

(19) 日本国特許庁(JP)

(12) 公表特許公報(A)

(11) 特許出願公表番号

特表2004-534080

(P2004-534080A)

(43) 公表日 平成16年11月11日(2004.11.11)

(51) Int.Cl.⁷

C07K 16/12
A61K 39/00
A61K 39/395
A61P 31/04
G01N 33/53

F I

C O 7 K 16/12 Z N A
 A 6 1 K 39/00 H
 A 6 1 K 39/395 D
 A 6 1 K 39/395 R
 A 6 1 P 31/04

テーマコード (参考)

4 B O 6 4
 4 C O 8 5
 4 H O 4 5

審査請求 未請求 予備審査請求 未請求 (全 171 頁) 最終頁に続く

(21) 出願番号 特願2003-506301 (P2003-506301)
 (86) (22) 出願日 平成14年6月17日 (2002.6.17)
 (85) 翻訳文提出日 平成15年12月12日 (2003.12.12)
 (86) 国際出願番号 PCT/US2002/019220
 (87) 国際公開番号 W02002/102829
 (87) 国際公開日 平成14年12月27日 (2002.12.27)
 (31) 優先権主張番号 60/298,098
 (32) 優先日 平成13年6月15日 (2001.6.15)
 (33) 優先権主張国 米国 (US)

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(54) 【発明の名称】 コアグラゼ陰性ブドウ球菌および黄色ブドウ球菌由来の表面蛋白質を認識する、交差反応性モノクローナルおよびポリクローナル抗体

(57) 【要約】

両方のコアグラゼ陽性ブドウ球菌細菌（例えば、*S. hemolyticus*）に交差反応するポリクローナルおよびモノクローナル抗体が提供され、これらは、コアグラゼ陽性およびコアグラゼ陰性の両方のブドウ球菌細菌由来の表面蛋白質を認識し得る。該抗体は、*S. aureus*とコアグラゼ陰性ブドウ球菌との間で共通であり得る特徴に基づいて単離された表面蛋白質から産生され得、そしてこれらの組換え表面蛋白質は、本発明の抗体を産生するために使用される。広範囲のブドウ球菌感染に対する処置または予防のためにこれらの蛋白質および抗体を使用する、ワクチンおよび方法もまた提供される。

【特許請求の範囲】

【請求項 1】

配列番号 2、4、6、8、10、12、14、16、17、18、19 および 21 からなる群から選択されるブドウ球菌表面蛋白質へ結合する、単離抗体。

【請求項 2】

該抗体が表面蛋白質の A ドメインに対して惹起される、請求項 1 に記載の抗体。

【請求項 3】

該抗体がヒトまたは動物における *S. aureus* 感染を処置または予防する、請求項 1 に記載の抗体。

【請求項 4】

該抗体がヒトまたは動物における非経口、経口、鼻内、皮下、エアゾール化または静脈内投与に好適である、請求項 1 に記載の抗体。

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【請求項 5】

前記抗体がモノクローナル抗体である、請求項 1 に記載の抗体。

【請求項 6】

前記抗体がポリクローナル抗体である、請求項 1 に記載の抗体。

【請求項 7】

前記モノクローナル抗体が、マウス、キメラ、ヒト化およびヒトモノクローナル抗体からなる群から選択されるタイプのものである、請求項 5 に記載の抗体。

【請求項 8】

該抗体が一本鎖モノクローナル抗体である、請求項 5 に記載の抗体。

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【請求項 9】

配列番号 2、4、6、8、10、12、14、16、17、18、19 および 21 からなる群から選択される配列を有するブドウ球菌表面蛋白質へ結合する抗体と同一の結合特性を有する抗体フラグメントを含む、請求項 1 に記載の抗体。

【請求項 10】

配列番号 2、4、6、8、10、12、14、16、17、18、19 および 21 からなる群から選択されるアミノ酸配列を有する蛋白質に対して惹起される、請求項 1 に記載の抗体。

【請求項 11】

前記表面蛋白質が、核酸配列 配列番号 1、3、5、7、9、11、13、15、20 および Aap 蛋白質の A ドメインまたはその縮重物をコードする核酸配列からなる群から選択される核酸配列によってコードされるアミノ酸配列を有する、請求項 1 に記載の抗体。

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【請求項 12】

請求項 1 に記載の抗体を含有する、単離抗血清。

【請求項 13】

請求項 1 に記載の抗体およびその抗体による結合を検出するための手段を含む、診断キット。

【請求項 14】

前記結合を検出するための手段が、前記抗体へ連結される検出可能な標識を含む、請求項 13 に記載の診断キット。

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【請求項 15】

請求項 1 に記載の抗体を、*S. aureus* に感染していると疑われるサンプルへ添加すること、および抗体が該サンプルへ結合したかどうかを測定することを包含する、*S. aureus* の感染を診断するための方法。

【請求項 16】

有効量の請求項 1 の抗体および薬学的に許容されるビヒクル、担体または賦形剤を含む、*S. aureus* の感染を処置または予防するための薬学的組成物。

【請求項 17】

有効量の請求項 1 に記載の抗体をヒトまたは動物患者へ投与することを包含する、*S. a*

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ureus の感染を処置または予防するための方法。

【請求項 18】

アミノ酸配列 配列番号 2、4、6、8、10、12、14、16、17、18、19 および 21 からなる群から選択される免疫原性量の単離蛋白質を、ヒトまたは動物へ投与することを包含する、免疫学的応答を誘発させる方法。

【請求項 19】

配列番号 1、3、5、7、9、11、13、15、20 の核酸配列および Aap 蛋白質の A ドメインまたはその縮重物をコードする核酸配列によってコードされるアミノ酸へ結合する能力を有する、請求項 1 に記載の単離抗体。

【請求項 20】

DsqA 蛋白質の A ドメイン由来の単離された活性フラグメント。

【請求項 21】

生理学的に許容される抗生物質を更に含む、請求項 1 に記載の単離抗体。

【請求項 22】

免疫応答を誘発するに有効な量の配列番号 2、4、6、8、10、12、14、16、17、18、19 および 21 からなる群から選択される蛋白質配列、ならびに薬学的に許容されるビヒクル、担体または賦形剤を含む、*S. aureus* の感染を処置または予防するためのワクチン。

【発明の詳細な説明】

【技術分野】

【0001】

〔関連出願の相互参照〕

本願は、2001 年 6 月 15 日に出願された米国仮出願第 60/298,098 の利益を主張する。

【0002】

〔発明の分野〕

本発明は、一般的に、*Staphylococcus aureus* (黄色ブドウ球菌) 由来の表面蛋白質およびそれらの活性領域 (例えば、それらの A ドメイン) [これは、コアグラゼ陰性ブドウ球菌 (例えば、*S. epidermidis* および *S. hemolyticus*) における相同蛋白質 (homologue proteins) を有する]、ならびに該蛋白質を認識する抗体に関し、そして特に、*Staphylococcus aureus* およびコアグラゼ陰性ブドウ球菌由来の特定の蛋白質を認識し、そして *S. aureus* およびコアグラゼ陰性ブドウ球菌に対して交差反応性であり、そして従ってブドウ球菌細菌によって引き起こされる広範囲の感染を予防または処置するために有用なワクチンおよび方法において使用され得る、単離されたモノクローナルおよびポリクローナル抗体に関する。

【背景技術】

【0003】

〔発明の背景〕

宿主の成功的定着 (colonization) は、動物およびヒトにおいて感染を生じさせる大抵の微生物について必要とされるプロセスである。微生物付着 (microbial adhesion) は、最終的に疾患へと導き得る一連の事象における第一の重要な工程である。病原性微生物は、細菌の表面上に存在する特定の付着因子を介して、宿主組織または血清条件化された (conditioned) インプラントされた生体材料 (例えば、カテーテル、人工関節、および脈管移植片) へ付着することによって、宿主に定着する (colonize)。MSCRAMM (登録商標) (Microbial Surface Components Recognizing Adhesive Matrix Molecules (付着性マトリックス分子を認識する細菌性表面成分)) は、宿主細胞外マトリックス中の個々の成分を認識しそしてこれに特異的に結合する、細胞表面付着因子のファミリーである。一旦該細菌が宿主組織に首尾良く付着し (adhered) そして定着する (colonized) と、それらの生理学は

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劇的に変化され、そして有害な成分（例えば、毒素および蛋白質分解酵素）が分泌される。更に、付着している細菌は、しばしば、バイオフィルムを作製し、そして迅速に大抵の抗生物質の殺傷効果に対してより耐性となる。

【0004】

S. aureus は、皮膚病巣（例えば、創傷感染、膿痂疹、およびフルンケル）から、生命を脅かす状態（肺炎、敗血性関節炎（septic arthritis）、敗血症（sepsis）、心内膜炎、および生体材料関連感染（biomaterial related infections）を含む）にわたる、広範囲な感染を引き起こす。*S. aureus* は、特定の宿主組織成分への細菌付着を促進するように個々にまたは協力して作用し得る、異なる MSCRAMM のレパートリーを発現することが知られている。更に、別のタイプのブドウ球菌細菌が、コアグラーゼ陰性細菌として同定されており、*S. epidermidis* および *S. hemolyticus* のような種を含み、これらはまた、MSCRAMM を発現することが知られておりそして広範囲の細菌感染および関連疾患を担う。この点で、MSCRAMM は、一般的に、抗体（ポリクローナルおよびモノクローナル抗体の両方）による免疫学的攻撃のための優れた標的を提供する。

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【0005】

しかし、抗体は本質的に非常に特異的であるので、そして異なるタイプのブドウ球菌の場合〔例えば、一方で *S. aureus*（コアグラーゼ陽性）および他方で *S. epidermidis* および *S. hemolyticus*（コアグラーゼ陰性）〕、異なるタイプの細菌に渡って交差反応性を示す抗体を開発することは、依然として重要な課題のままである。ヒトおよび動物患者を免疫化し、そして両タイプのブドウ球菌細菌〔即ち、コアグラーゼ陽性細菌（例えば、*S. aureus*）およびコアグラーゼ陰性細菌（例えば、*S. epidermidis* および *S. hemolyticus*）〕によって引き起こされる感染に対する保護を提供することにおけるそれらの可能性のために、このような交差反応性抗体は特に望ましい。従って、このような抗体は、ブドウ球菌細菌によって引き起こされる広範囲の感染を予防または処置することにおいて極めて有用である。

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【発明の開示】

【発明が解決しようとする課題】

【0006】

〔発明の要旨〕

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従って、コアグラーゼ陽性細菌（例えば、*S. aureus*）由来の MSCRAMM（登録商標）ならびにコアグラーゼ陰性細菌（例えば、*S. epidermidis* および *S. hemolyticus*）由来の MSCRAMM（登録商標）の両方を認識するモノクローナル抗体を提供することが、本発明の主題である。

【0007】

ブドウ球菌細菌由来の MSCRAMM（登録商標）、ならびにそれらの活性領域（例えば、Aドメイン）を同定および単離することがまた、本発明の主題であり、これらは、コアグラーゼ陽性およびコアグラーゼ陰性の両方のブドウ球菌に対して交差反応性であるモノクローナルおよびポリクローナル抗体を産生するために使用され得る。

【0008】

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コアグラーゼ陰性ブドウ球菌由来の表面蛋白質（例えば、DgsK蛋白質）のAドメインを認識しそして同時に *Staphylococcus aureus* 由来の表面蛋白質（例えば、SasA蛋白質）を認識し得る単離抗体を提供することが、本発明のなお更なる主題である。

【0009】

本発明の単離蛋白質、Aドメインおよび抗体を使用してブドウ球菌感染の処置または予防に有用なワクチンを作製すること、ならびに本発明のワクチンおよび抗体を使用してブドウ球菌感染を予防または処置する方法を提供することが、本発明のなお別の主題である。

【0010】

これらおよび他の主題は本発明によって提供され、これは、両タイプのブドウ球菌の表面

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蛋白質を認識し得、従って広範囲のブドウ球菌感染を処置または予防するワクチンおよび方法において使用され得る、交差反応性抗体を惹起させ得る、1タイプのブドウ球菌細菌（例えば、コアグラゼ陰性またはコアグラゼ陽性ブドウ球菌）由来の表面蛋白質の同定および単離を含む。本発明はまた、これらの表面蛋白質からのポリクローナルおよびモノクローナル抗体の両方の産生、ならびにブドウ球菌感染を予防または処置することにおけるそれらの使用に関する。

【0011】

これらの実施形態ならびに開示される本発明の精神および範囲内の他の変形および修飾は、本明細書および／または本明細書中で引用される参考文献（これら全てが参考として援用される）を読むことにより、当業者に容易に明らかとなるであろう。

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【課題を解決するための手段】

【0012】

〔好ましい実施形態の詳細な説明〕

本発明によれば、コアグラゼ陽性ブドウ球菌細菌（例えば、*S. aureus*）ならびにコアグラゼ陰性ブドウ球菌（例えば、*S. epidermidis* および *S. hemolyticus*）由来の特定の表面蛋白質〔その活性フラグメント、例えば、これらの蛋白質のAドメイン、または該全蛋白質を認識する抗体を産生し得る他のエピトピック（epitopic）領域を含む〕が、提供される。本発明によれば、候補ペプチド配列および蛋白質の同定および単離は、多くの場合において細胞壁ペプチドグリカンへ共有結合されている、MSCRAMM（登録商標）（Microbial Surface Components Recognizing Adhensive Matrix Molecules）の共通特徴のいくつかに基づいて行われた。これらの表面蛋白質は以下の共通特徴を有し、これらは本発明の配列を同定および単離する際に使用された、即ち：（i）Sec-依存分泌（Sec-dependent secretion）に必要とされる、N末端シグナルペプチド（約40残基長）、（ii）プロリンおよびグリシン残基に富んでいるかまたはセリンおよびアスパルテートジペプチド繰り返しから構成される、壁スパニングドメイン（wall spanning domain）、（iii）ペプチドグリカンにおけるペンタグリシン架橋への該蛋白質の共有結合的固定（covalent anchoring）のために必要とされる、LPXTGモチーフ、（iv）疎水性膜スパニングドメイン（hydrophobic membrane-spanning domain）、続いて（v）いくつかの正に帯電した残基。

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【0013】

本発明によれば、上述のような特性の点で*S. aureus*の全ゲノムを利用することによって、MSCRAMMの指標である分泌（secretion）および固定（anchorage）モチーフを有する（即ち、N末端シグナルペプチドおよびC末端LPXTGモチーフ続いて疎水性ドメインおよび正に帯電したテイル（tail）を有する）蛋白質をコードする、少なくとも8つの新規のオープンリーディングフレームを同定した。以下の表は、*S. aureus*ゲノムにおけるそれらの分布、それらの蛋白質サイズおよびC末端細胞壁ソーティング配列（C-terminal cell wall sorting sequence）を含む、同定された蛋白質のリストを示す。

【0014】

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【表1】

表 1.

名称	分布	サイズ	C末端
EkeS	ENCSJM	2189 aa	LPNTGSEEMDLPLKELALITGAALLARRRS KKEKES
DsqA	ENCSJM	~1363- 2283 aa	LPDTGDSIKQNGLLGGVMTLLVGLGLMKR KKKKDENDQDDSQA
KesK	ENCSJM	~909 aa	LPKTGETTSSQSWWGLYALLGMLALFIPK FRKESK
KrkN2	ENCSJM (Cowan)	~278 aa	LPKTGLTSVDNFISTVAFATLALLGSLSLLLF KRKESK
KrkN	ENCSJM	~661 aa	LPQTGEESNKDMTLPMLIALSSIVAFVLP RKRKN
RkaS	ENCSJM	~801 aa	LPKTGTNQSSSPEAMFVLLAGIGLIATVRR RKAS
RrkN	NCSJM	1629 aa	LPKTGLESTQKGLIFSSIIGIAGLMLLARRRK N
KnkA	NCSJM	629 aa	LPKAGETIKEHWLPISVIVGAMGVLMIWLS RRNKLKNKA

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略字：e M R S A - 1 6 ; N , 8 3 2 5 ; C , C O L ; S , M S S A ; J , N 3 1 5 , M , M u 5 0 .

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8つのうち6つは、現在配列決定されている6つのブドウ球菌ゲノムの全てにおいて保存されており、そして残りの2つは、これらのゲノムの5/6において存在している。

【0015】

本発明によれば、上記蛋白質をコードするアミノ酸および核酸配列が得られ、そしてこれらは以下の通りであった：E k e s M R S A - 配列番号1 (DNA配列)； E k e S M R S A - 配列番号2 (蛋白質配列)； D s q A (8325) - 配列番号3 (DNA配列)； D s q A (8325) - 配列番号4 (蛋白質配列)； K e s K 1 (8325) - 配列番号5 (DNA配列)； K e s K 1 (8325) - 配列番号6 (蛋白質配列)； K r k N 2 (8325) - 配列番号7 (DNA配列)； K r k N 2 (8325) - 配列番号8 (蛋白質配列)； K r k N (8325) - 配列番号9 (DNA配列)； K r k N (8325) - 配列番号10 (蛋白質配列)； R k a S (COL) - 配列番号11 (DNA配列)； R k a S (COL) - 配列番号12 (蛋白質配列)； R r k N (8325) - 配列番号13 (DNA配列)； R r k N (8325) - 配列番号14 (蛋白質配列)； K n k A (8325) - 配列番号15 (DNA配列)； K n k A (8325) - 配列番号16 (蛋白質配列)。

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【0016】

本発明によれば、単離された抗体は、該蛋白質および/または該ドメインを認識し得るように、上記蛋白質またはそれらの活性領域 (例えば、Aドメイン) から産生され得る。これらの抗体は、モノクローナルまたはポリクローナルのいずれかであり得る。ポリクローナル抗体が望ましい場合、これらは、当該分野に周知の多数の慣用的様式で産生され得る。典型的なプロセスにおいて、所望の表面蛋白質またはその活性領域は、好適な宿主動物 (例えば、マウスまたはウサギ) へ注射され得、そして好適な時間後、抗体が、宿主動物から単離および回収され得る。モノクローナル抗体に関して、本発明によれば、これらは、多数の好適な様式 [例えば、Kohler and Milstein, Nature 256:495-497 (1975) の周知の方法、または当該分野において公知の他の好適な様式 (例えば、米国特許6,331,415; 5,981,216; 5,807,715; および4,816,567; 欧州特許出願519,596; ならびにPCT公開公報W0 00/71585に開示されている方法) を含む; これらの特許公開公報の全ては、本明細書中で参考として援用される] で、作製され得る。これらの方法は、当該分野に周知である様式での、キメラ、ヒト化、またはヒトモノクローナル抗体としてのそれらの製造を含む。なお更に、モノクローナル抗体は、軽鎖または重鎖のような一本鎖から製造され

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得、そして更に、全抗体の結合特徴（例えば、特異性および／または親和性）を保持する抗体活性フラグメントから製造され得る。活性フラグメント（active fragments）とは、異なるタイプのブドウ球菌細菌（即ち、コアグラールゼ陰性またはコアグラールゼ陽性）由来の特定の表面蛋白質またはその相同体（homologue）へ結合する完全抗体と同一の結合特異性を有する、抗体フラグメントを意味し、そして用語“抗体”は、本明細書中で使用される場合、該フラグメントを含むように意味される。更に、本発明に従うモノクローナルまたはポリクローナル抗体を使用して作製される抗血清がまた意図され、そして当業者に認識されるような多数の好適な様式で作製され得る。

【0017】

上述されるように、本発明に従う単離された表面蛋白質および／またはそれらの活性領域に対する抗体は、当該分野において周知である多数の好適な様式、例えば、モノクローナル抗体を産生するために使用され得る、上述の十分確立されたK o h l e rおよびM i l s t e i nの方法で作製され得る。例えば、このようなプロセスで使用される予備工程において、マウスに、本発明に従う精製組換えM S C R A M M（登録商標）またはその活性部分が、長期間にわたって一週間に一回腹腔内注射され得、続いて該精製蛋白質に対する反応性を測定するために、該免疫化マウスから得た血液の検査が行われる。該蛋白質に反応性であるマウスの同定に続いて、マウス脾臓から単離したリンパ球をマウスメラノーマ細胞と融合させて、本発明の表面蛋白質に対する抗体について陽性であるハイブリドーマを作製し、これを次いで単離および培養し、続いて精製およびアイソタイピング（isotyping）する。

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【0018】

本発明に従うモノクローナル抗体を産生するために、これらが、本発明に従う組換えで作製されたM S C R A M M（登録商標）を使用して産生されることが好ましく、そしてこれらの組換え体は、当該分野に周知の多数の標準方法を使用して産生および単離され得る。例えば、このような方法の1つは、組換え蛋白質およびペプチドをクローン化および発現するために発現ベクターとしてE. c o l i発現ベクターp Q E-30を使用する。1つの好ましい方法において、P C Rを使用して、D g s KまたはS a s Aと同定された表面蛋白質のAドメインを上述の配列から増幅し、そしてE. c o l i発現ベクターP Q E-30（Q i a g e n）〔これは、6つのヒスチジン残基を含有する組換え融合蛋白質の発現を可能にする〕へサブクローン化する。引き続いて、このベクターを、E. c o l i株A T C C 5 5 1 5 1へ形質転換し、0.7の光学密度（O D₆₀₀）まで15リットル発酵槽において増殖させ、そして4時間0.2 mMイソプロピル-1-ベーターDガラクトシド（I P T G）で誘導した。細胞を、A G T e c h n o l o g i e s h o l l o w - f i b e r a s s e m b l y（細孔サイズ（pore size）0.45 μm）を使用して収穫し、そして細胞ペーストを-80℃で凍結させた。細胞を、1100 p s iでt h e F r e n c h P r e s sを通過させる2回パスを使用して1×P B S（10 mL緩衝液／1 g細胞ペースト）において溶解させた。溶解した細胞を、30分間17,000 r p mでスピンドウンさせて（spun down）、細胞残骸を除去した。上清みを、0.1 M N i C l₂を充填させた5-mL H i T r a p C h e l a t i n g（P h a r m a c i a）カラムに通過させた。ローディング（loading）後、カラムを、10 mM T r i s（p H 8.0）、100 mM N a C l（緩衝液A）の5カラム容積で洗浄した。蛋白質を、30カラム容積に渡って、10 mM T r i s（p H 8.0）、100 mM N a C l、200 mM イミダゾール（緩衝液B）の0～100%勾配を使用して溶出した。S d r G N 1 N 2 N 3またはS d r G N 2 N 3は、～13%緩衝液B（～26 mMイミダゾール）で溶出した。280 nmでの吸光度をモニタリングした。S d r G N 1 N 2 N 3またはS d r G N 2 N 3を含有する分画を、1×P B Sで透析した。

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【0019】

次に、各蛋白質を、次いで、内毒素除去プロトコル（endotoxin removal protocol）に通した。このプロトコルの間に使用した緩衝液は、5-mL M o n o - Q s e p h a r o s e（P h a r m a c i a）カラムを通過させることによって、内毒素フリーにさせた

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。蛋白質を、 4×15 mLチューブに等しく分割した。各チューブの容積は、緩衝液Aを用いて9 mLにされた。1 mLの10% Triton X-114を各チューブに添加し、そして4°Cで1時間回転しながらインキュベートした。チューブを37°C水浴に配置して相分離させた。チューブを10分間2,000 rpmでスピンドウンさせ、そして各チューブからの上部の水相を回収し、そして洗剤抽出 (detergent extraction) を繰り返した。第2番目の抽出からの水相を合わせ、そして0.1 M NiCl_2 をチャージした5-mL IDA chelating (Sigma) カラムを通過させて、残っている洗剤を除去した。カラムを緩衝液Aの9カラム容積で洗浄し、その後、該蛋白質を緩衝液Bの3カラム容積で溶出した。溶離液 (eluant) を、5-mL Detoxigel (Sigma) カラムを通過させ、フロースルー (flow-through) を回収し、そしてカラムへ再適用した。第2通過からのフロースルーを回収し、そして1×PBSにおいて透析した。精製産物を、濃度、純度および内毒素レベルについて分析し、その後マウスへ投与した。

【0020】

好ましいプロセスにおいて、本発明に従うモノクローナル抗体は、以下の様式において上記に同定される組換え蛋白質から作製され得る。このプロセスにおいて、E. coli 発現および精製された組換えSasAおよびDsgK蛋白質は、マウスモノクローナル抗体のパネル (panel) を産生するために使用し、一方、マウス血清は、ポリクローナル抗体のソース (source) として使用した。手短に言えば、Balb/CまたはSJLマウスのグループに、表2において以下に記載されるように溶液状態のまたはアジュバントと混合されている1~10 mgの蛋白質の一連の皮下免疫化 (subcutaneous immunizations) を受容させた。

【0021】

【表2】

表2. 免疫化スキーム

RIMMS 注射	日	量 (μg)	経路	アジュバント
#1	0	5	皮下	FCA/RIBI
#2	2	1	皮下	FCA/RIBI
#3	4	1	皮下	FCA/RIBI
#4	7	1	皮下	FCA/RIBI
#5	9	1	皮下	FCA/RIBI
慣用的な注射	日	量 (μg)	経路	アジュバント
初期	0	5	皮下	FCA
ブースト # 1	14	1	腹腔内	RIBI
ブースト # 2	28	1	腹腔内	RIBI
ブースト # 3	42	1	腹腔内	RIBI

【0022】

犠牲 (sacrifice) (RIMMS) 時またはブースト (boost) (慣用的) 後7日目に、血清を回収し、そしてMSCRAMM (登録商標) 蛋白質に対するまたは全細胞 (S. epidermidis および S. aureus) についてのELISAアッセイにおいて力価を滴定した。最終ブースト3日後に、脾臓またはリンパ節を除去し、単一細胞懸濁液へ掻き裂き (teased)、そしてリンパ球を収獲した。次いで、リンパ球をP3X63Ag8.653メラノーマ細胞系 (ATCC#CRL-1580) へ融合させた。細胞融合、引き続いてプレート化 (plating) および栄養補給 (feeding) を、Current Protocols in Immunologyからのthe Production of Monoclonal Antibodies protocol (Chapter 2, Unit 2) [本明細書中で参考として援用される] に従って行った。

【0023】

次いで、該融合から産生された任意のクローンを、標準ELISAアッセイを使用して特異的抗-SasA抗体産生についてスクリーンした (screened)。陽性クローンを増やし

そしてフローサイトメトリーにより全細菌細胞結合アッセイにおける活性についてそして Biacore 分析により SasA 結合について更に試験した。Biacore 分析の間、流速 (flow rate) は 10 ml / 分で一定のままであった。SasA または DgsK 注射の前に、試験抗体を、RAM-Fc 結合を介してチップ (chip) へ吸着させた。時間 0 の際、30 mg / ml の濃度の SasA または DgsK を、3 分間チップ上に注射し、続いて 2 分間解離した (dissociation)。この段階の分析によって、Mab / SasA または DgsK 相互作用の相対的結合および解離動力学 (the relative association and dissociation kinetics) が測定された。

【0024】

次に、上述のように作製された抗体を、全細菌 (whole bacteria) への結合について試験した。この試験において、細菌サンプル *S. aureus* Newman、*S. aureus* 67-0、*S. aureus* 397 (Sal6)、*S. aureus* Wood、*S. aureus* 8325-4、メチシリン耐性 *S. aureus* MRSA 16、*S. epidermidis* ATCC 35984、*S. epidermidis* HB、*S. epidermidis* CN-899 および *S. haemolyticus* ATCC 43253 を回収し、洗浄し、そしてウサギ IgG (50 mg / ml) でブロッキングした後の 2 μg / ml の濃度の Mab または PBS のみ (コントロール) と共にインキュベートした。抗体と共のインキュベーション後、細菌細胞を、ヤギー F_(ab')₂ - 抗マウス F_(ab')₂ - FITC (これは、検出抗体として役立った) と共にインキュベーションした。抗体標識後、細菌細胞を、FACS カリバーフローサイトメーター (FACScaliber flow cytometer) を介して吸引して、蛍光放出を分析した (励起: 488、放出: 570)。各細菌株について、10,000 事象 (events) を回収しそして測定した。これらのデータは、*S. aureus* SasA に対する抗体がコアグラゼ陰性ブドウ球菌の表面上の相同な蛋白質を認識し得たことを示す。該データは、*S. aureus* SasA に対するウサギポリクローナル抗体が *S. epidermidis* HB の細胞表面から放出された蛋白質ならびに *S. epidermidis* からクローン化された DsgK 由来の組換え A-領域と交差反応することを実証するウェスタンブロット分析を支持する (下記の表 3 および図 6 を参照のこと)。

【0025】

【表 3】

表 3. ポリクローナル血清反応性

	New man	67-0	397 (SAL 6)	Wood 46	8325 -4	MRS A 16	ATC C 3598	HB	CN-899	ATC C 4325
							4			3
正常マウス血清	-	-	-	-	-	-	-	-	-	-
マウス抗-SasA	+	+	+/-	-	+	+	+	+	+	+

【0026】

本発明の表面蛋白質の組換え形態を使用するの抗体の産生が好ましいが、抗体は、これらの蛋白質またはそれらの活性領域 (例えば、A ドメイン) の天然の単離および精製されたバージョンから産生され得、そしてモノクローナルまたはポリクローナル抗体は、このような抗体を得るための上述の同一様式でこれらの蛋白質または活性領域を使用して産生さ

れ得る。なお他の慣用的な様式が、当業者に認識されるように、組換えまたは天然の精製された蛋白質またはそれらの活性領域を使用して、本発明の抗体を産生するために利用可能である。

【0027】

当業者に認識されるように、本発明の抗体はまた、ブドウ球菌細菌によって引き起こされる感染を処置または予防するために、ヒトまたは動物患者への投与に好適な薬学的組成物に形成され得る。本発明の抗体（または、その有効なフラグメント）を含有する薬学的組成物は、当該分野において一般的に使用される任意の好適な薬学的ビヒクル（vehicle）、賦形剤または担体（例えば、生理食塩水、デキストロース、水、グリセロール、エタノールを含む）、他の治療化合物、ならびにそれらの組み合わせと組み合わせて処方され得る。当業者に認識されるように、使用される特定のビヒクル、賦形剤または担体は、患者および患者の状態に依存して変化し、そして様々な投与様式が、当業者に認識されるように、本発明の組成物に好適である。本願に開示される任意の薬学的組成物を投与する好適な様式としては、局所、経口、肛門、膣、静脈内、腹腔内、筋肉内、皮下、鼻内および皮内投与が挙げられるが、これらに限定されない。

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【0028】

局所投与について、組成物は、軟膏、クリーム、ゲル、ローション、ドロップ（例えば、点眼剤および点耳剤（ear drops））、または溶液（例えば、マウスウォッシュ）の形態で処方される。創傷または手術包帯（dressings）、縫合糸およびエアゾール（aerosols）が、該組成物で含浸され得る。該組成物は、保存剤、浸透を促進するための溶媒、および軟化剤（emollients）のような慣用の添加物を含有し得る。局所製剤はまた、クリームもしくは軟膏ベース、エタノール、またはオレイルアルコールのような慣用の担体を含有し得る。抗体組成物の更なる形態、ならびに他のM S C R A M M（登録商標）についての組成物、ワクチン、方法および適用に関する他の情報がまた、一般的に、上述のM S C R A M M（登録商標）およびそれらの活性領域ならびにそれらに対する抗体を包含する本発明に適用可能であり得、そしてこれらの他のM S C R A M M（登録商標）は、例えば、米国特許5,175,096; 5,320,951; 5,416,021; 5,440,014; 5,571,514; 5,652,217; 5,707,702; 5,789,549; 5,840,846; 5,980,908; 6,086,895; 6,008,341; 6,177,084; 5,851,794 および 6,288,214において開示されており；これらの特許の全ては、本明細書中で参考として援用される。

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【0029】

本発明の抗体組成物はまた、免疫原性応答を増強するに有効な量の好適なアジュバントと共に投与され得る。例えば、好適なアジュバントとしては、ミョウバン（リン酸アルミニウムまたは水酸化アルミニウム）（これは、ヒトにおいて広く使用される）、ならびに他のアジュバント、例えば、サポニンおよびその精製成分Q u i l A、フロイント完全アジュバント、R I B B I アジュバント、ならびに研究および獣医学適用において使用される他のアジュバントが挙げられ得る。なお他の化学的に規定される調製物、例えばムラミールジペプチド、モノホスホリルリピドA、リン脂質複合体（例えば、Goodman-Snitkoff et al. J. Immunol. 147: 410-415 (1991) によって記載されそして本明細書中で参考として援用されるもの）、Miller et al., J. Exp. Med. 176: 1739-1744 (1992) によって記載されそして本明細書中で参考として援用されるようなプロテオリポソーム（proteoliposome）中の該複合体の封入物（encapsulation）、ならびに脂質小胞中の該蛋白質の封入物〔例えば、N o v a s o m e TM 脂質小胞（Micro Vascular Systems, Inc., Nashua, NH）〕もまた、有用であり得る。

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【0030】

任意の事象において、上述の蛋白質またはそれらの活性領域を認識する本発明の抗体組成物は、ブドウ球菌感染を予防または処置する方法において、ならびに宿主組織および／または細胞へのブドウ球菌細菌の結合を阻害することにおいて有用である。本発明によれば、ブドウ球菌感染を処置または予防するために本明細書に開示された表面蛋白質またはそれらの活性サブ領域（subregions）に対する有効量の抗体を投与することを包含する、ブ

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ドウ球菌感染を予防または処置するための方法が提供される。更に、これらのモノクローナル抗体は、宿主細胞へのブドウ球菌細菌の結合を弱める (impairing) ことにおいて有用である。

【0031】

従って、本発明によれば、上記の任意の慣用的な様式 (例えば、局所、非経口、筋肉内など) での本発明の抗体の投与は、有効量の該抗体組成物がヒトまたは動物患者に投与されると、ヒトまたは動物患者におけるブドウ球菌感染を処置または予防する極めて有用な方法を提供するであろう。有効量とは、細菌の付着を予防するか、宿主細胞へのブドウ球菌細菌の結合を阻害するに十分であり、そして従ってブドウ球菌感染の処置または予防に有用である、使用のレベル (例えば、抗体力価) を意味する。当業者に認識されるように、ブドウ球菌感染を処置または予防することにおいて有効であるために必要とされる抗体力価のレベルは、患者の性質および状態、ならびに／あるいは既存のブドウ球菌感染の重篤度に依存して変化する。

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【0032】

ブドウ球菌感染を処置または予防する方法における使用に加えて、本発明の抗体はまた、ブドウ球菌蛋白質の特異的検出のために、または研究ツールとして、使用され得る。用語“抗体”は、本明細書中で使用される場合、モノクローナル、ポリクローナル、キメラ、一本鎖 (single chain)、二重特異的 (bispecific)、サル化 (simianized)、およびヒト化または霊長類化 (primatized) 抗体ならびに F a b フラグメント [例えば、上記に特定される表面蛋白質に対する該抗体の結合特異性を保持しているフラグメント、F a b 免疫グロブリン発現ライブラリーのプロダクトを含む] を含む。従って、本発明は、該抗体の可変重鎖および軽鎖のような一本鎖 (single chain) の使用を意図する。これらのタイプのいずれの抗体または抗体フラグメントの産生も、当業者に周知である。この場合において、上記へ参照されるような表面蛋白質またはそれらの活性領域に対する抗体が、産生、単離および／または精製され、そして次いでブドウ球菌感染に対して処置または保護するために使用され得る。

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【0033】

上述の抗体のいずれもが、ブドウ球菌細菌の同定および定量のために、検出可能な標識で直接標識化され得る。イムノアッセイにおける使用のための標識は、一般的に、当業者に公知であり、そして酵素、ラジオアイソトープ、および蛍光、発光および色素産生性物質 (コロイダルゴールドまたはラテックスビーズのような着色された粒子を含む) を含む。好適なイムノアッセイとしては、酵素結合イムノソルベント検定法 (E L I S A) が挙げられる。

