



US007381793B2

(12) **United States Patent**  
**Patti et al.**

(10) **Patent No.:** **US 7,381,793 B2**  
(45) **Date of Patent:** **Jun. 3, 2008**

(54) **EXTRACELLULAR MATRIX-BINDING PROTEINS FROM *STAPHYLOCOCCUS AUREUS***

(75) Inventors: **Joseph M. Patti**, Cumming, GA (US); **Timothy J. Foster**, Dublin (IE); **Elisabet Josefsson**, Gothenburg (SE); **Deidre Ni Eidhin**, Dublin (IE); **Magnus A. O. Hook**, Houston, TX (US); **Samuel E. Perkins**, Houston, TX (US)

(73) Assignees: **Inhibitex, Inc.**, Alpharetta, GA (US); **BioResearch Ireland**, Dublin (IE); **The Texas A&M University System**, College Station, TX (US)

(\* ) Notice: Subject to any disclaimer, the term of this patent is extended or adjusted under 35 U.S.C. 154(b) by 439 days.

(21) Appl. No.: **10/744,672**

(22) Filed: **Dec. 24, 2003**

(65) **Prior Publication Data**  
US 2004/0254354 A1 Dec. 16, 2004

**Related U.S. Application Data**

(62) Division of application No. 09/200,650, filed on Nov. 25, 1998, now Pat. No. 6,680,195.  
(60) Provisional application No. 60/066,815, filed on Nov. 26, 1997, provisional application No. 60/036,139, filed on Jan. 21, 1997, provisional application No. 60/098,427, filed on Aug. 31, 1998.

(51) **Int. Cl.**  
**C07K 1/00** (2006.01)  
(52) **U.S. Cl.** ..... **530/350; 435/975**  
(58) **Field of Classification Search** ..... None  
See application file for complete search history.

(56) **References Cited**  
U.S. PATENT DOCUMENTS

6,593,114 B1 7/2003 Kunsch et al.  
6,737,248 B2\* 5/2004 Kunsch et al. .... 435/69.1  
6,753,149 B2 6/2004 Bailey et al.  
6,833,253 B2 12/2004 Choi  
2002/0010338 A1 1/2002 Hull et al.

2003/0054436 A1 3/2003 Kunsch et al.  
2003/0186364 A1 10/2003 Bailey et al.  
2004/0043037 A1 3/2004 Kunsch et al.  
2004/0265962 A1 12/2004 Bailey et al.  
2005/0106597 A1 5/2005 Choi

**FOREIGN PATENT DOCUMENTS**

EP 0 786 519 \* 7/1997  
WO WO 95/34655 12/1995

**OTHER PUBLICATIONS**

McDevitt et al "Molecular characterization of the clumping factor (fibrinogen receptor) of *Staphylococcus aureus*" 1994, pp. 237-248, Molecular Microbiology.

\* cited by examiner

*Primary Examiner*—Patricia A Duffy  
(74) *Attorney, Agent, or Firm*—B. Aaron Schulman; Stites & Harbison PLLC

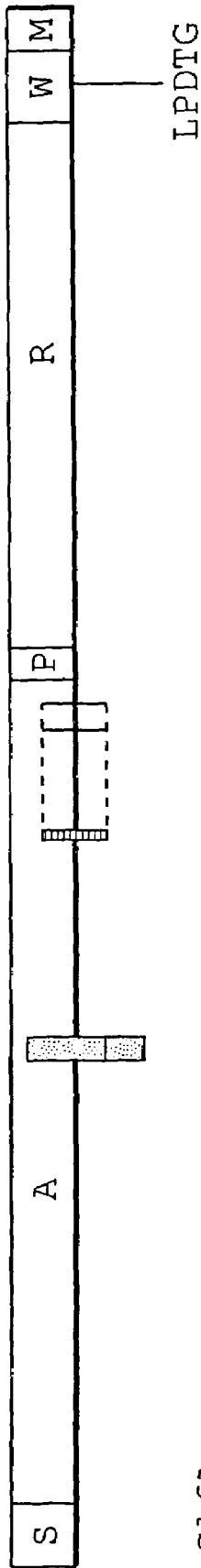
(57) **ABSTRACT**

Isolated extracellular matrix-binding proteins, designated ClfB, SdrC, SdrD and SdrE, and their corresponding amino acid and nucleic acid sequences and motifs are described. The proteins, peptides, fragments thereof or antigenic portions thereof are useful for the prevention, inhibition, treatment and diagnosis of *S. aureus* infection and as scientific research tools. Further, antibodies or antibody fragments to the proteins, peptides, fragments thereof or antigenic portions thereof are also useful for the prevention, inhibition, treatment and diagnosis of *S. aureus* infection. In particular, the proteins or antibodies thereof may be administered to wounds or used to coat biomaterials to act as blocking agents to prevent or inhibit the binding of *S. aureus* to wounds or biomaterials.

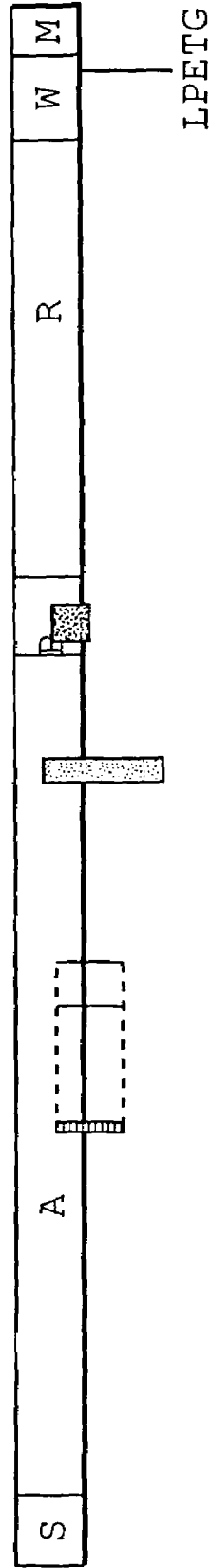
ClfB is a cell-wall associated protein having a predicted molecular weight of approximately 88 kDa and an apparent molecular weight of approximately 124 kDa, which binds both soluble and immobilized fibrinogen. ClfB binds both the alpha and beta chains of fibrinogen and acts as a clumping factor. SdrC, SdrD and SdrE are cell-wall associated proteins that exhibit cation-dependent ligand binding to the extracellular matrix. It has been discovered that in the A region of SdrC, SdrD, SdrE, ClfA and ClfB, there is a highly conserved amino acid sequence that can be used to derive a consensus motif of TYTFTDYVD.

**9 Claims, 34 Drawing Sheets**

ClfA

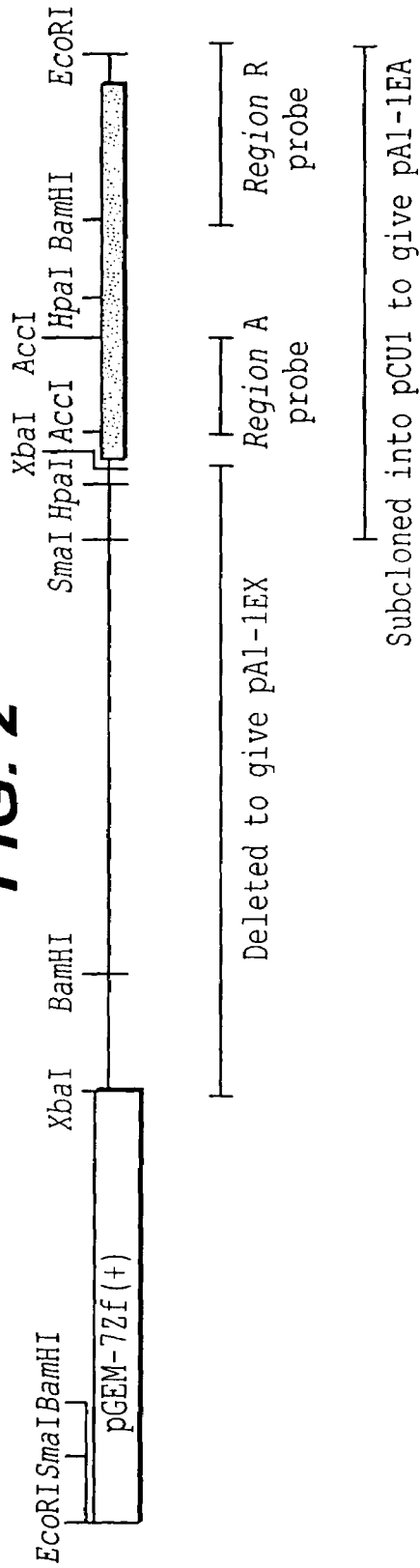


ClfA



**FIG. 1**

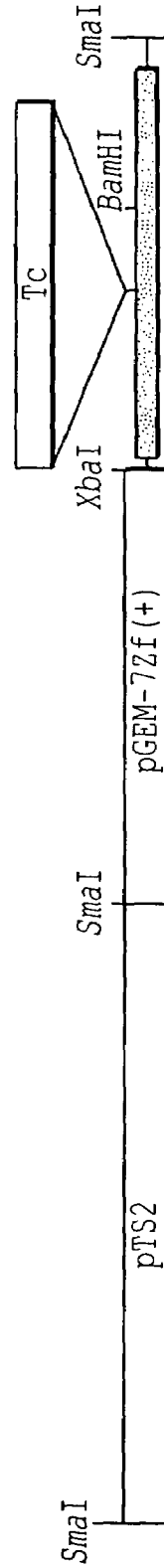
**FIG. 2**

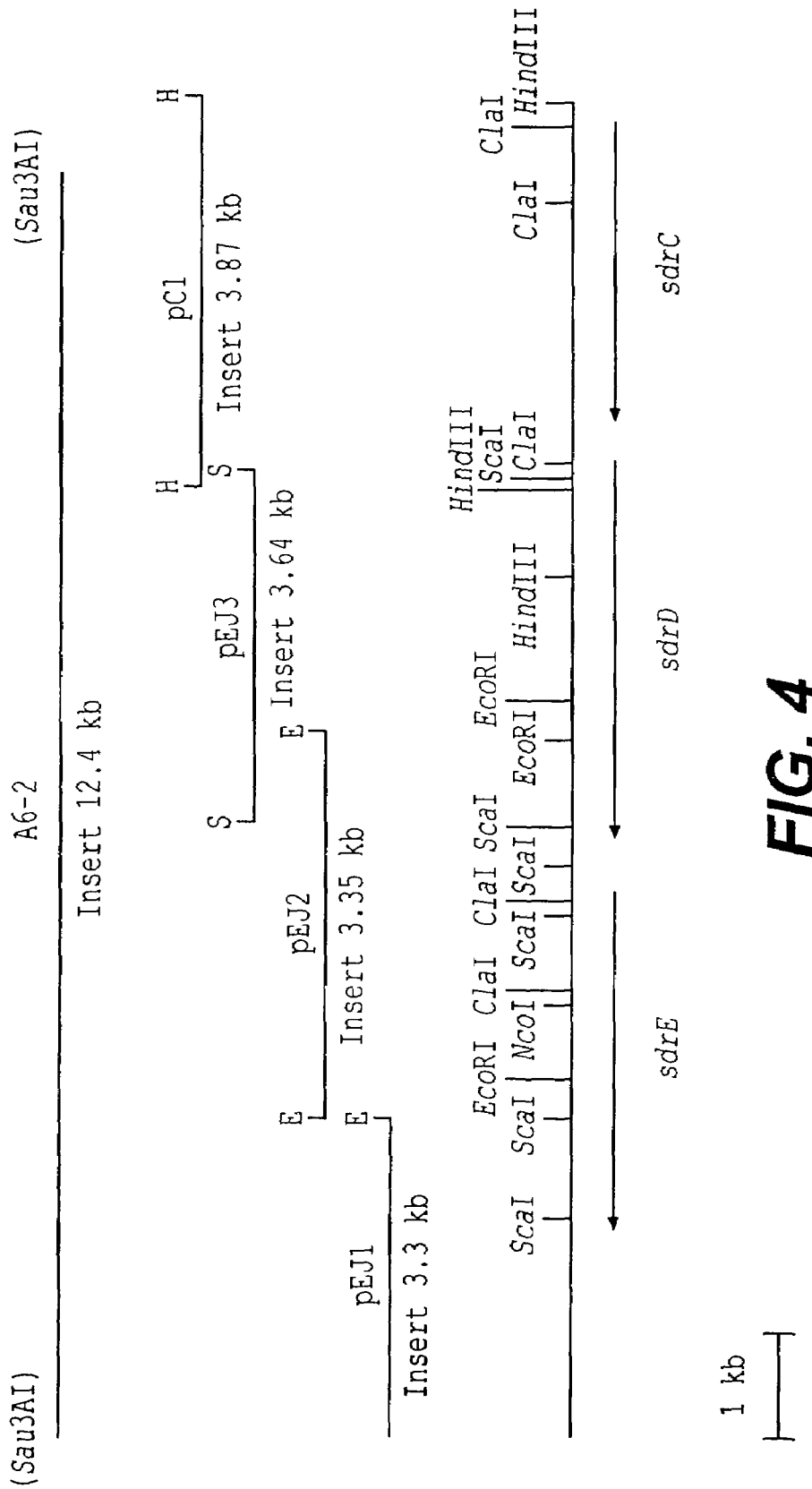


Probe used to  
screen mutants

Fragment modified in mutants

**FIG. 3**





S&gt;

TAGAAATTGAAATGGAGTAATATTTTTGAAAAAAGAATTGATTATTTGTCGAATAAGCAGAATAAGTATTCGATTAGACGTTTTCAGT

N G V I F L K K R I D Y L S N K Q N K Y S I R R F

T V 22

A&gt;

AGGTACCACATCAGTAATAGTAGGGGCAACTATACTATTTGGGATAGGCAATCATCAAGCACAACTTCAGAACAATCGAACGATCAAC

G T T S V I V G A T I L F G I G N H Q A Q A S E Q S N D

T T 52

GCAATCTTCGAAAAATAATGCAAGTGCAGATTCGAAAAACAATATGATAGAAACACCTCAATTAATACAACGGCTAATGATACATC

Q S S K N N A S A D S E K N N M I E T P Q L N T T A N D

T S 82

TGATATTAGTGCAAAACACAAACAGTGCAGATGTAGATAGCACAAACCAATGTCTACACAAACGAGCAATACCACTACAACAGAGCC

D I S A N T N S A N V D S T T K P M S T Q T S N T T T T

E P 112

AGCTTCAACAAATGAAACACCTCAACCGACGGCAATTAATAATCAAGCAACTGCTGCAAAAAATGCAAGATCAAACCTGTCTCTCAAGAAGG

A S T N E T P Q P T A I K N Q A T A A K M Q D Q T V P Q

E G 142

AAATCTCAAGTAGATAATAAAACAACCAATGATGCTAATAGCATAGCAACAAACAGTGAGCTTAAAAATCTCAACATTAGATTACC

N S Q V D N K T T N D A N S I A T N S E L K N S Q T L D

L P 172

ACAATCATCACCACAAACGATTTCCAATGCGCAAGGAAGTAAACCAAGTGTAGAACGAGAGCTGTACGTAGTTAGCTCTTGCTGA

Q S S P Q T I S N A Q G T S K P S V R T R A V R S L A V

A E 202

ACCGGTAGTAAATGCTGCTGATGCTAAAGGTACAATGTAATGATAAAGTTACGGCAAGTAATTTCAAGTTACAAAAGACTACATTTGA

P V V N A A D A K G T N V N D K V T A S N F K L E K T T

F D 232

CCCTAATCAAAGTGGTAACACATTTATGGCGGCAATTTTACAGTGACAGATAAAGTCAAATCAGGGGATTATTTTACAGCGAAGTTACC

P N Q S G N T F M A A N F T V T D K V K S G D Y F T A K

L P 262

AGATAGTTTAACTGGTAATGGACACGTGGATTATCTAATCAAATAATACGATGCCAATGCAGACATTAAGTACGAATGGCGATGT

D S L T G N G D V D Y S N S N N T M P I A D I K S T N G

D V 292

TGTAGCTAAAGCAACATATGATATCTTGACTAAGACGTATACATTTGCTTTACAGATTATGTAATAATAAAGAAAATATTAACG

**FIG. 5A**

GACA  
 V A K A T Y D I L T K T Y T F V F T D Y V N N K E N I N  
 G Q 322  
 ATTTTCATTACCTTTATTACAGACCGAGCAAAGGCACCTAAATCAGGAACATATGATGCGAATATTAATATTCCGGATGAAATGT  
 TTAA  
 F S L P L E T D R A K A P K S G T Y D A N I N I A D E M  
 F N 352  
 TAATAAAATTACTTATAACTATAGTTCGCCAATTGCAGGAATTGATAACCAATGGCGGAACATTTCTTCTCAAATTATTGCTG  
 TAGA  
 N K I T Y N Y S S P I A G I D K P N G A N I S S Q I I G  
 V D 382  
 TACAGCTTCAGGTCAAAACACATACAGCAAACAGTATTTGTTAACCTAAGCAACGAGTTTTAGCTAATACGTCGGTGTATATTA  
 AAGG  
 T A S G Q N T Y K Q T V F V N P K Q R V L G N T W V Y I  
 K G 412  
 CTACCAAGATAAAAATCGAAGAAAGTTCGGTAAAGTAAGTGCTACAGATACAAAACACTGAGAATTTTTGAAGTCAATGATACATCTA  
 AATT  
 Y Q D K I E E S S G K V S A T D T K L R I F E V N D T S  
 K L 442  
 ATCAGATAGCTACTATGCAGATCCAAATGACTCTAACCTTAAAGAAGTAACAGACCAATTTAAAATAGAATCIATTATGACCATC  
 CAAA  
 S D S Y Y A D P N D S N L K E V T D Q F K N R I Y Y E H  
 P N 472  
 TGTAGCTAGTATTAATTTGGTGAATTTACTAAAACATATGTAGTATTAGTAGAAGGCCATTACGACAATACAGGTAACAACCTAA  
 AAC  
 V A S I K E G D I T K T Y V V L V E G H Y D N T G K N L  
 K T 502  
 TCAGGTTATTCAAGAAAATGTTGATCCTGTAACAAATAGAGACTACAGTATTTCCGGTTGGAATAATGAGAATGTTGTACGTTATG  
 GTGG  
 Q V I Q E N V D P V T N R D Y S I F G W N N E N V V R Y  
 G G 532

P>

TGGAAGTGCTGATGGTCAATCAGCACTAAATCCGAAAGACCCAACCTCCAGGGCCGCCGGTTGACCCAGAACCAAGTCCAGACCCAG  
 AACC  
 G S A D G D S A V N P K D P T P G P P V D P E P S P D P  
 E P 562

R>

AGAACCAACGCCAGATCCAGAACCAGTCCAGACCCAGAACCGGAACCAAGCCAGACCCGGATCCGGATTCCGATTCCAGACAGTG  
 ACTC  
 E P T P D P E P S P D P E P E P S P D P D P D S D S D S  
 D S 592  
 AGGCTCAGACAGCCACTCAGGTTCCAGATAGGACTCAGAATCAGATAGCGATTCCGGATTCCAGACAGTGATTCCAGATTCCAGACAGC  
 ACTC

FIG. 5B

G S D S D S G S D S D S E S D S D S D S D S D S D S D S  
D S 622  
AGAATCAGATAGCGATTGAGAATCAGATAGCGACTCAGATTGAGATAGCGATTGAGATAGCGATTGAGATAGCGATTGAGATAGCG  
ATTC  
E S D S D S E S D S D S D S D S D S D S D S D S D S D S  
D S 652  
GGATTCAGACAGTCAATTCAGATTCAGACAGCGACTCAGAATCAGATAGCGACTCAGAATCAGATAGTCAATTCAGATTCAGACAGT  
ACTC  
D S D S D S D S D S D S E S D S D S E S D S E S D S E S D S D S  
D S 682  
GGACTCAGACAGTCAATTCAGACTCAGATAGCGATTGAGACTCAGATAGCGATTGAGATTCAGACAGCGACTCAGATTCAGACAGCG  
ACTC  
D S D S D S D S D S D S D S D S D S D S D S D S D S D S  
D S 712  
AGACTCAGATAGCGACTCAGACTCAGACAGCGACTCAGATTCAGATAGCGATTGAGACTCAGACAGCGACTCAGACTCAGACAGCG  
ACTC  
D S D S D S D S D S D S D S D S D S D S D S D S D S D S  
D S 742  
AGACTCAGATAGCGACTCAGATTCAGATAGCGATTGAGACTCAGACAGCGACTCAGATTCAGATAGCGATTGAGACTCAGACAGCG  
ATTC  
D S D S D S D S D S D S D S D S D S D S D S D S D S D S  
D S 772  
AGATTCAGACAGCGACTCAGACTCGGATAGCGATTGAGATTCAGATAGCGATTGAGATTCAGACAGTCAATTCAGATTCAGACAGCG  
ACTC  
D S D S D S D S D S D S D S D S D S D S D S D S D S D S  
D S 802  
AGACTCGGATAGCGACTCAGACTCAGACAGCGATTGAGACTCAGATAGCGACTCAGACTCGGATAGCGACTCGGATTCAGATAGCG  
ACTC  
D S D S D S D S D S D S D S D S D S D S D S D S D S D S  
D S 832

W>

AGACTCAGATAGTCACTCCGATTCAAGAGTTACACCACCAATAATGAACAGAAACCACCATCAAATCCTAAAGGTGAAGTAAACC  
ATTC  
D S D S D S D S R V T P P N N E Q K A P S N P K G E V N  
H S 862

M>

TAATAAGGTATCAAAACAACACAAAACACTGATGCTTTACCAGAAACAGGAGATAAGAGCGAAAACACAAATGCAACTTTATTTGGTG  
CAAT  
N K V S K Q H K T D A L P E T G D K S E N T N A I L F G  
A M 892  
GATGCCATTATTAGGATCATTACTATTGTTAGAAAACGCAAGCAAGATCATAAAGAAAAGCGTAAATACTTTTTAGGCCGAAT  
ACAT  
M A L L G S L L L F R K R K Q D H K E K A

FIG. 5C

913

TTGTATTCGGTTTTTTGTGAAAATGATTTTAAAGTGAATTGATTAACCGTAAAATCTTGATAAAGTAGAATTAGAAAGGGTCA  
TGAC

GTATGCCTTATATTCATTA~~A~~CTATCATTACCAACAATTGCTATGCATCAA~~A~~ATTGACAGTCATTTTACCGGAACAACCAGAA  
TTC

**FIG. 5D**



clfB  
clfA

DVDYNSNNTMPIADIKSTNGDVVAKATYDILTKTYTFVFTDYVNNKENINGQFSLPLFT 329  
-VTSTAKVPPIMAGDQVLANGVIDSDG-----NVIYTFDYVNTKDDVKATLTPAYI 339  
\* . . . \* .\*\* . . . . \*\*\*\*\*.\*. . . \* .

**FIG. 6**

S>  
 ATGAATAATAAAAAGACAGCAACAAATAGAAAAGGCATGATACCAAATCGATTAACAAATTTTCGATAAGAACTATTCTGTAGG  
 TACT  
 M N N K K T A T N R K G M I P N R L N K F S I R K Y S V G  
 T 30

A>  
 GCTTCAATTTTAGTAGGGACAACATTGATTTTGGGTAAAGTGGTCATGAAGCTAAAGCGGCAGAACATACGAATGGAGAATTAAA  
 TCAA  
 A S I L V G T T L I F G L S G H E A K A A E H T N G E L N  
 Q 60  
 TCAAAAAATGAAACGACAGCCCCAAGTGAATAAAAACAACATAAAAAGTTGATAGTCGTCAACTAAAAGACAAATACCCAACTGC  
 AACT  
 S K N E T T A P S E N K T T K K V D S R Q L K D N T Q F A  
 T 90  
 GCAGATCAGCCTAAAGTGACAATGAGTGAATGCAACAGTTAAAGAACTAGTAGTAACATGCAATCACCACAAAACGCTACAGC  
 TAAT  
 A D Q P K V T M S D S A T V K E T S S N M Q S P Q N A T A  
 N 120  
 CAATCTACTACAAAAAGTAGCAATGTACAACAAATGATAAATCATCAACTACATATAGTAATGAAACTGATAAAAAGTAATTTAAC  
 ACAA  
 Q S T T K T S N V T T N D K S S T T Y S N E F D K S N L T  
 Q 150  
 GCAAAGATGTTTCAACTACACCTAAACAACGACTATTAACCAAGAACITTAATCGCATGCCAGTGAATACTGTTCAGCTCC  
 ACAA  
 A K D V S T T P K T T T I K P R T L N R M A V N T V A A P  
 Q 180  
 CAAGGAACAAATGTTAATGATAAAGTACATTTTCAAATATTGACATTGCGATTGATAAAGGACATGTTAATCAGACTACTGCTAA  
 AACT  
 Q G T N V N D K V H F S N I D I A I D K G H V N Q I T G K  
 T 210  
 GAATTTGGGCAACTTCACTGATGTTTTAAATTAAGCAAATTACACAATCGATGATTCTGTTAAAGAGGGCGATACATTTAC  
 TTTT  
 E F W A T S S D V L K L K A N Y T I D D S V K E G D T F T  
 F 240  
 AAATATGGTCAATATTTCCGTCCAGGATCAGTAAGATTACCTTCACAACTCAAAATTTATATAATGCCCAAGGTAATATTATTGC  
 AAAA  
 K Y G Q Y F R P E S V R L P S Q T Q N L Y N A Q G N I I A  
 K 270  
 GGTATTTATGATAGTACAACAAACACAACAACATATACTTTTACGAACTATGTAGATCAATATACAAATGTTAGAGGTAGCTTTGA  
 ACAA  
 G I Y D S T T N T T T Y T F T N Y V D Q Y T N V R G S F E  
 Q 300  
 GTTGCATTTCCGAAACGTAAAAATGCACAACACTGATAAAACAGCTTATAAAATGGAAGTAACCTTTAGCTAATGATACATATACCGA

**FIG. 7A**

AGAA

V A F A K R K N A T T D K T A Y K M E V T L G N D T Y S E  
E 330

ATCATTGTCGATTATGGTAATAAAAAAGCACACCCTTATTTCAAGTACAACTATATTAACAATGAAGATTTATCGCGTAATAT  
GACT

I I V D Y G N K K A Q P L I S S T N Y I N N E D L S R N M  
T 360

GCATATGTAAATCAACCTAAAAATACATATACTAAACAAACGTTTGTACTAATTTAACTGGATATAAATTTAATCCAAATGCAAA  
AAAC

A Y V N Q P K N T Y T K Q T F V T N L I G Y K F N P N A K  
N 390

TTCAAAATTTACCAAGTGACAGATCAAATCAATTTGTGGATAGTTTCACCCCTGATACTTCAAACCTTAAAGATGTTACTGATCA  
ATTC

F K I Y E V T D Q N Q F V D S F T P D T S K L K D V T D Q  
F 420

GATGTTATTTATAGTAATGATAATAAACAGCTACAGTCGATTTAATCAAAGGCCAAACAAGCAGCAATAACAATACATCATTCA  
ACAA

D V I Y S N D N K T A T V D L M K G Q T S S N K Q Y I I Q  
Q 450

GTTGCTTATCCAGATAATAGTTCAACAGATAATGGAAAAATTGATTATACTTTAGACACTGACAAACTAAATATAGTTGGTCAAA  
TAGT

V A Y P D N S S T D N G K I D Y T L D T D K T K Y S W S N  
S 480

B1>

TATTCAAATGTGAATGGCTCATCAACTGCTAATGGCGACCAAAAGAAATATAATCTAGGTGACTATGTATGGGAAGATACAAATAA  
AGAT

Y S N V N G S S T A N G D Q K K Y N L G D Y V W E D T N K  
D 510

GGTAAACAAGATGCCAATGAAAAGGGATTAAGGTGTTTATGTCATTCTTAAAGATAGTAACGGTAAAGAATTAGATCGTACGAC  
AACA

G K Q D A N E K G I K G V Y V I L K D S N G K E L D R T T  
T 540

GATGAAAATGGTAAATATCAGTTCAGTCTGCTTTAAGCAATGGAAGTTATAGTGTAGAGTTTTCAACACCAGCCGTTATACACCGAC  
AACT

D E N G K Y Q F T G L S N G T Y S V E F S T P A G Y T P T  
T 570

GCAAATGTAGGTACAGATGATGCTGTAGATTCTGATGGACTAACTACAACAGGTGTCATTAAGACCGCTGACAACATGACATTAGA  
TAGT

A N V G T D D A V D S D G L T T T G V I K D A D N M T L D  
S 600

B2>

GGATTCTACAAAACACCAAAATATAGTTTAGGTGATTATGTTTGGTACGACAGTAATAAAGATGGTAAACGAGATTCGACTGAAAA  
AGGA

**FIG. 7B**

G F Y K T P K Y S L G D Y V W Y D S N K D G K R D S T E K  
G 630

ATTAAAGGTGTTAAAGTTACTTTGCAAACGAAAAAGGCGAAGTAATTGGTACAACGAAACAGATGAAAATGGTAAATACCGCTT  
TGAT

I K G V K V T L Q N E K G E V I G T T E T D E N G K Y R F  
D 660

AATTTAGATAGTGGTAAATACAAAGTTATCTTTGAAAAACCTGCTGGCTTAACTCAAACAGGTACAATACAACGAAAGATGATAA  
AGAT

N L D S G K Y K V I F E K P A G L T Q T G T N T T E D D K  
D 690

R>

GCCGATGGTGGCGAAGTTGATGTAACAATTACGGATCATGATGATTCACACTTGATAATGGCTACTACGAAGAAGAAACATCAGA  
TAGC

A D G G E V D V T I T D H D D F T L D N G Y Y E E E T S D  
S 720

GACTCAGATTCTGACAGCGATTGACTCAGATAGCGACTCAGATTCAGATAGCGACTCAGATTCAGACAGCGATTGACTCAGACAGCGA  
CTCA

D S D  
S 750

GACTCAGATAGCGATTGACTCAGATTCAGACAGCGACTCAGACTCAGACAGCAATTCAGACTCGGATAGCGACTCAGACTCAGATAGCGA  
CTCA

D S D S D S D S D S D S D S D S D S N S D S D S D S D S D S D S D S D S D S D S D S D S D S D S D  
S 780

GATTCGGATAGCGACTCAGACTCAGATAGCGATTGACTCAGATTCAGATAGCGATTGCGACTCAGACAGTGATTGACTCAGACTCAGA  
TAGC

D S D  
S 810

GACTCAGATTCTGACAGCGATTGACTCAGACATCGACTCAGACTCAGACAGTGATTGACTCAGATTCAGACAGCGACTCAGATTCAGA  
TAGC

D S D  
S 840

GACTCAGACTCAGATAGCGACTCAGACTCAGATAGCGACTCAGACTCGGATAGCGATTGACTCAGACAGCGACTCAGATTCAGA  
TAGC

D S D  
S 870

W>

GATTCGGACTCAGACAACGACTCAGATTCAGATAGCGATTGACTCAGATTCAGATGCAGGTAACATACTCCGGCTAAACCAATGAGTAC  
GGTT

D S D S D N D S D S D S D S D S D S D A G K H T P A K P M S T  
V 900

M>

AAAGATCAGCATAAACAGCTAAAGCATTACCAGAAACAGGTAGTGAAAATAATAATTCAAATAATGCCACATTATTCGGTGGATT  
ATTC

**FIG. 7C**

K D Q H K T A K A L P E T G S E N N N S N N G T L F G G L  
F 930  
GCGGCATTAGGATCATTATTGTCATTCCGGTCGTCGTAATAAAACAAAATAAA  
A A L G S L L S F G R R K K Q N K  
947

