to (iii); (v) a nucleic acid encoding the polypeptide of any one of (i) to (iv); or (vi) any combination of (i) to (v); and (b) instructions to use the kit for the prevention and/or treatment of Staphylococcal IMI.

[0015] In another aspect, the present invention provides a method of diagnosing Staphylococcal IMI in a
mammal, said method comprising: determining a level of expression of at least one gene, wherein said gene is SACOL0029, SACOL0264, SACOL0442, SACOL0718, SACOL0720, SACOL1353, SACOL1416, SACOL1611, SACOL1944, SACOL2144, SACOL2365 or SACOL2599, or the level of activity of a polypeptide encoded by said one or more genes, in a biological sample from said mammal; and comparing said level of expression or activity to a reference level of expression or activity; wherein a higher expression or activity in said biological sample relative to said reference expression or activity is indicative that said mammal has staphylococcal IMI.

[0016] In another aspect, the present invention provides a kit for the diagnosis of Staphylococcal IMI, comprising (a) at least one ligand, wherein said at least one ligand binds to: (i) a polypeptide encoded by a gene, wherein said gene is SACOL0029, SACOL0264, SACOL0442, SACOL0718, SACOL0720, SACOL1353, SACOL1416, SACOL1611, SACOL1944, SACOL2144, SACOL2365 or SACOL2599, based on the gene nomenclature from the Staphylococcus aureus COL (SACOL) genome set forth in NCBI Reference Sequence NC_002951.2; (ii) a polypeptide encoded by a gene from a same operon as one of the genes of (a); (iii) an immunogenic fragment of (i) or (ii); (iv) an immunogenic variant of any one of (i) to (iii); (v) a nucleic acid encoding the polypeptide of any one of (i) to (iv); or (vi) any combination of (i) to (v); and (b) instructions to use the kit for the diagnosis of Staphylococcal IMI.

[0017] In another aspect, the present invention provides a method for preventing and/or treating Staphylococcal intramammary infection (IMI) in a mammal, said method comprising administrating to said mammal an effective amount of at least one agent, wherein said agent is a live attenuated form of Staphylococcus aureus comprising a mutation in a gene, wherein said gene is SACOL0029, SACOL0264, SACOL0442, SACOL0718, SACOL0720, SACOL1353, SACOL1416, SACOL1611, SACOL1944, SACOL2144, SACOL2365 or SACOL2599, based on the gene nomenclature from the Staphylococcus aureus COL (SACOL) genome set forth in NCBI Reference Sequence NC_002951.2, and wherein the mutation is a deletion or an insertion.

[0018] In another aspect, the present invention provides a use of an agent, wherein said agent is a live attenuated form of Staphylococcus aureus comprising a mutation in a gene, wherein said gene is SACOL0029, SACOL0264, SACOL0442, SACOL0718, SACOL0720, SACOL1353, SACOL1416, SACOL1611, SACOL1944, SACOL2144, SACOL2365 or SACOL2599, based on the gene nomenclature from the Staphylococcus aureus COL (SACOL) genome set forth in NCBI Reference Sequence NC_002951.2, and wherein the mutation is a deletion, an insertion or a substitution of one or more nucleotides, for preventing and/or treating Staphylococcal intramammary infection (IMI) in a mammal or for the preparation of a medicament for preventing and/or treating Staphylococcal intramammary infection (IMI) in a mammal.

[0019] In another aspect, the present invention provides a pharmaceutical composition for preventing and/or treating Staphylococcal intramammary infection (IMI) in a mammal, said composition comprising an agent, wherein said agent is a live attenuated form of Staphylococcus aureus comprising a mutation in a gene, wherein
said gene is SACOL029, SACOL0264, SACOL0442, SACOL0718, SACOL0720, SACOL1353, SACOL1416, SACOL1611, SACOL1944, SACOL2144, SACOL2365 or SACOL2599, based on the gene nomenclature from the *Staphylococcus aureus* COL (SACOL) genome set forth in NCBI Reference Sequence NC_002951.2, and wherein the mutation is a deletion, an insertion or a substitution of one or more nucleotides.

[0020] In another aspect, the present invention provides a kit for the prevention and/or treatment of *Staphylococcal* IMI, comprising at least one agent, wherein said agent is a live attenuated form of *Staphylococcus aureus* comprising a mutation in a gene, wherein said gene is SACOL0029, SACOL0264, SACOL0442, SACOL0718, SACOL0720, SACOL1353, SACOL1416, SACOL1611, SACOL1944, SACOL2144, SACOL2365 or SACOL2599, based on the gene nomenclature from the *Staphylococcus aureus* COL (SACOL) genome set forth in NCBI Reference Sequence NC_002951.2, and wherein the mutation is a deletion, an insertion or a substitution of one or more nucleotides.

[0021] In an embodiment, the above-mentioned gene from the same operon as one of the genes of (a) is SACOL0720, and wherein said one or more genes of (a) is SACOL0718.

[0022] In an embodiment, the above-mentioned one or more genes is SACOL0442, SACOL0718, SACOL0720 or any combination thereof. In a further embodiment, the above-mentioned one or more genes is SACOL0442, SACOL0720 or both.

[0023] In another embodiment, the above-mentioned methods, uses, pharmaceutical compositions or kits comprise a combination of agents. In a further embodiment, the above-mentioned combination of agents comprises: (i) a first agent, wherein said first agent is (a) a polypeptide encoded by SACOL0442, (b) an immunogenic fragment of (a); (c) an immunogenic variant of (a) or (b); (d) a nucleic acid encoding the polypeptide of any one of (a) to (c); or (e) any combination of (a) to (d); and (ii) a second agent, wherein said second agent is (a) a polypeptide encoded by SACOL0720, (b) an immunogenic fragment of (a); (c) an immunogenic variant of (a) or (b); (d) a nucleic acid encoding the polypeptide of any one of (a) to (c); or (e) any combination of (a) to (d).

[0024] In an embodiment, the above-mentioned gene is SACOL0442 and the immunogenic fragment comprises one or more of the following amino acid sequences: TFGSIYPKADASTQN (SEQ ID NO: 17), KDTINGKSNKSRNW (SEQ ID NO: 18) or KDGGKYTLESHKELQ (SEQ ID NO: 19).

[0025] In another embodiment, the above-mentioned gene is SACOL0720 and the immunogenic fragment comprises one or more of the following amino acid sequences: QFGFDLKHKKDALA (SEQ ID NO: 20), TIKQQKQANSALS (SEQ ID NO: 21), KDINKYFMTDVDL (SEQ ID NO: 22) or DVDLGGPTFVLD (SEQ ID NO: 23).

[0026] In an embodiment, the above-mentioned *Staphylococcal* intramammary infection is caused by one or
more Staphylococcus aureus strains.

[0027] In an embodiment, the above-mentioned methods, uses, pharmaceutical compositions or kits further comprise an adjuvant. In a further embodiment, the above-mentioned adjuvant is alum, Emulsigen™ D, cyclic-diguanosine-5′-monophosphate (c-di-GMP), polyphosphasine or pathogen-associated molecular patterns (PAMPS). In yet a further embodiment, the above-mentioned PAMPS is unmethylated dinucleotides (CpG) or microbial polysaccharides.

[0028] In an embodiment, the above-mentioned (i) agent, (ii) adjuvant, or both (i) and (ii) are comprised in a pharmaceutical composition.

[0029] In an embodiment, the above-mentioned pharmaceutical composition further comprises one or more pharmaceutically acceptable excipients.

[0030] In an embodiment, the above-mentioned mammal is a cow.

[0031] In an embodiment, the above-mentioned IMI is associated with bovine mastitis.

[0032] In an embodiment, the above-mentioned reference expression or activity is a level of expression or activity determined in a corresponding biological sample from a mammal known to not having staphylococcal IMI. In another embodiment, the above-mentioned level of expression is determined by measuring the level of expression of a mRNA transcribed from said one or more genes. In another embodiment, said level of expression is determined by measuring the level of expression of a polypeptide encoded by said one or more genes.

[0033] In an embodiment, the above-mentioned biological sample is milk.

[0034] In an embodiment, the above-mentioned kit comprises a combination of ligands.

[0035] In an embodiment, the above-mentioned combination of ligands comprises ligands which bind to: (i) a first agent, wherein said first agent is (a) a polypeptide encoded by SACOL0442, (b) an immunogenic fragment of (a); (c) an immunogenic variant of (a) or (b); (d) a nucleic acid encoding the polypeptide of any one of (a) to (c); or (e) any combination of (a) to (d); and (ii) a second agent, wherein said second agent is (a) a polypeptide encoded by SACOL0720, (b) an immunogenic fragment of (a); (c) an immunogenic variant of (a) or (b); (d) a nucleic acid encoding the polypeptide of any one of (a) to (c); or (e) any combination of (a) to (d).

**BRIEF DESCRIPTION OF THE DRAWINGS**

[0036] In the appended drawings:

[0037] FIG. 1 shows the types of Staphylococcus aureus strains isolated from cows: chronic and systematically
isolated strains from cows with clinical symptoms. Chronic isolates were isolated from cows shedding a genetically identical *S. aureus* strain >55 days apart, between dry off and calving as illustrated (1st and 2nd samples). Systematically isolated strains were taken at calving or in the lactation period from cows shedding high somatic cell counts (SCC) in milk or having signs of inflammation (mastitis). Somatic cells are leukocytes (white blood cells). The SCC is an indicator of the quality of milk. The number of somatic cells increases in response to pathogenic bacteria like *S. aureus*.

[0038] FIG. 2 shows the genetic relatedness of the *S. aureus* isolates used in the studies described herein as determined by comparative genomic DNA hybridization data obtained for 530 genes printed on DNA arrays. Underlined isolates are chronic strains. Unrelated reference strains and isolates randomly/systematically picked from bovine mastitis cases with clinical symptoms during lactation are shown for comparison.

[0039] FIG. 3 shows Q-PCR analyses reporting the relative level of gene expression for indicators of virulence: *hid* (Agr-dependent exotoxin production), icaC (ica-dependent biofilm production) and overall biofilm production (measured by a spectrophotometric method with crystal violet) in *S. aureus* isolates grown in a cultivation medium in vitro. Chronic isolates #3 (black inverted triangle), #557 (black circle) and #1290 (black diamond) are compared to a collection of systematically isolated strains from bovine mastitis with clinical signs (black squares, where isolate SHY97-3906, a previously described strain isolated from a typical mastitis case with clinical signs, is represented as the open square). Q-PCR results are presented as fold-expression compared to the reference strain Newbould (ATCC 29740) and biofilm production is reported as a percentage of that produced by strain SHY97-3906. All Q-PCR results are normalized using the level of expression of *gyr*.

[0040] FIG. 4 shows the description of the method used for isolating bacteria from mastitis milk samples. (A) Milk before first centrifugation with (Prot (+)) or without (Prot (-)) casein protease. (B) After first centrifugation. (C) After “RNA Protect” treatment and centrifugation (last step). A large bacterial pellet is recovered from milk treated with casein protease.

[0041] FIG. 5 shows experimental infection profiles caused by chronic strains #3, #557 and #1290 and by a strain isolated from a typical mastitis case SHY97-3906 in cows reported as a function of bacterial (CFU) (left Y axis) or somatic cell counts (SCC) (right Y axis) over the infection period. FIGs. 5A, 5B and 5C represent cows #313, cow #307 and cow #5325, respectively. The four different *S. aureus* strains used in this study are represented as black bars and dots (SHY97-3906), white bars and dots (chronic isolate #3), grey bars and dots (chronic isolate #557) and shaded bars and star-shaped dots (chronic isolate #1290). Cow #5325 was euthanized at day 15.

[0042] FIG. 6 shows a Venn diagram of the genes differentially expressed in the chronic strains taken all together (isolates #3, #557 and #1290) versus SHY97-3906 isolated from a typical mastitis case with clinical signs.
[0043] FIG. 7 shows a Venn diagram of the 43 genes found to be strongly expressed in microarray experiments using bacterial samples from cow #307 at day 8 (A) and day 10 (B) of infection, and in cow #5325 at day 10 of infection (C). The number of bacterial samples in which the genes were shown to be expressed is indicated in parenthesis and the gene names in bold characters were selected for Q-PCR analyses (see FIG. 8).

[0044] FIG. 8 shows quantitative PCR analyses of genes found to be strongly expressed by *S. aureus* collected from cow’s IMI (cow). Gene expression was compared to that measured in *S. aureus* cultivated *in vitro* in Mueller-Hinton broth supplemented with iron (broth + iron), in iron-restricted broth (broth – iron), and in freshly collected non-mastitis milk *in vitro* (milk *in vitro*). All results are normalized using the level of expression of *gyrB* and are presented as Log10 values of the relative expression ratios. The horizontal bar represents the median ratio value of all samples. Significant differences between the median and a ratio of zero (representing no change in the expression of the gene) are shown (* = P<0.05; ** = P<0.01; *** = P<0.005; unpaired t-test). RNA samples from *S. aureus* grown in two different animals were analyzed: cow #5325 at day 10 of infection with isolates SHY97-3906 (○), #3 (△), #557 (◇), and #1290 (□), cow #307 at day 8 of infection (same symbol shapes, black symbols) and cow #307 at day 14 of infection (same symbol shapes, grey symbols). For the sample collected from strain #1290 in cow #5325 at day 10 of infection (○), the error bars are shown. Relative gene expression is shown for a capsular biosynthesis gene (*capM*), a gene of unknown function (SACOL2171), a transcriptional regulator of unknown function (SACOL2325), an ABC transporter of unknown function (SACOL0718) and a chromosomally encoded gene not previously characterized (SACOL0442).

[0045] FIG. 9 shows the organization of the SACOL0718-720 predicted operon in *S. aureus* strains COL, N315, RF122, USA300 and MSSA476. The two genes overlap by 10 nucleotides. The arrow indicates the direction of transcription. The genome position of the predicted -10 and -35 boxes of the promoter region is also indicated.

[0046] FIG 10 shows the growth kinetics of the *S. aureus* strain ATCC 29213 and the isogenic three mutants ATCC 29213ΔSACOL0442a (∆442a), ATCC 29213ΔSACOL0442b (∆442b) and ATCC29213 ΔSACOL0720 (∆720). Mutants for genes SACOL0442 and SACOL0720 were produced by gene replacement (∆442a) or by intron insertion (∆442b and ∆720). Prior to experimental bovine IMI, the relative growth of the parental strain and the mutants was evaluated *in vitro* in freshly collected milk (FIG. 10A). In FIG 10A, the mean CFU/ml (log10) for the 4 strains is represented over time following a small or large inoculum (left and right panels, respectively). FIG. 10B shows the mean bacterial counts recovered from the milk of eight (8) experimentally infected multiparous Holstein cows in mid lactation as a function of time. Each of the 8 cows was infused intra-mammary with the four *S. aureus* strains (ATCC 29213 and mutants ∆442a, ∆442b, and ∆720) and the position of each strain in each of the four mammary gland quarters alternated between the animals. The infections were carried out for 21 days. Milk of the infected quarters was collected and the determination of viable bacterial counts was performed. Solid circles and open line represent growth of the parent strain and the open symbols and solid lines the growth of the
three mutants as indicated on the graph.

[0047] FIGs. 11A-11D show a nucleic acid (FIGs. 11A-C) and amino acid (FIG. 11D) sequence alignment of SACOL0442 from various Staphylococcus aureus strains (nucleic acid sequences: MW0345 (MW2) (SEQ ID NO: 24); SAS0347 (MSSA476) (SEQ ID NO: 25); SACOL0442 (Col) (SEQ ID NO: 26); SAOUHSC_00354 (ncto8325) (SEQ ID NO: 27); NWMN_0362 (NEWMAN) (SEQ ID NO: 28); SAUSA300_0370 (USA300-FPR3757) (SEQ ID NO: 29); SaurJH1_0429 (JH1) (SEQ ID NO: 30); SAHV_0367 (Mu3) (SEQ ID NO: 31); SaurJH9_0419 (JH9) (SEQ ID NO: 32); SAV0370 (Mu50) (SEQ ID NO: 33); SA0357 (N315) (SEQ ID NO: 34); SAB0321 (RF122) (SEQ ID NO: 35); and consensus (SEQ ID NO: 36); amino acid sequences: SACOL0442 (Col) (SEQ ID NO: 37); SAOUHSC_00354 (ncto8325) (SEQ ID NO: 38); NWMN_0362 (NEWMAN) (SEQ ID NO: 39); SAUSA300_0370 (USA300-FPR3757) (SEQ ID NO: 40); SaurJH1_0429 (JH1) (SEQ ID NO: 41); SAHV_0367 (Mu3) (SEQ ID NO: 42); SaurJH9_0419 (JH9) (SEQ ID NO: 43); SAV0370 (Mu50) (SEQ ID NO: 44); SA0357 (N315) (SEQ ID NO: 45); SAB0347 (MSSA476) (SEQ ID NO: 46); SAB0321 (RF122) (SEQ ID NO: 47); and consensus (SEQ ID NO: 48). Alignments are based on the sequences available from multiple Staphylococcus aureus strains, including the bovine mastitis isolate RF122. The amino acid sequence of MW0345 (MW2) (SEQ ID NO: 75) is not included in the alignment. The characteristics of the compared strains are provided in FIG. 13. Under each alignment, an asterisk (*) indicates a 100% match of nucleotide or amino acid between all strains compared; a double-dot (::) indicates that conserved substitutions have been observed and a single dot (·) means that semi-conserved substitutions are observed.

[0048] FIGs. 12A-12K show a nucleic acid (FIGs. 12A-H) and amino acid (FIG. 12I-K) sequence alignment of SACOL0720 from various Staphylococcus aureus strains. (nucleic acid sequences: SaurJH1_0700 (JH1) (SEQ ID NO: 49); SaurJH9_0685 (JH9) (SEQ ID NO: 50); SAHV_0669 (Mu3) (SEQ ID NO: 51); SAV0662 (Mu50) (SEQ ID NO: 52); SAV0617 (N315) (SEQ ID NO: 53); MW0624 (MW2) (SEQ ID NO: 54); SAV0627 (MSSA476) (SEQ ID NO: 55); SACOL0720 (SEQ ID NO: 56); SAUSA300_0648 (USA300-FPR3757) (SEQ ID NO: 57); SAOUHSC_00668 (NCTC8325) (SEQ ID NO: 58); NWMN_0631 (Newman) (SEQ ID NO: 59); SAB0611 (RF122) (SEQ ID NO: 60); consensus (SEQ ID NO: 61); amino acid sequence: SACOL0720 (SEQ ID NO: 62); SAUSA300_0648 (USA300-FPR3757) (SEQ ID NO: 63); SAOUHSC_00668 (NCTC8325) (SEQ ID NO: 64); NWMN_0631 (Newman) (SEQ ID NO: 65); SaurJH1_0700 (JH1) (SEQ ID NO: 66); SaurJH9_0685 (JH9) (SEQ ID NO: 67); SAHV_0669 (Mu3) (SEQ ID NO: 68); SAV0662 (Mu50) (SEQ ID NO: 69); SAV0617 (N315) (SEQ ID NO: 70); MW0624 (MW2) (SEQ ID NO: 71); SAV0627 (MSSA476) (SEQ ID NO: 72); SAB0611 (RF122) (SEQ ID NO: 73); consensus (SEQ ID NO: 74). Alignments are based on the sequences available from multiple Staphylococcus aureus strains, including the bovine mastitis isolate RF122. The characteristics of the compared strains are provided in FIG. 13. Under each alignment, an asterisk (*) indicates a 100% match of nucleotide or amino acid between all strains compared; a double-dot (::) indicates that conserved substitutions have been observed and a single dot (·) means that semi-conserved substitutions are observed.
[0049] FIG. 13 shows the characteristics of the Staphylococcus aureus strains whose sequences are aligned at FIGS. 11A-D and 12A-K.