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【0034】

あるいは、抗体は、免疫グロブリンについて親和性を有する標識化物質との反応によって、間接的に標識化され得る。抗体は、第2物質と複合体化されて、そして抗体へ複合体化された第2物質について親和性を有する標識化された第3物質で検出され得る。例えば、抗体はビオチンへ複合体化され、そして抗体-ビオチン複合体が、標識化されたアビジンまたはストレプトアビジンを使用して検出され得る。同様に、抗体はハプテンへ複合体化され、そして抗体-ハプテン複合体が、標識化された抗-ハプテン抗体を使用して検出され得る。抗体およびアッセイ複合体を標識するこれらおよび他の方法は、当業者に周知である。

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【0035】

本発明によれば、ブドウ球菌感染に対して処置または保護するために設計された能動または受動免疫化のためのワクチンがまた提供され、そしてこれらのワクチンは、当該分野に周知の多数の慣用的ワクチン調製方法を使用して、上述の表面蛋白質またはそれらの活性領域から作製され得る。典型的なワクチンにおいて、免疫原性量の好適な表面蛋白質またはその活性フラグメントは、好適な薬学的に許容されるビヒクル、担体または賦形剤と組み合わされ、そしてヒトまたは動物患者を免疫化するに有効な量のこのワクチンが、必要に応じて投与され得る。免疫原性量 (immunogenic amount) とは、これが、ヒトまたは動

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物患者において免疫原性応答 (immunogenic response) を惹起させ得る該蛋白質またはその活性フラグメントもしくはサブ領域の任意の量をいうことが、当業者によって理解される。

【0036】

本発明に従う免疫原性量の蛋白質または活性フラグメントの導入または投与により患者において抗体が産生される、能動ワクチン (active vaccines) に加えて、本発明の単離抗体 (または、その活性フラグメント) はまた、ブドウ球菌感染に対する受動免疫化のためのワクチン開発において使用され得る。このような場合、上述のような抗体組成物、即ち、有効量の抗体および薬学的に許容されるビヒクル、担体または賦形剤が、必要に応じて、ヒトまたは動物患者へ投与され得る。

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【0037】

従って、本発明によれば、該蛋白質またはその活性フラグメントは、能動ワクチンとして使用され得、そして本発明の抗体は、ブドウ球菌感染を処置または予防する好適な抗体を提供することにおいて有用である受動ワクチンとして使用され得る。当業者に認識されるように、ワクチンは、多数の好適な様式での投与、例えば非経口 (即ち、筋肉内、皮内または皮下) 投与または鼻咽喉頭 (即ち、鼻内) 投与のために包装され得る。1つのこのような様式は、ワクチンが筋肉内に (例えば、三角筋へ) 注射される場合であるが、投与の特定のモードは、扱われる細菌感染の性質および患者の状態に依存する。ワクチンは、好ましくは、投与を促進するために薬学的に許容されるビヒクル、担体または賦形剤と組み合わせられ、そして担体は、通常、保存剤を含むかまたは含まない、水または緩衝化生理食塩水である。ワクチンは、投与時の再懸濁のために凍結乾燥されるかまたは溶液状態であり得る。

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【0038】

更に、特定の場合において、本発明の抗体は、それが投与される患者において必要であるならばそれらがより少ない免疫原性であるように、必要である場合には修飾され得る。例えば、患者がヒトである場合、抗体は、例えば Jones et al., Nature 321:522-525 (1986) または Tempest et al. Biotechnology 9:266-273 (1991) によって記載されるように、ハイブリドーマ由来抗体の相補性決定領域をヒトモノクローナル抗体へ移植することによって“ヒト化”され得、あるいは、例えば Padlan, Molecular Imm. 28: 489-498 (1991) によって記載されるように、免疫グロブリン可変領域における表面曝露マウスフレームワーク残基を、対応の相同ヒトフレームワークを模倣するように変化させることによって“化粧張り (veneered)”され得、これらの文献は本明細書中で参考として援用される。なお更に、そのように望まれる場合、本発明のモノクローナル抗体は、必要である場合には細菌感染と戦う本発明の組成物の能力を更に増強するために、好適な抗生物質と組み合わせて投与され得る。

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【0039】

ヒトまたは動物患者を処置することに加えて、本発明の組成物はまた、医療装置または他の生体材料 (例えば、インプラント) の感染を停止 (halt) または予防するために使用され得る。ここで記載される抗体、蛋白質および活性フラグメントでコーティングされる医療装置またはポリマー性生体材料としては、ステープル (staples)、縫合糸、代替心臓弁 (replacement heart valves)、心臓補助装置 (cardiac assist devices)、ハードおよびソフトコンタクトレンズ、眼内レンズインプラント (前房 (anterior chamber) または後房)、他のインプラント、例えば、角膜インレー (corneal inlays)、人工角膜 (kerato-prostheses)、脈管ステント、エピケラトファリア (epikeratophalia) 装置、緑内障シャント (glaucoma shunts)、網膜ステープル (retinal staples)、強膜バックル (scleral buckles)、歯科プロテーゼ (dental prostheses)、甲状腺軟骨形成装置、喉頭形成装置、脈管グラフト、軟および硬組織プロテーゼ (ポンプを含むが、これに限定されない)、電気装置 (スティミュレーターおよびレコーダーを含む)、耳プロテーゼ、ペースメーカー、人工喉頭、歯科インプラント、乳房インプラント、陰茎インプラント、頭蓋／顔の腱、人工関節、腱、靱帯、半月板、および円板、人工骨、人工器官 (人工脾臓、人

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工心臓、人工肢、および心臓弁を含む）、ステント、ワイヤー、ガイドワイヤー、静脈内および中心静脈カテーテル、レーザーおよびバルーン血管形成装置、脈管および心臓装置（チューブ、カテーテル、バルーン）、心室補助器（ventricular assists）、血液透析コンポーネント、血液オキシジェネーター、尿道／尿管／尿装置（Foleyカテーテル、ステント、チューブおよびバルーン）、気道カテーテル（気管内および気管開口チューブおよびカフ（cuffs））、経腸供給チューブ（経鼻胃管、胃内および空腸チューブを含む）、創傷ドレナージチューブ、体腔（例えば、胸膜、腹膜、頭蓋、および心膜腔）を空にするために使用されるチューブ、血液バック、試験管、採血チューブ、ヴァキュエーター（vacutainers）、注射器、針、ピペット、ピペットチップ、および血液チュービングが挙げられるが、これらに限定されない。

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【0040】

用語“コーティングされた（coated）”または“コーティング（coating）”は、本明細書中で使用される場合、抗体または活性フラグメント、あるいはそれから誘導される薬学的組成物を、装置の表面、好ましくはブドウ球菌細菌感染へ曝される外部表面に適用することを意味することが、当業者に理解される。装置の表面は、該蛋白質、抗体または活性フラグメントによって完全にカバーされる必要はない。

【0041】

本発明に従う抗体組成物の投与のために好ましい用量は、量がブドウ球菌感染を予防または処置することにおいて有効であることであり、そしてこの量は、感染の性質および患者の状態に依存して大いに変化することが容易に認識されるだろう。上述のように、本発明に従って使用される“有効量”の抗体または薬学的薬剤は、所望の予防または治療効果が引き起こされるような、非毒性であるが十分な量の薬剤を意味するように意図される。以下に指摘されるように、必要とされる抗体または特定の薬剤の正確な量は、被験体の種、年齢、および一般的状態、処置される状態の重篤度、使用される特定の担体またはアジュバントおよびその投与モードなどに依存して、被験体ごとに変化する。従って、任意の特定の抗体組成物の“有効量”は、特定の状況に基づいて変化し、そして好適な有効量は、ルーチンの実験のみを使用して、当業者によって、適用の各場合において決定され得る。用量は、組成物が投与される個体に合うように調節されるべきであり、そして個体の年齢、体重および代謝で変化する。組成物はまた、安定剤または薬学的に許容される保存剤（例えば、チメロサル（エチル（2-メルカプトベンゾエート-S）水銀ナトリウム塩）（Sigma Chemical Company, St. Louis, MO））を含有し得る。

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【0042】

好適な標識または他の好適な検出可能な生体分子または化学物質と共に使用される場合、ここで記載されるモノクローナル抗体は、ブドウ球菌感染のインビボおよびインビトロ診断あるはブドウ球菌細菌の検出のような目的のために有用である。実験室研究はまた、このような抗体の使用によって促進され得る。例えば以下に記載されるものような、種々のタイプの標識および本発明の抗体へ該標識を複合体化させる方法が、当業者に周知である。

【0043】

例えば、抗体は、これらに限定されないが³²P、³H、¹⁴C、³⁵S、¹²⁵I、または¹³¹Iのような放射標識へ複合体化され得る。標識の検出は、シンチレーションカウンティング、ガンマ線分光法またはオートラジオグラフィーのような方法により可能である。生物発光標識（例えば、ホタルルシフェリンの誘導体）がまた有用である。生物発光物質が、慣用方法で該蛋白質へ共有結合され、そして該標識化された蛋白質は、ルシフェラーゼのような酵素がATPとの反応を触媒して生物発光分子に光の光子を放出させる際に、検出される。フルオロゲン（fluorogen）がまた、蛋白質を標識するために使用され得る。フルオロゲンの例としては、フルオレセインおよび誘導体、フィコエリトリン、アローフィコシアニン（allo-phycoyanin）、フィコシアニン、ローダミン、およびテキサスレッドが挙げられる。フルオロゲンは、一般的に、蛍光検出器によって検出される。

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【0044】

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細胞中のリガンドの位置は、上述の抗体を標識しそして当業者に周知の方法〔例えば、Warrenら (Mol. Cell. Biol., 7:1326-1337, 1987) によって記載されるもののような手順を使用する免疫蛍光顕微鏡検査法〕に従って標識を検出することによって測定され得る。

【0045】

上述のように、本発明のモノクローナル抗体（またはその活性な部分またはフラグメント）は、感染を担うブドウ球菌病原体と哺乳動物宿主との間の初期物理的相互作用を妨害するために特に有用であり、そして該物理的相互作用のこの妨害は、患者を処置することおよび使用をより安全にするために留置（in-dwelling）医療装置における細菌感染を予防または軽減することの両方に、有用であり得る。

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【0046】

本発明の別の実施形態において、ブドウ球菌細菌および感染を単離および同定することに有用であり得るキットが提供され、これは、単一容器に凍結乾燥されて次いでブドウ球菌細菌を含有すると疑われる水性サンプルの添加によって活性となるような、好適な形態で本発明の抗体を含む。このようなキットは、典型的に、本発明の表面蛋白質または抗体へ結合する複合体（complexes）の同定を可能にする好適な免疫検出試薬と共に、好適な形態の抗体を収容するための好適な容器を含む。一般的に、これらのキットは、本発明に従う抗体と、患者由来のサンプルが該抗体に導入された場合に該抗体の結合を同定するための手段とを含み得る。例えば、好適な免疫検出試薬は、好適な検出可能なシグナルまたは標識（例えば、ビオチンまたは検出可能な色を生成する酵素など）を含み得、これは、通常、抗体へ結合され得るか、または抗体が抗原へ結合する場合に検出可能な結果を提供するような他の好適な様式で使用され得る。

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【0047】

手短に言えば、本発明の表面蛋白質（またはその活性フラグメント）を認識しそしてこれに結合する本発明の抗体は、従って、ヒトおよび動物患者ならびに医療または他の留置装置における、広範囲のブドウ球菌感染を処置することに有用である。本発明によれば、これらの蛋白質の性質、ならびにそれらが他のタイプのブドウ球菌細菌の蛋白質と共通するエピトープを含有する、即ち、コアグラゼ陰性ブドウ球菌由来の蛋白質が *S. aureus* 由来の相同蛋白質を認識する抗体を惹起する（逆も同様）という事実のために、本発明の抗体は、交差反応性を示し、そして広範囲のブドウ球菌感染に対して有効である。従

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【発明を実施するための最良の形態】

【0048】

〔実施例〕

以下の実施例が提供され、これは本発明の好ましい実施形態の局面を例証する。続く実施例において開示される技術は、本発明者らによって本発明の実施において十分に機能すると発見された技術を示し、そして従ってその実施のために好ましいモードを構成すると考えられ得ることが、当業者によって認識されるべきである。しかし、当業者は、本開示を考慮して、多くの変化が、本発明の精神および範囲から逸脱することなしに、開示される特定の

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【0049】

〔実施例1. *S. Aureus* 由来の MSCRAMM 's の単離および配列決定〕

Staphylococcus aureus は、細菌が宿主防衛を回避することを可能にしてそして付着因子として作用することにより病原性において重要な役割を果たす、表面結合蛋白質のクラスを発現することが公知である。これらの蛋白質は、MSCRAMM s (Microbial Surface Components Recognizing Adhesive Matrix Molecules (付着性マトリックス分子を認識する細菌性表面成分)) として公知であり、そして大抵の場合において、細胞壁ペ

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プチドグリカンに共有結合的に固定されている。それらは、いくつかの共通の特徴を有する：(i) Sec-依存分泌に必要とされる、N末端シグナルペプチド(約40残基長)、(ii) プロリンおよびグリシン残基に富んでいるかまたはセリンおよびアスパルテートジペプチド繰り返しから構成される、壁スパニングドメイン、(iii) ペプチドグリカンにおけるペンタグリシン架橋への該蛋白質の共有結合的固定のために必要とされる、LPXTGモチーフ、(iv) 疎水性膜スパニングドメイン、続いて(v) いくつかの正に帯電した残基。

【0050】

S. aureus の全ゲノム配列を利用することによって、MSCRAMMの指標である分泌および固定モチーフを有する(即ち、N末端シグナルペプチドおよびC末端LPXTGモチーフ続いて疎水性ドメインおよび正に帯電したテイル(tail)を有する)蛋白質をコードする、8つの新規のオープンリーディングフレームを同定した。以下の表は、*S. aureus* ゲノムにおけるそれらの分布、それらの蛋白質サイズおよびC末端細胞壁ソ

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【0051】

【表4】

名称	分布	サイズ	C末端
EkeS	ENCSJM	2189 aa	LPNTGSEMDLPLKELALITGAALLARRRS KKEKES
DsqA	ENCSJM	~1363- 2283 aa	LPDTGDSIKQNGLLGGVMTLLVGLGLMKR KKKKDENDQDDSQA
KesK	ENCSJM	~909 aa	LPKTGETTSSQSWWGLYALLGMLALFIPK FRKESK
KrkN2	ENCSJM (Cowan)	~278 aa	LPKTGLTSVDNFISTVAFATLALLGSLSLLLF KRKESK
KrkN	ENCSJM	~661 aa	LPQTGEESNKM LPLMALIALSSIVAFVLP RKRKN
RkaS	ENCSJM	~801 aa	LPKTGTNQSSSPEAMFVLLAGIGLIATVRR RKAS
RrkN	NCSJM	1629 aa	LPKTGLESTQKGLIFSSIIGIAGLMLLARRRK N
KnkA	NCSJM	629 aa	LPKAGETIKEHWLPISVIVGAMGVLMIWLS RRNKLKKA

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略字：eM RSA-16; N, 8325; C, COL; S, MSSA; J, N315, M, Mu50。

【0052】

8つのうち6つは、現在配列決定されている6つのブドウ球菌ゲノムの全てにおいて保存されており、そして残りの2つは、これらのゲノムの5/6において存在している。

【0053】

以下は、DNAおよび蛋白質配列のリストである：

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【0054】

【数1】

Ekes MRSA (配列番号 1)

acaacacagcagagaatagacaaccaggaggaaaaacgaaatgaatttgtaaagaaaaataaatatagtattag
aaaatataaagtagggatattctactttaatcgggacagttttattactttcaaaccctaaatggtgcacaagcttaac
tacggatcataatgtgcaaggtggttcaaatacagcattacctggcaactcacaatacaaatgccgatactaatac
gagacatagtaaatgattcgcaaaatactcctaatagcacatgcaacagacaatacatcaacaaatcaagcattgac
taatcatcaaaacggtgatgtggcaaatcaagtcgggcctgctccaatacagcctagcgctgcctgcgcaaaata
ataataattctaataatcaacagcaacagagccagcggaatacaataataatttagcatcaataaacaat
acattaaacgctgctaataatacagataacaatgattcagcgctcatctgactttaaaagaaattcaagaagatgtt
cgctattcgtctgataagccagagtagttgctgattgctgaagaagcatctaataagaccgaaaaagagaagcagac
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【 0 0 5 5 】

【 数 2 】

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【 0 0 5 6 】

【 数 3 】

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EkeS_MRSA (配列番号 2)

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【 0 0 5 7 】

【 数 4 】

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DsqA (8325) (配列番号 3)

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【 0 0 5 8 】

【 数 5 】

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【数 6】

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DsqA (8325) (配列番号 4)

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【 0 0 6 0 】

【 数 7 】

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KesK1 (8325) (配列番号 5)

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 aagaaaaagccggcacaccatcgaaagaaaacaaacttagtcaatctaaaatgtacaaaaactggagaa

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【 0 0 6 1 】

【 数 8 】

acaactcaagccaatcatggtggggccttatatgcgttattaggtatgtagctttattcattcctaaattcagaaaagaat
ctaaataa

KesK1 (8325) (配列番号 6)

LLSIKYNLIGVVNNMNKHHPKLRSFYSSIRKSTLGVASVIVSTLFLITSQHQAAEAENT
NTSDKISENQNNNATTTQPPKDTNQTQPATQPANTAKNYPAADESLKDAIKDPALE
NKEHDIGPREQVNFQLLDKNNETQYYHFFSIKDPADVYYTKKKAEVELDINTASTW
KKFEVYENNQKLPVRLVSYSVPEDHAYIRFPVSDGTQELKIVSSTQIDDGEETNY
DYTKLVFAKPIYNDPSLVKSDTNDVVTNDQSSSVASNQNTNTNTSNQNIISTINNAN
NQPPATTNMSQPAQPKSSTNADQASSQPAHETNSNGTNDKTNESSNQSDVNQ
QYPPADESLQDAIKNPAIIDKEHTADNWRPIDFQMKNDKGERQFYHYASTVEPATV
IFTKTGPPIELGLKASTWKKFEVYEGDKKLPVELVSYSDKDYAYIRFPVSNGTRE
VKIVSSIEYGENIHEDYDYTLMVFAQPITNNPDDYVDEETYNLQKLLAPYHKAKTLE
RQVYELEKLQEKLPEKYKAEYKKKLDQTRVELADQVKSATFEFENVPTNDQLTD
LQEAHFVVFEESEENSESVMDFVEHPFYTATLNGQKYVMKTKDDSYWKDLIVEG
KRVTTVSKDPKNNRSLIFPYIPDKAVYNAIVKVVANIGYEGQYHVRINQDINTKD
DDTSQNNTSEPLNVQTGQEGKVADTDVAENSSTATNPKDASDKADVIEPESDVVK
DADNNIDKDVQHDVDHLSMDSDNNHFDKYDLKEMDTQIAKDTDRNVDKDADNSV
GMSSNVDTDKDSNKNKDKVIQLNHIADKNNHTGKAALKDVVKQNYNNTDKVTDKK
TTEHLPSDIHKTVDKTVKTKKAGTPSKENKLSQSKMLPKTGETTSSQSWWGLYA
LLGMLALFIPKFRKESK

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KrkN2 (8325) (配列番号 7)

gaggaaaacaacatgacaaaacattatttaaacagtaagtatcaatcagaacaacgttca
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caaagcacacaagtttctcaagcaacatcacaaccaattaatttccaagtgcaaaaagat
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gaaaaagttaaaccctactgtaactacaacaagcaagtgagacaatcactctactaaa
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gttacaaaacataacgaaacgcctaaacaagcatctaaagctaaagaattaccaaaaact
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KrkN2 (8325) (配列番号 8)

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【 0 0 6 2 】

【 数 9 】

EENNMTKHYLNSKYQSEQRSSAMKKITMGTASIILGSLVYIGADSQQVNAATEATN
 ATNNQSTQVSQATSQPINFQVQKDGSSSEKSHMDDYMQHPGKVIKQNNKYFQTV
 LNNASFWKEYKFYNANNQELATTVNDNKKADTRTINVAVEPGYKSLTTKVHIVP
 QINYNHRYTTTHLEFEKAIPTLADAAKPNNVKPVQPKPAQPKTPTEQTKPVQPKVEK
 VKPTVTTTTSKVEDNHSTKVVDSTDTTKDQTKTQTAHTVKTAAQTAQEKNKVQTPVKD
 VATAKSESNNQAVSDNKSQQTNKVTKHNETPKQASKAKELPKTGLTSVDNFISTV
 AFATLALLGSLSLLLFKRKESK

KrkN (8325) (配列番号 9)

tatacaattaggaggtgtttctacaacatgaacaaacagcaaaaagaatttaaatcattttattcaattagaaagtcac
 actaggcgttgcatctgttagcaatttagcacattttattattaatgtcaaatggcgaagcacaagcagcagctgaaga
 aacagggtggtacaaatacagaagcacaacaaaaactgaagcagttgcaagtcacaacaacatctgaaaaa
 gctccagaaactaaaccagtagctaattgctgtctcagtatctaataaagaagttgaggccctacttctgaaacaaa
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 aaaatatatggtcatggaaactactaatgacgattactggaagatttcatggtgaaggtaacggtttagaactata
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 ggtgaagtagaatcaagtagtacaactccaactaaggttagtatctacgactcaaaatgttgcaaaaaccaacaactg
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 gcaaaatcattaccacaaaactgggaagaatcaaataaagatatgacattaccattaatggcattattagctttaagta
 gcatcgttgattcgtattacctagaaaacgtaaaaactaa

KrkN (8325) (配列番号 10)

YTIRSCFYNNMKQKQKEFKSFYSIRKSSLGVASVAISTLLLLMSNGEAQAAAEETGG
 TNTÉAQPKTEAVASPTTTSEKAPETKPVANAVSVSNKEVEAPTSETKEAKEVKEV
 KAPKETKEVKPAKATNNTYPILNQELREAIKNPAIKDKDHSAPNSRPIDFEMKKKD
 GTQQFYHYASSVKPARVIFTDSKPEIELGLQSGQFWRKFVYEGDKKLPIKLVSYD
 TVKDYAYIRFSVSNGTAKVKIVSSTHFNNKEEKYDYTLMEFAQPIYNSADKFKTEED
 YKAEKLLAPYKKAKTLERQVYELNKIQDKLPEKLAKEYKKKLEDTKKALDEQVKSAL
 TEFQNVQPTNEKMTDLQDTKYVVYESVENNESMMDTFVKHPIKTGMLNGKKYMV

【0063】

【数10】

METTNDYWKDFMVEGQVRVTRISKDAKNNTRTIIFPYVEGKTLYDAIVKVHVKTIDY
DGQYHVRIVDKAFTKANTDKSNKKEQQDNSAKKEATPATPSKPTSPVEKESQK
QDSQKDDNKQLPSVEKENDASSESGDKTPATKPTKGEVSSSTTPTKVSTTQ
NVAKPTTASSKTTKDVVQTSAGSSEAKDSAPLQKANIKNTNDGHTQSQNNKNTQE
NKAKSLPQTGEESNKDMTLPMLALLLSSIVAFVLPKRKN

RkaS (COL) (配列番号 1 1)

tttataaataatttacataaaatcaatcattttaataataaggattatgataatatattggtgtatgacagttaatggaggga
acgaaatgaaagctttattacttaaaacaagtgatggctgctttttagtgtaatgggattatggcaagtctcgaa
cgcggtgagcagcatcaccaatgaaagcacatgcagtaacaacgatagacaaagcaacaacagataagca
acaagtaccgccaacaaaggaagcggtcatcttgccaaagaagcggaaccaacgatcagcatcagcg
cagggaacagctgatgatacaaacagcaagtaacatccaacgcaccatctaacaaccatctacagtagtttca
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gttcaaatatcaaatgctaaacagcatcatttcaccacgaatgtttgctgctaattgcaccacaaacaacaacaca
taaaatattacatacaaatgatatccatggcggactagccgaagaaaaagggcggtcatcggtatggctaaattaa
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ccagttcaaaaggtgaagaaatggctaaagcaatgaatgcagtagggtatgatgctatggcagtcggtaccatgaat
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atcaaagttcaagcccagaagcgatgtttgtatttagcaggtatagggttaatcgcgactgtacgacgtagaaaag
ctagctaa

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RkaS (COL) (配列番号 1 2)

FINNLHKINHFNIRIMIIYWCMTVNNGNEMKALLKTSWWLVLLFSVMGLWQVSNA
EQHTPMKAHAVTTIDKATTDKQVPPTKEAAHHSKGKAATNVSASAQGTADDTN

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【 0 0 6 4 】

【 数 1 1 】

SKVTSNAPS NKPSTVVSTKVN ETRD VDTQQASTQKPTH TATFKLSNAKTASLSPR
MFAANAPQTTTHKILHTNDI HGR LAEEKGRVIGMAKLKTVKEQEKPD LMLDAGDAF
QGLPLSNQSKGEEMAKAMNAVGYDAMAVGNHEFDFGYDQLKKLEGMLDFPMLS
TNVYKDGKRAF KPSTIVTKNGIRYGIIGVTTPETKTKTRPEGIKGVEFRDPLQSVTA
EMMRYIKDVDTFVVISHLGIDPSTQETWRGDYLVKQLSQNPQLKKRITVIDGHSH
VLQNGQIYNNDALAQGTALANIGKITFN YRNGEVSNIKPSLINVKDVENVT PNKAL
AEQINQADQTFRAQTAEVIIPNNTIDFKGERDDVRTRETN LGNAIADAMEAYGVKN
FSKKTDFAVTNGGGIRASIAKGKVTRYDLISVLPFGNTIAQIDVKGSDVWTAFEHSL
GAPTTQKD GKT VLTANGLLHISDSIRVYYDINKPSGKRINAIQILNKETGKFENIDL
KRVYHVTMNDFTASGGDGYSMFGGPREEGISLDQVLASYLKTANLAKYDTTEPQR
MLLGKPAVSEQPAKGQQGSKGSKSGKDTQPIGDDKVM DPAKKPAPGKV VLLLAH
RGTVSSGTEGSGRTIEGATVSSSGKQLARMSV PKGSAHEKQLPKTGTNQSSSP
EAMFVLLAGIGLIATVRRRKAS

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RrkN (8325) (配列番号 1 3)

agtggaaaatattgaaaaaggagtagcaaatgagagataagaaaggaccggtaaataaaagagtagatttct
atcaataaattgaataaattcaataagaaaatttacagttggaacagcatctatttaattggctcactaatgtatttg
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gagcttttgtaaaaatgacagttctggaattcacaaatggttgagaaaaatgataaatcaaaaactaatttttaa
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gggacagaaaaagtaacaagagaaggacaaaaaggtagagaagacaataacgacaccaacactaaaaatc

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【 0 0 6 5 】

【 数 1 2 】

cattaactggagtaattattagtaaaggtgaacccaaaagaagaatcacaaaagatccgattaatgaattaacaga
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 cgagcagaattacaaaaacagggttagaaagcacgcaaaaaggttgatcttagtagtataattggaattgctgga
 ttaatgtattggctcgtagaagaagaattaa

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RrK N (8325) (配列番号 1 4)

SGKYGKRSMQMRD KKG PVNKR VDFLSNKL NKYSIRKFTVGTASILIGSLMYLG TQ
 QEAEAAENNIENPTTLKDNVQSKEVKIEEVTNKDTAPQGV EAKSEVTSNKDTIEHE
 PSVKAEDISKKEDTPKEVADVAEVQPKSSVTHNAETPKVRKARSVDEGSFDITRDS
 KNVVESTPITIQQKEHFEGYGSVDIQKKPTDLGVSEVTRFNVGNESNGLIGALQLK
 NKIDFSKDFNFKVRVAN NHQSNTTGADGWGFLFSKGNAEEYLTNGGILGDKGLVN
 SGGFKIDTGYIYTSSMDKTEKQAGQGYRGYGA FVKNDSSGNSQMVG ENIDKSKT

【 0 0 6 6 】

【 数 1 3 】

NFLNYADNSTNTSDGKFHGQRLNDVILTYVASTGKMRAEYAGKTWETSITDLGLS
 KNQAYNFLITSSQRWGLNQQGINANGWMRTDLKGSEFTFTPEAPKTITELEKKVEEI
 PFKKERKFNPDLAPGTEKVTREGQKGEKTITPTLKNPLTGVIISKGEPKEEITKDPI
 NELTEYGPETIAPGHRDEFDPKLPTGEKEEVPGKPGIKNPETGDVVRPPVDSVTKY
 GPVKGDSIVEKEEIPFEKERKFNPDLAPGTEKVTREGQKGEKTITPTLKNPLTGEII
 SKGESKEEITKDPINELTEYGPETITPGHRDEFDPKLPTGEKEEVPGKPGIKNPETG
 DVVRPPVDSVTKYGPVKGDSIVEKEEIPFEKERKFNPDLAPGTEKVTREGQKGEK
 TITPTLKNPLTGVIISKGEPKEEITKDPINELTEYGPETITPGHRDEFDPKLPTGEKE
 EVPGKPGIKNPETGDVVRPPVDSVTKYGPVKGDSIVEKEEIPFKKERKFNPDLAPG
 TEKVTREGQKGEKTITPTLKNPLTGEIISKGESKEEITKDPINELTEYGPETITPGH
 RDEFDPKLPTGEKEEVPGKPGIKNPETGDVVRPPVDSVTKYGPVKGDSIVEKEEIP
 FEKERKFNPDLAPGTEKVTREGQKGEKTITPTLKNPLTGEIISKGESKEEITKDPIN
 ELTEYGPETITPGHRDEFDPKLPTGEKEEVPGKPGIKNPETGDVVRPPVDSVTKYG
 PVKGDSIVEKEEIPFKKERKFNPDLAPGTEKVTREGQKGEKTITPTLKNPLTGEIIS
 KGESKEEITKDPINELTEYGPETITPGHRDEFDPKLPTGEKEEVPGKPGIKNPETGD
 VVRPPVDSVTKYGPVKGDSIVEKEEIPFEKERKFNPDLAPGTEKVTREGQKGEKTI
 TPTLKNPLTGEIISKGESKEEITKDPINELTEYGPETITPGHRDEFDPKLPTGEKEE
 VPGKPGIKNPETGDVVRPPVDSVTKYGPVKGDSIVEKEEIPFEKERKFNPDLAPGT
 EKVTREGQKGEKTITPTLKNPLTGEIISKGESKEEITKDPVNELTEFGGEKIPQGH
 KDIFDPNLPTDQTEKVPKPGIKNPDTGKVIIEPVDDVIKHGPKTGT PETKTVEIPF
 ETKREFNPKLQPGEEVRVKQEGQPGSKTITPTVNPLTGEKVGEQGPTEEITKQPV
 DKIVEFGGEKPKDPKGPENPEKPSRPTHPSGPVNPNNPGLSKDRAKPNGPVHSM
 DKNDKVKSKIAKESVANQEKKRAELPKTGLESTQKGLIFSSIIGIAGLMLLARRRK
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KnkA (8325) (配列番号 1 5)

ggaaggagtatgtgatggcctaaatcgcagggaaaccgtttcaattatgtaaagttatcggttcgacaatgatggc
 gacaagtatcatcttaacgaatatcttgccgtacgatgcccaagctgcatctgaaaaggatactgaaattacaaaaga
 gatattatctaagcaagatttattagacaaagttgacaaggcaattcgtcaaatgagcaattaaaacagttatcggtc
 catctaaagaacattataaagcacactaaatgaagcgaaaacagcatcgcaaatagatgaaatcataaaacga
 gctaattgagttgtagcaaagacaataaaagttctcactgaaatgaacgggtcaaagtgatatagacagtaaatt
 agatcaattgcttaaagatttaaatgaggtttctcaaatgttgataggggtcaacaaagtggcgaggacgatctaat
 gcaatgaaaaatgatatgcacaaacggctacaacaaacatggagaaaaagatgataaaaatgatgaagca
 atggttaaataaggcgtagaagacctagaccattgaatcagcaaaatacacaaatcgaaagatgcatcgaaagat
 acatcggaagatccagcagtgctacacagataataatcatgaagtagctaaaacgccaataatgatggtctg
 gacatgttggttaaataaattcctttcaaatgaagagaatcaaagccatagtaatcgactcactgataaattacaagg
 aagcgataaaatcatgctatgattgaaaaattagctaaaagtaatgcctcaacgcaacattacacatatcataa
 actgaatacgttacaatcttagatcaacgtattgcaataacgcaacttctaaaaatcaaaaatcagacttaatgagc
 gaagtaaataagacgaaagagcgtataaaaagtcaacgaaatatttttgaagaactgcacgtactgatgata
 aaaagtatgctacacaaagcatttagaaagtattttaataaagacgaggcagttaaaattctaaaagatatagct
 gttgatggtaaaacagatcaacaaatgcagatcaaaactcgtcatattgatcaattatctctgacaacgagtgatg
 atttataacgtcattgatgatcaatcacaagataagtcgctattgatttctcaaatcttacaacgaaattaggaaaag
 ctgaagcagataaattggctaaagattggacgaataaaggattatcaaatcgccaaatcggtgaccaattgaagaa
 acattttgcatcaactggcgacacgtctcagatgatataaaaagcaattttgaataatgccaagataaaaaaca
 agcaattgaaacgattttagcaacacgtatagaaagacaaaaggcaaaattactggcagatttaattactaaaata

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【 0 0 6 7 】

【 数 1 4 】

gaaacagatcaaaataaaatttttaatttagttaaatcggcattgaatggtaaagcggatgatttattgaattacaaaa
 gagactcaatcaaacgaaaaaagatatagattatattttatcaccaatagtaaatcgccaagttactagatcgattg
 aataaaaaatgggaaacgacagatttaataagtttagcaaatatgaatcaaggatcagatttattagacagtatt
 ccagatataccacaccaaagccagaaaagacggttaacacttggttaaaggtaatggattgtaagtggattattaa
 tgctgatggtaatgtatctttgctaaagcgggggaaacgataaaagaacattggtgccgatctgtaattgttggtg
 caatgggtgtactaatgatttggttatcacgacgcaataagttgaaaaataaagcataa

KnkA (8325) (配列番号 16)

GRSMLMAKYRGKPFQLYVKLSCSTMATSIIILNIPYDAQAASEKDTEITKEILSK
 QDLLDKVDKAIKQIEQLKQLSASSKEHYKAQLNEAKTASQIDEIHKRANELDSKDNK
 SSHTMNGQSDIDSKLDQLLKDLNEVSSNVDRGQQSGEDDLNAMKNDMSQTATT
 KHGEKDDKNDEAMVNKALEDLDHLNQIHKSKDASKDTSSEDPVSTTDNNHEVA
 KTPNNDGSGHVVLNKFLSNEENQSHSNRLTDKLQGS DKINHAMEIKLAKSNASTQ
 HYTYHKLNTLQSLDQRIANTQLPKNQKSDLMSEVNKTKERIKSQRNIIIEELARTDD
 KKYATQSILESIFNKDEAVKILKDIRVDGKTDDQIADQITRHHIDQLSLTSSDDLTLSD
 QSQDKSLLISQILQTKLGAEADKLAKDWTNKGSLNRQIVDQLKKHFASTGDTSSD
 DILKAILNNAKDKKQAIETILATRIERQKAKLLADLITKIETDQNKIFNLVKSALNGKAD
 DLLNLQKRLNQTKKIDIDYILSPIVNRPSLLDRLNKNKGTDDLNLKLANLMNQGSDDL
 SIPDIPTPKPEKTLTLGKGNGLLSGLLNADGNVSLPKAGETIKEHWLPISVIVGAMG
 VLMIWLSRRNKLKNA