**FIG. 7D**

S&gt;

ATGCTAAACAGAGAAAATAAACCGCAATAACAAGGAAAGGCATGGTATCCAATCGATTAAATAAATTTTCGATTAGAAAGTACAC  
AGTG

M L N R E N K T A I T R K G M V S N R L N K F S I R K Y T  
V 30

A&gt;

GGAACAGCATCAATTTTAGTAGGTACAACATTAATTTTTGGTCTGGGAACCAAGAAGCAAAGGCTGCAGAAAGTACTAATAAAGA  
ATTG

G T A S I L V G T T L I F G L G N Q E A K A A E S T N K E  
L 60

AACGAAGCGACAACCTTCAGCAAGTGATAATCAATCGAGTGATAAAGTTGATATGCAGCAACTAAATCAAGAAGACAATACTAAAAA  
TGAT

N E A T T S A S D N Q S S D K V D M Q Q L N Q E D N T K N  
D 90

AATCAAAAAGAAATGGTATCATCTCAAGGTAATGAAACGACTTCAAATGGGAATAAATTAATAGAAAAAGAAAGTGTACAATCTAG  
CACT

N Q K E M V S S Q G N E T T S N G N K L I E K E S V Q S T  
T 120

GGAATAAAGTTGAAGTTCAACTGCCAATCAGATGAGCAAGCTTCAACAAAATCTACGAATGAAGATTTAAACACTAAACAAAC  
TATA

G N K V E V S T A K S D E Q A S P K S T N E D L N T K Q T  
I 150

AGTAATCAAGAAGCGTTACAACCTGATTGCAAGAGAATAAATCAGTGGTAAATGTTCAACCAACTAATGAGGAAAACAAAAGGT  
AGAT

S N Q E A L Q P D L Q E N K S V V N V Q P T N E E N K K V  
D 180

GCCAAAACCTGAATCAACTACATTAATGTTAAAAGTGATGCTATCAACAGTAATGATGAAACTCTTGTGATAACAATAGTAATTC  
AAAT

A K T E S T T L N V K S D A I K S N D E T L V D N N S N S  
N 210

AATGAAAATAATGCAGATATCATTTTGCCAAAAGTACAGCACCTAAACGTTTGAATACAAGAATGCGTATAGCAGCAGTACAGCC  
ATCA

N E N N A D I I L P K S T A P K R L N T R M R I A A V Q P  
S 240

TCAACAGAGGCTAAAAATGTTAATGATTTAATCACATCAAATACAACATTAAGTGTGCTGATGCAGATAAAAAACAATAAAATCGT  
ACCA

S T E A K N V N D L I T S N T T L T V V D A D K N N K I V  
P 270

GCCCAAGATTATTTATCATTAAAATCACAATTACAGTTGATGACAAAAGTTAAATCAGGTGATTATTTACAAATTAATACTCAGA  
TACA

A Q D Y L S L K S Q I T V D D K V K S G D Y F T I K Y S D  
T 300

GTACAAGTATATGGATTGAATCCGGAAGATATTAATAATTTGGTGATATTAAGATCCAATAATGGTGAACAATTGCCACTGC

**FIG. 8A**

AAAA

V Q V Y G L N P E D I K N I G D I K D P N N G E T I A T A  
K 330CATCATACTGCAAATAATTTAATTACATATACATTTACAGATTATGTTGATCGATTAATCTGTACAAATGGGAATTAATTATTC  
AATTH D T A N N L I T Y T F T D Y V D R F N S V Q M G I N Y S  
I 360TATATGCATGCTGATACAATTCCTCTTAGTAAAAACGATGTTGAGTTTAATGTTACGATAGGTAATACTACAACAAAACAACACTGC  
TAACY M D A D T I P V S K N D V E F N V T I G N T T T K T T A  
N 390ATCAATATCCAGATTATGTTGTAATGAGAAAAATTC AATTGGATCAGCCTTCACTGAAACAGTTTCACATGTTGAAATAAAGA  
AAATI Q Y P D Y V V N E K N S I G S A F T E T V S H V G N K E  
N 420CCAGGGTACTATAAACAAACGATTTATGTAATCCATCGGAAAATTC TTTAACAATGCCAACTAAAAGTTCAAGCTTACCACCTC  
AAGTP G Y Y K Q T I Y V N P S E N S L T N A K L K V Q A Y H S  
S 450TATCCTAATAATATCGGGCAAATAAATAAAGATGTAACAGATATAAAAATATATCAAGTTCCTAAAGGTTATACATTAATAAAGG  
ATACY P N N I G Q I N K D V T D I K I Y Q V P K G Y T L N K G  
Y 480GATGTGAATACTAAAGAGCTTACAGATGTAACAAATCAATACTTGCAGAAAATTACATATGGCGACAACAATAGCCCTGTTATTGA  
TTTTD V N T K E L T D V T N Q Y L Q K I T Y G D N N S A V I D  
F 510GGAAATGCAGATTCTGCTTATGTTGTAATGGTTAATAACAAAATTC AATATAACAAATAGCGAAAGCCCAACTTCTTCAAATGCC  
TACTG N A D S A Y V V M V N T K F Q Y T N S E S P T L V Q M A  
T 540

B1&gt;

TTATCTTCAACAGGTAATAAATCCGTTTCTACTGGCAATGCTTTAGGATTTACTAATAACCAAAGTGGCGGAGCTGGTCAAGAAGT  
ATATL S S T G N K S V S T G N A L G F T N N Q S G G A G Q E V  
Y 570AAAATTGCTAACTACGTATGGGAAGATACTAATAAAAACGGTGTTCAGAATTAGGAGAAAAAGCGTTGGCAATGTAAGTGAAC  
TGTAK I G N Y V W E D T N K N G V Q E L G E K G V G N V T V T  
V 600TTTGATAATAATACAAATACAAAAGTAGGAGAAGCAGTTACTAAAGAAGATGGGTCATACTTGATTCCAAACTTACCTAATGGAGA  
TTAC

F D N N T N T K V G E A V T K E D G S Y L I P N L P N G D

**FIG. 8B**

Y 630  
 CGTGTAGAATTTTCAAACCTTACCAAAGGTTATGAAGTAACCCCTTCAAACAAGGTAATAACGAAGAATTAGATTCAAACGGCTT  
 ATCT  
 R V E F S N L P K G Y E V T P S K Q G N N E E L D S N G L  
 S 660

B2>

TCAGTTATTACAGTTAATGGCAAAGATAACTTATCTGCAGACTTAGGTATTTACAAACCTAAATACAACCTTAGGTCACTATCTCTG  
 GGAA  
 S V I T V N G K D N L S A D L G I Y K P K Y N L G D Y V W  
 E 690  
 GATACAAATAAAAATCGTATCCAAGACCAAGATGAAAAAGGTATATCTGGCGTAACGGTAACATTAAAAGATGAAAACGGTAACGT  
 GTTA  
D T N K N G I Q D Q D E K G I S G V T V T L K D E N G N V  
 L 720  
 AAAACAGTTACAACAGACGCTGATGGCAAATATAAATTTACTGATTTAGATAATGGTAATTATAAACTTGAATTTACTACACCAGA  
 AGGC  
 K T V T T D A D G K Y K F T D L D N G N Y K V E F T T P E  
 G 750  
 TATACACCGACTACAGTAACATCTGGTAGCGACATTGAAAAAGACTCTAATGGTTAACAACAACAGGTGTTATTAATGGTGCTGA  
 TAAC  
 Y T P T T V T S G S D I E K D S N G L T T T G V I N G A D  
 N 780

B3>

ATGACATTAGATAGTGGATTCTACAAAACACCAAAATATAATTTAGGTAATTATGTATGGGAAGATACAAATAAAGATGGTAAGCA  
 GGAT  
 M T L D S G F Y K T P K Y N L G N Y V W E D T N K D G K Q  
D 810  
 TCAACTGAAAAGGTATTTCAAGCGTAACAGTTACATTGAAAATGAAAACGGTGAAGTTTACAAACAACCTAAAACAGATAAAGA  
 TGGT  
S T E K G I S G V T V T L K N E N G E V L Q T T K T D K D  
 G 840  
 AAATATCAATTTACTGGATTAGAAAATGGAACCTATAAAGTTGAATTCGAAACACCATCAGGTTACACACCAACACAAGTAGGTT  
 AGGA  
 K Y Q F T G L E N G T Y K V E F E T P S G Y T P T Q V G S  
 G 870  
 ACTGATGAAGGTATAGATTCAAATGGTACATCAACAACAGGTGTCATTAAGATAAAGATAACGATACTATTGACTCTGGTTTCTA  
 CAAA  
 T D E G I D S N G T S T T G V I K D K D N D T I D S G F Y  
 K 900

B4>

CCGACTTACAACCTTAGGTGACTATGTATGGGAAGATACAAATAAAAACGGTGTTCAGATAAAGATGAAAAGGCATTTCAGGTGT  
 AACA  
 P T Y N L G D Y V W E D T N K N G V Q D K D E K G I S G V

**FIG. 8C**



T 930  
 GTTACGTTAAAAGATGAAAACGACAAAGTTTTAAAAACAGTTACAACAGATGAAAATGGTAAATATCAATTCACTCATTAAACAA  
 TGGA  
 V T L K D E N D K V L K T V T T D E N G K Y Q F T D L N N  
 G 960  
 ACTTATAAAGTTGAATTCGAGACACCATCAGTTATACACCAACTTCAGTAACTTCTCGAAATGATACTGAAAAACATTCTAATGG  
 TTTA  
 T Y K V E F E T P S G Y T P T S V T S G N D T E K D S N G  
 L 990

B5>

ACAACAACAGGTGTCATTAAAGATGCAGATAACATGACATTAGACAGTGGTTTCTATAAAAACACCAAAATATAGTTAGGTGATTA  
 TGTT  
 T T T G V I K D A D N M T L D S G F Y K T P K Y S L G D Y  
 V 1020  
 TGGTACGACACTAATAAAGACCGCAAACAAGATTCAACTGAAAAGGTATCAAAGATGTTAAAGTTACTTTATTAATGAAAAGC  
 CGAA  
 W Y D S N K D G K O D S T E K G I K D V K V T L L N E K G  
 E 1050  
 GTAATTGGAACAACATAAACAGATGAAAATGGTAAATACTGCTTTGATAATTTAGATAGCGGTAATAACAAAGTTATTTTTGAAAA  
 GCCT  
 V I G T I K T D E N G K Y C F D N L D S G K Y K V I F E K  
 P 1080  
 GCTGCTTAACACAAAACAGGTACAAATACAACACTGAAGATGATAAAGATGCAGATGGTGGCGAAGTTCACGTAACAATTACGGATCA  
 TGAT  
 A G L T Q T G T N T T E D D K D A D G G E V D V T I T D H  
 D 1110  
 R>  
 GATTCACACTTGATAATGGCTACTACGAAGAAGAAACATCAGATAGCCACTCAGATTCGGACAGCGACTCAGATTCAGACAGAGA  
 CTCA  
 D F T L D N G Y Y E E E T S D S D S D S D S D S D S D S D R D  
 S 1140  
 GACTCAGATAGTTCAGACTCGGATAGCGATTTCAGATTCAGACAGCGATTTCAGATTCAGATAGCGATTTCAGATTCAGACAGAGA  
 CTCA  
 D S D S D S D S D S D S D S D S D S D S D S D S D S D S D R D  
 S 1170  
 GATAGTGATTCAGACTCAGATAGCGACTCAGATTCAGACAGCGACTCAGATTCAGACAGCGACTCAGACTCAGATAGTGATTCAGA  
 CTCA  
 D S D S D S D S D S D S D S D S D S D S D S D S D S D S D S D  
 S 1200  
 GATAGCGACTCAGATTCGGATAGCGACTCAGATTCAGACAGCGACTCAGACTCGGATAGTGATTCAGACTCAGATAGCGACTCAGA  
 CTCA  
 D S D S D S D S D S D S D S D S D S D S D S D S D S D S D S D  
 S 1230

FIG. 8D

W>  
GATAGCGATTGAGATTGAGATAGCGACTCAGACTCAGACAGCGATTGAGACTCAGACAGCGACTCAGACTCAGATGCAGGTAAGCA  
CACA  
D S D S D S D S D S D S D S D S D S D S D S D S D S D S D A G K H  
T 1260  
CCTGTAAACCAATGAGTACTACTAAAGACCATCACAATAAAGCAAAGCATTACCAGAAACAGGTAATGAAAATAGCGGCTCAA  
TAAC  
P V K P M S T T K D H H N K A K A L P E T G N E N S G S N  
N 1290  
M>  
GCAACGTTATTTGGCGGATTATTCGCAGCATTAGGATCATTATTGTTATTCGGTCGTAATAAAACAAAATAAA  
A T L F G G L F A A L G S L L L F G R R K K Q N K  
1315

**FIG. 8E**

S&gt;

ATGATTAACAGGGATAATAAAAAGGCAATAACAAAAAGGGTATGATTTCAAATCGCTTAAACAAATTTTCGATTAGAACTATAC  
TGTA

M I N R D N K K A I T K K G M I S N R L N K F S I R K Y T  
V 30

A&gt;

GGAAGTCATCGATTTTAGTAGGTACGACATTGATTTTTGGTCTAGGGAACCAAGAAGCTAAAGCTGCTGAAAACACTAGTACAGA  
AAAT

G T A S I L V G T T L I F G L G N Q E A K A A E N T S T E  
N 60

GCAAAACAAGATGATGCAACGACTAGTGATAATAAAGAAGTAGTGTGCGAAACTGAAAATAATTCGACAACAGAAAATAATTCAAC  
AAAT

A K Q D D A T T S D N K E V V S E T E N N S T T E N N S T  
N 90

CCAATTAAGAAAGAAACAAATACTGATTCACAACCAGAAGCTAAAAAGAATCAACTTCATCAAGTACTCAAAAACAGCAAAATAA  
CGTT

P I K K E T N T D S Q P E A K K E S T S S S T Q K Q Q N N  
V 120

ACAGCTACAAGTAACTAAGCCTCAAAACATGAAAAAGAAAATGTTAAACCTTCAACTGATAAACTGCGACAGAAGATACATC  
TGTT

T A T T E T K P Q N I E K E N V K P S T D K T A T E D T S  
V 150

ATTTTAGAAGAGAAGAAAGCACCAATAATACAAATAACGATGTAACACTACAAAACCTCTACAAGTGAACCATCTACAAGTGAAT  
TCAA

I L E E K K A P N N T N N D V T T K P S T S E P S T S E I  
Q 180

ACAAAACCAACTACACCTCAAGAATCTACAAATATTGAAAATTCACAACCGCAACCAACGCCTTCAAAGTAGACAATCAAGTTAC  
AGAT

T K P T T P Q E S T N I E N S Q P Q P T P S K V D N Q V T  
D 210

GCAACTAATCCAAAAGAACCAGTAAATGTGTCAAAGAAGAACTTAAAAATAATCCTCAGAAATTAAGAATGGTTAGAAATGA  
TAGC

A T N P K E P V N V S K E E L K N N P E K L K E L V R N D  
S 240

AATACAGATCATTCAACTAAACCAGTTGCTACAGCTCCAACAAGTGTGCACCAAAACGTGTAACGCAAAAATGCGCTTTGCAGT  
TGCA

N T D H S T K P V A T A P T S V A P K R V N A K M R F A V  
A 270

CAACCAGCAGCAGTTGCTTCAAACAATGTAATGATTTAATTAAGTGACGAAGCAACAATCAAAGTTGGCGATGGTAAAGATAA  
TGTG

Q P A A V A S N N V N D L I K V T K Q T I K V G D G K D N  
V 300

GCAGCAGCGCATGACGGTAAAGATATTGAATATGATACAGAGTTTACAATTGACAATAAAGTCAAAAAAGGCGATACAATGACGAT

**FIG. 9A**

TAAT  
A A A H D G K D I E Y D T E F T I D N K V K K G D T M T I  
N 330  
TATGATAAGAATGTAATTCCTTCGGATTAAACAGATAAAAATGATCCTATCGATATTACTGATCCATCAGGAGAGGTTCATTECTAA  
AGGA  
Y D K N V I P S D L T D K N D P I D I T D P S G E V I A K  
G 360  
ACATTTGATAAAGCAACTAAGCAAATCACATATACATTTACAGACTATGTAGATAAAATATGAAGATATAAAATCACCCTTAACTCT  
ATAT  
T F D K A T K Q I T Y T F T D Y V D K Y E D I K S R L T L  
Y 390  
TCGTATATTGATAAAAAACAGTTCCAAATGAGACAAGTTTGAATTTAACATTTGCTACAGCAGGTAAAGAAACAAGCCAAAATGT  
CACT  
S Y I D K K T V P N E T S L N L T F A T A G K E T S Q N V  
T 420  
GTTGATTATCAAGATCCAATGGTCCATGGTGATTCAAACATTCATCTATCTTTACAAAATTAGATGAAGATAAGCAAATATTGA  
ACAA  
V D Y Q D P M V H G D S N I Q S I F T K L D E D K Q T I E  
Q 450  
CAAATTTATGTTAACCCATTGAAAAAATCAGCAACCAACACTAAAGTTGATATAGCTGGTAGTCAAGTAGATGATTATGGAAATAT  
TAAA  
Q I Y V N P L K K S A T N T K V D I A G S Q V D D Y G N I  
K 480  
CTAGGAAATGGTAGCACCATTATTGACCAAAATACAGAAATAAAGGTTATAAAGTTAACTCTGATCAACAATTGCCTCAAAGTAA  
TAGA  
L G N G S T I I D Q N T E I K V Y K V N S D Q Q L P Q S N  
R 510  
ATCTATGATTTTGTAGTCAATACGAAGATGTAACAAGTCAATTTGATAATAAAAAATCATTGTAATAATGTAGCAACATTGGATTT  
TGGT  
I Y D F S Q Y E D V T S Q F D N K K S F S N N V A T L D F  
G 540  
GATATTAATTCAGCCTATATTATCAAAGTTGTTAGTAAATATACACCTACATCAGATGGCGAAGTACTAGATATTGCCCAAGSTACTAG  
TATG  
D I N S A Y I I K V V S K Y T P T S D G E L D I A Q G T S  
M 570  
AGAACAACCTGATAAATATGGTTATTATAATTATGCAGGATATTCAAACCTTCATCGTAACTTCTAATGACACTGGCGGTGGCGACGG  
TACT  
R T T D K Y G Y Y N Y A G Y S N F I V T S N D T G G G D G  
T 600

B1>  
GTAAACCTGAAGAAAAGTTATACAAAATTGGTGAATGATGTTGGGAAAGACGTTGATAAAGACGGTGTTCAGGTACAGATTCAA  
AGAA  
V K P E E K L Y K I G D Y V W E D V D K D G V Q G T D S K

**FIG. 9B**

E 630  
AAACCAATGGCAAACGTTTTAGTTACATTAACCTACCCGGACGGTACTACAAAATCAGTAAGAACAGATGCTAATGGTCATTATGA  
ATTC  
K P M A N V L V T L T Y P D G T T K S V R T D A N G H Y E  
F 660  
GGTGGTTTGAAGACGGAGAAACTTATACAGTTAAATTCGAAACGCCAACTGGATATCTCCAACAAAAGTAAATCGAACAACCTGA  
TGCT  
G G L K D G E T Y T V K F E T P T G Y L P T K V N G T T D  
G 690  
B2>  
GAAAAAGACTCAAATGGTAGTTCGGTTACTGTTAAAATTAATGGTAAAGATGATATGCTTTAGATACTGGTTTTTACAAGAACC  
TAAA  
E K D S N G S S V T V K I N G K D D M S L D T G F Y K E P  
K 720  
TACAACCTTAGGTGACTATGTATGGGAAGATACTAATAAAGATGGTATCCAAGATGCAAATGAGCCAGGAATCAAAGATGTTAAGGT  
TACA  
Y N L G D Y V W E D T N K D G I Q D A N E P G I K D V K V  
T 750  
TTAAAAGATACTACTGGAAAAGTTATTGGTACAACACTACTGATGCCTCGGGTAAATATAAATTTACAGATTTAGATAATGGTAA  
CTAT  
L K D S T G K V I G T T T T D A S G K Y K F T D L D N G N  
Y 780  
ACAGTAGAATTTGAAACACCAGCAGGTTACACGCCAACGGTTAAAATACTACAGCTGATGATAAAGATTCTAATGGTTTAAACAAC  
AACA  
T V E F E T P A G Y T P T V K N T T A D D K D S N G L T T  
T 810  
B3>  
GGTGTCAATTAAGATGCAGATAATATGACATTAGACAGGGGTTTCTATAAAACACCCAAAATACAGTTTAGGTGATTATGTTTGGTA  
CGAC  
G V I K D A D N M T L D R G F Y K T P K Y S L G D Y V W Y  
D 840  
AGTAATAAAGACGGCAAACAAGATTCAACTGAAAAAGGTATCAAAGATGTGACAGTTACATTGCAAAACGAAAAAGCGGAAGTAAT  
TGGA  
S N K D G K Q D S T E K G I K D V T V T L Q N E K G E V I  
G 870  
ACAACATAAACAGATGAAAATGGTAAATATCGTTTCGATAATTTAGATAGCGGTAAATACAAAGTTATTTTTGAAAAGCCTGCTGG  
CTTA  
T T K T D E N G K Y R F D N L D S G K Y K V I F E K P A G  
L 900  
ACACAAACAGTTACAATACTGAAGATGATAAAGATGCAGATGGTGGCGAAGTTGACGTAACAATTACGGATCATGATGATT  
CACA  
T Q T V T N T T E D D K D A D G G E V D V T I T D H D D F  
T 930