[0050] FIG. 14A-14C show the predicted cellular localization of proteins SACOL0442 (FIG. 14A), SACOL0718 (FIG. 14B) and SACOL0720 (FIG. 14C), and FIG. 14D shows the predicted transmembrane helices of protein SACOL0720 (SEQ ID NO: 62). In FIG. 14D, “I” and “i” represent intracellular amino acids, “H” and “h” represent amino acids part of helix and are localized in the membrane, “O” and “o” represent amino acids that are extracellular, i.e., localized outside the cytoplasmic membrane (capital letters indicate a stronger prediction relative to lower case letters). The highlighted and boxed sequence is the longest extracellular sequence of the protein that was used as a vaccine component in Example 7. Cellular localization was determined using the web site: http://psort.org/index.html (Gardy J. L. et al., Bioinformatics 2005 21(5):617-623; doi:10.1093/bioinformatics/bti057) and amino acid composition at the web site: http://www.expasy.ch/tools/protparam.html (Gasteiger E. et al., The Proteomics Protocols Handbook, Humana Press (2005), pp. 571-607). The localization of the transmembrane helix was provided by the server ExPASy™ proteomic server: http://www.enzim.hu/hmmtop/index.html (G.E Tusnády and I. Simon (2001), Bioinformatics 17, 849-850).

[0051] FIG. 15 shows total IgG titers in sera of mice vaccinated with SACOL0442 and SACOL0720. Antibody titers were determined by ELISA. One group of animals (10 mice per group) received two injections of saline, one group received 2 injections of 100 µg of polypeptide SACOL0442, one group received 2 injections of 100 µg of polypeptide SACOL0720, one group received 2 injections of 100 µg of polypeptide SACOL1781, and one group received 2 injections of 100 µg of each of all three polypeptides premixed together (SACOL0442, SACOL0720, and SACOL1781). The 2 injections were performed 3 weeks apart. Three weeks after the second immunization, mice were euthanized and blood collected for the determination of total IgG titers by ELISA. Each dot represents the serum titer of one mouse. Horizontal bars are the means for each group (dotted grey lines, antigens injected individually; solid black lines, antigens injected in combination).

[0052] FIG. 16 shows total IgG antibody titers in sera of dairy cows vaccinated with SACOL0442 and SACOL0720. One group of animals (5 cows per group) received two injections of saline, one group received 2 injections of 300 µg of polypeptide SACOL0442, one group received 2 injections of 300 µg of polypeptide SACOL0720 and one group received 2 injections of 300 µg of each of the two polypeptides premixed together (SACOL0442, SACOL0720). The 2 injections were performed 10 weeks apart. Blood was collected every two weeks for the determination of the total IgG antibody titers by ELISA. Data represent the mean of each group. Solid lines and solid symbols represent total IgG antibody titer against SACOL0720 and open lines and open symbols present total IgG antibody titers against SACOL0442. Circles (solid for SACOL0720 and open for SACOL0442) represent the antibody titers for the group that received saline. Triangles (solid for SACOL0720 and open for SACOL0442) represent the antibody titers for the two groups that received each one of the two
polypeptides. Squares (solid for SACOL0720 and open for SACOL0442) represent the antibody titers for the group that received both polypeptides.

[0053] FIG. 17 shows IgG1 and IgG2 antibody titers at week 16, in sera of dairy cows vaccinated with SACOL0442 and SACOL0720 (total IgG titers from the same samples at week 16 were shown in FIG. 16). As described in FIG. 16, one group of animals (5 cows per group) received two injections of saline, one group received 2 injections of 300 μg of polypeptide SACOL0442, one group received 2 injections of 300 μg of polypeptide SACOL0720 and one group received 2 injections of 300 μg of each of the two polypeptides premixed together (SACOL0442, SACOL0720). The 2 injections were performed 10 weeks apart. Blood was collected at week 16 for the determination of IgG1 and IgG2 antibody titers by ELISA. FIG. 17A and B show IgG1 and IgG2 isotypes titers, respectively against polypeptide SACOL0442, FIG. 17C and D show IgG2 and IgG1 isotypes titers, respectively against polypeptide SACOL0720. Each dot on the graphs represents the serum titer of one cow (black squares, week 16; open circles, pre-immune titers before immunization). Horizontal bars are the medians for each group (solid lines, immune sera at week 16; open lines, pre-immune sera before immunizations).

[0054] Figure 18 shows the amino acid (SEQ ID NO: 76) sequence of SACOL01781 from S. aureus MW2 strain.

DESCRIPTION OF ILLUSTRATIVE EMBODIMENTS

[0055] In a first aspect, the present invention provides a method for preventing and/or treating Staphylococcal intramammary infection (IMI) in a mammal, said method comprising administrating to said mammal an effective amount of an agent, wherein said agent is: (a) a polypeptide encoded by a gene, wherein said gene is SACOL0029, SACOL0100, SACOL0101, SACOL0105, SACOL0148, SACOL0154, SACOL0204, SACOL0205, SACOL0264, SACOL0442, SACOL0461, SACOL0608, SACOL0660, SACOL0688, SACOL0690, SACOL0704, SACOL0718, SACOL0720, SACOL0829, SACOL1054, SACOL1142, SACOL1145, SACOL1320, SACOL1353, SACOL1416, SACOL1611, SACOL1637, SACOL1680, SACOL1781, SACOL1812, SACOL1867, SACOL1912, SACOL1944, SACOL2092, SACOL2144, SACOL2169, SACOL2171, SACOL2321, SACOL2325, SACOL2342, SACOL2365, SACOL2379, SACOL2385 or SACOL2599, based on the gene nomenclature from the Staphylococcus aureus COL (SACOL) genome set forth in NCBI Reference Sequence NC_002951.2; (b) a polypeptide encoded by a gene from a same operon as one of the genes of (a); (c) an immunogenic fragment of (a) or (b); (d) an immunogenic variant of any one of (a) to (c); (e) a nucleic acid encoding the polypeptide of any one of (a) to (d); or (f) any combination of (a) to (e).

[0056] In another aspect, the present invention provides a use of an agent, wherein said agent is: (a) a polypeptide encoded by a gene, wherein said gene is SACOL0029, SACOL0100, SACOL0101, SACOL0105, SACOL0148, SACOL0154, SACOL0204, SACOL0205, SACOL0264, SACOL0442, SACOL0461, SACOL0608,
SACOL0660, SACOL0688, SACOL0690, SACOL0704, SACOL0718, SACOL0720, SACOL0829, SACOL1054, SACOL1142, SACOL1145, SACOL1320, SACOL1353, SACOL1416, SACOL1611, SACOL1637, SACOL1680, SACOL1781, SACOL1812, SACOL1867, SACOL1912, SACOL1944, SACOL2092, SACOL2144, SACOL2169, SACOL2171, SACOL2231, SACOL2325, SACOL2342, SACOL2365, SACOL2379, SACOL2385 or SACOL2599, based on the gene nomenclature from the Staphylococcus aureus COL (SACOL) genome set forth in NCBI Reference Sequence NC_002951.2; (b) a polypeptide encoded by a gene from a same operon as one of the genes of (a); (c) an immunogenic fragment of (a) or (b); (d) an immunogenic variant of any of (a) to (c); (e) a nucleic acid encoding the polypeptide of any one of (a) to (d); or (f) any combination of (a) to (e), for preventing and/or treating a Staphylococcal intramammary infection (IMI) in a mammal.

[0057] In another aspect, the present invention provides a use of an agent, wherein said agent is: (a) a polypeptide encoded by a gene, wherein said agent is SACOL0029, SACOL0100, SACOL0101, SACOL0105, SACOL0148, SACOL0154, SACOL0204, SACOL0205, SACOL0264, SACOL0442, SACOL0461, SACOL0608, SACOL0660, SACOL0688, SACOL0690, SACOL0704, SACOL0718, SACOL0720, SACOL0829, SACOL1054, SACOL1142, SACOL1145, SACOL1320, SACOL1353, SACOL1416, SACOL1611, SACOL1637, SACOL1680, SACOL1781, SACOL1812, SACOL1867, SACOL1912, SACOL1944, SACOL2092, SACOL2144, SACOL2169, SACOL2171, SACOL2231, SACOL2325, SACOL2342, SACOL2365, SACOL2379, SACOL2385 or SACOL2599, based on the gene nomenclature from the Staphylococcus aureus COL (SACOL) genome set forth in NCBI Reference Sequence NC_002951.2; (b) a polypeptide encoded by a gene from a same operon as one of the genes of (a); (c) an immunogenic fragment of (a) or (b); (d) an immunogenic variant of any one of (a) to (c); (e) a nucleic acid encoding the polypeptide of any one of (a) to (d); or (f) any combination of (a) to (e), for the preparation of a medicament for preventing and/or treating Staphylococcal intramammary infection (IMI) in a mammal.

[0058] In another aspect, the present invention provides a pharmaceutical composition (e.g., a vaccine) for preventing and/or treating Staphylococcal intramammary infection (IMI) in a mammal, said composition comprising an agent, wherein said agent is: (a) a polypeptide encoded by a gene, wherein said gene is SACOL0029, SACOL0100, SACOL0101, SACOL0105, SACOL0148, SACOL0154, SACOL0204, SACOL0205, SACOL0264, SACOL0442, SACOL0461, SACOL0608, SACOL0690, SACOL0688, SACOL0690, SACOL0704, SACOL0718, SACOL0720, SACOL0829, SACOL1054, SACOL1142, SACOL1145, SACOL1320, SACOL1353, SACOL1416, SACOL1611, SACOL1637, SACOL1680, SACOL1781, SACOL1812, SACOL1867, SACOL1912, SACOL1944, SACOL2092, SACOL2144, SACOL2169, SACOL2171, SACOL2231, SACOL2325, SACOL2342, SACOL2365, SACOL2379, SACOL2385 or SACOL2599, based on the gene nomenclature from the Staphylococcus aureus COL (SACOL) genome set forth in NCBI Reference Sequence NC_002951.2; (b) a polypeptide encoded by a gene from a same operon as one of the genes of (a); (c) an immunogenic fragment of (a) or (b); (d) an immunogenic variant of any one of (a) to (c); (e) a nucleic acid encoding the polypeptide of any one of (a) to (d); or (f) any combination of (a) to (e), and optionally one or more pharmaceutically acceptable
excipients/carriers.

[0059] The Genbank accession numbers for the above-mentioned S. aureus genes and encoded polypeptides are depicted in Table I below:

Table I: Genbank accession numbers for the IMI-associated S. aureus genes and encoded polypeptides described herein.

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[0060] In an embodiment, the above-mentioned gene is SACOL0029, SACOL0264, SACOL0442, SACOL0718, SACOL0720, SACOL1353, SACOL1416, SACOL1611, SACOL1944, SACOL2144, SACOL2365 or SACOL2599.

[0061] As used herein, the term "vaccine" refers to any compound/agent ("vaccine component"), or combinations thereof, capable of inducing/eliciting an immune response in a host and which permits to treat and/or prevent an infection and/or a disease. Therefore, non-limiting examples of such agent include proteins, polypeptides, protein/polypeptide fragments, immunogens, antigens, peptide epitopes, epitopes, mixtures of proteins, peptides or epitopes as well as nucleic acids, genes or portions of genes (encoding a polypeptide or a protein of interest or a fragment thereof) added separately or in a contiguous sequence such as in nucleic acid vaccines, and the like.

[0062] An immunogenic fragment of a protein/polypeptide is defined as a part of a protein/polypeptide which is capable of inducing/eliciting an immune response in a host. In an embodiment, the immunogenic fragment is capable of eliciting the same immune response in kind, albeit not necessarily in amount, as the protein/polypeptide. An immunogenic fragment of a protein/polypeptide preferably comprises one or more epitopes of said protein/polypeptide. An epitope of a protein/polypeptide is defined as a fragment of said protein/polypeptide of at least about 4 or 5 amino acids in length, capable of eliciting a specific antibody and/or an immune cell (e.g., a T cell or B cell) bearing a receptor capable of specifically binding said epitope. Two different kinds of epitopes exist: linear epitopes and conformational epitopes. A linear epitope comprises a stretch of consecutive amino acids. A conformational epitope is typically formed by several stretches of consecutive amino acids that are folded in position and together form an epitope in a properly folded protein. An immunogenic fragment as used herein refers to either one, or both, of said types of epitopes. In an embodiment, the immunogenic fragment of a protein/polypeptide comprises at least 4 or 5 amino acid residues. In a further embodiment, the immunogenic fragment comprises at least 6, 7, 8, 9, 10, 13, 14, 15, 20, 25, 30, 50 or 100 consecutive amino acids of the native protein/polypeptide.

[0063] As will be understood by the person of ordinary skill, agents (proteins/polypeptides, fragments thereof) having non-naturally occurring modifications (e.g., immunogenic variants) and which are capable of inducing an immune response specific for the unmodified agent (e.g., capable of inducing the production of antibodies capable of recognizing the unmodified agent) are also within the scope of the term "vaccine component". For example, the vaccine components of the present invention can be modified to enhance their activity, stability, and/or bioavailability, and/or to reduce their toxicity. Conservative amino acid substitutions may be made, like for example replacement of an amino acid comprising an acidic side chain by another amino acid comprising an
acidic side chain, replacement of a bulky amino acid by another bulky amino acid, replacement of an amino acid comprising a basic side chain by another amino acid comprising a basic side chain, and the like. A person skilled in the art is well able to generate variants of a protein/polypeptide. This is for instance done through screening of a peptide library or by peptide changing programs. An immunogenic variant according to the invention has essentially the same immunogenic properties of said protein in kind, not necessarily in amount. An immunogenic variant of a protein/polypeptide of the invention may for instance comprise a fusion protein and/or chimeric protein. For example, the biological function of protein SACOL0442 identified herein is predicted to be an exotoxin, enterotoxin or superantigen and it could potentially interfere with the mammalian immune system and antibody production, and/or show some toxicity in the host. Although such interference was not observed when the SACOL0442 polypeptide was used in combination with for example SACOL0720 during immunization (FIG. 15), it may be useful to modify the protein or polypeptide used for vaccination so that the biological activity of the exotoxin is decreased. For such a purpose, it is possible to inactivate the exotoxin with chemicals (e.g., formaldehyde). It is also possible to use molecular biology techniques to delete or mutate the putative region(s) involved in exotoxin activity without loosing immunogenicity (Chang et al., 2008). Another example is the conjugation or mixture of amino acid-based components with nucleic acids (e.g., genes or portions of genes added separately or in a contiguous sequence) carbohydrates such as those found in microbial polysaccharide capsules or biofilms.

[0064] In an embodiment, the above-mentioned polypeptide is a polypeptide normally secreted or expressed at the surface of the bacteria (e.g., Staphylococcus aureus).

[0065] In another embodiment, the above-mentioned polypeptide, or a polypeptide substantially identical to said polypeptide, is expressed in at least two different strains of Staphylococcus aureus. Substantially identical as used herein refers to polypeptides having at least 60% of similarity, in embodiments at least 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% of similarity in their amino acid sequences. In further embodiments, the polypeptides have at least 60%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% of identity in their amino acid sequences.

[0066] In an embodiment, the above-mentioned immunogenic fragment comprises a sequence that is conserved (i.e. identical) in at least two different strains of Staphylococcus aureus. In further embodiments, the above-mentioned immunogenic fragment comprises a sequence that is conserved (i.e. identical) in at least 3, 4, 5, 6, 7, 8, 9 or 10 different strains of Staphylococcus aureus. In another embodiment, the above-mentioned strains of Staphylococcus aureus are COL, RF122, NCTC 8325, JH1, JH9, Newman, Mu3, Mu50, USA300-FPR3757, N315, MW2 or MSSA476. In an embodiment, the above-mentioned strains of Staphylococcus aureus is associated with bovine mastitis (e.g., RF122).

[0067] The similarity and identity between amino acid or nucleotide sequences can be determined by comparing each position in the aligned sequences. Optimal alignment of sequences for comparisons of similarity
and/or identity may be conducted using a variety of algorithms, for example using a multiple sequence alignment program/software well known in the art such as ClustalW™, SAGA™, UGENE™ or T-coffee™. Examples of multiple sequence alignments are described in the examples below and depicted in FIGs. 10A-D and FIGs.11A-K.

[0068] Also within the context of the present invention is the in vivo administration of a nucleic acid of the invention to a mammal so that one or more proteins/polypeptides (or a fragment thereof) of interest is/are expressed in the mammal (e.g., nucleic acid vaccine, DNA or RNA vaccine).

[0069] The nucleic acid of the present invention preferably comprises a nucleotide sequence that encodes one or more proteins/polypeptides noted above (or fragments thereof) operably linked to regulatory elements needed for gene expression, such as a promoter, an initiation codon, a stop codon, enhancers, and a polyadenylation signal. Regulatory elements are preferably selected that are operable in the species to which they are to be administered.

[0070] The nucleic acid of the present vaccine can be "naked" DNA or can be operably incorporated in a vector. Nucleic acids may be delivered to cells in vivo using methods well known in the art such as direct injection of DNA, receptor-mediated DNA uptake, viral-mediated transfection or non-viral transfection and lipid-based transfection, all of which may involve the use of vectors. Direct injection has been used to introduce naked DNA into cells in vivo (see e.g., Acsadi et al. (1991) Nature 332:815-818; Wolff et al. (1990) Science 247:1465-1468). A delivery apparatus (e.g., a "gene gun") for injecting DNA into cells in vivo may be used. Such an apparatus may be commercially available (e.g., from BioRad). Naked DNA may also be introduced into cells by complexing the DNA to a cation, such as polylysine, which is coupled to a ligand for a cell-surface receptor (see for example Wu, G. and Wu, C. H. (1988) J. Biol. Chem. 263: 14621; Wilson et al. (1992) J. Biol. Chem. 267: 963-967; and U.S. Pat. No. 5,166,320). Binding of the DNA-ligand complex to the receptor may facilitate uptake of the DNA by receptor-mediated endocytosis. A DNA-ligand complex linked to adenovirus capsids which disrupt endosomes, thereby releasing material into the cytoplasm, may be used to avoid degradation of the complex by intracellular lysosomes (see for example Curiel et al. (1991) Proc. Natl. Acad. Sci. USA 88: 8850; Cristiano et al. (1993) Proc. Natl. Acad. Sci. USA 90:2122-2126).

[0071] Useful delivery vectors include biodegradable microcapsules, immuno-stimulating complexes (ISCOMs) or liposomes, and genetically engineered attenuated live vectors such as viruses or bacteria. Examples of suitable attenuated live bacterial vectors include Salmonella typhi/murium, Salmonella typhi, Shigella, Bacillus, Lactobacillus, Bacille Calmette-Guerin (BCG), Escherichia coli, Vibrio cholerae, Campylobacter, or any other suitable bacterial vector, as is known in the art. Methods of transforming live bacterial vectors with an exogenous DNA construct are well described in the art. See, for example, Joseph Sambrook and David W. Russell, Molecular Cloning, A Laboratory Manual, 3rd Ed., Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y. (2001).
[0072] Preferred viral vectors include Bacteriophages, Herpes virus, Adenovirus, Polio virus, Vaccinia virus, defective retroviruses, adeno-associated virus (AAV) and Avipox. Methods of transforming viral vector with an exogenous DNA construct are also well described in the art. See Sambrook and Russell, above.

[0073] Liposome vectors are unilamellar or multilamellar vesicles, having a membrane portion formed of lipophilic material and an interior aqueous portion. The aqueous portion is used in the present invention to contain the polynucleotide material to be delivered to the target cell. It is generally preferred that the liposome forming materials have a cationic group, such as a quaternary ammonium group, and one or more lipophilic groups, such as saturated or unsaturated alkyl groups having about 6 to about 30 carbon atoms. One group of suitable materials is described in European Patent Publication No. 0187702, and further discussed in U.S. Pat. No. 6,228,844 to Wolff et al., the pertinent disclosures of which are incorporated by reference. Many other suitable liposome-forming cationic lipid compounds are described in the literature. See, e.g., L. Stamatas, et al., Biochemistry 27:3917-3925 (1988); and H. Eibl, et al., Biophysical Chemistry 10:261-271 (1979). Alternatively, a microsphere such as a polylactide-coglycolide biodegradable microsphere can be utilized. A nucleic acid construct is encapsulated or otherwise complexed with the liposome or microsphere for delivery of the nucleic acid to a tissue, as is known in the art.

[0074] Alternatively, the nucleic acid (e.g., DNA or RNA) may be incorporated in a cell in vitro or ex vivo by transfection or transformation, and the transfected or transformed cell (e.g., an immune cell such as a dendritic cell), which expresses the protein or polypeptide of interest (or a fragment thereof), may be administered to the host. Following administration, the cell will express the protein or polypeptide of interest (or a fragment thereof) in the host, which will in turn lead to the induction of an immune response directed against the protein, polypeptide or fragment thereof.