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【0068】

〔一次構造分析：〕

バイオインフォマティクスのアプローチを、一次構造および機能予測のために使用した (図1)。蛋白質 RrkN および DsqA は、前述の MSCRAMM と類似した構造組織 (structural organization) を有した。RrkN は、S. aureus および S. epidermidis それぞれの Pls/Aap 蛋白質と構造において類似していた。それは、Pls および Aap と 40% 同一性を示す 200 残基ドメインをその N 末端に含む。該蛋白質の C 末端は、主に、128 残基繰り返しドメイン (repeat domain) (これは、株ごとに繰り返し数が増加する) から構成されている。これらの繰り返しはまた、Pls および Aap に存在している。推定 sar 相同体ならびに fnbpA および fnbpB は、該ゲノムにおいて RrkN から直接上流に位置している。

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【0069】

DsqA は、蛋白質の Sdr ファミリーと構造組織において類似している。それは、典型的な A ドメイン、続いて TYYFTDVK モチーフ (これは、Sdr 蛋白質の全てにおいて見られる保存された TYTF TVYVD モチーフに類似している) を含んでいる。このモチーフの機能は、まだ、決定されなければならない。2つの 88 残基繰り返しドメインが、該蛋白質の中央に存在し、これに、Sdr 蛋白質において見られる SD-繰り返しモチーフと類似した C-末端 SX-繰り返しモチーフが続く。この繰り返しのサイズは、株ごとに変化する。DsqA は、該ゲノムにおいて secY および secA に隣接している。DsqA 相同体 (>90% 同一) はまた、S. epidermidis において見られる。

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【0070】

KnkA は、その配列に繰り返しドメインを含まない。二次構造予測分析は、この蛋白質が、主としてアルファヘリックスから構成されることを示す。

【0071】

RkaS は、その配列に繰り返しドメインを含まない。BLAST 分析は、それが、5' ヌクレオチダーゼ UDP-糖ヒドロラーゼと類似していることを示す。RkaS をコードする遺伝子は、orfX (mec エLEMENT の挿入部位) から直接上流に位置する。

【0072】

KesK は、38% 同一である 2つの 140 残基繰り返しドメインを該蛋白質の N 末端に

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含む。ヒドロパシープロット分析 (hydropathy plot analysis) (Kyte and Doolittle, 1982) は、該蛋白質の中央に大きな親水性ドメインが存在することを示す (残基 500-560)。

【0073】

E k e S は、38% 同一である 2 つの 300 残基繰り返しドメインを該蛋白質の中央に含む。ブラスト分析 (Blast analysis) は、該蛋白質の N 末端 (残基 1-1268、両方の繰り返しを有する) が F m t B [17 タンデム繰り返し (tandem repeats) を有する L P X T G 蛋白質] と 49% 同一であることを示す。f m t B の不活性化がメチシリン耐性を撤廃する (abolishes) ために、F m t B は、メチシリン耐性に間接的に関与していると提案されている。これは、細胞壁前駆体グルコサミン-1-ホスフェートの産生を増加させることによってメチシリン感受性が緩和され得るので、細胞壁組成に影響を与えることに起因するようである (Komatsuzawa et al., 2000)。

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【0074】

K r k N および K r k N 2 は、ゲノムにおいて互いに隣接している。

【0075】

〔発現分析：〕

蛋白質データベースに配列相同性が不足しているために、これらの蛋白質の各々についての推定機能を予測することができず、そして従って、分子のアプローチを行った。o r f s の 4 つの特有の領域を、Q i a g e n p Q E-30 発現系を使用して、組換え h i s タグ融合蛋白質 (recombinant his-tagged fusion proteins) として、E. c o l i に おいて発現させた。図 2 は、精製 N 末端 h i s タグ融合蛋白質のクマシー染色された (C o m a s s i e s t a i n e d) S D S-P A G E ゲルを示す。組換え蛋白質 R r k N 1、D s q A 2、K e s K 1 および K n k A を使用し、ウサギにおいて抗体を産生させた。S. a u r e u s 細胞壁抽出物のウェスタンブロッティング分析は、K e s K、K n k A および D s q A が発現されそして細胞壁結合されている (cell wall-associated) ことを示した (図 3)。株 e M R S A-16 は、k n k A 遺伝子を欠いているために、k n k A 陰性株を示す。65 k D a の免疫反応性バンドは、株 8325-4 の指数増殖期および定常期の両方の細胞由来の細胞壁分画と反応する (図 3、B)。株 e M R S A-16 においてこのバンドが存在しないことは、それが k n k A の遺伝子産物を示すことを示唆している。

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【0076】

抗-K e s K 抗体を使用する株 8325-4 の細胞壁分画のウェスタンイムノブロッティングは、指数増殖期および定常期の両方の培養物において 150 k D a 免疫反応性バンドを同定した。発現プラスミド (p K S 80) において全長 K e s K を発現する L a c t o c o c c u s l a c t i s の細胞壁分画から放出された同様なサイズの免疫反応性蛋白質は、150 k D a バンドが k e s K 遺伝子産物を示すことを示唆する (データを示していない)。S. a u r e u s における k e s K ノックアウト突然変異体が、細胞壁放出される K e s K 蛋白質のサイズを確認するために必要とされるだろう。

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【0077】

抗-D s q A 抗体を使用する S. a u r e u s 株 M S S A および e M R S A-16 の細胞壁分画のウェスタンイムノブロッティングは、130 k D a 免疫反応性バンドを同定した。発現レベルは、定常期細胞においてより高い。

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【0078】

〔L a c t o c o c c u s l a c t i s における異種発現：〕

L a c t o c o c c u s l a c t i s (L. l a c t i s) における S. a u r e u s 表面蛋白質の異種発現は、蛋白質機能を研究するためのツールとして既に使用されている (Sinha et al., 2000)。この研究において、この代替系 (surrogate system) は、L. l a c t i s の表面において、インシリコ予測された (in silico-predicted) M S C R A M M の各々を発現させて、機能を探るために使用される。K e s K および K n k A は、L. l a c t i s へクローン化され、そしてドットブロッティング (dot blotting) によって表面発現されることが示された (図 4)。交差反応は、ネガティブコントロール (挿

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入物 (insert) を含まない p K S 8 0 プラスミド) を用いて観察されず、これが特異的反応であることを示す。p K S - K n k A および p K S - K e s K を有する *L a c t o c o c c u s l a c t i s* の細胞壁およびプロトプラスト分画を、リゾチームおよびムタノリシン (mutanolysin) を用いての細胞の消化によって作製し、そしてそれぞれ、抗-K n k A および抗-K e s K 抗体を使用してのウェスタンブロッティング研究において使用した。*S. a u r e u s* において観察されたものとは異なり、K n k A は、*L. l a c t i s* の細胞壁分画において検出されなかったが、プロトプラスト分画と結合されることが判った。K n k A の固着モチーフ (anchoring motif) は、それがスレオニンの代わりにアラニン残基を含有する (即ち、L P K A G) という点で、コンセンサス L P X T G 配列とは異なる (表 1)。*S. a u r e u s* は 2 つのソルターゼ (sortase) 遺伝子、s r t A および s r t B を含むことが、最近発表された (Pallen, 2001)。*L P X T G* モチーフのこの変異形態は第二ソルターゼ遺伝子 (これは、*L. l a c t i s* には存在しない) によって処理されることが可能である。これはまた、細胞壁ソーティングシグナル (cell wall sorting signal) は切断されていなかったもので、プロトプラスト分画において観察された K n k A 蛋白質のサイズの僅かな増加を説明する。

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【0079】

K e s K は、*L. l a c t i s* の細胞壁分画において検出されたが、*S. a u r e u s* の細胞壁から放出された K e s K 蛋白質よりも少ない分子量で移動した。*L. l a c t i s* の表面において発現される M S C R A M M の大部分は、細胞壁抽出手順の間に、蛋白質分解の傾向がある (Louise O'Brien, personal communication)。従って、*L. l a c t i s* の表面から放出された K e s K 蛋白質が K e s K の先を切り取った形態 (truncated form) を示すことが可能である。リゾチームおよびムタノリシンでのより短い消化時間が、蛋白質分解の程度を制限することが示された。

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【0080】

〔インビボにおけるインシリコ予測される M S C R A M M の発現：〕

S. a u r e u s 感染から回復した 33 患者からの回復期 (convalescent-phase) 血清を、E L I S A アッセイにおいて精製 N 末端 h i s タグ融合蛋白質 (purified N-terminal his-tag fusion proteins) を認識するそれらの能力を試験した。子供および健康な血液ドナーからのプールした血清を、ネガティブコントロールとして使用した。陽性反応は、該ネガティブコントロールの値の二倍に等しいか又はそれ以上の値の場合に採用した。図 5 A ~ 5 D は、全ての蛋白質が、患者の 27 ~ 42 % によって認識されたことを示し、これらの蛋白質がインビボで発現されそして宿主の感染の間に免疫原性であることを示す。

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【0081】

〔参考文献：〕

【0082】

【表 5】

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【0083】

〔実施例2：S. Aureusおよびコアグララーゼ陰性ブドウ球菌からの交差反応性蛋白質の単離および配列決定〕 20

S. epidermidisは、S. aureus MSCRAMM（登録商標）蛋白質と構造的に関連する表面蛋白質を含有することが、最近示された（US 09/386,962）。S. aureus由来の1つの蛋白質は、それがS. epidermidisにおけるものに近い相同体を有するので、特に興味深い。該蛋白質は、DsqaまたはSasa（S. aureus）ならびにDgsk（S. epidermidis）と呼ばれる。それらは、約500アミノ酸残基の典型的な“A”ドメイン、続いて88残基の2つのB繰り返し（これらは、～40%同一である）、および特有のSXSXジペプチド繰り返し（これは、株に依存して長さが変化し得る）によって特徴付けられる。S. aureus Dsqa / SasaのAドメイン内に、S. aureus蛋白質RrkN、PlsおよびS. ep 30
i d e r m i d i s蛋白質Aapの領域A内の類似のサイズのドメインとの～40%同一性を有する180残基領域が含まれる。Dsqa / SasaおよびDgsk蛋白質のA領域は、アミノ酸レベルで46%同一であり、BB繰り返しは50%同一である。S. aureusおよびコアグララーゼ陰性ブドウ球菌蛋白質の両方を認識する、ワクチン、ポリクローナルおよびモノクローナル抗体を含む、能動および受動免疫化戦略は、本発明の主題である。

【0084】

コアグララーゼ陰性ブドウ球菌およびS. aureusと交差反応する抗体の具体例。

【0085】

【数15】

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コアグラゼ陰性ブドウ球菌 D g s K Aドメイン:

アミノ酸配列 (配列番号 17)

ASETPITSEISSNSETVANQNSTTIKNSQKETVNSTLESNHSNSTNKQMSSEVTN
TAQSSEKAGISQQSSETSNQSSKLNTYASTDHVESTTINNDNTAQDQNKSSNVT
SKSTQSNTSSSEKNISSNLQSIETKATDSLATSEARTSTNQISNLTSTSTSNQSSP
TSFANLRTFSRFTVLNTMAAPTTTSTTTTSSLTSNSVVVNKDNFNEHMLSGSATY
DPKTGIATLTPDAYSQKGAISLNRDLSNRSFRFIGKVN LGNRYEGYSPDGVAGGD
GIGFAFSPGPLGQIGKEGA AVGIGGLNNAFGFKLD TYHNTSTPRSDAKAKADPRN
VGGGGAFGAFVSTDRNGMATTEESTA AKLNVQPTDNSFQDFVIDYNGDTKVMTV
TYAGQTFTRNLTDWIKNSGGTTFSLSMTASTGGAKNLQQVQFGTFEYTESAVAKV
RYVDANTGKDIIPPKTIAGEVDGTVNIDKQLNNFKNLGYSYVGTDALKAPNYTETSG
TPTLKL TNSSQTVIYKFKDVQ

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S. aureus S a s A Aドメイン:

アミノ酸配列 (配列番号 18)

ASDAPLTSELNTQSETVGNQNSTTIEASTSTADSTSVTKNSSSVQTSNSDTVSEK
SEKVTSTTNSTSNQQEKLSTSESTSSKNTTSSSDTKSVASTSSTEQPINTSTNQS
TASNNTSQSTTPSSVNLNKTSTTSTSTAPVKLRTFSRLAMSTFASAATTTAVTANTI
TVNKDNLKQYMTTSGNATYDQSTGIVTLTQDAYSQKGAILGTRIDSNKSFHFSGK
VNLGNKYEGHGNNGDGIGFAFSPGVLGETGLNGAAVGIGGLSNAFGFKLD TYHNT
SKPNSAAKANADPSNVAGGGAFGAFVTTDSYGVATTYTSSSTADNA AKLNVQPT
NNTFQDFDINYNNGDTKVMTVKYAGQWTRNISDWIAKSGTTNFSLSMTASTGGAT
NLQQVQFGTFEYTESAVTQVRYVDVTTGKDIIPPKTYS GNVDQVVTIDNQQSALTA
KGYNYTSVDSSYASTYNDTNKTVKMTNAGQSVTYFTDVV

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【0086】

A a p 蛋白質およびそれをコードするDNAの全配列を (Aドメインの存在を示しながら)
)、以下に示す:

【0087】

【数16】

S. epidermidis Aap 蛋白質 (Aドメインに下線) (配列番号 19)

MGKRRQGPINKKVDFLPNKLNKYSIRKFTVGTASILLGSTLIFGSSSHEAKAAEEKQ
VDPITQANQNDSSERSLENTNQPTVNNEAPQMSSTLQAEEGSNAEAPQSEPTKA
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NDTQTQFSEAPTRNDLARKEDIPAVSKNEELQSSQPNTDSKIEPTTSEPVNLNYSS
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KDGDDFLKNGGILREKGTSAAGFRIDTGYYNNDPLDKIQKQAGQGYRGYGTFFV
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ATYAGKTWTATLSELGLSPTDSYNFLVTSSQYGNNGNSGTYSVGMRADLDGATL
TYTPKAVDGDPIISTKEIPFNKKREFDPNLAPGTEKVQKGEPGIETTTTPTYVNP
TGEKVGEGETEKITKQPVDEIVHYGGEEIKPGHKDEFDPNAPKGSQTTQPGKPG
VKNPDTGEVTPPVDDVTKYGPVDGDPITSTEEIPFDKKREFNPDLKPGEERVKQ
KGEPGKTITPTTKNPLTGEKVGEGETEKITKQPVDEITEYGGEEIKPGHKDEFD
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REFNPDLKPGEERVKQKGEPGKTITPTTKNPLTGEKVGEGETEKITKQPVDEI
VHYGGEQIPQGHKDEFDPNAPVDSKTEVPKPGVKNPDTGEVTPPVDDVTKYG
PVDGDSITSTEEIPFDKKREFDPNLAPGTEKVQKGEPGKTITPTTKNPLTGEKV
GEGKSTEVTKQPVDEIVEYGPTKAEPGKPAEPGKPAEPGKPAEPGTPAEPGKPA
EPGTPAEPGKPAEPGKPAEPGKPAEPGKPAEPGTPAEPGTPAEPGKPAEPGTPA
EPGKPAEPGTPAEPGKPAESGKPVPGTPAQSGAPEQPNRSMHSTDNKNQLPD
TGENRQANEGTLVGSLLAIVGSLFIFGRRKKGNEK

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S. epidermidis aap DNA (配列番号 20)

atgggcaaac gtagacaagg tcctattaat aaaaaagtgg

【 0 0 8 8 】

【 数 1 7 】

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 caatattact tggttcgaca cttatttttg gaagtagtag ccatgaagcg aaagctgcag
 aagaaaaaca agttgatcca attacacaag ctaatcaaaa tgatagtagt gaaagatcac
 ttgaaaacac aaatcaacct actgtaaaca atgaagcacc acagatgtct tctacattgc
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 gaggcaatgc agaagcagct caatctgagc caacgaaggc agaagaagga ggcaatgcag
 aagcacctca atctgagcca acgaaggcag aagaaggagg caatgcagaa gcagctcaat
 ctgagccaac gaagacagaa gaaggaagca acgtaaaagc agctcaatct gagccaacga
 aggcagaaga aggaagcaat gcagaagcac ctcaatctga gccaacgaag acagaagaag
 gaagcaacgc aaaagcagct caatctgagc caacgaaggc agaagaagga ggcaatgcag
 aagcagctca atctgagcca acgaagacag aagaaggaag caatgcagaa gcacctcaat
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 ttaactacgc agataatact actaatgatt tagatggtaa attccatggt caaaaattaa
 ataatgttaa ttigaaatat aatgcttcaa atcaaacttt tacagctact tatgctggta
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【 0 0 8 9 】

【 数 1 8 】

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 aaccaatag atcaatgcat tcaacagata ataaaaatca attacatgat acagggtgaa

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【 0 0 9 0 】

【 数 1 9 】

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tcatatttgg tcgtcgtaaa aaaggaatg aaaaaataatt tcatataaaa actttctgcc
attaa

S. epidermidis Aap由来のAドメイン (アミノ酸55-600)
(配列番号21)

⁵⁵EKQVDPITQANQNDSSERSLENTNQPTVNNEAPQMSSTLQAEEGSNAEAPQSE
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GSNVKAAQSEPTKAEEGSNAEAPQSEPTKTEEGSNAKAAQSEPTKAEEGGNAEA
AQSEPTKTEEGSNAEAPQSEPTKAEEGGNAEAPQSEPTKTEEGGNAEAPNVPTIK
ANSNDNTQTQFSEAPTRNDLARKEDIPAVSKNEELQSSQPNTDSKIEPTTSEPVNL
NYSSPFMSLLSMPADSSSNNTKNTIDIPPTTVKGRDNYDFYGRVDIESNPTDLNAT
NLTRYNYGQPPGTTTAGAVQFKNQVSFDKDFDNIRVANNRQSNTTGADGWGF
MFSKKDGDDFLKNGGILREKGTSAAGFRIDTGYYNNDPLDKIQKQAGQGYRGYG
TFVKNSQGNSTKVGSGTPSTDFLNYADNTTNDLDGKFHGQKLNNVNLKYNASN
QTFTATYAGKTWTATLSELGLSPTDSYNFLVTSSQYGNNGSGTYASGVMRADLD
GA⁶⁰⁰

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【0091】

〔蛋白質産生および精製〕

PCRを使用して、DgsKまたはSasAのAドメインを、上記の配列から増幅させ、そして*E. coli*発現ベクターPQE-30 (Qiagen) [これは、6つのヒスチジン残基を含む組換え融合蛋白質の発現を可能にする]へサブクローン化した。引き続いて、このベクターを*E. coli*株ATCC 55151へ形質転換し、0.7の光学密度 (OD₆₀₀)まで15リットル発酵槽において増殖させ、そして4時間0.2mMイソプロピル-1-ベーターDガラクトシド (IPTG)で誘導した。細胞を、AG Technologies中空糸アセンブリ (細孔サイズ (pore size) 0.45μm)を使用して収獲し、そして細胞ペーストを-80℃で凍結させた。細胞を、1100psiでthe French Pressを通過させる2回パスを使用して1×PBS (10mL緩衝液/1g細胞ペースト)において溶解させた。溶解した細胞を、30分間17,000rpmでスピンドウンさせて (spun down)、細胞残骸を除去した。上清みを、0.1M NiCl₂を充填させた5-mL HiTrap Chelating (Pharmacia)カラムに通過させた。ローディングした (loading) 後、カラムを、10mM Tris (pH8.0)、100mM NaCl (緩衝液A)の5カラム容積で洗浄した。蛋白質を、30カラム容積に渡って、10mM Tris (pH8.0)、100mM NaCl、200mM イミダゾール (緩衝液B)の0~100%勾配を使用して溶出した。SdrGN1N2N3またはSdrGN2N3は、~13%緩衝液B (~26mMイミダゾール)で溶出した。280nmでの吸光度をモニターした。SdrGN1N2N3またはSdrGN2N3を含有する分画を、1×PBSで透析した。

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【0092】

次いで、各蛋白質を、エンドトキシン除去プロトコル (endotoxin removal protocol)に通した。このプロトコルの間に使用した緩衝液は、5-mL Mono-Q sepharose (Pharmacia)カラムを通過させることによって、内毒素フリーにさせた。蛋白質を、4×15mLチューブに等しく分割した。各チューブの容積は、緩衝液Aを用いて9mLにされた。1mLの10% Triton X-114を各チューブに添加し、そして4℃で1時間回転しながらインキュベートした。チューブを37℃水浴に配置

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して相分離させた。チューブを10分間2,000rpmでスピンドウンさせ、そして各チューブからの上部の水相を回収し、そして洗剤抽出(detergent extraction)を繰り返した。第2番目の抽出からの水相を合わせ、そして0.1M NiCl_2 をチャージした5-mL IDA chelating (Sigma) カラムを通過させて、残っている洗剤を除去した。カラムを緩衝液Aの9カラム容積で洗浄し、その後、該蛋白質を緩衝液Bの3カラム容積で溶出した。溶離液(eluant)を、5-mL Detoxigel (Sigma) カラムを通過させ、フロースルー(flow-through)を回収し、そしてカラムへ再適用した。第2通過からのフロースルーを回収し、そして1×PBSにおいて透析した。精製産物を、濃度、純度およびエンドトキシンレベルについて分析し、その後マウスへ投与した。

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【0093】

〔モノクローナル抗体産生〕

E. coli 発現および精製された組換えSasAおよびDsgK蛋白質を、マウスモノクローナル抗体のパネル(panel)を産生するために使用し、一方、マウス血清を、ポリクローナル抗体のソース(source)として使用した。手短に言えば、Balb/CまたはSJLマウスのグループに、以下の表において以下に記載されるように溶液状態のまたはアジュバントと混合されている1~10mgの蛋白質の一連の皮下免疫化(subcutaneous immunizations)を受容させた。

【0094】

〔免疫化スキーム〕

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【0095】

【表6】

RIMMS 注射	日	量	(μg)	経路	アジュバント
#1	0	5		皮下	FCA/RIBI
#2	2	1		皮下	FCA/RIBI
#3	4	1		皮下	FCA/RIBI
#4	7	1		皮下	FCA/RIBI
#5	9	1		皮下	FCA/RIBI

慣用的な注射	日	量	(μg)	経路	アジュバント
初期	0	5		皮下	FCA
ブースト#1	14	1		腹腔内	RIBI
ブースト#2	28	1		腹腔内	RIBI
ブースト#3	42	1		腹腔内	RIBI

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【0096】

犠牲(sacrifice) (RIMMS) 時またはブースト(boost) (慣用的) 後7日に、血清を回収し、そしてMSCRAMM (登録商標) 蛋白質に対するまたは全細胞(S. epidermidis および S. aureus) についてのELISAアッセイにおいて力価を測定した。最終ブースト3日後に、脾臓またはリンパ節を除去し、単一細胞懸濁へ掻き裂き(teased)、そしてリンパ球を収獲した。次いで、リンパ球をP3X63Ag8.653メラノーマ細胞系(ATCC#CRL-1580)へ融合させた。細胞融合、引き続いてプレート化(plating)および栄養補給(feeding)を、Current Protocols in Immunologyからのthe Production of Monoclonal Antibodies protocol (Chapter 2, Unit 2) [本明細書中で参考として援用される]に従って行った。

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【0097】

次いで、該融合物から産生されたクローンを、標準ELISAアッセイを使用して特異的抗-SasA抗体産生についてスクリーンした(screened)。陽性クローンを増やしそしてフローサイトメトリーにより全細菌細胞結合アッセイにおける活性についてそしてBiacore分析によりSasA結合について更に試験した。

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【0098】

〔Biacore 分析〕

該分析の間、流速 (flow rate) は 10 ml / 分で一定のままであった。SasA または DgsK 注射の前に、試験抗体を、RAM-Fc 結合を介してチップ (chip) へ吸着させた。時間 0 の際、30 mg / ml の濃度の SasA または DgsK を、3 分間チップ上に注射し、続いて 2 分間解離した (dissociation)。この段階の分析によって、Mab / SasA または DgsK 相互作用の相対的結合および解離動力学 (the relative association and disassociation kinetics) が測定された。

【0099】

〔全細菌への結合〕

細菌サンプル *S. aureus* Newman、*S. aureus* 67-0、*S. aureus* 397 (Sal 6)、*S. aureus* Wood、*S. aureus* 8325-4、メチシリン耐性 *S. aureus* MRSA 16、*S. epidermidis* ATCC 35984、*S. epidermidis* HB、*S. epidermidis* CN-899 および *S. haemolyticus* ATCC 43253 を回収し、洗浄し、そしてウサギ IgG (50 mg / ml) でブロッキングした後の 2 μg / ml の濃度の Mab または PBS のみ (コントロール) と共にインキュベートした。抗体と共のインキュベーション後、細菌細胞を、ヤギ F_(ab')2-抗マウス-F_(ab')2-FITC (これは、検出抗体として役立った) と共にインキュベーションした。抗体標識後、細菌細胞を、FACS カリバーフローサイトメーター (FACScaliber flow cytometer) を介して吸引して、蛍光放出を分析した (励起: 488、放出: 570)。各細菌株について、10,000 事象 (events) を回収しそして測定した。これらのデータは、*S. aureus* SasA に対する抗体がコアグラゼ陰性ブドウ球菌の表面上の相同な蛋白質を認識し得たことを示す。該データは、*S. aureus* SasA に対するウサギポリクローナル抗体が *S. epidermidis* HB の細胞表面から放出された蛋白質ならびに *S. epidermidis* からクローン化された DgsK 由来の組換え A-領域と交差反応することを実証するウェスタンブロット分析を支持する (下記の表および図 6 を参照のこと)。

【0100】

【表 7】

ポリクローナル血清反応性

	Newman	67-0	397 (SAL 6)	Wood 46	8325-4	MRSA 16	ATCC 35984	HB	CN-899	ATCC 43253
正常マウス血清	-	-	-	-	-	-	-	-	-	-
マウス抗-SasA	+	+	+/-	-	+	+	+	+	+	+

【図面の簡単な説明】

【0101】

【図 1】

図 1 は、本発明に従うインシリコ予測される蛋白質の一次構造の描写である。

【図 2】

図 2 は、本発明の orfs を発現する精製 N 末端組換え His-タグ蛋白質のクマシーゲルを示す。

【図 3】図 3 A～3 C は、それぞれ抗-KesK 抗体（図 3 A）、抗-KnkA 抗体（図 3 B）および抗-DsqA 抗体（図 3 C）でのプロービング（probing）を示す、*S. aureus* 細胞壁抽出物のウェスタンブロッティングを示す。

【図 4】

図 4 A～4 B は、*S. aureus* MSCRAMM（登録商標）、即ち KnkA（図 4 A）および KesK（図 4 B）を発現する *Lactococcus lactis* のドットブロッティングおよびウェスタンイムノブロッティングを示す。

【図 5】

図 5 A～5 D は、RrKn および RrKN2（図 5 A）、KesK1 および KesK2A（図 5 B）、KnkA（図 5 C）ならびに DsqA2（図 5 D）を含む、インビボ発現を検査する回復期血清を用いての、本発明に従う組換え LPXTG 蛋白質のプロービングを示す。

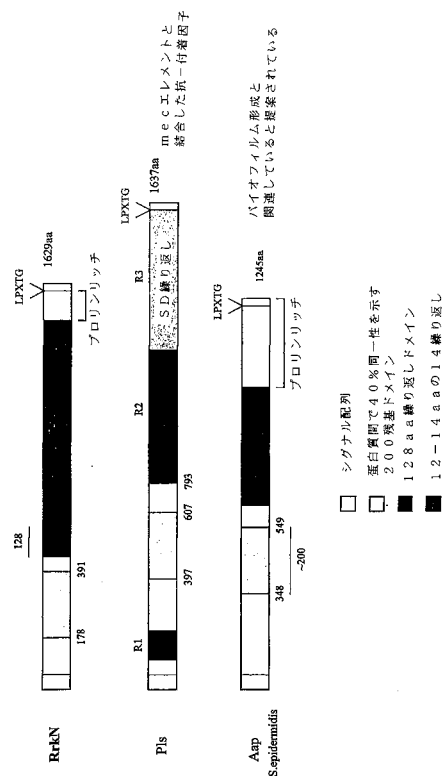
【図 6】

図 6 は、*S. aureus* SasA に対するウサギポリクローナル抗体は、*S. epidermidis* HB の細胞表面から放出される蛋白質ならびに *S. epidermidis* からクローン化された DsgK 由来の組換え A-領域と交差反応することを実証する、ウェスタンブロット分析を示す。

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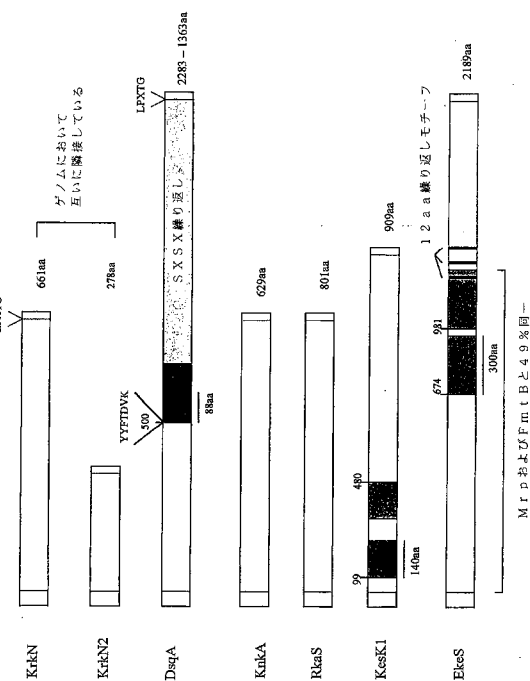
【図 1-1】

Figure 1. インシリコ予測される LPXTG 蛋白質の一次構造



【図 1-2】

Figure 1 (続き)



【図 2】

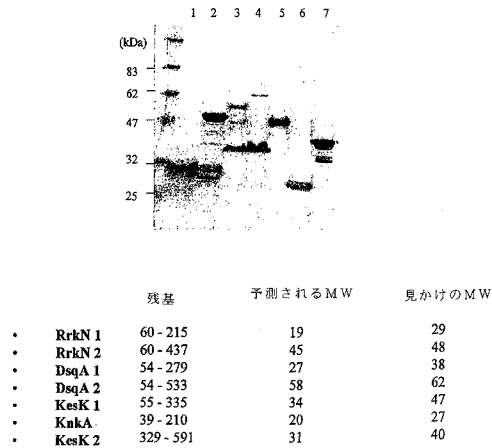


Figure 2. 精製N末端Hisタグ融合蛋白質のクマシーゲル

【図 3】

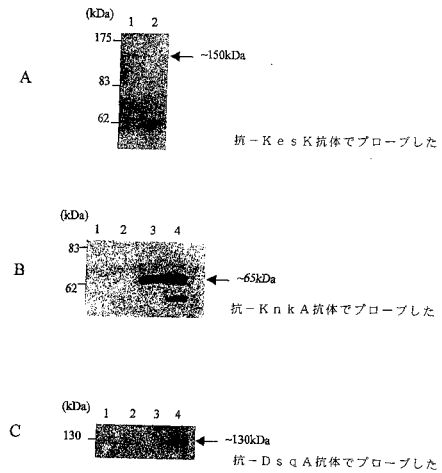


Figure 3. *S. aureus* 細胞壁抽出物のウェスタンブロッティング。
細菌細胞を50単位のOD₆₀₀で標準化し、そして細胞壁を、安定化プロトプラストのリゾスタフィン消化によって単離した。
A. レーン1、8325-4（初期指数増殖期）；レーン2、8325-4（定常期）。
B. レーン1および2、eMRSA-16；レーン3および4、8325-4；レーン1および3は初期指数増殖期細胞を示し、そしてレーン2および4は定常期細胞を示す。
C. レーン1および2、MSSA；レーン3および4、eMRSA-16；レーン1および3は初期指数増殖期細胞を示し、そしてレーン2および4は定常期細胞を示す。

【図 4】

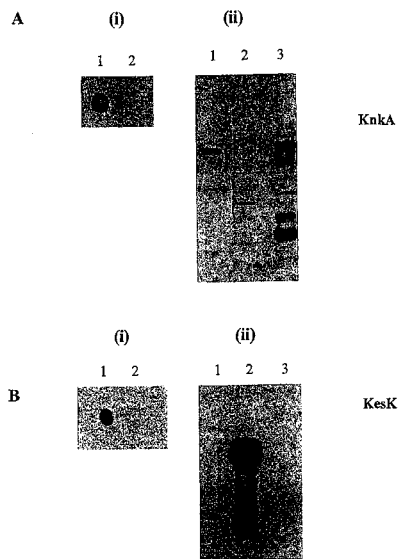


Figure 4. *S. aureus* MSCRAMMを発現する *Lactococcus lactis* のドットブロッティングおよびウェスタンブロッティング。
全長 knkA および kesK を、*L. lactis* 発現プラスミド pKS80 にクローニングし、そしてコンピテントな (competent) *L. lactis* MG1363 細胞へエレクトロポレーションした。陽性 KnkA および KesK 発現クローンを、それぞれ、抗-KnkA (A) および抗-KesK (B) 抗体を用いてドットブロッティングを使用して検出した。pKS80 を有する *L. lactis* を、ネガティブコントロールとして使用した。A. (i) レーン1、*L. lactis* pKS-KnkA；レーン2、*L. lactis* pKS80。B. (i) レーン1、*L. lactis* pKS-KnkA；レーン2、*L. lactis* pKS80。ウェスタンブロッティングを使用して、*S. aureus* および *L. lactis* において、KesK および KnkA の発現を検査した。A (ii)。レーン1、指数増殖期 *S. aureus* 株 8325-4 由来の細胞壁抽出物；レーン2、pKS80 を有する *L. lactis* 由来のプロトプラスト分画；レーン3、pKS-KnkA を有する *L. lactis* 由来のプロトプラスト分画。B. (ii) レーン1、指数増殖期 *S. aureus* 株 8325-4 由来の細胞壁抽出物；レーン2、pKS-KesK を有する *L. lactis* 由来の細胞壁抽出物；レーン3、pKS80 を有する *L. lactis* 由来の細胞壁抽出物。

【図 5 A】

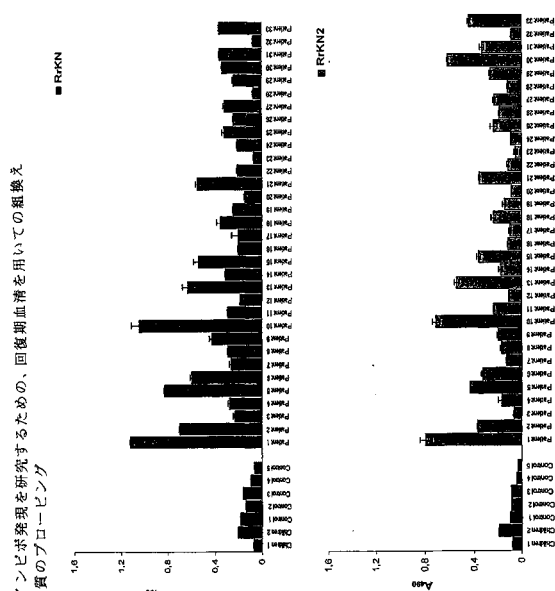


Figure 5A. インビトロ研究するための、回復期血清を用いての組織換え LPTX 蛋白質のプロローピング

【図 5 B】

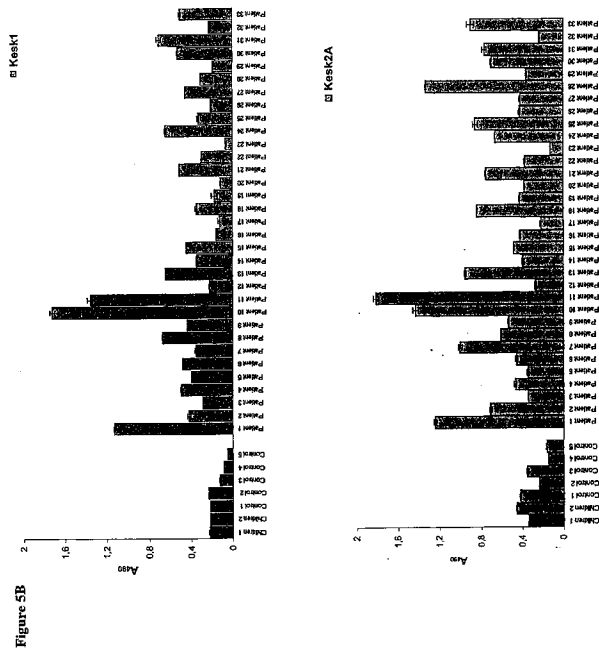


Figure 5B

【図 5 C】

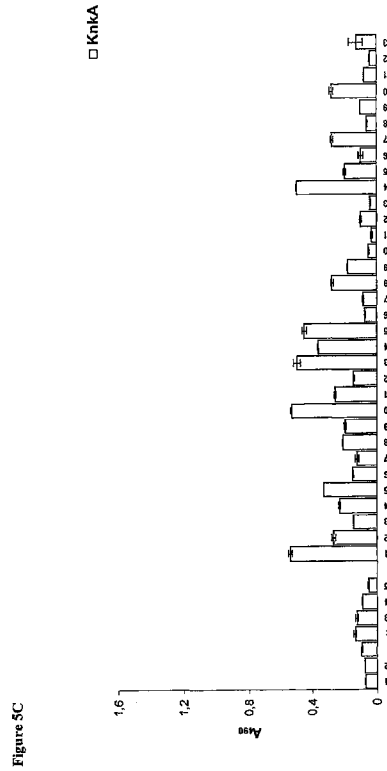


Figure 5C

【図 5 D】

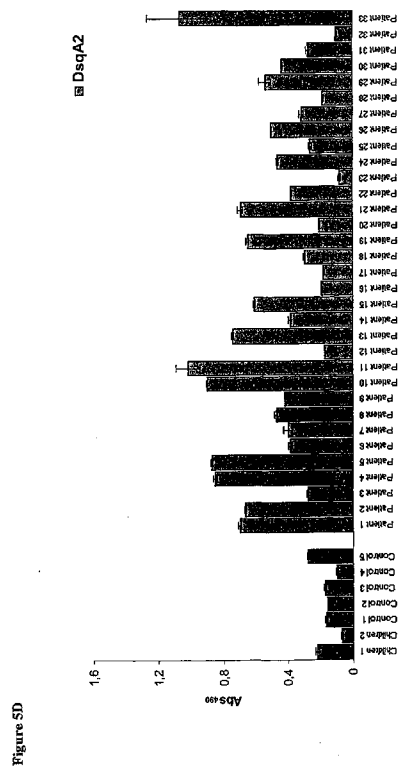


Figure 5D

【図 6】

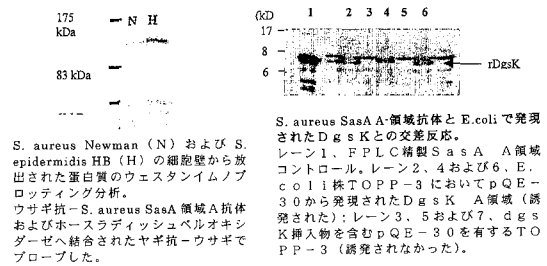


FIGURE 6

【国際公開パンフレット】

(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau(43) International Publication Date
27 December 2002 (27.12.2002)

PCT

(10) International Publication Number
WO 02/102829 A2

- (51) International Patent Classification: C07K
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(21) International Application Number: PCT/US02/19220
SPEZIALE, Pietro; c/o Università Degli Studi Di Pavia,
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(22) International Filing Date: 17 June 2002 (17.06.2002)
(25) Filing Language: English
(26) Publication Language: English
(30) Priority Data: 60/298,098 15 June 2001 (15.06.2001) US
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(81) Designated States (national): AL, AG, AL, AM, AI, AU,
AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU,
CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH,
GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC,
LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW,
MX, MY, NZ, OM, PH, PL, PT, RO, RU, SD, SE, SG,
SI, SK, SL, TJ, TM, TN, TR, TT, TZ, UA, UG, UZ, VN,
YU, ZA, ZM, ZW.
(84) Designated States (regional): ARIPO patent (GH, GM,
KE, LS, MW, MZ, SD, SI, SZ, TZ, UG, ZM, ZW),
Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM),
European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR,
GB, GR, IE, IT, LU, MC, NL, PT, SE, TR), OAPI patent
(BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR,
NI, SN, TD, TG).
Published:
— without international search report and to be republished
upon receipt of that report
For two-letter codes and other abbreviations, refer to the "Guid-
ance Notes on Codes and Abbreviations" appearing at the begin-
ning of each regular issue of the PCT Gazette.