FIG. 9C

R>

CTTGATAACGGATACTTCGAAGAAGATACATCAGACAGCGATTTCAGACTCAGATAGTGACTCAGACAGCGACTCAGACTCAGACAG  
CGAC

L D N G Y F E E D T S D S D S D S D S D S D S D S D S D S D S D S  
D 960

TCAGACTCAGACAGTGATTTCAGATTTCAGACAGCGACTCAGATTTCAGATAGCGACTCAGATTTCGGACAGCGATTTCAGACTCAGATAG  
CGAC

S D S D S D S D S D S D S D S D S D S D S D S D S D S D S D S D S D S  
D 990

TCAGATTTCAGATAGCGATTTCAGACTCAGACAGCGACTCAGATTTCAGATAGCGATTTCGGACTCAGACAGCGATTTCAGACTCAGATAG  
CGAC

S D S D S D S D S D S D S D S D S D S D S D S D S D S D S D S D S D S  
D 1020

TCAGACTCAGACAGCGACTCAGATTTCAGATAGCGATTTCGGACTCAGATAGCGACTCAGATTTCAGACAGCGATTTCAGACTCAGATAG  
CGAC

S D S D S D S D S D S D S D S D S D S D S D S D S D S D S D S D S D S  
D 1050

TCAGATTTCAGACAGCGATTTCAGACTCAGATAGCGACTCAGACTCAGACAGTGATTTCAGATTTCAGACAGCGACTCAGACTCAGATAG  
CGAC

S D S D S D S D S D S D S D S D S D S D S D S D S D S D S D S D S D S  
D 1080

W>

TCAGATTTCAGACAGCGACTCAGACTCAGATAGCGACTCAGACTCAGACAGTGATTTCAGACAGCGATTTCAGACTTCGGATGCAGGAAA  
ACAT

S D S D S D S D S D S D S D S D S D S D S D S D S D S D S D A G K  
H 1110

ACACCTGTAAACCAATGAGTACTACTAAAGACCATCACAATAAAGCAAAGCATTACCAGAAACAGGTAGTAAAATAACGGCTC  
AAAT

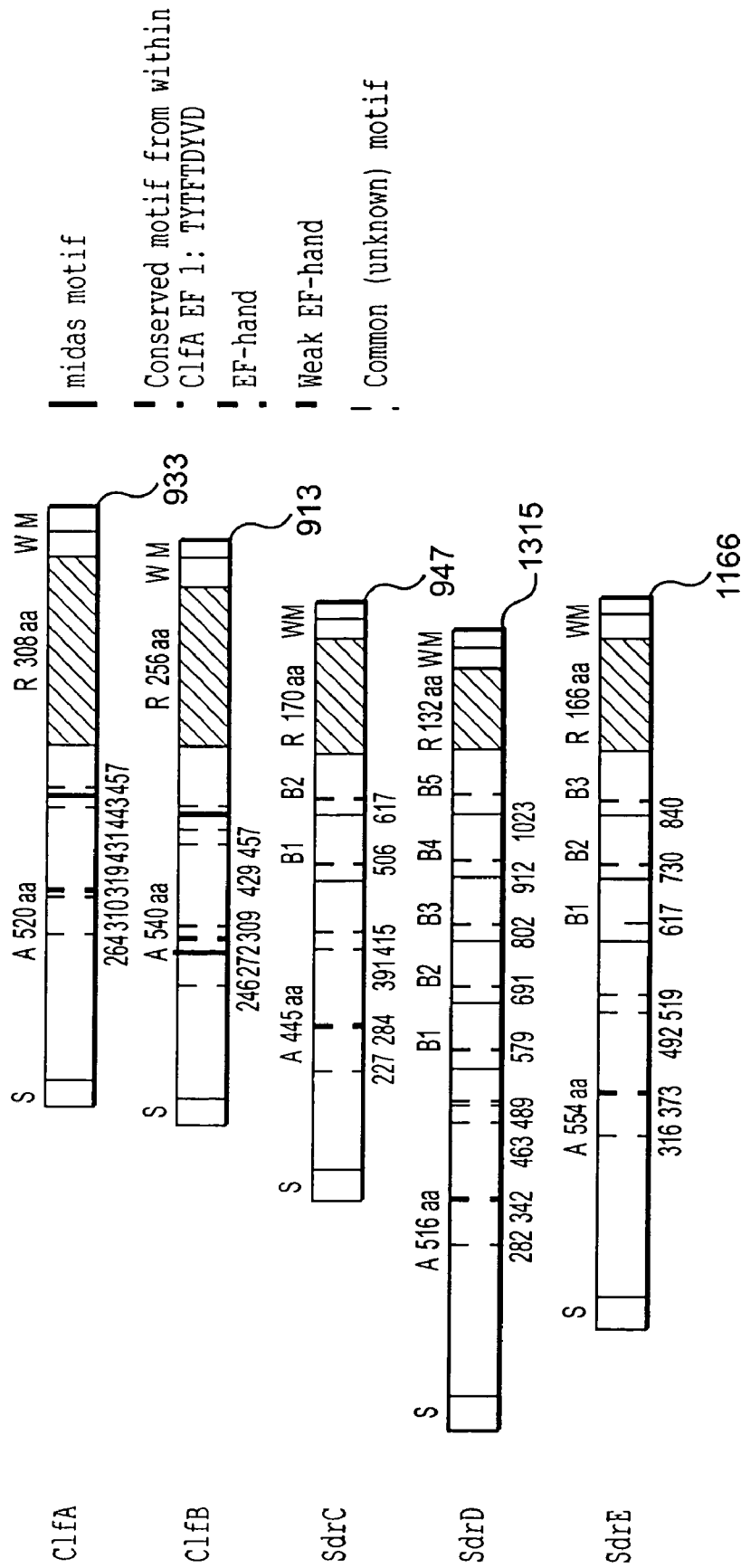
T P V K P M S T T K D H H N K A K A L P E T G S E N N G S  
N 1140

M>

AACGCAACGTTATTTGGTGGATTATTTGCAGCATTAGGTTTCATTATTGTTATTCGGTCGTCGCAAAAAACAAAACAAA

N A T L F G G L F A A L G S L L L F G R R K K Q N K  
1166

FIG. 9D



**FIG. 10**

%	ClfB	SdrC	SdrD	SdrE
ClfA	27	20	24	25
ClfB		24	25	23
SdrC			24	23
SdrD				30

**FIG. 11**

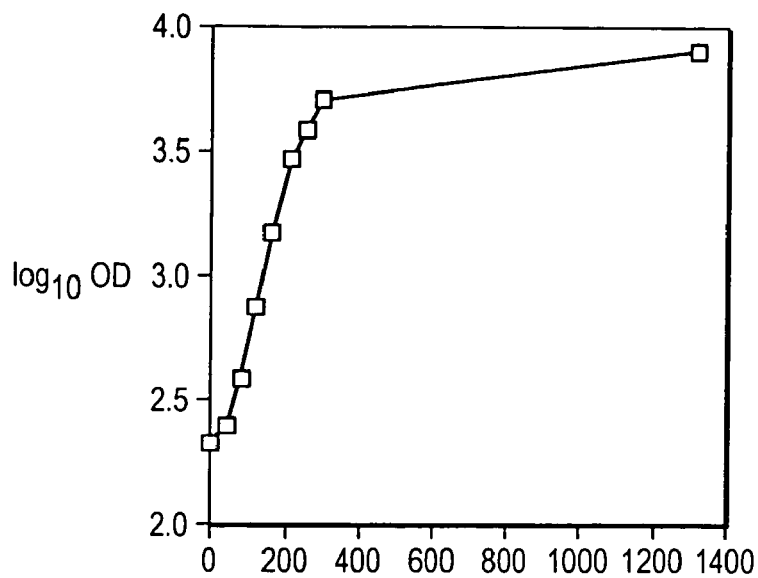


1.			
SdrD	282	TVDDKVKSGDYFTIK	296
SdrE	316	TIDNKVKKGD <del>T</del> MTIN	330
ClfB	246	TVTDKVKSGDYFTAK	269
SdrC	227	TIDDSVKEGDTFTFK	241
ClfA	264	SVPNSAVKGD <del>T</del> FKIT	278
		:: :... .** :. .	
2.			
SdrD	324	ETIATAKHDTANNLITYTFTDYVD	347
SdrE	355	EVIAGTFDKATKQITYTFTDYVD	378
ClfB	291	DVVAKATYDILTKYTFVFTDYVN	314
SdrC	266	NIIAKGIYDSTNTT <del>T</del> YFTNYVD	289
ClfA	302	QVLANGVIDSDGN-VIY <del>T</del> FTDYVN	324
		: :*.. * : : **:**:	
3.			
SdrD	463	TDIKIYQVPK	472
SdrE	492	TEIKVYKVNS	501
ClfB	429	TKLRIFEVND	438
SdrC	391	--FKIYEVTD	398
ClfA	431	TSIKVYKVDN	440
		: : : : * .	
4.			
SdrD	489	DVTNQ	493
SdrE	519	DVTSQ	523
ClfB	457	EVTDQ	461
SdrC	415	DVTDQ	419
ClfA	457	DVTNS	461
		:**..	
5.			
SdrCB1	496	KYNLG <del>D</del> YVWED <del>T</del> NKDGKQ--DANEKGIKGVYVILKDSNGK-ELDR <del>T</del> TTDENGKYQFTGLS	552
SdrCB2	607	KYSLG <del>D</del> YVWYDSNKG <del>G</del> KR--DSTEKGIKGVKVTLQNEKGE-VIG <del>T</del> TETDENGKYRFDNLD	663
SdrDB1	569	VYKIGNYVWED <del>T</del> NKNGVQ--ELGEKGVGNVTVTFDNNTN <del>T</del> KVGEAVTKEDGSYLIPNLP	626
SdrDB2	681	KYNLG <del>D</del> YVWED <del>T</del> NKNGIQ--DQDEKGISGVTVTLKDENG <del>N</del> -VLKT <del>V</del> TTDADGKYKFTDL <del>D</del>	737
SdrDB3	792	KYNLGNYVWED <del>T</del> NKDGKQ--DSTEKGISGVTVTLK <del>N</del> ENGE-VLQ <del>T</del> TKTDKDGKYQFTGLE	848
SdrDB4	902	TYNLG <del>D</del> YVWED <del>T</del> NKNGVQ--DKDEKGISGVTVTLK <del>D</del> ENDK-VLKT <del>V</del> TTDENGKYQFTDL <del>N</del>	958
SdrDB5	1013	KYSLG <del>D</del> YVWYDSNKG <del>G</del> KQ--DSTEKGIKDVKVTLLNEKGE-VIG <del>T</del> TKTDENGKYCFDNLD	1069
SdrEB1	607	LYKIG <del>D</del> YVWEDV <del>D</del> KDGVQGTDSKEKPMANVLVTLTYPDG--TTKSV <del>R</del> TDANGHYEFGGLK	664
SdrEB2	720	KYNLG <del>D</del> YVWED <del>T</del> NKDGIQ--DANE <del>P</del> GIKDVKVT <del>L</del> KDSTGK-VIG <del>T</del> TTT <del>D</del> ASGKYKFTDL <del>D</del>	776
SdrEB3	830	KYSLG <del>D</del> YVWYDSNKG <del>G</del> KQ--DSTEKGIKDV <del>T</del> VTLQNEKGE-VIG <del>T</del> TKTDENGKYRFDNLD	886
		*.:*:* ** * :*: * : : * :.* * : . * . * * : . *	

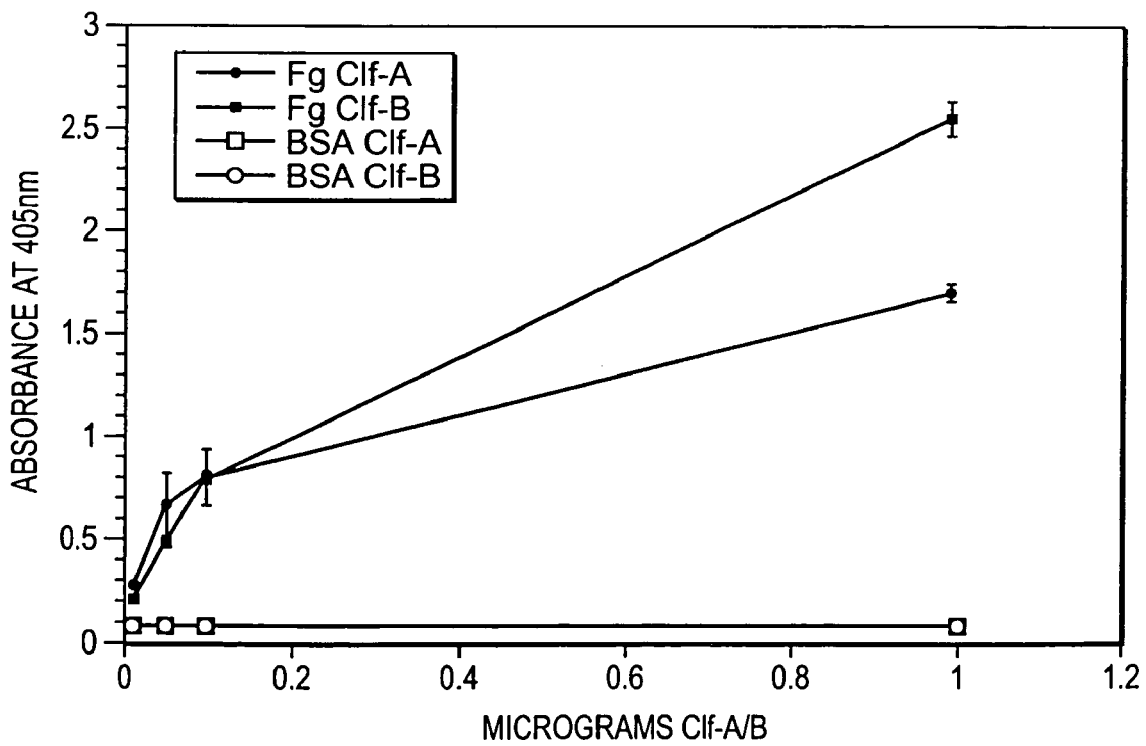
FIG. 12A

SdrCB1	553	NG-TYSVEFST-PAGYTPTTANVGTDDAVDSGLTTTGVIKDADNMTLDSGFYKTP-	606
SdrCB2	664	SG-KYKVIFEK-PAGLTQTGTNTTEDD-KDADGGEVDVTITDHDDFTLDNGYEEET	717
SdrDB1	627	NG-DYRVEFSNLPKGYEVTPSKQGNNEELDSNGLSSVITVNGKDNLSADLGIYKP--	680
SdrDB2	738	NG-NYKVEFTT-PEGYTPTTVTSGSDIEKDSNGLTTTGVIINGADNMTLDSGFYKTP-	791
SdrDB3	849	NG-TYKVEFET-PSGYTPTQVSGTDEGIDSNGTSTTGVIKDKDNDTIDSGFYKP--	901
SdrDB4	959	NG-TYKVEFET-PSGYTPTSVTSGNDEKDSNGLTTTGVIKDADNMTLDSGFYKTP-	1012
SdrDB5	1070	SG-KYKVIFEK-PAGLTQTGTNTTEDD-KDADGGEVDVTITDHDDFTLDNGYEEET	1123
SdrEB1	665	DGETYTVKFET-PTGYLPTKVNGTTDGEKDSNGSSVTVKINGKDDMSLDTGFYKEP-	719
SdrEB2	777	NG-NYTVFEFET-PAGYTPTVKNTTADD-KDSNGLTTTGVIKDADNMTLDRGFYKTP-	829
SdrEB3	887	SG-KYKVIFEK-PAGLTQTVTNTTEDD-KDADGGEVDVTITDHDDFTLDNGYFEEDT	940
		. * * * * . * * * * : * : * * : . . . * : : * * : :	

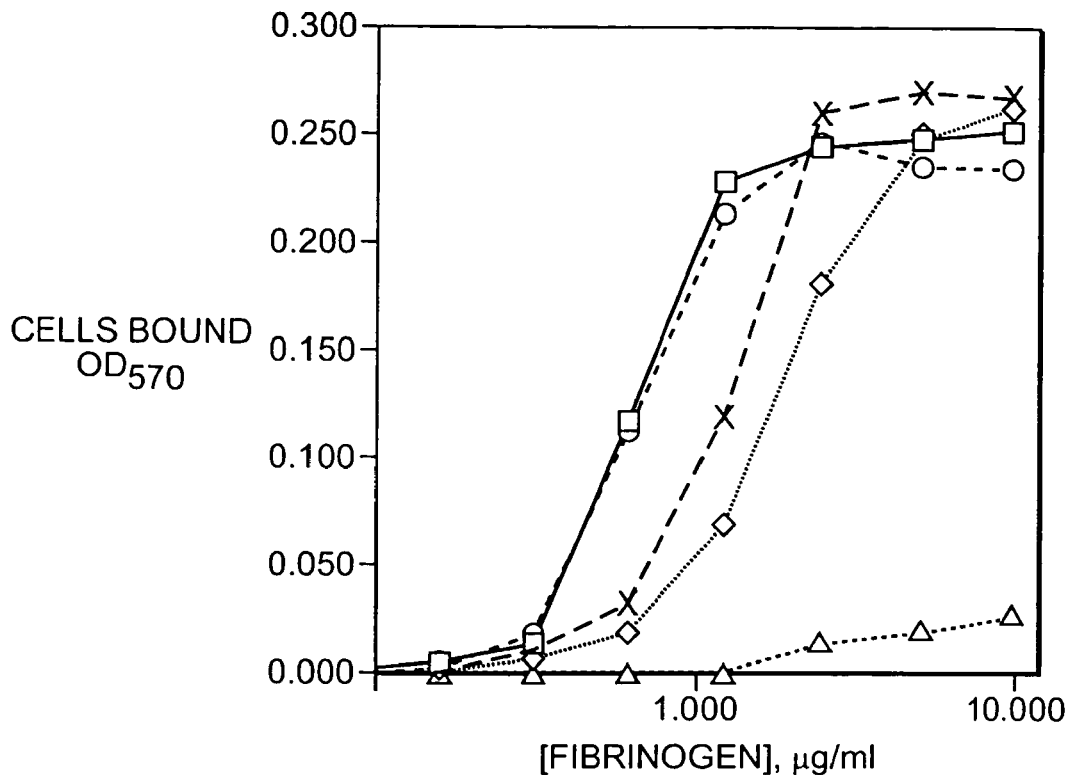
**FIG. 12B**



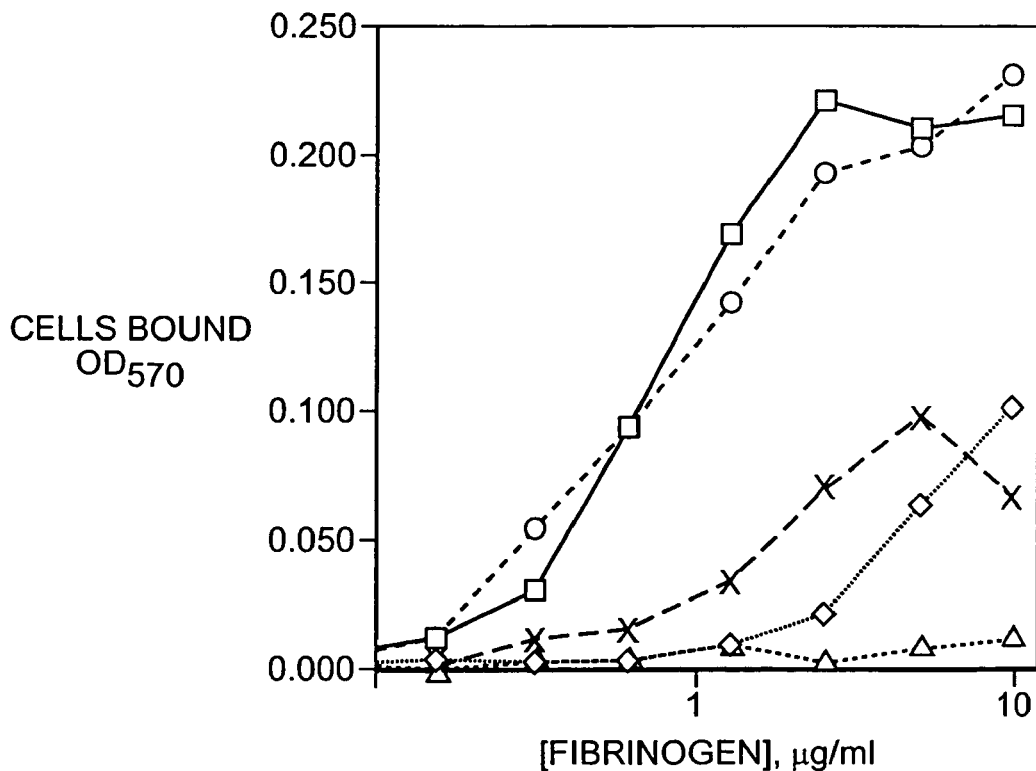
**FIG. 13**



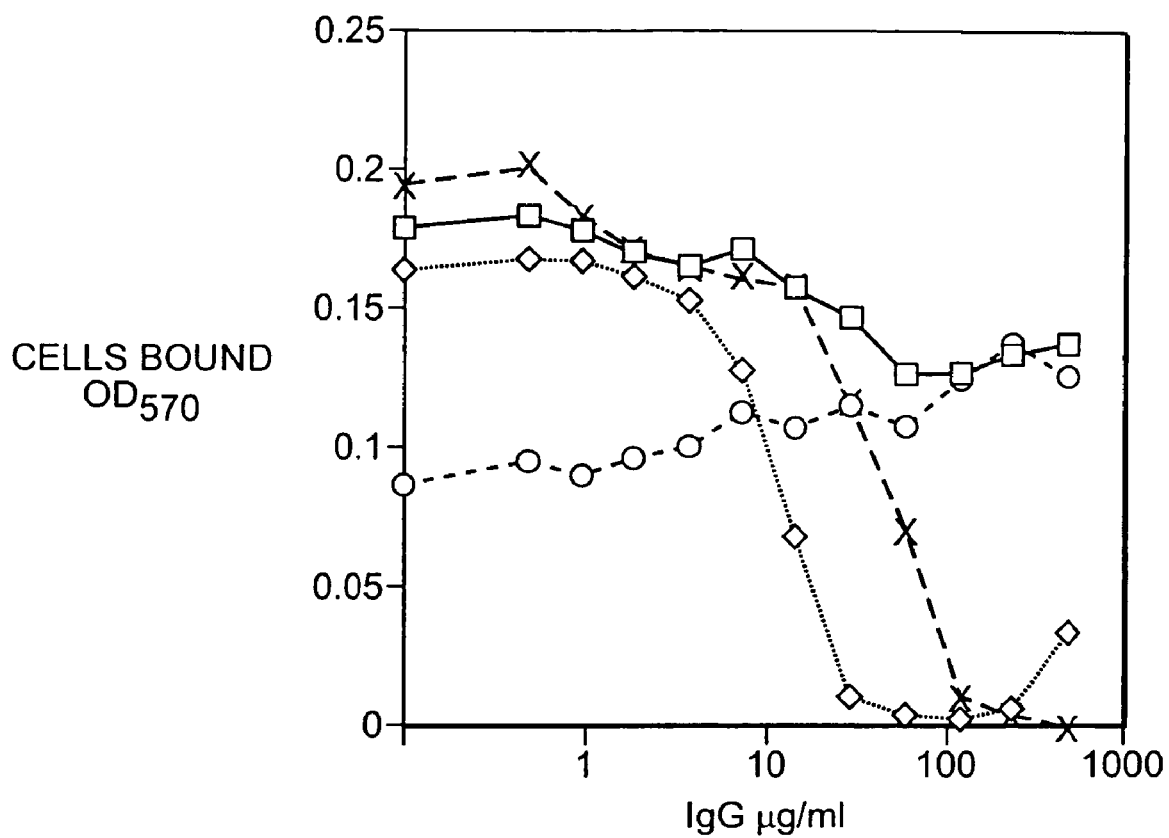
**FIG. 14**



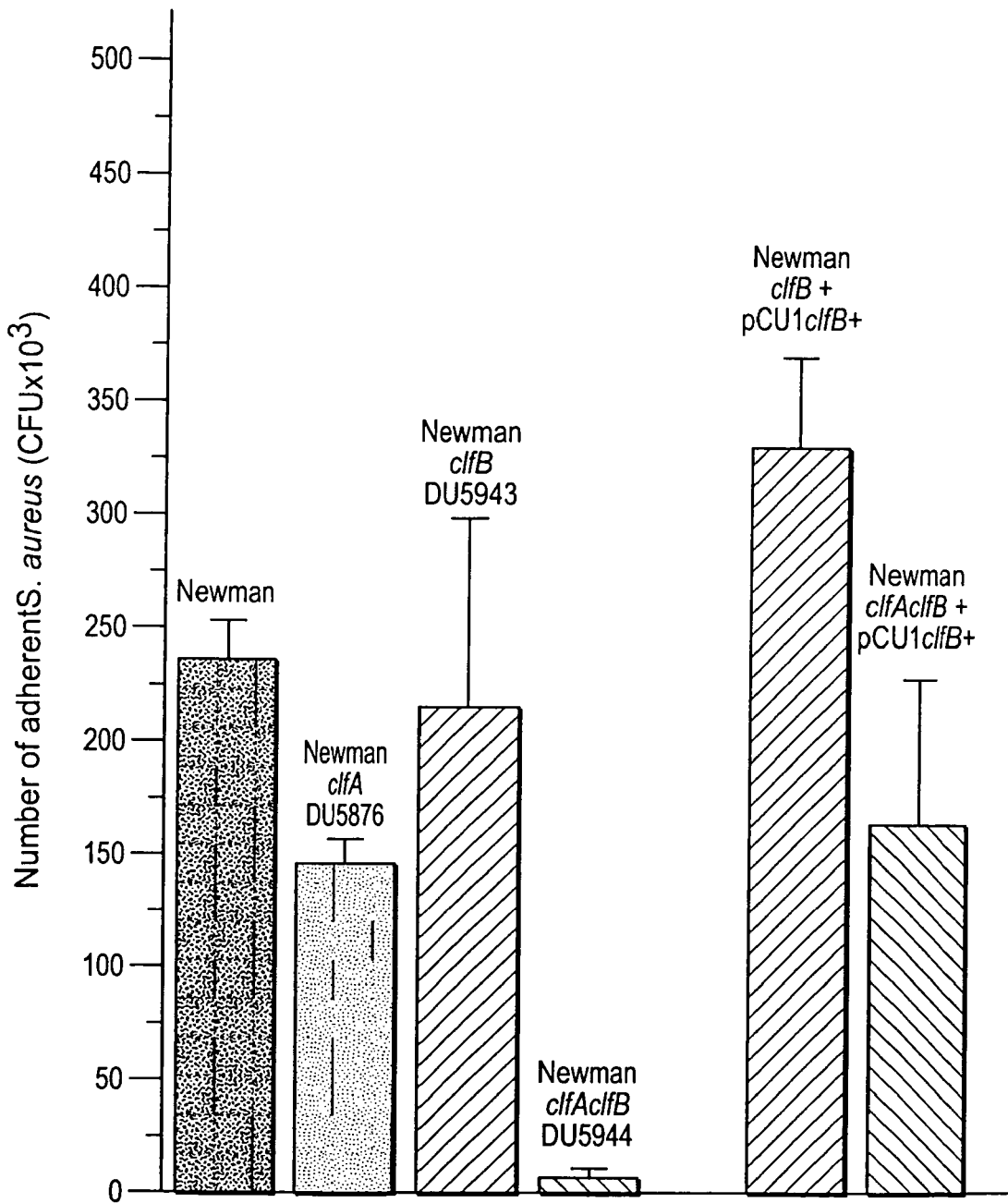
**FIG. 15**



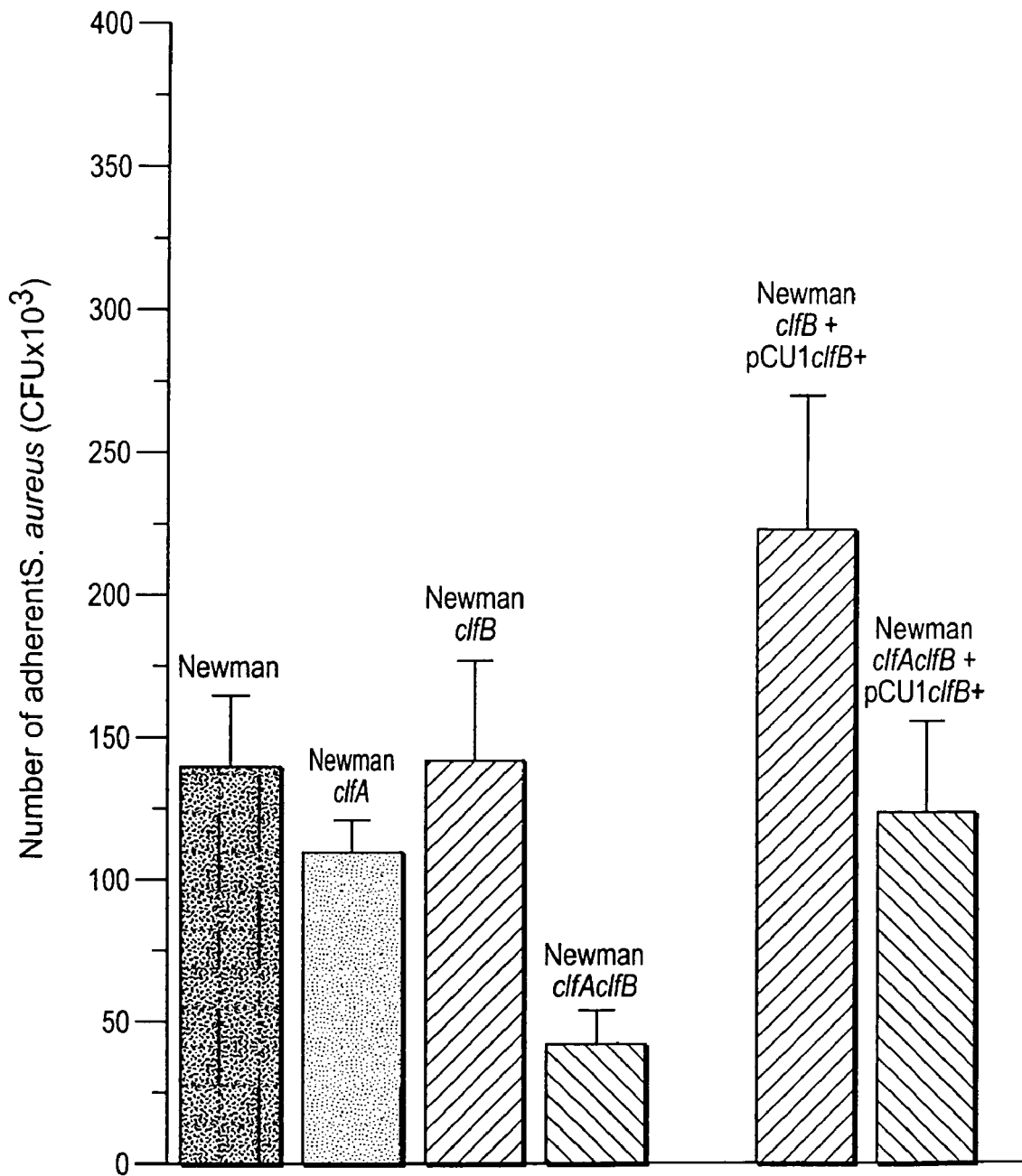
**FIG. 16**



**FIG. 17**



**FIG. 18**



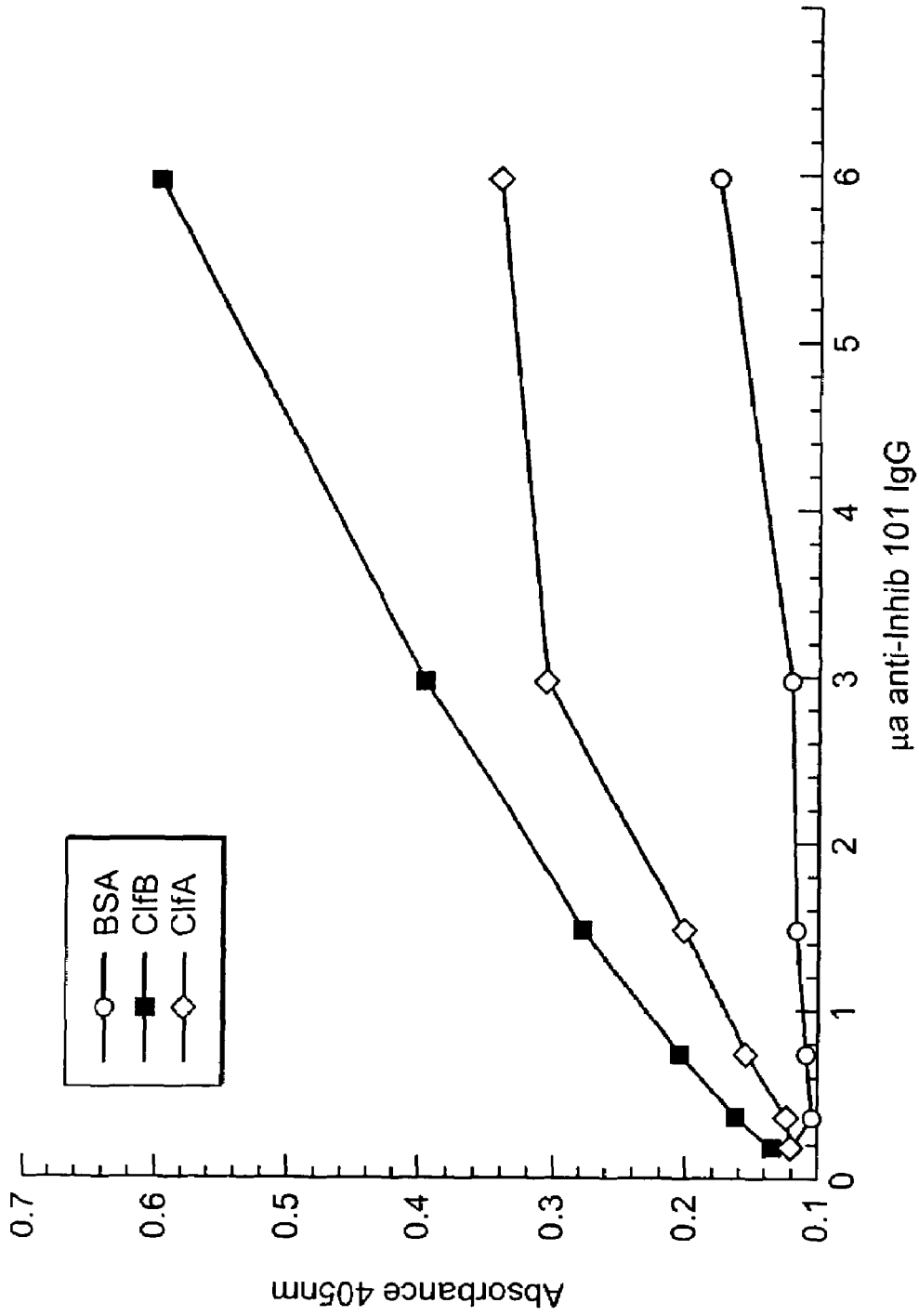
**FIG. 19**

*Staphylococcus aureus* Consensus and Variable Motif

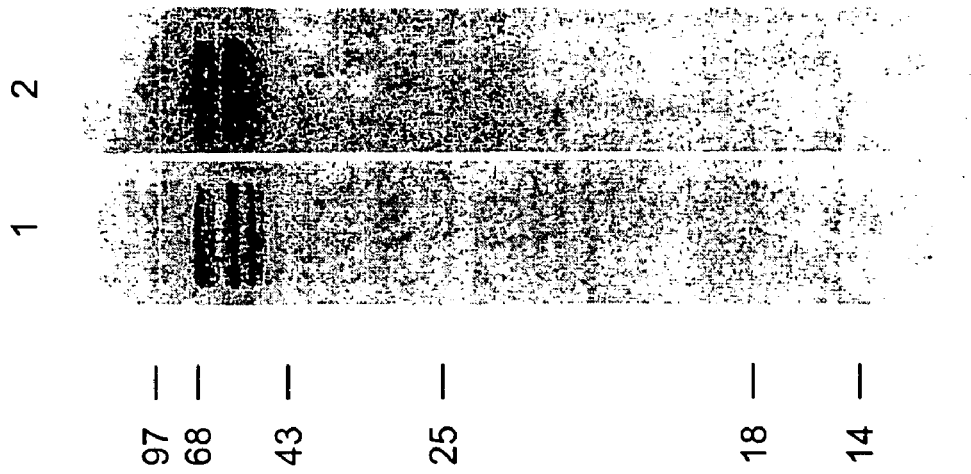
Protein	Motif									
ClfA	I	Y	T	F	T	D	Y	V	N	
ClfB	T	F	V	F	T	D	Y	V	N	
SdrC	T	Y	T	F	T	D	Y	V	D	
SdrD	T	Y	T	F	T	D	Y	V	D	
SdrE	T	Y	T	F	T	D	Y	V	D	
Consensus	T	Y	T	F	T	D	Y	V	D	
Variable Motif	T/I	Y/F	T/V	F	T	D/N	Y	V	D/N	