[0075] Also encompassed by the methods, uses, pharmaceutical compositions and kits of the present invention is passive immunization, which is the injection of antibodies or antiserum, previously generated against the pathogen, in order to protect or cure a recipient animal of an infection or future infection. Protection fades over the course of a few weeks during which time the active immunization with protein and/or DNA (as described above) will have time to generate a lasting protective response. Serum for passive immunization can be generated by immunization of donor animals using the S. aureus antigens (proteins, polypeptides or nucleic acids), as described above. This serum, which contains antibodies against the antigens, can be used immediately or stored under appropriate conditions. It can be used to combat acute infections (IMI) or as a prophylactic (Tuchscher et al., 2008). Use of antibodies or serums in a passive immunization can be combined with other agents such as an antibiotic to increase the cure rate of an infection currently in progress or to increase protection against an imminent infection.

[0076] Also encompassed by the methods, uses, pharmaceutical compositions and kits of the present invention is immunization with the Staphylococcus aureus bacteria in attenuated live or inactivated form (e.g., S.
aureus having at least one of the genes of the present invention mutated (e.g., Δ442a, Δ442b and Δ720 of SACOL442 and SACOL720, as described in Example 6). Mutation as used herein includes a substitution, a deletion and/or an insertion of one or more nucleotides that prevents expression of the polypeptide encoded by a gene of the present invention or that prevents expression of a functional polypeptide. In a preferred embodiment, the mutation prevents expression of the polypeptide (e.g., Δ442a, Δ442b and Δ720 of SACOL442 and SACOL720, as described in Example 6). In another specific embodiment, the mutation is a deletion or an insertion. It is expected that a mutated strain of *S. aureus* having a mutation at any position of one of the genes of the present invention that prevents expression of the polypeptide can be used as an attenuated live vaccine in accordance with the present invention. Attenuated live vaccines, i.e. vaccines comprising the bacterium according to the invention in a live attenuated form, have the advantage over inactivated vaccines that they best mimic the natural way of infection. In addition, their replicating abilities allow vaccination with low amounts of bacteria; their number will automatically increase until it reaches the trigger level of the immune system. From that moment on, the immune system will be triggered and will finally eliminate the bacteria. A minor disadvantage of the use of live attenuated bacteria however might be that inherently there is a certain level of virulence left. This need not be a real disadvantage as long as the level of virulence is acceptable, i.e. as long as the vaccine at least decreases the mammal IMI symptoms. Of course, the lower the rest virulence of the live attenuated vaccine is, the less influence the vaccination has on weight gain during/after vaccination.

[0077] The components identified in accordance with the teachings of the present invention have a prophylactic and/or therapeutic value such as they can be used to raise an immune response to prevent and/or combat diseases or conditions, and more particularly diseases or conditions related to microbial infections.

[0078] The terms “treat/treating/treatment” and “prevent/preventing/prevention” as used herein, refers to eliciting the desired biological response, i.e., a therapeutic and prophylactic effect, respectively. In accordance with the subject invention, the therapeutic effect comprises one or more of a decrease/reduction in the severity of the disease (e.g., a reduction or inhibition of infection), a decrease/reduction in symptoms and disease-related effects, an amelioration of symptoms and disease-related effects, and an increased survival time of the affected host animal, following administration of the at least one agent (or of a composition comprising the agent). In accordance with the invention, a prophylactic effect may comprise a complete or partial avoidance/inhibition or a delay of infection, and an increased survival time of the affected host animal, following administration of the at least one agent (or of a composition comprising the agent).

[0079] As used herein, the term “pharmaceutically acceptable” refers to vaccine components (e.g., excipients, carriers) and compositions that are physiologically tolerable and do not typically produce an allergic or similar untoward reaction, such as gastric upset, dizziness and the like, when administered to a subject. Preferably, as used herein, the term “pharmaceutically acceptable” means approved by regulatory agency of the federal or state government or listed in the U.S. Pharmacopeia or other generally recognized pharmacopeia for use in animals,
and in humans. The term “excipient” refers to a diluent, carrier, or vehicle with which the vaccine components of the present invention may be administered. Sterile water or aqueous saline solutions and aqueous dextrose and glycerol solutions may be employed as carriers, particularly for injectable solutions.

[0080] In an embodiment, the agent of the present invention is administered in combination with an adjuvant or immunostimulant. Suitable adjuvant or immunostimulant that may improve the efficacy of components to raise an immune response include but is not limited to oils (e.g., mineral oils, emulsified oil such as EMULSISEN™-D), metallic salts (e.g., alum, aluminum hydroxide or aluminum phosphate), natural and artificial microbial components (e.g., bacterial liposaccharides, Freund’s adjuvants, muramyl dipeptide (MDP), cyclo-diguanosine-5’-monophosphate (c-di-GMP), pathogen-associated molecular patterns (PAMPs)), plant components (e.g., Quil A), and/or one or more substances that have a carrier effect (e.g., bentonite, latex particles, liposomes, ISCOM™ and polyphosphazene (PCPP) copolymers). Immunization with synthetic nanoparticles (such as those made from a biodegradable synthetic polymer like poly(D,L-lactic-glycolic acid)) containing antigens plus ligands that signal through TLR to stimulate proinflammatory cytokines is also possible (Kasturi et al, 2011).

[0081] Vaccine components of the invention may be administered in a pharmaceutical composition. Pharmaceutical compositions may be administered in unit dosage form. Any appropriate route of administration may be employed, for example, parenteral, subcutaneous, intramuscular, intramammary, intracranial, intraorbital, ophthalmic, intraventricular, intracapsular, intraarticular, intraspinal, intracisternal, intraperitoneal, intranasal, aerosol, or oral administration. Examples of specific routes of administration include parenteral, e.g., intravenous, intradermal, subcutaneous, intramammary; oral (e.g., inhalation); transdermal (topical); transmucosal, and rectal administration.

[0082] Conventional pharmaceutical practice may be employed to provide suitable formulations or compositions to administer such vaccine components with or without adjuvants to subjects. Methods well known in the art for making pharmaceutical compositions and formulations are found in, for example, Remington: The Science and Practice of Pharmacy, (20th ed.) ed. A. R. Gennaro A R., 2000, Lippincott: Philadelphia. Formulations for parenteral administration may, for example, contain excipients, sterile water, or saline, polyalkylene glycols such as polyethylene glycol, miglyol, oils of vegetable origin, or hydrogenated naphthalenes. Biocompatible, biodegradable lactide polymer, lactide/glycolide copolymer, or polyethylene-polyoxpropylene copolymers may be used to control the release of the compounds. Other potentially useful parenteral delivery systems for compounds of the invention include ethylenevinyl acetate copolymer particles, osmotic pumps, implantable infusion systems, and liposomes. Formulations for inhalation or intramammary injection may contain excipients, for example, lactose, or may be aqueous solutions containing, for example, polyethylene-9-lauryl ether, miglyol, glycocholate and deoxycholate, or may be oily solutions (e.g., paraffin oil) for administration in the form of nasal drops, or as a gel.

[0083] Therapeutic formulations may be in the form of liquid solutions or suspension; for oral administration,
formulations may be in the form of tablets or capsules; and for intranasal formulations, in the form of powders, nasal drops, or aerosols. Solutions or suspensions used for parenteral, intradermal, intramammary or subcutaneous application can include the following components: a sterile diluent such as water for injection, saline solution, fixed oils (e.g., paraffin oil), polyethylene glycols, glycerine, propylene glycol, miglyol or other synthetic solvents; antibacterial agents such as benzyl alcohol or methyl parabens; antioxidants such as ascorbic acid or sodium bisulfite; chelating agents such as ethylenediaminetetraacetic acid; reducing agents such as dithiothreitol, buffers such as acetates, citrates or phosphates and agents for the adjustment of tonicity such as sodium chloride or dextrose. The pH can be adjusted with acids or bases, such as hydrochloric acid or sodium hydroxide. The parenteral preparation can be enclosed in ampoules, disposable syringes or multiple dose vials made of glass or plastic.

[0084] Pharmaceutical compositions suitable for injectable use include sterile aqueous solutions (where water soluble) or dispersions and sterile powders for the extemporaneous preparation of sterile injectable solutions or dispersion. For intravenous or intramammary administration, suitable carriers include physiological saline, bacteriostatic water, Cremophor™ ELTM (BASF, Parsippany, N.J.) or phosphate buffered saline (PBS).

[0085] Oral compositions generally include an inert diluent or an edible carrier. They can be enclosed in gelatin capsules or compressed into tablets or feed. For the purpose of oral vaccine administration, the active components can be incorporated with excipients and used in the form of tablets, troches, capsules or in feed. Pharmaceutically compatible binding agents, and/or adjuvant materials can be included as part of the composition. The tablets, pills, capsules, troches and the like can contain any of the following ingredients, or compounds of a similar nature: a binder such as microcrystalline cellulose, gum tragacanth or gelatin; an excipient such as starch or lactose, a disintegrating agent such as alginic acid, Primogel™, or corn starch; a lubricant such as magnesium stearate or Sterotes; a glidant such as colloidal silicon dioxide; a sweetening agent such as sucrose or saccharin; or a flavoring agent such as peppermint, methyl salicylate, or orange flavoring.

[0086] For administration by inhalation, the vaccine components are delivered in the form of an aerosol spray from pressured container or dispenser which contains a suitable propellant, e.g., a gas such as carbon dioxide, or a nebulizer.

[0087] Systemic administration can also be by transmucosal or transdermal means. For transmucosal or transdermal administration, penetrants appropriate to the barrier to be permeated are used in the formulation. Such penetrants are generally known in the art, and include, for example, for transmucosal administration, detergents, bile salts, and fusidic acid derivatives. Transmucosal administration can be accomplished through the use of nasal sprays or suppositories. For transdermal administration, the active compounds are formulated into ointments, salves, gels, or creams as generally known in the art.

[0088] Liposomal suspensions (including liposomes targeted to specific cell types) can also be used as
pharmaceutically acceptable carriers.

[0089] The pharmaceutical compositions may also contain preserving agents, solubilising agents, stabilising agents, wetting agents, emulsifiers, sweeteners, colorants, odorants, salts for the variation of osmotic pressure, buffers, coating agents or antioxidants. They may also contain other therapeutically valuable agents.

[0090] Intravenous, intramuscular, intramammary or oral administration is a preferred form of use. The dosages in which the components of the present invention are administered in effective amounts depend on the nature of the specific active ingredient, the host and the requirements of the subject and the mode of application. In general, an amount of about 0.01 mg - 500 mg per dose, come into consideration.

[0091] Toxicity or efficacy of vaccine components to elicit an immune response can be determined by standard procedures in cell cultures or experimental animals. The dose ratio between toxic and immune stimulatory effects can be measured. Components that exhibit large ratios are preferred. While components that exhibit toxic side effects may be used, care should be taken to design a delivery system in order to minimize potential damage to cells and, thereby, reduce side effects.

[0092] Data obtained from cell culture assays and laboratory animal studies can be used in formulating a range of dosage for use in large animals and humans. The dosage of such components lies preferably within a range of administered concentrations that include efficacy with little or no toxicity. The dosage may vary within this range depending upon the dosage form employed and the route of administration utilized.

[0093] The skilled artisan will appreciate that certain factors may influence the dosage required to effectively raise an immune response in a subject. Moreover, the therapeutically effective amount of a component of the present invention may require a series of doses.

[0094] The present invention also encompasses kits comprising the components of the present invention. For example, the kit can comprise one or more components. The components can be packaged in a suitable container and device for administration. The kit can further comprise instructions for using the kit.

[0095] The present invention also provides a method of diagnosing Staphylococcal IMI in a mammal, said method comprising: determining a level of expression of at least one gene, wherein said gene is SACOL0029, SACOL0100, SACOL0101, SACOL0105, SACOL0148, SACOL0154, SACOL0204, SACOL0205, SACOL0264, SACOL0442, SACOL0461, SACOL0608, SACOL0660, SACOL0688, SACOL0690, SACOL0704, SACOL0718, SACOL0720, SACOL0829, SACOL1054, SACOL1142, SACOL1145, SACOL1320, SACOL1353, SACOL1416, SACOL1611, SACOL1637, SACOL1680, SACOL1781, SACOL1812, SACOL1887, SACOL1912, SACOL1944, SACOL2092, SACOL2144, SACOL2169, SACOL2171, SACOL2321, SACOL2325, SACOL2342, SACOL2365, SACOL2379, SACOL2385 or SACOL2599, based on the gene nomenclature from the Staphylococcus aureus COL (SACOL) genome set forth in NCBI Reference Sequence NC_002951.2, or the level of activity of a
polypeptide encoded by said one or more genes (at least one gene), in a biological sample from said mammal; and comparing said level of expression or activity to a reference level of expression or activity; wherein a higher expression or activity in said biological sample relative to said reference expression or activity is indicative that said mammal has staphylococcal IMI.

[0096] In an embodiment, the above-mentioned reference expression or activity is a level of expression or activity determined in a corresponding biological sample from a mammal known to not having staphylococcal IMI. Such reference expression or activity may be an expression or activity corresponding to an average or median expression or activity calculated based on measurements made in several subjects not suffering from the condition (e.g., known to not having staphylococcal IMI). The reference expression or activity may be adjusted or normalized for age, gender, race, or other parameters.

[0097] In an embodiment, the above-mentioned at least one gene is SACOL0029, SACOL0264, SACOL0442, SACOL0718, SACOL0720, SACOL1353, SACOL1416, SACOL1611, SACOL1944, SACOL2144, SACOL2365 or SACOL2599.

[0098] “Sample” or “biological sample” refers to any solid or liquid sample isolated from a live being. In a particular embodiment, it refers to any solid (e.g., tissue sample) or liquid sample isolated from a mammal, such as milk, a biopsy material (e.g., solid tissue sample), blood (e.g., plasma, serum or whole blood), saliva, synovial fluid, urine, amniotic fluid and cerebrospinal fluid. Such sample may be, for example, fresh, fixed (e.g., formalin-, alcohol- or acetone-fixed), paraffin-embedded or frozen prior to analysis of the infectious agent’s expression level.

[0099] In an embodiment, the above-mentioned biological sample is milk.

[0100] In an embodiment, the above-mentioned mammal is a cow.

[0101] In an embodiment, the above-mentioned level of expression is determined by measuring the level of expression of a polypeptide/protein encoded by said one or more genes. Methods to measure the amount/level of selected polypeptides/proteins of this invention (one or more of the polypeptides noted above) are well known in the art. Protein/polypeptide levels may be detected either directly using affinity reagents, such as an antibody or a fragment thereof (for methods, see for example Harlow, E. and Lane, D (1988) Antibodies : A Laboratory Manual, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY), or a ligand (natural or synthetic) which binds the protein. Protein/polypeptide levels may be detected based on other properties, for example by measurement of the protein’s activity, which may entail enzymatic activity to produce a detectable product (e.g., with altered spectroscopic properties) or a detectable phenotype (e.g., alterations in cell growth/function).

[0102] Examples of methods to measure the amount/level of selected proteins/polypeptides include, but are
not limited to: Western blot, immunoblot, enzyme-linked immunosorbent assay (ELISA), radioimmunoassay (RIA), immunoprecipitation, surface plasmon resonance, chemiluminescence, fluorescent polarization, phosphorescence, immunohistochemical analysis, matrix-assisted laser desorption/ionization time-of-flight (MALDI-TOF) mass spectrometry, microcytometry, microarray, microscopy, flow cytometry, and assays based on a property of the protein including but not limited to DNA binding, ligand binding, interaction with other protein partners or enzymatic activity.

[00103] In an embodiment, the amount of the polypeptide/protein within the methods of the present invention is detected using antibodies that are directed specifically against the polypeptide/protein. The term "antibody" as used herein encompasses monoclonal antibodies, polyclonal antibodies, multispecific antibodies (e.g., bispecific antibodies), and antibody fragments, so long as they exhibit the desired biological activity or specificity. "Antibody fragments" comprise a portion of a full-length antibody, generally the antigen binding or variable region thereof. Interactions between antibodies and a target polypeptide are detected by radiometric, colorimetric, or fluorometric means. Detection of antigen-antibody complexes may be accomplished by addition of a secondary antibody that is coupled to a detectable tag, such as for example, an enzyme, fluorophore, or chromophore.

[00104] Methods for making antibodies are well known in the art. Polyclonal antibodies can be prepared by immunizing a suitable subject (e.g., rabbit, goat, mouse, or other mammal) with the polypeptide/protein of interest or a fragment thereof as an immunogen. A polypeptide/protein "fragment" "portion" or "segment" is a stretch of amino acid residues of at least about 5, 7, 10, 14, 15, 20, 21 or more amino acids of the polypeptide noted above. The antibody titer in the immunized subject can be monitored over time by standard techniques, such as with an enzyme linked immunosorbent assay (ELISA) using immobilized exosomal marker polypeptide or a fragment thereof. At an appropriate time after immunization, e.g., when the antibody titers are highest, antibody-producing cells can be obtained from the animal, usually a mouse, and can be used to prepare monoclonal antibodies by standard techniques, such as the hybridoma technique originally described by Kohler and Milstein (1975) Nature 256: 495-497, the human B cell hybridoma technique (Kozbor et al. (1983) Immunol. Today 4: 72), the EBV-hybridoma technique (Cole et al. (1985) in Monoclonal Antibodies and Cancer Therapy, ed. Reisfeld and Sell (Alan R. Liss, Inc., New York, NY), pp. 77-96) or trioma techniques. The technology for producing hybridomas is well known (see generally Coligan et al., eds. (1994) Current Protocols in Immunology, John Wiley & Sons, Inc., New York, NY).

[00105] Alternatively to preparing monoclonal antibody-secreting hybridomas, a monoclonal antibody can be identified and isolated by screening a recombinant combinatorial immunoglobulin library (e.g., an antibody phage display library) with a polypeptide or a fragment thereof to thereby isolate immunoglobulin library members that bind the polypeptide. Kits for generating and screening phage display libraries are commercially available (e.g., the Pharmacia Recombinant Phage Antibody System™, Catalog No. 27-9400-01; and the Stratagene SurfZAP™ Phage Display Kit, Catalog No. 240612).
[00106] Furthermore, antibodies directed against one or more of the polypeptides/proteins described herein may be obtained from commercial sources.

[00107] The use of immobilized antibodies specific for the polypeptides/proteins is also contemplated by the present invention and is well known by one of ordinary skill in the art. The antibodies could be immobilized onto a variety of solid supports, such as magnetic or chromatographic matrix particles, the surface of an assay place (such as microtiter wells), pieces of a solid substrate material (such as plastic, nylon, paper), and the like. An assay strip could be prepared by coating the antibody or a plurality of antibodies in an array on solid support. This strip could then be dipped into the test sample and then processed quickly through washes and detection steps to generate a measurable signal, such as a colored spot.

[00108] The analysis of a plurality (2 or more) of polypeptides/proteins may be carried out separately or simultaneously with one test sample. Several polypeptides/proteins may be combined into one test for efficient processing of a multiple of samples.

[00109] The analysis of polypeptides/proteins could be carried out in a variety of physical formats as well. For example, the use of microtiter plates or automation could be used to facilitate the processing of large numbers of test samples. Alternatively, single sample formats could be developed to facilitate immediate treatment and diagnosis in a timely fashion. Particularly useful physical formats comprise surfaces having a plurality of discrete, addressable locations for the detection of a plurality of different analytes. Such formats include protein microarrays, or "protein chips" (see, e.g., Ng and Illag, J. Cell Mol. Med. 6: 329-340, 2002) and capillary devices.

[00110] In an embodiment, the above-mentioned level of expression is determined by measuring the level of expression of a mRNA transcribed from said one or more genes.