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(54) Title: CROSS-REACTIVE MONOCLONAL AND POLYCLONAL ANTIBODIES WHICH RECOGNIZE SURFACE PRO-
TEINS FROM COAGULASE-NEGATIVE STAPHYLOCOCCI AND STAPHYLOCOCCUS AUREUS(57) Abstract: Polyclonal and monoclonal antibodies which are cross-reactive to both coagulase-positive staphylococcus bacteria,
such as *S. hemolyticus*, are provided which can recognize surface proteins from both coagulase-positive and coagulase negative staph-
bacteria. The antibodies may be generated from surface proteins that have been isolated on the basis of characteristics that may be
common between *S. aureus* and coagulase-negative staphylococci, and these recombinant surface proteins are used to generate the
antibodies of the invention. There is also provided vaccines and methods which utilize these proteins and antibodies for the treatment
or protection against a wide variety of staphylococcal infections.

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**CROSS-REACTIVE MONOCLONAL AND POLYCLONAL ANTIBODIES
WHICH RECOGNIZE SURFACE PROTEINS FROM COAGULASE-NEGATIVE
STAPHYLOCOCCI AND STAPHYLOCOCCUS AUREUS**

Cross Reference to Related Applications

5 The present application claims the benefit of U.S. provisional application Ser.
No. 60/298,098 filed June 15, 2001.

Field of the Invention

10 The present invention relates in general to surface proteins from
Staphylococcus aureus and their active regions such as their A domains which have
homologue proteins on coagulase-negative Staphylococci such as *S. epidermidis*
and *S. hemolyticus* as well as antibodies which recognize said proteins, and in
particular to isolated monoclonal and polyclonal antibodies which recognize specific
15 proteins from *Staphylococcus aureus* and coagulase-negative Staphylococci and
which are cross-reactive against *S. aureus* and coagulase-negative Staphylococci
and can thus be utilized in vaccines and methods useful for preventing or treating a
wide variety of infections caused by staphylococcal bacteria.

Background of the Invention

20 The successful colonization of the host is a process required for most
microorganisms to cause infections in animals and humans. Microbial adhesion is
the first crucial step in a series of events that can eventually lead to disease.
Pathogenic microorganisms colonize the host by attaching to host tissues or serum
conditioned implanted biomaterials, such as catheters, artificial joints, and vascular
grafts, through specific adhesins present on the surface of the bacteria.
25 MSCRAMMs (Microbial Surface Components Recognizing Adhesive Matrix
Molecules) are a family of cell surface adhesins that recognize and specifically bind
to distinct components in the host's extracellular matrix. Once the bacteria have
successfully adhered and colonized host tissues, their physiology is dramatically
altered and damaging components such as toxins and proteolytic enzymes are
30 secreted. Moreover, adherent bacteria often produce a biofilm and quickly become
more resistant to the killing effect of most antibiotics.

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S. aureus causes a spectrum of infections that range from cutaneous lesions such as wound infections, impetigo, and furuncles to life-threatening conditions that include pneumonia, septic arthritis, sepsis, endocarditis, and biomaterial related infections. *S. aureus* is known to express a repertoire of different MSCRAMMs that can act individually or in concert to facilitate microbial adhesion to specific host tissue components. In addition, another type of staphylococcus bacteria is identified as the coagulase-negative bacteria, including such species as *S. epidermidis* and *S. hemolyticus* which are also have been known to express MSCRAMMs, and which also are responsible for a wide range of bacterial infections and related diseases. In this regard, MSCRAMMs generally provide an excellent target for immunological attack by antibodies, both polyclonal and monoclonal antibodies.

However, because antibodies by nature are very specific and in the case of different types of Staphylococci, such as *S. aureus* on one hand (coagulase-positive) and *S. epidermidis* and *S. hemolyticus* on the other (coagulase-negative), it has still remained a significant problem to develop antibodies that exhibit cross-reactivity across the different types of bacteria. Such cross-reactive antibodies are particularly desirable because of their potential in immunizing human and animal patients and providing protection against infections caused by both types of Staphylococcal bacteria, namely coagulase-positive bacteria such as *S. aureus* and the coagulase-negative bacteria, such as *S. epidermidis* and *S. hemolyticus*. Such antibodies would thus be extremely useful in preventing or treating a wide variety of the infections caused by staphylococcal bacteria.

Summary of the Invention

Accordingly, it is an object of the present invention to provide monoclonal antibodies that recognize MSCRAMM's from both coagulase-positive bacteria such as *S. aureus* as well as MSCRAMM's from coagulase-negative bacteria, such as *S. epidermidis* and *S. hemolyticus*.

It is also an object of the present invention to identify and isolate MSCRAMM's from staphylococcal bacteria, as well as their active regions such as the A domain, which can be used to generate monoclonal and polyclonal antibodies that will be cross-reactive against both coagulase-positive and coagulase-negative staphylococci.

It is still further an object of the present invention to provide isolated antibodies that can recognize the A domain of surface proteins such as the DgsK protein from coagulase-negative staphylococci and at the same time recognize surface proteins such as the SasA protein from *Staphylococcus aureus*.

It is yet another object of the present invention to utilize the isolated proteins, A domains and antibodies of the invention to produce vaccines useful in the treatment or prevention of staphylococcal infections, and to provide methods wherein the vaccines and antibodies of the invention are used to prevent or treat a staphylococcal infection.

These and other objects are provided by virtue of the present invention which comprises the identification and isolation of surface proteins from one type of staphylococcal bacteria, such as coagulase-negative or coagulase-positive staph, which can give rise to cross-reactive antibodies which can recognize surface proteins of both types of staph and which can thus be utilized in vaccines and methods of treating or preventing a wide range of staphylococcal infections. The present invention also relates to the generation of both polyclonal and monoclonal antibodies from these surface proteins and their use in preventing or treating staphylococcal infections.

These embodiments and other alternatives and modifications within the spirit and scope of the disclosed invention will become readily apparent to those skilled in the art from reading the present specification and/or the references cited herein, all of which are incorporated by reference.

Brief Description of the Drawing Figures

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Figure 1 is a depiction of the primary structure of the in silico-predicted proteins in accordance with the present invention.

Figure 2 shows a Coomassie gel of the purified N-terminal recombinant His-tagged proteins expressing the orfs of the present invention.

5 Figures 3A-3C show Western blotting of *S. aureus* cell wall extracts showing probing with anti-KesK antibodies (Fig. 3A), anti-KnkA antibodies (Fig. 3B) and anti-DsqA antibodies (Fig. 3C), respectively.

10 Figures 4A-4B show Dot-blotting and Western immunoblotting of *Lactococcus lactis* expressing *S. aureus* MSCRAMMs, namely KnkA (Fig. 4A) and KesK (Fig. 4B).

Figures 5A-5D representing the probing of recombinant LPXTG proteins in accordance with the present invention with convalescent sera examining *in vivo* expression, including RrKn and RrKN2 (Fig. 5A), KesK1 and KesK2A (Fig. 5B), KnkA (Fig. 5C) and DsqA2 (Fig. 5D).

15 Figure 6 shows a Western blot analysis demonstrating that rabbit polyclonal antibodies against *S. aureus* SasA cross-react with a protein released from the cell surface of *S. epidermidis* HB as well as the recombinant A-region from DsgK cloned from *S. epidermidis*.

20 DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENTS

In accordance with the present invention, there are provided specific surface proteins from coagulase-positive staphylococcal bacteria, such as *S. aureus* as well as from coagulase-negative staph such as *S. epidermidis* and *S. hemolyticus*, including active fragments thereof such as the A domains of these proteins or other
25 epitopic regions which can generate antibodies that recognize the whole protein. In accordance with the invention, the identification and isolation of candidate peptide sequences and proteins was carried out based on some of the common features of the MSCRAMMs ((Microbial Surface Components Recognizing Adhesive Matrix Molecules) which are in most cases are covalently anchored to the cell wall
30 peptidoglycan. These surface proteins had the following common features which

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were utilized in identifying and isolated the sequences of the present invention, namely: (i) an N-terminal signal peptide (approximately 40 residues in length) required for Sec-dependent secretion, (ii) a wall spanning domain either rich in proline and glycine residues or composed of serine and aspartate dipeptide repeats, (iii) an LPXTG motif required for covalent anchoring of the protein to the pentaglycine crossbridge in peptidoglycan, (iv) a hydrophobic membrane-spanning domain followed by (v) several positively charged residues.

In accordance with the invention, by exploiting the whole genome of *S. aureus* in light of the properties as set forth above, at least eight novel open reading frames encoding proteins with secretion and anchorage motifs indicative of MSCRAMMs were identified (i.e. bearing an N-terminal signal peptide and a C-terminal LPXTG motif followed by a hydrophobic domain and a positively charged tail). Table 1 illustrates the list of proteins identified including their distribution among *S. aureus* genomes, their protein size and C-terminal cell wall sorting sequence.

Table 1.

Name	Distribution	Size	C-terminus
EkeS	ENCSJM	2189 aa	LPNTGSEEMDLPLKELALITGAALLARRRS KKEKES
DsqA	ENCSJM	~1363- 2283 aa	LPDTGDSIKQNGLLGGVMTLLVGLGLMKR KKKKDENDQDDSQA
KesK	ENCSJM	~909 aa	LPKTGETTSSQSWWGLYALLGMLALFIPK FRKESK
KrkN2	ENCSJM (Cowan)	~278 aa	LPKTGLTSVDNFISTVAFATLALLGSLSLLLF KRKESK
KrkN	ENCSJM	~661 aa	LPQTGEESNKMNTLPLMALIALSSIVAFVLP RKRN
RkaS	ENCSJM	~801 aa	LPKTGTNQSSSPEAMFVLLAGIGLIATVRR RKAS
RrkN	NCSJM	1629 aa	LPKTGLESTQKGLIFSSIIIGIAGLMLLARRRK N
KnkA	NCSJM	629 aa	LPKAGETIKEHWLPISVIVGAMGVLMIWLS RRNKLKKA

Abbreviations: eMRSA-16; N, 8325; C, COL; S, MSSA; J, N315; M, Mu50.

Six out of eight are conserved in all of the six staphylococcal genomes currently sequenced and the remaining two are present in 5/6 of these genomes.

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In accordance with the invention, amino acid and nucleic acid sequences coding for the above proteins were obtained, and these were as follows: Ekes MRSA – SEQ ID NO:1 (DNA sequence); EkeS_MRSA – SEQ ID NO:2 (Protein sequence); DsqA (8325) – SEQ ID NO:3 (DNA sequence); DsqA (8325) – SEQ ID NO:4 (Protein sequence); KesK1 (8325) – SEQ ID NO:5 (DNA sequence); KesK1 (8325) – SEQ ID NO:6 (Protein sequence); KrkN2 (8325) – SEQ ID NO:7 (DNA sequence); KrkN2 (8325) – SEQ ID NO:8 (Protein sequence); KrkN (8325) – SEQ ID NO:9 (DNA sequence); KrkN (8325) – SEQ ID NO:10 (Protein sequence); RkaS (COL) – SEQ ID NO:11 (DNA sequence); RkaS (COL) – SEQ ID NO:12 (Protein sequence); RrkN (8325) – SEQ ID NO:13 (DNA sequence); RrkN (8325) – SEQ ID NO:14 (Protein sequence); KnkA (8325) – SEQ ID NO:15 (DNA sequence); KnkA (8325) – SEQ ID NO:16 (Protein sequence).

In accordance with the present invention, isolated antibodies may be generated from the above proteins or their active regions such as the A domain so as to be able to recognize said proteins and/or said domains. These antibodies may be either monoclonal or polyclonal. If polyclonal antibodies are desired, these may be generated in any of a number of conventional ways well known in the art. In a typical process, the desired surface protein or active region thereof may be injected into a suitable host animal, e.g., a mouse or rabbit, and after a suitable time period, antibodies may be isolated and recovered from the host animal. With regard to monoclonal antibodies, in accordance with the present invention, these may be produced in any number of suitable ways including, e.g., the well known method of Kohler and Milstein, Nature 256:495-497 (1975), or other suitable ways known in the field, such as those methods disclosed in U.S. Pat. Nos. 6,331,415; 5,981,216; 5,807,715; and 4,816,567; Eur. Pat. App. 519,596; and PCT publication WO 00/71585, all of these patent publications incorporated herein by reference. These methods include their preparation as chimeric, humanized, or human monoclonal antibodies in ways that would be well known in this field. Still further, monoclonal antibodies may be prepared from a single chain, such as the light or heavy chains, and in addition may be prepared from active fragments of an

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antibody which retain the binding characteristics (e.g., specificity and/or affinity) of the whole antibody. By active fragments is meant an antibody fragment which has the same binding specificity as a complete antibody which binds to the particular surface protein or its homologue from the different type of staph bacteria (i.e.,
5 coagulase negative or coagulase-positive), and the term "antibody" as used herein is meant to include said fragments. Additionally, antisera prepared using monoclonal or polyclonal antibodies in accordance with the invention are also contemplated and may be prepared in a number of suitable ways as would be recognized by one skilled in the art.

10 As indicated above, antibodies to the isolated surface proteins and/or their active regions in accordance with the invention may be prepared in a number of suitable ways that would be well known in the art, such as the well-established Kohler and Milstein method described above which can be utilized to generate monoclonal antibodies. For example, in preliminary steps utilized in such a
15 process, mice may be injected intraperitoneally once a week for a prolonged period with a purified recombinant MSCRAMM® in accordance with the invention or an active portion thereof, followed by a test of blood obtained from the immunized mice to determine reactivity to the purified protein. Following identification of mice reactive to the proteins, lymphocytes isolated from mouse spleens are fused to
20 mouse myeloma cells to produce hybridomas positive for the antibodies against the surface proteins of the invention which are then isolated and cultured, following by purification and isotyping.

In order to generate monoclonal antibodies in accordance with the invention, it is preferred that these be generated using recombinantly prepared MSCRAMM®s
25 in accordance with the invention, and these recombinants may be generated and isolated using a number of standard methods well known in the art. For example, one such method employs the use of *E. coli* expression vector pQE-30 as an expression vector for cloning and expressing recombinant proteins and peptides. In one preferred method, using PCR, the A domain of the surface protein identified as
30 DgsK or SasA was amplified from the sequences described above and subcloned

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into the *E. coli* expression vector PQE-30 (Qiagen), which allows for the expression of a recombinant fusion protein containing six histidine residues. This vector was subsequently transformed into *E. coli* strain ATCC 55151, grown in a 15-liter fermentor to an optical density (OD₆₀₀) of 0.7 and induced with 0.2 mM isopropyl-1-beta-D galactoside (IPTG) for 4 hours. The cells were harvested using an AG Technologies hollow-fiber assembly (pore size 0.45 µm) and the cell paste frozen at -80° C. Cells were lysed in 1X PBS (10 mL buffer/1 g of cell paste) using 2 passes through the French Press @ 1100psi. Lysed cells were spun down at 17,000rpm for 30 minutes to remove cell debris. Supernatant was passed over a 5-mL HiTrap Chelating (Pharmacia) column charged with 0.1M NiCl₂. After loading, the column was washed with 5 column volumes of 10mM Tris, pH 8.0, 100mM NaCl (Buffer A). Protein was eluted using a 0-100% gradient of 10mM Tris, pH 8.0, 100mM NaCl, 200 mM imidazole (Buffer B) over 30 column volumes. SdrGN1N2N3 or SdrGN2N3 eluted at ~13% Buffer B (~26mM imidazole). Absorbance at 280nm was monitored.

15 Fractions containing SdrGN1N2N3 or SdrGN2N3 were dialyzed in 1x PBS.

Next, each protein was then put through an endotoxin removal protocol. Buffers used during this protocol were made endotoxin free by passing over a 5-mL Mono-Q sepharose (Pharmacia) column. Protein was divided evenly between 4x 15mL tubes. The volume of each tube was brought to 9mL with Buffer A. 1mL of 10% Triton X-114 was added to each tube and incubated with rotation for 1 hour at 4°C. Tubes were placed in a 37°C water bath to separate phases. Tubes were spun down at 2,000rpm for 10 minutes and the upper aqueous phase from each tube was collected and the detergent extraction repeated. Aqueous phases from the 2nd extraction were combined and passed over a 5-mL IDA chelating (Sigma) column, charged with 0.1M NiCl₂ to remove remaining detergent. The column was washed with 9 column volumes of Buffer A before the protein was eluted with 3 column volumes of Buffer B. The eluant was passed over a 5-mL Detoxigel (Sigma) column and the flow-through collected and reapplied to the column. The flow-through from the second pass was collected and dialyzed in 1x PBS. The

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purified product was analyzed for concentration, purity and endotoxin level before administration into the mice.

In the preferred process, monoclonal antibodies in accordance with the present invention may be prepared from the recombinant proteins identified above in the following manner. In this process, *E. coli* expressed and purified recombinant SasA and DsgK proteins were used to generate a panel of murine monoclonal antibodies while the mouse sera was used as a source of polyclonal antibodies. Briefly, a group of Balb/C or SJL mice received a series of subcutaneous immunizations of 1-10 mg of protein in solution or mixed with adjuvant as described below in Table 2.

Table 2. Immunization Schemes

RIMMS				
Injection	Day	Amount (ug)	Route	Adjuvant
#1	0	5	Subcutaneous	FCA/RIBI
#2	2	1	Subcutaneous	FCA/RIBI
#3	4	1	Subcutaneous	FCA/RIBI
#4	7	1	Subcutaneous	FCA/RIBI
#5	9	1	Subcutaneous	FCA/RIBI

Conventional				
Injection	Day	Amount (ug)	Route	Adjuvant
Primary	0	5	Subcutaneous	FCA
Boost #1	14	1	Intraperitoneal	RIBI
Boost #2	28	1	Intraperitoneal	RIBI
Boost #3	42	1	Intraperitoneal	RIBI

At the time of sacrifice (RIMMS) or seven days after a boost (conventional) serum was collected and titrated in ELISA assays against MSCRAMM[®] proteins or on whole cells (*S. epidermidis* and *S. aureus*). Three days after the final boost, the spleens or lymph nodes were removed, teased into a single cell suspension and the lymphocytes harvested. Lymphocytes were then fused to a P3X63Ag8.653 myeloma cell line (ATCC #CRL-1580). Cell fusion, subsequent plating and feeding were performed according to the Production of Monoclonal Antibodies protocol from Current Protocols in Immunology (Chapter 2, Unit 2.), incorporated herein by reference.

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Any clones that were generated from the fusion were then screened for specific anti-SasA antibody production using a standard ELISA assay. Positive clones were expanded and tested further for activity in a whole bacterial cell binding assay by flow cytometry and SasA binding by Biacore analysis. Throughout the Biacore analysis, the flow rate remained constant at 10 ml/min. Prior to the SasA or DgsK injection, test antibody was adsorbed to the chip via RAM-Fc binding. At time 0, SasA or DgsK at a concentration of 30 mg/ml was injected over the chip for 3 min followed by 2 minutes of dissociation. This phase of the analysis measured the relative association and disassociation kinetics of the Mab/SasA or DgsK interaction.

Next, the antibodies prepared as set forth above were tested for binding to whole bacteria. In these tests, bacterial samples *S. aureus* Newman, *S. aureus* 67-0, *S. aureus* 397 (Sal6), *S. aureus* Wood, *S. aureus* 8325-4, methicillin resistant *S. aureus* MRSA 16, *S. epidermidis* ATCC 35984, *S. epidermidis* HB, *S. epidermidis* CN-899 and *S. haemolyticus* ATCC 43253 were collected, washed and incubated with Mab or PBS alone (control) at a concentration of 2 µg/ml after blocking with rabbit IgG (50 mg/ml). Following incubation with antibody, bacterial cells were incubated with Goat-F_{(ab)₂}-Anti-Mouse-F_{(ab)₂}-FITC which served as the detection antibody. After antibody labeling, bacterial cells were aspirated through the FACScaliber flow cytometer to analyze fluorescence emission (excitation: 488, emission: 570). For each bacterial strain, 10,000 events were collected and measured. These data indicate that antibodies against *S. aureus* SasA were able to recognize a homologous protein on the surface of coagulase-negative staphylococci. The data support Western blot analysis demonstrating that rabbit polyclonal antibodies against *S. aureus* SasA cross-react with a protein released from the cell surface of *S. epidermidis* HB as well as the recombinant A-region from DgsK cloned from *S. epidermidis* (see Figure 6 and Table 3 below).

Table 3. Polyclonal Sera Reactivity

	New man	67-0	397 (SAL 6)	Wo od 46	8325 -4	MRS A 16	ATC C 3598	HB	CN- 899	ATC C 4325
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Normal Mouse Sera	-	-	-	-	-	-	-	-	-	-
Mouse anti-SasA	+	+	+/-	-	+	+	+	+	+	+

Although production of antibodies using recombinant forms of the surface proteins of the present invention is preferred, antibodies may be generated from natural isolated and purified versions of these proteins or their active regions such as the A domain, and monoclonal or polyclonal antibodies can be generated using these proteins or active regions in the same manner as described above to obtain such antibodies. Still other conventional ways are available to generate the antibodies of the present invention using recombinant or natural purified proteins or their active regions, as would be recognized by one skilled in the art.

As would be recognized by one skilled in the art, the antibodies of the present invention may also be formed into suitable pharmaceutical compositions for administration to a human or animal patient in order to treat or prevent an infection caused by staphylococcal bacteria. Pharmaceutical compositions containing the antibodies of the present invention, or effective fragments thereof, may be formulated in combination with any suitable pharmaceutical vehicle, excipient or carrier that would commonly be used in this art, including such as saline, dextrose, water, glycerol, ethanol, other therapeutic compounds, and combinations thereof. As one skilled in this art would recognize, the particular vehicle, excipient or carrier used will vary depending on the patient and the patient's condition, and a variety of modes of administration would be suitable for the compositions of the invention, as would be recognized by one of ordinary skill in this art. Suitable methods of administering any pharmaceutical composition disclosed in this application include,

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but are not limited to, topical, oral, anal, vaginal, intravenous, intraperitoneal, intramuscular, subcutaneous, intranasal and intradermal administration.

For topical administration, the composition is formulated in the form of an ointment, cream, gel, lotion, drops (such as eye drops and ear drops), or solution (such as mouthwash). Wound or surgical dressings, sutures and aerosols may be impregnated with the composition. The composition may contain conventional additives, such as preservatives, solvents to promote penetration, and emollients. Topical formulations may also contain conventional carriers such as cream or ointment bases, ethanol, or oleyl alcohol. Additional forms of antibody compositions, and other information concerning compositions, vaccines, methods and applications with regard to other MSCRAMM@s will generally also be applicable to the present invention involving the aforementioned MSCRAMM@s and their active regions and antibodies thereto, and these other MSCRAMM@s are disclosed, for example, in U.S. patents 5,175,096; 5,320,951; 5,416,021; 5,440,014; 5,571,514; 5,652,217; 5,707,702; 5,789,549; 5,840,846; 5,980,908; 6,086,895; 6,008,341; 6,177,084; 5,851,794 and 6,288,214; all of these patents incorporated herein by reference.

The antibody compositions of the present invention may also be administered with a suitable adjuvant in an amount effective to enhance the immunogenic response. For example, suitable adjuvants may include alum (aluminum phosphate or aluminum hydroxide), which is used widely in humans, and other adjuvants such as saponin and its purified component Quil A, Freund's complete adjuvant, RIBBI adjuvant, and other adjuvants used in research and veterinary applications. Still other chemically defined preparations such as muramyl dipeptide, monophosphoryl lipid A, phospholipid conjugates such as those described by Goodman-Snitkoff *et al.* *J. Immunol.* 147:410-415 (1991) and incorporated by reference herein, encapsulation of the conjugate within a proteoliposome as described by Miller *et al.*, *J. Exp. Med.* 176:1739-1744 (1992) and incorporated by reference herein, and encapsulation of the protein in lipid

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vesicles such as Novasome™ lipid vesicles (Micro Vascular Systems, Inc., Nashua, NH) may also be useful.

In any event, the antibody compositions of the present invention which recognize the proteins or their active regions as set forth above will be useful in methods of preventing or treating staphylococcal infection, and in inhibiting binding of staphylococcal bacteria to host tissue and/or cells. In accordance with the present invention, methods are provided for preventing or treating a staphylococcal infection which comprise administering an effective amount of an antibody to the surface proteins as set forth herein or their active subregions so as to treat or prevent a staphylococcal infection. In addition, these monoclonal antibodies will be useful in impairing the binding of staphylococcal bacteria to host cells

Accordingly, in accordance with the invention, administration of the antibodies of the present invention in any of the conventional ways described above (e.g., topical, parenteral, intramuscular, etc.), and will thus provide an extremely useful method of treating or preventing staphylococcal infections in human or animal patients when an effective amount of the antibody compositions are administered to a human or animal patient. By effective amount is meant that level of use, such as of an antibody titer, that will be sufficient to either prevent adherence of the bacteria, to inhibit binding of staph bacteria to host cells and thus be useful in the treatment or prevention of a staph infection. As would be recognized by one of ordinary skill in this art, the level of antibody titer needed to be effective in treating or preventing staphylococcal infection will vary depending on the nature and condition of the patient, and/or the severity of the pre-existing staphylococcal infection.

In addition to use in methods of treating or preventing a staphylococcal infection, the antibodies of the invention may also be used for the specific detection of staphylococcal proteins, or as research tools. The term "antibodies" as used herein includes monoclonal, polyclonal, chimeric, single chain, bispecific, simianized, and humanized or primatized antibodies as well as Fab fragments, such as those fragments which maintain the binding specificity of the antibodies to the

surface proteins specified above, including the products of an Fab immunoglobulin expression library. Accordingly, the invention contemplates the use of single chains such as the variable heavy and light chains of the antibodies. Generation of any of these types of antibodies or antibody fragments is well known to those skilled in the art. In the present case, antibodies to the surface proteins or their active regions as referred to above can be generated, isolated and/or purified, and then used to treat or protect against staphylococcal infection.

Any of the above described antibodies may be labeled directly with a detectable label for identification and quantification of staph bacteria. Labels for use in immunoassays are generally known to those skilled in the art and include enzymes, radioisotopes, and fluorescent, luminescent and chromogenic substances, including colored particles such as colloidal gold or latex beads. Suitable immunoassays include enzyme-linked immunosorbent assays (ELISA).

Alternatively, the antibody may be labeled indirectly by reaction with labeled substances that have an affinity for immunoglobulin. The antibody may be conjugated with a second substance and detected with a labeled third substance having an affinity for the second substance conjugated to the antibody. For example, the antibody may be conjugated to biotin and the antibody-biotin conjugate detected using labeled avidin or streptavidin. Similarly, the antibody may be conjugated to a hapten and the antibody-hapten conjugate detected using labeled anti-hapten antibody. These and other methods of labeling antibodies and assay conjugates are well known to those skilled in the art.

In accordance with the present invention, there are also provided vaccines for either active or passive immunization designed to treat or protect against staphylococcal infections, and these vaccines may be prepared from the surface proteins or their active regions as set forth above using a number of the conventional vaccine preparation methods well known in this field. In the typical vaccine, an immunogenic amount of a suitable surface protein or active fragment thereof is combined with a suitable pharmaceutically acceptable vehicle, carrier or excipient, and an amount of this vaccine effective to immunize a human or animal

patient may be administered as appropriate. By immunogenic amount it would be understood by one of ordinary skill in this art that this refers to any amount of the protein or active fragment or subregion thereof which is able to raise an immunogenic response in the human or animal patient.

5 In addition to active vaccines wherein antibodies are generated in the patient by virtue of the introduction or administration of an immunogenic amount of a protein or active fragment in accordance with the present invention, the isolated antibodies of the present invention, or active fragments thereof, may also be utilized in the development of vaccines for passive immunization against staph infections. In
10 such a case, the antibody compositions as described above, namely an effective amount of the antibody and a pharmaceutically acceptable vehicle, carrier or excipient, may be administered as appropriate to a human or animal patient.

Accordingly, in accordance with the invention, the proteins or active fragments thereof may be utilized as active vaccines, and the antibodies of the
15 invention may be used as a passive vaccine which will be useful in providing suitable antibodies to treat or prevent a staphylococcal infection. As would be recognized by one skilled in this art, a vaccine may be packaged for administration in a number of suitable ways, such as by parenteral (i.e., intramuscular, intradermal or subcutaneous) administration or nasopharyngeal (i.e., intranasal) administration.
20 One such mode is where the vaccine is injected intramuscularly, e.g., into the deltoid muscle, however, the particular mode of administration will depend on the nature of the bacterial infection to be dealt with and the condition of the patient. The vaccine is preferably combined with a pharmaceutically acceptable vehicle, carrier or excipient to facilitate administration, and the carrier is usually water or a
25 buffered saline, with or without a preservative. The vaccine may be lyophilized for resuspension at the time of administration or in solution.

In addition, in certain cases, the antibodies of the present invention may be modified as necessary so that, when necessary, they become less immunogenic in the patient to whom it is administered. For example, if the patient is a human, the
30 antibody may be "humanized" by transplanting the complementarity determining

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regions of the hybridoma-derived antibody into a human monoclonal antibody as described, e.g., by Jones *et al.*, *Nature* 321:522-525 (1986) or Tempest *et al. Biotechnology* 9:266-273 (1991) or "veneered" by changing the surface exposed murine framework residues in the immunoglobulin variable regions to mimic a homologous human framework counterpart as described, e.g., by Padlan, *Molecular Imm.* 28:489-498 (1991), these references incorporated herein by reference. Even further, when so desired, the monoclonal antibodies of the present invention may be administered in conjunction with a suitable antibiotic to further enhance the ability of the present compositions to fight bacterial infections when necessary.

10 In addition to treating human or animal patients, the present compositions may also be used to halt or prevent infection of a medical device or other biomaterials such as an implant. Medical devices or polymeric biomaterials to be coated with the antibodies, proteins and active fragments described herein include, but are not limited to, staples, sutures, replacement heart valves, cardiac assist
15 devices, hard and soft contact lenses, intraocular lens implants (anterior chamber or posterior chamber), other implants such as corneal inlays, kerato-prostheses, vascular stents, epikeratophalia devices, glaucoma shunts, retinal staples, scleral buckles, dental prostheses, thyroplastic devices, laryngoplastic devices, vascular grafts, soft and hard tissue prostheses including, but not limited to, pumps, electrical
20 devices including stimulators and recorders, auditory prostheses, pacemakers, artificial larynx, dental implants, mammary implants, penile implants, cranio/facial tendons, artificial joints, tendons, ligaments, menisci, and disks, artificial bones, artificial organs including artificial pancreas, artificial hearts, artificial limbs, and heart valves; stents, wires, guide wires, intravenous and central venous catheters,
25 laser and balloon angioplasty devices, vascular and heart devices (tubes, catheters, balloons), ventricular assists, blood dialysis components, blood oxygenators, urethral/ureteral/urinary devices (Foley catheters, stents, tubes and balloons), airway catheters (endotracheal and tracheostomy tubes and cuffs), enteral feeding tubes (including nasogastric, intragastric and jejunal tubes), wound drainage tubes,
30 tubes used to drain the body cavities such as the pleural, peritoneal, cranial, and

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pericardial cavities, blood bags, test tubes, blood collection tubes, vacutainers, syringes, needles, pipettes, pipette tips, and blood tubing.

It will be understood by those skilled in the art that the term "coated" or "coating", as used herein, means to apply the antibody or active fragment, or pharmaceutical composition derived therefrom, to a surface of the device, preferably an outer surface that would be exposed to streptococcal bacterial infection. The surface of the device need not be entirely covered by the protein, antibody or active fragment.