**FIG. 20**

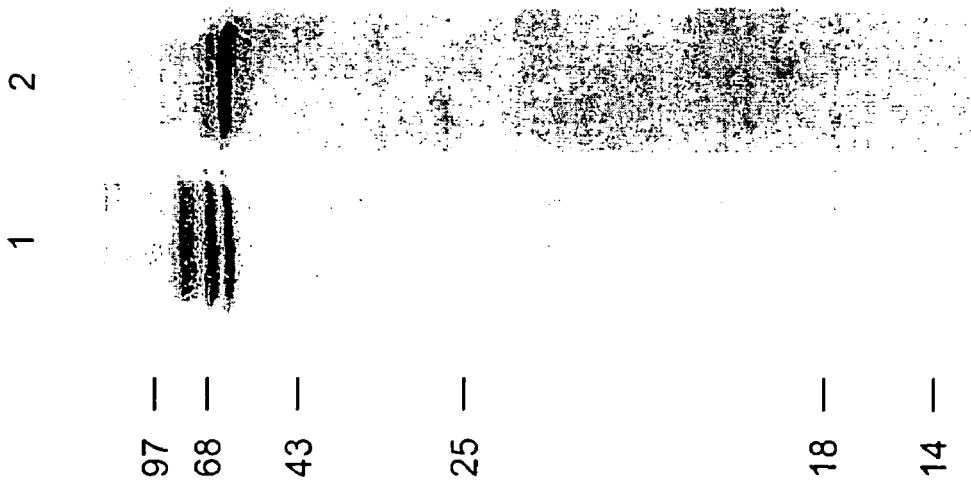




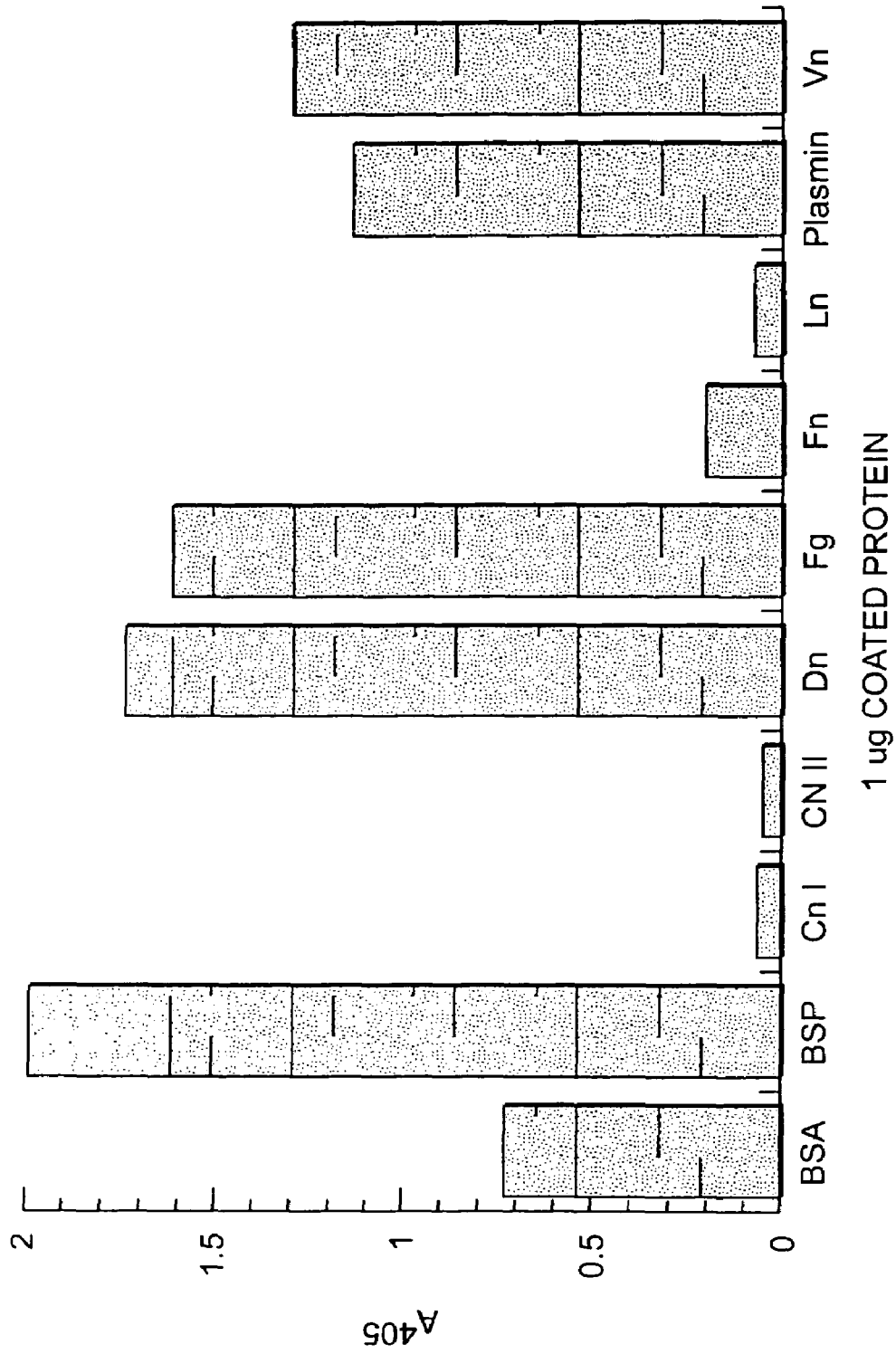
**FIG. 21**



**FIG. 22B**



**FIG. 22A**



**FIG. 23**

1

**EXTRACELLULAR MATRIX-BINDING  
PROTEINS FROM *STAPHYLOCOCCUS  
AUREUS***

CROSS-REFERENCE TO RELATED  
APPLICATIONS

The present application is a divisional application of U.S. application Ser. No. 09/200,650, filed Nov. 25, 1998, now U.S. Pat. No. 6,680,195 and claims the benefit of U.S. Provisional Applications Ser. No. 60/066,815 filed Nov. 26, 1997, 60/036,139, filed Jan. 21, 1997, and Ser. No. 60/098,427, filed Aug. 31, 1998.

This application claims priority to U.S. provisional application Nos. 60/098,427, filed Aug. 31, 1998 and 60/066,815, filed Nov. 26, 1997.

The U.S. Government has rights in this invention arising out of National Institutes of Health grant number AI20624.

FIELD OF THE INVENTION

The present invention is in the fields of microbiology and molecular biology. The invention includes the isolation and use of extracellular matrix-binding proteins and genes that express the proteins from *Staphylococcus aureus* to inhibit, prevent and diagnose *S. aureus* infection.

BACKGROUND OF THE INVENTION

In hospitalized patients *Staphylococcus aureus* is a major cause of infections associated with indwelling medical devices, such as catheters and prostheses, and related infections of surgical wounds. A significant increase in *Staphylococcus aureus* isolates that exhibit resistance to most known antibiotics has been observed in hospitals throughout the world. The recent emergence of resistance to vancomycin, the last remaining antibiotic for treating methicillin-resistant *Staphylococcus aureus* (MRSA) infections, has emphasized the need for alternative prophylactic or vaccine strategies to reduce the risk of nosocomial *S. aureus* infections.

Initial localized infections of wounds or indwelling medical devices can lead to serious invasive infections such as septicemia, osteomyelitis, and endocarditis. In infections associated with medical devices, plastic and metal surfaces become coated with host plasma and extracellular matrix proteins such as fibrinogen and fibronectin shortly after implantation. The ability of *S. aureus* to adhere to these proteins is of crucial importance for initiating infection. Vascular grafts, intravenous catheters, artificial heart valves, and cardiac assist devices are thrombogenic and prone to bacterial colonization. *S. aureus* is the most damaging pathogen to cause such infections.

Fibrin is the major component of blood clots, and fibrinogen/fibrin is one of the major plasma proteins deposited on implanted biomaterials. Considerable evidence exists to suggest that bacterial adherence to fibrinogen/fibrin is important in the initiation of device-related infection. For example, as shown by Vaudaux et al., *S. aureus* adheres to in vitro plastic that has been coated with fibrinogen in a dose-dependent manner (*J. Infect. Dis.* 160:865-875 (1989)). In addition, in a model that mimics a blood clot or damage to a heart valve, Herrmann et al. demonstrated that *S. aureus* binds avidly via a fibrinogen bridge to platelets adhering to surfaces (*J. Infect. Dis.* 167:312-322 (1993)). *S. aureus* can adhere directly to fibrinogen in blood clots formed in vitro, and can adhere to cultured endothelial cells

2

via fibrinogen deposited from plasma acting as a bridge (Moreillon et al., *Infect. Immun.* 63:4738-4743 (1995); Cheung et al., *J. Clin. Invest.* 87:2236-2245 (1991)). As shown by Vaudaux et al. and Moreillon et al., mutants defective in the fibrinogen-binding protein clumping factor (ClfA) exhibit reduced adherence to fibrinogen in vitro, to explanted catheters, to blood clots, and to damaged heart valves in the rat model for endocarditis (Vaudaux et al., *Infect. Immun.* 63:585-590 (1995); Moreillon et al., *Infect. Immun.* 63:4738-4743 (1995)).

An adhesin for fibrinogen, often referred to as "clumping factor," is located on the surface of *S. aureus* cells. The interaction between the clumping factor on bacteria and fibrinogen in solution results in the instantaneous clumping of bacterial cells. The binding site on fibrinogen is located in the C-terminus of the gamma chain of the dimeric fibrinogen glycoprotein. The affinity is very high and clumping occurs in low concentrations of fibrinogen. Scientists have recently shown that clumping factor also promotes adherence to solid phase fibrinogen, to blood clots, and to damaged heart valves (McDevitt et al., *Mol. Microbiol.* 11:237-248(1994); Vaudaux et al., *Infect. Immun.* 63:585-590 (1995); Moreillon et al., *Infect. Immun.* 63:47384743 (1995)).

The gene for a clumping factor protein, designated ClfA, has been cloned, sequenced and analyzed in detail at the molecular level (McDevitt et al., *Mol. Microbiol.* 11:237-248 (1994); McDevitt et al., *Mol. Microbiol.* 16:895-907 (1995)). The predicted protein is composed of 933 amino acids. A signal sequence of 39 residues occurs at the N-terminus followed by a 520 residue region (region A), which contains the fibrinogen binding domain. A 308 residue region (region R), composed of 154 repeats of the dipeptide serine-aspartate, follows. The R region sequence is encoded by the 18 basepair repeat GAYTCNGAYT CNGAYAGY (SEQ ID NO: 9) in which Y equals pyrimidines and N equals any base. The C-terminus of ClfA has features present in many surface proteins of Gram-positive bacteria such as an LPDTG (SEQ ID NO: 10) motif, which is responsible for anchoring the protein to the cell wall, a membrane anchor, and positive charged residues at the extreme C-terminus.

The platelet integrin alpha IIb $\beta$ 3 recognizes the C-terminus of the gamma chain of fibrinogen. This is a crucial event in the initiation of blood clotting during coagulation. ClfA and alpha IIb $\beta$ 3 appear to recognize precisely the same sites on fibrinogen gamma chain because ClfA can block platelet aggregation, and a peptide corresponding to the C-terminus of the gamma chain (198-411) can block both the integrin and ClfA interacting with fibrinogen (McDevitt et al., *Eur. J. Biochem.* 247:416-424 (1997)). The fibrinogen binding site of alpha IIb $\beta$ 3 is close to, or overlaps, a Ca<sup>2+</sup> binding determinant referred to as an "EF hand". ClfA region A carries several EF hand-like motifs. A concentration of Ca<sup>2+</sup> in the range of 3-5 mM blocks these ClfA-fibrinogen interactions and changes the secondary structure of the ClfA protein. Mutations affecting the ClfA EF hand reduce or prevent interactions with fibrinogen. Ca<sup>2+</sup> and the fibrinogen gamma chain seem to bind to the same, or to overlapping, sites in ClfA region A.

The alpha chain of the leucocyte integrin, alpha M $\beta$ 2, has an insertion of 200 amino acids (A or I domain) which is responsible for ligand binding activities. A novel metal ion-dependent adhesion site (MIDAS) motif in the I domain is required for ligand binding. Among the ligands recognized is fibrinogen. The binding site on fibrinogen is in the gamma chain (residues 190-202). It was recently reported that *Candida albicans* has a surface protein, alpha Int1p,

having properties reminiscent of eukaryotic integrins. The surface protein has amino acid sequence homology with the I domain of alpha M $\beta$ 2, including the MIDAS motif. Furthermore, alpha Int1p binds to fibrinogen.

ClfA region A also exhibits some degree of sequence homology with alpha Int1p. Examination of the ClfA region A sequence has revealed a potential MIDAS motif. Mutations in supposed cation coordinating residues in the DXSXS (SEQ ID NO: 13) portion of the MIDAS motif in ClfA results in a significant reduction in fibrinogen binding. A peptide corresponding to the gamma-chain binding site for alpha M $\beta$ 2 (190-202) has been shown by O'Connell et al. to inhibit ClfA-fibrinogen interactions (O'Connell et al., *J. Biol. Chem.*, in press). Thus it appears that ClfA can bind to the gamma-chain of fibrinogen at two separate sites. The ligand binding sites on the ClfA are similar to those employed by eukaryotic integrins and involve divalent cation binding EF-hand and MIDAS motifs.

Scientists have recently shown that *S. aureus* expresses proteins other than ClfA that may bind fibrinogen (Boden and Flock, *Mol. Microbiol.* 12:599-606 (1994)). One of these proteins is probably the same as the broad spectrum ligand-binding protein reported by Homonylo-McGavin et al., *Infect. Immun.* 61:2479-2485 (1993). Another is coagulase, as reported by Boden and Flock, *Infect. Immun.* 57:2358-2363 (1989), a predominantly extracellular protein that activates the plasma clotting activity of prothrombin. Coagulase binds prothrombin at its N-terminus and also interacts with soluble fibrinogen at its C-terminus. Cheung et al., *Infect. Immun.* 63:1914-1920 (1995) have described a variant of coagulase that binds fibrinogen. There is some evidence that coagulase can contribute, in a minor way, to the ability of *S. aureus* cells to bind fibrinogen. As shown by Wolz et al., *Infect. Immun.* 64:3142-3147 (1996), in an agr regulatory mutant, where coagulase is expressed at a high level, coagulase appears to contribute to the binding of soluble fibrinogen to bacterial cells. Also, as shown by Dickinson et al., *Infect. Immun.* 63:3143-3150 (1995), coagulase contributes in a minor way to the attachment of *S. aureus* to plasma-coated surfaces under flow. However, it is clear that clumping factor ClfA is the major surface-located fibrinogen-binding protein responsible for bacterial attachment to immobilized fibrinogen/fibrin.

The identification and isolation of additional *S. aureus* extracellular matrix binding proteins would be useful for the development of therapies, diagnosis, prevention strategies and research tools for *S. aureus* infection.

Accordingly it is an object of the present invention to provide isolated cell-wall associated extracellular matrix-binding proteins of *S. aureus* and active fragments thereof.

It is a further object of the invention to provide methods for preventing, diagnosing, treating or monitoring the progress of therapy for bacterial infections caused by *S. aureus*.

It is a further object of the present invention to provide isolated *S. aureus* surface proteins that are related in amino acid sequence to ClfA and are able to promote adhesion to the extracellular matrix or host cells.

It is another object of the present invention to generate antisera and antibodies to cell-wall associated extracellular matrix-binding proteins of *S. aureus*, or active fragments thereof.

It is a further object of the present invention to provide *S. aureus* vaccines, including a DNA vaccine.

It is a further object of the present invention to provide improved materials and methods for detecting and differentiating *S. aureus* organisms in clinical and laboratory settings.

5 It is a further object of the invention to provide nucleic acid probes and primers specific for *S. aureus*.

It is a further object of the invention to provide isolated extracellular matrix-binding proteins or peptides of *S. aureus*.

#### 10 SUMMARY OF THE INVENTION

Isolated extracellular matrix-binding proteins, designated ClfB, SdrC, SdrD and SdrE, and their corresponding amino acid and nucleic acid sequences and motifs are described. 15 The proteins, peptides, fragments thereof or antigenic portions thereof are useful for the prevention, inhibition, treatment and diagnosis of *S. aureus* infection and as scientific research tools. Further, antibodies or antibody fragments to the proteins, peptides, fragments thereof or antigenic portions thereof are also useful for the prevention, inhibition, treatment and diagnosis of *S. aureus* infection. The proteins, peptides, peptide fragments, antibodies, or antibody fragments can be administered in an effective amount to a patient 25 in need thereof in any appropriate manner, preferably intravenously or otherwise by injection, to impart active or passive immunity. In an alternative embodiment, the proteins or antibodies thereof can be administered to wounds or used to coat biomaterials to act as blocking agents to prevent or inhibit the binding of *S. aureus* to wounds or biomaterials.

Specifically, extracellular matrix-binding proteins from *S. aureus* designated as ClfB, SdrC, SdrD, and SdrE are provided.

ClfB is a fibrinogen binding protein. The nucleic acid and amino acid sequences of ClfB are provided in FIG. 5. The amino acid sequence of ClfB is SEQ ID NO:1, and the nucleic acid sequence of ClfB is SEQ ID NO:2.

SdrC has been discovered to bind to several extracellular matrix proteins of the host, including for example, bone sialoprotein (BSP), decorin, plasmin, fibrinogen and vitronectin. The amino acid and nucleic acid sequences of SdrC are SEQ ID NOS:3 and 4 respectively and are provided in FIG. 7.

Another of the discovered proteins, SdrD, binds to at least vitronectin. The amino acid and nucleic acid sequences of SdrD are SEQ ID NOS:5 and 6 respectively and are provided in FIG. 8.

SdrE binds to extracellular matrix proteins, for example, bone sialoprotein (BSP). The amino acid and nucleic acid sequences of SdrE are SEQ ID NOS:7 and 8 respectively and are provided in FIG. 9.

ClfB has a predicted molecular weight of approximately 88 kDa and an apparent molecular weight of approximately 124 kDa. ClfB is a cell-wall associated protein and binds both soluble and immobilized fibrinogen. In addition, ClfB binds both the alpha and beta chains of fibrinogen and acts as a clumping factor. SdrC, SdrD and SdrE are cell-wall associated proteins that exhibit cation-dependent ligand binding of extracellular matrix proteins such as decorin, plasmin, fibrinogen, vitronectin and BSP.

It has been discovered that in the A region of SdrC, SdrD, SdrE, ClfA, and ClfB, there is highly conserved amino acid sequence that can be used to derive a consensus TYTFT-DYVD (SEQ ID NO: 18) motif (see FIG. 20). The motif can be used in vaccines to impart broad spectrum immunity 65 against bacterial infections. The motif can also be used as an antigen in the production of monoclonal or polyclonal

antibodies to impart broad spectrum passive immunity. In an alternative embodiment, any combination of the variable sequence motif (T/I) (Y/F) (T/V) (F) (T) (D/N) (Y) (V) (D/N) can be used as an immunogen or antigen, or in the preparation of antibodies.

The ClfB, SdrC, SdrD and SdrE proteins or the consensus or variable motifs thereof are useful as scientific research tools to identify *S. aureus* binding sites on the extracellular matrix. They are further useful as research tools to promote an understanding of the mechanisms of bacterial pathology and the development of antibacterial therapies.

The ClfB, SdrC, SdrD and SdrE nucleic acid sequences or selected fragments thereof, including the sequences encoding the consensus or variable motifs, are useful as nucleic acid probes for the identification of other *S. aureus* extracellular matrix-binding proteins. Alternatively, the amino acid sequences of the proteins, or selected fragments thereof, can be used as probes to identify the corresponding nucleic acid sequences.

The ClfB, SdrC, SdrD and SdrE nucleic acid sequences or the sequences encoding the consensus or variable motifs are further useful as polynucleotides which comprise contiguous nucleic acid sequences capable of being expressed. The nucleic acid sequences may be inserted into a vector and placed in a microorganism for the production of recombinant ClfB, SdrC, SdrD and SdrE proteins or the variable or consensus amino acid motifs. This allows for the production of the gene product upon introduction of said polynucleotide into eukaryotic tissues *in vivo*. The encoded gene product preferably either acts as an immunostimulant or as an antigen capable of generating an immune response. Thus, the nucleic acid sequences in this embodiment encode an MSCRAMM (Microbial Surface Components Recognising Adhesive Matrix Molecules) immunogenic epitope, and optionally a cytokine or a T-cell costimulatory element, such as a member of the B7 family of proteins.

There are several advantages of immunization with a gene rather than its gene product. The first is the relative simplicity with which native or nearly native antigen can be presented to the immune system. A second advantage of DNA immunization is the potential for the immunogen to enter the MHC class I pathway and evoke a cytotoxic T cell response. Cell-mediated immunity is important in controlling infection. Since DNA immunization can evoke both humoral and cell-mediated immune responses, its greatest advantage may be that it provides a relatively simple method to survey a large number of *S. aureus* genes for their vaccine potential.

Antibodies immunoreactive with ClfB, SdrC, SdrD and SdrE proteins, or their active fragments, including with the consensus or variable amino acid motifs, are provided herein. Vaccines or other pharmaceutical compositions containing the proteins or amino acid motifs are additionally provided herein.

Antibodies and antisera to the consensus TYTFTDYVD (SEQ ID NO: 18) sequence epitope or the variable (T/I) (Y/F) (T/N) (F) (D/N) (Y) (V) (D/N) sequence, specifically TYTFTNYVD (SEQ ID NO: 19) in SdrC, TYTFTDYVD (SEQ ID NO: 18) in SdrD and SdrE, TFVFTDYVN (SEQ ID NO: 20) in ClfB or IYTFTDYVN (SEQ ID NO: 21) in ClfA are provided herein. Vaccines or other pharmaceutical compositions containing the epitopes are also provided herein.

In addition, diagnostic kits containing nucleic acid molecules, the proteins, antibodies or antisera to ClfB, SdrC, SdrD, SdrE or their active fragments, including the consen-

sus or variable amino acid motifs and the appropriate reagents for reaction with a sample are also provided.

In one embodiment of the invention, the diagnostic kit is used to identify patients or animals that have levels of antibodies to ClfB ClfB, SdrC, SdrD, or SdrE that are above a population norm. The plasma of the patients or animals can be obtained, processed, and administered to a host in need of passive immunity to *S. aureus* infection.

#### BRIEF DESCRIPTION OF THE FIGURES

FIG. 1 is a schematic representation comparing features of unprocessed ClfA and ClfB proteins. S indicates the signal sequence. A indicates the conserved region (region A). P indicates the proline-rich region (repeats are indicated by gray boxes). R indicates the SD repeat region (region R). W indicates the wall-spanning region. M indicates the membrane spanning and anchoring regions. EF hand I of ClfA and its partial homologue on ClfB are indicated by black vertical bars. The MIDAS motifs are indicated by hatched (DXSXS) (SEQ ID NO: 13) and narrow vertical lines (downstream T and D residues) connected by dashed lines.

FIG. 2 is a schematic representation of general plasmid and probe constructions for sequencing clfB. A repeat-carrying EcoRI fragment was cloned from phage clone A1-1 into pGEM 7Z (f)+ to give pA1-1E (top), and subsequently reduced by deletion of an XbaI fragment to give pA1-1EX, which contains the entire clfB gene. A SmaI fragment containing clfB and 500 bp of upstream DNA was cloned into pCU1 for overexpression and complementation work (pA1-1EA). The HpaI probe used to screen mutants, and the hybridizing BamHI fragment are also indicated.

FIG. 3 is a schematic representation showing construction of a cassette for allele replacement clfB was interrupted by blunt-end cloning the Tc determinant from pT181 into the HpaI site in the middle of the gene in pA1-1EX. pTS2 was then cloned into the SmaI site of the cassette to enable temperature sensitive propagation in *S. aureus*.

FIG. 4 is a schematic representation of a physical map of the sdrC sdrD sdrE locus in *S. aureus* strain Newman. The extents of the plasmid clones are delineated. A6-2 is a LambdaGEM®-12 clone. pEJ1, pEJ2 and pEJ3 are A6-2 fragments subcloned in the pGEM 7Z (f)+ (pEJ1 and pEJ2) and the pBluescript KS+ vector (pEJ3). pC1 is a HindIII fragment directly cloned from strain Newman in the pBluescript KS+ vector. Arrows indicate the direction of transcription of sdrC, sdrD and sdrE.

FIG. 5 is the nucleic acid sequence of clfB and flanking DNA, and amino acid translation of the ORF. The likely start codon is double underlined, and the principal regions indicated using the abbreviations of FIG. 1. Two salient features of region A, the DYSNS (SEQ ID NO: 11) of the putative MIDAS motif, and the sequence FTDYVN (SEQ ID NO: 12), the longest region of identity with ClfB, are underlined. Vertical bars indicate the repeats in the proline-rich region. An inverted repeat specifying a possible transcription termination signal is underlined.

FIG. 6 is an amino acid sequence alignment of part of region A of the ClfB and ClfA proteins in the region of strongest similarity. EF hand I of ClfA is underlined. Identical residues are denoted by an asterisk; conservative substitutions are denoted by a period. The DXSXS (SEQ ID NO: 13) portion of the MIDAS motif of ClfB is double underlined.

FIG. 7 is the nucleic acid sequence and amino acid translation of the sdrC gene. The consensus TYTFTDYVD (SEQ ID NO: 18) motif, expressed in SdrC as TYTFT-

7

NYVD (SEQ ID NO: 19), the EF hands in the B repeats, and the LPXTG (SEQ ID NO: 14) motif are underlined. Major regions, such as the signal sequence (S), region A (A), B repeats (B) region R (R), the wall-spanning domain (W), and the membrane-anchoring domain (M), are indicated.

FIG. 8 is the nucleic acid sequence and amino acid translation of the *sdrD* gene. The consensus TYTFTDYVD (SEQ ID NO: 18) motif, the EF hands in the B repeats, and the LPXTG (SEQ ID NO: 14) motif are underlined. Major regions, such as the signal sequence (S), region A (A), B repeats (B) region R (R), the wall-spanning domain (W), and the membrane-anchoring domain (M), are indicated.

FIG. 9 is the nucleic acid sequence and amino acid translation of the *sdrE* gene. The consensus TYTFTDYVD (SEQ ID NO: 18) motif, the EF hands in the B repeats, and the LPXTG (SEQ ID NO: 14) motif are underlined. Major regions, such as the signal sequence (S), region A (A), B repeats (B) region R (R), the wall-spanning domain (W), and the membrane-anchoring domain (M), are indicated.

FIG. 10 is a schematic diagram of the region R-containing proteins. Numerals over the proteins denote numbers of amino acids in the regions, numerals under the proteins denote the location on the amino acid sequence of the motifs counted from the beginning of the signal peptide. Abbreviations: S: Signal peptide; A: Region A; B: B repeat; R: Region R; W.M: Wall and membrane spanning regions.

FIG. 11 is a chart showing similarities between A regions ClfA, ClfB, SdrC, SdrD and SdrE. Each sequence was aligned in pairwise combinations and the percent identical residues given.

FIG. 12 indicates Clustal™ multiple sequence alignments of areas of similarity of the A and B regions of the region R containing genes of strain Newman. An asterisk denotes identity of amino acids, and a colon represents increasing similarity of polarity and hydrophobicity/hydrophilicity of side chains of amino acids. Alignments 1-4 show areas from region A. Alignments 1, 3 and 4 show the common motifs. Alignment 2 shows homology in the vicinity of the ClfA EF-hand (underlined), with the consensus TYTFTDYVD (SEQ ID NO: 18) sequence conserved in all five genes. Alignment 5 shows the B repeats of proteins SdrD, SdrD and SdrE with possible EF hands underlined.

FIG. 13 is a time-course graph of ClfB expression in *S. aureus* Newman versus time, monitored by Western blotting. Shake flask cultures were sampled at specific time intervals. A standard number of cells was used to prepare lysates.

FIG. 14 is a graph of absorbance versus concentration of ClfA/ClfB comparing the binding of increasing concentrations of biotinylated recombinant region A from ClfA and ClfB to fibrinogen coated plates. Binding to BSA-coated plates is shown as a control. The closed square symbol represents fibrinogen-ClfA; the closed circle symbol represents fibrinogen-ClfB; the open square symbol represents BSA-ClfA; the open circle symbol represents BSA-ClfB.