[00111] Methods to determine nucleic acid (mRNA) levels are known in the art, and include for example polymerase chain reaction (PCR), reverse transcriptase-PCR (RT-PCR), SAGE, quantitative PCR (q-PCR), Southern blot, Northern blot, sequence analysis, microarray analysis, detection of a reporter gene, or other DNA/RNA hybridization platforms. For RNA expression, preferred methods include, but are not limited to: extraction of cellular mRNA and Northern blotting using labeled probes that hybridize to transcripts encoding all or part of one or more of the nucleic acids encoding the protein/polypeptide of this invention; amplification of mRNA expressed from one or more of the nucleic acids encoding the proteins/polypeptides of this invention using specific primers, polymerase chain reaction (PCR), quantitative PCR (q-PCR), and reverse transcriptase-polymerase chain reaction (RT-PCR), followed by quantitative detection of the product by any of a variety of means; extraction of total RNA from the biological sample, which is then labeled and used to probe cDNAs or oligonucleotides encoding all or part of the nucleic acids encoding the proteins/polypeptides of this invention, arrayed on any of a variety of surfaces.
The present invention also provides a kit or package comprising reagents useful for determining the amount/level of one or more proteins/polypeptides of the present invention, for example a ligand that specifically bind to proteins/polypeptides, such as a specific antibody, or to a nucleic acid encoding proteins/polypeptides, such as an oligonucleotide (e.g., primer or probe). Such kit may further comprise, for example, instructions for the diagnosis of Staphylococcal IMI, control samples (e.g., samples to which the test sample may be compared to establish the diagnostic), containers, reagents useful for performing the methods (e.g., buffers, enzymes, immunodetection reagents, etc). The kit may further include where necessary agents for reducing background interference in a test, agents for increasing signal, software and algorithms for combining and interpolating marker values to produce a prediction of clinical outcome of interest, apparatus for conducting a test, calibration curves and charts, standardization curves and charts, and the like. The present invention also provides a kit or package comprising one or more agents of the present invention for treating and/or preventing Staphylococcal IMI. Such kit may further comprise, for example, instructions for the prevention and/or treatment of IMI in a mammal.

The use of the word “a” or “an” when used in conjunction with the term “comprising” in the claims and/or the specification may mean “one” but it is also consistent with the meaning of “one or more”, “at least one”, and “one or more than one”.

As used in this specification and claim(s), the words “comprising” (and any form of comprising, such as “comprise” and “comprises”), “having” (and any form of having, such as “have” and “has”), “including” (and any form of including, such as “includes” and “include”) or “containing” (and any form of containing, such as “contains” and “contain”) are inclusive or open-ended and do not exclude additional, un-recited elements or method steps.

MODE(S) FOR CARRYING OUT THE INVENTION

The present invention is illustrated in further details by the following non-limiting examples.

EXAMPLE 1: Materials and methods

Two types of Staphylococcus aureus isolates from cows were used in this study: chronic and systematically isolated strains (i.e. strains isolated from bovine mastitis with clinical signs (high somatic cell counts (SCC) in milk or signs of inflammation). Chronic isolates were from cows shedding a genetically identical S. aureus strain >55 days apart, between dry off and calving as illustrated in FIG. 1 (1st and 2nd samples). Systematically isolated strains were taken at calving or in the lactation period from cows shedding high somatic cell counts (SCC) in milk or signs of inflammation (mastitis). For the experimental infections described further below, 3 chronic strains were used (#3, #557 and #1290) and were compared to SHY97-3906, a previously described strain isolated from a typical mastitis case with clinical signs (Diarra et al., 2002) and of a
known in vitro transcriptome (Allard et al., 2006). The collection of isolates used in this study is shown in Table II below.

**Table II.** *Staphylococcus aureus* mastitis isolates used in the studies described herein

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<tr>
<th>Isolate</th>
<th>Type</th>
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<tr>
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<td>83</td>
<td>4</td>
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<td>4210</td>
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<tr>
<td>G3</td>
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<td>SHY97-3906</td>
<td>R</td>
<td>From mastitis; Diarra, MS et al., 2002; Allard et al., 2006</td>
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<td>Newbould</td>
<td>R</td>
<td>From mastitis; Prasad and Newbould, 1968 (ATCC 29740)</td>
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<tr>
<td>N315</td>
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<td>Reference MRSA strain from human</td>
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</tbody>
</table>
Chr, chronic; R, random; C, mix of all quarters; NA, not available

[00117] Comparative genomic hybridization of S. aureus isolates. The genetic relatedness of S. aureus isolates was determined by comparative genomic DNA hybridization data obtained for 530 genes printed on arrays as described previously (Atalla et al., 2008) and is shown in FIG. 2.

[00118] Production of biofilms by S. aureus isolates. Biofilm formation was evaluated by spectrophotometry in microplates using crystal violet staining, as previously described with a few modifications (Brouillette et al., 2005). Briefly, strains were cultured from frozen stocks onto BHI agar plates and incubated overnight at 35°C. Three colonies were then inoculated into 7 ml of BHI containing 0.25% of supplemental glucose and incubated at 35°C for 18 h with shaking at 225 rpm. This culture was then diluted to 0.5 McFarland in BHI 0.25% glucose and transferred into wells of a flat-bottom polystyrene microtiter plate half full of the same medium. The plates were then incubated at 35°C for 24 or 48 h. The supernatant was then discarded and the wells were delicately washed three times with 200 µl of PBS. The plates were dried, stained for 30 min with crystal violet, washed twice with 200 µl of water and allowed to dry again. A volume of 200 µl of 95% ethanol was added to each well and plates were incubated at room temperature for 1 h with frequent agitation. The absorbance of each well was then measured at 560 nm using a plate reader (Bio-Tek Instruments). The results were collected from at least three independent experiments in which the biofilm formation of each culture tested was evaluated in four replicates.

[00119] In vitro culture conditions. For bacterial growth in low and high iron concentrations, bacteria were first grown in Mueller-Hinton broth (MHB, Becton Dickinson Sparks, MD, USA) in an orbital shaker (225 RPM) at 35°C. At an A_{600nm} of 0.6 (approx. 1 x 10^8 CFU/ml), the culture was divided into two pre-warmed sterile flasks. Iron limitation was induced by addition of 2,2'-dipyridyl (Sigma Chemicals, St-Louis, MO) at 600 µM to one culture, whereas FeCl_3 was added to the other culture at 10 µM. The growth rate of S. aureus in the presence of supplemental 2,2'-dipyridyl or FeCl_3 was equivalent in both test conditions and these supplements did not affect the exponential growth during the one-hour treatment period. After 1 h, the cultures reached an A_{600nm} of 1.0 (approx. 10^9 CFU/ml) and 5 ml of each culture were treated with RINaprotect™ (Qiagen, Mississauga, ON, Canada) for 10 min before harvesting the cells by centrifugation. For bacterial growth in freshly collected non-mastitic milk, S. aureus SHY97-3906, #3, #557 and #1290 were first grown overnight in MHB in an orbital shaker (225 RPM) at 35°C. In the morning, 250 ml of fresh milk was inoculated with bacteria from the overnight culture to obtain a bacterial concentration of approximately 10^4 CFU/ml. Bacterial growth was allowed for 7h in an orbital shaker (225 RPM) at 35°C before isolating the bacteria from milk as described below. For bacterial growth destined to the qPCR amplification of icaC and hld genes, S. aureus was grown in brain heart infusion (BHI) broth (BD, ON, Canada) until the cultures reached an A_{600nm} of 0.6.

[00120] Animals. All animal experiments were approved by local institutional animal care committees and conducted in accordance with the guidelines of the Canadian Council on Animal Care. Animals were kept in a
level 2 confinement barn for the entire duration of each trial. Eight Multiparous Holstein cows in mid lactation were housed at the Dairy and Swine Research and Development Centre of Agriculture and Agri-Food Canada in Sherbrooke, QC, Canada. Cows were selected as not infected before the experiment by bacterial analysis of aseptic milk samples and somatic cell count (SCC) determination.

**00121** Experimental infections. Before the animal trials, the relation between the absorbance of bacterial cultures ($A_{600\text{nm}}$) and CFU was determined as previously described (Petitclerc et al., 2007). The morning of the challenge, a volume of the overnight culture of *S. aureus* in MHB was transferred to 200 ml of fresh MHB to obtain an $A_{600\text{nm}}$ of 0.1 and grown at 35°C without shaking until the $A_{600\text{nm}}$ reached a value corresponding to $10^8$ CFU/ml in the exponential phase of growth. Bacteria were then diluted in sterile physiological saline (Baxter Healthcare Corporation, Deerfield, IL) to obtain 50 CFU in 3 ml. Intramammary (IM) infusions were performed the same day immediately after the late evening milking. Each individual mammary gland quarter was infused with 3 ml of a bacterial suspension. Each of the 8 cows was infused with the four different *S. aureus* strains and the position of each strain in the four quarters alternated between the animals. Infusion of mammary quarters with bacteria was performed according to the procedure described by Nickerson et al. (1999) with few modifications. All infusions were performed after milking. Before inoculation, the teat end of each quarter was thoroughly wiped to remove gross contamination and dipped in a solution of iodine. After a minimum of 30 second contact time, teats were wiped dry and subsequently scrubbed with gauzes soaked in 70% ethanol. Teats were allowed to air-dry. Foremilk was then discarded and the IM infusion was performed. Immediately afterwards, all quarters were thoroughly massaged and teats dipped again with an iodine solution. Disposable gloves were worn throughout the procedure and changed before proceeding to the next animal.

**00122** Milk samples. Milk samples were always aseptically collected before milking the experimentally infected cows using the procedure suggested by the National Mastitis Council (1996). After foremilk was discarded, a 10 ml milk sample was collected for each individual quarter in a 50 ml sterile vial. Milk samples were serially diluted and 200 µl plated on TSA and on mannitol salt agar plates (MSA; Becton Dickinson Sparks, MD, USA) for *S. aureus* identification. Plates were then incubated for 24h at 35°C before colony counting. The dilution that showed between 30 and 300 colonies was the one considered for the calculation of bacterial concentration. Considering the wide range of dilutions plated and the great number of samples to be tested, only one plate per sample was considered. Samples that showed 0 colonies for the undiluted milk was considered to have a concentration of ≤ 5 CFU/ml. The concentration of lactose, protein, fat and SCC in milk which indicates the presence of leukocytes in response to an infection were determined in a commercial laboratory (Valacta Inc., Ste-Anne-de-Bellevue, QC, Canada). This was done every two days over the 18-day period of experimental infections.

**00123** Milk collection for bacterial isolation. Milk was collected from each quarter of each cow every 2-4 days in the morning for a total of 18 days. Milk was harvested using individual quarter milking units. Prior to milking,
the four reservoirs were disinfected with 70% ethanol. A maximum of one litre of milk was collected for centrifugation and isolation of bacteria.

[00124] Bacterial isolation from milk. Mastitic milk from experimentally infected cows or freshly collected milk from non-infected cows used for bacterial growth in vitro was treated with 200 \( \mu \)g/ml of protease from bovine pancreas (Sigma) for 10 min in an orbital shaker (100 RPM) at 35°C. After the treatment, the milk was centrifuged 15 minutes at 4000 g. The supernatant was discarded and the pellet was washed with PBS and centrifuged. The supernatant was discarded and the bacterial cell pellet was suspended in 1 ml PBS and treated with RNAprotect™ for 10 min before harvesting the cells by centrifugation. The cell pellet was then stored frozen at -80°C.

[00125] RNA extraction and purification. Bacterial pellets from in vitro and in vivo growth conditions were suspended in 200\( \mu \)l of TE buffer containing 200\( \mu \)g/ml lysostaphin™ (Sigma). Cell lysis was allowed for 1 h at room temperature before RNA extraction with the TRizol™ Max bacterial RNA isolation Kit (Invitrogen, Carlsbad, CA, USA) followed by a DNase treatment with TURBO™ DNase (Ambion, Austin, TX, USA). RNA from bacteria isolated from the milk of infected cows underwent an additional purification step using the MICROBEnrich™ Kit from Ambion followed by a second round of DNase treatment with TURBO™ DNase (Ambion). The RNA concentration in samples was determined by an \( A_{260nm} \) reading and the samples were stored at -80°C until used.

[00126] cDNA probe synthesis. Fluorescent probes for hybridization to DNA arrays were generated through an aminoallyl cDNA labelling procedure. Briefly, 2.5-5 \( \mu \)g of total RNA was mixed with 5 \( \mu \)g of random hexamers (Amersham Biosciences, Piscataway, NJ, USA). This mixture was denatured at 70°C for 10 min. Reverse transcription was carried out in the presence of RT buffer (Invitrogen), 10 mM DDT, dNTP mix (final concentration: 500 \( \mu \)M dATP, dCTP, dGTP, 300 \( \mu \)M dTTP and 200 \( \mu \)M 5-[3-aminolallyl]-2-deoxyuridine (Sigma)) and 400 U of Superscript™ II RT was added to the RNA preparation and the reaction was allowed to occur for 2h at 42°C. The RNA was hydrolyzed after transcription with 200 mM NaOH and 100 mM EDTA at 65°C for 15 min. The reaction was neutralized with 333 \( \mu \)M HEPES pH 7.5. The cDNAs were purified before fluorescent labeling through three passages on a Microcon™ YM30 (Millipore). The resulting aadUTP-cDNA was coupled with NHS-Cy5 (Fluorolink™ Cy5 monoreactive pack, Amersham Biosciences) in the presence of 100 \( \mu \)M NaHCO\(_3\), pH 9.0 for 1 h at room temperature. The reactions were quenched with 1.25 \( \mu \)M hydroxylamine for 15 min at room temperature. The fluorescent cDNAs were purified by using a QIAquick™ PCR purification kit (QIAGen), including three washing steps with buffer PE, before eluting in water.

[00127] DNA arrays. Arrays were previously described (Allard et al., 2006; Moisan et al., 2006) and contained a selection of 530 known or putative genes implicated in iron/cation-transport and acquisition systems, virulence (biofilm genes, adhesins, toxins and homologs of such genes), secretion, general stress responses, sensory/regulator systems, antibiotic resistance and various biosynthesis and metabolism genes. Genes were first amplified by PCR using Sigma Genosys™ (Oakville, ON, Canada) primers based on the published genome
sequence of the *S. aureus* COL genome as well as other primers that were designed using the Primer3 software (primer3-www.cgi v 0.2). PCR products were then purified using the QIAquick PCR purification kit, precipitated, suspended at a concentration of 150 ng/μl in 50% DMSO and printed in triplicate on Corning GAPS II slides (Corning, Comin, NY, USA) with the help of the Microarray printing platform of the Biotechnology Research Institute of Montreal (Montreal, QC, Canada). Control spots were from the Lucidea Universal Scorecard (Amersham, Piscataway, NJ).

[00128] Hybridization to DNA arrays and analysis. The probes were suspended in 18.5 μl of hybridization buffer (5X SSC, 0.1% SDS, 25% formamide). The prehybridization, hybridization and washing steps were done as prescribed for Corning Gaps II Slides. Hybridization signals for each spot were quantified with the ScanArrayExpress Microarray Scanner and the ScanArrayExpress software V 2.2.0.0022 (Perkin Elmer, Wellesley, MA, USA). A mean intensity value was calculated as the: Σ (intensity of every spot) / number of genes on array = 100%. Only genes with a Cy5 signal intensity of ≥100%, i.e., greater or equal to the mean Cy5 intensity of the entire array were analyzed. Thus, this report identifies only genes that were strongly expressed *in vivo* during mastitis because their signal intensities on arrays were higher than average.

[00129] Quantitative PCR (qPCR). Additional RNA preparations were obtained for qPCR analyses. Bacteria were collected from broth cultures (low-iron and iron-rich) as well as from milk (*in vitro and in vivo*) as described above. Also, RNA was extracted as mentioned earlier. Total RNA (2-5 μg) was reversely transcribed with 0.5 mM dNTP, 50 ng random hexamers and 200 U of Invitrogen Superscript II Reverse Transcriptase according to the manufacturer recommendations. RNA was denatured and the cDNAs were purified with QIAquick PCR purification kit. One μl of cDNA was amplified on the Stratagene MX3000P Real-Time PCR (Stratagene, LaJolla, CA USA) with a master mix composed of 6 mM Tris-HCl pH 8.3, 25 mM KCl, 4 mM MgCl2, 75 mM trehalose, 0.1% (v/v) Tween™ 20, 0.1 mg/ml nonacetylated BSA, 0.07x SYBR green (Invitrogen), 125 mM dNTPs and 0.5 U JumpStart™ Taq DNA Polymerase (Sigma), and 100 nM of the primers listed in Table III below. Reaction mixtures were denatured for 10 min at 95°C, followed by 40 cycles of 1 min at 60°C, 1 min at 72°C and finished with a dissociation ramp from 55°C to 95°C. The level of expression of each gene was calculated by using the Ct of the *in vitro* experiments as the calibrator (expression fold = 2^ΔCt, where ΔCt represents the difference between the Ct of the *in vitro* and *in vivo* conditions). The fold expression of genes from each experiment was then normalized with their respective gyrB expression level. The gyrB gene was found to be constitutively expressed during growth up to the early stationary phase (Goerke et al., 2000), which is well within the boundaries of the growth experiments described herein. Also, it was found that the expression of gyrB in the *in vitro* as well as in the *in vivo* conditions was not significantly modulated.

**Table III:** Sequence of primers used for quantitative PCR (qPCR).

<table>
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<th>ORF</th>
<th>Gene</th>
<th>Description</th>
<th>Forward sequence</th>
<th>Reverse sequence</th>
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<tbody>
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<td></td>
<td></td>
<td>DNA gyrase, B subunit</td>
<td>DNA gyrase, B subunit</td>
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<td>---</td>
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<tr>
<td>SACOL005</td>
<td>gyrB</td>
<td>DNA gyrase, B subunit</td>
<td>GGTGCTGGGCAAATACAAAGT (SEQ ID NO: 1)</td>
<td>TCCACACTAAATGGTGCAA (SEQ ID NO: 2)</td>
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<tr>
<td>SACOL0148</td>
<td>capM</td>
<td>Capsular polysaccharide biosynthesis</td>
<td>AGCTTCCATAGGGGAGGGGCTT (SEQ ID NO: 3)</td>
<td>TCTTCCATACCTTGAGC (SEQ ID NO: 4)</td>
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<td>SACOL0442</td>
<td>Exotoxin, putative</td>
<td>CATAACAGGCTGGCAGAG (SEQ ID NO: 5)</td>
<td>CAAGGATAGGAAAATATGAGCA (SEQ ID NO: 6)</td>
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<tr>
<td>SACOL0718</td>
<td>ABC transporter, unknown function</td>
<td>GCACAAAGGTTGGCAGA (SEQ ID NO: 7)</td>
<td>GTCGTTTTCCAGATCCAGA (SEQ ID NO: 8)</td>
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<td>SACOL2022</td>
<td>hld</td>
<td>Delta-hemolysin, RNA III</td>
<td>TAATTTAGGAAAGGTCTGTAC (SEQ ID NO: 9)</td>
<td>TTTTATGGAATTGGTACTC (SEQ ID NO: 10)</td>
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<tr>
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<td>Unknown function, possibly iron-related</td>
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<td>GCTTAGCTTGTTGGAACCTGG (SEQ ID NO: 12)</td>
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<td>SACOL2325</td>
<td>Transcriptional regulator, LysR family</td>
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<td>SACOL269</td>
<td>icaA</td>
<td>Biosynthesis of polysaccharides, biofilms</td>
<td>TTGGCTTAGCAATTGGAGAC (SEQ ID NO: 15)</td>
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[00130] **Sequence alignments.** Nucleic acid and amino acid sequences of *S. aureus* genes (including SACOL0442 and SACOL0718, as well as other genes) and encoded proteins from *Staphylococcus aureus* strains COL, RF122, NCTC 8325, JH1, JH9, Newman, Mu3, Mu50, USA300-FPR3757, N315, MW2 or MSSA476 were obtained from the Comprehensive Microbial Resource (CMR) of the J. Craig Venter™ Institute at http://cmr.jcvi.org/tigr-scripts/CMR/CmrHomePage.cgi (Peterson, J. D., et al., *Nucleic Acids Res.* 2001 29(1): 123-5). The sequences were submitted to a multiple sequence alignment program for DNA or proteins, ClustalW2™, available online for free from the European Bioinformatics Institute (www.ebi.ac.uk; Larkin M.A. et al., 2007. Bioinformatics 23(21): 2947-2948).

[00131] **Purification of proteins encoded by *S. aureus* genes expressed during IMI.** Genes or part of the genes were cloned into the vector pQE-30 (Qiagen) downstream to a polyhistidine signal to allow protein expression in *Escherichia coli* and purification of the expressed his-tagged polypeptides using a nickel affinity column (Qiagen Ni-NTA 1018244). Expression of the recombinant proteins and their purification was performed according to the manufacturer’s recommendations (Qiagen).