The preferred dose for administration of an antibody composition in accordance with the present invention is that amount will be effective in preventing of treating a staphylococcal infection, and one would readily recognize that this amount will vary greatly depending on the nature of the infection and the condition of a patient. As indicated above, an "effective amount" of antibody or pharmaceutical agent to be used in accordance with the invention is intended to mean a nontoxic but sufficient amount of the agent, such that the desired prophylactic or therapeutic effect is produced. As will be pointed out below, the exact amount of the antibody or a particular agent that is required will vary from subject to subject, depending on the species, age, and general condition of the subject, the severity of the condition being treated, the particular carrier or adjuvant being used and its mode of administration, and the like. Accordingly, the "effective amount" of any particular antibody composition will vary based on the particular circumstances, and an appropriate effective amount may be determined in each case of application by one of ordinary skill in the art using only routine experimentation. The dose should be adjusted to suit the individual to whom the composition is administered and will vary with age, weight and metabolism of the individual. The compositions may also contain stabilizers or pharmaceutically acceptable preservatives, such as thimerosal (ethyl(2-mercaptobenzoate-S)mercury sodium salt) (Sigma Chemical Company, St. Louis, MO).

When used with suitable labels or other appropriate detectable biomolecule or chemicals, the monoclonal antibodies described herein are useful for purposes

such as *in vivo* and *in vitro* diagnosis of staphylococcal infections or detection of staphylococcal bacteria. Laboratory research may also be facilitated through use of such antibodies. Various types of labels and methods of conjugating the labels to the antibodies of the invention are well known to those skilled in the art, such as the ones set forth below.

For example, the antibody can be conjugated (directly or via chelation) to a radiolabel such as, but not restricted to, ^{32}P , ^3H , ^{14}C , ^{35}S , ^{125}I , or ^{131}I . Detection of a label can be by methods such as scintillation counting, gamma ray spectrometry or autoradiography. Bioluminescent labels, such as derivatives of firefly luciferin, are also useful. The bioluminescent substance is covalently bound to the protein by conventional methods, and the labeled protein is detected when an enzyme, such as luciferase, catalyzes a reaction with ATP causing the bioluminescent molecule to emit photons of light. Fluorogens may also be used to label proteins. Examples of fluorogens include fluorescein and derivatives, phycoerythrin, allo-phycoyanin, phycocyanin, rhodamine, and Texas Red. The fluorogens are generally detected by a fluorescence detector.

The location of a ligand in cells can be determined by labeling an antibody as described above and detecting the label in accordance with methods well known to one skilled in the art, such as immunofluorescence microscopy using procedures such as those described by Warren et al. (*Mol. Cell. Biol.*, 7: 1326-1337, 1987).

As indicated above, the monoclonal antibodies of the present invention, or active portions or fragments thereof, are particularly useful for interfering with the initial physical interaction between a staphylococcal pathogen responsible for infection and a mammalian host, and this interference with the physical interaction may be useful both in treating patients and in preventing or reducing bacteria infection on in-dwelling medical devices to make them safer for use.

In another embodiment of the present invention, a kit which may be useful in isolating and identifying staphylococcal bacteria and infection is provided which comprises the antibodies of the present invention in a suitable form, such as lyophilized in a single vessel which then becomes active by addition of an aqueous

sample suspected of containing the staphylococcal bacteria. Such a kit will typically include a suitable container for housing the antibodies in a suitable form along with a suitable immunodetection reagent which will allow identification of complexes binding to the surface proteins or the antibodies of the invention. In general, these kits may contain an antibody in accordance with the invention and means to identify binding of that antibody when a sample from a patient is introduced to the antibody. For example, a suitable immunodetection reagent may comprise an appropriate detectable signal or label, such as a biotin or enzyme that produces a detectable color, etc., which may be linked to the antibody or utilized in other suitable ways so as to provide a detectable result when the antibody binds to the antigen.

In short, the antibodies of the present invention which recognize and bind to the surface proteins of the invention, or active fragments thereof, will thus be useful in treating a wide variety of staphylococcal infections in human and animal patients and in medical or other in-dwelling devices. In accordance with the invention, because of the nature of these proteins and the fact that they contain epitopes in common with proteins of the other type of staphylococcal bacteria, i.e., a protein from a coagulase-negative staph will raise antibodies that recognize a homologous protein from *S. aureus* and vice versa, the antibodies of the invention will exhibit cross-reactivity and should be effective against a broad range of staphylococcal infections. Accordingly, the present invention provides methods and compositions for improved methods of treating or protecting against a wide range of staphylococcal infections.

EXAMPLES

The following examples are provided which exemplify aspects of the preferred embodiments of the present invention. It should be appreciated by those of skill in the art that the techniques disclosed in the examples which follow represent techniques discovered by the inventors to function well in the practice of the invention, and thus can be considered to constitute preferred modes for its practice. However, those of skill in the art should, in light of the present disclosure,

appreciate that many changes can be made in the specific embodiments which are disclosed and still obtain a like or similar result without departing from the spirit and scope of the invention.

5 **Example 1. Isolation and Sequencing of MSCRAMM's from *S. Aureus***

Staphylococcus aureus is known to express a class of surface-associated proteins which play important roles in pathogenicity by allowing bacteria to avoid host defenses and by acting as adhesins. These proteins are known as MSCRAMMs (Microbial Surface Components Recognizing Adhesive Matrix Molecules) and in most cases are covalently anchored to the cell wall peptidoglycan. They have several common features: (i) an N-terminal signal peptide (approximately 40 residues in length) required for Sec-dependent secretion, (ii) a wall spanning domain either rich in proline and glycine residues or composed of serine and aspartate dipeptide repeats, (iii) an LPXTG motif required for covalent anchoring of the protein to the pentaglycine crossbridge in peptidoglycan, (iv) a hydrophobic membrane-spanning domain followed by (v) several positively charged residues.

By exploiting the whole genome sequences of *S. aureus*, eight novel open reading frames encoding proteins with secretion and anchorage motifs indicative of MSCRAMMs were identified (i.e. bearing an N-terminal signal peptide and a C-terminal LPXTG motif followed by a hydrophobic domain and a positively charged tail). The following Table illustrates the list of proteins identified including their distribution among *S. aureus* genomes, their protein size and C-terminal cell wall sorting sequence.

Name	Distribution	Size	C-terminus
EkeS	ENCSJM	2189 aa	LPNTGSEEMDLPLKELALITGAALLARRRS KKEKES
DsqA	ENCSJM	~1363- 2283 aa	LPDTGDSIKQNGLLGGVMTLLVGLGLMKR KKKKDENDQDDSQA
KesK	ENCSJM	~909 aa	LPKTGETTSSQSWWGLYALLGMLALFIPK FRKESK

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KrkN2	ENCSJM (Cowan)	~278 aa	LPKTGLTSVDNFISTVAFATLALLGSLSLLLF KRKESK
KrkN	ENCSJM	~661 aa	LPQTGEESNKDMTLPLMALIALSSIVAFVLP RKRRKN
RkaS	ENCSJM	~801 aa	LPKTGTNQSSSPEAMFVLLAGIGLIATVRR RKAS
RrkN	NCSJM	1629 aa	LPKTGLESTQKGLIFSSIIIGIAGMLLARRRK N
KnkA	NCSJM	629 aa	LPKAGETIKEHWLPISVIVGAMGVLMIWLS RRNKLKNKA

Abbreviations: eMRSA-16; N, 8325; C, COL; S, MSSA; J, N315; M, Mu50.
Six out of eight are conserved in all of the six staphylococcal genomes currently
sequenced and the remaining two are present in 5/6 of these genomes.

5

The following is a list of the DNA and protein sequences:

Ekes MRSA (SEQ ID NO:1)

10 acaacacagcagagaatagacaaccaggaggaaaacgaatgaattgttaagaaaaaataatagattag
aaaaataaagtagggataitctctacttaacgggacagttttactttcaaacccaaatggtgcacaagcttaac
tacggatcataatgtgcaagggtgttcaaatcaagcattaccctggcaactcacaatacaaatccgatactaact
gagacatagtaaatgattcgcaaaatactcctaafgcacatgcaacagacaatacatcaacaatcaagcattgac
taatcatcaaaacgttgatggcaaatcaagtcggccctgctccaatacagcctagcgcgtcgcgcgcaaaata
ataataattctaagctaatcaacagcaacagagccagcggcgaatacaataataatttagcatcaataacaat
15 acattaaacgtgcctaataacagataacaatgattcagcgcgtcatctgacttaaaagaaatcaagaagatgtt
ogtcatctcgtgataagccagagtttagtgcgattgctgaagaagcatctaatagaccgaaaaagagagaagcagac
gtgctgcgcaacagatcctaatagcaacaccagcagatccaacggctacaccagcagatccaacggcaggaaat
ggtagtgcacacgttgcaattacagcgcatacagccaacactgatccaatgccaataataggacaaaatg
20 caactaaccgaagtgcttctattgatgatacaacacatagaccaagtacgaacggctctgctcactagtaactgtgtt
gataattacaggctacacactgattaatggtgtaagtaggggtgtttagtcatgcaatggttaagaacgagcatgt
ttgattcaggagatccaagaactatcaagcgaaggcaatgtaattgcatgggtcgtattagaggaaatgataca
aatgatcatgcgattttaaaggatcgagaaacattacagtaaatccgaattcgaattaatcttgaatttaatact
atgactactaaaaactcaaggatgacaaaatttaactataaaaatgctgataacgatactgttattggtgaaaaag
25 tagttgcttatggtcgtattggcgttataaaagtaacctgaaaaatgttagtcatcaaaatcaattgtacctaaaaat
gacgcaataacagatgcacgtgtattatcaattacgagatggatataaatactatgactttagactcaatcggtct
tcattctgggtcacatgtctatgttgaagacgtacaatggagccaacagcaacaataataaagaatttacagttac
aacgtcattaaagaataatggttaacttggcgtctcatcaatacagatgattttgtatataaaattcaattacctgaaggt
gttgaatgttaataattcattgactaaagatttctcagcggtaattcaggtgtgataataatgataatgtagcgtat
30 tagcagcagcaaatcgaattattcaataaaagtaactggtgaggtacagggaattcgcgcgcagcactaagcgtg
ataaaattattggaattgaagtataagctacgtgtgaacaatgtgccaacaccaagaacagtaacatttaacgatacat
taacgtataaaacataattcacaagattttattatcacctgctgaaagtcatactgtaagtacaaatccatatacaattg
atatcatcatgaataaagacgcattgcaagccgaagtcgatagacgaattcaacaagcggattatcattgcatcat
tagatatattttaatgatcttaaaagacgcgcacaacaatttttagatgaaaacgtaacaatgtacctttaacaaaag
35 agtttctcagcagatagcattcattagcaaatcagatgcaacatacgttaattcagcagtgtagcgtgaaatgccc

[illegible]

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5 aaaataatttaataaagcaataacgaagcgacagatgaagaacaaaatgctgcaatagtacaagtgaaaaa
gagtaattaaagctaaacacaaatgctggtgcagtgactaatgctgagtgcatatttattgcatgtgggaaaa
acgaaatcgtgaaatcgaaacctgtattataaaaaagcaactgcgcgagaacaaataacacattattcaacgat
aagaaacaagcaattgaagcgaattgtcaagcaacagtagaagaagaagaatgatttttagcacagttacaaaa
catttatgacactgctattggacaaatgatcaagatcgtagcaatgcacaagttgataaaacagcaacattaaatct
10 acaaaaacatacatgatttagacgtacatcctattaaaaagccagatgctgaaaaaacgattaatgatcttgcac
gtgttacacatttagtgcataatgatgaaaagtaagtgatcglataaggtgatgataaaagctataactgcatt
aaaattacaaatggatgaagaattaaaaacagcacgcactaalgctgattgagcagttttaaagcatttaattgtt
gcattaggcgatatagaagcagtaattactgaaaaagaaaatagcttactgcgcattgataacattgclcaacaaac
15 atatcgcaaatcaaaagcgatcgcaacaccagaacaattagctaaagtaaaagcattaatgatcaatatgttcag
atggcaatagaattggttgatgaagatcgacattaaatgacatcaaaaaagatacgcaactcattattgatgaaattt
tagcaattaaattacctgctgaagtataaaagctcaccaaaagtggtggcaccctgctccaaaagttgtacgcct
attaaaaaagaagataaacaagaagtcgaagattgtaaaagaactccaaatactggttctgaagaatggatt
taccattaaaaagattagcactaattacaggcgcagcattattagctagaagacgttctaaaaaagaaaaaatc
ataa

EkeS_MRSA (SEQ ID NO:2)

20 MNLKKNKYSIRKYKVGIFSTLIGTVLLSNPNQAQALTTDHNVQGGSNQALPGNS
QNTNADTNRDIVNDSQNTPNHAHATDNTSTNQALTNHQNVQVQVPAPIQPSA
SPAQNNNNSNANSTATEPAANTNNNLASNNTLNVPNNNDNNSARHLTLKEIQE
DVRHSSDKPELVIAIEEASNRPKKRSRRAPDTPNATPADPTATPADPTAGNGSA
PVAITAPYPTTDDPNANNIGQNPAPNEVLSFDDNNIRPSTNRSVPTVTVDNLPGYTL
25 INGGKVGVSFHAMVRTSMFDSGDAKNYQAQGNVIALGRIRGNDTNDHGDGFGNIEK
TLTVNPNSLIFEFTMTTKNYQGMNLINKNADNDTVIGEKVVAYGPIWRLLKVPE
NVSHLKIQFVPKNDAITDARGIYQLRDGYKYDFVDSIGLHSGSHVYVERRTMEPT
ATNNKEFTVTTSLKNNGNFGASFNTDDFVYKQLPEGVEYVNNSLTKDFPSGNSG
VDINDMNVTYDAANRIITIKSTGGGTGNSPARLMPDKILDLYKLRVNNVPTPTVT
30 FNDTLTYKTSQDFINSFAESHTVSTNPHYIDIIMNKDALQAEVDRIQQADYTFASL
DIFNDLKRRAQTILDENRNNVPLNKRVSQADIDSLANQMQLTLIRSVDAENAVNRK
VDDMEDLVNQNDLTDDEEKQAAIQVIEEHKNEIIGNIGDQTTDDGVTRIKDQGIQL
SGDTATPVVKPNAKQAIRDKAAKQREINHPTDATQDEIQDALNQLTTDETDAIDNV
TNATTNADVETAKNNGINTIGAVAPQVTHKQAARDAINQATATKRQQINSNREATQ
35 EEKNAALNELTQATNHALEQINQATTNDDVDVTAKGDLNAINPIAPVTVVKQAARD
AVSHDAQQHIAIEINANPDATQEERQAAIEKVYAAVAVANTNLANNTNADVEQVKT
NAIQGQIAIEPATKVKTDAKNAIDQSAETQHNAIFNNNDATLEEQQAQQLDQAVA
TAKQNIINAADTNQEVQAQKDGQTQNIIVVIQPATQVKTDARNVNEKAREAITNINA
TPGATREEKQEAIRNVNTLNKRALNDIGVTSTTAMVNSIRDDAVNQIGAVOPHVT
40 KQTATGVLTDLATAKKQEINQNTNATTEEKQVALNQVDQDLATAINNINQADTNAE
VDQAQQLGKTKAINAIQPNIVKPAALAQTNQHSYAKLVEINATPDATDDEKNAAINT
LNQDRQQAIESIKQANTNAEVDQAAQVAENNIDAVQVDVVKQAARDKITAIEVAKR
IEAVKQTPNATDEEKQAAVNVQINQLKDQAFNQINQNTNDQVDATTNQAINAIDNV
EAEVVIKPAIADIEKAVKEKQQIDNSLDSTDNEKEVALQALAKEKEKALAAIDQA
45 QTSQVNVQAATNGVSAIKIIPETKIKPAAREKINQKANELRAQINQDKEATEAERQ
AALDKINDLYAKAMTNITNDRTNQQVNDSTNQALDDIALVTPDHIVRAAARDVAKQ
QYEAKEHEIEQAEHATDEEKQVALNQLANNEKRALQINQAIANNNDVKRVESNGIA

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TLKGVPEHVIVKPEAQEAIKASADNQVESIKDTPHATTDELDEANQQINDTLKQGG
QDIDNTTQDAAVNDVRNQTIKAEQIKPKVRRKRAALNDIDESNNNQDLAIRNTLDT
TQDERNVAIALNKNVNAIKNDIAQNKNTAEVDQTEADGNNNIKVILPKVQVKAAR
QSVSAKAEALQNALIDQSDLSTEEERLAAKHLVEQALNQAIQINHADKTAQVNS
5 IDAQNIISKIPATTVKATALQQIQNIAIKNLIKANNEATDEEQNAIVQVEKELIKA
KQQIAGAVTNADVAYLLHDGKNEIREIEPVINKKATAREQLTTLFNDKKQAIENVQ
ATVEERNISILAQLNQIYDTAIGQIDQDRSNAQVDKTATLNLQTIHDLVHPKPPDAE
KTINDDLARVTHLVQNYRKVSDRNKADALKAITALKLQMDDELKTARTNADVAVL
10 KRFNVALGDIEAVITEKENSLLRIDNIAQQTAKFKAIATPEQLAKVKALIDQYVADG
NRMVDEDATLNDIKKDTQLIDEILAIKLP AEVIKASPKVGGPAPKVCPIKKEDKQEV
RKVVKELPNTGSEEMDLPLKELALITGAALLARRRSKKEKES

DsqA (8325) (SEQ ID NO:3)

15 tctaataagataaagataaagaggtattacatgagtaaaagacagaagcattcatgacagctagcaaa
cgaaaaaacaagagtaagactttataaatcggaaaaaatgggtaaaatccggaattaaagaaatagaatgttc
aaaattatgggctacacattattatgctagatttagtgagtaagataatcaaaagcattagtaaaaaatgacgggat
acggactgaaaaactacgcggtattgtgtgctacacggtataatgttgcatgaccagcaagctttgcggtct
gatgcaccattactctgaattaaacacacaaaagtgaaacagtaggtaataaaactcaacgacaatcgaagcat
20 caactcaacacgacgattcccaagtgtaacgaaaaatagtagtgcgttcaaaacataaatgacacagctc
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DsqA (8325) (SEQ ID NO:4)

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KesK1 (8325) (SEQ ID NO:6)

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KrkN2 (8325) (SEQ ID NO:7)

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caagtggttgaaaaagaaaaatgacgcatctagttagtcaggtaagacaaaacgctgctacaaaaccaactaaa
gggtgaagtgaatgaatgatacaactccaactaaggtagtatctcagactcaaatgttgcaaaccaacactg
cttcatcaaaaacaacaaaagattgtttcaaacctcagcaggttctagcgaagcaaaagatagtgctccattacaa
35 aaagcaaacattaaaaacacaaatgatggacacactcaaaagccaaaaataaaaaatacacaagaaaataaa
gcaaaaatcattaccacaacactgggtgaagaatcaataaagatatgacattaccattatggtgcattattgattaaagta
gcactgttcattcgtattacctagaaaaacgtaaaaactaa

KrKN (8325) (SEQ ID NO:10)

40 YTIRSCFYNNMKQQKEFKSFYSIRKSSLGVASVAISTLLLLMSNGEAQAAAAEETGG
TNTAEPKTEAVASPTTTSEKAPETKPVANAVSVSNKEVEAPTSETKEAKEVKEV
KAPKETKEVKPAAKATNNTYPILNQELREAIKNPAIKDKDHSAPNSRPIDFEMKKKD
GTQQFYHYASSVKPARVIFTDSKPEIELGLQSGQFWRKFEVYEGDKKLPIKLVSYD
TVKDYFYIRFSVNGTKAVKIVSSTHFNNKEEKYDYLMEFAQPIYNSADKFKTEED
45 YKAELKLAPYKKAKTLEKQVYELNKIQDKLPEKLKAEYKKKLEDTKKALDEQVKSAI
TEFQNVQPTNEKMTDLQDTKYVVYVESVENNESMMDTFVKHPKIGMLNGKKYMV

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METTNDYDWDKDFMVEGQVRVTRISKDAKNNTRIIFPYVEGKTLYDAIVKVHVKTIDY
DGYHYHVRIVDKAFTKANTDKSNKKEQODNSAKKEATPATPSKPTSPVEKESQK
QDSQKDDNKQLPSVEKENDASSESGKDKTPATKPTKGEVSSSTTPTKVVSSTQ
NVAKPTTASSKTTKDVVQTSAGSSEAKDSAPLQKANIKNTNDGHTQSQNNKNTQE
5 NKAKSPLQTGEESNKDMTLPMLALLSSIVAFVLPKRKN

RkaS (COL) (SEQ ID NO:11)

10 tttataaataattacataaaatcaatcattttaataataaggattatgataataatgttggtatgacagttaatggaggga
acgaaatgaagctttattacttaaaacagtgatgctcgttttgcttttagtgtaattgggattatggcaagctcga
cgcggtgagcagcagatacacaatgaaagcacatgcagtaacaacgatatagacaaagcaacaacagataagca
acaagctaccccaacaaaggaagcggtcctcatctctgcaaaagacggcaaccaacgtatcagcatcagcg
cagggaacagctgatgatacaaacagcaaaagtaacatccaacgacacatctaacaacacatctacagtagttca
acaaaagtaaacgaaacacgacgtagatatacacaacagcctcaacacaaaaacaaactcacacagcaac
15 gttaaatattacaatgctaaacacagcatcattcaccacgaatgtttgctgctaagcaccacaaacaacaacaca
taaaatattacatacaaatgataatccatgacgactagcgaagaaaaagggcgtgcatcggtatggctaaattaa
aaacagtaaaagaaacaaagaaagcctgatttaattgtagcgcaggagacgcctccaaggtttaccatttcaaa
ccagctaaagggaagaaatggctaaagcaatgaatgcagtaggttatgctatggcagtcggttaacctgaat
20 ttgacttggatcagcagtgtaaaaagttaggggtatgttagactcccgatgctaagtactaacgtttataaagatg
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gaaatgatgctgattataaaagcgtgatacattgtttatatacattaggaaatgatcctcaacacaaagaaaca
tggtggtggtgattacttagtgaacaattaagtcaaaatcccaatgaagaaacgtattacagttatgtgctattc
25 acatacagctactcaaaatggcaaatataacaatgatgcattggcacaacaggtacagcacttgcgaatatcgg
taagattacatttaattatcgcaatggagaggtatcgaatataaacccgctattgataatgttaaaagcgttgaaatgt
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30 ttctgctctatcgcacaaaggaagtgacacgctatgatttaattctcagttattaccatttggaaatacagattgcgcaa
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gcaaacgaatattgctattcaaattttaataaaagagacaggttaagttgaaatattgatttaaaacgctgtatcac
35 gtaacgatgaatgacttcacagcatcaggtggcgacggaatagtagtgcgttggtcctagagaagaaggtatttca
ttagatcaagctactagcaagttatttaaaacagctaactagctaagtagatagcagacgaaccacaacgtatgtat
taggttaaacacagcagtaagtgaaacacagctaaaggacaacaaaggtagcaaggttagtaagctcgttaagat
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cgcatagaaagcattgttagcgggtacagaaggttctggtgcacacatagaaggagctactgtatcaagcaaga
40 gtgggaacaaatggctagaatgtcagtgccctaaaggtagcgcgcatgagaacaggtacccaaaaactggaacta
atcaagttcaagcccagaagcgaatgtttgtattatagcaggtatagggttaacgcgactgtacgacgtagaaaag
ctagctaa

RkaS (COL) (SEQ ID NO:12)

45 FINNLHKINHFNRIMIIYWCMTVNGGNEMKALLLKTSVWLVLFSVMGLWQVSNAA
EQHTPMKAHAVTTIDKATTDKQKQVPPTKEAAHSGKEAATNVSASAQGTADDTN

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SKVTSNAPSINKPSTVSTKVNTRDVTQQASTQKPTHATFKLSNAKTASLSPR
MFAANAPQTTTHKILHTNDIHRGLAEKGRVIGMAKLKTVKEQEKPDMLDAGDAF
QGLPLSNQSKGEEMAKAMNAVGYDAMAVGNHEFDGYDQLKKLEGMDFPMLS
TNVYKDGKRAFKPSTIVTKNGIRYGIIVTTPETKTKTRPEGIKGEVFRDPLQSVTA
5 EMMRIYKDVDTFVVISHLGIDPSTQE TWRGDYLVKQLSQNPQLKKRITVIDGHSHT
VLQNGQIYNNDALAQGTALANIGKITFNRYRNGEVSNIKPSLINVKDVENVTPNKAL
AEQINQADQTFRAQTAEVIPNNITDFKGERDDVTRTRETNLGNAIADAMEAYGVKN
FSKKTDFAVTNGGGIRASIAKGKVTRYDLISVLPFGNTIAQIDVKGSDVWTAFAHSL
GAPTQQKDGKTVLTANGLLHISDSIRVYYDINKPSGKRINAIQILNKETGKFENIDL
10 KRVIYHVTMNDFTASGGDGYSMFGGPREEGISLDQVLASYLKTANLAKYDITTEPQR
MLLGKPAVSEQPAKGQGSKSGSKGKDTQPIGDDKVMDBAKKPAPGKVVLLLAH
RGTVSSGTEGSGRTIEGATVSSKSGKQLARMSVPKGSHEKQLPKTGTNQSSSP
EAMFVLLAGI GLIATVRRRKAS

15 RrKN (8325) (SEQ ID NO:13)

agtggaatatgaaaaaggagtagcaaatgagagataagaaaggaccggtaataaaaggtagattttct
atcaataaatgataaatattcaataagaaaattacagttggaacagcatctatttaattggctcactaatgtatttg
20 ggaactcaacaagaggcagagcagctgaaaacaatattgagaatccaactacattaaagataatgccaatc
aaaagaagtgaagattgaagaagtaacaaacaagacactgcaccaagggtgtagaagctaaatctgaagta
acttcaacaagaacacaatgaacatgaacatcagtaaaagctgaagataatcaaaaaaggaggatacac
caaaagaagtagctgtgtctgaagttcagccgaatcgtcagtcactacacgcagagacacctaagggttag
aaaagctcgttctgtgatgaaggctctttgatattacaagagattctaaaaatgtagtgaattcccccaattacaatt
25 caaggtaagaacattttgaagggttacggaagttgtgatatacaaaaaaaccaacagatttgggtatcagagg
taaccaggtttaattgttggaatgaaagtaattgttgtagggagctttacaattaaaaataaaatagatttttagtaag
gatttcaatttaaagttgaggtggcaataaccatcaatacaacacaggtgctgagttgggttcttatttagt
aaaggaatgcagaagaataatttaactaatgttggaatccttgggataaaggtcgtgtaaatcaggcggtatttaa
aattgatactgatactattatacaagttccatggcaaaaactgaaaagcaagctggcaaggttatagaggatacgc
30 gagctttgtgaaaaatgacagttctggttaattcacaatggttgagaaaaatattgataaatcaaaaactaatttttaa
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tcaggcatataatttcttaattacatctagtaaaagatggggccttaacaggataaatgcaaatgggtggatgaga
actgacttgaaggttcagagtttattttacaccagaagcgccaaaaacaataacagaaattagaaaaaaagttg
35 aagagattccattcaagaagaacgttaatttaacccggttagcaccagggaacagaaaaagtaacaagagaa
ggcaaaaaagggtgagaagacaataacgcacccaactaaaaatccattaacgttgagtaattattgtaagggt
gaocaaaaagagagattacaaaagatccgattaatgaattaaacagaatacggacctgaaacaatagcccgag
gtcatcgagacgaatttgcgaagtacccaacaggagagaaagaggagttccaggttaaacagggaatttaag
aatccagaacacaggagagctgtagtaccgcccgtcgatagcgttaacaaaatagacctgttaaaaggagactc
40 gattgtagaaaaagaagagattccattcgagaagaacgttaatttaactctgatttagcaccagggaacagaaaaa
gtacaagagaaggacaaaaagggtgagaagacaataacgcacccaactaaaaatccattaacgttgagaa
attattagtaaagggtgaatcgaaagaagaatcacaaaagatccgattaatgaattaaacagaatacggaccagaa
acgataacaccagggtcatcgagacgaatttgcgaagtacccaacaggagagaaagagggaaggttccaggttaa
accaggaattagaatccagaacacaggagagtagtagaccacgggtcgatagcgttaacaaaatattggacctgt
45 aaaaggagactcgatgttagaaaaagaagagattccattcgagaagaacgttaatttaactctgatttagcacca
gggacagaaaaagtaacaagagaaggacaaaaagggtgagaagacaataacgcacccaactaaaaatccattaac

RrkN (8325) (SEQ ID NO:14)

SGKYGKRSMMQMRDCKGVPNKRVDFLSNLKNKYSIRKFTVGTASILIGSLMYLGTQ
QEAAEANNENIPTTLDKNVQSKVEKIEEVTNKDAPQGVAEKSEVTSNKDITIEHE
PSVKAEDISKEDTPKEADVAEVOVKSSVTHNAETPKVRKARSDVEGSFDITRDS
KNVVESTPIITQKGEHFEFGYSQSDGKKPTDLDGVSEVTRFNVGNESNGLIGALQLK
NKIDFSKDNFKFVRVANNHQSNTDQAGWGFLFSKGNAAEYLTNGGILGDKGLVN
SGGFKIDTGYIYTSMSMDKTEKQAGQGYRGYGAFFVNDSSNGSNVGENIDKSVT

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NFLNYADNSTNTSDGKFHGRQRLNDVILTYVASTGKMRAEYAGKTWETSITDLGLS
KNQAYNFLITSSQRWGLNQGINANGWMRTDLKGSEFTFTPEAPKTITELEKKVEEI
PFKKERKFNPDLAPGTEKVTREGQKGEKITTPTLKNPLTGVISKGEPEEITKDP
NELTEYGPETIAPGHRDEFDPKLPTEKEEVPKGPGIKNPETGDVVRPPVDSVTKY
5 GPVKGDSIVEKEEIPFEKERKFNPDLAPGTEKVTREGQKGEKITTPTLKNPLTGEI
SKGESKEEITKDPINELTEYGPETITPGHRDEFDPKLPTEKEEVPKGPGIKNPETG
DVVRPPVDSVTKYGPVKGDSIVEKEEIPFEKERKFNPDLAPGTEKVTREGQKGEK
TITPTLKNPLTGVISKGEPEEITKDPINELTEYGPETITPGHRDEFDPKLPTEKEE
EVPKGPGIKNPETGDVVRPPVDSVTKYGPVKGDSIVEKEEIPFEKERKFNPDLAPG
10 TEKVTREGQKGEKITTPTLKNPLTGEIISKGESKEEITKDPINELTEYGPETITPGH
RDEFDPKLPTEKEEVPKGPGIKNPETGDVVRPPVDSVTKYGPVKGDSIVEKEEIP
FEKERKFNPDLAPGTEKVTREGQKGEKITTPTLKNPLTGEIISKGESKEEITKDPIN
ELTEYGPETITPGHRDEFDPKLPTEKEEVPKGPGIKNPETGDVVRPPVDSVTKYG
PVKGDSIVEKEEIPFEKERKFNPDLAPGTEKVTREGQKGEKITTPTLKNPLTGEIIS
15 KGESKEEITKDPINELTEYGPETITPGHRDEFDPKLPTEKEEVPKGPGIKNPETGD
VVRPPVDSVTKYGPVKGDSIVEKEEIPFEKERKFNPDLAPGTEKVTREGQKGEKTI
TPTLKNPLTGEIISKGESKEEITKDPINELTEYGPETITPGHRDEFDPKLPTEKEE
VPGKGPNPETGDVVRPPVDSVTKYGPVKGDSIVEKEEIPFEKERKFNPDLAPGT
EKVTREGQKGEKITTPTLKNPLTGEIISKGESKEEITKDPINELTEFGGEKIPQGH
20 KDIFDPNLPDQTEKVPKGPGIKNPDTGKVIIEPVDVVKHGPKTGTPTKTVEIPI
FKREFNPKLQPGEEERVKQEGQPGSKTITPTITVNPVTGEKVGEGQPTTEITKQPV
DKIVEFGGEKPKDPKGPENPEKPSRPTHPSGPVNPNNPGLSKDRAKPNPVGHS
M
N

25 Kna (8325) (SEQ ID NO:15)

ggaaggagtagtgatggcctaaatcgagggaacggttcaattatgtaaagttatcggttcgacaatgatggc
gacaagtatcatittaaagaatatctgacgatgcccaagctgcatcgaaaaggatgactgaaattacaaaaga
30 gatattatcaagcaagattatagacaaagtgcacaaggcaattogtcaaatgagcaattaaaacagttatcggtt
catctaaagaacattataaagcacaactaaatgaagcgaaacagcatcgcaaatagatgaaatcataaaacga
gctaagtagttgtagcaaaagacaataaaagtctcacactgaaatgaacggtcaaaagtatagacagtaaat
agatcaattgcttaagatttaaagtgaggttctcaaatgttgatgggtcaacaaagtggcgaggacgatcta
gcaatgaaaaatgatgtcacaacggtctacacaaaacatggagaaaaagatgataaaatgatgaagca
35 atggtaataaaggcgttagaagacctagaccatttgaatcagcaatacacaaatcgaaagatgcatgaaagat
acatcggaagatccagcagtgctctacaacagataataatcatgaagtagctaaaacgccaataatgatggttctg
gacatgttggttaataaattccttcaaatgaagagaatcaaaagccatagtaacgactcactgataaatacaagg
aagcgataaaatcaatcatgtatgattgaaaaatagctaaaagtaatgcctcaacgcaacattacacatacataa
40 actgaatacgttacaacttttagatcaacgtattgcaaatacgcaacttctaaaaatcaaaatcagacttaatgagc
gaagtaataaagacgaagagcgtataaaagtaacgaataatatttgggaagactgcaogtactgatgata
aaaagtagctacacaaagcattttagaagtatattataaaagacgaggcagttaaaatctaaaagataatcgt
gttgatgtaaaacagatcaacaaattgcagatcaaatctgcataatgatcaattatctcgacaacgagtgatg
atttattacgtcattgattgatcaatcaagataagtcgctattgatttctcaaatittacaacgaaataggaaaag
ctgaagcagataaattggctaaagattggcgaataaaggattatcaaatcgccaaatcgttgaccaattgaagaa
45 acatttgcatacaactggcgacacgtctcagatgatattaaaagcaatttgaataatgccaaagataaaaaaca
agcaattgaaacgattttagcaacacglatagaaagacaaaaggcaaaattactggcagatttaattactaaaaa

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gaaacagatcaaaaataatttttaatttagttaaatcgccatgaatggtaaacgggatgatattgaatttcaaaa
 gagactcaatcaaacgaaaaagatatagattatattatcaccaatagtaaatcgccaagttaactagatcgattg
 aataaaaatgggaaacgacagatttaataagttagcaaatttaatgaatcaaggatcagattttagacagtatt
 ccagatataccacaccaaagccagaaaagacgttaacactggtaaggttaaggattgtaagtggtattataaa
 5 tgcgtggtgaatgtatcttgcctaagcgggggaaacgataaaagaacattggtgacgatactgtaattgtggtg
 caatgggtgtactaaatgattggtatcacgacgcaataagtgaaaaataaagcataa

KnkA (8325) (SEQ ID NO:16)

10 GRSMMLAKYRGKPFQLYVKLSCSTMMATSILTNILPYDAQAAASEKDETEITKEILSK
 QDLLDKVDKAIHQIEQLKQLSASSKEHYKAQLNEAKTASQIDEIKRANELDSKDNK
 SSHTMNGQSDIDSKLDQLKDLNEVSSNVDRGQQSGEDDLNAMKNDMSQTATT
 KHGEKDDKNDEAMVNKALEDLDHLNQIHKSKDASKDTSDEPAVSTTDNNHEVA
 15 KTPNNDGSGHVVNLNKFSLNEENQSHSNRLTDKLQSGDKINHAMEIKLAKSNASTQ
 HYTYHKLNTLQSLDQRIANTQLPKNQKSDLMSEVNKTKERIKSQRNILEELARTDD
 KKYATQSILESFINKDEAVKILKDIRVDGKTDQQIADQITRHHIDQLSLTTSDDLTSIID
 QSQDKSLISQILQTKLGKAEADKLAKDWTNKGLSNRQIVDQLKKHFASTGDTSSD
 DILKAILNNAKDKKQAIETILATRIERQKAKLLADLITKIETDQNKIFNLVKSALNGKAD
 20 DLLNLQKRLNQTKKIDIDYILSPIVNRPSLLDRLNKNKGTDTDLNKLNLNMNQGSDLLD
 SIPDIPTPKPEKTLTLGKGNGLSGLLNADGNVSLPKAGETIKEHWLPISVIVGAMG
 VLMIVLSRRNKLKNKA

Primary structure analysis:

25 A bioinformatic approach was used for primary structure and function prediction
 (Figure 1). Proteins RrkN and DsqA possessed a similar structural organization to
 previously described MSCRAMMs. RrkN is similar in structure to the Pls/Aap
 proteins of *S. aureus* and *S. epidermidis*, respectively. It contains a 200-residue
 30 domain at its N-terminus showing 40% identity to Pls and Aap. The C-terminus of
 the protein is predominantly composed of a 128 residue repeat domain, which
 varies in the numbers of repeats from strain to strain. These repeats are also
 present in Pls and Aap. A putative *sar* homolog and *fnbA* and *fnbB* lie directly
 upstream from RrkN on the genome.