FIG. 15 is a graph of cells bound versus fibrinogen concentration showing adherence of *S. aureus* Newman and mutants to fibrinogen immobilized on ELISA plates. Increasing amounts of fibrinogen were used to coat the plates, and a fixed concentration of cells from exponential phase cultures were added. The square symbol represents wild-type; the diamond symbol represents *clfA*; the circle symbol represents *clfB*; the triangle symbol represents *clfAclfB*; the x symbol represents *clfAclfB,clfB*<sup>+</sup>.

FIG. 16 is a graph of cells bound versus fibrinogen concentration showing adherence of *S. aureus* Newman and mutants to fibrinogen immobilized on ELISA plates. Increasing amounts of fibrinogen were used to coat the

8

plates, and a fixed concentration of cells from stationary phase cultures added. The square symbol represents wild-type; the diamond symbol represents *clfA*; the circle symbol represents *clfB*; the triangle symbol represents *clfAclfB*; the x symbol represents *clfAclfB,clfB*<sup>+</sup>.

FIG. 17 is a graph of cells bound versus IgG concentration showing effects of preincubation with anti-ClfB IgG on adherence of *S. aureus* Newman and mutants to immobilized fibrinogen. The square symbol represents wild-type; the diamond symbol represents *clfA*; the circle symbol represents *clfB*; the x symbol represents *clfAclfB,clfB*<sup>+</sup>.

FIG. 18 is a bar graph showing adherence of *S. aureus* Newman and mutants to explanted hemodialysis tubing. Cells from two hour shake-flask cultures were used. The graph provides the means and SEM of three experiments.

FIG. 19 is a bar graph showing adherence of *S. aureus* Newman and mutants to fibrinogen immobilized on PMMA (polymethylmethacrylate) coverslips. Cells from two hour shake-flask cultures were used. The graph provides the means and SEM of three experiments.

FIG. 20 is a table which shows the highly conserved amino acid sequences in the A region of ClfA, ClfB, SdrC, SdrD and SdrE, which are used to provide consensus and variable motifs.

FIG. 21 is a graph of absorbance versus concentration of anti-TYTFTDYVD (SEQ ID NO: 18) antibodies, demonstrating the binding of increasing concentrations of the antibodies to ClfA, ClfB or BSA coated plates. BSA-coated plates are used as a control, and no significant binding is observed. The closed square symbol represents antibody bound to ClfB; the open diamond symbol represents antibody bound to ClfA; the open circle symbol represents BSA.

FIG. 22 is a Western Blot which illustrates the differing specificities of ClfA and ClfB in the binding of human fibrinogen. The Western Blot was created by the separation of human fibrinogen, and later, the incubation of the nitrocellulose membrane with the A region of either biotinylated ClfA or ClfB. Biotinylated ClfA region A binds the  $\gamma$  chain of fibrinogen, as is seen in lane A2. Biotinylated ClfB region A binds to both the  $\alpha$  and  $\beta$  chains of fibrinogen, as seen in lane B2.

FIG. 23 is a bar graph showing adherence of recombinant SdrC region A (SdrCA) to ten different extracellular matrix proteins, BSA, BSP, two forms of collagen, decorin, fibrinogen, fibronectin, laminin, plasmin and vitronectin. The extracellular matrix proteins were immobilized on microtiter wells. Absorbance tests revealed reactivity of SdrCA with fibrinogen, BSP, decorin, plasmin and vitronectin.

#### DETAILED DESCRIPTION OF THE INVENTION

Isolated extracellular matrix-binding proteins, designated ClfB, SdrC, SdrD and SdrE, and their corresponding amino acid and nucleic acid sequences and motifs are described. The proteins, peptides, fragments thereof or antigenic portions thereof are useful for the prevention, inhibition, treatment and diagnosis of *S. aureus* infection and as scientific research tools. Further, antibody or antibody fragments to the proteins, peptides, fragments thereof or antigenic portions thereof are also useful for the prevention, inhibition, treatment and diagnosis of *S. aureus* infection. In particular, the proteins or antibodies, or active fragments thereof may be administered as vaccines to induce either passive or cellular immunity.

ClfB binds to at least fibrinogen.

SdrC has been discovered to bind to extracellular matrix proteins of the host, including for example, BSP, decorin, plasmin, vitronectin and fibrinogen. SdrD binds to at least vitronectin. SdrE binds to extracellular matrix proteins, for example, bone sialoprotein (BSP).

The amino acid sequence of ClfB is SEQ ID NO:1. The nucleic acid sequence encoding ClfB is SEQ ID NO:2. The nucleic acid and amino acid sequences of ClfB are also provided in FIG. 5. The amino acid and nucleic acid sequences of SdrC are SEQ ID NOS:3 and 4 respectively and are provided in FIG. 7. The amino acid and nucleic acid sequences of SdrD are SEQ ID NOS:5 and 6 respectively and are provided in FIG. 8. The amino acid and nucleic acid sequences of SdrE are SEQ ID NOS:7 and 8 respectively and are provided in FIG. 9. The term "isolated" is defined herein as free from at least some of the components with which it naturally occurs. In a preferred embodiment, an isolated component is at least 90% pure, and more preferably 95%.

ClfB has a predicted molecular weight of approximately 88 kDa and an apparent molecular weight of approximately 124 kDa. ClfB is a cell-wall associated protein and binds both soluble and immobilized fibrinogen. In addition, ClfB binds both the alpha and beta chains of fibrinogen and acts as a clumping factor. Despite the low level of identity between ClfA and ClfB, both proteins bind fibrinogen (on different chains) by a mechanism that is susceptible to inhibition by divalent cations, despite not sharing obvious metal binding motifs. The ClfB protein has been demonstrated to be a virulence factor in experimental endocarditis.

The SdrC, SdrD and SdrE proteins are related in primary sequence and structural organization to the ClfA and ClfB proteins and are localized on the cell surface. The SdrC, SdrD and SdrE proteins are cell wall-associated proteins, having a signal sequence at the N-terminus and an LPXTG (SEQ ID NO: 14) motif, hydrophobic domain and positively charged residues at the C-terminus. Each also has an SD repeat containing region R of sufficient length to allow, along with the B motifs, efficient expression of the ligand binding domain region A on the cell surface. With the A region of the SdrC, SdrD and SdrE proteins located on the cell surface, the proteins can interact with proteins in plasma, the extracellular matrix or with molecules on the surface of host cells. The Sdr proteins share some limited amino acid sequence similarity with ClfA and ClfB. Like ClfA and ClfB, SdrC, SdrD and SdrE also exhibit cation-dependent ligand binding of extracellular matrix proteins.

It was surprising to learn that the disclosed extracellular matrix-binding proteins share a unique dipeptide repeat region (region R) including predominately aspartate and serine residues. It had been reported by McDevitt et al., *Mol. Microbiol.* 11:237-248 (1994); McDevitt et al., *Mol. Microbiol.* 16:895-907 (1995) that ClfA also has this R repeat region. He reported that there were genes in *S. epidermidis* that hybridized to the gene encoding the R domain containing protein. However, McDevitt et al did not know the function of the R region and had not discovered that other cell surface proteins from *S. aureus*, *S. hemolyticus*, *S. lugdenensis*, *S. schleriferi* share this unusual motif. Therefore, in one aspect of this invention, a method is provided for the identification of genes and encoding proteins from *S. aureus* (other than ClfA), *S. hemolyticus*, *S. lugdenensis*, *S. schleriferi* useful for the prevention, treatment, and diagnosis of bacterial infection that includes using the R repeat region as an identifying probe.

The DS repeat is encoded by 18 nucleotide repeats with the consensus (where Y equals pyrimidines and N equals any base) GAYTCNGAYT CNGAYACY (SEQ ID NO: 9), with TCN as the first and second serine codons and AGY as the third serine codon. The R region is near the C-terminus of the proteins and typically contains between 40 and 300 DS residues, or more particularly, greater than 40, 60, 80, 100, 125, 150, 200 or 250 repeating units, of which greater than 90, 95 or even 98% of the amino acids are D or S. The R region DS repeat varies in length between proteins, and while the R region itself does not bind extracellular matrix proteins, the R region enables the presentation of the binding regions of the protein on the cell surface of *S. aureus*. Thus, probes to the consensus DNA encoding the DS repeat (see above) can be used to identify other genes encoding different binding proteins essential to the attachment of *S. aureus* to host tissues. Antibodies to an R region can be used to discover such additional binding proteins as well.

The sdr genes are closely linked and tandemly arrayed. The Sdr proteins have both organizational and sequence similarity to ClfA and ClfB. At the N-terminus secretory signal sequences precede A regions which are approximately 500 residues in length. The A regions of the Sdr and Clf proteins exhibit only 20-30% residue identity when aligned with any other member of the family.

It has been discovered that in the A region of SdrC, SdrD, SdrE, ClfA, and ClfB, there is highly conserved amino acid sequence that can be used to derive a consensus TYTFT-DYVD (SEQ ID NO: 18) motif. The motif exhibits slight variation between the different proteins. This variation, along with the consensus sequence of the motif is depicted in FIG. 20. In the Clf-Sdr proteins, this motif is highly conserved. The motif can be used in vaccines to impart broad spectrum cellular immunity to bacterial infections, and also can be used as an antigen in the production of monoclonal or polyclonal antibodies. Such an antibody can be used to impart broad spectrum passive immunity.

The Sdr proteins differ from ClfA and ClfB by having two to five additional 110-113 residue repeated sequences (B-motifs) located between region A and the R-region. Each B-motif contains a consensus Ca<sup>2+</sup>-binding EF-hand loop normally found in eukaryotic proteins. The structural integrity of a recombinant protein comprising the five B-repeats of SdrD was shown by bisANS fluorescence analysis to be Ca<sup>2+</sup>-dependent, suggesting that the EF-hands are functional. When Ca<sup>2+</sup> was removed the structure collapsed to an unfolded conformation. The original structure was restored by addition of Ca<sup>2+</sup>. The C-terminal R-domains of the Sdr proteins contain 132-170 SD residues. These are followed by conserved wall-anchoring regions characteristic of many surface proteins of Gram positive bacteria. The sdr locus was present in all 31 *S. aureus* strains from human and bovine sources tested by Southern hybridization, although in a few strains it contained two rather than three genes.

In the Sdr and Clf proteins this B motif is highly conserved while a degenerate version occurs in fibronectin binding MSCRAMMS, as well as the collagen binding protein Cna. The B motifs, in conjunction with the R regions, are necessary for displaying the ligand-binding domain at some distance from the cell surface.

The repeated B motifs are one common denominator of the sub-group of SD repeat proteins described herein. These motifs are found in different numbers in the three Sdr proteins from strain Newman. There are clear distinctions between the individual B motifs. The most conserved units are those located adjacent to the R regions (SdrC B2, SdrD B5 and SdrE B3). They differ from the rest at several sites,



especially in the C-terminal half. A noteworthy structural detail is that adjacent B repeats are always separated by a proline residue present in the C-terminal region, but a proline never occurs between the last B repeats and the R region. Instead this linker is characterized by a short acidic stretch. These differences are evidence that the end units have a different structural or functional role compared to the other B motifs. The N-terminal B motifs of SdrD and SdrE have drifted apart from the others, and there are numerous amino acid alterations, including small insertions and deletions whereas the remaining internal B motifs are more highly conserved. Note that each of the three Sdr proteins has at least one B motif of each kind.

The C-terminal R-domains of the Sdr proteins contain 132-170 SD residues. These are followed by conserved wall-anchoring regions characteristic of many surface proteins of Gram positive bacteria.

ClfB, SdrC, SdrD and SdrE subdomains are shown in FIG. 10 and, depending on the protein, include subdomains A and B1-B5.

The terms ClfB protein, SdrC protein, SdrD protein and SdrE protein are defined herein to include ClfB, SdrC, SdrD and SdrE subdomains, and active or antigenic fragments of ClfB, SdrC, SdrD and SdrE proteins, such as consensus or variable sequence amino acid motifs. Active fragments of ClfB, SdrC, SdrD, SdrE and consensus or variable sequence amino acid motifs peptides or proteins are defined herein as peptides or polypeptides capable of blocking the binding of *S. aureus* to extracellular matrix proteins. Antigenic fragments of ClfB, SdrC, SdrD, SdrE proteins or the consensus or variable amino acid motifs are defined herein as peptides or polypeptides capable of producing an immunological response.

#### Nucleic Acid Sequences

The nucleic acid sequences encoding ClfB, SdrC, SdrD, SdrE and the consensus or variable sequence amino acid motifs are useful for the production of recombinant extracellular matrix-binding proteins. They are further useful as nucleic acid probes for the detection of *S. aureus*-binding proteins in a sample or specimen with high sensitivity and specificity. The probes can be used to detect the presence of *S. aureus* in the sample, diagnose infection with the disease, quantify the amount of *S. aureus* in the sample, or monitor the progress of therapies used to treat the infection. The nucleic acid and amino acid sequences are also useful as laboratory research tools to study the organism and the disease, thus furthering the development of therapies and treatments for the disease.

It will be understood by those skilled in the art that ClfB, SdrC, SdrD, SdrE and the consensus or variable sequence amino acid motifs are also encoded by sequences substantially similar to the nucleic acid sequences provided in the sequence listing. By "substantially similar" is meant a DNA sequence which, by virtue of the degeneracy of the genetic code, is not identical with that shown in any of SEQ ID NOS:2, 4, 6, and 8, but which still encodes the same amino acid sequence; or a DNA sequence which encodes a different amino acid sequence but retains the activities of the proteins, either because one amino acid is replaced with another similar amino acid, or because the change (whether it be substitution, deletion or insertion) does not affect the active site of the protein. In the latter case, the sequence has substantial homology to the disclosed sequence if it encodes a protein with at least 70% 80%, 90%, 95% or even 98% of the same amino acids.

Also provided herein are sequences of nucleic acid molecules that selectively hybridize with nucleic acid molecules encoding the extracellular matrix-binding proteins from *S. aureus* described herein or complementary sequences thereof. By "selective" or "selectively" is meant a sequence which does not hybridize with other nucleic acids to prevent adequate detection of ClfB, SdrC, SdrD, SdrE or the consensus or variable sequence amino acid motifs. Therefore, in the design of hybridizing nucleic acids, selectivity will depend upon the other components present in a sample. The hybridizing nucleic acid should have at least 70% complementarity with the segment of the nucleic acid to which it hybridizes. As used herein to describe nucleic acids, the term "selectively hybridizes" excludes the occasional randomly hybridizing nucleic acids, and thus, has the same meaning as "specifically hybridizing". The selectively hybridizing nucleic acids of the invention can have at least 70%, 80%, 85%, 90%, 95%, 97%, 98%, and 99% complementarity with the segment of the sequence to which it hybridizes.

The invention contemplates sequences, probes and primers which selectively hybridize to the encoding DNA or the complementary, or opposite, strand of DNA as those specifically provided herein. Specific hybridization with nucleic acid can occur with minor modifications or substitutions in the nucleic acid, so long as functional species-specific hybridization capability is maintained. By "probe" is meant nucleic acid sequences that can be used as probes or primers for selective hybridization with complementary nucleic acid sequences for their detection or amplification, which probes can vary in length from about 5 to 100 nucleotides, or preferably from about 10 to 50 nucleotides, or most preferably about 18-24 nucleotides. Therefore, the terms "probe" or "probes" as used herein are defined to include "primers". Isolated nucleic acids are provided herein that selectively hybridize with the species-specific nucleic acids under stringent conditions and should have at least 5 nucleotides complementary to the sequence of interest as described by Sambrook, J., E. F. Fritsch, and T. Maniatis. 1989. MOLECULAR CLONING: A LABORATORY MANUAL, 2nd ed. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.

If used as primers, the composition preferably includes at least two nucleic acid molecules which hybridize to different regions of the target molecule so as to amplify a desired region. Depending on the length of the probe or primer, the target region can range between 70% complementary bases and full complementarity and still hybridize under stringent conditions. For example, for the purpose of diagnosing the presence of the *S. aureus*, the degree of complementarity between the hybridizing nucleic acid (probe or primer) and the sequence to which it hybridizes (e.g., *S. aureus* DNA from a sample) is at least enough to distinguish hybridization with a nucleic acid from other bacteria.

The nucleic acid sequences encoding ClfB, SdrC, SdrD, SdrE active fragments thereof or consensus or variable sequence amino acid motifs can be inserted into a vector, such as a plasmid, and recombinantly expressed in a living organism to produce recombinant ClfB, SdrC, SdrD and SdrE proteins or fragments thereof, such as consensus or variable sequence amino acid motifs. For example, DNA molecules producing recombinant ClfB, SdrC, and both SdrD and SdrE were deposited in plasmids pA1-1EX, pC1 and lambda phage A6-2, respectively, at the NCIMB under the Accession Nos. 40903, 40902 and 40904, respectively on Oct. 13, 1997.

Methods for the Detection and Identification of *S. aureus*

Methods of using the nucleic acids described herein to detect and identify the presence of *S. aureus* are provided. The methods are useful for diagnosing *S. aureus* infections and disease such as upper respiratory tract infections (such as otitis media, bacterial tracheitis, acute epiglottitis, thyroiditis), lower respiratory infections (such as emphysema, lung abscess), cardiac (such as infective endocarditis), gastrointestinal (such as secretory diarrhea, splenic abscess, retroperitoneal abscess), central nervous system (such as cerebral abscess), ocular (such as blepharitis, conjunctivitis, keratitis, endophthalmitis, preseptal and orbital cellulitis, dacryocystitis), kidney and urinary tract (such as epididymitis, intrarenal and perinephric abscess, toxic shock syndrome), skin (such as impetigo, folliculitis, cutaneous abscesses, cellulitis, wound infection, bacterial myositis, bone and joint (such as septic arthritis, osteomyelitis).

The method involves the steps of obtaining a sample suspected of containing *S. aureus*. The sample may be taken from an individual, such as a wound, blood, saliva, tissues, bone, muscle, cartilage, or skin. The cells can then be lysed, and the DNA extracted, precipitated and amplified. Detection of *S. aureus* DNA is achieved by hybridizing the amplified DNA with a *S. aureus* probe that selectively hybridizes with the DNA as described above. Detection of hybridization is indicative of the presence of *S. aureus*.

Preferably, detection of nucleic acid (e.g. probes or primers) hybridization can be facilitated by the use of detectable moieties. For example, the probes can be labeled with biotin and used in a streptavidin-coated microtiter plate assay. Other detectable moieties include radioactive labeling, enzyme labeling, and fluorescent labeling, for example.

DNA may be detected directly or may be amplified enzymatically using polymerase chain reaction (PCR) or other amplification techniques prior to analysis. RNA or cDNA can be similarly detected. Increased or decreased expression of ClfB, SdrC, SdrD, SdrE and consensus or variable sequence amino acid motifs can be measured using any of the methods well known in the art for the quantitation of nucleic acid molecules, such as, amplification, PCR, RT-PCR, RNase protection, Northern blotting, and other hybridization methods.

Diagnostic assays which test for the presence of the ClfB or SdrC, SdrD or SdrE proteins, peptides, motifs, fragments thereof or antibodies to any of these may also be used to detect the presence of an infection. Assay techniques for determining protein or antibody levels in a sample are well known to those skilled in the art and include methods such as radioimmunoassay, Western blot analysis and ELISA (Enzyme-Linked Immunosorbant Assay) assays.

## Amino Acid Sequences

It will be understood by those skilled in the art that minor amino acid substitutions or deletions may be present in functional ClfB, SdrC, SdrD, SdrE and consensus or variable sequence amino acid motifs, peptides, proteins, or fragments thereof. The amino acid sequences set forth herein and substantially similar amino acid sequences can be used to produce synthetic ClfB, SdrC, SdrD, SdrE and consensus or variable sequence amino acid motifs, peptides, proteins or active fragments thereof. Active ClfB, SdrC, SdrD, SdrE or consensus or variable sequence amino acid motifs, peptide or protein fragments are defined herein as ClfB, SdrC, SdrD, SdrE or consensus or variable sequence amino acid motifs, portions or peptides that bind to extracellular matrix proteins or compete with or prevent *S. aureus* organisms from

binding to extracellular matrix proteins such as decorin, plasmin, fibrinogen, vitronectin or bone sialoprotein.

When used in conjunction with amino acid sequences, the term "substantially similar" means an amino acid sequence which is not identical to SEQ ID NOS:1, 3, 5, or 7, but which produces a protein having the same functionality and retaining the activities of ClfB, SdrC, SdrD, SdrE and consensus or variable sequence amino acid motifs, either because one amino acid is replaced with another similar amino acid, or because the change (whether it be substitution, deletion or insertion) does not affect the active site of the protein or peptide. Two amino acid sequences are "substantially homologous" when at least about 70%, (preferably at least about 80%, and most preferably at least about 90% or 95%) of the amino acids match over the defined length of the sequences.

## Extracellular Matrix-Binding Protein Antibodies

The isolated, recombinant or synthetic ClfB, SdrC, SdrD, SdrE or consensus or variable sequence amino acid motifs, or peptides or active fragments thereof or fusion proteins thereof, are useful as scientific research tools to identify *S. aureus* binding sites on the extracellular matrix. This will promote an understanding of the mechanisms of bacterial pathology and the development of antibacterial therapies. Furthermore, the isolated, recombinant or synthetic protein, or antigenic portions thereof (including epitope-bearing fragments), or fusion proteins thereof can be administered to humans or animals as immunogens or antigens. It can be administered alone or in combination with an adjuvant, for the production of antisera reactive with ClfB, SdrC, SdrD, SdrE or motifs or peptides thereof. In addition, the peptides or proteins can be used to screen antisera for hyperimmune patients from whom can be derived antibodies having a very high affinity for the proteins.

Antibodies isolated from the antisera are useful for the specific detection of *S. aureus* or *S. aureus* extracellular matrix-binding proteins or as research tools. The term "antibodies" as used herein includes monoclonal antibodies, polyclonal, chimeric, single chain, bispecific, simianized, and humanized antibodies as well as Fab fragments, including the products of an Fab immunoglobulin expression library.

Monoclonal antibodies are generated by methods well known to those skilled in the art. The preferred method is a modified version of the method of Kearney, et al., *J. Immunol.* 123:1548-1558 (1979), which is incorporated by reference herein. Briefly, animals such as mice or rabbits are inoculated with the immunogen in adjuvant, and spleen cells are harvested and mixed with a myeloma cell line, such as P3X63Ag8,653. The cells are induced to fuse by the addition of polyethylene glycol. Hybridomas are chemically selected by plating the cells in a selection medium containing hypoxanthine, aminopterin and thymidine (HAT). Hybridomas producing the preferred antibodies are cloned, expanded and stored frozen for future production.

Techniques for the production of single chain antibodies are known to those skilled in the art and described in U.S. Pat. No. 4,946,778 and can be used to produce single chain antibodies to the proteins described herein. Phage display technology may be used to select antibody genes having binding activities for ClfB, SdrC, SdrD, SdrE, and consensus or variable sequence amino acid motifs, or antigenic portions thereof, from PCR-amplified *v* genes of lymphocytes from humans screened for having antibodies to ClfB, SdrC, SdrD, SdrE or consensus or variable sequence amino

acid motifs or naive libraries. Bispecific antibodies have two antigen binding domains wherein each domain is directed against a different epitope.

The antibody may be labeled directly with a detectable label for identification and quantitation of *S. aureus*. 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 and 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, such as protein A or G or second antibodies. 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.

Antibodies to the disclosed proteins may also be used in production facilities or laboratories to isolate additional quantities of the protein, such as by affinity chromatography.

The proteins, or antigenic portions thereof, are useful in the diagnosis of *S. aureus* bacterial infections and in the development of anti-*S. aureus* vaccines for active or passive immunization. When administered to a wound or used to coat polymeric biomaterials in vitro and in vivo, both the proteins and antibodies thereof are useful as blocking agents to prevent or inhibit the initial binding of *S. aureus* to the wound site or biomaterials. Preferably, the antibody is modified so that it is less immunogenic in the patient to whom it is administered. For example, if the patient is a human, the antibody may be "humanized" by transplanting the complementarity determining regions of the hybridoma-derived antibody into a human monoclonal antibody as described by Jones et al., *Nature* 321:522-525 (1986) or Tempest et al. *Biotechnology* 9:266-273 (1991).

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 devices, hard and soft contact lenses, intraocular lens implants (anterior chamber, posterior chamber or phakic), 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 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, 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, tubes used to drain the

body cavities such as the pleural, peritoneal, cranial, and 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 protein, antibody, or active fragment to a surface of the device, preferably an outer surface that would be exposed to *S. aureus* infection. The surface of the device need not be entirely covered by the protein, antibody or active fragment.

#### Immunological and Pharmaceutical Compositions

Immunological compositions, including vaccine, and other pharmaceutical compositions containing the ClfB, SdrC, SdrD, SdrE or consensus or variable sequence amino acid motif, peptides or proteins are included within the scope of the present invention. One or more of the ClfB, SdrC, SdrD, SdrE or consensus or variable sequence amino acid motif, peptides, proteins, or active or antigenic fragments thereof, or fusion proteins thereof can be formulated and packaged, alone or in combination with other antigens, using methods and materials known to those skilled in the art for vaccines. The immunological response may be used therapeutically or prophylactically and may provide antibody immunity or cellular immunity such as that produced by T lymphocytes such as cytotoxic T lymphocytes or CD4<sup>+</sup> T lymphocytes.

The immunological compositions, such as vaccines, and other pharmaceutical compositions can be used alone or in combination with other blocking agents to protect against human and animal infections caused by *S. aureus*. In particular, the compositions can be used to protect humans against endocarditis or to protect humans or ruminants against mastitis caused by *S. aureus* infections. The vaccine can also be used to protect canine and equine animals against similar *S. aureus* infections.

To enhance immunogenicity, the proteins may be conjugated to a carrier molecule. Suitable immunogenic carriers include proteins, polypeptides or peptides such as albumin, hemocyanin, thyroglobulin and derivatives thereof, particularly bovine serum albumin (BSA) and keyhole limpet hemocyanin (KLH), polysaccharides, carbohydrates, polymers, and solid phases. Other protein derived or non-protein derived substances are known to those skilled in the art. An immunogenic carrier typically has a molecular weight of at least 1,000 daltons, preferably greater than 10,000 daltons. Carrier molecules often contain a reactive group to facilitate covalent conjugation to the hapten. The carboxylic acid group or amine group of amino acids or the sugar groups of glycoproteins are often used in this manner. Carriers lacking such groups can often be reacted with an appropriate chemical to produce them. Preferably, an immune response is produced when the immunogen is injected into animals such as mice, rabbits, rats, goats, sheep, guinea pigs, chickens, and other animals, most preferably mice and rabbits. Alternatively, a multiple antigenic peptide comprising multiple copies of the protein or polypeptide, or an antigenically or immunologically equivalent polypeptide may be sufficiently antigenic to improve immunogenicity without the use of a carrier.

The ClfB, SdrC, SdrD, SdrE or consensus or variable sequence amino acid motif, peptide, protein or proteins may be administered with an adjuvant in an amount effective to enhance the immunogenic response against the conjugate. At this time, the only adjuvant widely used in humans has been alum (aluminum phosphate or aluminum hydroxide). Saponin and its purified component Quil A, Freund's com-

plete adjuvant and other adjuvants used in research and veterinary applications have toxicities which limit their potential use in human vaccines. However, 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 vesicles such as Novasome™ lipid vesicles (Micro Vesicular Systems, Inc., Nashua, N.H.) may also be useful.

The term "vaccine" as used herein includes DNA vaccines in which the nucleic acid molecule encoding ClfB, SdrC, SdrD, SdrE and consensus or variable sequence amino acid motifs, or nucleic acid molecules which are not identical to the disclosed sequences, but which are substantially homologous thereto and encode peptides or proteins which have the same functionality and activities, or antigenic portions thereof in a pharmaceutical composition is administered to a patient. For genetic immunization, suitable delivery methods known to those skilled in the art include direct injection of plasmid DNA into muscles (Wolff et al., *Hum. Mol. Genet.* 1:363 (1992)), delivery of DNA complexed with specific protein carriers (Wu et al., *J. Biol. Chem.* 264:16985 (1989), coprecipitation of DNA with calcium phosphate (Benvenisty and Reshef, *Proc. Natl. Acad. Sci.* 83:9551 (1986)), encapsulation of DNA in liposomes (Kaneda et al., *Science* 243:375 (1989)), particle bombardment (Tang et al., *Nature* 356:152 (1992) and Eisenbraun et al., *DNA Cell Biol.* 12:791 (1993)), and in vivo infection using cloned retroviral vectors (Seeger et al., *Proc. Natl. Acad. Sci.* 81:5849, 1984).

#### Methods of Administration and Dose of Pharmaceutical Compositions

Pharmaceutical compositions containing the ClfB, SdrC, SdrD or SdrE proteins, nucleic acid molecules, antibodies, or fragments thereof may be formulated in combination with a pharmaceutical carrier such as saline, dextrose, water, glycerol, ethanol, other therapeutic compounds, and combinations thereof. The formulation should be appropriate for the mode of administration. The compositions are useful for interfering with, modulating, or inhibiting *S. aureus* host cell binding interactions with the extracellular matrix.

Suitable methods of administration include, 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.

In a preferred embodiment, a vaccine is packaged in a single dosage for immunization by parenteral (i.e., intramuscular, intradermal or subcutaneous) administration or nasopharyngeal (i.e., intranasal) administration. The vaccine is most preferably injected intramuscularly into the deltoid muscle. The vaccine is preferably combined with a pharmaceutically acceptable carrier to facilitate administration. The carrier is usually water or a buffered saline, with or without

a preservative. The vaccine may be lyophilized for resuspension at the time of administration or in solution.