[00132] **Immunization of mammalian species and measurement of antibody titers.** Mice were immunized with the antigens (purified recombinant proteins, polypeptides or epitopes of interest, alone or in combination). For example, each animal group composed of ten mice received a different antigen (100 µg per injection), a combination of antigens (100 µg of each per injection) or saline (i.e. the control non-immunized group). Mice were immunized twice 3 weeks apart. The antigens or saline was combined with the adjuvant Emulsigen®-D (MVP Technologies, Omaha, USA). Injections were performed subcutaneously in 400 µl on the back of the mice. Blood samples were performed in the mandibular vein before each injection and, 3 weeks after the second

injection, mice were euthanized and maximum blood was sampled. The levels of specific antibodies against the immunizing antigens were determined. Levels of antibodies were evaluated using standard ELISA methodology (Loiselle et al., 2009). Briefly 96-well plates were coated with individual purified antigen and then saturated with non-specific protein. After incubation with serial dilutions of the sera and washes, a secondary antibody conjugated to an enzyme (HRP) was added and the presence of antibodies was detected with a colorimetric reaction.

[00133] Immunization in cows. Each animal group composed of 5 cows receives a different antigen (300 µg per injection), a combination of antigens (300 µg of each per injection) or saline (i.e. the control non-immunized group). The antigens or saline is combined with the adjuvant Emulsigen®-D. After blood samplings for the determination of pre-immune levels of antibodies, a final volume of 3 ml per dose of antigens or saline is injected subcutaneously in the neck of the cows. Blood samplings is performed every 2 weeks. Ten weeks after the first injection, the second injection is performed subcutaneously in the neck on the other side of the animals. The levels of the specific antibodies is determined as described for the mice immunization.

[00134] Evaluation of antibody binding on bacterial surface. Bacteria were incubated at 4°C, under gentle agitation, with a solution of PBS-2% BSA containing a 1/500 dilution of rabbit serum to block staphylococcal protein A, which can bind non specifically the Fc fragment of immunoglobulins. After 2 washes with PBS-2% BSA-0.02% tween20™, bacteria were incubated at 4°C, under gentle agitation, in PBS-2% PBS containing 10 µl of bovine pre immune or immune serum against the antigen of interest. After 2 washes with PBS-2% BSA-0.02% tween20™, bacteria were incubated for one hour at 4°C, under gentle agitation, in PBS-2% PBS containing a 1/1000 dilution of FITC-conjugated goat anti-bovine IgG. After 3 washes with PBS-2% BSA-0.02% tween20™, bacteria were suspended in PBS with 1% formaldehyde. Surface labeling was then analyzed by flow cytometry using a BD FACSCalibur™ instrument and the CellQuest™ Pro software.

[00135] Identification of B cell epitopes. With a combination of prediction software including BCPred Predictions (EL-Manzalawy et al., 2008a), AAP Predictions (Chen J et al., 2007), FBCPred Prediction (EL-Manzalawy et al., 2008b) and ABCPred (Saha, S. and Raghava G.P.S., 2006), available at http://bicionf.bgu.ac.il/bsu/immunology/epitope_pred/index.htm, http://allab.cs.iastate.edu/bcpreds/index.html and elsewhere, B cell epitopes, i.e., short amino acid sequences that will be recognized by B cells, thus inducing the production of antibodies by B cells, were determined for several vaccine components.

[00136] Identification of T cell epitopes. Computer driven algorithms can also be used to facilitate identification of T cell epitopes i.e., short amino acid sequences that will bind MHC molecules (MHC class I and/or II) and be recognized by T cells, thus inducing a cellular immune response. The antigens may be subjected to analysis by the Epimatrix™ System (http://www.epivax.com/platform/) to identify putative T cell epitopes. This in-silico technique divides the total sequence of the antigen into fragments of 9 amino acids overlapping by 8 amino acids. This pool of 9-mer peptides is then screened for predicted affinity against a group of known MHC class I
and class II alleles. The resulting scores can be used to rate putative epitopes on a common scale which can then be tested in vitro. The technique is applicable to any animal for which a sufficient knowledge of MHC sequences is available. (De Groot et al., 2006).

EXAMPLE 2: Validation of chronic S. aureus strains

[00137] Comparative genomic hybridization data for the members of chronic isolate pairs collected from cows >55 days apart between dry-off and calving (FIG. 2, underlined isolates), show a high genetic relatedness. Unrelated reference strains (S. aureus N315, MRSACOL, Newbould, ATCC 49775, ATCC 51811 and SHY973906) and isolates randomly or systematically picked from bovine mastitis cases with clinical symptoms during lactation (annoted "R" in Table I above) are also shown in FIG. 2 for comparison. This analysis confirmed that the isolates that were collected from the same cow and the same quarter >55 days apart, were genetically identical. It is clear that the chronic isolates #3, #557, and #1290 used in the studies described herein have the ability to cause an IMI and persist in the mammary gland for a long period of time (i.e., are able to cause a chronic IMI).

[00138] FIG. 3 shows Q-PCR analyses reporting the relative level of gene expression for indicators of virulence such as hld (Agr-dependent exotoxin production), icaC (ica-dependent biofilm production) and overall biofilm production (measured by a spectrophotometric method with crystal violet) for S. aureus isolates grown in a cultivation medium in vitro. Chronic isolates (#3, 557, 1290) were compared to a collection of systematically isolated strains from bovine mastitis with clinical signs (where isolate SHY97-3906 is represented as the open square). Q-PCR results are presented as fold-expression relative to the reference strain Newbould (ATCC 29740) and biofilm production is reported as a percentage of that produced by strain SHY97-3906. All Q-PCR results were normalized based on the level of expression of gyrA. The primers used for the analysis are shown in Table II above. This analysis confirmed that the chronic isolates used in the Examples described herein substantially differ in their basal level of gene expression for known virulence determinants and for biofilm production compared with the population of systematically collected isolates from clinical mastitis cases during lactation. These characteristics described for the chronic isolates resemble those reported for S. aureus strains isolated from persistent bovine IMI (Melchior et al., 2009).

EXAMPLE 3: Efficient isolation of bacteria from the milk of experimentally infected cows

[00139] The method used for isolating bacteria from mastitis milk samples is illustrated in FIG. 4. The bacterial pellet recovered from milk treated with proteases (prot+, FIG. 4) was much larger and allowed greater amounts of microbial RNA to be isolated for DNA microarray and qPCR experiments compared to that obtained from the untreated bacterial pellet (prot-, FIG. 4). A DNA microarray experiment comparing the transcriptional profiles of S. aureus grown in vitro in milk treated with casein protease to that of S. aureus cells grown in untreated milk did not show significant gene modulation. Quantitative PCR analysis for 4 genes expressed in S. aureus grown in
an iron-restricted medium in vitro and under iron-rich conditions show that a 2-hour delay before RNA extraction (time period between milking and RNA extraction) did not affect the observed modulations in expression of iron-regulated genes (isdB, ferritin and SACOL2170) and did not affect expression of the housekeeping gene gyrB. The integrity of bacterial messenger RNA directly isolated from mastitis milk should therefore not be affected by the time required for isolation of bacteria after milking of the infected cow.

**EXAMPLE 4: Experimental infection profiles in cows**

[00140] Experimental infection profiles for strain SHY97-3906 and the 3 chronic strains (#3, 557, 1290) in 3 different cows are reported in FIG. 5 as a function of bacterial (left Y axis) or somatic cell counts (right Y axis) over the infection period. Data show that all bacterial isolates are able to establish an intra-mammary infection in cows although the host (cow) seems to influence the level of bacterial counts, cow #313 showing low bacterial counts vs. cow #5325 showing high counts for all tested isolates. Milk samples with high bacterial counts (obtained from cows #307 and 5325) were thereafter used for transcriptional analyses.

**EXAMPLE 5: S. aureus genes expressed during IMI in cows**

[00141] The transcriptional profile of *S. aureus* strains infecting the mammary glands of cows was determined by DNA microarray experiments. The relative levels of expression of the differentially expressed genes and the 20 genes expressed by both of the two groups of isolates (i.e., from chronic or acute mastitis) are reported in Table IV below. FIG. 6 shows the Venn diagram of the genes differentially expressed in the chronic strains taken all together (isolates #3, #557 and #1290) versus SHY97-3906 isolated from a typical mastitis case with clinical signs (acute mastitis). This analysis shows that the two types of isolates (chronic vs. typical mastitis isolate (acute)) present different gene expression profiles and that specific genes may be more strongly expressed in each group. These specific sub-groups of genes constitute therapeutic or vaccine targets to treat specific clinical cases. Also of interest are the genes commonly expressed by both types of isolates (20 genes in this case, identified by a plus [+]+ sign in Table IV below), which may be used to treat acute and/or chronic cases.

[00142] FIG. 7 shows the Venn diagram of the 43 genes found to be strongly expressed in microarray experiments (Table IV below) using bacterial samples from cow #307 at day 8 (A) and day 10 (B) of infection, and in cow #5325 at day 10 of infection (C). The number of bacterial samples in which the genes were shown to be expressed is indicated in parenthesis and the gene names that are represented in bold characters were chosen for qPCR analyses (FIG. 8). This analysis allows identification of genes that are expressed by one or more isolates in one or more cows at one or more time points during the infection.

[00143] Several genes shown in FIG. 7 were thus expressed in one of the following situations: (i) expressed in more than one strain, (ii) observed in more than one cow and/or (iii) at more than one time point. The expression of 5 such interesting *S. aureus* genes in 5 to 12 independent samples collected from cows with IMI was thus
verified and confirmed by qPCR (FIG. 8). These included the capsular biosynthesis gene (cap), a gene of unknown function (SACOL2171), a transcriptional regulator of unknown function (SACOL2325), an ABC transporter of unknown function (SACOL0716) and a chromosomally encoded gene not previously characterized (SACOL0442). In parallel, gene expression was compared to that measured in S. aureus cultivated in vitro in Muller-Hinton broth supplemented with iron (broth + iron), in iron-restricted broth (broth – iron), and in freshly collected non-mastitic milk in vitro (milk in vitro) in order to identify the environmental stimuli involved in gene expression (FIG. 8).

[00144] It was observed (i) that the expression of capM was reduced in cows and in milk compared to that seen in vitro, (ii) that gene SACOL2171 was up-regulated by iron restriction either in cows, in milk or in iron-restricted broth in vitro, (iii) that the expression of SACOL0718 and SACOL2325 were specifically induced by the milk environment (i.e. up-regulated in cows compared to any broth in vitro but equivalent to that seen in fresh milk) and (iv) that gene SACOL0442 was exclusively expressed during infection in the cow, i.e., more expressed in cows compared to any other environment. The summary of the expression profile determined by DNA array and qPCR analyses for genes SACOL0442 and SACOL0718 in different strains, cows and time points during infection is reported in Table V below. As seen, SACOL442 and SACOL0718 are representative examples of S. aureus genes that exhibit sustained expression during IMI and this independently of individual S. aureus strains. Table VI below lists 11 genes (i.e. SACOL442, SACOL0718 and 9 other genes) for which expression had never been reported before, when S. aureus was grown in “other” mammalian environments (i.e. different from the bovine mammary gland environment, as used herein) (Allard et al., 2006; Burlak et al., 2007; Goerke et al., 2000; Garzoni et al., 2007) or in surrogate cultivation media such as in human neutrophils in vitro (Voyich et al., 2005), an iron-restricted medium in vitro (Allard et al., 2006; Maresso et al., 2006), in milk in vitro (Lammers et al., 2000) or when S. aureus mastitis isolates were grown in vitro (Taverna et al., 2007). The genes depicted in Table VI thus represent excellent targets for prevention and/or treatment of S. aureus IMI, for example as components for a vaccine composition aimed at preventing S. aureus IMI. Also, reports of S. aureus genes expressed in surrogate media or in mammalian environment other than the mammary gland can actually lead away from what is reported here for S. aureus genes expressed during bovine IMI. For example, the gene capM (SACOL0148) was reported to be expressed in a mastitis isolate grown on a blood agar plate in vitro (Taverna et al., 2007) but is shown here to be less expressed during bovine IMI than that measured after growth in vitro (FIG. 8). It has been previously demonstrated that the genes capM, csb33, csb28, pflB, gliP, and SACOL0154 (listed in Table III above) were all less expressed during growth of S. aureus in tissue cages implanted in the peritoneal cavity of mice than when measured in vitro (Allard et al., 2006), whereas a strong expression of all these genes during bovine IMI is shown in the instant studies. Indeed, the host defense barriers and immune response, the infected mammary gland tissue and tissue damage, as well as the altered composition and low oxygen tension of the mastitis milk of cows suffering from IMI all create a unique and complex environment that would be difficult to mimic in other animal models of infection, in surrogate systems or other cultivation media (Mayer et al., 1988; Park et al., 2007).
Gene SACOL0718 identified in Tables III and V is part of an operon comprising genes SACOL0718-SACOL0720 as illustrated in FIG. 9 and as determined by programs known by those in the field (Prediction of operon: www.microbesonline.org, Dehal P.S. et al., Nucleic Acids Res. 2010 Jan; 38(Database issue): D396-400. Epub 2009 Nov 11; Promoter search: www.softberry.com, Srivastava S et al., Bioinformation 2008; 3(4):173-6. Epub 2008 Dec 6). The predicted function of these genes is the formation of an ABC transporter composed of an ATP-binding protein and a permease (Table V below). SACOL0720 was not detected in the microarray experiments (Table III above) because it was not included in the composition of the DNA array (Allard et al., 2006). However, it is well known that genes from operons are expressed from the same promoter sequence and are translated into proteins from the same messenger RNA. Therefore, given that expression of SACOL0718 is detected during IMI, it may be predicted that expression of SACOL0720 certainly also occurs and thus that both SACOL0718 and SACOL0720 represent targets for prevention and/or treatment of S. aureus IMI.

Table IV: S. aureus genes (43 genes) with significant levels of expression (intensity >100%) during bovine IMI as determined in microarray experiments. Genes are listed by name (if attributed) as well as by open reading frame (ORF) numbers for three different S. aureus strains for which the genome is sequenced (MRSA COL, N315 and the mastitis isolate RF122). Such genes are also reported in the Venn diagrams of FIGs. 6 and 7. The 20 genes expressed by both chronic strains as well as by strain SHY97-3906 (common) are indicated by a plus (+) sign.
<table>
<thead>
<tr>
<th>Gene</th>
<th>ORF</th>
<th>ORF</th>
<th>ORF</th>
<th>Description</th>
<th>Cow 307, Day 8</th>
<th>Cow 307, Day 10</th>
<th>Cow 5325, Day 10</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>C10L</td>
<td>RF122</td>
<td>RS315</td>
<td></td>
<td>SHY97</td>
<td>03</td>
<td>#557</td>
</tr>
<tr>
<td>sblA</td>
<td>0029</td>
<td>-</td>
<td>35</td>
<td>Biosynthesis of cofactors</td>
<td>+</td>
<td>106.6</td>
<td>195.4</td>
</tr>
<tr>
<td>sblF</td>
<td>0100</td>
<td>55</td>
<td>112</td>
<td>Staphylokinase biosynthesis</td>
<td>+</td>
<td>440.5</td>
<td></td>
</tr>
<tr>
<td>sblB</td>
<td>0101</td>
<td>56</td>
<td>113</td>
<td>Staphylokinase biosynthesis</td>
<td>+</td>
<td>559.4</td>
<td></td>
</tr>
<tr>
<td>sblE</td>
<td>0105</td>
<td>60</td>
<td>117</td>
<td>Staphylokinase biosynthesis</td>
<td>+</td>
<td>395.7</td>
<td></td>
</tr>
<tr>
<td>copB</td>
<td>0146</td>
<td>102</td>
<td>156</td>
<td>Capsular polysaccharide biosynthesis</td>
<td>+</td>
<td>1187.2</td>
<td>291.6</td>
</tr>
<tr>
<td>adh</td>
<td>0154</td>
<td>108</td>
<td>162</td>
<td>Aldol dehydrogenase</td>
<td>+</td>
<td>136.8</td>
<td></td>
</tr>
<tr>
<td>pgb</td>
<td>0204</td>
<td>164</td>
<td>218</td>
<td>Fumarate acetyltransferase</td>
<td>+</td>
<td>1825.5</td>
<td>465.6</td>
</tr>
<tr>
<td>pgbL</td>
<td>0205</td>
<td>165</td>
<td>219</td>
<td>Fumarate-lyase activating enzyme</td>
<td>+</td>
<td>1300.8</td>
<td>231.8</td>
</tr>
<tr>
<td>mnuC</td>
<td>0264</td>
<td>216c</td>
<td>266</td>
<td>ABC transporter, unknown function</td>
<td>+</td>
<td>111.8</td>
<td></td>
</tr>
<tr>
<td>gicA</td>
<td>0432</td>
<td>321</td>
<td>357</td>
<td>Exotoxin, putative</td>
<td>+</td>
<td>383.8</td>
<td>115.3</td>
</tr>
<tr>
<td>gicC</td>
<td>0461</td>
<td>341</td>
<td>376</td>
<td>GicB synthase</td>
<td>+</td>
<td>396.3</td>
<td>213.0</td>
</tr>
<tr>
<td>nudC</td>
<td>0608</td>
<td>513</td>
<td>519</td>
<td>Virulence adhesin</td>
<td>+</td>
<td>173.6</td>
<td>312.1</td>
</tr>
<tr>
<td>nudH</td>
<td>0660</td>
<td>557</td>
<td>562</td>
<td>Allool dehydrogenase, Zn containing</td>
<td>+</td>
<td>2228.3</td>
<td>168.2</td>
</tr>
<tr>
<td>mnnC</td>
<td>0688</td>
<td>581c</td>
<td>587</td>
<td>Manganese ABC transporter</td>
<td>+</td>
<td>273.8</td>
<td></td>
</tr>
<tr>
<td>mnnA</td>
<td>0690</td>
<td>583c</td>
<td>589</td>
<td>Manganese ABC transporter</td>
<td>+</td>
<td>168.8</td>
<td></td>
</tr>
<tr>
<td>fnuC</td>
<td>0704</td>
<td>596</td>
<td>602</td>
<td>Ferrichrome transport ATP-binding protein</td>
<td>+</td>
<td>162.1</td>
<td></td>
</tr>
<tr>
<td>fnuB</td>
<td>0718</td>
<td>610</td>
<td>616</td>
<td>ABC transporter, unknown function</td>
<td>+</td>
<td>171.5</td>
<td>110.6</td>
</tr>
<tr>
<td>mnnB</td>
<td>0829</td>
<td>717</td>
<td>719</td>
<td>Thioester reductase</td>
<td>+</td>
<td>171.5</td>
<td>110.6</td>
</tr>
<tr>
<td>mnnD</td>
<td>1054</td>
<td>912</td>
<td>898</td>
<td>Enoyl-CoA hydratase/isomerase family</td>
<td>+</td>
<td>226.6</td>
<td></td>
</tr>
<tr>
<td>sldD</td>
<td>1142</td>
<td>996</td>
<td>979</td>
<td>Iron transport from heme</td>
<td>+</td>
<td>142.5</td>
<td>200.0</td>
</tr>
<tr>
<td>sldA</td>
<td>1145</td>
<td>999</td>
<td>982</td>
<td>Solute B</td>
<td>+</td>
<td>434.1</td>
<td>672.1</td>
</tr>
<tr>
<td>glpK</td>
<td>1320</td>
<td>1161</td>
<td>1141</td>
<td>Glycolaldehyde kinase</td>
<td>+</td>
<td>1115.5</td>
<td></td>
</tr>
<tr>
<td>glpC</td>
<td>1353</td>
<td>-</td>
<td>1157</td>
<td>ABC transporter, unknown function</td>
<td>+</td>
<td>117.1</td>
<td></td>
</tr>
<tr>
<td>glpB</td>
<td>1416</td>
<td>1236c</td>
<td>1213</td>
<td>ABC transporter, unknown function</td>
<td>+</td>
<td>117.1</td>
<td></td>
</tr>
<tr>
<td>glpD</td>
<td>1611</td>
<td>1426c</td>
<td>1383</td>
<td>Transcription regulatory homolog</td>
<td>+</td>
<td>118.0</td>
<td></td>
</tr>
<tr>
<td>dnaF</td>
<td>1637</td>
<td>1452c</td>
<td>1409</td>
<td>Chaperone protein</td>
<td>+</td>
<td>264.1</td>
<td></td>
</tr>
<tr>
<td>dnaH</td>
<td>1680</td>
<td>-</td>
<td>1452</td>
<td>Conserved protein</td>
<td>+</td>
<td>120.9</td>
<td></td>
</tr>
<tr>
<td>dnaG</td>
<td>1781</td>
<td>1500c</td>
<td>1552</td>
<td>Iron transport from heme</td>
<td>+</td>
<td>120.9</td>
<td></td>
</tr>
<tr>
<td>roc</td>
<td>1812</td>
<td>1622c</td>
<td>1583</td>
<td>Regulator of toxin, Rot</td>
<td>+</td>
<td>116.2</td>
<td></td>
</tr>
<tr>
<td>apf</td>
<td>1867</td>
<td>1671c</td>
<td>1629</td>
<td>Serine protease</td>
<td>+</td>
<td>111.0</td>
<td></td>
</tr>
<tr>
<td>apf2</td>
<td>1912</td>
<td>1788c</td>
<td>1631</td>
<td>Glucosamine-6-phosphate isomerase</td>
<td>+</td>
<td>118.6</td>
<td>176.5</td>
</tr>
<tr>
<td>mnuC</td>
<td>2092</td>
<td>1984c</td>
<td>1902</td>
<td>UDP-Nacetyl-1-carboxyvinyltransferase</td>
<td>+</td>
<td>116.4</td>
<td></td>
</tr>
<tr>
<td>mnuA</td>
<td>2144</td>
<td>2033c</td>
<td>1958</td>
<td>ABC transporter, unknown function</td>
<td>+</td>
<td>377.6</td>
<td></td>
</tr>
<tr>
<td>mnuB</td>
<td>2169</td>
<td>2060c</td>
<td>1981</td>
<td>Siderophore biosynthesis, putative</td>
<td>+</td>
<td>177.3</td>
<td></td>
</tr>
<tr>
<td>mnuB2</td>
<td>2171</td>
<td>2062</td>
<td>1983</td>
<td>Siderophore biosynthesis, putative</td>
<td>+</td>
<td>128.4</td>
<td></td>
</tr>
<tr>
<td>mnuC2</td>
<td>2321</td>
<td>2205e</td>
<td>2139</td>
<td>Glutathione-S-transferase/dihydroxyacid/dihydroxyacid reductase</td>
<td>+</td>
<td>129.9</td>
<td></td>
</tr>
<tr>
<td>mnuD</td>
<td>2325</td>
<td>2209</td>
<td>2123</td>
<td>Transcriptional regulator, Lysf, family</td>
<td>+</td>
<td>242.1</td>
<td>364.9</td>
</tr>
<tr>
<td>corA</td>
<td>2342</td>
<td>2226e</td>
<td>2137</td>
<td>Magnesium and cobalt transport protein</td>
<td>+</td>
<td>106.9</td>
<td></td>
</tr>
<tr>
<td>corB</td>
<td>2345</td>
<td>2238e</td>
<td>2158</td>
<td>Hypothetical protein</td>
<td>+</td>
<td>106.9</td>
<td></td>
</tr>
<tr>
<td>corD</td>
<td>2379</td>
<td>2261</td>
<td>2170</td>
<td>Conserved protein</td>
<td>+</td>
<td>116.2</td>
<td></td>
</tr>
<tr>
<td>corE</td>
<td>2385</td>
<td>2266</td>
<td>2175</td>
<td>HisP20 family protein</td>
<td>+</td>
<td>123.7</td>
<td></td>
</tr>
<tr>
<td>corF</td>
<td>2599</td>
<td>2457e</td>
<td>2369</td>
<td>Homolog to FeoB, Fe2+ transport protein</td>
<td>+</td>
<td>101.8</td>
<td></td>
</tr>
</tbody>
</table>