35 DsqA is similar in structural organization to the Sdr family of proteins. It contains a
 typical A domain followed by a TYYFTDVK motif which is similar to a conserved
 TYTFTVYVD motif found in all of the Sdr proteins. The function of this motif has yet
 to be determined. Two 88 residue repeat domains reside in the centre of the protein

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followed by a C-terminal SX-repeat motif similar to the SD-repeat motif found in the Sdr proteins. The size of this repeat varies from strain to strain. DsqA neighbors *secY* and *secA* on the genome. A DsqA homolog (>90% identical) is also found in *S. epidermidis*.

5

KrkA contains no repeat domains in its sequence. Secondary structure prediction analysis indicate that this protein is predominantly composed of alpha-helices.

10 RkaS contains no repeat domains in its sequence. BLAST analysis indicates that it is similar to a 5' nucleotidase UDP-sugar hydrolase. The gene encoding RkaS lies directly upstream from *orfX*, the insertion site of the *mec* element.

15 KesK contains two 140 residue repeat domains at the N-terminus of the protein which are 38% identical. Hydropathy plot analysis (Kyte and Doolittle, 1982) indicates that there is a large hydrophilic domain in the center of the protein (residue 500-560).

20 EkeS contains two 300 residue repeat domains in the center of the protein which are 38% identical. Blast analysis indicates that the N-terminus of the protein (residues 1-1268, bearing both repeats) is 49% identical to FmtB, an LPXTG protein with 17 tandem repeats. FmtB is proposed to be involved indirectly in methicillin resistance as inactivation of *fmtB* abolishes methicillin resistance. This appears to be due to affecting cell wall composition as methicillin sensitivity can be relieved by increasing the production of the cell wall precursor glucosamine-1-phosphate (Komatsuzawa *et al.*, 2000).

25

KrkN and KrkN2 neighbor each other on the genome.

Expression analysis:

30

Due to lack of sequence homology with protein databases, a putative function for each of these proteins could not be predicted and hence a molecular approach was taken. Unique regions of four of the *orfs* were expressed in *E. coli* as recombinant his-tagged fusion proteins using the Qiagen pQE-30 expression system. Figure 2. represents a Coomassie stained SDS-PAGE gel of the purified N-terminal his-tag fusion proteins. The recombinant proteins RrkN1, DsqA2, KesK1 and KnkA were used to generate antibodies in rabbits. Western blotting analysis of *S. aureus* cell wall extracts revealed that KesK, KnkA and DsqA are expressed and cell wall-associated (Figure 3). Strain eMRSA-16 represents a *knkA*-negative strain since it lacks the *knkA* gene. An immunoreactive band of 65kDa reacts with the cell wall fraction from both exponential and stationary phase cells of strain 8325-4 (Figure 3, B). The absence of this band in strain eMRSA-16 suggests that it represents the gene product of *knkA*.

Western immunoblotting of the cell wall fraction of strain 8325-4 using anti-KesK antibodies identified a 150kDa immunoreactive band in both exponential and stationary phase cultures. A similar sized immunoreactive protein released from the cell wall fraction of *Lactococcus lactis* expressing full length KesK on an expression plasmid (pKS80) suggests that the 150kDa band represents the *kesK* gene product (data not shown). A *kesK* knockout mutant in *S. aureus* would be required to confirm the size of the cell wall-released KesK protein.

Western immunoblotting of the cell wall fraction of *S. aureus* strain MSSA and eMRSA-16 using anti-DsqA antibodies identified a 130kDa immunoreactive band. Expression levels are higher in stationary phase cells.

Heterologous expression in *Lactococcus lactis*:

Heterologous expression of *S. aureus* surface proteins in *Lactococcus lactis* (*L. lactis*) has previously been used as a tool to study protein function (Sinha *et al.*, 2000). In this study this surrogate system will be used to express each of the in

silico-predicted MSCRAMMs on the surface of *L. lactis* to fish for a function. KesK and KnkA have been cloned into *L. lactis* and shown by dot blotting to be surface expressed (Figure 4). No cross reaction was observed with the negative control (pKS80 plasmid without an insert) indicating that this is a specific reaction. Cell wall and protoplast fractions of *Lactococcus lactis* bearing pKS-KnkA and pKS-KesK were generated by digestion of cells with lysozyme and mutanolysin and used in Western blotting studies using anti-KnkA and anti-KesK antibodies, respectively. Unlike what was observed in *S. aureus*, KnkA was not detected in the cell wall fraction of *L. lactis* but found to be associated with the protoplast fraction. The anchoring motif of KnkA differs from the consensus LPXTG sequence in that it contains an Alanine residue instead of a Threonine (i.e. LPKAG) (Table 1). It has been recently been published that *S. aureus* contains two sortase genes, *srtA* and *srtB* (Pallen, 2001). It is possible that this variant form of the LPXTG motif is processed by the second sortase gene, which is absent in *L. lactis*. This would also explain the slight increase in size of the KnkA protein observed in the protoplast fraction, as the cell wall sorting signal has not been cleaved.

KesK was detected in the cell wall fraction of *L. lactis* but migrated at a smaller molecular weight than the KesK protein released from the cell wall of *S. aureus*. The majority of MSCRAMMs expressed on the surface of *L. lactis* are prone to proteolysis during the cell wall extraction procedure (Louise O'Brien, personal communication). Therefore, it is possible that the KesK protein released from the surface of *L. lactis* represents a truncated form of KesK. Shorter digestion times with lysozyme and mutanolysin has been shown to limit the extent of proteolysis.

Expression of in silico-predicted MSCRAMMs in vivo:

Convalescent-phase sera from 33 patients recovering from *S. aureus* infections were tested in their ability to recognize the purified N-terminal his-tag fusion proteins in an ELISA assay. Pooled sera from children and healthy blood donors were used

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as negative controls. A positive reaction was taken as a value equal to or greater than twice the value of the negative control. Figures 5A-5D illustrate that all of the proteins were recognized by 27-42% of the patients suggesting that these proteins are expressed *in vivo* and are immunogenic during infection of the host.

5

References:

- 10 Komatsuzawa, H., Ohta, K., Sugai, M., Fujiwara, T., Glanzmann, P., Berger-Bachi, B., Suganaka, H. (2000) Tn551-mediated insertional inactivation of the *fmtB* gene encoding a cell wall-associated protein abolishes methicillin resistance in *Staphylococcus aureus*. J. Antimicrob. Chemother. **45**: 421-31.
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- 20 Pallen, M.J., Lam, A.C., Antonio, M., Dunbar, K. (2000) An embarrassment of sortases - a richness of substrates? Trends. Microbiol. **9**: 97-101

Example 2. Isolation and Sequencing of Cross-Reactive Proteins from *S. Aureus* and from Coagulase-Negative Staphylococci

25

It has been recently shown that *S. epidermidis* contains surface proteins structurally related to *S. aureus* MSCRAMM[®] proteins (US 09/386,962). One protein from *S. aureus* is of particular interest since it has a close homologue in *S. epidermidis*. The protein is called DsqA or SasA (*S. aureus*) and DgsK (*S. epidermidis*). They are characterized by a typical "A" domain of approximately 500 amino acid residues,

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followed by two B repeats of 88 residues that are ~40% identical, and a unique SXSX dipeptide repeat that can vary in length depending on the strain. Contained within the A domain of the *S. aureus* DsqA/SasA is a 180 residue region that has ~40% identity to a similar sized domain within region A of *S. aureus* proteins RrkN, Pls and *S. epidermidis* protein Aap. The A regions of the DsqA/SasA and DgsK proteins are 46 % identical at the amino acid level, the BB repeats are 50% identical. Active and passive immunization strategies that include; vaccines, polyclonal and monoclonal antibodies recognizing both *S. aureus* and coagulase-negative staphylococcal proteins are the subject of this invention.

Specific Examples of Antibodies that Cross-React with Coagulase-Negative Staphylococci and *S. aureus*.

Coagulase-negative staphylococcal DgsK A-Domain:

Amino Acid Sequence (SEQ ID NO:17)

ASEPTITSEISSNSETVANQNSTTIKNSQKETVNSTSLSNHSNSTNKQMSSEVTN
TAQSSEKAGISQQSSETSNQSSKLNITYASTDHVESTTINNDNTAQDQNKSSNVT
SKSTQSNSTSSSEKNISSNLTSIETKATDSLATSEARTSTNQISNLTSTSTSNQSSP
TSFANLRTFSRFTVLNTMAAPTSTTTTSSLTNSVSVNKNDFNEHMLNLSGSA
20 DPKTGIALTPDAYSQKGAISLNLRLDSNRFRFIGKVNLGNNRYEGYSPDGVAGGD
GIGFAFSPGPLQGIGKEGAAGVIGGLNNAFGFKLDYHNTSTPRSDAKAKADPRN
VGGGGAGAFVSTDRNGMATTEESTAALKLVQPTDNSFQDFVIDYNGDTKVMVT
TYAGQFTFRNLTDWIKNSGGTTFSLSMTASTGGAKNLQQVQFGTFEYTESAVAKV
RYVDANTGKDIIPKTIAGEVDGTVNIDKQLNFKNLGYSYVGTDLKAPNYTETSG
25 TPTLKLNTSSQTVIYKFKDVQ

***S. aureus* SasA A-domain:**

Amino Acid Sequence (SEQ ID NO:18)

30 ASDAPLTSELNTQSETVGNQNSTTIEASTSTADSTSVTKNSSSVQTSNSDVTVSSEK
SEKVTSTTNSTSNQKEKLTSTSESTSSKNNTSSSDTKSVASTSSTEQPISTNQ
TASNNTSQSTTPSSVNLNKTSTTSTSTAPVKLRFTSRSLAMSTFASAAATTTAVTANT
TVNKDNLKQYMTTSGNATYDQSTGIVTLTQDAYSQKGAITLGTIRIDSNKSFHFSK
VNLGNKYEGHNGGDDGIGFAFSPGVLGETGLNGAAGVIGGLSNAFGFKLDYHNT
35 SKPNSAAKANADPSNVAGGGAGFAFVTTDSYGVATTYTSSTADNAAKLVQPT
NNTFQDFDINYNGDTKVMVTVKYAGQWTRNISDWIAKSGTTNFSLSMTASTGGAT
NLQQVQFGTFEYTESAVTQVRYVDVTTGKDIIPPKTYSGNVQVVTIDNQSSALTA
KGYNYTSVDSSYASTYNDTNKTVKMTNAGQSVTYFTDVV

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The entire sequence of the Aap protein and the DNA coding therefor (with an indication of the presence of the A domain) is shown below:

***S. epidermidis* Aap Protein (A-domain underlined)** (SEQ ID NO:19)

5

MGKRRQGPINKKVDLPLNKLNKYSIRKFTVGTASILLGSTLIFGSSSSHEAKAAEEKQ
VDPIQANQNDSERSLENTNQPTVNNEAPQMSSTLQAEEGSNAEAPQSEPTKA
EEGGNAEAAQSEPTKAEEGGNAEAPQSEPTKAEEGGNAEAAQSEPTKTEEGSNV
KAAQSEPTKAEEGSNAEAPQSEPTKTEEGSNAKAAQSEPTKAEEGGNAEAAQSE
10 PTKTEEGSNAEAPQSEPTKAEEGGNAEAPQSEPTKTEEGGNAEAPNVPTIKANS
NDTQTQFSEAPTRNDLARKEDIPAVSKNEELQSSQPNNTDSKIEPTTSEPVNLNYSS
PFMSLLSMPADSSSNNTKNTIDIPPTTVKGRDNYDFYGRVDIESNPTDLNATNLTR
YNYGQPPGTTTAGAVQFKNQVSFDKDFDNIRVANNRQSNTTGADGWGFMFSK
KDGDDEFKNGGILREKGTSAAGFRIDTGYNNNDPLDKIQKQAGQGYRGYGTFFV
15 NDSQGNSTKVGSGTPSTDFLNYADNTTNDLDGKFHGGKLNNVNLKYNASNQFTT
ATYAGKTWTATLSELGLSPTDSYNFLVTSSQYGNNGSGTYASGVMRADLDGATL
TYTPKAVDGDPITSTKEIPFNKKREFDPNLAPGTEKVVQKGEPIETTTTPTYVNP
TGEKVGEGETEKITKQPVDEIVHYGGEEIKPGHKDEFDPNAPKGSQTTQPGKPG
VKNPDTGEVVTTPVDDVTKYGPVDGDPITSTEEIPFDKKREFNPDLKPGEERVQ
20 KGEPGKTITPTTKNPLTGEKVGEGETEKITKQPVDEITEYGGEEIKPGHKDEF
PNAPKGSQEDVPGKPGVKNPVTGEVVTTPVDDVTKYGPVDGDPITSTEEIPFDK
REFNPDLKPGEERVQKQGEPTGKTITPTTKNPLTGEKVGEGETEKITKQPVDEI
VHYGGEQIPQGHKDEFDPNAPVDSKTEVPGKPGVKNPDTGEVVTTPVDDVTKY
PVDGDSITSTEEIPFDKKREFDPNLAPGTEKVVQKGEPTGKTITPTTKNPLTGEK
25 GEGKSTEVTKQPVDEIVEYGPTKAEPGKPAEPGKPAEPGKPAEPGTPAEPGKPA
EPGTPAEPGKPAEPGKPAEPGKPAEPGKPAEPGTPAEPGTPAEPGKPAEPGTPA
EPGKPAEPGTPAEPGKPAESGKPVETPAQSGAPEQPNRSMHSTDNKNQLPD
TGENRQANEGTLVGSLLAIVGSLFIFGRRKKGNEK

30 ***S. epidermidis* aap DNA** (SEQ ID NO:20)

atgggcaaac gtagacaagg tcctattaat aaaaaagtgg

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attttttacc taacaaatta aacaagtatt ctataagaaa attcactgtt ggtaaggcct
 caatattact tggttcgaca ctatttttg gaagtagtag ccatgaagcg aaagctgcag
 aagaaaaaca agttgatoca attacacaag ctaatcaaaa tgatagtagt gaaagatcac
 ttgaaaacac aaatcaacct actgtaaca atgaagcacc acagatgtct tctacattgc
 5 aagcagaaga aggaagcaat gcagaagcac ctcaatctga gccaacgaag gcagaagaag
 gaggcaatgc agaagcagct caatctgagc caacgaaggc agaagaagga ggcaatgcag
 aagcacctca atctgagcca acgaaggcag aagaaggagg caatgcagaa gcagctcaat
 ctgagccaac gaagacagaa gaaggaagca acglaaaaag agctcaatct gagccaacga
 aggcagaaga aggaagcaat gcagaagcac ctcaatctga gccaacgaag acagaagaag
 10 gaagcaacgc aaaagcagct caatctgagc caacgaaggc agaagaagga ggcaatgcag
 aagcagctca atctgagcca acgaagacag aagaagggaag caatgcagaa gcacotcaat
 ctgagccaac gaaggcagaa gaaggaggca atgcagaagc acctcaatct gagccaacga
 agacagaaga agggaggcaat gcagaagcac cgaatgttcc aactcaaaa gctaattcag
 ataagatac acaaacacaa ttctcagaag cccctacaag aaatgacct gctagaaaag
 15 aagatatcc tgcgtttct aaaaacgagg aattacaatc atcacaacca aacactgaca
 gtaaaataga acctacaact tcagaacctg tgaatttaaa ttatagttct ccgtttatgt
 ccttattaag catgcctgct gatagttcat ccaataacac taaaaataca atagatatac
 cgccaactac ggttaaaggt agagataatt acgattttta cggtagagta gatatcgaaa
 gtaatcctac agatttaaat gcgacaatt taacgagata taattatgga cagccacctg
 20 gtacaacaac agctgggtgca gtccaattta aaaatcaagt tagttttgat aaagatttcg
 acittaacat tagagtagca aacaatcgtc aaaglaatac aactgggtgca gatggttggg
 gctttatgt cagcaagaaa gatgggggat atttctaaa aaacgggtgt atcttacgtg
 aaaaaggtag acctagtgca gctggtttca gaattgatac aggatattat aataacgac
 cattagataa aatacagaaa caagctgtgc aaggctatag agggatggg acatttgita
 25 aaaatgactc ccaaggtaat acttctaag taggatcagg tactccatca acagattttc
 ttaactcgc agataatact actaatgati tagatggtaa atccatggt caaaaattaa
 ataatttaa ttgaaatat aatgoticaa atcaaaactt tacagciact tatgtggta
 aaactggac ggctacgtta tctgaattag gattgagtc aactgatatg tacaatttt
 tagttacatc aagtaatat ggaaatggta atagtggtag atacgcaagt gccgttatga
 30 gagctgattt agatgggtgca acattgacat acactcctaa agcagtgat ggagatccaa

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ttatatcaac taaggaaata ccatttaata agaaacgtga atttgatcca aacttagccc
 caggtagaca aaaagtagtc caaaaagggtg aaccaggaat tgaacaaca acaacaccaa
 cttatgtcaa tctaatata ggagaaaaag ttggcgaagg tgaaccaaca gaaaaataa
 caaacaacc agtggatgaa atcggtcatt atggfggcga agaatcaag ccaggccata
 5 aggatgaatt tgaatccaaat gcacgaaaag gtagtcaaac aacgcaacca ggtaagccgg
 gggtaaaaa tctgataca ggcgaagtag ttactccacc tgggatgat gtgacaaat
 atggtcaggt tgaaggagat ccgatcacgt caacggaaga aattccattc gacaagaaac
 gtgaattcaa tctgalita aaaccagggt aagagcgtgt taaacaaaaa ggtgaaccag
 gaacaaaaac aattacaaca ccaacaacta agaaccatt aacaggggaa aaagtggcg
 10 aaggltgaacc aacagaaaaa ataacaaaac aaccagtaga tgaatcaca gaatatgtg
 gcgaagaaat caagccaggc cataaggatg aattgatcc aaatgcaccg aaaggtagcc
 aagaggacgt tccaggtaaa ccaggagtta aaaacctgg aacaggcgaa gtagtcacac
 caccagtgga tgaatgaca aaatatggtc cagttgatgg agatccgac acgtcaacgg
 aagaaattcc attcgacaag aaacgtgaat tcaatcctga tttaaaacca ggtgaagagc
 15 cgttaaaaca gaaagggtgaa ccaggaacaa aaacaattac aacgccaaca actaagaacc
 cattaacagg agaaaaagtt ggcgaagggt aaccaacaga aaaaataaca aaacaaccag
 tggatgagat tgttcattat ggtggtgaac aaataccaca aggtcataaa gatgaattg
 atccaaatgc acctgtagat agtaaaactg aagttccagg taaaccagga gttaaaaatc
 ctgatacagg tgaagttgtt accccaccag tggatgatgt gacaaaatat ggtccagttg
 20 atggagattc gattacgtca acggaagaaa ttccgtttga taaaaaacgc gaatttgatc
 caaacttagc gccagggtaca gagaaagtgc ttcaaaaagg tgaaccagga acaaaaaaca
 ttacaacgcc aacaactaag aaccattaa caggagaaaa agttggcgaa ggtaaatcaa
 cagaaaaagt cactaaacaa cctgttgacg aaattgtga gtatggtcca acaaaagcag
 aaccaggtaa accagcgga ccaggtaaac cagcgggaacc aggtaaacca gcggaaccag
 25 gtacgccagc agaaccaggt aaaccagcgg aaccaggtag gccagcagaa ccaggtaaac
 cagcgggaacc aggtaaacca gcggaaccag glaaaccagc ggaaccaggt aaaccagcgg
 aaccaggtag gccagcagaa ccaggtagc cagcagaacc aggtaaacca gcggaaccag
 gtacgccagc agaaccaggt aaaccagcgg aaccaggtag gccagcagaa ccaggtaaac
 cagcgggaatc aggtaaacca gtggaaccag gtacgccagc acaatcaggt gcaccagaac
 30 aaccaaatag atcaatgcat tcaacagata ataaaaatca attacctgat acagggtgaa

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atcgtcaagc taatgagga accttagtcg gatctctatt agcaattgtc ggatcattgt
 tcataattgg tcgtcgtaaa aaaggtaatg aaaaataatt tcataaaaa acittctgcc
 attaa

- 5 **A-Domain from *S. epidermidis* Aap (amino acids 55-600)** (SEQ ID NO:21)
⁵⁵EKQVDPITQANQNDSSERSLENTNQPTVNNEAPQMSSTLQAEEGSNAEAPQSE
 PTKAEEGGNAEAAQSEPTKAEEGGNAEAPQSEPTKAEEGGNAEAAQSEPTKTEE
 GSNVKAQSEPTKAEEGSNAEAPQSEPTKTEEGSNAKAAQSEPTKAEEGGNAEA
 AQSEPTKTEEGSNAEAPQSEPTKAEEGGNAEAPQSEPTKTEEGGNAEAPNVPTIK
 10 ANSDNDTQTQFSEAPTRNDLARKEDIPAVSKNEELQSSQPNTDSKIEPTTSEPVNL
 NYSSPFMSLLSMPADSSSNNTKNTIDIPPTTVKGRDNYDFYGRVDIESNPTDLNAT
 NLTRYNYGQPPGTTTAGAVQFKNQVSFDKDFDNIRVANNRQSNTTGADGWGF
 MFSKKDGGDFLKNGGILREKGTSAAGFRIDTGYNNNDPLDKIQKQAGQGYRGYG
 TFVKNSQGNSTSKVSGTSPSTDFLNYADNTTNDLDGKFHGQKLNNVNLKYNASN
 15 QTFTATYAGKTWTATLSELGLSPTDSYNFLVTSSQYGNGNSGTYASGVMRADLD
 GA⁶⁰⁰

Protein Production and Purification

- 20 Using PCR, the A domain of DgsK or SasA was amplified from the sequences
 described above and subcloned into the *E. coli* expression vector PQE-30 (Qiagen),
 which allows for the expression of a recombinant fusion protein containing six
 histidine residues. This vector was subsequently transformed into the *E. coli* strain
 25 ATCC 55151, grown in a 15-liter fermentor to an optical density (OD₆₀₀) of 0.7 and
 induced with 0.2 mM isopropyl-1-beta-D galactoside (IPTG) for 4 hours. The cells
 were harvested using an AG Technologies hollow-fiber assembly (pore size of 0.45
 μm) and the cell paste frozen at -80° C. Cells were lysed in 1X PBS (10 mL of
 buffer/1 g of cell paste) using 2 passes through the French Press @ 1100psi.
 30 Lysed cells were spun down at 17,000rpm for 30 minutes to remove cell debris.
 Supernatant was passed over a 5-mL HiTrap Chelating (Pharmacia) column
 charged with 0.1M NiCl₂. After loading, the column was washed with 5 column

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volumes of 10mM Tris, pH 8.0, 100mM NaCl (Buffer A). Protein was eluted using a 0-100% gradient of 10mM Tris, pH 8.0, 100mM NaCl, 200 mM imidazole (Buffer B) over 30 column volumes. SdrGN1N2N3 or SdrGN2N3 eluted at ~13% Buffer B (~26mM imidazole). Absorbance at 280nm was monitored. Fractions containing

5 SdrGN1N2N3 or SdrGN2N3 were dialyzed in 1x PBS.

Each protein was then put through an endotoxin removal protocol. Buffers used during this protocol were made endotoxin free by passing over a 5-mL Mono-Q sepharose (Pharmacia) column. Protein was divided evenly between 4x 15mL tubes. The volume of each tube was brought to 9mL with Buffer A. 1mL of 10%

10 Triton X-114 was added to each tube and incubated with rotation for 1 hour at 4°C. Tubes were placed in a 37°C water bath to separate phases. Tubes were spun down at 2,000rpm for 10 minutes and the upper aqueous phase from each tube was collected and the detergent extraction repeated. Aqueous phases from the 2nd extraction were combined and passed over a 5-mL IDA chelating (Sigma) column,

15 charged with 0.1M NiCl₂ to remove remaining detergent. The column was washed with 9 column volumes of Buffer A before the protein was eluted with 3 column volumes of Buffer B. The eluant was passed over a 5-mL Detoxigel (Sigma) column and the flow-through collected and reapplied to the column. The flow-through from the second pass was collected and dialyzed in 1x PBS. The purified

20 product was analyzed for concentration, purity and endotoxin level before administration into the mice.

Monoclonal Antibody Production

25 *E. coli* expressed and purified recombinant SasA and DsgK proteins were used to generate a panel of murine monoclonal antibodies while the mouse sera was used as a source of polyclonal antibodies. Briefly, a group of Balb/C or SJL mice received a series of subcutaneous immunizations of 1-10 mg of protein in solution or mixed with adjuvant as described in the Table below.

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Immunization Schemes

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RIMMS				
Injection	Day	Amount (µg)	Route	Adjuvant
#1	0	5	Subcutaneous	FCA/RIBI
#2	2	1	Subcutaneous	FCA/RIBI
#3	4	1	Subcutaneous	FCA/RIBI
#4	7	1	Subcutaneous	FCA/RIBI
#5	9	1	Subcutaneous	FCA/RIBI

Conventional				
Injection	Day	Amount (µg)	Route	Adjuvant
Primary	0	5	Subcutaneous	FCA
Boost #1	14	1	Intraperitoneal	RIBI
Boost #2	28	1	Intraperitoneal	RIBI
Boost #3	42	1	Intraperitoneal	RIBI

At the time of sacrifice (RIMMS) or seven days after a boost (conventional) serum was collected and titered in ELISA assays against MSCRAMM[®] proteins or on whole cells (*S. epidermidis* and *S. aureus*). Three days after the final boost, the spleens or lymph nodes were removed, teased into a single cell suspension and the lymphocytes harvested. The lymphocytes were then fused to a P3X63Ag8.653 myeloma cell line (ATCC #CRL-1580). Cell fusion, subsequent plating and feeding were performed according to the Production of Monoclonal Antibodies protocol from Current Protocols in Immunology (Chapter 2, Unit 2.).

Any clones that were generated from the fusion were then screened for specific anti-SasA antibody production using a standard ELISA assay. Positive clones were expanded and tested further for activity in a whole bacterial cell binding assay by flow cytometry and SasA binding by Biacore analysis.

Biacore Analysis

Throughout the analysis, the flow rate remained constant at 10 ml/min. Prior to the SasA or DgsK injection, test antibody was adsorbed to the chip via RAM-Fc binding.

At time 0, SasA or DgsK at a concentration of 30 mg/ml was injected over the chip for 3 min followed by 2 minutes of dissociation. This phase of the analysis

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measured the relative association and disassociation kinetics of the Mab / SasA or DgsK interaction.

Binding to Whole Bacteria

5 Bacterial samples *S. aureus* Newman, *S. aureus* 67-0, *S. aureus* 397 (Sal6), *S. aureus* Wood, *S. aureus* 8325-4, methicillin resistant *S. aureus* MRSA 16, *S. epidermidis* ATCC 35984, *S. epidermidis* HB, *S. epidermidis* CN-899 and *S. haemolyticus* ATCC 43253 were collected, washed and incubated with Mab or PBS alone (control) at a concentration of 2 µg/ml after blocking with rabbit IgG (50 mg/ml). Following incubation with antibody, bacterial cells were incubated with Goat-F_(ab)2-Anti-Mouse-F_(ab)2-FITC which served as the detection antibody. After antibody labeling, bacterial cells were aspirated through the FACScaliber flow cytometer to analyze fluorescence emission (excitation: 488, emission: 570). For 15 each bacterial strain, 10,000 events were collected and measured. These data indicate that antibodies against *S. aureus* SasA were able to recognize a homologous protein on the surface of coagulase-negative staphylococci. The data support Western blot analysis demonstrating that rabbit polyclonal antibodies against *S. aureus* SasA cross-react with a protein released from the cell surface of 20 *S. epidermidis* HB as well as the recombinant A-region from DsgK cloned from *S. epidermidis* (see Table below and Figure 6).

Polyclonal Sera Reactivity

	New man	67-0	397 (SAL 6)	Wo od 46	8325 -4	MRS A 16	ATC C 3598 4	HB	CN- 899	ATC C 4325 3
Normal Mouse Sera	-	-	-	-	-	-	-	-	-	-
Mouse anti- SasA	+	+	+/-	-	+	+	+	+	+	+

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What is claimed is:

1. An isolated antibody which binds to a staphylococcal surface protein selected from the group consisting of SEQ ID NOS. 2, 4, 6, 8, 10, 12, 14, 16, 17,
5 18, 19 and 21.
2. The antibody according to Claim 1 wherein the antibody is raised against the A domain of the surface protein.
- 10 3. The antibody according to Claim 1, wherein the antibody treats or prevents *S. aureus* infection in a human or animal.
4. The antibody according to Claim 1, wherein the antibody is suitable for parenteral, oral, intranasal, subcutaneous, aerosolized or intravenous administration
15 in a human or animal.
5. The antibody according to Claim 1, wherein said antibody is a monoclonal antibody.
- 20 6. The antibody according to Claim 1, wherein said antibody is a polyclonal antibody.
7. The antibody according to Claim 5 wherein the monoclonal antibody is of a type selected from the group consisting of murine, chimeric, humanized and
25 human monoclonal antibodies.
8. The antibody according to Claim 5 wherein the antibody is a single chain monoclonal antibody.

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9. The antibody according to Claim 1 which comprises an antibody
fragment having the same binding specificity of an antibody which binds to a
staphylococcal surface protein having the sequence selected from the group
5 consisting of SEQ ID NOS. 2, 4, 6, 8, 10, 12, 14, 16, 17, 18, 19 and 21.

10. The antibody according to Claim 1 that is raised against a protein
having an amino acid sequence selected from the group consisting of SEQ ID NOS.
2, 4, 6, 8, 10, 12, 14, 16, 17, 18, 19 and 21.
10

11. The antibody according to Claim 1 wherein the surface protein has an
amino acid sequence encoded by a nucleic acid sequence selected from the group
consisting of nucleic acid sequences SEQ ID NOS. 1, 3, 5, 7, 9, 11, 13, 15, 20 and
the nucleic acid sequences coding for the A domain of the Aap protein or
15 degenerates thereof.

12. Isolated antisera containing an antibody according to Claim 1.

13. A diagnostic kit comprising an antibody according to Claim 1 and
20 means for detecting binding by that antibody.

14. A diagnostic kit according to Claim 13 wherein said means for
detecting binding comprises a detectable label that is linked to said antibody.

25 15. A method of diagnosing an infection of *S. aureus* comprising adding
an antibody according to Claim 1 to a sample suspected of being infected with *S.*
aureus, and determining if antibodies have bound to the sample.

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16. A pharmaceutical composition for treating or preventing an infection of *S. aureus* comprising an effective amount of the antibody of Claim 1 and a pharmaceutically acceptable vehicle, carrier or excipient.
- 5 17. A method of treating or preventing an infection of *S. aureus* comprising administering to a human or animal patient an effective amount of an antibody according to Claim 1.
- 10 18. A method of inducing an immunological response comprising administering to a human or animal an immunogenic amount of an isolated protein selected from the group consisting of the amino acid sequences SEQ ID NOS. 2, 4, 6, 8, 10, 12, 14, 16, 17, 18, 19 and 21.
- 15 19. An isolated antibody according to Claim 1 that has the ability to bind to an amino acid sequence coded by the nucleic acid sequence of SEQ ID NOS. 1, 3, 5, 7, 9, 11, 13, 15, 20 and the nucleic acid sequences coding for the A domain of the Aap protein or degenerates thereof.
- 20 20. An isolated active fragment from the A domain of the DsqA protein.
21. An isolated antibody according to Claim 1 further comprising a physiologically acceptable antibiotic.
- 25 22. A vaccine for treating or preventing an infection of *S. aureus* comprising an amount of a protein sequence selected from the group consisting of SEQ ID NOS. 2, 4, 6, 8, 10, 12, 14, 16, 17, 18, 19 and 21 in an amount effective to elicit an immune response, and a pharmaceutically acceptable vehicle, carrier or excipient.

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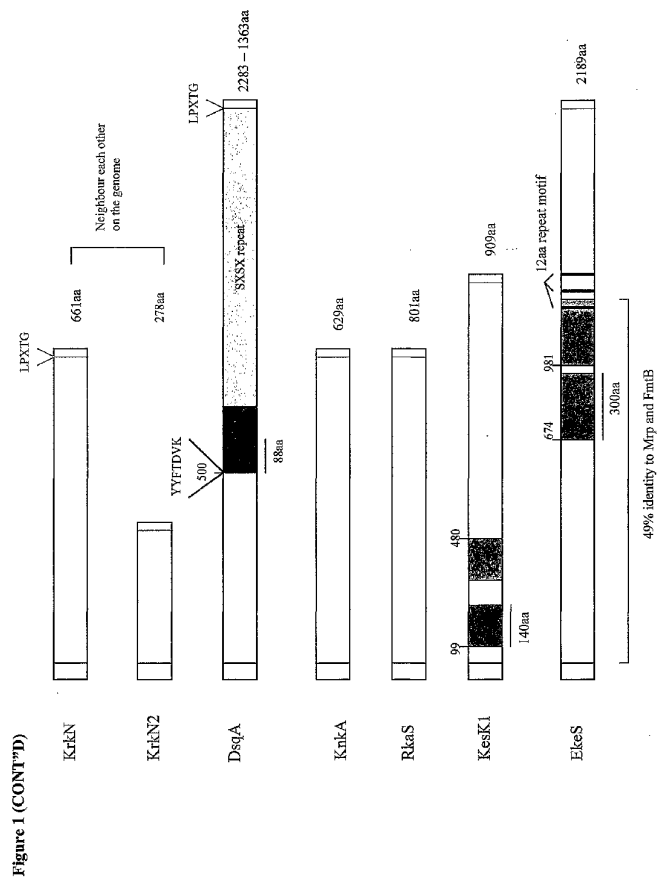
The diagram illustrates the domain structure of the RrKn protein. It is divided into three main regions: R1, R2, and R3. R1 contains a proline-rich region (indicated by a bracket) and a signal sequence (indicated by a triangle). R2 is a large domain (indicated by a bracket) and is associated with anti-adhesin activity (indicated by a triangle). R3 contains a proline-rich region (indicated by a bracket) and a signal sequence (indicated by a triangle). The total length of the protein is 1629 aa. The R1 region is 178 aa long, R2 is 793 aa long, and R3 is 1637 aa long. The R2 region is associated with anti-adhesin activity. The R3 region is associated with proline-richness. The R1 region is associated with a signal sequence. The R2 region is associated with a signal sequence. The R3 region is associated with a signal sequence.