The carrier to which the protein may be conjugated may also be a polymeric delayed release system. Synthetic polymers are particularly useful in the formulation of a vaccine to effect the controlled release of antigens. For example, the polymerization of methyl methacrylate into spheres having diameters less than one micron has been reported by Kreuter, J., *MICROCAPSULES AND NANOPARTICLES IN MEDICINE AND PHARMACOLOGY*, M. Donbrow (Ed). CRC Press, p. 125-148.

Microencapsulation of the protein will also give a controlled release. A number of factors contribute to the selection of a particular polymer for microencapsulation. The reproducibility of polymer synthesis and the microencapsulation process, the cost of the microencapsulation materials and process, the toxicological profile, the requirements for variable release kinetics and the physicochemical compatibility of the polymer and the antigens are all factors that must be considered. Examples of useful polymers are polycarbonates, polyesters, polyurethanes, polyorthoesters polyamides, poly (d,l-lactide-co-glycolide) (PLGA) and other biodegradable polymers. The use of PLGA for the controlled release of antigen is reviewed by Eldridge, J. H., et al. *CURRENT TOPICS IN MICROBIOLOGY AND IMMUNOLOGY*, 146: 59-66 (1989).

One typical dose for human administration is from 0.01 mg/kg to 10 mg/kg. Based on this range, equivalent dosages for heavier body weights can be determined. 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 vaccine may additionally contain stabilizers such as thimerosal (ethyl(2-mercaptobenzoate-S)mercury sodium salt) (Sigma Chemical Company, St. Louis, Mo.) or physiologically acceptable preservatives.

#### Protein-Label Conjugates

When labeled with a detectable biomolecule or chemical, the extracellular matrix-binding proteins described herein are useful for purposes such as in vivo and in vitro diagnostics and laboratory research. Various types of labels and methods of conjugating the labels to the proteins are well known to those skilled in the art. Several specific labels are set forth below. The labels are particularly useful when conjugated to a protein such as an antibody or receptor.

For example, the protein can be conjugated to a radiolabel such as, but not restricted to, <sup>32</sup>P, <sup>3</sup>H, <sup>14</sup>C, <sup>35</sup>S, <sup>125</sup>I, or <sup>131</sup>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, phycoyanin, rhodamine, and Texas Red. The fluorogens are generally detected by a fluorescence detector.

The protein can alternatively be labeled with a chromogen to provide an enzyme or affinity label. For example, the protein can be biotinylated so that it can be utilized in a biotin-avidin reaction, which may also be coupled to a label such as an enzyme or fluorogen. For example, the protein can be labeled with peroxidase, alkaline phosphatase or other enzymes giving a chromogenic or fluorogenic reaction

upon addition of substrate. Additives such as 5-amino-2,3-dihydro-1,4-phthalazinedione (also known as Lumino<sup>TM</sup>) (Sigma Chemical Company, St. Louis, Mo.) and rate enhancers such as p-hydroxybiphenyl (also known as p-phenylphenol) (Sigma Chemical Company, St. Louis, Mo.) can be used to amplify enzymes such as horseradish peroxidase through a luminescent reaction; and luminogenic or fluorogenic dioxetane derivatives of enzyme substrates can also be used. Such labels can be detected using enzyme-linked immunoassays (ELISA) or by detecting a color change with the aid of a spectrophotometer. In addition, proteins may be labeled with colloidal gold for use in immunoelectron microscopy in accordance with methods well known to those skilled in the art.

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 those skilled in the art, such as immunofluorescence microscopy using procedures such as those described by Warren and Nelson, *Mol. Cell. Biol.* 7:1326-1337 (1987).

#### Screening Methods

The ClfB, SdrC, SdrD and SdrE proteins, or fragments thereof, such as consensus or variable amino acid motifs, are useful in a method for screening materials to identify substances that inhibit *S. aureus* host cell binding interactions with the extracellular matrix. In accordance with the method for screening, the substance of interest is combined with one or more of the ClfB, SdrC, SdrD, or SdrE proteins, or fragments thereof, such as consensus or variable sequence amino acid motif peptides, and the degree of binding of the molecule to the extracellular matrix is measured or observed. If the presence of the substance results in the inhibition of binding, then the substance may be useful for inhibiting *S. aureus* in vivo or in vitro. The method could similarly be used to identify substances that promote *S. aureus* interactions with the extracellular matrix.

The method is particularly useful for identifying substances having bacteriostatic or bacteriocidal properties.

For example, to screen for *S. aureus* agonists or antagonists, a synthetic reaction mixture, a cellular compartment (such as a membrane, cell envelope or cell wall) containing one or more of the ClfB, SdrC, SdrD, SdrE proteins, or fragments thereof, such as consensus or variable sequence amino acid motifs, and a labeled substrate or ligand of the protein is incubated in the presence of a substance under investigation. The ability of the substance to agonize or antagonize the protein is shown by a decrease in the binding of the labeled ligand or decreased formation of substrate product. Substances that bind well and increase the rate of product formation from substrate are agonists. Detection of the rate or level of formation of product from substrate may be enhanced by use of a reporter system, such as a calorimetric labeled substrate converted to product, a reporter gene that is responsive to changes in ClfB, SdrC, SdrD, SdrE or consensus or variable amino acid sequence motifs' nucleic acid or protein activity, and binding assays known to those skilled in the art. Competitive inhibition assays can also be used.

Potential antagonists include small organic molecules, peptides, polypeptides and antibodies that bind to ClfB, SdrC, SdrD, SdrE or consensus or variable sequence amino acid motifs' nucleic acid molecules or proteins and thereby inhibit their activity or bind to a binding molecule (such as fibrinogen) to prevent the binding of the ClfB, SdrC, SdrD, SdrE or consensus or variable sequence amino acid motifs' nucleic acid molecules or proteins to the binding molecule. For example, a compound that inhibits ClfB, SdrC, SdrD, SdrE or consensus or variable sequence amino acid motifs' activity may be a small molecule that binds to and occupies

the binding site of the ClfB, SdrC, SdrD, SdrE or consensus or variable sequence amino acid motif peptide or protein, thereby preventing binding to cellular binding molecules. Examples of small molecules include, but are not limited to, small organic molecules, peptides or peptide-like molecules. Other potential antagonists include antisense molecules. Preferred antagonists include compounds related to and variants or derivatives of ClfB, SdrC, SdrD, SdrE or consensus or variable sequence amino acid motif peptides or proteins.

The nucleic acid molecules described herein may also be used to screen compounds for antibacterial activity.

#### Therapeutic Applications

In addition to the therapeutic compositions and methods described above, the ClfB, SdrC, SdrD, SdrE or consensus or variable amino acid motifs, peptides or proteins, nucleic acid molecules or antibodies are useful for interfering with the initial physical interaction between a pathogen and mammalian host responsible for infection, to mammalian extracellular matrix proteins on indwelling devices or to extracellular matrix proteins in wounds they are further useful to block ClfB, SdrC, SdrD, SdrE, or active fragments thereof, including consensus or variable amino acid motifs, peptide or protein-mediated mammalian cell invasion. In addition, these molecules are useful to mediate tissue damage and to block the normal progression of pathogenesis in infections.

#### *S. aureus* Detection Kit

The invention further contemplates a kit containing one or more ClfB, SdrC, SdrD, SdrE proteins, peptides, or active fragments thereof, including consensus or variable amino acid motif-encoding nucleic acid probes. These probes can be used for the detection of *S. aureus* or *S. aureus* extracellular matrix-binding proteins in a sample. Such a kit can also contain the appropriate reagents for hybridizing the probe to the sample and detecting bound probe.

In an alternative embodiment, the kit contains one or more ClfB, SdrC, SdrD, or SdrE proteins, peptides or consensus or variable amino acid motif-specific antibodies, which can be used for the detection of *S. aureus* organisms or *S. aureus* extracellular matrix-binding proteins in a sample.

In yet another embodiment, the kit contains one or more ClfB, SdrC, SdrD or SdrE-proteins, or active fragments thereof, such as the consensus or variable sequence amino acid motifs, which can be used for the detection of *S. aureus* organisms or *S. aureus* extracellular matrix-binding antibodies in a sample.

The kits described herein may additionally contain equipment for safely obtaining the sample, a vessel for containing the reagents, a timing means, a buffer for diluting the sample, and a calorimeter, reflectometer, or standard against which a color change may be measured.

In a preferred embodiment, the reagents, including the protein or antibody, are lyophilized, most preferably in a single vessel. Addition of aqueous sample to the vessel results in solubilization of the lyophilized reagents, causing them to react. Most preferably, the reagents are sequentially lyophilized in a single container, in accordance with methods well known to those skilled in the art that minimize reaction by the reagents prior to addition of the sample.

#### EXAMPLES

The present invention is further illustrated by the following non-limiting examples, which are not to be construed in any way as imposing limitations upon the scope thereof. On the contrary, it is to be clearly understood that resort may be had to various other embodiments, modifications, and equivalents thereof which, after reading the description

## 21

herein, may suggest themselves to those skilled in the art without departing from the spirit of the present invention.

## Example 1

## Gene Cloning, Sequencing and Expression

A fibrinogen-binding protein gene, designated *clfB*, was isolated, cloned and sequenced as follows:

## 22

Bacterial Strains and Growth Conditions The *E. coli* and *S. aureus* strains used for the cloning and sequencing of *clfB* are listed in Table 1, below. *Escherichia coli* was routinely grown on L-broth or agar. *S. aureus* was routinely grown on trypticase soy broth (Oxoid) or agar. The following antibiotics were incorporated into media where appropriate: ampicillin (Ap), 100 µg/ml; tetracycline (Tc), 2 µg/ml; chloramphenicol (Cm), 5 µg/ml; erythromycin (Em) 10 µg/ml.

TABLE 1

Bacterial strains used in the present study			
Bacterial strain	Genotype	Relevant properties/ Use in present study	Source/reference
<i>E. coli</i>			
C600	F <sup>-</sup> , lacY1, leuB6, supE44, thi-1, thr-1, tonA21	Propagation of lambda recombinants	Appleyard, Genetics 39: 440-452 (1954)
DH5α	F <sup>-</sup> , ø80dlacZM15, deoR, endA1, gyrA96, hsdR17, (r <sub>k</sub> <sup>-</sup> , m <sub>k</sub> <sup>+</sup> ), (lacZYA-argF)U169, recA1, relA1, supE44, thi-1	Recombination deficient, host strain for plasmids and for DNA sequencing	Hanahan et al., J. Mol. Biol. 166: 557-580 (1983)
JM101	supE, thi-1, (lac-proAB), [F' traD36, proAB, lacI <sup>q</sup> ZM15]	Host strain for plasmid bank and for sequencing	Stratagene (La Jolla, CA)
LE392	F <sup>-</sup> , (r <sub>k</sub> <sup>-</sup> , m <sub>k</sub> <sup>+</sup> ), galK2, galT22, hsdR574, lacY1 or (lacIZY)6, metB1, supE44, supF58, trpR55	Propagation of lambda recombinants	Promega Corp. (Madison, WI)
XL-1 Blue		[F' proAB, lacI <sup>q</sup> ZM15, Tn10(tc <sup>r</sup> ), endA1, gyrA96, hsdR17, lac, recA1, relA1, supE44, thi-1	Propagation of plasmidsStratagene
<i>S. aureus</i>			
Newman		Strong adherence to fibrinogen	NCTC 8178; Duthie and Lorenz, J. Gen. Microbiol. 6: 95-107 (1952)
DU5876		<i>clfA2</i> ::Tn917, Em <sub>r</sub>	McDevitt et al., Mol. Microbiol. 11: 237-248 (1994)
DU5943		<i>clfB</i> ::Tc <sub>r</sub> , Tc <sub>r</sub>	described herein
DU5944		<i>clfA</i> <i>clfB</i> , Em <sub>r</sub> , Tc <sub>r</sub>	described herein
DU5874		<i>spa</i> ::Tc <sub>r</sub>	Protein A-defective mutant of NewmanMcDevitt et al., Mol. Microbiol. 16: 895-907 (1995)
Δ map			McDevitt, unpublished
8325-4		NCTC 8325 cured of prophages	Novick, Virology 33: 155-166 (1967)
ISP546		<i>agr</i> ::Tn551	8325-4 agrBrown and Pattee, Infect. Immun. 30: 36-42 (1980)
RN4220		Restriction deficient derivative of 8325-4	Kreiswirth et al., Nature 305: 709-712 (1983)
V8		Classic V8 protease producer, produces PV leukocidin	ATCC 27733
Cowan 1		Classic protein A producer, adheres well to fibrinogen and fibronectin	ATCC 12598
RN4282		TSST-1 producer	Kreiswirth et al., 1983 (as 3-14)

TABLE 1-continued

Bacterial strains used in the present study			
Bacterial strain	Genotype	Relevant properties/ Use in present study	Source/reference
Phillips		Collagen binding strain	Patti et al., <i>Infect. Immun.</i> 62: 152-161 (1994)
V13		Septicaemia isolate	O'Reilly et al., <i>Mol. Microbiol.</i> 4: 1947-1955 (1990)
GH13		Methicillin resistant	Poston and Li Saw Hee, <i>J. Med. Microbiol.</i> 34: 193-201 (1991)
P1		Rabbit virulent strain	Sherertz et al., <i>J. Infect. Dis.</i> 167: 98-106 (1993)
M60		Bovine mastitis isolate	Anderson, <i>Zentralbl. Bakteriologie, Parasitenkunde, Infektionskrankheiten und Hygiene</i> 176: 783-790 (1976)

#### DNA Manipulation

Unless otherwise specified, DNA manipulations were done according to standard methods as described by Ausubel et al., *CURRENT PROTOCOLS IN MOLECULAR BIOLOGY*. New York, John Wiley and Sons (1987) and Sambrook et al., *MOLECULAR CLONING: A LABORATORY MANUAL*, 2nd ed. Cold Spring Harbour, N.Y., Cold Spring Harbour Laboratory Press (1989). Enzymes for DNA manipulation were obtained from New England Biolabs (Beverly, Mass.) and Promega (Madison, Wis.), and used as directed by the manufacturer. Genomic DNA from *S. aureus* Newman was prepared according to methods of Muller et al., *Infect. Immun.* 61:551-558 (1993).

Smaller scale preparations were made by lysing cells in phosphate buffered saline (PBS) containing 12 µg/ml lyso-staphin and 20 mM EDTA (ethylenediaminetetraacetic acid), followed by protease K treatment (500 µg/ml in 1% SDS) for 1 hour at 60° C., extraction with phenol and chloroform, and dialysis against 10 mM Tris HCL, pH 8.0, 1 mM EDTA. Plasmid DNA was prepared from *S. aureus* according to the method of Vriesema et al., *Appl. Environ. Microbiol.* 62:3527-3529 (1996). *E. coli* plasmid DNA for use in polymerase chain reaction (PCR) and sequencing was routinely made by the modified alkaline lysis method of Feliciello and Chinali, *Anal. Biochem.* 212:394-401 (1993), and occasionally by large scale isolation and dye-buoyant density centrifugation. Screening of *E. coli* transformants for chimeric plasmids was routinely done by the rapid colony lysis procedure of Le Gouill and Dery, *Nucl. Acids Res.* 19:6655 (1994).

#### Cloning of Repeat-containing Loci

A genomic library of *S. aureus* Newman was constructed in the LambdaGEM-12 replacement vector (obtained as prepared XhoI half-site arms from Promega Madison, Wis.) according to the manufacturer's instructions. Oligonucleotide probes specific for regions A and R of *S. aureus* Newman were made by polymerase chain amplification of these regions from the cloned gene on pCF14, as described by McDevitt and Foster, *Microbiology* 141:937-943 (1995), and random-primer labeled with [alpha-<sup>32</sup>P]dATP using the Promega Prime-a-Gene™ kit (Promega). The bank was

25

screened by Southern blotting, using an overnight hybridization temperature of 65° C. Selected clones were single plaque purified twice, and plate-lysate stocks made for storage and for inoculation of liquid cultures for the large-scale preparation of phage for DNA isolation.

30

A 3.87-kb HindIII fragment containing homology to region R DNA was cloned from the genome of *S. aureus*. HindIII-cleaved genomic DNA in the range of 3-4 kb was excised from an agarose gel, purified, and ligated to the pBluescript cloning vector. Plasmids were transformed into *E. coli* JM101 and identification of a recombinant *E. coli* containing a region R DNA insert was identified by PCR screening. PCR products were generated using primers specific for region R DNA. Individual colonies within a pool producing a positive PCR reaction were then analyzed for their potential to generate a PCR product. One transformant, pC1, was identified and found to contain the 3.87-kb fragment with homology to region R.

35

#### DNA Sequencing

The DNA sequence of *clfB* was obtained from pA1-1EX, a plasmid containing a fragment subcloned from recombinant phage A1-1 into pGEM 7Z (f)+. Nested deletions were made using the Erase-a-Base™ Kit (Promega). The Flash Dye Primer Sequencing Kit (Genpak) was used for sequencing in a Model 373A sequencing system (Applied Biosystems, Foster City, Calif.). Confirmatory sequencing in the forward direction was carried out. Double stranded sequencing of *sdrD* and *sdrE* was done on the subclones pEJ1, pEJ2 and pEJ3, containing fragments subcloned from recombinant phage A6-2 in pGEM 7Z (f)+, by nested deletions and primer walking. Automated sequence analysis of *sdrC* and the 5' end of *sdrD* on plasmid clone pC1 was performed. Sequence analysis was performed on both strands by primer extension to known sequences.

40

#### Screening of *S. aureus* Strains for *clfB* Homologues

A probe specific for the region A-encoding portion of *clfB* was made by excising a 614 bp internal *AccI* fragment from pA1-1EX, purifying from an agarose gel using the GENECLEAN II™ kit (BIO 101 Inc., La Jolla, Calif.), and labeling with [alpha-<sup>32</sup>P]dATP as described in FIG. 2. A

45

50

55

60

65

## 25

probe was similarly made to distal regions of the gene (encoding region R, the wall and membrane-spanning regions, and about 100 bp of downstream DNA), using a 1.2 kb BamHI fragment from pA1-1EX. HindIII digests of genomic DNA from a panel of strains were Southern blotted and screened using these probes.

Expression of *clfB* Region A

Region A (encoding residues S45 to N542) of *clfB* was amplified from pA1-1EX by PCR using the following primers:

## Forward:

5' CGAGGATCCTCAGGACAATCGAACGATACAACG (SEQ ID NO:15)  
3'

## Reverse:

5' CGAGGTACCATTACTGCTGAATCACC 3'. (SEQ ID NO:16)

Cleavage sites for BamHI and KpnI (underlined) were appended to the 5' ends of the respective primers to introduce these sites into the product and facilitate its cloning into expression vectors. The forward primer was subsequently found to include a single base mismatch (G, underlined), changing an E codon to a G codon. Reaction mixtures (50 µl) contained 2 mM dNTPs, 1.5 mM MgCl<sub>2</sub>, 1 ng pA1-1EX, 50 nM primers and 1.25 U Taq polymerase in standard Promega (Madison, Wis.) Taq reaction buffer. Amplification proceeded in a Perkin Elmer Cetus (Foster City, Calif.) thermocycler with an initial denaturation at 94° C. for 4 minutes, followed by 30 cycles with denaturation at 94° C. for 1 minute, annealing at 50° C., and extension at 72° C. for 1.5 minutes, with minimum heating and cooling between steps. The final extension was for 10 minutes. A single product was obtained, which was purified using the Wizard<sup>TM</sup> PCR purification kit (Promega). The product was initially cloned into the His-tag expression vector pQE30. However, because high-level expression was not obtained in this system, the product was recloned into an alternative vector, the GST fusion vector pGEX-KG, between the BamHI and HindIII sites. The recombinant protein was recovered from lysates by affinity chromatography on glutathione-sepharose (GST Gene Fusion System<sup>TM</sup>, Pharmacia, Piscataway, N.J.) and from the glutathione-S-transferase fusion partner by thrombin cleavage.

## Cloning of Repeat Carrying Loci

A library of *S. aureus* Newman genomic DNA was made using the replacement lambda vector LambdaGEM<sup>TM</sup>-12. About 10 000 plaques were screened using the region R-specific probe. Of the 60 positive plaques retained, 26 were purified and counter-screened with a *clfA* region A-specific probe. One plaque hybridized with the latter, indicating that it contained the *clfA* gene; of the remaining, non-hybridizing plaques, three were selected at random, and the DNA isolated. The DNA was cut with several restriction enzymes and analyzed by Southern blotting using the region R probe. Clones A1-1 and A2-3 appeared to contain overlapping sequences. Restriction mapping and Southern blotting indicated that these clones contained a single region R homologue. Clone A6-2 was found to contain three region R homologues, since cleavage with EcoRV yielded three fragments hybridizing to the region R probe.

Clone A1-1 was chosen for more detailed study, as the hybridizing fragment was slightly longer than in clone A2-3. A 7.4 kb EcoRI fragment containing the repeat region was subcloned from lambda clone A1-1 into plasmid pGEM 7Z f(+) to generate plasmid pA1-1E. This insert was reduced to

## 26

approximately 3 kb by excision of a 4.4 kb XbaI segment to form pA1-1EX as shown in FIGS. 2 and 3.

Clone A6-2 was restriction mapped and fragments subcloned into plasmid vectors for sequencing as shown in FIG. 4. Southern blotting with the region R probe and preliminary sequencing suggested that there were three tandemly arrayed genes carrying region R encoding sequences. On A6-2 there were two complete ORFs, *sdrD* and *sdrE*, and one incomplete ORF, *sdrC*.

The two complete ORFs were sequenced on fragments subcloned from lambdaA6-2 into plasmid vectors pGEM7Z f(+) (subclones pEJ1 and pEJ2) and pBluescript KS+ (subclone pEJ3). *sdrC* was cloned separately from *S. aureus* genomic DNA. A 3.87-kb HindIII fragment of strain Newman was cloned directly into plasmid pBluescript KS+, generating clone pC1 (FIG. 4). This clone, containing a region R DNA insert, was identified by PCR screening. The sequence of *sdrC* and the 162 bp at the 5' end of *sdrD* were determined from pC1.

Plasmid pA1-1EX, carrying the *clfB* gene, was deposited at the National Collections of Industrial and Marine Bacteria on Oct. 13, 1997 under the Accession No. 40903. Plasmid pC1, carrying the gene for *sdrC*, was deposited at the National Collections of Industrial and Marine Bacteria under the Accession No. NCIMB 40902 on Oct. 13, 1997 and a recombinant lambda phage A6-2, carrying the *sdrD* and *sdrE* genes, was deposited at the NCIMB on Oct. 13, 1997 under the Accession No. NCIMB 40904. All deposits comply with the terms of the Budapest Treaty.

## Features of ClfB

The translated open reading frame (ORF) contained within pA1-1EX is shown in FIG. 5. The ORF shows features reminiscent of secreted proteins of Gram positive cocci. Although the entire ORF is shown in FIG. 5, the start codon is unlikely to be the N codon. There is no ATG codon at the 5' end of the ORF. However, GTG and TTG are occasionally used as translational start codons in *S. aureus*, although methionine is the actual amino acid residue inserted, e.g., the fibronectin binding proteins (GTG), and protein A (TTG). The first TTG codon (L) may well be the initiation codon, as a possible ribosome binding site, GGAG, is suitably located upstream, starting at position-12. The N-terminal 44 amino acid residue region thus predicted has properties similar to signal sequences of secreted proteins of Gram positive cocci, i.e., an initial stretch of 19 mostly polar residues, with an overall positive charge, followed by 18 neutral residues with a high content of hydrophobic residues, and finally a short stretch of mainly polar residues with a good consensus cleavage site, AQA-S.

If the above prediction of the signal sequence is correct, region A of ClfB is 498 residues long, and shows 26.3% residue identity with the equivalent region in ClfA, or 44.4% homology when conservative substitutions are included. The most marked stretch of amino acid similarity between ClfA and ClfB occurs between residues 314-329 (ClfA) and 304-319 (ClfB), with 7 identical and 5 conserved residues. In ClfA, the stretch overlaps the C-terminal half of a putative Ca<sup>2+</sup> binding loop, EF hand I, required for fibrinogen binding as shown in FIG. 6. The sequence DYSNS (SEQ ID NO:11), which obeys the consensus for the N-terminal moiety of a MIDAS motif, occurs a short distance upstream. Accordingly, the downstream sequence was inspected for D and T residues to complete the motif. D and T occur frequently throughout the protein, and T 339 is suitably located, 63 residues downstream. However, the consensus would require a D residue 14-23 residues downstream from



the T, and in the present case, the nearest D residues are 9 or 28 residues away (D 348 and D 367).

At the C-terminal end of region A, a prominent proline-rich region occurs (21/42 residues are P; as shown in FIG. 5). There is a 14-residue repeat within this sequence. The DNA encoding the P-rich repeats is highly conserved. Of the three base substitutions, only one results in an amino acid replacement, a conservative substitution of S for T.

Region R is somewhat shorter in *clfB* than in *clfA* (272 residues instead of 308). The region R encoding sequence comprises the 18-bp consensus repeat observed in the equivalent part of *clfA*.

Following region R is a short stretch of predominantly hydrophilic residues, containing the distinctive LPETG (SEQ ID NO: 22) motif near its C-terminal end, presumably the cell-sorting signal. The C-terminal region of the predicted protein shows strong homology with the corresponding region in *ClfA*, with an initial stretch of mostly hydrophobic residues in a final stretch rich in positively charged residues, reminiscent of membrane spanning and anchoring domains, respectively. The general organization of *ClfA* and *ClfB* is compared in FIG. 1.

A putative transcription termination signal occurs 3' to *clfB*. No open reading frames occur within 260 bp 5' or 200 bp 3', suggesting that the gene is not part of an operon.

#### Features of SdrC, SdrD and SdrE

The DNA sequences and the translated amino acid sequences of *sdrC*, *sdrD* and *sdrE* are shown in FIGS. 7, 8 and 9. Each predicted protein has a putative signal sequence, an approximately 500 residue "region A" with limited homology to region A of *ClfA* (see FIG. 10), variable numbers of B repeats, an SD repeat containing region F, and LPXTG (SEQ ID NO: 14) cell wall sorting motif, a hydrophilic membrane anchor, and positively charged residues at the extreme C terminus.

The organization of the five region R containing proteins is shown in FIG. 10. The A regions of *SdrC*, *SdrD* and *SdrE* have limited sequence similarity to each other and to those of *ClfA* and *ClfB* as shown in FIG. 11. Alignments of those sequences more strongly conserved between all five proteins are shown in FIG. 12. The consensus motif TYTFTDYVD (SEQ ID NO: 18) overlaps the EF hand 1 motif of *ClfA* (alignment 2, FIG. 12). This region of *ClfA* has been shown to be of crucial significance in its ligand (fibrinogen) binding activity as described by O'Connell et al., *J. Biol. Chem.*, 273:6821-6829 (1998), and may also be of importance in the biological activity of the new proteins.

The three proteins *SdrC*, *SdrD* and *SdrE* form a separate subgroup of region R containing proteins: in addition to regions R and A they contain variable numbers of B repeats, located between region A and region R. The B repeats are 110-113 amino acids long and show considerable similarity (alignment 5, FIG. 12). The repeats *SdrC* B2, *SdrD* B5 and *SdrE* B3 adjacent to region R are 93-95% identical. There is a strongly conserved EF hand near the N-terminal end of each repeat.

#### *clfB* Homologues in Other *S. aureus* Strains

Nine strains of *S. aureus* were screened for the *clfB* gene by Southern blotting. Genomic DNA was cut to completion with *Hind*III, and probed with an internal 0.6 kb *Acc*I fragment of the region A coding sequence of *clfB*, shown in FIG. 2. The probe recognized a single *Hind*III fragment varying from 2 to 3 kb in length in all nine strains, indicating that each possesses a single *clfB* allele. A probe made from the region R and distal regions of *clfB* recognized an

identical band in all strains, indicating that the *clfB* homologues in other strains also contain region R.

#### Expression of *clfB*

The portion of *clfB* encoding region A was amplified by PCR using primers incorporating suitable 5' restriction sites, and cloned into the *E. coli* expression vector pGEX-KG. A protein of 94.3 kDa was detected in lysates in induced bacteria. The GST-*ClfB* fusion protein was immobilized on a glutathione sepharose affinity column, cleaved with thrombin, and examined by SDS-PAGE. The predominant band was 42 kDa, whereas the calculated molecular weight of region A is 54 kDa. This protein was used to raise antibody in rabbits, to probe Western blots of cell lysates made from strain Newman grown under a variety of conditions, as described below. The antibody failed to detect any antigens in lysates made from plate cultures, statically grown broth cultures, or shake-flask cultures grown to stationary phase. A single 124-kDa band was detected in lysates made from exponential phase shake-flask cultures of strain Newman and derivatives. If it is assumed that processing removes the signal sequence and the C-terminal portion of the protein from the last G of the LPETG (SEQ ID NO:22), the predicted molecular weight of *ClfB* is 88.3 kDa. In a time-course of *ClfB* production by a shake-flask culture of strain Newman, the *ClfB* protein was most abundant in the early exponential phase and showed a sharp decline toward the end of exponential phase, after which levels became undetectable. The results of the time-course study are shown in FIG. 13.

#### Example 2

##### Production of Anti-*ClfB* Serum

Antibodies to recombinant region A were raised in two young New Zealand white rabbits (2 kg) showing no prior reaction with *E. coli* or *S. aureus* antigens in Western blots. Injections, given subcutaneously, contained 25 µg of the antigen, diluted to 500 µl in phosphate buffered saline (PBS) emulsified with an equal volume of adjuvant. The initial injection contained Freund's complete adjuvant; the two to three subsequent injections, given at two-week intervals, contained Freund's incomplete adjuvant. When the response to the recombinant protein was judged adequate, the rabbits were bled, serum recovered, and total IgG purified by affinity chromatography on protein A sepharose (Sigma Chemical Co., St. Louis, Mo.).