Proportion of genes (%) with significant level of expression (intensity >100%) on arrays

|       | 0.6 | 5.4 | 7.5 | 5.9 | 16.7 | 16.7 | 16.1 | 15.4 | 7.6 | 8.3 |

For Table IV, please refer to the full document for more details.
Table V: Mastitic milk samples in which the expression of SACOL0442 (upper panel) or SACOL0718 (lower panel) was detected on DNA array or by qPCR for 4 different S. aureus strains at 3 different time points in two cows.

<table>
<thead>
<tr>
<th>Gene SACOL0442</th>
<th>S. aureus strains</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cow</td>
<td>Day of infection</td>
</tr>
<tr>
<td>307</td>
<td>8</td>
</tr>
<tr>
<td>307</td>
<td>10</td>
</tr>
<tr>
<td>307</td>
<td>14</td>
</tr>
<tr>
<td>5325</td>
<td>10</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Gene SACOL0718</th>
<th>S. aureus strains</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cow</td>
<td>Day of infection</td>
</tr>
<tr>
<td>307</td>
<td>8</td>
</tr>
<tr>
<td>307</td>
<td>10</td>
</tr>
<tr>
<td>307</td>
<td>14</td>
</tr>
<tr>
<td>5325</td>
<td>10</td>
</tr>
</tbody>
</table>

ND, not detected.
Table VI: Names and annotations for a selection of 11 genes or operons taken from the 43 genes found to be strongly expressed in microarray experiments (Table III above) and for which expression had never been reported when *S. aureus* was grown in a different mammalian environment or in surrogate cultivation media such as in human neutrophils *in vitro*, in iron-restricted media or in milk *in vitro* or when *S. aureus* mastitis isolates were grown *in vitro*. Annotations are compared for representatives of the *S. aureus* sequenced genomes [MRSA COL, N315, RF122 [a mastitis isolate], USA300, MSSA476].

<table>
<thead>
<tr>
<th>Gene SACOL</th>
<th>COL</th>
<th>N315</th>
<th>RF122</th>
<th>USA300</th>
<th>MSSA476</th>
</tr>
</thead>
<tbody>
<tr>
<td>0442</td>
<td>enterotoxin, putative</td>
<td>similar to exotoxin 2</td>
<td>hypothetical protein</td>
<td>enterotoxin, putative</td>
<td>putative exported protein</td>
</tr>
<tr>
<td>0718-0720</td>
<td>ABC transporter, ATP-binding protein and permease</td>
<td>ABC transporter, ATP-binding protein and permease</td>
<td>ABC transporter, ATP-binding protein and permease</td>
<td>ABC transporter, ATP-binding protein and permease</td>
<td>putative ABC transporter protein and permease</td>
</tr>
<tr>
<td>2365</td>
<td>lipoprotein, putative</td>
<td>hypothetical protein, similar to TpgX protein</td>
<td>lipoprotein, putative</td>
<td>lipoprotein, putative</td>
<td>lipoprotein, putative</td>
</tr>
<tr>
<td>0029</td>
<td>HMG-CoA synthase, truncation</td>
<td>probable HMG-CoA synthase</td>
<td>-</td>
<td>conserved hypothetical protein</td>
<td>-</td>
</tr>
<tr>
<td>1416</td>
<td>peptide ABC transporter, permease protein, putative</td>
<td>oligopeptide transporter membrane permease domain (opp2c)</td>
<td>probable oligopeptide membrane permease</td>
<td>peptide ABC transporter, permease protein</td>
<td>putative oligopeptide transport system permease</td>
</tr>
<tr>
<td>1944</td>
<td>conserved hypothetical protein</td>
<td>conserved hypothetical protein</td>
<td>conserved hypothetical protein</td>
<td>conserved hypothetical protein</td>
<td>putative membrane protein</td>
</tr>
<tr>
<td>1611</td>
<td>transcriptional regulator, Fur family</td>
<td>ferric uptake regulator homolog</td>
<td>zinc-specific metalloregulator (fur)</td>
<td>ferric uptake regulation protein (fur)</td>
<td>zinc-specific metalloregulatory protein</td>
</tr>
<tr>
<td>2599</td>
<td>conserved domain protein</td>
<td>hypothetical protein, similar to ferrous iron transporter</td>
<td>probable membrane protein</td>
<td>transporter gate domain protein</td>
<td>putative membrane protein</td>
</tr>
<tr>
<td>1353</td>
<td>ABC transporter, permease protein, putative</td>
<td>hypothetical protein, similar to ABC transporter integral</td>
<td>-</td>
<td>ABC transporter, permease protein</td>
<td>putative membrane protein</td>
</tr>
<tr>
<td>0264</td>
<td>ABC transporter, ATP-binding protein</td>
<td>conserved hypothetical protein</td>
<td>probable ABC transporter ATP binding protein</td>
<td>ABC transporter, ATP-binding protein</td>
<td>putative ABC transporter ATP-binding protein</td>
</tr>
</tbody>
</table>

**EXAMPLE 6: Attenuation of S. aureus virulence**

[00147] Mutants for genes SACOL0442 and SACOL0720 were produced by gene replacement for mutant
Δ442a, (Mitchell et al., 2008) and by intron insertion for mutants Δ442b and Δ720 (TargeTron Gene Knockout System, Sigma Aldrich (Chen et al., 2007)). The mutants were carried out in the S. aureus parental strain ATCC 29213 that could be easily transformed by electroporation. For creating mutant Δ442a, a 223-pb fragment of gene SACOL0442 in strain ATCC 29213 was deleted and replaced by insertion of the 1300-bp erythromycin resistance gene ermA between positions 188 and 411 of the nucleotide sequence of SACOL0442. For creating mutants Δ442b and Δ720, the Group II intron (fragment size of approx. 2Kb) from the TargeTron Gene Knockout System inserted itself into the target chromosomal gene between nucleotide positions 45 and 46 for gene SACOL0442 and between positions 803 and 804 for gene SACOL0720, respectively. Prior to experimental IMI with the mutants, their growth, compared to the parental strain, was evaluated in vitro in freshly collected milk (FIG. 10A). No difference between the growth of the 3 mutants and the parental strain was observed. Eight healthy lactating cows were then inoculated intramammary with 25-250 CFU of the parental strain and of the three mutants and the infection was followed for 21 days. Each of the 8 cows was infused with the four S. aureus strains and the position of each strain in the four quarters alternated between the animals. Milk of the infected quarters was collected and the determination of viable bacterial counts was performed. Each of the three mutants showed a significant reduction of bacterial counts in milk compared to the parental strain (FIG. 10B). The virulence of each of the mutants is thus attenuated compared to the parental strain. These results demonstrate the importance of the expression of genes SACOL0442 and SACOL0718-720 for the infection process. Antibodies directed toward their gene products should greatly impair the ability of S. aureus to cause bovine IMI (see Examples 8-11). Besides, attenuated bacterial strains have been used as live vaccines (for example, PRIORIX® is a combined measles, mumps and rubella, live, attenuated vaccine; VARILRIX®, is a varicella virus vaccine, live, attenuated (Oka-strain); BCG, is a vaccine for tuberculosis using the attenuated live bacteria Mycobacterium bovis) and thus, the use of the mutants described herein (e.g., Δ442a, Δ442b and Δ720 mutants) for immunization of cows is another approach to stimulate immunity and to protect the animal against a future infection by a fully virulent strain. Hence, a mutation/deletion of any of the genes identified here as being expressed by S. aureus during bovine IMI may attenuate virulence and such resulting attenuated mutants could be used in a live attenuated vaccine method for immunization.

EXAMPLE 7: Relatedness of some S. aureus genes and proteins

[00148] FIG. 11 shows the nucleic acid (FIGs. 11A-11C) and amino acid (FIG. 11D) alignments of vaccine components SACOL0442 and SACOL0720 of all Staphylococcus aureus sequenced strains, including the strain RF122 isolated from bovine mastitis. The sequences for SACOL0442 and SACOL0720 show a similarity of about 94 to 100% among the compared strains and are thus considered as highly conserved among these representative S. aureus strains. The fact that these genes/proteins could be found in strains isolated from multiple sources strengthens their potential as targets (e.g., vaccine candidates) as it would target most S. aureus udder infections (IMI infections).
Similarly, Table VII below shows the percentage of similarity and identity of the amino acid sequences corresponding to some of the S. aureus genes expressed in vivo during bovine IMI (Table VI above) for some representatives of the sequenced S. aureus genomes. Again, a high degree of similarity and identity was observed (> 92.7%), confirming that these genes and encoded proteins represent good targets for protection against multiple S. aureus strains. There is also about 40% identity and about 60% similarity between the amino acid sequence of SACOL0442 and that of other putative exotoxins such as SACOL0469, SACOL0470, SACOL0472 and SACOL0473 (also known as SA0383 exotoxin 7 [set7], SA0384 exotoxin 8 [set8], SA0385 exotoxin 9 [set9] and SA0389 exotoxin 13 [set13] in strain N315, respectively) (www.jcvi.org). Although these components are not the same genes or proteins, it is possible to find common protein regions, fragments or epitopes for use in vaccines with broader applications and thus aim at the prevention and control of many types of S. aureus infections in addition to IMI. Some genetically related bacterial species or genus such as Staphylococcus epidermidis, Streptococcus, Listeria and others may also have homologs of these genes or proteins. Thus it may also be possible to find common protein regions, fragments or epitopes for use in vaccines with broader applications aimed at the prevention and control of many types of bacterial infections. For example, the S. aureus gene SACOL1416 shows about 30% sequence homology to Streptococcus agalactiae gene SAJ1496 and Listeria gene EW10119 and the S. aureus gene SACOL0718 shows about 40-50% sequence homologies to Streptococcus agalactiae gene SAJ1013 and Listeria gene EW1764 (www.jcvi.org). Noteworthy, Streptococcus agalactiae is also a pathogen involved in IMI and Listeria is a pathogen often contaminating milk products (Bradley, 2002; Jayarao et al., 2001).

Table VII: Percentage similarity (%sim) and identity (%ide) of the amino acid sequences corresponding to some of the S. aureus genes expressed in vivo during bovine IMI (Table IV above) for some representatives of the sequenced Staphylococcus aureus genomes (strains N315, RF122, USA300-FPR3757 and MSSA476 compared to the MRSA COL strain).

<table>
<thead>
<tr>
<th>Gene</th>
<th>COL</th>
<th>N315</th>
<th>RF122</th>
<th>USA300</th>
<th>MSSA476</th>
</tr>
</thead>
<tbody>
<tr>
<td>SACOL</td>
<td>% ide</td>
<td>% sim</td>
<td>% ide</td>
<td>% sim</td>
<td>% ide</td>
</tr>
<tr>
<td>0442</td>
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<td>94.6</td>
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</tr>
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<td>0029</td>
<td>100</td>
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<td>100</td>
<td>100</td>
<td>-</td>
</tr>
<tr>
<td>1416</td>
<td>100</td>
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<td>99.6</td>
<td>100</td>
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<td>100</td>
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<td>2144</td>
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<td>94.6</td>
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<td>92.7</td>
</tr>
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<td>-</td>
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<tr>
<td>0264</td>
<td>100</td>
<td>100</td>
<td>99.5</td>
<td>99.5</td>
<td>99.1</td>
</tr>
</tbody>
</table>

EXAMPLE 8: Preparation of vaccines
[00150] Bioinformatic software provided sequence and structural information on proteins SACOL0718, SACOL0720 and SACL0442 that were useful for preparing such proteins in vaccine compositions (FIG. 14). For example, protein SACOL0442 was determined to be extracellular (secreted bacterial protein). The cellular localization of protein SACOL0718 and its amino acid composition showing 45% of hydrophobic amino acids suggest that it is associated with the bacterial cytoplasmic membrane. Protein SACOL0720 was also predicted to be associated with the bacterial cytoplasmic membrane. Protein SACOL0720 contains 10 transmembrane helices and it was possible to identify a region exposed at the surface of the bacterium.

[00151] For vaccine preparation, most of the SACOL0442 protein was used (polypeptide comprising amino acids 44 to 159 in the sequence depicted at FIG. 11D (the full sequence of SACOL0442 is set forth in SEQ ID NO: 37)) and as such, excluded its transport signal (amino acids 1 to 35). The predicted extracellular region of protein SACOL0720 (α-annotated amino acids 309 to 508 in the sequence depicted at FIG. 14D (the full sequence of SACOL0720 is set forth in SEQ ID NO: 62)) was also used in a vaccine composition. In the same way, the extracellular region of the SACOL1781 protein (polypeptide comprising amino acids 41 to 895 of protein IsdH (the full sequence of SACOL1781 is set forth in SEQ ID NO: 76), see also Figure 18; (www.jcvi.org)) was used as an additional vaccine component.

EXAMPLE 9: Immunogenicity of vaccine of the present invention in mice

[00152] Each of the purified polypeptides derived from SACOL0442, SACOL0720 and SACOL1781, independently or all together in combination, were tested for antibody production in mice. Antibody titers in sera of mice vaccinated with SACOL042, SACOL0720 and SACOL1781 (in the presence of the adjuvant Emulsigen®-D) are shown in FIG. 15. One group of animals (10 animals per group) twice received saline, one received 2 injections of 100 μg of polypeptide SACOL0442, one group received 2 injections of 100 μg of polypeptide SACOL0720, one group received 2 injections of 100 μg of polypeptide SACOL1781, and one group received 2 injections of 100 μg of each three polypeptides SACOL0442, SACOL0720 and SACOL1781 premixed together in a combination. The 2 injections were performed 3 weeks apart, and 3 weeks after the second immunization mice were euthanized and blood collected for the determination of antibody titers by ELISA. Results from FIG. 15 show that the polypeptides used for immunization were indeed immunogenic (i.e., able to stimulate an immune response and antibody production). Results also show that the combination of polypeptides SACOL0442 and SACOL0720 to another antigen such as SACOL1781 did not reduce or alter antibody production compared to that measured when SACOL0442 and SACOL0720 were injected independently. Such a vaccine composition (SACOL0442 and/or SACOL0720 with or without other antigens) is thus a practical useful approach for raising antibodies against multiple antigens of interest. Such a combination vaccine could then provide protection against bovine IMI as well as protection against other diseases that may require other vaccine components for immunization. EXAMPLE 10: Immunogenicity of vaccine of the present invention in cows

[00154] Immunizations were also performed in dairy cows. Antibody titers in sera of cows vaccinated with the
polypeptide fragments of SACOL0442, SACOL0720 described in Example 8 (in the presence of the adjuvant Emulsigen®-D) are shown in FIG. 16. One group of animals (5 animals per group) received 2 injections of saline, one group received 2 injections of 300 μg of polypeptide SACOL0442, one group received 2 injections of 300 μg of polypeptide SACOL0720 and one group received 2 injections of 300 μg of each of the two polypeptides SACOL0442 and SACOL0720 premixed together in a combination. The 2 injections were performed 10 weeks apart, and blood was collected for the determination of total IgG antibody titers by ELISA. Results from FIG. 16 show that the polypeptides used for immunization were also highly immunogenic in cows and that combining the antigens for immunization also does not significantly modify the immune response compared to that obtained using individual antigens. The determination of the isotypes is presented in FIG 17. Immunization of cows leads to the induction of an immune response with the presence of both IgG1 and IgG2. Isotype IgG2 is known to be helpful for opsonization of S. aureus and to increase bovine neutrophil functions (Guidry et al., 1993; Barrio et al., 2003). It is known that using different types of adjuvant and/or vaccine administration vehicles or routes can modulate the resulting balance of IgG1 and IgG2 for specific needs (Spickler and Roth, 2003). The capacity of bovine antibodies induced by immunization to bind their target proteins (e.g., SACOL0720) at the bacterial surface was evaluated. Bacteria grown for 8 hours in freshly collected milk were used for this assay as this condition was shown to allow expression of SACOL0718-720 as measured by qPCR (FIG. 8). Evaluation of antibody binding on the bacterial surface was done using flow cytometry as described in “Example 1 materials and methods”. It was found that 22.2% more bacteria were bound by labeled antibodies in the presence of the bovine immune serum raised against SACOL0720 in comparison to the labeling obtained in the presence of the control pre-immune serum. This demonstrates that bovine antibodies induced against SACOL0720 are able to bind to the protein at the surface of the bacteria. Such antibody binding (opsonization) is known to help neutrophils phagocytic and killing activity (Guidry et al., 1993; Barrio et al., 2003).

**EXAMPLE 11: Epitopes of interest**

[00156] As an alternative of using the entire proteins or a long region of the polypeptides of interest for vaccination, it is also possible to specifically used small peptide regions predicted to be recognized by the B or T cells from the mammalian immune system. Identification of the B cell epitopes (that is to say short amino acid sequences that will be recognized by the immune system and able to induce the production of antibodies by the B cells) among some of the proteins of interest such as SACOL0442 and SACOL0720 are shown in Table VIII below. For each protein, the predicted B cell epitopes are presented with their position in the protein sequence. The score was obtained from 4 distinct programs: BCPred Predictions, AAP Predictions, FBCPred Predictions and ABCPred.