Anti-adhesin, associated with the mec element

Proposed to be involved in biofilm formation

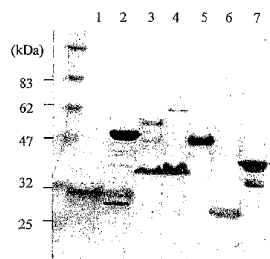
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	Residues	Predicted MW	Apparent MW
• RrkN 1	60 - 215	19	29
• RrkN 2	60 - 437	45	48
• DsqA 1	54 - 279	27	38
• DsqA 2	54 - 533	58	62
• KesK 1	55 - 335	34	47
• KnkA	39 - 210	20	27
• KesK 2	329 - 591	31	40

Figure 2. Coomassie gel of the purified N-terminal His-tagged fusion proteins.

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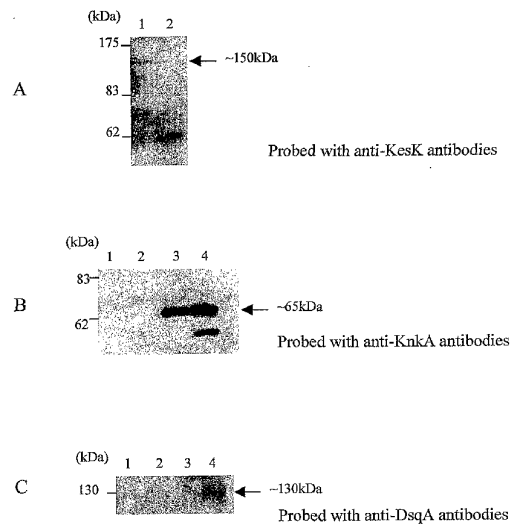


Figure 3. Western blotting of *S. aureus* cell wall extracts. Bacterial cells were standardised to an OD₆₀₀ of 50 units and cell walls were isolated by lysostaphin digestion of stabilised protoplasts. A. Lane 1, 8325-4 (early exponential phase); lane 2, 8325-4 (stationary phase). B. Lanes 1 and 2, eMRSA-16; lanes 3 and 4, 8325-4; lanes 1 and 3 represent early exponential phase cells and lanes 2 and 4 represent stationary phase cells. C. Lanes 1 and 2, MSSA; lanes 3 and 4, eMRSA-16; lanes 1 and 3 represent early exponential phase cells and lanes 2 and 4 represent stationary phase cells.

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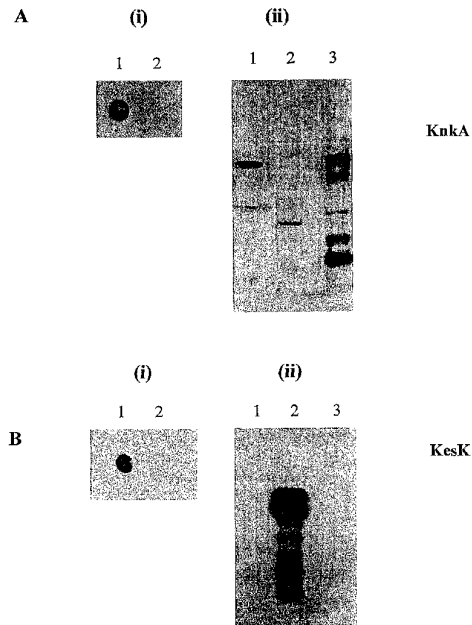
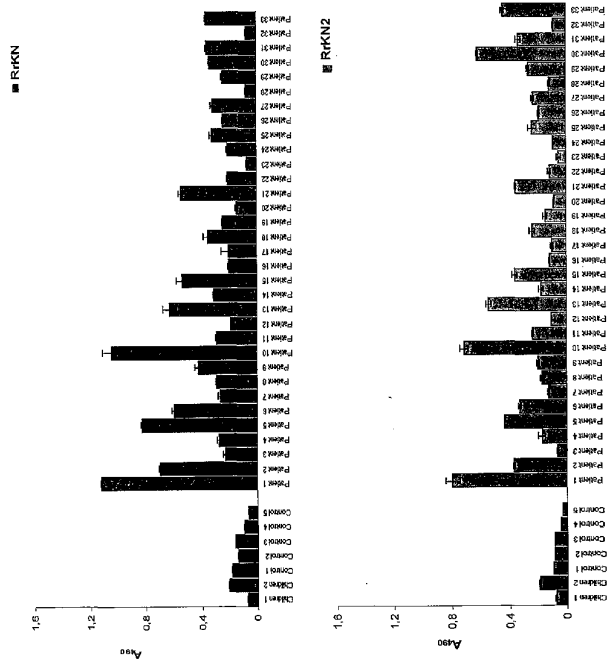


Figure 4. Dot blotting and Western immunoblotting of *Lactococcus lactis* expressing *S. aureus* MSCRAMMs. Full length *knkA* and *kesK* were cloned into the *L. lactis* expression plasmid pKS80 and electroporated into competent *L. lactis* MG1363 cells. Positive KnkA and KesK expressing clones were detected using dot blotting with anti-KnkA (A) and anti-KesK (B) antibodies, respectively. *L. lactis* bearing pKS80 was used as a negative control. A. (i) lane 1, *L. lactis* pKS-KnkA; lane 2, *L. lactis* pKS80. B. (ii) lane 1, *L. lactis* pKS-KesK; lane 2, *L. lactis* pKS80. Western immunoblotting was used to examine the expression of KesK and KnkA in *S. aureus* and *L. lactis*. A. (ii). Lane 1, cell wall extract from exponential phase *S. aureus* strain 8325-4, lane 2, protoplast fraction from *L. lactis* bearing pKS80; lane 3, protoplast fraction from *L. lactis* bearing pKS-KnkA. B. (ii) Lane 1, cell wall extract from exponential phase *S. aureus* strain 8325-4; lane 2, cell wall extract from *L. lactis* bearing pKS-KesK; lane 3, cell wall extract from *L. lactis* bearing pKS80.

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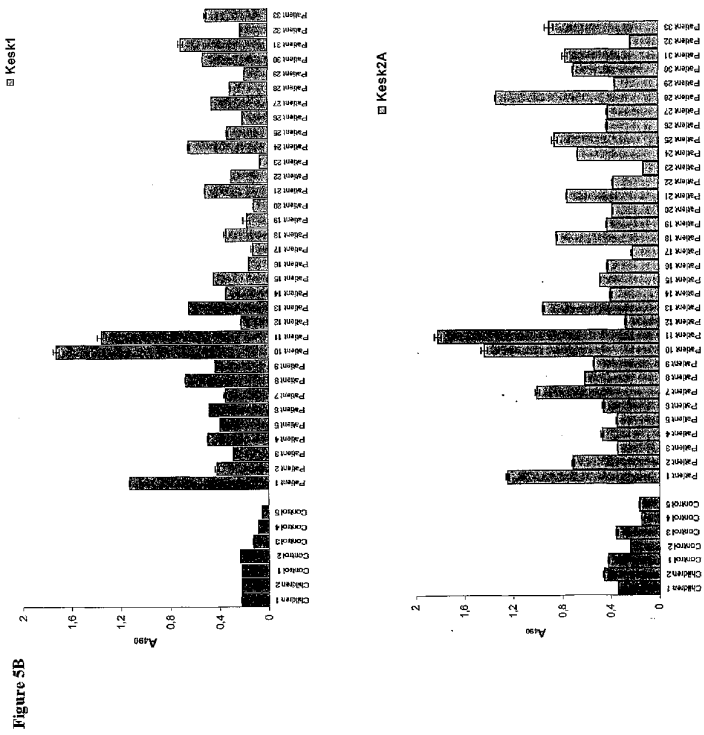
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Figure 5A. Probing recombinant LPXIG proteins with convalescent sera to study *in vivo* expression.



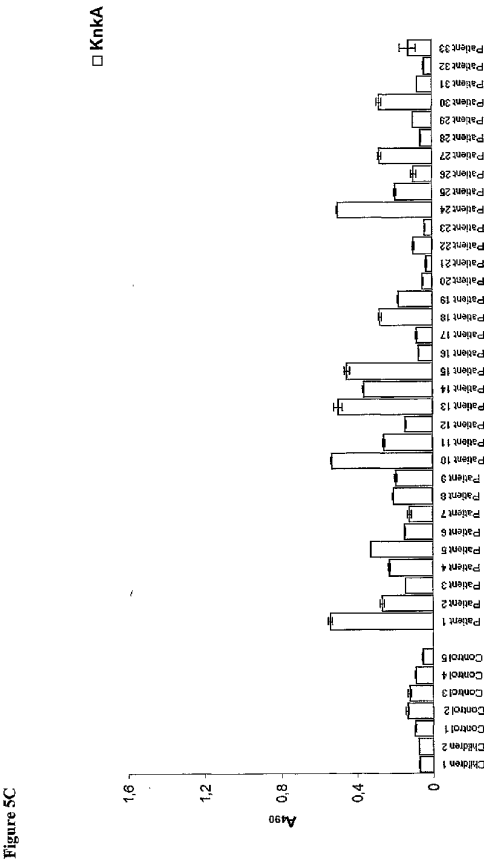
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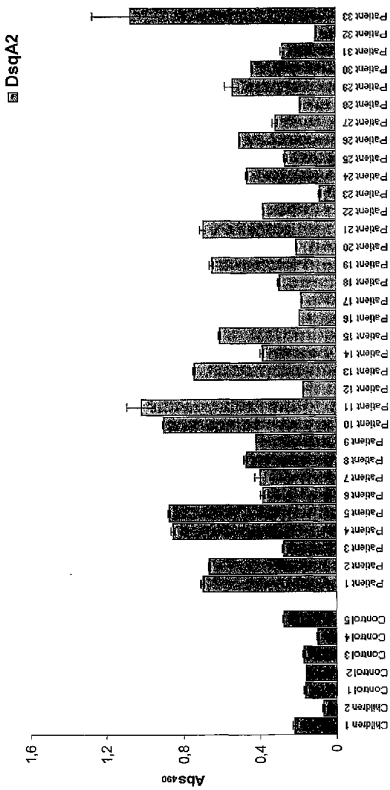
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Figure 5D



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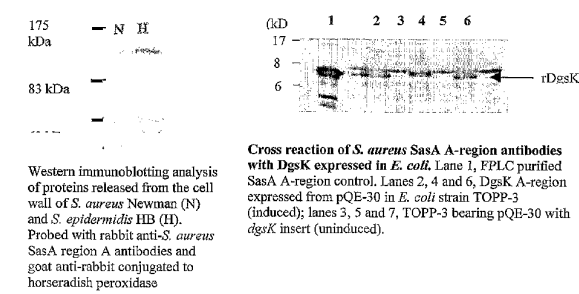


FIGURE 6

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	Asp Ser Thr Asn Gln Ala Leu Asp Asp Ile Ala Leu Val Thr Pro		
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5	Asp Glu Glu Lys Gln Val	Ala Leu Asn Gln Leu	Ala Asn Asn Glu
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10	Lys Arg Ala Leu Gln Asn	Ile Asn Gln Ala Ile	Ala Asn Asn Asp
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	Val Lys Arg Val Glu Ser	Asn Gly Ile Ala Thr	Leu Lys Gly Val
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15	Glu Pro His Ile Val Val	Lys Pro Glu Ala Gln	Glu Ala Ile Lys
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	Ala Ser Ala Asp Asn Gln	Val Glu Ser Ile Lys	Asp Thr Pro His
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25	Ser Ile Leu Ala Gln Leu Gln	Asn Ile Tyr Asp Thr	Ala Ile Gly
	1925	1930	1935
	Gln Ile Asp Gln Asp Arg Ser	Asn Ala Gln Val Asp	Lys Thr Ala
	1940	1945	1950
30	Thr Leu Asn Leu Gln Thr Ile	His Asp Leu Asp Val	His Pro Ile
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	Lys Lys Pro Asp Ala Glu Lys	Thr Ile Asn Asp Asp	Leu Ala Arg
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	1985	1990	1995
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10	Ser Leu	Ser Glu	Ser Asn	Ser Thr	Ser Asp	Ser Glu	Ser Gln	Ser
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	Ala Ser	Ala Phe	Leu Ser	Glu Ser	Leu Ser	Glu Ser	Thr Ser	Glu
	1220			1225			1230	
15	Ser Thr	Ser Glu	Ser Val	Ser Ser	Ser Thr	Ser Glu	Ser Thr	Ser
	1235			1240			1245	
20	Leu Ser	Asp Ser	Thr Ser	Glu Ser	Gly Ser	Thr Ser	Thr Ser	Leu
	1250			1255			1260	
	Ser Asn	Ser Thr	Ser Gly	Ser Thr	Ser Ile	Ser Thr	Ser Thr	Ser
	1265			1270			1275	
25	Ile Ser	Glu Ser	Thr Ser	Thr Phe	Lys Ser	Glu Ser	Val Ser	Thr
	1280			1285			1290	
	Ser Leu	Ser Met	Ser Thr	Ser Thr	Ser Leu	Ser Asp	Ser Thr	Ser
	1295			1300			1305	
30	Leu Ser	Thr Ser	Leu Ser	Asp Ser	Thr Ser	Asp Ser	Lys Ser	Asp
	1310			1315			1320	
	Ser Leu	Ser Thr	Ser Met	Ser Thr	Ser Asp	Ser Ile	Ser Thr	Ser
	1325			1330			1335	
35	Lys Ser	Asp Ser	Ile Ser	Ser Thr	Ser Ser	Leu Ser	Gly Ser	Thr
	1340			1345			1350	
40	Ser Glu	Ser Glu	Ser Asp	Ser Thr	Ser Ser	Ser Glu	Ser Lys	Ser
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	Asp Ser	Thr Ser	Met Ser	Ile Ser	Met Ser	Gln Ser	Thr Ser	Gly
	1370			1375			1380	
45	Ser Thr	Ser Thr	Ser Thr	Ser Thr	Ser Leu	Ser Asp	Ser Thr	Ser
	1385			1390			1395	
	Thr Ser	Leu Ser	Leu Ser	Ala Ser	Met Asn	Gln Ser	Gly Val	Asp
	1400			1405			1410	
50	Ser Asn	Ser Ala	Ser Gln	Ser Ala	Ser Asn	Ser Thr	Ser Thr	Ser
	1415			1420			1425	
55	Thr Ser	Glu Ser	Asp Ser	Gln Ser	Thr Ser	Ser Tyr	Thr Ser	Gln
	1430			1435			1440	
	Ser Thr	Ser Gln	Ser Glu	Ser Thr	Ser Thr	Ser Thr	Ser Leu	Ser
	1445			1450			1455	
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Asp Ser Thr Ser Ile Ser Lys Ser Thr Ser Gln Ser Gly Ser Val
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 5 Ser Thr Ser Ala Ser Leu Ser Gly Ser Glu Ser Glu Ser Asp Ser
 1475 1480 1485
 Gln Ser Ile Ser Thr Ser Ala Ser Glu Ser Thr Ser Glu Ser Ala
 1490 1495 1500
 10 Ser Thr Ser Leu Ser Asp Ser Thr Ser Thr Ser Asn Ser Gly Ser
 1505 1510 1515
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 1520 1525 1530
 15 Ser Asp Leu Ser Ser Thr Ser Leu Ser Asp Ser Thr Ser Ala Ser
 1535 1540 1545
 20 Met Gln Ser Ser Glu Ser Asp Ser Gln Ser Thr Ser Ala Ser Leu
 1550 1555 1560
 Ser Asp Ser Leu Ser Thr Ser Thr Ser Asn Arg Met Ser Thr Ile
 1565 1570 1575
 25 Ala Ser Leu Ser Thr Ser Val Ser Thr Ser Glu Ser Gly Ser Thr
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 1670 1675 1680
 45 Ser Thr Ser Gly Ser Met Ser Val Ser Ile Ser Leu Ser Asp Ser
 1685 1690 1695
 Thr Ser Thr Ser Thr Ser Ala Ser Glu Val Met Ser Ala Ser Ile
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 50 Ser Asp Ser Gln Ser Met Ser Glu Ser Val Asn Asp Ser Glu Ser
 1715 1720 1725
 Val Ser Glu Ser Asn Ser Glu Ser Asp Ser Lys Ser Met Ser Gly
 1730 1735 1740
 Ser Thr Ser Val Ser Asp Ser Gly Ser Leu Ser Val Ser Thr Ser
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Leu Arg Lys Ser Glu Ser Val Ser Glu Ser Ser Ser Leu Ser Cys
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 1835 1840 1845
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 1865 1870 1875
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 1880 1885 1890
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 30 Thr Ser Asn Ser Gln Phe Asp Ser Met Ser Ile Ser Ala Ser Glu
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 35 Ser Asp Ser Met Ser Thr Ser Asp Ser Ser Ser Ile Ser Gly Ser
 1925 1930 1935
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 40 Ser Val Ser Val Ser Thr Ser Thr Ser Leu Ser Asp Ser Ile Ser
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 1970 1975 1980
 45 Ser Leu Ser Asp Ser Met Ser Gln Ser Gln Ser Thr Ser Thr Ser
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 50 Ala Ser Gly Ser Leu Ser Thr Ser Ile Ser Thr Ser Met Ser Met
 2000 2005 2010
 Ser Ala Ser Thr Ser Ser Ser Gln Ser Thr Ser Val Ser Thr Ser
 2015 2020 2025
 55 Leu Ser Thr Ser Asp Ser Ile Ser Asp Ser Thr Ser Ile Ser Ile
 2030 2035 2040
 Ser Gly Ser Gln Ser Thr Val Glu Ser Glu Ser Thr Ser Asp Ser
 2045 2050 2055
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Thr Ser Ile Ser Asp Ser Glu Ser Leu Ser Thr Ser Asp Ser Asp
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 15 Ser Glu Ser Val Ser Thr Ser Thr Ser Thr Ser Leu Ser Thr Ser
 2135 2140 2145
 Asp Ser Thr Ser Thr Ser Glu Ser Leu Ser Thr Ser Met Ser Gly
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 Ser Gln Ser Ile Ser Asp Ser Thr Ser Thr Ser Met Ser Gly Ser
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 2180 2185 2190
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 2225 2230 2235
 35 Lys Arg Leu Pro Asp Thr Gly Asp Ser Ile Lys Gln Asn Gly Leu
 2240 2245 2250
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 55 agtacactat ttttaattac ttctcaaat caagcacaag cagcagaaaa tacaataact 180
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 60 acaaatcaaa cacaacctgc taogcaacca gcaaacacty cgaataacta toctgcagcg 300

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 5 ggccaagag aacaagtcaa ttccagtta ttagataaaa acaatgaaac gcagtactat 420
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 20 25 30
 Leu Gly Val Ala Ser Val Ile Val Ser Thr Leu Phe Leu Ile Thr Ser
 35 40 45
 40 Gln His Gln Ala Gln Ala Ala Glu Asn Thr Asn Thr Ser Asp Lys Ile
 50 55 60
 45 Ser Glu Asn Gln Asn Asn Asn Ala Thr Thr Thr Gln Pro Pro Lys Asp
 65 70 75 80
 Thr Asn Gln Thr Gln Pro Ala Thr Gln Pro Ala Asn Thr Ala Lys Asn
 85 90 95
 50 Tyr Pro Ala Ala Asp Glu Ser Leu Lys Asp Ala Ile Lys Asp Pro Ala
 100 105 110
 Leu Glu Asn Lys Glu His Asp Ile Gly Pro Arg Glu Gln Val Asn Phe
 115 120 125
 55 Gln Leu Leu Asp Lys Asn Asn Glu Thr Gln Tyr Tyr His Phe Phe Ser
 130 135 140
 60 Ile Lys Asp Pro Ala Asp Val Tyr Tyr Thr Lys Lys Lys Ala Glu Val
 145 150 155 160

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Glu Leu Asp Ile Asn Thr Ala Ser Thr Trp Lys Lys Phe Glu Val Tyr
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 5 Glu Asn Asn Gln Lys Leu Pro Val Arg Leu Val Ser Tyr Ser Pro Val
 180 185 190
 Pro Glu Asp His Ala Tyr Ile Arg Phe Pro Val Ser Asp Gly Thr Gln
 195 200 205
 10 Glu Leu Lys Ile Val Ser Ser Thr Gln Ile Asp Asp Gly Glu Glu Thr
 210 215 220
 Asn Tyr Asp Tyr Thr Lys Leu Val Phe Ala Lys Pro Ile Tyr Asn Asp
 225 230 235 240
 15 Pro Ser Leu Val Lys Ser Asp Thr Asn Asp Ala Val Val Thr Asn Asp
 245 250 255
 Gln Ser Ser Ser Val Ala Ser Asn Gln Thr Asn Thr Ser Asn
 260 265 270
 20 Gln Asn Ile Ser Thr Ile Asn Asn Ala Asn Asn Gln Pro Gln Ala Thr
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 290 295 300
 25 Gln Ala Ser Ser Gln Pro Ala His Glu Thr Asn Ser Asn Gly Asn Thr
 305 310 315 320
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 325 330 335
 35 Tyr Pro Pro Ala Asp Glu Ser Leu Gln Asp Ala Ile Lys Asn Pro Ala
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 355 360 365
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 420 425 430
 Lys Asp Tyr Ala Tyr Ile Arg Phe Pro Val Ser Asn Gly Thr Arg Glu
 435 440 445
 55 Val Lys Ile Val Ser Ser Ile Glu Tyr Gly Glu Asn Ile His Glu Asp
 450 455 460
 Tyr Asp Tyr Thr Leu Met Val Phe Ala Gln Pro Ile Thr Asn Asn Pro
 465 470 475 480

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Asp Asp Tyr Val Asp Glu Glu Thr Tyr Asn Leu Gln Lys Leu Leu Ala
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 5 Pro Tyr His Lys Ala Lys Thr Leu Glu Arg Gln Val Tyr Glu Leu Glu
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 25 Lys Asp Leu Ile Val Glu Gly Lys Arg Val Thr Thr Val Ser Lys Asp
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 Pro Lys Asn Asn Ser Arg Thr Leu Ile Phe Pro Tyr Ile Pro Asp Lys
 625 630 635 640
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 645 650 655
 35 Glu Gly Gln Tyr His Val Arg Ile Ile Asn Gln Asp Ile Asn Thr Lys
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 40 Thr Gly Gln Glu Gly Lys Val Ala Asp Thr Asp Val Ala Glu Asn Ser
 690 695 700
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 Glu Pro Glu Ser Asp Val Val Lys Asp Ala Asp Asn Asn Ile Asp Lys
 725 730 735
 50 Asp Val Gln His Asp Val Asp His Leu Ser Ser Asp Met Ser Asp Asn Asn
 740 745 750
 His Phe Asp Lys Tyr Asp Leu Lys Glu Met Asp Thr Gln Ile Ala Lys
 755 760 765
 55 Asp Thr Asp Arg Asn Val Asp Lys Asp Ala Asp Asn Ser Val Gly Met
 770 775 780
 Ser Ser Asn Val Asp Thr Asp Lys Asp Ser Asn Lys Asn Lys Asp Lys
 785 790 795 800

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Val Ile Gln Leu Asn His Ile Ala Asp Lys Asn Asn His Thr Gly Lys
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820 825 830

Val Thr Asp Lys Lys Thr Thr Glu His Leu Pro Ser Asp Ile His Lys
835 840 845

10 Thr Val Asp Lys Thr Val Lys Thr Lys Glu Lys Ala Gly Thr Pro Ser
850 855 860

15 Lys Glu Asn Lys Leu Ser Gln Ser Lys Met Leu Pro Lys Thr Gly Glu
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    35      40      45
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    65      70      75      80
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    85      90      95
30 Lys Val Ile Lys Gln Asn Asn Lys Tyr Tyr Phe Gln Thr Val Leu Asn
    100      105      110
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    165      170      175
45 His Leu Glu Phe Glu Lys Ala Ile Pro Thr Leu Ala Asp Ala Ala Lys
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Asp Ala Lys Asn Asn Thr Arg Thr Ile Ile Phe Pro Tyr Val Glu Gly
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 705 710 715 720

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Glu Gly Gln Lys Gly Glu Lys Thr Ile Thr Thr Pro Thr Leu Lys Asn
 725 730 735
 5 Pro Leu Thr Gly Val Ile Ile Ser Lys Gly Glu Pro Lys Glu Glu Ile
 740 745 750
 Thr Lys Asp Pro Ile Asn Glu Leu Thr Glu Tyr Gly Pro Glu Thr Ile
 755 760 765
 10 Thr Pro Gly His Arg Asp Glu Phe Asp Pro Lys Leu Pro Thr Gly Glu
 770 775 780
 Lys Glu Glu Val Pro Gly Lys Pro Gly Ile Lys Asn Pro Glu Thr Gly
 785 790 795 800
 15 Asp Val Val Arg Pro Pro Val Asp Ser Val Thr Lys Tyr Gly Pro Val
 805 810 815
 Lys Gly Asp Ser Ile Val Glu Lys Glu Glu Ile Pro Phe Lys Lys Glu
 820 825 830
 20 Arg Lys Phe Asn Pro Asp Leu Ala Pro Gly Thr Glu Lys Val Thr Arg
 835 840 845
 25 Glu Gly Gln Lys Gly Glu Lys Thr Ile Thr Thr Pro Thr Leu Lys Asn
 850 855 860
 Pro Leu Thr Gly Glu Ile Ile Ser Lys Gly Glu Ser Lys Glu Glu Ile
 865 870 875 880
 30 Thr Lys Asp Pro Ile Asn Glu Leu Thr Glu Tyr Gly Pro Glu Thr Ile
 885 890 895
 35 Thr Pro Gly His Arg Asp Glu Phe Asp Pro Lys Leu Pro Thr Gly Glu
 900 905 910
 Lys Glu Glu Val Pro Gly Lys Pro Gly Ile Lys Asn Pro Glu Thr Gly
 915 920 925
 40 Asp Val Val Arg Pro Pro Val Asp Ser Val Thr Lys Tyr Gly Pro Val
 930 935 940
 Lys Gly Asp Ser Ile Val Glu Lys Glu Glu Ile Pro Phe Glu Lys Glu
 945 950 955 960
 Arg Lys Phe Asn Pro Asp Leu Ala Pro Gly Thr Glu Lys Val Thr Arg
 965 970 975
 50 Glu Gly Gln Lys Gly Glu Lys Thr Ile Thr Thr Pro Thr Leu Lys Asn
 980 985 990
 Pro Leu Thr Gly Glu Ile Ile Ser Lys Gly Glu Ser Lys Glu Glu Ile
 995 1000 1005
 55 Thr Lys Asp Pro Ile Asn Glu Leu Thr Glu Tyr Gly Pro Glu Thr
 1010 1015 1020
 Ile Thr Pro Gly His Arg Asp Glu Phe Asp Pro Lys Leu Pro Thr
 1025 1030 1035
 60

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	Gly Glu	Lys Glu Glu Val	Pro	Gly Lys Pro Gly Ile	Lys Asn Pro
	1040		1045	1050	
5	Glu Thr	Gly Asp Val Val Arg	Pro Pro Val Asp Ser	Val Thr Lys	
	1055		1060	1065	
	Tyr Gly	Pro Val Lys Gly Asp	Ser Ile Val Glu Lys	Glu Glu Ile	
	1070		1075	1080	
10	Pro Phe	Lys Lys Glu Arg Lys	Phe Asn Pro Asp Leu	Ala Pro Gly	
	1085		1090	1095	
	Thr Glu	Lys Val Thr Arg Glu	Gly Gln Lys Gly Glu	Lys Thr Ile	
	1100		1105	1110	
15	Thr Thr	Pro Thr Leu Lys Asn	Pro Leu Thr Gly Glu	Ile Ile Ser	
	1115		1120	1125	
20	Lys Gly	Glu Ser Lys Glu Glu	Ile Thr Lys Asp Pro	Ile Asn Glu	
	1130		1135	1140	
	Leu Thr	Glu Tyr Gly Pro Glu	Thr Ile Thr Pro Gly	His Arg Asp	
	1145		1150	1155	
25	Glu Phe	Asp Pro Lys Leu Pro	Thr Gly Glu Lys Glu	Glu Val Pro	
	1160		1165	1170	
	Gly Lys	Pro Gly Ile Lys Asn	Pro Glu Thr Gly Asp	Val Val Arg	
	1175		1180	1185	
30	Pro Pro	Val Asp Ser Val Thr	Lys Tyr Gly Pro Val	Lys Gly Asp	
	1190		1195	1200	
35	Ser Ile	Val Glu Lys Glu Glu	Ile Pro Phe Glu Lys	Glu Arg Lys	
	1205		1210	1215	
	Phe Asn	Pro Asp Leu Ala Pro	Gly Thr Glu Lys Val	Thr Arg Glu	
	1220		1225	1230	
40	Gly Gln	Lys Gly Glu Lys Thr	Ile Thr Thr Pro Thr	Leu Lys Asn	
	1235		1240	1245	
45	Pro Leu	Thr Gly Glu Ile Ile	Ser Lys Gly Glu Ser	Lys Glu Glu	
	1250		1255	1260	
	Ile Thr	Lys Asp Pro Ile Asn	Glu Leu Thr Glu Tyr	Gly Pro Glu	
	1265		1270	1275	
50	Thr Ile	Thr Pro Gly His Arg	Asp Glu Phe Asp Pro	Lys Leu Pro	
	1280		1285	1290	
	Thr Gly	Glu Lys Glu Glu Val	Pro Gly Lys Pro Gly	Ile Lys Asn	
	1295		1300	1305	
55	Pro Glu	Thr Gly Asp Val Val	Arg Pro Pro Val Asp	Ser Val Thr	
	1310		1315	1320	
60	Lys Tyr	Gly Pro Val Lys Gly	Asp Ser Ile Val Glu	Lys Glu Glu	
	1325		1330	1335	

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	Ile Pro	Phe Glu Lys Glu Arg	Lys Phe Asn Pro Asp	Leu Ala Pro
	1340	1345	1350	
5	Gly Thr	Glu Lys Val Thr Arg	Glu Gly Gln Lys Gly	Glu Lys Thr
	1355	1360	1365	
	Ile Thr	Thr Pro Thr Leu Lys	Asn Pro Leu Thr Gly	Glu Ile Ile
	1370	1375	1380	
10	Ser Lys	Gly Glu Ser Lys Glu	Glu Ile Thr Lys Asp	Pro Val Asn
	1385	1390	1395	
	Glu Leu	Thr Glu Phe Gly Gly	Glu Lys Ile Pro Gln	Gly His Lys
	1400	1405	1410	
15	Asp Ile	Phe Asp Pro Asn Leu	Pro Thr Asp Gln Thr	Glu Lys Val
	1415	1420	1425	
20	Pro Gly	Lys Pro Gly Ile Lys	Asn Pro Asp Thr Gly	Lys Val Ile
	1430	1435	1440	
	Glu Glu	Pro Val Asp Asp Val	Ile Lys His Gly Pro	Lys Thr Gly
	1445	1450	1455	
25	Thr Pro	Glu Thr Lys Thr Val	Glu Ile Pro Phe Glu	Thr Lys Arg
	1460	1465	1470	
30	Glu Phe	Asn Pro Lys Leu Gln	Pro Gly Glu Glu Arg	Val Lys Gln
	1475	1480	1485	
	Glu Gly	Gln Pro Gly Ser Lys	Thr Ile Thr Thr Pro	Ile Thr Val
	1490	1495	1500	
35	Asn Pro	Leu Thr Gly Glu Lys	Val Gly Glu Gly Gln	Pro Thr Glu
	1505	1510	1515	
	Glu Ile	Thr Lys Gln Pro Val	Asp Lys Ile Val Glu	Phe Gly Gly
	1520	1525	1530	
40	Glu Lys	Pro Lys Asp Pro Lys	Gly Pro Glu Asn Pro	Glu Lys Pro
	1535	1540	1545	
45	Ser Arg	Pro Thr His Pro Ser	Gly Pro Val Asn Pro	Asn Asn Pro
	1550	1555	1560	
	Gly Leu	Ser Lys Asp Arg Ala	Lys Pro Asn Gly Pro	Val His Ser
	1565	1570	1575	
50	Met Asp	Lys Asn Asp Lys Val	Lys Lys Ser Lys Ile	Ala Lys Glu
	1580	1585	1590	
	Ser Val	Ala Asn Gln Glu Lys	Lys Arg Ala Glu Leu	Pro Lys Thr
	1595	1600	1605	
55	Gly Leu	Glu Ser Thr Gln Lys	Gly Leu Ile Phe Ser	Ser Ile Ile
	1610	1615	1620	
60	Gly Ile	Ala Gly Leu Met Leu	Leu Ala Arg Arg Arg	Lys Asn
	1625	1630	1635	

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5 <213> Staphylococcus epidermidis

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caagotgcat ctgaaaagga taotgaaatt acaaaagaga tattatctaa gcaagattta    180
15 ttgacaaaag ttgacaaagg aattcgtcaa attgagcaat taaaacagtt atcggtctca    240
tctaaagaac attataaagc acaactaat gaagcganaa cagcatogca aatagatgaa    300
atcataaaac gagctaata gttgtagatg aaagacaata aaagtcttca cactgaaatg    360
20 aacggtcaaa gtgatataga cagtaatta gatcaattgc ttaagattt aaatgaggtt    420
tcttcaaatg ttgatagggg tcaacaaagt ggcgaggacg atcttaatgc aatgaaaaat    480
25 gatattgcac aaacggctac acaaaaacat ggagaaaaag atgataaaaa tgatgaagca    540
atggtaaata aggcgttaga agacctagac catttgaaatc agcaaataca caaatcgaaa    600
gatgcacoga aagataacac ggaagatcca gcagtgtcta caacagataa taatcatgaa    660
30 gtagctaaaa cgccaaataa tgatggttct ggacatgttg tgttaataa attcctttca    720
aatgaagaga atcaaaagcca tagtaatoga ctcaatgata aattacaagg aagcgataaa    780
attaatcatg ctatgattga aaaattagct aaaagtaatg cctcaacgca acattacaca    840
35 tatcataaac tgaatacgtt acaatcttta gatcaacgta ttgcaaatac gcaacttctc    900
aaaaatcaaa aatcagactt aatgagcgaa gtaaaataga cgaagagagc tataaaaagt    960
40 caacgaataa ttatttttga agaaotttga cgtactgatg ataaaaagta tgctacacaa    1020
agcatttttag aaagtatatt taataaagac gaggcagtta aaattctaaa agatatacgt    1080
45 gttgatggta aaacagatca acaaatgca gatcaatta ctggtcatat tgatcaatta    1140
tctctgacaa cgagtgatga tttattaacg tcaattgattg atcaatcaca agataagtcg    1200
ctattgattt ctcaaatctt acaaacgaaa ttaggaaaag ctgaagcaga taaattggct    1260
50 aaagatttga cgaataaagg attatcaaat cgcgaatcg ttgaccaatt gaagaaacat    1320
tttgatcaa ctggcgacac gtcttcagat gatattatga aagcaatttt gaataatgcc    1380
aaagataaaa acaacgcaat tgaacgatt ttgcaaacac gtatagaag acaaaaggca    1440
55 aaattactgg cagatttaat tactaaaata gaaacagatc aaaataaaat ttttaattta    1500
gttaaatcgg cattgaatgg taaagcggat gatatttga atttacaaaa gagactcaat    1560
60 caaacgaaaa aagatataga ttatatttta tcaccaatag taaatcgicc aagtttacta    1620

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gatcgattga ataaaaatgg gaaaacgaca gatttaaata agtttagcaaa tttaatgaat 1680
5 caaggatcag atttattaga cagtattcca gatataccca caccasagcc agaaaagacg 1740
ttaacacattg gtaaaggtaa tggattgtta agtggattat taaatgctga tggtaattga 1800
tctttgccta aagcggggga aacgataaaa gaacatttgt tgccgataac tgtaattggt 1860
10 ggtgcaatgg gtgtactaat gatttgggta tcacgacgca ataagttgaa aaataaagca 1920
taa 1923

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<213> Staphylococcus epidermidis