##### SDS-PAGE and Western Blotting

Samples were analyzed by SDS-PAGE in 10 or 12% acrylamide gels. Isolated proteins and *E. coli* cell lysates were prepared for electrophoresis by boiling for five minutes in denaturation buffer. For *S. aureus*, cells were suspended to an OD<sub>600</sub> of 40 units in 100 mM PBS containing 10 mM EDTA. To each 500 µl sample, 40 µl protease inhibitors (Complete™ cocktail, Boehringer Mannheim, Indianapolis, Ind.), 5 µl each of DNase and RNase (from 10 mg/ml stocks, Sigma Chemical Co.), and 60 µl of a 2 mg/ml lysostaphin stock (Ambicin L™ recombinant lysostaphin, Applied Microbiology Inc., Tarrytown, N.Y.) were then added and the suspension incubated in a 37° C. water bath until it cleared. The samples were then processed as usual. Gels were stained with Coomassie blue or transferred to Nytran™ membrane by Western blotting in the Bio-Rad Semidry™ system (Bio-Rad Laboratories, Richmond, Calif.). For detection of native *ClfB* in *S. aureus*, blots were processed using the BM Chemiluminescence Detection Sys-

29

tem™ (POD) of Boehringer Mannheim, according to the manufacturer's instructions. Primary anti-ClfB antibody was used at a 1/1000 dilution, for a two hour incubation at room temperature. Protein A conjugated with horse radish peroxidase (Sigma Chemical Co.) was used to detect bound antibody, diluted 1/2000 for a one hour incubation at room temperature. Blots requiring less sensitivity were treated in a similar way, except that 5% skim milk was used as a blocking agent, and the blots were developed using chloronaphthol and hydrogen peroxide.

To determine whether ClfB is cell wall-associated, whole cells from an exponential phase culture were treated with lysostaphin in buffer supplemented with 30% raffinose to stabilize the protoplasts. The protoplasts were harvested, and the protoplasts and supernatant analyzed separately by Western blotting. ClfB protein was detected only in the supernatant, indicating that all ClfB was cross-linked to the peptidoglycan, and could be released by lysostaphin without disruption of the protoplast.

ClfB expression was enhanced by growth in rich media, such as tryptone soy broth or brain heart infusion.

Several *S. aureus* strains known to contain *clfB* alleles were screened for ClfB production by Western blotting. Cultures were harvested in early exponential phase to maximize expression. Of the nine strains examined, 8325-4, RN4282, and V13 expressed immunoreactive antigens of similar size and intensity to that of Newman, whereas strains GH13 and P1 had very weak bands of this size. Strains P1, Cowan and M60 expressed smaller immunoreactive antigens which may be degradation products. Strains V8 and Phillips expressed no detectable ClfB protein. Strain RN4220, which was derived from 8325-4, expressed exceptionally high levels of ClfB.

#### Example 3

##### Immunoassay for ClfB Using Biotinylated Recombinant ClfB Region A

The DNA encoding region A of *clfB* (encoding residues S45 to N542) was amplified from genomic DNA of *S. aureus* Newman using the following primers:

Forward:

5' CGAAAGCCTTGTTCAGAACAAATCGAACGATACAAC (SEQ ID NO:17)  
G 3'

Reverse:

5' CGAGGATCCATTTACTGCTGAATCACC 3' (SEQ ID NO:16)

Cleavage sites for HindIII and BamHI (underlined) were appended to the 5' ends of the respective primers to facilitate cloning of the product into the His-tag expression vector pV4. Cloning employed *E. coli* JM101 as a host strain. The recombinant region A was purified by nickel affinity chromatography.

##### Enzyme Linked Immunosorbent Assay (ELISA)

Immulon 1™ plates (Dynatech™, Dynal, Inc., Great Neck, N.Y.) were coated overnight with 100 µl of 10 µg/ml human fibrinogen (Chromogenix). They were then blocked with 200 µl of 5 mg/ml bovine serum albumin (BSA) for one hour. The plates were then incubated for three hours with 100 µl biotinylated ClfB (His-tag recombinant region A) diluted to 0.1-10 µg/ml. They were then given three five-minute washes with PBS containing 0.02% Tween 20 and 1 mg/ml BSA. The plates were then incubated for one hour with 100 µl of a 1/10 000 dilution of streptavidin conjugated

30

with alkaline phosphatase (Boehringer Mannheim, Indianapolis, Ind.), and washed as before. The plates were then developed for 30 minutes at 37° C. with 100 µl of 1 M diethanolamine, pH 9.8, containing 1 mg/ml p-nitrophenyl phosphate (Sigma Chemical Co.). Plates coated with BSA only were used as negative controls. The absorbance was measured at 405 nm.

##### Western Affinity Blotting

A 20 µg quantity of human fibrinogen (Chromogenix) was subjected to SDS-PAGE on a 15% acrylamide gel for two hours. Proteins were transferred to nitrocellulose at 100 V for two hours. The membrane was blocked overnight in PBS containing 10% nonfat dry milk. The blot was then incubated with 2.5 µg/ml biotinylated ClfB (His-tag recombinant region A) for one hour with shaking, the biotinylation being performed with EZ link-sulfo-NHS-LC-Biotin™ (Pierce, Rockford, Ill.). The blot was then given three five-minute washes in PBS containing 0.1% Tween 20. The blot was then incubated for one hour with avidin conjugated with horseradish peroxidase (Boehringer Mannheim) at a 1/200,000 dilution. The blot was then washed as before, and developed using the enhanced chemiluminescence system of Amersham (Little Chalfont, Bucks, UK). The band profile was compared with that obtained by subjecting fibrinogen to SDS-PAGE and Coomassie Blue staining.

In a Western affinity blot, in which biotinylated purified ClfB region A was used to probe blotted fibrinogen, a comparison with a lane of stained fibrinogen indicated that ClfB bound the alpha and beta-chains of fibrinogen. No bands were seen when ClfB was omitted. This experiment shows an important difference with ClfA, which is known to bind to the gamma-chain of fibrinogen.

#### Example 4

##### Mutagenesis of *clfB*

An insertion mutation in *clfB* was created by introducing a fragment containing a Tc resistance marker into the middle of the gene on pA1-1EX as shown in FIG. 3. The 2.35-kb HindIII fragment from pT181 was filled in with Klenow enzyme, and blunt-end ligated into the HpaI site of pA1-1EX. Plasmid pTS2, with temperature sensitive replication and a Cm<sup>r</sup> marker, was cloned into this construct at the SmaI site by cleaving with Aval. This cloning step was carried out in *E. coli*, and transformants were selected on Ap and incubated at 30° C. to avoid selection of revertants to temperature independence. The plasmid was then purified and transformed into *S. aureus* RN4220 by electroporation and Tc<sup>r</sup> transformants selected at 30° C. Five independent broth cultures grown at 30° C. were diluted 1/100 in fresh medium without antibiotics, and grown at 42° C. for six hours or 18 hours. The cultures were then diluted 1/100 and incubated at 42° C. for another time period. Six such dilutions and incubations were made, by which time Tc resistance had declined to approximately 1/1000 colony forming units (CFU). The cultures were then diluted to give approximately 100 CFU per plate on medium containing Tc, and incubated overnight at 37° C. Colonies which were Tc<sup>r</sup> but Cm<sup>s</sup> were presumed to have undergone a double crossover event between the plasmid and host genome, leading to replacement of the wild-type gene with the mutated one, with subsequent loss of the plasmid. Five hundred colonies were screened per culture. Eleven presumptive mutants were isolated from four of the five cultures. Four representative mutants were selected and genomic DNA isolated. Mutant

DU5944, deficient in both *clfA* and *clfB*, was constructed by transducing *clfA2::Tn917* from strain DU5876 into *clfB* mutant DU5943, selecting for *Em<sup>r</sup>*.

To determine whether mutations known to affect exoprotein expression influenced *clfB*, strain 8325-4 and the agr mutant ISP546 were compared. No significant differences in the level or dynamics of ClfB expression were noted.

To determine the role of ClfB in bacteria-fibrinogen interactions, a *clfB* mutant of strain Newman was constructed by allele replacement as shown in FIG. 2. Genomic DNA of the mutant was digested with BamHI and subjected to Southern blotting with a labeled 1.3 kb HpaI fragment from plasmid pA1-1E containing the 5' half of *clfB* and about 150 bp of upstream sequence. A single band hybridized in each case, but as expected, the band was 2.35 kb longer in the mutant than in the wild-type. The mutation was initially isolated in RN4220 and then transduced into strain Newman, forming strain DU5943.

#### Overexpression of ClfB and Complementation of *clfB* Mutation

Overproduction of ClfB was enabled by subcloning a SmaI fragment containing the *clfB* gene and 500 bp of upstream DNA from pA1-1E into the high copy number shuttle plasmid pCU1. The construct was then transformed into strain RN4220 and transduced into strain Newman. Transductants were selected on Cm. Southern and Western blotting confirmed that the high copy number was maintained in strain Newman, and that ClfB was produced at higher than wild-type levels, indicating that the upstream DNA contained the promoter necessary for expression of the *clfB* gene. Transduction of the construct into *clfB* mutants restored ClfB synthesis to higher than wild-type levels. The construct was also transduced into *clfAclfB* double mutants for use in complementation studies.

To create a *clfAclfB* double mutant, a *clfA::Tn917* mutation was transferred by transduction from strain DU5876 into the *clfB::Tc<sup>r</sup>* mutant DU5943, forming DU5944. The wild-type *clfB<sup>+</sup>* gene was cloned into shuttle plasmid pCU1 to give plasmid pA1-1EA, which was introduced into the *clfAclfB* mutant by transduction to test complementation. Western blotting with anti-ClfB serum showed that the ClfB protein was missing in mutant DU5943. It was expressed at a higher level than the wild-type in mutants carrying the complementing plasmid pA1-1EA, indicating overexpression of the protein due to gene dosage effect.

#### Example 5

##### ClfB Binding Assays

##### Clumping Assays

The role of ClfB in binding of *S. aureus* cells to soluble fibrinogen was investigated in clumping assays. Clumping assays were carried out in Sarstedt™ flat-bottomed multi-well test plates, using 50- $\mu$ l volumes of human fibrinogen (Calbiochem Corp. (San Diego, Calif.) plasminogen free, >95% pure), diluted serially two-fold in PBS from a starting concentration of 1 mg/ml. *S. aureus* cultures were washed once in PBS, resuspended to a final OD<sub>600</sub> of 6, and 20  $\mu$ l added to each well. Control wells contained PBS only. The plates were agitated briskly for five minutes and visually examined for clumping. The clumping titer was the lowest concentration of fibrinogen at which clumping occurred. The results are set forth in Table 2, below. Results are the mean of concurrent duplicate assays.

TABLE 2

Strain	Clumping titres of <i>S. aureus</i> Newman and mutants from different culture phases	
	Exponential phase	Stationary phase
Wild-type	0.98	0.98
<i>clfA</i>	3.91	>1000.00
<i>clfB</i>	1.95	0.98
<i>clfA clfB</i>	>1000.00	>1000.00
<i>clfA clfB</i> (pA1-1EA; <i>clfB<sup>+</sup></i> )	2.93	250.00

The clumping titers of *clfA* and *clfB* single mutants were very similar to wild-type when exponential phase cultures were used. However, the double *clfAclfB* mutant failed to form clumps, even at the highest fibrinogen concentration. In contrast, the double mutant carrying the wild-type *clfB* gene on pA1-1EA formed clumps with almost the same avidity as the wild-type. These data show unambiguously that ClfB is a clumping factor.

The difference in clumping titer between the single mutants was much greater when stationary phase cultures were used, where only ClfA is present on cells. The wild-type strain and single *clfB* mutant had identical titers. The single *clfA* mutant failed to clump, and was thus indistinguishable from the double mutant. Interestingly, there was a slight restoration of clumping when the double mutant was complemented with the overexpressed *clfB<sup>+</sup>* gene. This probably reflects over expression of the protein.

##### Plate Adherence Assays

To determine whether ClfB can promote bacterial attachment to immobilized fibrinogen, strains were tested for fibrinogen binding in a microtiter plate adherence assay. Binding of cells to fibrinogen immobilized on plates was measured by the assay of Wolz et al., *Infect. Immun.* 64:3142-3147 (1996). Fibrinogen was diluted in carbonate buffer (15 mM Na<sub>2</sub>CO<sub>3</sub>, 35 mM NaHCO<sub>3</sub>, 3.2  $\mu$ M NaN<sub>3</sub>, pH 9.6) and 100  $\mu$ l used to coat 96-well flat-bottomed ELISA plates (Immulon 4™, Dynatech) overnight at 4° C. Control wells contained carbonate buffer only. After washing in 150 mM NaCl, 0.05% Tween 20™ surfactant, the plates were blocked for one hour at 37° C. in 1% BSA, 0.05% Tween in PBS. After washing as before, 100  $\mu$ l of a cell suspension (OD<sub>600</sub> of 0.4 in PBS) was added, and the plates incubated for two hours at 37° C. After gentle washing by hand, adherent cells were fixed by adding 100  $\mu$ l of 25% aqueous formaldehyde, and incubating at room temperature for at least 30 minutes. The plates were then washed gently once more, stained with crystal violet, washed again, and the plates read by ELISA reader at 570 nm. To avoid inter-assay variation, experiments were designed so that a single plate provided a complete set of results.

The pattern of adherence strongly reflected that obtained in clumping assays (FIG. 15). Assays in which the concentration of cells was varied indicated that binding was approximately half the maximum value at a cell density of 0.4 OD (except for the double mutant), and this cell density was subsequently used routinely. Wild-type, *clfA*, *clfB* mutants and *clfAclfB* (pA1-1EA) showed a fibrinogen concentration-dependent increase in binding (FIG. 16). This increase was less marked for the *clfB* mutant (expressing ClfA) than for the *clfA* mutant (expressing ClfB), suggesting that ClfB may be a less avid and/or abundant receptor. With

stationary phase cells, the *clfB* mutant continued to behave like the wild-type strain, whereas the *clfA* mutant bound much less avidly. As with clumping, adherence was slightly higher with the complemented double mutant, presumably due to a gene dosage effect.

The clumping and adherence assays show that ClfB mediates binding both to soluble and immobilized fibrinogen, closely resembling the activity of ClfA.

The binding of increasing concentrations of biotinylated purified region A from ClfA and ClfB to solid phase fibrinogen was compared in a direct ELISA. The results are shown in FIG. 14. The adherence profiles of the two proteins were very similar, especially at the lower concentrations. At the highest concentration, binding of ClfA was approximately 50% greater than that of ClfB. Neither protein bound to BSA.

#### Effect of Anti-ClfB Antibody on Bacterial Adherence to Immobilized Fibrinogen

To study inhibition of fibrinogen binding by IgG, the cells used for the assay were preincubated with serial two-fold dilutions of purified IgG in PBS, starting with a concentration of 500 µg/ml. Preincubation was for two hours at 37° C. in Sarstedt™ multiwell test plates, and the cells were then transferred to ELISA plates coated with fibrinogen (2.5 µg/ml) and blocked as before. The rest of the assay was as before.

Cells from exponential phase cultures of wild-type and mutant Newman strains were preincubated with increasing concentrations of purified anti-ClfB IgG, and adherence to plastic surfaces coated with 2.5 µg/ml fibrinogen examined. The results are shown in FIG. 17. Binding of the *clfB* mutant was not inhibited, and binding of wild-type cells was almost unaffected, even at the highest antibody concentration. However, binding of the *clfA* mutant showed an IgG concentration-dependent decrease, with an  $IC_{50}$  of 16 µg/ml. The double mutant carrying *clfB*<sup>+</sup> on a complementing plasmid was also inhibited by the antibody, although the  $IC_{50}$  was higher (50 µg/ml), presumably because more ClfB was being expressed on the cell surface.

#### Effect of Divalent Cations on Bacterial Adherence to Immobilized Fibrinogen

The effect of metal ions on fibrinogen binding was studied in a similar manner, preincubating the cells with serial two-fold dilutions of MgCl<sub>2</sub>, MnCl<sub>2</sub> or MgCl<sub>2</sub> in TBS (50 mM Tris HCl, pH 7.5, 150 mM NaCl), starting with a concentration of 50 mM. TBS was used instead of PBS, which causes precipitation of both calcium and manganese. Since the cells bound less well under these conditions, the starting cell concentration was doubled.

It is known that the interaction of ClfA and fibrinogen is inhibited by Ca<sup>2+</sup> and Mn<sup>2+</sup>, but not Mg<sup>2+</sup> ions. The effect of divalent cations on ClfB-promoted adherence to fibrinogen was thus tested. Preincubation of exponential phase cells of the wild-type strain and the *clf* mutants with CaCl<sub>2</sub> inhibited binding to fibrinogen. Those strains expressing ClfB alone showed greater sensitivity than the mutant expressing ClfA alone (*clfB*). The  $IC_{50}$  for the wild-type strain and the *clfB* mutant were 17 and 14 mM, respectively, whereas for the *clfA* mutant and the *clfB*<sup>+</sup> complemented double mutant the  $IC_{50}$  was 1.05 and 0.60 mM, respectively. MnCl<sub>2</sub> also inhibited attachment of the wild-type strain and mutants, with a stronger effect on strains expressing only *clfB*. The  $IC_{50}$  for the wild-type and the *clfB* mutant was 3.3 and 6.4 mM, respectively, whereas for the *clfA* mutant and the double mutant carrying *clfB*<sup>+</sup> on a complementing

plasmid the  $IC_{50}$  was 0.35 and 1.26 mM respectively. MgCl<sub>2</sub> had no effect on binding below 12.5 mM.

Thus, *clfB* promoted adherence of bacteria to immobilized fibrinogen is inhibited by Ca<sup>2+</sup> and Mn<sup>2+</sup> at similar concentrations to ClfA-promoted adherence. However, the mechanisms are likely to be different since ClfB does not contain a homologue of EF hand I implicated in Ca<sup>2+</sup> promoted modulation of ClfA-fibrinogen interactions.

#### Platelet-fibrin Clot Adherence Assay

Adherence to platelet-fibrin clots was measured using a modification of an assay employed by Moreillon et al., *Infect. Immun.* 63:47384743 (1995). Fresh canine blood was collected on 10% sodium citrate buffer (Sigma Chemical Co.), and centrifuged at 3000×g for 10 minutes at room temperature. The plasma fraction was removed and placed in a clean tube. Platelet-fibrin clots were made by mixing 0.5 ml volumes of plasma with 0.1 ml volumes of 0.2 mM CaCl<sub>2</sub> in 35 mm petri dishes. Thrombin (0.1 ml of 500 U/ml Sigma bovine thrombin) was then added, mixed in quickly, and the clots allowed to form. To measure bacterial adherence, 2 ml of PBS containing 5×10<sup>3</sup> cfu/ml of bacteria (from a BHI-grown exponential phase culture) was added to each dish, and the dishes shaken for three minutes on an orbital shaker. The inoculum was drained off and the clots washed twice for five minutes each with 2 ml of PBS. The clots were then overlaid with 3 ml of molten TSA, incubated for 15 hours at 37° C., and the colonies counted. The bacterial suspension used as an inoculum was spread on TSA plates to obtain a total viable count, and the percentage of bound inoculum calculated. Results represent means of 6-10 plates per strain, and were analyzed statistically using the student's T test.

The *clfB* mutation reduced adherence when compared to the wild-type strain Newman, as did the *clfA* mutation which was previously shown by Moreillon et al. to have significantly reduced adherence in this model.

#### Assay for Adherence to Haemodialysis Tubing

In order to demonstrate that ClfB could serve as an adhesin for *S. aureus* in biomaterial-related infections, explanted human haemodialysis tubing was tested for promotion of bacterial adherence in vitro. The tubing was coated with a complex mixture of host plasma proteins including fibrinogen and fibronectin.

These experiments employed sections of haemodialysis tubing removed from patients 3 to 3.5 hours after implantation. Cultures were grown for two hours with shaking. Results, showing means with SEM of three experiments, are shown in FIG. 19.

#### Assay for Adherence to Fibrinogen-coated PMMA Coverslips

Adherence of *S. aureus* Newman and mutants to fibrinogen-coated polymethylmethacrylate (PMMA) coverslips was measured as described by Greene et al., *Mol. Microbiol.* 17:1143-1152 (1995), except that the coverslips were coated with pure fibrinogen (1 µg/ml). Cultures for the assay were grown for two hours with shaking. Results, showing the means and SEM of triplicate experiments, are shown in FIG. 18.

The pattern of adherence to the tubing segments resembled the pattern of binding seen for immobilized fibrinogen in a parallel assay for adherence to fibrinogen immobilized on PMMA coverslips. The single *clfA* mutants had slightly lower levels of adherence compared to the wild-type whereas the double *clfAclfB* mutant was reduced to approximately 30% of wild-type level. Complementation of the single *clfB* mutant with the *clfB* gene on pA1-1EA

## 35

restored binding to greater than wild-type levels, whereas complementation of the double mutant with the same plasmid restored binding only to the same level as the single *clfA* mutant.

## Example 6

## ClfB as a Virulence Factor in Experimental Endocarditis

Clumping factor A was shown to be a virulence factor promoting adherence to damaged heart valves in the rat model of experimental endocarditis of Moreillon et al., *Infect. Immun.* 63:4738-4743 (1995). Therefore, the role of ClfB in this infection was tested by comparing the infection rate of a *clfB* mutant and the mutant carrying the complementing *clfB+* plasmid. Rats were infected intravenously at an ID<sub>60</sub> with  $5 \times 10^3$  cfu. 61% of the wild-type control animals' valves were infected (n=13), whereas only 30% of the *clfB* mutant infected animals were colonized (n=20). In contrast 77% (n=9) of the complemented mutant became infected. This clearly shows that ClfB is an adhesin and potential virulence factor in the endocarditis model.

## Example 7

## Generation of TYTFTDYVD (SEQ ID NO:18) Peptide Antibodies

The nanopeptide, TYTFTDYVD (SEQ ID NO:18), was synthesized in multiple antigen peptide format (MAP; Research Genetics, Inc., Huntsville, Ala.). The peptide was conjugated to KLH according to manufacturers' directions (Pierce). Two female New Zealand White rabbits were immunized subcutaneously with the KLH-TYTFTDYVD (SEQ ID NO:18) conjugate emulsified with Freund's Complete Adjuvant. The rabbits were boosted 3 weeks later by subcutaneous injection of KLH-TYTFTDYVD (SEQ ID NO: 18) adjuvanted with Freund's Incomplete. A third boost was administered subcutaneously with KLH-TYTFTDYVD (SEQ ID NO:18) in PBS. The animals were analyzed for TYTFTDYVD (SEQ ID NO:18) specific antibodies 21 days after the final boost. For purification of antibodies, antisera was diluted 1:1 with Tris-HCl pH 8.0 and passed over a Protein A-Sepharose® column. After sequential washes with Tris-HCl pH 8.0, 0.5 M sodium chloride, the bound antibodies were eluted in 3.5 M MgCl<sub>2</sub>, and dialyzed into PBS.

Immulon-2 microtiter plates (Dynex Technologies, Chantilly, Va.) were coated for 2 hr at room temperature with 1 µg ClfA, ClfB, or BSA. The protein coated plates were washed three times with PBS, 0.05% Tween 20 and then blocked with PBS, 1% BSA. The blocked plates were washed three times with PBS, 0.05% Tween 20. Fifty µl of the purified rabbit KLH-TYTFTDYVD (SEQ ID NO:18) antibodies were serially diluted in PBS and added to the microtiter plate and incubated at 25° C. on a rocker platform. The wells were washed three times with PBS, 0.05% Tween 20 and the secondary antibody was added to the wells and incubated for 1 hr at room temperature. The secondary antibody was alkaline phosphatase-conjugated goat anti-rabbit IgG (Bio-Rad), diluted 3000-fold in PBS. ELISA plates were developed for 1 hr at 37° C. with 1 mg/ml p-nitrophenyl phosphate (Sigma) in 1 M diethanolamine, 0.5 mM MgCl<sub>2</sub>, pH 9.8, and quantified at 405 nm on a Perkin Elmer HTS 7000 Bio-Assay reader.

## 36

The data is shown in FIG. 21. These data indicate that the anticonsensus sequence TYTFTDYVD (SEQ ID NO: 18) antibodies significantly bind to ClfA and ClfB proteins, but not the control protein, BSA.

## Example 8

## Passive Immunization with Rabbit ClfB IgG

The DNA encoding region A of *clfB* (encoding residues S45 to N542) was amplified from genomic DNA of *S. aureus* Newman using the following primers:

Forward:  
5' CGAAAGCTTGTCAGAACCAATCGAACGATACAAC (SEQ ID NO:17)  
G 3'

Reverse:  
5' CGAGGATCCATTTACTGCTGAATCACC 3' (SEQ ID NO:16)

Cleavage sites for HindIII and BamHI (underlined) were appended to the 5' ends of the respective primers to facilitate cloning of the product into the His-tag expression vector pV4. Cloning employed *E. coli* JM101 as a host strain. The recombinant region A was purified by nickel affinity chromatography. Antibodies were raised in rabbits with the purified recombinant A region according to standard procedures. Anti-ClfB A region IgG was purified by affinity chromatography on a Protein A sepharose column.

Twenty Swiss Webster mice (23-28 g) were used to determine if passive immunization with purified rabbit anti-ClfB A region IgG could prevent infection mediated by a methicillin resistant *S. aureus*.

Methicillin resistant *S. aureus* strain 601 was cultured on blood agar plates. A single colony was then inoculated into 10 mls of BHI broth and incubated at 37° C. overnight. The culture was diluted to a 1:100 dilution, placed into 10 ml of fresh BHI and grown to an optical density (O.D.) of 1.5-2.0. The culture was then centrifuged and washed in 1×PBS. The culture was re-suspended in 1×PBS containing 5% BSA and 10% dimethyl sulfoxide (DMSO) and kept frozen at -20° C. The bacterial solution was thawed, washed, diluted in PBS, and adjusted to the appropriate concentrations before dosing the mice.

The mice were divided into four treatment groups (5 mice per treatment group). Mice were assigned to treatment groups as follows:

Antibody/Bacteria	Dose CFU/mouse	No. of Mice
1 Normal rabbit IgG/ <i>S. aureus</i>	$3.81 \times 10^7$	5
2 Normal rabbit IgG/ <i>S. aureus</i>	$7.62 \times 10^7$	5
3 Rabbit anti-ClfB IgG/ <i>S. aureus</i>	$3.81 \times 10^7$	5
4 Rabbit anti-ClfB IgG/ <i>S. aureus</i>	$7.62 \times 10^7$	5

On day -1, ten mice were administered 10 mg rabbit anti-ClfB region A IgG and 10 mice were given 10 mg normal rabbit IgG. Both antibodies were given via intraperitoneal (i.p.) injection. On day 0, all mice were infected intravenously (i.v.) with either  $3.81 \times 10^7$  CFU *S. aureus* or  $7.62 \times 10^7$  CFU *S. aureus*.

Systemic infection was measured by evaluation of body weight loss. Body weight loss is one of the primary parameters that is evaluated when cases of illness and injury are being assessed in mice. The body weight of each animal was

recorded on Day -1 and every other day thereafter, including terminal sacrifice. The animals were weighed to the nearest 0.1 gram.

Mice injected with normal rabbit IgG displayed a significantly larger weight loss at the end of the experiment compared to mice passively immunized with rabbit anti-C1fB region A IgG (see table below). In addition, pathological evaluation of the mice at necropsy revealed a greater number of lesions and foci of infection in the kidneys from the mice receiving normal rabbit IgG compared to the kidneys from mice that were immunized with anti-C1fB region A IgG.

Day of Study	% Change in body weight (mean)			
	Normal IgG/ <i>S. aureus</i> $3.81 \times 10^7$	Anti-C1fB IgG/ <i>S. aureus</i> $3.81 \times 10^7$	Normal IgG/ <i>S. aureus</i> $7.62 \times 10^7$	Anti-C1fB IgG/ <i>S. aureus</i> $7.62 \times 10^7$
-1	0	0	0	0
1	2.9	3.6	3.9	5.8
3	10	5.1	8.5	8.2
5	8.3	1.5	8.0	6.6

Example 9

CUB Region A Binds  $\alpha$  and  $\beta$  Chains of Human Fibrinogen

Human fibrinogen (20  $\mu$ g; Chromogenix) was separated by SDS-PAGE on a 15% acrylamide gel for 2 hours. Proteins were transferred to nitrocellulose at 100 V for 2 h.

The membranes were blocked overnight in PBS containing 10% non-fat dry milk and then incubated with 2.5  $\mu$ g/ml biotinylated C1fB or C1fA region A protein for 1 h with shaking. They were then given 3x5 mm washes with PBS containing 0.1% Tween 20 and incubated for 1 hr with avidin conjugated horseradish peroxidase (Boehringer Mannheim: 1:100,000 dilution). The filters were washed as before and developed using enhanced chemilluminescence (Amersham). The Western Blot (FIG. 22) illustrates the binding of biotinylated C1fA to the  $\gamma$  chain of fibrinogen and the binding of biotinylated C1fB to the  $\alpha$  and  $\beta$  chains of fibrinogen.

Example 10

C1fB Region A Binds 75 kD and 50 kD Proteins from Human Rhabdomyosarcoma Cell Line

Human Rhabdomyosarcoma Cells were lysed with the SDS-PAGE running buffer and varying amounts (2-10  $\mu$ l) of the protein lysate were separated by SDS-PAGE on a 15% acrylamide gel for 2 h. Proteins were transferred to nitrocellulose at 100 V for 2 h. The membranes were blocked overnight in PBS containing 10% non-fat dry milk and then incubated with 2.5  $\mu$ g/ml biotinylated C1fB or C1fA region A protein for 1 hr with constant shaking. They were then given 3x5 min washes with PBS containing 0.1% Tween 20 and incubated for 1 hr with avidin conjugated horseradish peroxidase (Boehringer Mannheim; 1:100,000 dilution). The filters were washed as before and developed using enhanced chemilluminescence (Amersham). Two major bands were seen at 50 kD and 75 kD that reacted with the biotinylated C1fB region A protein.