[00157] Similarly, computer driven algorithms can also be used to facilitate the identification of T cell epitopes (that is to say short amino acid sequences that will be recognized by the immune system and able to induce a cellular response by T cells) for use as vaccines against Staphylococcus aureus infection. The proteins of interest can be subjected to analysis by the Epimatrix™ system to identify putative T cell epitopes.
technique divides the total sequence of the antigen into fragments of 9 amino acids overlapping by 8 amino acids. This pool of 9-mer is screened for predicted affinity against a group of known MHC class I and class II alleles. The resulting scores can be used to rate putative epitopes on a common scale which can then be tested in vitro. The technique is applicable to any animal for which a sufficient knowledge of MHC sequences is available. (De Groot et al., 2008)

[00158] The B or T cell epitopes can therefore be used in vaccine compositions alone or in combination with an assemblage of proteins, peptides or other epitopes. In addition, any B or T cell epitopes as well as any other epitopes can be presented in a contiguous sequence (such as in a protein fusion approach) by using genetic and protein engineering methods.

Table VIII. Identification of B cell epitopes among some of the proteins of interest. (A) SACOL0442 and (B) SACOL0720. For each protein, the predicted B cell epitopes are presented with their position in the protein sequence and the prediction score they obtained using 4 distinct softwares: BCPred Predictions, AAP Predictions, FBCPred Predictions and ABCPred.

(A) SACOL0442

<table>
<thead>
<tr>
<th>Potential B cell epitope</th>
<th>Position into the sequence</th>
<th>score</th>
</tr>
</thead>
<tbody>
<tr>
<td>TFGIYPKADASTQN (SEQ ID NO: 17)</td>
<td>26</td>
<td>0.840</td>
</tr>
<tr>
<td>KDTINGKSNSKSRNW (SEQ ID NO: 18)</td>
<td>72</td>
<td>0.848</td>
</tr>
<tr>
<td>KDGKHYTLESHKELQ (SEQ ID NO: 19)</td>
<td>159</td>
<td>1.000</td>
</tr>
</tbody>
</table>

(B) SACOL0720

<table>
<thead>
<tr>
<th>Potential B cell epitope</th>
<th>Position into the sequence</th>
<th>score</th>
</tr>
</thead>
<tbody>
<tr>
<td>QFGFDLKHKKDLA (SEQ ID NO: 20)</td>
<td>468</td>
<td>0.981</td>
</tr>
<tr>
<td>TIKKDKQKANGASL (SEQ ID NO: 21)</td>
<td>325</td>
<td>0.898</td>
</tr>
<tr>
<td>KDINYYFMTDVDL (SEQ ID NO: 22)</td>
<td>428</td>
<td>0.890</td>
</tr>
<tr>
<td>DVLGPGTFVLND (SEQ ID NO: 23)</td>
<td>436</td>
<td>0.993</td>
</tr>
</tbody>
</table>

EXAMPLE 12: Use of S. aureus genes expressed during IMI as diagnostic tools

[00159] The diagnosis of S. aureus IMI is difficult and requires time. Traditionally, milk samples are taken and shipped to a microbiology laboratory where cultivation of S. aureus is achieved using various artificial growth media. Following growth and if growth occur (usually 24h after sample arrival), the microorganism need to be identified as S. aureus among other possible pathogens by a variety of biochemical tests which could take up an additional 24h. For milk producers, this delay represents a serious economic loss as cows suspected to have acquired an IMI need to be removed from the milk production herd while cows not tested for S. aureus but that have subclinical IMI may continue to contaminate the bulk milk tank. It would thus be highly desirable to develop a novel tool for rapid detection of S. aureus in milk to permit a rapid intervention by milk producers or veterinarians.
[00160] As an alternative of using traditional microbial cultures to identify S. aureus in milk samples of cows with or without clinical signs of IMI and mastitis, the products of the S. aureus genes identified as expressed during IMI (either the messenger RNA, the protein or the metabolic product subsequent to the protein activity) may be used as diagnostic tools. Indeed, the detection of such specific products, for example in milk, blood or biopsies, would indicate the presence of S. aureus. Since such products are strongly expressed during IMI, their detection would also strongly correlate with this specific type of infection.

[00161] For example, detection of the putative exotoxin SACOL0442 that is secreted in the extracellular milieu, i.e., in milk during mastitis, would be a strong indication that the cow is infected by S. aureus since the gene is only expressed during IMI. The detection of the putative exotoxin SACOL0442 can be easily achieved by the use of a specific antibody and an ELISA technique or a dip stick approach or the like and the signal of detection can be easily amplified by a variety of signal amplification techniques. Such techniques could rapidly be performed by the microbiology laboratory or even on-farm by the milk producer himself, hence gaining valuable time. Alternatively, detection of messenger RNA (mRNA) from the genes expressed during IMI would also indicate the presence of S. aureus in milk. Detection of mRNA is possible after its release from bacteria by a cell lysis step, copying mRNA into complementary DNA by reverse transcription and by PCR amplification.
REFERENCES


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CLAIMS

1. A method for preventing and/or treating Staphylococcal intramammary infection (IMI) in a mammal, said method comprising administrating to said mammal an effective amount of at least one agent, wherein said agent is:
   (a) a polypeptide encoded by a gene, wherein said gene is SACOL0029, SACOL0264, SACOL0442, SACOL0718, SACOL0720, SACOL1353, SACOL1416, SACOL1611, SACOL1944, SACOL2144, SACOL2365 or SACOL2599, based on the gene nomenclature from the Staphylococcus aureus COL (SACOL) genome set forth in NCBI Reference Sequence NC_002951.2;
   (b) a polypeptide encoded by a gene from a same operon as one of the genes of (a);
   (c) an immunogenic fragment of (a) or (b);
   (d) an immunogenic variant of any one of (a) to (c);
   (e) a nucleic acid encoding the polypeptide of any one of (a) to (d); or
   (f) any combination of (a) to (e).

2. The method of claim 1, wherein said gene from the same operon as one of the genes of (a) is SACOL0720, and wherein said gene of (a) is SACOL0718.

3. The method of claim 1, wherein said gene is SACOL0442, SACOL0718, SACOL0720 or any combination thereof.

4. The method of claim 3, wherein said gene is SACOL0442, SACOL0720 or both.

5. The method of any one of claims 1 to 4, comprising administrating to said mammal an effective amount of a combination of agents.

6. The method of claim 5, wherein said combination of agents comprises:
   (i) a first agent, wherein said first agent is (a) a polypeptide encoded by SACOL0442, (b) an immunogenic fragment of (a); (c) an immunogenic variant of (a) or (b); (d) a nucleic acid encoding the polypeptide of any one of (a) to (c); or (e) any combination of (a) to (d); and
   (ii) a second agent, wherein said second agent is (a) a polypeptide encoded by SACOL0720, (b) an immunogenic fragment of (a); (c) an immunogenic variant of (a) or (b); (d) a nucleic acid encoding the polypeptide of any one of (a) to (c); or (e) any combination of (a) to (d).

7. The method of claim 3, wherein said gene is SACOL0442 and wherein said immunogenic fragment comprises one or more of the following amino acid sequences: TFGIYPKADASTQN (SEQ ID NO: 17), KDTINGKSNKSRNW (SEQ ID NO: 18) or KDGGKYTEHSHKELQ (SEQ ID NO: 19).
8. The method of claim 4, wherein said gene is SACOL0720 and wherein said immunogenic fragment comprises one or more of the following amino acid sequences: QFGFDLKHKKDALA (SEQ ID NO: 20), TIKDQKANQLAS (SEQ ID NO: 21), KDINKYFMTDVL (SEQ ID NO: 22) or DVLGGPTFLNLD (SEQ ID NO: 23).

9. The method of any one of claims 1 to 8, wherein said Staphylococcal intramammary infection is caused by one or more Staphylococcus aureus strains.

10. The method of any one of claims 1 to 9, further comprising administering to said mammal an effective amount of an adjuvant.

11. The method of claim 10, wherein said adjuvant is alum, Emulsigen™ D, cyclic-diguanosine-5'-monophosphate (c-di-GMP), polyphosphasine or pathogen-associated molecular patterns (PAMPS).

12. The method of claim 11, wherein said PAMPS is unmethylated dinucleotides (CpG) or microbial polysaccharides.

13. The method of any one of claims 10 to 12, wherein said (i) agent, (ii) adjuvant, or both (i) and (ii) are comprised in a pharmaceutical composition.

14. The method of claim 13, wherein said pharmaceutical composition further comprises one or more pharmaceutically acceptable excipients.

15. The method of any one of claims 1 to 14, wherein said mammal is a cow.

16. The method of claim 15, wherein said IMI is associated with bovine mastitis.

17. Use of an agent, wherein said agent is:

   (a) a polypeptide encoded by a gene, wherein said gene is SACOL0029, SACOL0264, SACOL0442, SACOL0718, SACOL0720, SACOL1353, SACOL1416, SACOL1611, SACOL1944, SACOL2144, SACOL2366 or SACOL2599 based on the gene nomenclature from the Staphylococcus aureus COL (SACOL) genome set forth in NCBI Reference Sequence NC_002951.2;

   (b) a polypeptide encoded by a gene from a same operon as one of the genes of (a);

   (c) an immunogenic fragment of (a) or (b);

   (d) an immunogenic variant of any one of (a) to (c);

   (e) a nucleic acid encoding the polypeptide of any one of (a) to (d); or

   (f) any combination of (a) to (e),

for preventing and/or treating Staphylococcal intramammary infection (IMI) in a mammal.
18. Use of at least one agent, wherein said agent is:
   (a) a polypeptide encoded by a gene, wherein said gene is SACOL0029, SACOL0264, SACOL0442, SACOL0718, SACOL0720, SACOL1353, SACOL1416, SACOL1611, SACOL1944, SACOL2144, SACOL2365 or SACOL2599, based on the gene nomenclature from the *Staphylococcus aureus* COL (SACOL) genome set forth in NCBI Reference Sequence NC_002951.2;
   (b) a polypeptide encoded by a gene from a same operon as one of the genes of (a);
   (c) an immunogenic fragment of (a) or (b);
   (d) an immunogenic variant of any one of (a) to (c);
   (e) a nucleic acid encoding the polypeptide of any one of (a) to (d); or
   (f) any combination of (a) to (e),

for the preparation of a medicament for preventing and/or treating *Staphylococcal* intramammary infection (IMI) in a mammal.

19. The use of claim 17 or 18, wherein said gene from the same operon as one of the gene of (a) is SACOL0720, and wherein said gene of (a) is SACOL0718.

20. The use of claim 17 or 18, wherein said gene is SACOL0442, SACOL0718, SACOL0720 or a combination thereof.

21. The use of claim 20, wherein said gene is SACOL0442, SACOL0720 or both.

22. The use of any one of claims 17 to 21, comprising the use of a combination of agents.

23. The use of claim 22, wherein said combination of agents comprises:

   (i) a first agent, wherein said first agent is (a) a polypeptide encoded by SACOL0442, (b) an immunogenic fragment of (a); (c) an immunogenic variant of (a) or (b); (d) a nucleic acid encoding the polypeptide of any one of (a) to (c); or (e) any combination of (a) to (d); and

   (ii) a second agent, wherein said second agent is (a) a polypeptide encoded by SACOL0720, (b) an immunogenic fragment of (a); (c) an immunogenic variant of (a) or (b); (d) a nucleic acid encoding the polypeptide of any one of (a) to (c); or (e) any combination of (a) to (d).

24. The use of claim 21, wherein said gene is SACOL0442 and wherein said immunogenic fragment comprises one or more of the following amino acid sequences: TFGiYPKADASTQN (SEQ ID NO: 17), KDTINGKNSRNRW (SEQ ID NO: 18) or KDGGKYTiEShKELQ (SEQ ID NO: 19).

25. The use of claim 21, wherein said gene is SACOL0720 and wherein said immunogenic fragment
comprises one or more of the following amino acid sequences: QFGFDLKHKKDALA (SEQ ID NO: 20), TIKDQKQANQLAS (SEQ ID NO: 21), KDINKIFMTDVL (SEQ ID NO: 22) or DVDLGPGPTFVLND (SEQ ID NO: 23).

26. The use of any one of claims 17 to 25, wherein said Staphylococcal intramammary infection is caused by one or more Staphylococcus aureus strains.

27. The use of any one of claims 17 to 26, further comprising administering to said mammal an effective amount of an adjuvant.

28. The use of claim 27, wherein said adjuvant is alum, Emulsigen™ D, cyclic-diguanosine-5'-monophosphate (c-di-GMP), polyphosphasine or pathogen-associated molecular patterns (PAMPS).

29. The use of claim 28, wherein said PAMPS is unmethylated dinucleotides (CpG) or microbial polysaccharides.

30. The use of any one of claims 27 to 29, wherein said (i) agent, (ii) adjuvant, or both (i) and (ii), are comprised in a pharmaceutical composition.

31. The use of claim 30, wherein said pharmaceutical composition further comprises one or more pharmaceutically acceptable excipients.

32. The use of any one of claims 27 to 31, wherein said mammal is a cow.

33. The use of claim 32, wherein said IMI is associated with bovine mastitis.

34. A pharmaceutical composition for preventing and/or treating Staphylococcal intramammary infection (IMI) in a mammal, said composition comprising:

(a) at least one agent, wherein said agent is:

(i) a polypeptide encoded by a gene, wherein said gene is SAC0L0029, SAC0L0264, SAC0L0442, SAC0L0718, SAC0L0720, SAC0L1353, SAC0L1416, SAC0L1611, SAC0L1944, SAC0L2144, SAC0L2365 or SAC0L2599, based on the gene nomenclature from the Staphylococcus aureus COL (SACOL) genome set forth in NCBI Reference Sequence NC_002951.2;

(ii) a polypeptide encoded by a gene from a same operon as one of the genes of (a);

(iii) an immunogenic fragment of (i) or (ii);

(iv) an immunogenic variant of any one of (i) to (iii);

(v) a nucleic acid encoding the polypeptide of any one of (i) to (iv); or

(vi) any combination of (i) to (v).
35. The pharmaceutical composition of claim 34, wherein said gene from the same operon as one of the
gene of (a) is SACOL0720, and wherein said gene of (a) is SACOL0718.

36. The pharmaceutical composition of claim 34, wherein said gene is SACOL0442, SACOL0718,
SACOL0720, or any combination thereof.

37. The pharmaceutical composition of claim 34, wherein said gene is SACOL0442, SACOL0720 or both.

38. The pharmaceutical composition of any one of claims 34 to 37, comprising a combination of agents.

39. The pharmaceutical composition of claim 38, wherein said combination of agents comprises:

   (i) a first agent, wherein said first agent is (a) a polypeptide encoded by SACOL0442, (b) an
       immunogenic fragment of (a); (c) an immunogenic variant of (a) or (b); (d) a nucleic acid
       encoding the polypeptide of any one of (a) to (c); or (e) any combination of (a) to (d); and

   (ii) a second agent, wherein said second agent is (a) a polypeptide encoded by
       SACOL0720, (b) an immunogenic fragment of (a); (c) an immunogenic variant of (a) or (b); (d) a
       nucleic acid encoding the polypeptide of any one of (a) to (c); or (e) any combination of (a) to
       (d).

40. The pharmaceutical composition of claim 37, wherein said gene is SACOL0442 and wherein said
    immunogenic fragment comprises one or more of the following amino acid sequences:
    TFGIYPKADASTQN (SEQ ID NO: 17), KDIINGKSNKSRNW (SEQ ID NO: 18) or
    KDGGKYKTESHELQ (SEQ ID NO: 19).

41. The pharmaceutical composition of claim 37, wherein said gene is SACOL0720 and wherein said
    immunogenic fragment comprises one or more of the following amino acid sequences:
    QFGFDLKHKKDALA (SEQ ID NO: 20), TKDOQKANQLAS (SEQ ID NO: 21), KDINKYFMTDVDL
    (SEQ ID NO: 22) or DVLGGPTFVLND (SEQ ID NO: 23).

42. The pharmaceutical composition of any one of claims 34 to 41, wherein said Staphylococcal
    intramammary infection is caused by one or more Staphylococcus aureus strains.

43. The pharmaceutical composition of any one of claims 34 to 42, further comprising an adjuvant.

44. The pharmaceutical composition of claim 43, wherein said adjuvant is alum, Emulsigen™ D, cyclic-
    diguanosine-5′-monophosphate (c-di-GMP), polyphosphasine or pathogen-associated molecular
    patterns (PAMPs).

45. The pharmaceutical composition of claim 44, wherein said PAMPS is unmethylated dinucleotides (CpG)
or microbial polysaccharides.

46. The pharmaceutical composition of any one of claims 34 to 45, wherein said pharmaceutical composition further comprises one or more pharmaceutically acceptable excipients.

47. The pharmaceutical composition of any one of claims 34 to 46, wherein said mammal is a cow.

48. The pharmaceutical composition of claim 47, wherein said IMI is associated with bovine mastitis.

49. A pharmaceutical composition comprising:

(a) at least one agent, wherein said agent is:

(i) a polypeptide encoded by a gene, wherein said gene is SACOL029, SACOL0264, SACOL0442, SACOL0718, SACOL0720, SACOL1353, SACOL1416, SACOL1611, SACOL1944, SACOL2144, SACOL2365 or SACOL2599, based on the gene nomenclature from the *Staphylococcus aureus* COL (SACOL) genome set forth in NCBI Reference Sequence NC_002951.2;

(ii) a polypeptide encoded by a gene from a same operon as one of the genes of (a);

(iii) an immunogenic fragment of (i) or (ii);

(iv) an immunogenic variant of any one of (i) to (iii);

(v) a nucleic acid encoding the polypeptide of any one of (i) to (iv); or

(vi) any combination of (i) to (v);

and

(b) a pharmaceutically acceptable excipient.

50. The pharmaceutical composition of claim 49, wherein said gene from the same operon as one of the gene of (a) is SACOL0720, and wherein said gene of (a) is SACOL0718.

51. The pharmaceutical composition of claim 49, wherein said gene is SACOL0442, SACOL0718, SACOL0720, or any combination thereof.

52. The pharmaceutical composition of claim 51, wherein said gene is SACOL0442, SACOL0720 or both.

53. The pharmaceutical composition of any one of claims 49 to 52, comprising a combination of agents.

54. The pharmaceutical composition of claim 49, wherein said combination of agents comprises:

(i) a first agent, wherein said first agent is (a) a polypeptide encoded by SACOL0442, (b) an immunogenic fragment of (a); (c) an immunogenic variant of (a) or (b); (d) a nucleic acid encoding the polypeptide of any one of (a) to (c); or (e) any combination of (a) to (d); and
(ii) a second agent, wherein said second agent is (a) a polypeptide encoded by SACOL0720, (b) an immunogenic fragment of (a); (c) an immunogenic variant of (a) or (b); (d) a nucleic acid encoding the polypeptide of any one of (a) to (c); or (e) any combination of (a) to (d).

55. The pharmaceutical composition of claim 49, wherein said gene is SACOL0442 and wherein said immunogenic fragment comprises one or more of the following amino acid sequences: TFGIYPKADASTQN (SEQ ID NO: 17), KDTINGKSNKSRNW (SEQ ID NO: 18) or KDGGKYTELISHKELO (SEQ ID NO: 19).

56. The pharmaceutical composition of claim 49, wherein said gene is SACOL0720 and wherein said immunogenic fragment comprises one or more of the following amino acid sequences: QFDFDLKHKKDLAL (SEQ ID NO: 20), TIKDOQKANQLAS (SEQ ID NO: 21), KDINKIYFMTDVL (SEQ ID NO: 22) or DVDDLGGPTFVLND (SEQ ID NO: 23).

57. The pharmaceutical composition of any one of claims 49 to 56, further comprising an adjuvant.

58. The pharmaceutical composition of claim 57, wherein said adjuvant is alum, Emulsigen™ D, cyclic-diguanosine-5'-monophosphate (c-di-GMP), polyphosphasine or pathogen-associated molecular patterns (PAMPS).

59. The pharmaceutical composition of claim 58, wherein said PAMPS is unmethylated dinucleotides (CpG) or microbial polysaccharides.