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25 20 25 30
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35 40 45
30 Glu Ile Thr Lys Glu Ile Leu Ser Lys Gln Asp Leu Leu Asp Lys Val
50 55 60
Asp Lys Ala Ile Arg Gln Ile Glu Gln Leu Lys Gln Leu Ser Ala Ser
65 70 75 80
35 Ser Lys Glu His Tyr Lys Ala Gln Leu Asn Glu Ala Lys Thr Ala Ser
85 90 95
Gln Ile Asp Glu Ile Ile Lys Arg Ala Asn Glu Leu Asp Ser Lys Asp
40 100 105 110
Asn Lys Ser Ser His Thr Glu Met Asn Gly Gln Ser Asp Ile Asp Ser
115 120 125
45 Lys Leu Asp Gln Leu Leu Lys Asp Leu Asn Glu Val Ser Ser Asn Val
130 135 140
Asp Arg Gly Gln Gln Ser Gly Glu Asp Asp Leu Asn Ala Met Lys Asn
145 150 155 160
50 Asp Met Ser Gln Thr Ala Thr Thr Lys His Gly Glu Lys Asp Asp Lys
165 170 175
Asn Asp Glu Ala Met Val Asn Lys Ala Leu Glu Asp Leu Asp His Leu
55 180 185 190
Asn Gln Gln Ile His Lys Ser Lys Asp Ala Ser Lys Asp Thr Ser Glu
195 200 205
60 Asp Pro Ala Val Ser Thr Thr Asp Asn Asn His Glu Val Ala Lys Thr

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      210              215              220
Pro Asn Asn Asp Gly Ser Gly His Val Val Leu Asn Lys Phe Leu Ser
225
5  Asn Glu Glu Asn Gln Ser His Ser Asn Arg Leu Thr Asp Lys Leu Gln
   245
Gly Ser Asp Lys Ile Asn His Ala Met Ile Glu Lys Leu Ala Lys Ser
10  260              265              270
   Asn Ala Ser Thr Gln His Tyr Thr Tyr His Lys Leu Asn Thr Leu Gln
   275              280              285
15  Ser Leu Asp Gln Arg Ile Ala Asn Thr Gln Leu Pro Lys Asn Gln Lys
   290              295              300
   Ser Asp Leu Met Ser Glu Val Asn Lys Thr Lys Glu Arg Ile Lys Ser
   305              310              315
20  Gln Arg Asn Ile Ile Leu Glu Glu Leu Ala Arg Thr Asp Asp Lys Lys
   325              330              335
   Tyr Ala Thr Gln Ser Ile Leu Glu Ser Ile Phe Asn Lys Asp Glu Ala
   340              345              350
25  Val Lys Ile Leu Lys Asp Ile Arg Val Asp Gly Lys Thr Asp Gln Gln
   355              360              365
30  Ile Ala Asp Gln Ile Thr Arg His Ile Asp Gln Leu Ser Leu Thr Thr
   370              375              380
   Ser Asp Asp Leu Leu Thr Ser Leu Ile Asp Gln Ser Gln Asp Lys Ser
   385              390              395
35  Leu Leu Ile Ser Gln Ile Leu Gln Thr Lys Leu Gly Lys Ala Glu Ala
   405              410              415
   Asp Lys Leu Ala Lys Asp Trp Thr Asn Lys Lys Gly Leu Ser Asn Arg Gln
   420              425              430
   Ile Val Asp Gln Leu Lys Lys His Phe Ala Ser Thr Gly Asp Thr Ser
   435              440              445
45  Ser Asp Asp Ile Leu Lys Ala Ile Leu Asn Asn Ala Lys Asp Lys Lys
   450              455              460
   Gln Ala Ile Glu Thr Ile Leu Ala Thr Arg Ile Glu Arg Gln Lys Ala
   465              470              475
50  Lys Leu Leu Ala Asp Leu Ile Thr Lys Ile Glu Thr Asp Gln Asn Lys
   485              490              495
   Ile Phe Asn Leu Val Lys Ser Ala Leu Asn Gly Lys Ala Asp Asp Leu
   500              505              510
   Leu Asn Leu Gln Lys Arg Leu Asn Gln Thr Lys Lys Asp Ile Asp Tyr
   515              520              525
60  Ile Leu Ser Pro Ile Val Asn Arg Pro Ser Leu Leu Asp Arg Leu Asn

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[illegible]

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Phe Ser Arg Phe Thr Val Leu Asn Thr Met Ala Ala Pro Thr Thr Thr
 180 185 190
 5 Ser Thr Thr Thr Ser Ser Leu Thr Ser Asn Ser Val Val Val Asn
 195 200 205
 Lys Asp Asn Phe Asn Glu His Met Asn Leu Ser Gly Ser Ala Thr Tyr
 210 215 220
 10 Asp Pro Lys Thr Gly Ile Ala Thr Leu Thr Pro Asp Ala Tyr Ser Gln
 225 230 235 240
 Lys Gly Ala Ile Ser Leu Asn Thr Arg Leu Asp Ser Asn Arg Ser Phe
 245 250 255
 15 Arg Phe Ile Gly Lys Val Asn Leu Gly Asn Arg Tyr Glu Gly Tyr Ser
 260 265 270
 20 Pro Asp Gly Val Ala Gly Gly Asp Gly Ile Gly Phe Ala Phe Ser Pro
 275 280 285
 Gly Pro Leu Gly Gln Ile Gly Lys Glu Gly Ala Val Gly Ile Gly
 290 295 300
 25 Gly Leu Asn Asn Ala Phe Gly Phe Lys Leu Asp Thr Tyr His Asn Thr
 305 310 315 320
 Ser Thr Pro Arg Ser Asp Ala Lys Ala Lys Ala Asp Pro Arg Asn Val
 325 330 335
 30 Gly Gly Gly Gly Ala Phe Gly Ala Phe Val Ser Thr Asp Arg Asn Gly
 340 345 350
 35 Met Ala Thr Thr Glu Glu Ser Thr Ala Ala Lys Leu Asn Val Gln Pro
 355 360 365
 Thr Asp Asn Ser Phe Gln Asp Phe Val Ile Asp Tyr Asn Gly Asp Thr
 370 375 380
 40 Lys Val Met Thr Val Thr Tyr Ala Gly Gln Thr Phe Thr Arg Asn Leu
 385 390 395 400
 Thr Asp Trp Ile Lys Asn Ser Gly Gly Thr Thr Phe Ser Leu Ser Met
 405 410 415
 45 Thr Ala Ser Thr Gly Gly Ala Lys Asn Leu Gln Gln Val Gln Phe Gly
 420 425 430
 Thr Phe Glu Tyr Thr Glu Ser Ala Val Ala Lys Val Arg Tyr Val Asp
 435 440 445
 50 Ala Asn Thr Gly Lys Asp Ile Ile Pro Pro Lys Thr Ile Ala Gly Glu
 450 455 460
 55 Val Asp Gly Thr Val Asn Ile Asp Lys Gln Leu Asn Asn Phe Lys Asn
 465 470 475 480
 Leu Gly Tyr Ser Tyr Val Gly Thr Asp Ala Leu Lys Ala Pro Asn Tyr
 485 490 495
 60

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Thr Glu Thr Ser Gly Thr Pro Thr Leu Lys Leu Thr Asn Ser Ser Gln
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 5 Thr Val Ile Tyr Lys Phe Lys Asp Val Gln
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 35 40 45
 Ser Asp Thr Val Ser Ser Glu Lys Ser Glu Lys Val Thr Ser Thr Thr
 50 55 60
 25 Asn Ser Thr Ser Asn Gln Gln Glu Lys Leu Thr Ser Thr Ser Glu Ser
 65 70 75 80
 Thr Ser Ser Lys Asn Thr Thr Ser Ser Ser Asp Thr Lys Ser Val Ala
 85 90 95
 30 Ser Thr Ser Ser Thr Glu Gln Pro Ile Asn Thr Ser Thr Asn Gln Ser
 100 105 110
 35 Thr Ala Ser Asn Asn Thr Ser Gln Ser Thr Thr Pro Ser Ser Val Asn
 115 120 125
 Leu Asn Lys Thr Ser Thr Thr Ser Thr Ser Thr Ala Pro Val Lys Leu
 130 135 140
 40 Arg Thr Phe Ser Arg Leu Ala Met Ser Thr Phe Ala Ser Ala Ala Thr
 145 150 155 160
 Thr Thr Ala Val Thr Ala Asn Thr Ile Thr Val Asn Lys Asp Asn Leu
 165 170 175
 Lys Gln Tyr Met Thr Thr Ser Gly Asn Ala Thr Tyr Asp Gln Ser Thr
 180 185 190
 50 Gly Ile Val Thr Leu Thr Gln Asp Ala Tyr Ser Gln Lys Gly Ala Ile
 195 200 205
 Thr Leu Gly Thr Arg Ile Asp Ser Asn Lys Ser Phe His Phe Ser Gly
 210 215 220
 55 Lys Val Asn Leu Gly Asn Lys Tyr Glu Gly His Gly Asn Gly Gly Asp
 225 230 235 240
 Gly Ile Gly Phe Ala Phe Ser Pro Gly Val Leu Gly Glu Thr Gly Leu
 245 250 255

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Asn Gly Ala Ala Val Gly Ile Gly Gly Leu Ser Asn Ala Phe Gly Phe
 260 265 270
 5 Lys Leu Asp Thr Tyr His Asn Thr Ser Lys Pro Asn Ser Ala Ala Lys
 275 280 285
 Ala Asn Ala Asp Pro Ser Asn Val Ala Gly Gly Gly Ala Phe Gly Ala
 290 295 300
 10 Phe Val Thr Thr Asp Ser Tyr Gly Val Ala Thr Thr Tyr Ser Ser
 305 310 315 320
 15 Ser Thr Ala Asp Asn Ala Ala Lys Leu Asn Val Gln Pro Thr Asn Asn
 325 330 335
 Thr Phe Gln Asp Phe Asp Ile Asn Tyr Asn Gly Asp Thr Lys Val Met
 340 345 350
 20 Thr Val Lys Tyr Ala Gly Gln Thr Trp Thr Arg Asn Ile Ser Asp Trp
 355 360 365
 Ile Ala Lys Ser Gly Thr Thr Asn Phe Ser Leu Ser Met Thr Ala Ser
 370 375 380
 25 Thr Gly Gly Ala Thr Asn Leu Gln Gln Val Gln Phe Gly Thr Phe Glu
 385 390 395 400
 Tyr Thr Glu Ser Ala Val Thr Gln Val Arg Tyr Val Asp Val Thr Thr
 405 410 415
 30 Gly Lys Asp Ile Ile Pro Pro Lys Thr Tyr Ser Gly Asn Val Asp Gln
 420 425 430
 35 Val Val Thr Thr Ile Asp Asn Gln Gln Ser Ala Leu Thr Ala Lys Gly Tyr
 435 440 445
 Asn Tyr Thr Ser Val Asp Ser Ser Tyr Ala Ser Thr Tyr Asn Asp Thr
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 Pro Asn Lys Leu Asn Lys Tyr Ser Ile Arg Lys Phe Thr Val Gly Thr
 20 25 30
 60 Ala Ser Ile Leu Leu Gly Ser Thr Leu Ile Phe Gly Ser Ser Ser His

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Glu Ala Lys Ala Ala Glu Glu Lys Gln Val Asp Pro Ile Thr Gln Ala
5      50          55          60
Asn Gln Asn Asp Ser Ser Glu Arg Ser Leu Glu Asn Thr Asn Gln Pro
65          70          75          80
Thr Val Asn Asn Glu Ala Pro Gln Met Ser Ser Thr Leu Gln Ala Glu
10     85          90          95
Glu Gly Ser Asn Ala Glu Ala Pro Gln Ser Glu Pro Thr Lys Ala Glu
100          105          110
Glu Gly Gly Asn Ala Glu Ala Ala Gln Ser Glu Pro Thr Lys Ala Glu
15     115          120          125
Glu Gly Gly Asn Ala Glu Ala Pro Gln Ser Glu Pro Thr Lys Ala Glu
130          135          140
Glu Gly Gly Asn Ala Glu Ala Ala Gln Ser Glu Pro Thr Lys Thr Glu
20     145          150          155
Glu Gly Ser Asn Val Lys Ala Ala Gln Ser Glu Pro Thr Lys Ala Glu
25     165          170          175
Glu Gly Ser Asn Ala Glu Ala Pro Gln Ser Glu Pro Thr Lys Thr Glu
180          185          190
Glu Gly Ser Asn Ala Lys Ala Ala Gln Ser Glu Pro Thr Lys Ala Glu
30     195          200          205
Glu Gly Gly Asn Ala Glu Ala Ala Gln Ser Glu Pro Thr Lys Thr Glu
210          215          220
Glu Gly Ser Asn Ala Glu Ala Pro Gln Ser Glu Pro Thr Lys Ala Glu
35     225          230          235
Glu Gly Gly Asn Ala Glu Ala Pro Gln Ser Glu Pro Thr Lys Thr Glu
40     245          250          255
Glu Gly Gly Asn Ala Glu Ala Pro Asn Val Pro Thr Ile Lys Ala Asn
260          265          270
Ser Asp Asn Asp Thr Gln Thr Gln Phe Ser Glu Ala Pro Thr Arg Asn
45     275          280          285
Asp Leu Ala Arg Lys Glu Asp Ile Pro Ala Val Ser Lys Asn Glu Glu
290          295          300
Leu Gln Ser Ser Gln Pro Asn Thr Asp Ser Lys Ile Glu Pro Thr Thr
50     305          310          315
Ser Glu Pro Val Asn Leu Asn Tyr Ser Ser Pro Phe Met Ser Leu Leu
55     325          330          335
Ser Met Pro Ala Asp Ser Ser Ser Asn Asn Thr Lys Asn Thr Ile Asp
340          345          350
Ile Pro Pro Thr Thr Val Lys Gly Arg Asp Asn Tyr Asp Phe Tyr Gly
60

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	355	360	365
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	385	390	395
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	405	410	415
	Ile Arg Val Ala Asn Asn Arg Gln Ser Asn Thr Thr Gly Ala Asp Gly		
	420	425	430
15	Trp Gly Phe Met Phe Ser Lys Lys Asp Gly Asp Asp Phe Leu Lys Asn		
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	Gly Gly Ile Leu Arg Glu Lys Gly Thr Pro Ser Ala Ala Gly Phe Arg		
	450	455	460
20	Ile Asp Thr Gly Tyr Tyr Asn Asn Asp Pro Leu Asp Lys Ile Gln Lys		
	465	470	475
	Gln Ala Gly Gln Gly Tyr Arg Gly Tyr Gly Thr Phe Val Lys Asn Asp		
	485	490	495
	Ser Gln Gly Asn Thr Ser Lys Val Gly Ser Gly Thr Pro Ser Thr Asp		
	500	505	510
30	Phe Leu Asn Tyr Ala Asp Asn Thr Thr Asn Asp Leu Asp Gly Lys Phe		
	515	520	525
	His Gly Gln Lys Leu Asn Asn Val Asn Leu Lys Tyr Asn Ala Ser Asn		
	530	535	540
35	Gln Thr Phe Thr Ala Thr Tyr Ala Gly Lys Thr Trp Thr Ala Thr Leu		
	545	550	555
	Ser Glu Leu Gly Leu Ser Pro Thr Asp Ser Tyr Asn Phe Leu Val Thr		
	565	570	575
40	Ser Ser Gln Tyr Gly Asn Gly Asn Ser Gly Thr Tyr Ala Ser Gly Val		
	580	585	590
	Met Arg Ala Asp Leu Asp Gly Ala Thr Leu Thr Tyr Thr Pro Lys Ala		
	595	600	605
	Val Asp Gly Asp Pro Ile Ile Ser Thr Lys Glu Ile Pro Phe Asn Lys		
	610	615	620
50	Lys Arg Glu Phe Asp Pro Asn Leu Ala Pro Gly Thr Glu Lys Val Val		
	625	630	635
	Gln Lys Gly Glu Pro Gly Ile Glu Thr Thr Thr Thr Pro Thr Tyr Val		
	645	650	655
55	Asn Pro Asn Thr Gly Glu Lys Val Gly Glu Gly Glu Pro Thr Glu Lys		
	660	665	670
60	Ile Thr Lys Gln Pro Val Asp Glu Ile Val His Tyr Gly Gly Glu Glu		

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	675	680	685
	Ile Lys Pro Gly His Lys Asp Glu Phe Asp Pro Asn Ala Pro Lys Gly		
	690	695	700
5	Ser Gln Thr Thr Gln Pro Gly Lys Pro Gly Val Lys Asn Pro Asp Thr		
	705	710	715
	Gly Glu Val Val Thr Pro Pro Val Asp Asp Val Thr Lys Tyr Gly Pro		
10		725	730
	Val Asp Gly Asp Pro Ile Thr Ser Thr Glu Glu Ile Pro Phe Asp Lys		
	740	745	750
15	Lys Arg Glu Phe Asn Pro Asp Leu Lys Pro Gly Glu Glu Arg Val Lys		
	755	760	765
	Gln Lys Gly Glu Pro Gly Thr Lys Thr Ile Thr Thr Pro Thr Thr Lys		
	770	775	780
20	Asn Pro Leu Thr Gly Glu Lys Val Gly Glu Gly Glu Pro Thr Glu Lys		
	785	790	795
	Ile Thr Lys Gln Pro Val Asp Glu Ile Thr Glu Tyr Gly Gly Glu Glu		
25		805	810
	Ile Lys Pro Gly His Lys Asp Glu Phe Asp Pro Asn Ala Pro Lys Gly		
	820	825	830
30	Ser Gln Glu Asp Val Pro Gly Lys Pro Gly Val Lys Asn Pro Gly Thr		
	835	840	845
	Gly Glu Val Val Thr Pro Pro Val Asp Asp Val Thr Lys Tyr Gly Pro		
	850	855	860
35	Val Asp Gly Asp Pro Ile Thr Ser Thr Glu Glu Ile Pro Phe Asp Lys		
	865	870	875
	Lys Arg Glu Phe Asn Pro Asp Leu Lys Pro Gly Glu Glu Arg Val Lys		
40		885	890
	Gln Lys Gly Glu Pro Gly Thr Lys Thr Ile Thr Thr Pro Thr Thr Lys		
	900	905	910
45	Asn Pro Leu Thr Gly Glu Lys Val Gly Glu Gly Glu Pro Thr Glu Lys		
	915	920	925
	Ile Thr Lys Gln Pro Val Asp Glu Ile Val His Tyr Gly Gly Glu Gln		
	930	935	940
50	Ile Pro Gln Gly His Lys Asp Glu Phe Asp Pro Asn Ala Pro Val Asp		
	945	950	955
	Ser Lys Thr Glu Val Pro Gly Lys Pro Gly Val Lys Asn Pro Asp Thr		
55		965	970
	Gly Glu Val Val Thr Pro Pro Val Asp Asp Val Thr Lys Tyr Gly Pro		
	980	985	990
60	Val Asp Gly Asp Ser Ile Thr Ser Thr Glu Glu Ile Pro Phe Asp Lys		

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【国際公開パンフレット（コレクトバージョン）】

(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property
Organization
International Bureau



(43) International Publication Date
27 December 2002 (27.12.2002)

PCT

(10) International Publication Number
WO 2002/102829 A3

(51) International Patent Classification: **G01N 33/569**,
C12N 5/06, 5/16, C07K 16/00

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(21) International Application Number:
PCT/US2002/019220

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(22) International Filing Date: 17 June 2002 (17.06.2002)

(81) Designated States (*national*): AE, AG, AL, AM, AT, AU,
AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU,
CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH,
GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC,
LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW,
MX, MZ, NO, NZ, OM, PH, PL, PT, RO, RU, SD, SE, SG,
SI, SK, SL, TJ, TM, TN, TR, TT, TZ, UA, UG, UZ, VN,
YU, ZA, ZM, ZW.

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
60/298,098 15 June 2001 (15.06.2001) US

(84) Designated States (*regional*): ARIPO patent (GH, GM,
KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW),
Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM),
European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR,
GB, GR, IE, IT, LU, MC, NL, PT, SE, TR), OAPI patent
(BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR,
NE, SN, TD, TG).

Published:

— with international search report
— before the expiration of the time limit for amending the
claims and to be republished in the event of receipt of
amendments

(88) Date of publication of the international search report:
25 March 2004

For two-letter codes and other abbreviations, refer to the "Guid-
ance Notes on Codes and Abbreviations" appearing at the begin-
ning of each regular issue of the PCT Gazette.

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C/o The Provost Fellows and Scholars of the Colleg, e of

(54) Title: CROSS-REACTIVE MONOCLONAL AND POLYCLONAL ANTIBODIES WHICH RECOGNIZE SURFACE PRO-
TEINS FROM COAGULASE-NEGATIVE STAPHYLOCOCCI AND STAPHYLOCOCCUS AUREUS

(57) Abstract: Polyclonal and monoclonal antibodies which are cross-reactive to both coagulase-positive staphylococcus bacteria,
such as *S. hemolyticus*, are provided which can recognize surface proteins from both coagulase-positive and coagulase negative staph
bacteria. The antibodies may be generated from surface proteins that have been isolated on the basis of characteristics that may be
common between *S. aureus* and coagulase-negative staphylococci, and these recombinant surface proteins are used to generate the
antibodies of the invention. There is also provided vaccines and methods which utilize these proteins and antibodies for the treatment
or protection against a wide variety of staphylococcal infections.

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【国際調査報告】

INTERNATIONAL SEARCH REPORT		International application No. PCT/US02/19220
A. CLASSIFICATION OF SUBJECT MATTER IPC(7) : G01 N 33/569; C12 N 5/06, 5/16; C07 K 16/00 US CL : 435/7.33, 326, 332, 530/388.2, 388.4 According to International Patent Classification (IPC) or to both national classification and IPC		
B. FIELDS SEARCHED Minimum documentation searched (classification system followed by classification symbols) U.S. : 435/7.33, 326, 332, 530/388.2, 388.4 Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched Electronic data base consulted during the international search (name of data base and, where practicable, search terms used) Please See Continuation Sheet		
C. DOCUMENTS CONSIDERED TO BE RELEVANT		
Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	Database SPTREMBL, Swiss Institute for Bioinformatics, SIB (Geneva, Switzerland), The European Bioinformatics Institute, EBI (Cambridge, UK) Accession number Q9L470, 100% identical to SEQ.ID.NO: 21, SEQ.ID.NO: 19.	1-16, 19 and 21
Y	Database SPTREMBL, Swiss Institute for Bioinformatics, SIB (Geneva, Switzerland), The European Bioinformatics Institute, EBI (Cambridge, UK) Accession number Q99QY4, 99.8% identical to SEQ.ID.NO: 18.	1-16, 19 and 21
Y	Database SPTREMBL, Swiss Institute for Bioinformatics, SIB (Geneva, Switzerland), The European Bioinformatics Institute, EBI (Cambridge, UK) Accession number Q99QZ2, 97.4% identical to SEQ.ID.NO: 16.	1-16, 19 and 21
Y	Database SPTREMBL, Swiss Institute for Bioinformatics, SIB (Geneva, Switzerland), The European Bioinformatics Institute, EBI (Cambridge, UK) Accession number Q99XB9, 92 % identical to SEQ.ID.NO: 12.	1-16, 19 and 21
☒ Further documents are listed in the continuation of Box C. ☐ See patent family annex.		
* Special categories of cited documents: "A" document defining the general state of the art which is not considered to be of particular relevance "B" earlier application or patent published on or after the international filing date "L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified) "O" document referring to an oral disclosure, use, exhibition or other means "P" document published prior to the international filing date but later than the priority date claimed "T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention "X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone "Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art "Z" document member of the same patent family		
Date of the actual completion of the international search 28 September 2003 (28.09.2003)		Date of mailing of the international search report 19 FEB 2004
Name and mailing address of the ISA/US Mail Stop PCT, Attn: ISA/US Commissioner for Patents P.O. Box 1450 Alexandria, Virginia 22313-1450 Facsimile No. (703)305-3230		Authorized officer Padmavathi v Baskar Telephone No. (703)308-0196

INTERNATIONAL SEARCH REPORT

PCT/US02/19220

C. (Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT		
Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	Database SPTREMBL, Swiss Institute for Bioinformatics, SIB (Geneva, Switzerland), The European Bioinformatics Institute, EBI (Cambridge, UK) Accession number Q99UX5, 97.8 % identical to SEQ.ID.NO: 10.	1-16, 19 and 21
Y	Database SPTREMBL, Swiss Institute for Bioinformatics, SIB (Geneva, Switzerland), The European Bioinformatics Institute, EBI (Cambridge, UK) Accession number Q99UX4, 98.8 % identical to SEQ.ID.NO: 8.	1-16, 19 and 21
Y	Database SPTREMBL, Swiss Institute for Bioinformatics, SIB (Geneva, Switzerland), The European Bioinformatics Institute, EBI (Cambridge, UK) Accession number Q931P4 96.7 % identical to SEQ.ID.NO: 6 and Accession number Q99TD3, 95.6 % identical to SEQ.ID.NO: 6	1-16, 19 and 21
Y ✓	Database SPTREMBL, Swiss Institute for Bioinformatics, SIB (Geneva, Switzerland), The European Bioinformatics Institute, EBI (Cambridge, UK) Accession number Q99QY4, 98.6 % identical to SEQ.ID.NO: 4.	1-16, 19 and 21
Y	Database SPTREMBL, Swiss Institute for Bioinformatics, SIB (Geneva, Switzerland), The European Bioinformatics Institute, EBI (Cambridge, UK) Accession number Q99TB0, 91.6 % identical to SEQ.ID.NO: 2.	1-16, 19 and 21
Y	OHLSEN, K. et al Effects of subinhibitory concentrations of antibiotics on alpha-toxin (hla) gene expression of methicillin-sensitive and methicillin-resistant Staphylococcus aureus isolates. Antimicrob Agents Chemother, November 1998, Vol 42, No.11, pages 2817-2823.	1-16, 19 and 21

INTERNATIONAL SEARCH REPORT		International application No. PCT/US02/19220
Box I Observations where certain claims were found unsearchable (Continuation of Item 1 of first sheet)		
This international report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:		
1.	☐	Claim Nos.: because they relate to subject matter not required to be searched by this Authority, namely:
2.	☐	Claim Nos.: because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:
3.	☐	Claim Nos.: because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).
Box II Observations where unity of invention is lacking (Continuation of Item 2 of first sheet)		
This International Searching Authority found multiple inventions in this international application, as follows: Please See Continuation Sheet		
1.	☐	As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2.	☐	As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3.	☒	As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.: Please See Continuation Sheet
4.	☐	No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:
Remark on Protest ☐ The additional search fees were accompanied by the applicant's protest.		
☐ No protest accompanied the payment of additional search fees.		

INTERNATIONAL SEARCH REPORT

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BOX II. OBSERVATIONS WHERE UNITY OF INVENTION IS LACKING

This application contains the following inventions or groups of inventions 1-58 which are not so linked as to form a single general inventive concept under PCT Rule 13.1. In order for all inventions to be examined, the appropriate additional examination fees must be paid.

Groups 1-21 Claim(s) 1-14, 16, 19, 21 and 15, drawn to an isolated antibodies that bind to SEQ.ID.NOS: 2, 4, 6,8,10, 12, 14, 16, 17, 18, 19, 21, nucleic acid sequence encoding amino acid sequences SEQ.ID.NOS: 1, 3, 5,7,9, 11, 13, 15, 20 and the nucleic sequences coding for the A domain of the Aap protein or degenerate.

Groups 22-33 Claims 20 and 22 drawn to fragment of the DspA protein and a vaccine comprising a protein SEQ.ID.NOS: 2, 4, 6,8,10, 12, 14, 16, 17, 18, 19 and 21

Groups 34-45 Claim 17 drawn to a method for treating or preventing *S.aureus* infection using antibodies that bind to SEQ.ID.NOS: 2, 4, 6,8,10, 12, 14, 16, 17, 18, 19 and 21.

Groups 46-57 Claim 18 drawn to a method inducing an immune response using protein SEQ.ID.NOS: 2, 4, 6,8,10, 12, 14, 16, 17, 18, 19 and 21.

The inventions listed as Groups 1-58 do not relate to a single general inventive concept under PCT Rule 13.1 because, under PCT Rule 13.2, they lack the same or corresponding special technical features for the following reasons:

Group 1, claim(s) 1-14, 16, 19, 21 and 15, claim(s) 1-14, 16, 19, 21 drawn to an isolated antibodies that bind to SEQ.ID.NOS: 2, diagnostic kit comprising antibody to SEQ.ID.NOS: 2, pharmaceutical composition comprising said antibody and a method of diagnosing *S.aureus* infection using said antibody which is the first product and first product of use.

Pursuant to PCT Rule 13.2 the ISA/US considers that where multiple products, processes and methods are claimed, the main invention shall consists of the first invention of the category first mentioned in the claims and the first recited invention of each of the other categories related thereto. Accordingly the main invention (Group 1) comprises the first product and a method of use.

Further pursuant to PCT Rule 13.2 the ISA/US considers that any feature which the subsequently recited products and methods share with the main invention does not constitute a special technical feature within the meaning of PCT Rule 13.2 and that each of such products and methods accordingly defines a separate invention. Therefore, the groups of inventions below do not constitute a special technical feature within the meaning of PCT Rule 13.2 and that each of such products and methods accordingly defines a separate invention.

Groups 2-21 drawn to different isolated antibodies that bind to SEQ.ID.NOS: 4, 6,8,10, 12, 14, 16, 17, 18, 19, 21, nucleic acid sequence encoding amino acid sequences SEQ.ID.NOS: 1, 3, 5,7,9, 11, 13, 15, 20 and the nucleic sequences coding for the A domain of the Aap protein or degenerate that are different to each other and lack the same or corresponding special technical features because each antibody bind to a protein having a specific amino acid sequence. They are structurally different to each other since each sequence has been identified with a specific sequence identification number that contains specific amino acids. In the instant case the different inventions represent structurally different antibodies that bind to different polypeptides. Therefore, where structural identity is required, such as for expression, the different sequences have different effects. Thus, each sequence is unique and lacks the same or corresponding special technical features.

Groups 22-33 drawn to fragment of the DspA protein and a vaccine comprising a protein SEQ.ID.NOS: 2, 4, 6,8,10, 12, 14, 16, 17, 18, 19, and 21. These proteins are different to each other and lack the same or corresponding special technical features because each protein contains a specific amino acid sequence. They are structurally different to each other since each sequence has been identified with a specific sequence identification number that contains specific amino acids. In the instant case the different inventions represent structurally different proteins. Therefore, where structural identity is required, such as for expression, the different sequences have different effects. Thus, each sequence is unique and lacks the same or corresponding special technical features

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Groups 34-45 and 46-57 are different methods utilizing different products of antibodies or proteins that are unique and lack the same or corresponding special technical features that result in a different outcome such as preventing an infection with antibody or inducing an immune response with specific protein. These methods are different to each other in utilizing different reagents such as different polypeptides and antibodies as discussed above and thus lack the same or special technical features as explained above.

Continuation of Box II Item 3:

1-16, 19 and 21 with respect to SEQ.ID.NOS: 2, 4, 6, 8, 10, 12, 16, 18, 19 and 21

Continuation of B. FIELDS SEARCHED Item 3:

SEQ.ID.NOS: 2, 4, 6, 8, 10, 12, 14, 16, 19, 17, 18 and 21 searched on MEDLINE, STN, A -GENSEQ, N-GENSEQ, EST, DERWENT, SWISS-PROT, PIR, USPTOWEST, SWISSPTREMBL, GENEMBL, PUBLISHED APPLICATIONS AND ISSUED PATENTS

フロントページの続き

(51)Int.Cl. ⁷	F I	テーマコード (参考)
G O 1 N 33/569	G O 1 N 33/53	D
// C 1 2 P 21/08	G O 1 N 33/569	E
	C 1 2 P 21/08	

(81)指定国 AP(GH,GM,KE,LS,MW,MZ,SD,SL,SZ,TZ,UG,ZM,ZW),EA(AM,AZ,BY,KG,KZ,MD,RU,TJ,TM),EP(AT,BE,CH,CY,DE,DK,ES,FI,FR,GB,GR,IE,IT,LU,MC,NL,PT,SE,TR),OA(BF,BJ,CF,CG,CI,CM,GA,GN,GQ,GW,ML,MR,NE,SN,TD,TG),AE,AG,AL,AM,AT,AU,AZ,BA,BB,BG,BR,BY,BZ,CA,CH,CN,CO,CR,CU,CZ,DE,DK,DM,DZ,EC,EE,ES,FI,GB,GD,GE,GH,GM,HR,HU,ID,IL,IN,IS,JP,KE,KG,KP,KR,KZ,LC,LK,LR,LS,LT,LU,LV,MA,MD,MG,MK,MN,MW,MX,MZ,NO,NZ,OM,PH,P L,PT,RO,RU,SD,SE,SG,SI,SK,SL,TJ,TM,TN,TR,TT,TZ,UA,UG,UZ,VN,YU,ZA,ZM,ZW

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Fターム(参考) 4B064 AG27 CC24 DA01 DA15

4C085 AA03 AA13 AA14 BA13 CC21 CC23

4H045 AA11 AA30 CA11 DA76 EA31 FA74

专利名称(译)	交叉反应性单克隆和多克隆抗体，可识别来自凝固酶阴性葡萄球菌和金黄色葡萄球菌的表面蛋白		
公开(公告)号	JP2004534080A	公开(公告)日	2004-11-11
申请号	JP2003506301	申请日	2002-06-17
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IPC分类号	G01N33/53 A61K39/00 A61K39/395 A61K39/40 A61P31/04 C07K14/31 C07K16/12 C12P21/08 G01N33/569		
CPC分类号	A61P31/04 C07K16/1271		
FI分类号	C07K16/12.ZNA A61K39/00.H A61K39/395.D A61K39/395.R A61P31/04 G01N33/53.D G01N33/569.E C12P21/08		
F-TERM分类号	4B064/AG27 4B064/CC24 4B064/DA01 4B064/DA15 4C085/AA03 4C085/AA13 4C085/AA14 4C085 /BA13 4C085/CC21 4C085/CC23 4H045/AA11 4H045/AA30 4H045/CA11 4H045/DA76 4H045/EA31 4H045/FA74		
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优先权	60/298098 2001-06-15 US		
其他公开文献	JP4424984B2		
外部链接	Espacenet		

摘要(译)

提供了对两种凝固酶阳性葡萄球菌细菌（如溶血链球菌）都具有交叉反应性的多克隆抗体和单克隆抗体，它们可以识别凝固酶阳性葡萄球菌和凝固酶阴性葡萄球菌的表面蛋白。 可以从在金黄色葡萄球菌和凝固酶阴性葡萄球菌之间可能共有的特征的基础上分离的表面蛋白中产生抗体，这些重组表面蛋白用于产生本发明的抗体。 还提供了利用这些蛋白质和抗体来治疗或预防多种葡萄球菌感染的疫苗和方法。

名称	分布	サイズ	C末端
EkeS	ENCSJM	2189 aa	LPNTGSEMDLPLKELALITGAALLARRRS KKEKES
DsqA	ENCSJM	~1363- 2283 aa	LPDTGDSIKQNGLLGGVMTLLVGLGLMKR KKKKDENDQDDSQA
KesK	ENCSJM	~909 aa	LPKTGETTSSQSWWGLYALLGMLALFIPK FRKESK
KrkN2	ENCSJM (Cowan)	~278 aa	LPKTGLTSVDNFISTVAFATLALLGSLSLLLF KRKESK
KrkN	ENCSJM	~661 aa	LPQTGEESNKDMTLPMLIALSSIVAFVLP RKRKN
RkaS	ENCSJM	~801 aa	LPKTGTNQSSSPEAMFVLLAGIGLIATVRR RKAS
RrkN	NCSJM	1629 aa	LPKTGLESTQKGLIFSSIIIGIAGMLLARRRK N
KnkA	NCSJM	629 aa	LPKAGETIKEHWLPISVIVGAMGVLMIWLS RRNKLKNKA