60. The pharmaceutical composition of any one of claims 49 to 59, wherein said (i) agent, (ii) adjuvant, or both (i) and (ii), are comprised in a pharmaceutical composition.

61. The pharmaceutical composition of claim 60, wherein said pharmaceutical composition further comprises one or more pharmaceutically acceptable excipients.

62. A kit for the prevention and/or treatment of Staphylococcal IMI, comprising (a) at least one agent, wherein said agent is:

(i) a polypeptide encoded by a gene, wherein said gene is SACOL0029, SACOL0264, SACOL0442, SACOL0718, SACOL0720, SACOL1353, SACOL1416, SACOL1611, SACOL1944, SACOL2144, SACOL2365 or SACOL2599, based on the gene nomenclature from the Staphylococcus aureus COL (SACOL) genome set forth in NCBI Reference Sequence NC_002951.2;

(ii) a polypeptide encoded by a gene from a same operon as one of the genes of (a);

(iii) an immunogenic fragment of (i) or (ii);
(iv) an immunogenic variant of any one of (i) to (iii);
(v) a nucleic acid encoding the polypeptide of any one of (i) to (iv); or
(vi) any combination of (i) to (v);

and

(b) instructions to use the kit for the prevention and/or treatment of Staphylococcal IMI.

63. The kit of claim 62, wherein said gene from the same operon as one of the gene of (a) is SACOL0720, and wherein said gene of (a) is SACOL0718.

64. The kit of claim 63, wherein said gene is SACOL0442, SACOL0718, SACOL0720, or any combination thereof.

65. The kit of claim 64, wherein said gene is SACOL0442, SACOL0720 or both.

66. The kit of any one of claims 62 to 65, comprising a combination of agents.

67. The kit of claim 62, wherein said combination of agents comprises:

(i) a first agent, wherein said first agent is (a) a polypeptide encoded by SACOL0442, (b) an immunogenic fragment of (a); (c) an immunogenic variant of (a) or (b); (d) a nucleic acid encoding the polypeptide of any one of (a) to (c); or (e) any combination of (a) to (d); and

(ii) a second agent, wherein said second agent is (a) a polypeptide encoded by SACOL0720, (b) an immunogenic fragment of (a); (c) an immunogenic variant of (a) or (b); (d) a nucleic acid encoding the polypeptide of any one of (a) to (c); or (e) any combination of (a) to (d).

68. The kit of claim 62, wherein said gene is SACOL0442 and wherein said immunogenic fragment comprises one or more of the following amino acid sequences: TFGYPKADASTQN (SEQ ID NO: 17), KDTINGKSNKSRNW (SEQ ID NO: 18) or KDGGKYTELESHKELQ (SEQ ID NO: 19).

69. The kit of claim 62, wherein said gene is SACOL0720 and wherein said immunogenic fragment comprises one or more of the following amino acid sequences: QFGFDLHKKDALA (SEQ ID NO: 20), TIKDQQKANQLAS (SEQ ID NO: 21), KDINKIYFMTVDL (SEQ ID NO: 22) or DVDBGPTFVLD (SEQ ID NO: 23).

70. The kit of any one of claims 62 to 69, further comprising an adjuvant.

71. The kit of claim 70, wherein said adjuvant is alum, Emulsigen™ D, cyclic-diguanosine-5’-monophosphate (c-di-GMP), polyphosphasine or pathogen-associated molecular patterns (PAMPS).
72. The kit of claim 71, wherein said PAMPS is unmethylated dinucleotides (CpG) or microbial polysaccharides.

73. The kit of any one of claims 62 to 72, wherein said (i) agent, (ii) adjuvant, or both (i) and (ii), are comprised in a pharmaceutical composition.

74. The kit of claim 73, wherein said pharmaceutical composition further comprises one or more pharmaceutically acceptable excipients.

75. A method of diagnosing Staphylococcal IMI in a mammal, said method comprising:
   determining a level of expression of at least one gene, wherein said gene is SACOL0029, SACOL0264, SACOL0442, SACOL0718, SACOL0720, SACOL1353, SACOL1416, SACOL1611, SACOL1944, SACOL2144, SACOL2365 or SACOL2599, or the level of activity of a polypeptide encoded by said one or more genes, in a biological sample from said mammal; and comparing said level of expression or activity to a reference level of expression or activity;
   wherein a higher expression or activity in said biological sample relative to said reference expression or activity is indicative that said mammal has staphylococcal IMI.

76. The method of claim 75, wherein said reference expression or activity is a level of expression or activity determined in a corresponding biological sample from a mammal known to not having staphylococcal IMI.

77. The method of claim 75 or 76, wherein said level of expression is determined by measuring the level of expression of a mRNA transcribed from said one or more genes.

78. The method of claim 75 or 76, wherein said level of expression is determined by measuring the level of expression of a polypeptide encoded by said one or more genes.

79. The method of any one of claims 75 to 76, wherein said biological sample is milk.

80. The method of any one of claims 75 to 76, wherein said mammal is a cow.

81. A kit for the diagnosis of Staphylococcal IMI, comprising (a) at least one ligand, wherein said at least one ligand binds to:
   (i) a polypeptide encoded by a gene, wherein said gene is SACOL0029, SACOL0264, SACOL0442, SACOL0718, SACOL0720, SACOL1353, SACOL1416, SACOL1611, SACOL1944, SACOL2144, SACOL2365 or SACOL2599, based on the gene nomenclature from the Staphylococcus aureus COL (SACOL) genome set forth in NCBI Reference Sequence NC_002951.2;
   (ii) a polypeptide encoded by a gene from a same operon as one of the genes of (a);
(iii) an immunogenic fragment of (i) or (ii);
(iv) an immunogenic variant of any one of (i) to (iii);
(v) a nucleic acid encoding the polypeptide of any one of (i) to (iv); or
(vi) any combination of (i) to (v);

and

(b) instructions to use the kit for the diagnosis of Staphylococcal IMI.

82. The kit of claim 81, wherein said gene from the same operon as one of the gene of (a) is SACOL0720, and wherein said gene of (a) is SACOL0718.

83. The kit of claim 81, wherein said gene is SACOL0442, SACOL0718, SACOL0720, or any combination thereof.

84. The kit of claim 83, wherein said gene is SACOL0442, SACOL0720 or both.

85. The kit of any one of claims 81 to 84, comprising a combination of ligands.

86. The kit of claim 85, wherein said combination of ligands comprises ligands which bind to:

(i) a first agent, wherein said first agent is (a) a polypeptide encoded by SACOL0442, (b) an immunogenic fragment of (a); (c) an immunogenic variant of (a) or (b); (d) a nucleic acid encoding the polypeptide of any one of (a) to (c); or (e) any combination of (a) to (d); and

(ii) a second agent, wherein said second agent is (a) a polypeptide encoded by SACOL0720, (b) an immunogenic fragment of (a); (c) an immunogenic variant of (a) or (b); (d) a nucleic acid encoding the polypeptide of any one of (a) to (c); or (e) any combination of (a) to (d).

87. The kit of claim 81, wherein said gene is SACOL0442 and wherein said immunogenic fragment comprises one or more of the following amino acid sequences: TFGIYPKADASTQN (SEQ ID NO: 17), KDTINGKSNKSRNW (SEQ ID NO: 18) or KDGKHYTFHELQ (SEQ ID NO: 19).

88. The kit of claim 81, wherein said gene is SACOL0720 and wherein said immunogenic fragment comprises one or more of the following amino acid sequences: QFGFDLHKKDALA (SEQ ID NO: 20), TIKDQKANQLAS (SEQ ID NO: 21), KDINKYFMTDVVL (SEQ ID NO: 22) or DVDLGGPTVFVLND (SEQ ID NO: 23).

89. The kit of any one of claims 81 to 88, further comprising an adjuvant.

90. The kit of claim 89, wherein said adjuvant is alum, Emulsigen™ D, cyclic-diguanosine-5'-
monophosphate (c-di-GMP), polyphosphasine or pathogen-associated molecular patterns (PAMPS).

91. The kit of claim 90, wherein said PAMPS is unmethylated dinucleotides (CpG) or microbial polysaccharides.

92. The kit of any one of claims 81 to 91, wherein said (i) agent, (ii) adjuvant, or both (i) and (ii), are comprised in a pharmaceutical composition.

93. The kit of claim 93, wherein said kit further comprises one or more pharmaceutically acceptable excipients.

94. A method for preventing and/or treating Staphylococcal intramammary infection (IMI) in a mammal, said method comprising administrating to said mammal an effective amount of at least one agent, wherein said agent is a live attenuated form of Staphylococcus aureus comprising a mutation in a gene, wherein said gene is SACOL0029, SACOL0264, SACOL0442, SACOL0718, SACOL0720, SACOL1353, SACOL1416, SACOL1611, SACOL1944, SACOL2144, SACOL2365 or SACOL2599, based on the gene nomenclature from the Staphylococcus aureus COL (SACOL) genome set forth in NCBI Reference Sequence NC_002951.2, and wherein the mutation is a deletion, an insertion or a substitution of one or more nucleotides.

95. The method of claim 94, wherein said gene is SACOL0442 or SACOL0720.

96. Use of an agent, wherein said agent is a live attenuated form of Staphylococcus aureus comprising a mutation in a gene, wherein said gene is SACOL0029, SACOL0264, SACOL0442, SACOL0718, SACOL0720, SACOL1353, SACOL1416, SACOL1611, SACOL1944, SACOL2144, SACOL2365 or SACOL2599, based on the gene nomenclature from the Staphylococcus aureus COL (SACOL) genome set forth in NCBI Reference Sequence NC_002951.2, and wherein the mutation is a deletion, an insertion or a substitution of one or more nucleotides for preventing and/or treating Staphylococcal intramammary infection (IMI) in a mammal or for the preparation of a medicament for preventing and/or treating Staphylococcal intramammary infection (IMI) in a mammal.

97. The use of claim 96, wherein said gene is SACOL0442 or SACOL0720.

98. A pharmaceutical composition for preventing and/or treating Staphylococcal intramammary infection (IMI) in a mammal, said composition comprising an agent, wherein said agent is a live attenuated form of Staphylococcus aureus comprising a mutation in a gene, wherein said gene is SACOL0029,
SACOL0264, SACOL0442, SACOL0718, SACOL0720, SACOL1353, SACOL1416, SACOL1611, SACOL1944, SACOL2144, SACOL2365 or SACOL2599, based on the gene nomenclature from the *Staphylococcus aureus* COL (SACOL) genome set forth in NCBI Reference Sequence NC_002951.2, and wherein the mutation is a deletion, an insertion or a substitution of one or more nucleotides.

99. The pharmaceutical composition of claim 96, wherein said gene is SACOL0442 or SACOL0720.

100. A kit for the prevention and/or treatment of Staphylococcal IMI, comprising at least one agent, wherein said agent is a live attenuated form of *Staphylococcus aureus* comprising a mutation in a gene, wherein said gene is SACOL0029, SACOL0264, SACOL0442, SACOL0718, SACOL0720, SACOL1353, SACOL1416, SACOL1611, SACOL1944, SACOL2144, SACOL2365 or SACOL2599, based on the gene nomenclature from the *Staphylococcus aureus* COL (SACOL) genome set forth in NCBI Reference Sequence NC_002951.2, and wherein the mutation is a deletion, an insertion or a substitution of one or more nucleotides.

101. The kit of claim 100, wherein said gene is SACOL0442 or SACOL0720.
**FIG. 3**

**hld**

Expression vs. Newbould (x fold)

- Chronic
- Syst. isolated

**icaC**

Expression vs. Newbould (x fold)

- Chronic
- Syst. isolated

Biofilm formation of chronic mastitis isolates

- SHY97-3906
- #3
- #557
- #1290

S. aureus chronic strain

Biofilm formation (% of SHY97-3906)

- #3
- #557
- #1290
A  Treatment of milk with casein protease (200 µg/ml)

Prot (-)  Prot (+)

B

centrifugation

Prot (-)  Prot (+)

C

centrifugation

Prot (+)  Prot (-)

↓

Wash pellet with PBS
Centrifugation
RNA protect (QIAGen)

FIG. 4
FIG. 5A
FIG. 5B
FIG. 5C
**FIG. 6**

- **Left Circle**:
  - SACOL1611
  - SACOL2599
  - SACOL1416
  - SACOL2144
  - SACOL1353
  - SACOL0264
  - SACOL2385
  - splC
  - sbnA
  - sbnB
  - sbnF
  - csb8
  - csb28

- **Right Circle**:
  - fhuA
  - glpK
  - mntA
  - SACOL0154
  - mntC
  - csb19
  - corA
  - trxB
  - rot
  - dnaK

- **Intersection**:
  - 20 genes

- **Other Text**:
  - isolates #3, #557 and #1290
  - SHY97-3906
FIG. 7
**FIG. 8**

![Log10 of relative expression ratio for different conditions](image)

- **capM**
  - SACOL2325
  - SACOL0718
  - SACOL2171
  - SACOL0442

Conditions:
- cow broth + iron
- cow broth - iron
- milk in vitro
In vitro growth in milk

- ATCC 29213
- Δ442a
- Δ442b
- Δ720

FIG. 10A
Mean CFU in milk / *S. aureus* strain

- • ATCC29213
- ○ Δ442a
- ▲ Δ442b
- □ Δ720

Infection

![Graph showing Mean CFU in milk / *S. aureus* strain](image)

**FIG. 10B**
Multiple sequence alignment: SACOL0442
Nucleic acid

**FIG. 11A**
FIG. 11C
Multiple sequence alignment: SACOL0442
Amino acids

SACOL0442 (COL)
SACO006_0442 (NTC09325)
N3NN_0362 (Neeman)
SAMS300_0370 (USA300-PPK3757)
SavuJHO_0429 (JHO)
SAVY_0367 (JMS)
SavuJHO_0419 (JHO)
SAV0379 (JMS)
SA0037 (N315)
SA0037 (HSSA476)
SAB0321 (KRF122)

SACOL0442 (COL)
SACO006_0442 (NTC09325)
N3NN_0362 (Neeman)
SAMS300_0370 (USA300-PPK3757)
SavuJHO_0429 (JHO)
SAVY_0367 (JMS)
SavuJHO_0419 (JHO)
SAV0379 (JMS)
SA0037 (N315)
SA0037 (HSSA476)
SAB0321 (KRF122)

SACOL0442 (COL)
SACO006_0442 (NTC09325)
N3NN_0362 (Neeman)
SAMS300_0370 (USA300-PPK3757)
SavuJHO_0429 (JHO)
SAVY_0367 (JMS)
SavuJHO_0419 (JHO)
SAV0379 (JMS)
SA0037 (N315)
SA0037 (HSSA476)
SAB0321 (KRF122)

SACOL0442 (COL)
SACO006_0442 (NTC09325)
N3NN_0362 (Neeman)
SAMS300_0370 (USA300-PPK3757)
SavuJHO_0429 (JHO)
SAVY_0367 (JMS)
SavuJHO_0419 (JHO)
SAV0379 (JMS)
SA0037 (N315)
SA0037 (HSSA476)
SAB0321 (KRF122)

SACOL0442 (COL)
SACO006_0442 (NTC09325)
N3NN_0362 (Neeman)
SAMS300_0370 (USA300-PPK3757)
SavuJHO_0429 (JHO)
SAVY_0367 (JMS)
SavuJHO_0419 (JHO)
SAV0379 (JMS)
SA0037 (N315)
SA0037 (HSSA476)
SAB0321 (KRF122)

SACOL0442 (COL)
SACO006_0442 (NTC09325)
N3NN_0362 (Neeman)
SAMS300_0370 (USA300-PPK3757)
SavuJHO_0429 (JHO)
SAVY_0367 (JMS)
SavuJHO_0419 (JHO)
SAV0379 (JMS)
SA0037 (N315)
SA0037 (HSSA476)
SAB0321 (KRF122)

SACOL0442 (COL)
SACO006_0442 (NTC09325)
N3NN_0362 (Neeman)
SAMS300_0370 (USA300-PPK3757)
SavuJHO_0429 (JHO)
SAVY_0367 (JMS)
SavuJHO_0419 (JHO)
SAV0379 (JMS)
SA0037 (N315)
SA0037 (HSSA476)
SAB0321 (KRF122)

FIG. 11D
**Multiple sequence alignment: SACOL0720**

**Nucleic acid**

<table>
<thead>
<tr>
<th>Accession</th>
<th>Name</th>
<th>Sequence</th>
</tr>
</thead>
</table>
| Saur_JH1_0700 (JH1) |                      | ATGCTTTAAGGAAAATTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTT TT
FIG. 12D
FIG. 12E
Multiple sequence alignment: SACOL0720

Amino acids

![Sequence Alignment Diagram](image-url)
FIG. 12J
FIG. 12K
Description of *S. aureus* sequenced strains (Source: NCBI, The Genome Project)

<table>
<thead>
<tr>
<th>Strain</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>COL</td>
<td>Methicillin-resistant (MRSA) strain that is also resistant to several other antibiotics including penicillin and tetracycline.</td>
</tr>
<tr>
<td>RF122</td>
<td>Common strain associated with mastitis in cattle.</td>
</tr>
<tr>
<td>NCTC 8325</td>
<td>Prototypical strain originally used as a propagating strain for bacteriophage 47.</td>
</tr>
<tr>
<td>USA300-FPR3757</td>
<td>Methicillin resistant strain implicated in outbreaks of skin and soft tissue infections among healthy individuals in U.S., Canada and Europe.</td>
</tr>
<tr>
<td>N315</td>
<td>Methicillin-resistant (MRSA) strain isolated in 1982 from a pharyngeal smear of a Japanese patient.</td>
</tr>
<tr>
<td>Mu50</td>
<td>Methicillin-resistant (MRSA) strain with vancomycin resistance (VRSA) isolated in 1997 from the pus of a Japanese male baby with a surgical wound infection.</td>
</tr>
<tr>
<td>JH1</td>
<td>Vancomycin-intermediate <em>S. aureus</em> (VISA) isolate recovered from clinical material in Baltimore, Md.</td>
</tr>
<tr>
<td>JH9</td>
<td>Vancomycin-intermediate <em>S. aureus</em> (VISA) isolate recovered from clinical material in Baltimore, Md.</td>
</tr>
<tr>
<td>Mu3</td>
<td>Heterogeneous vancomycin-intermediate <em>Staphylococcus aureus</em> (hVISA).</td>
</tr>
<tr>
<td>MW2</td>
<td>Community-acquired methicillin-resistant (MSRA) strain responsible for several fatal infections in the late 1990s.</td>
</tr>
<tr>
<td>MSSA476</td>
<td>Hyper-virulent community acquired methicillin-susceptible (MSSA) strain isolated in the United Kingdom.</td>
</tr>
<tr>
<td>Newman</td>
<td>Isolated from a case of secondarily infected tubercular osteomyelitis in man, great free-coagulase producer.</td>
</tr>
</tbody>
</table>

**FIG. 13**
Cellular localization of SACOL0442

Localization Scores:
- Cytoplasmic: 0.24
- CytoplasmicMembrane: 0.01
- Cellwall: 0.93
- Extracellular: 8.82

Final Prediction:
- Extracellular: 8.82

**FIG. 14A**

Cellular localization of SACOL0718

Localization Scores:
- Cytoplasmic: 0.22
- CytoplasmicMembrane: 9.49
- Cellwall: 0.00
- Extracellular: 0.29

Final Prediction:
- CytoplasmicMembrane: 9.49

**FIG. 14B**

Cellular localization of SACOL0720

Localization Scores:
- Cytoplasmic: 0.00
- CytoplasmicMembrane: 9.99
- Cellwall: 0.00
- Extracellular: 0.00

Final Prediction:
- CytoplasmicMembrane: 9.99

**FIG. 14C**
Prediction of the transmembrane helices of protein SACOL0720

Number of transmembrane helices: 10

FIG. 14D
FIG. 15
FIG. 16
SACOL0442 - IgG1 isotype

**FIG. 17A**
FIG. 17B
SACOL0720 - IgG1 isotype

FIG. 17C
SACOL0720 - IgG2 isotype

FIG. 17D
FIG. 18
FIG. 16

- saline against SACOL0442
- △ 442 alone
- □ SACOL0442 in combo
- ⊙ saline against SACOL0720
- ● 720 alone
- ■ SACOL0720 in combo