SEQUENCE LISTING

```

<160> NUMBER OF SEQ ID NOS: 23
<210> SEQ ID NO 1
<211> LENGTH: 918
<212> TYPE: PRT
<213> ORGANISM: Staphylococcus aureus
<400> SEQUENCE: 1
Asn Gly Val Ile Phe Leu Lys Lys Arg Ile Asp Tyr Leu Ser Asn Lys
 1                               5                               10                               15
Gln Asn Lys Tyr Ser Ile Arg Arg Phe Thr Val Gly Thr Thr Ser Val
 20                               25                               30
Ile Val Gly Ala Thr Ile Leu Phe Gly Ile Gly Asn His Gln Ala Gln
 35                               40                               45
Ala Ser Glu Gln Ser Asn Asp Thr Thr Gln Ser Ser Lys Asn Asn Ala
 50                               55                               60
Ser Ala Asp Ser Glu Lys Asn Asn Met Ile Glu Thr Pro Gln Leu Asn
 65                               70                               75                               80
Thr Thr Ala Asn Asp Thr Ser Asp Ile Ser Ala Asn Thr Asn Ser Ala
 85                               90                               95
Asn Val Asp Ser Thr Thr Lys Pro Met Ser Thr Gln Thr Ser Asn Thr
 100                              105                              110
Thr Thr Thr Glu Pro Ala Ser Thr Asn Glu Thr Pro Gln Pro Thr Ala
 115                              120                              125
    
```

-continued

Ile	Lys	Asn	Gln	Ala	Thr	Ala	Ala	Lys	Met	Gln	Asp	Gln	Thr	Val	Pro
130						135					140				
Gln	Glu	Gly	Asn	Ser	Gln	Val	Asp	Asn	Lys	Thr	Thr	Asn	Asp	Ala	Asn
145					150					155					160
Ser	Ile	Ala	Thr	Asn	Ser	Glu	Leu	Lys	Asn	Ser	Gln	Thr	Leu	Asp	Leu
				165					170					175	
Pro	Gln	Ser	Ser	Pro	Gln	Thr	Ile	Ser	Asn	Ala	Gln	Gly	Thr	Ser	Lys
			180					185					190		
Pro	Ser	Val	Arg	Thr	Arg	Ala	Val	Arg	Ser	Leu	Ala	Val	Ala	Glu	Pro
		195					200					205			
Val	Val	Asn	Ala	Ala	Asp	Ala	Lys	Gly	Thr	Asn	Val	Asn	Asp	Lys	Val
	210					215					220				
Thr	Ala	Ser	Asn	Phe	Lys	Leu	Glu	Lys	Thr	Thr	Phe	Asp	Pro	Asn	Gln
225					230					235					240
Ser	Gly	Asn	Thr	Phe	Met	Ala	Ala	Asn	Phe	Thr	Val	Thr	Asp	Lys	Val
				245					250					255	
Lys	Ser	Gly	Asp	Tyr	Phe	Thr	Ala	Lys	Leu	Pro	Asp	Ser	Leu	Thr	Gly
			260					265					270		
Asn	Gly	Asp	Val	Asp	Tyr	Ser	Asn	Ser	Asn	Asn	Thr	Met	Pro	Ile	Ala
		275					280					285			
Asp	Ile	Lys	Ser	Thr	Asn	Gly	Asp	Val	Val	Ala	Lys	Ala	Thr	Tyr	Asp
	290					295					300				
Ile	Leu	Thr	Lys	Thr	Tyr	Thr	Phe	Val	Phe	Thr	Asp	Tyr	Val	Asn	Asn
305					310					315					320
Lys	Glu	Asn	Ile	Asn	Gly	Gln	Phe	Ser	Leu	Pro	Leu	Phe	Thr	Asp	Arg
				325					330					335	
Ala	Lys	Ala	Pro	Lys	Ser	Gly	Thr	Tyr	Asp	Ala	Asn	Ile	Asn	Ile	Ala
			340						345				350		
Asp	Glu	Met	Phe	Asn	Asn	Lys	Ile	Thr	Tyr	Asn	Tyr	Ser	Ser	Pro	Ile
		355					360					365			
Ala	Gly	Ile	Asp	Lys	Pro	Asn	Gly	Ala	Asn	Ile	Ser	Ser	Gln	Ile	Ile
	370					375					380				
Gly	Val	Asp	Thr	Ala	Ser	Gly	Gln	Asn	Thr	Tyr	Lys	Gln	Thr	Val	Phe
385					390					395					400
Val	Asn	Pro	Lys	Gln	Arg	Val	Leu	Gly	Asn	Thr	Trp	Val	Tyr	Ile	Lys
				405					410					415	
Gly	Tyr	Gln	Asp	Lys	Ile	Glu	Glu	Ser	Ser	Gly	Lys	Val	Ser	Ala	Thr
			420					425					430		
Asp	Thr	Lys	Leu	Arg	Ile	Phe	Glu	Val	Asn	Asp	Thr	Ser	Lys	Leu	Ser
		435				440						445			
Asp	Ser	Tyr	Tyr	Ala	Asp	Pro	Asn	Asp	Ser	Asn	Leu	Lys	Glu	Val	Thr
	450					455					460				
Asp	Gln	Phe	Lys	Asn	Arg	Ile	Tyr	Tyr	Glu	His	Pro	Asn	Val	Ala	Ser
465					470					475					480
Ile	Lys	Phe	Gly	Asp	Ile	Thr	Lys	Thr	Tyr	Val	Val	Leu	Val	Glu	Gly
				485					490					495	
His	Tyr	Asp	Asn	Thr	Gly	Lys	Asn	Leu	Lys	Thr	Gln	Val	Ile	Gln	Glu
			500					505					510		
Asn	Val	Asp	Pro	Val	Thr	Asn	Arg	Asp	Tyr	Ser	Ile	Phe	Gly	Trp	Asn
		515					520					525			
Asn	Glu	Asn	Val	Val	Arg	Tyr	Gly	Gly	Gly	Ser	Ala	Asp	Gly	Asp	Ser
	530					535					540				
Ala	Val	Asn	Pro	Lys	Asp	Pro	Thr	Pro	Gly	Pro	Pro	Val	Asp	Pro	Glu

-continued

---

```

545                550                555                560
Pro Ser Pro Asp Pro Glu Pro Glu Pro Thr Pro Asp Pro Glu Pro Ser
565                570                575
Pro Asp Pro Glu Pro Glu Pro Ser Pro Asp Pro Asp Pro Asp Ser Asp
580                585                590
Ser Asp Ser Asp Ser Gly Ser Asp Ser Asp Ser Gly Ser Asp Ser Asp
595                600                605
Ser Glu Ser Asp Ser Asp Ser Asp Ser Asp Ser Asp Ser Asp Ser Asp
610                615                620
Ser Asp Ser Glu Ser Asp Ser Asp Ser Glu Ser Asp Ser Asp Ser Asp
625                630                635                640
Ser Asp Ser Asp Ser Asp Ser Asp Ser Asp Ser Asp Ser Asp Ser Asp
645                650                655
Ser Asp Ser Asp Ser Asp Ser Asp Ser Asp Ser Asp Ser Asp Ser Glu Ser Asp
660                665                670
Ser Asp Ser Glu Ser Asp Ser Glu Ser Asp Ser Asp Ser Asp Ser Asp
675                680                685
Ser Asp Ser Asp Ser Asp Ser Asp Ser Asp Ser Asp Ser Asp Ser Asp
690                695                700
Ser Asp Ser Asp Ser Asp Ser Asp Ser Asp Ser Asp Ser Asp Ser Asp
705                710                715                720
Ser Asp Ser Asp Ser Asp Ser Asp Ser Asp Ser Asp Ser Asp Ser Asp
725                730                735
Ser Asp Ser Asp Ser Asp Ser Asp Ser Asp Ser Asp Ser Asp Ser Asp
740                745                750
Ser Asp Ser Asp Ser Asp Ser Asp Ser Asp Ser Asp Ser Asp Ser Asp
755                760                765
Ser Asp Ser Asp Ser Asp Ser Asp Ser Asp Ser Asp Ser Asp Ser Asp
770                775                780
Ser Asp Ser Asp Ser Asp Ser Asp Ser Asp Ser Asp Ser Asp Ser Asp
785                790                795                800
Ser Asp Ser Asp Ser Asp Ser Asp Ser Asp Ser Asp Ser Asp Ser Asp
805                810                815
Ser Asp Ser Asp Ser Asp Ser Asp Ser Asp Ser Asp Ser Asp Ser Asp
820                825                830
Ser Asp Ser Asp Ser Asp Ser Asp Ser Asp Ser Asp Ser Arg Val Thr
835                840                845
Pro Pro Asn Asn Glu Gln Lys Ala Pro Ser Asn Pro Lys Gly Glu Val
850                855                860
Asn His Ser Asn Lys Val Ser Lys Gln His Lys Thr Asp Ala Leu Pro
865                870                875                880
Glu Thr Gly Asp Lys Ser Glu Asn Thr Asn Ala Thr Leu Phe Gly Ala
885                890                895
Met Met Ala Leu Leu Gly Ser Leu Leu Leu Phe Arg Lys Arg Lys Gln
900                905                910
Asp His Lys Glu Lys Ala
915

```

&lt;210&gt; SEQ ID NO 2

&lt;211&gt; LENGTH: 2969

&lt;212&gt; TYPE: DNA

&lt;213&gt; ORGANISM: Staphylococcus aureus

&lt;400&gt; SEQUENCE: 2



-continued

---

tagaaattga aatggagtaa tatttttgaa aaaaagaatt gattatttgt cgaataagca	60
gaataagtat tcgattagac gttttacagt aggtaccaca tcagtaatag taggggcaac	120
tatactatatt gggataggca atcatcaagc acaagcttca gaacaatcga acgatacaac	180
gcaatcttcg aaaaaaatg caagtgcaga ttccgaaaaa aacaatatga tagaaacacc	240
tcaattaaat acaacggcta atgatacatc tgatattagt gcaaacacaa acagtgcgaa	300
tgtagatagc acaacaaaac caatgtctac acaaacgagc aataccacta caacagagcc	360
agcttcaaca aatgaaacac ctcaaccgac ggcaattnaa aatcaagcaa ctgctgcaaa	420
aatgcaagat caaactgttc ctcaagaagg aaattctcaa gtagataata aaacaacgaa	480
tgatgctaata agcatagcaa caaacagtga gcttaaaaa tctcaaacat tagatttacc	540
acaatcatca ccacaaaaga tttccaatgc gcaaggaact agtaaaccaa gtgttagaac	600
gagagctgta cgtagttag ctgttctgta accggtagta aatgctgctg atgctaaagg	660
tacaaatgta aatgataaag ttacggcaag taatttcaag ttagaaaaga ctacatttga	720
ccctaatcaa agtggtaaca catttatggc ggcaaatntt acagtgcacag ataaagttaa	780
atcaggggat tattttacag cgaagttacc agatagtnta actggtaatg gagacgtgga	840
ttattctaat tcaataata cgatgccaat tgcagacatt aaaagtacga atggcgatgt	900
tgtagctaaa gcaacatag atactctgac taagacgtat acatttgtct ttacagatta	960
tgtaataaat aaagaaaata ttaacggaca attttcatta cctttattta cagaccgagc	1020
aaaggcacct aaatcaggaa catatgatgc gaatattaat attgctgatg aaatgtttaa	1080
taataaaaatt acttataact atagttcgcc aattgcagga attgataaac caaatggcgc	1140
gaacatttct tctcaaatga ttgggtgtaga tacagcttca ggtcaaaaca catacaagca	1200
aacagtatnt gttaacctca agcaacgagt tttaggtaat acgtgggtgt atattaaggg	1260
ctaccaagat aaaatcgaag aaagtgcgg taaagtaagt gctacagata caaaactgag	1320
aatttttgaa gtgaatgata catctaaatt atcagatagc tactatgcag atccaaatga	1380
ctctaacctt aaagaagtaa cagaccaatt taaaaataga atctattatg agcatccaaa	1440
tgtagctagt attaaattg gtgatattac taaaacatat gtagtattag tagaagggca	1500
ttacgacaat acaggtaaga acttaaaaac tcaggttatt caagaaaatg ttgatcctgt	1560
aacaaataga gactacagta ttttcggttg gaataatgag aatgtgtac gttatggtag	1620
tggaaagtgt gatggtgatt cagcagtaaa tccgaaagac ccaactccag ggccgccggt	1680
tgaccagaa ccaagtccag acccagaacc agaaccaacg ccagatccag aaccaagtcc	1740
agaccagaa ccggaaccaa gccagacc ccgatccgat tcggattcag acagtgactc	1800
aggctcagac agcagactcag gttcagatag cgactcagaa tcagatagcg attcggattc	1860
agacagtgat tcagattcag acagcgactc agaatcagat agcgattcag aatcagatag	1920
cgactcagat tcagatagcg attcagattc agatagcgat tcagattcag atagcgattc	1980
ggattcagac agtgattcag attcagacag cgactcagaa tcagatagcg actcagaatc	2040
agatagttag tcagattcag acagtgactc ggactcagac agtgattcag actcagatag	2100
cgattcagac tcagatagcg attcagattc agacagcgac tcagattcag acagcgactc	2160
agactcagat agcagactcag actcagacag cgactcagat tcagatagcg attcagactc	2220
agacagcgac tcagactcag acagcgactc agactcagat agcagactcag attcagatag	2280
cgattcagac tcagacagcg actcagattc agatagcgat tcggactcag acagcgattc	2340
agattcagac agcagactcag actcggatag cgattcagat tcagatagcg attcggattc	2400

-continued

---

```

agacagtgat tcagattcag acagcgactc agactcggat agcgactcag actcagacag 2460
cgattcagac tcagatagcg actcagactc ggatagcgac tcggattcag atagcgactc 2520
agactcagat agtgactccg attcaagagt tacaccacca aataatgaac agaaagcacc 2580
atcaaatcct aaaggtgaag taaaccattc taataaggtg tcaaaacaac aaaaactga 2640
tgctttacca gaaacaggag ataagagcga aaacacaaat gcaactttat ttggtgcaat 2700
gatggcatta ttaggatcat tactattggt tagaaaacgc aagcaagatc ataaagaaaa 2760
agcgtaaata ctttttagg ccgaatacat ttgtattcgg ttttttgggt gaaaatgatt 2820
ttaaagttaa ttgattaagc gtaaaatggt gataaagtag aattagaaag gggatcatgac 2880
gtatggctta tatttcatta aactatcatt caccaacaat tggtatgcat caaaatttga 2940
cagtcatttt accggaagaa cgagaattc 2969

```

```

<210> SEQ ID NO 3
<211> LENGTH: 930
<212> TYPE: PRT
<213> ORGANISM: Staphylococcus aureus

```

```

<400> SEQUENCE: 3

```

```

Met Asn Asn Lys Lys Thr Ala Thr Asn Arg Lys Gly Met Ile Pro Asn
  1          5          10          15
Arg Leu Asn Lys Phe Ser Ile Arg Lys Tyr Ser Val Gly Thr Ala Ser
  20          25          30
Ile Leu Val Gly Thr Thr Leu Ile Phe Gly Leu Ser Gly His Glu Ala
  35          40          45
Lys Ala Ala Glu His Thr Asn Gly Glu Leu Asn Gln Ser Lys Asn Glu
  50          55          60
Thr Thr Ala Pro Ser Glu Asn Lys Thr Thr Lys Lys Val Asp Ser Arg
  65          70          75          80
Gln Leu Lys Asp Asn Thr Gln Thr Ala Thr Ala Asp Gln Pro Lys Val
  85          90          95
Thr Met Ser Asp Ser Ala Thr Val Lys Glu Thr Ser Ser Asn Met Gln
 100          105          110
Ser Pro Gln Asn Ala Thr Ala Asn Gln Ser Thr Thr Lys Thr Ser Asn
 115          120          125
Val Thr Thr Asn Asp Lys Ser Ser Thr Thr Tyr Ser Asn Glu Thr Asp
 130          135          140
Lys Ser Asn Leu Thr Gln Ala Lys Asp Val Ser Thr Thr Pro Lys Thr
 145          150          155          160
Thr Thr Ile Lys Pro Arg Thr Leu Asn Arg Met Ala Val Asn Thr Val
 165          170          175
Ala Ala Pro Gln Gln Gly Thr Asn Val Asn Asp Lys Val His Phe Ser
 180          185          190
Asn Ile Asp Ile Ala Ile Asp Lys Gly His Val Asn Gln Thr Thr Gly
 195          200          205
Lys Thr Glu Phe Trp Ala Thr Ser Ser Asp Val Leu Lys Leu Lys Ala
 210          215          220
Asn Tyr Thr Ile Asp Asp Ser Val Lys Glu Gly Asp Thr Phe Thr Phe
 225          230          235          240
Lys Tyr Gly Gln Tyr Phe Arg Pro Gly Ser Val Arg Leu Pro Ser Gln
 245          250          255
Thr Gln Asn Leu Tyr Asn Ala Gln Gly Asn Ile Ile Ala Lys Gly Ile
 260          265          270

```

-continued

---

Tyr Asp Ser Thr Thr Asn Thr Thr Thr Tyr Thr Phe Thr Asn Tyr Val  
 275 280 285  
 Asp Gln Tyr Thr Asn Val Arg Gly Ser Phe Glu Gln Val Ala Phe Ala  
 290 295 300  
 Lys Arg Lys Asn Ala Thr Thr Asp Lys Thr Ala Tyr Lys Met Glu Val  
 305 310 315  
 Thr Leu Gly Asn Asp Thr Tyr Ser Glu Glu Ile Ile Val Asp Tyr Gly  
 325 330 335  
 Asn Lys Lys Ala Gln Pro Leu Ile Ser Ser Thr Asn Tyr Ile Asn Asn  
 340 345 350  
 Glu Asp Leu Ser Arg Asn Met Thr Ala Tyr Val Asn Gln Pro Lys Asn  
 355 360 365  
 Thr Tyr Thr Lys Gln Thr Phe Val Thr Asn Leu Thr Gly Tyr Lys Phe  
 370 375 380  
 Asn Pro Asn Ala Lys Asn Phe Lys Ile Tyr Glu Val Thr Asp Gln Asn  
 385 390 395 400  
 Gln Phe Val Asp Ser Phe Thr Pro Asp Thr Ser Lys Leu Lys Asp Val  
 405 410 415  
 Thr Asp Gln Phe Asp Val Ile Tyr Ser Asn Asp Asn Lys Thr Ala Thr  
 420 425 430  
 Val Asp Leu Met Lys Gly Gln Thr Ser Ser Asn Lys Gln Tyr Ile Ile  
 435 440 445  
 Gln Gln Val Ala Tyr Pro Asp Asn Ser Ser Thr Asp Asn Gly Lys Ile  
 450 455 460  
 Asp Tyr Thr Leu Asp Thr Asp Lys Thr Lys Tyr Ser Trp Ser Asn Ser  
 465 470 475 480  
 Tyr Ser Asn Val Asn Gly Ser Ser Thr Ala Asn Gly Asp Gln Lys Lys  
 485 490 495  
 Tyr Asn Leu Gly Asp Tyr Val Trp Glu Asp Thr Asn Lys Asp Gly Lys  
 500 505 510  
 Gln Asp Ala Asn Glu Lys Gly Ile Lys Gly Val Tyr Val Ile Leu Lys  
 515 520 525  
 Asp Ser Asn Gly Lys Glu Leu Asp Arg Thr Thr Thr Asp Glu Asn Gly  
 530 535 540  
 Lys Tyr Gln Phe Thr Gly Leu Ser Asn Gly Thr Tyr Ser Val Glu Phe  
 545 550 555 560  
 Ser Thr Pro Ala Gly Tyr Thr Pro Thr Thr Ala Asn Val Gly Thr Asp  
 565 570 575  
 Asp Ala Val Asp Ser Asp Gly Leu Thr Thr Thr Gly Val Ile Lys Asp  
 580 585 590  
 Ala Asp Asn Met Thr Leu Asp Ser Gly Phe Tyr Lys Thr Pro Lys Tyr  
 595 600 605  
 Ser Leu Gly Asp Tyr Val Trp Tyr Asp Ser Asn Lys Asp Gly Lys Arg  
 610 615 620  
 Asp Ser Thr Glu Lys Gly Ile Lys Gly Val Lys Val Thr Leu Gln Asn  
 625 630 635 640  
 Glu Lys Gly Glu Val Ile Gly Thr Thr Glu Thr Asp Glu Asn Gly Lys  
 645 650 655  
 Tyr Arg Phe Asp Asn Leu Asp Ser Gly Lys Tyr Lys Val Ile Phe Glu  
 660 665 670  
 Lys Pro Ala Gly Leu Thr Gln Thr Gly Thr Asn Thr Thr Glu Asp Asp  
 675 680 685

-continued

---

Lys Asp Ala Asp Gly Gly Glu Val Asp Val Thr Ile Thr Asp His Asp  
 690 695 700

Asp Phe Thr Leu Asp Asn Gly Tyr Tyr Glu Glu Glu Thr Ser Asp Ser  
 705 710 715 720

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

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

Asp Ser Asp Ser Asp Ser Asp Ser Asp Ser Asp Ser Asp Ser Asp Ser Asn Ser  
 755 760 765

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

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

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

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

Asp Ser Asp Ser Asp Ser Asp Ser Asp Ser Asp Ser Asp Ser Asp Ser Asp Ser  
 835 840 845

Asp Ser Asp Ser Asp Ser Asp Ser Asp Ser Asp Ser Asp Ser Asp Ser Asp Ser  
 850 855 860

Asp Ser Asp Ser Asp Ser Asp Ser Asp Ser Asp Ser Asp Asn Asp Ser Asp Ser  
 865 870 875 880

Asp Ser Asp Ser Asp Ser Asp Ala Gly Lys His Thr Pro Ala Lys Pro  
 885 890 895

Met Ser Thr Val Lys Asp Gln His Lys Thr Ala Lys Ala Leu Pro Glu  
 900 905 910

Thr Gly Ser Glu Asn Asn Asn Ser Asn Asn Gly Thr Leu Phe Gly Gly  
 915 920 925

Leu Phe  
 930

<210> SEQ ID NO 4  
 <211> LENGTH: 2841  
 <212> TYPE: DNA  
 <213> ORGANISM: Staphylococcus aureus

<400> SEQUENCE: 4

atgaataata aaaagacagc aacaaataga aaaggcatga taccaaactcg attaaacaaa 60

ttttcgataa gaaagtattc tgtaggctact gttcaattt tagtagggac aacattgatt 120

tttgggttaa gtggtcatga agctaaagcg gcagaacata cgaatggaga attaaatcaa 180

tcaaaaaatg aaacgacagc cccaagttag aataaaacaa ctaaaaaagt tgatagtcgt 240

caactaaaag acaatacgca aactgcaact gcagatcagc ctaaagtgac aatgagtgat 300

agtgcaacag ttaagaagaac tagtagtaac atgcaatcac cacaaaacgc tacagctaat 360

caatctacta caaaaactag caatgtaaca acaaatgata aatcatcaac tacatatagt 420

aatgaaactg ataaaagtaa tttaacacaa gcaaaagatg tttcaactac acctaaaaca 480

acgactatta aaccaagaac tttaaatcgc atggcagtga atactgttgc agctccacaa 540

caaggaacaa atgttaatga taaagtacat ttttcaata ttgacattgc gattgataaa 600

ggacatgta atcagactac tggtaaaact gaattttggg caacttcaag tgatgtttta 660

aaattaaaag caaattacac aatcgatgat tctgttaaag agggcgatgc atttactttt 720

-continued

---

```

aaatatggtc aatatttccg tccaggatca gtaagattac cttcacaaac tcaaaattta 780
tataatgccc aaggaatat tattgcaaaa ggtatttatg atagtacaac aaacacaaca 840
acataactt ttacgaacta tgtagatcaa tacaacaatg ttagaggtag ctttgaacaa 900
gttgcaattg cgaaacgtaa aaatgcaaca actgataaaa cagcttataa aatggaagta 960
actttaggtg atgatacata tagcgaagaa atcattgtcg attatggtaa taaaaaagca 1020
caaccgctta tttcaagtac aaactatatt aacaatgaag atttatcgcg taatatgact 1080
gcatatgtaa atcaacctaa aaatacatat actaaacaaa cgtttggtac taatttaact 1140
ggatataaat ttaatccaaa tgcaaaaaac ttcaaaattt acgaagtgc agatcaaaat 1200
caatttggg atagtttcc ccttgatact tcaaaacta aagatgttac tgatcaattc 1260
gatgttattt atagtaatga taataaaaca gctacagtcg atttaatgaa aggcacaaca 1320
agcagcaata aacaatacat cattcaacaa gttgcttacc cagataatag ttcaacagat 1380
aatggaaaaa ttgattatac tttagacact gacaaaacta aatatagttg gtcaaatagt 1440
tattcaaatg tgaatggctc atcaactgct aatggcgacc aaaagaaata taatctaggt 1500
gactatgtat gggagatac aaataaagat ggtaaacaaag atgccaatga aaaagggatt 1560
aaagtggtt atgtcattct taaagatagt aacggtaaag aattagatcg tacgacaaca 1620
gatgaaaatg gtaaatatca gttcactggg ttaagcaatg gaacttatag tgtagagttt 1680
tcaacaccag ccggttatac accgacaact gcaaatgtag gtacagatga tgctgtagat 1740
tctgatggac taactacaac aggtgtcatt aaagacgctg acaacatgac attagatagt 1800
ggattctaca aaacacaaaa atatagttta ggtgattatg tttggtagca cagtaataaa 1860
gatggtaaac gagattcgac tgaaaaagga attaaaggtg ttaaagttac tttgcaaac 1920
gaaaaaggcg aagtaattgg tacaactgaa acagatgaaa atggtaata ccgctttgat 1980
aatttagata gtgtaaaata caaagtatc tttgaaaaac ctgctggctt aactcaacaa 2040
ggtaaaaata caactgaaga tgataaagat gccgatggtg gcgaagtga tgtaacaatt 2100
acggatcatg atgatttcc acttgataat ggctactacg aagaagaaac atcagatagc 2160
gactcagatt ctgacagcga ttcagactca gatagcgact cagattcaga tagcgactca 2220
gattcagaca gcgattcaga cagcgactca gactcagata gcgattcaga ttcagacagc 2280
gactcagact cagacagcaa ttcagactcg gatagcgact cagactcaga tagcgactca 2340
gattcggata gcgactcaga ctcagatagc gattcagatt cagatagcga ttcggactca 2400
gacagtgatt cagattcaga ctcagatagc gactcagatt ctgacagcga ttcagactca 2460
gacagcgact cagactcaga cagtgattca gattcagaca gcgactcaga ttcagatagc 2520
gactcagact cagatagcga ctcagactca gatagcgact cagactcggg tagcgattca 2580
gattcagaca gcgactcaga ttcagatagc gattcggact cagacaaaga ctcagattca 2640
gatagcgatt cagattcaga tgcaggtaaa catactccgg ctaaaccaat gagtacggtt 2700
aaagatcagc ataaaacagc taaagcatta ccagaaacag gtagtgaaaa taataattca 2760
aataatggca cattattcgg tggattatc gcggcattag gatcattatt gtcattcggg 2820
cgtcgtaaaa aacaaaataa a 2841

```

&lt;210&gt; SEQ ID NO 5

&lt;211&gt; LENGTH: 1315

&lt;212&gt; TYPE: PRT

&lt;213&gt; ORGANISM: Staphylococcus aureus

-continued

&lt;400&gt; SEQUENCE: 5

Met Leu Asn Arg Glu Asn Lys Thr Ala Ile Thr Arg Lys Gly Met Val  
1 5 10 15  
Ser Asn Arg Leu Asn Lys Phe Ser Ile Arg Lys Tyr Thr Val Gly Thr  
20 25 30  
Ala Ser Ile Leu Val Gly Thr Thr Leu Ile Phe Gly Leu Gly Asn Gln  
35 40 45  
Glu Ala Lys Ala Ala Glu Ser Thr Asn Lys Glu Leu Asn Glu Ala Thr  
50 55 60  
Thr Ser Ala Ser Asp Asn Gln Ser Ser Asp Lys Val Asp Met Gln Gln  
65 70 75 80  
Leu Asn Gln Glu Asp Asn Thr Lys Asn Asp Asn Gln Lys Glu Met Val  
85 90 95  
Ser Ser Gln Gly Asn Glu Thr Thr Ser Asn Gly Asn Lys Leu Ile Glu  
100 105 110  
Lys Glu Ser Val Gln Ser Thr Thr Gly Asn Lys Val Glu Val Ser Thr  
115 120 125  
Ala Lys Ser Asp Glu Gln Ala Ser Pro Lys Ser Thr Asn Glu Asp Leu  
130 135 140  
Asn Thr Lys Gln Thr Ile Ser Asn Gln Glu Ala Leu Gln Pro Asp Leu  
145 150 155 160  
Gln Glu Asn Lys Ser Val Val Asn Val Gln Pro Thr Asn Glu Glu Asn  
165 170 175  
Lys Lys Val Asp Ala Lys Thr Glu Ser Thr Thr Leu Asn Val Lys Ser  
180 185 190  
Asp Ala Ile Lys Ser Asn Asp Glu Thr Leu Val Asp Asn Asn Ser Asn  
195 200 205  
Ser Asn Asn Glu Asn Asn Ala Asp Ile Ile Leu Pro Lys Ser Thr Ala  
210 215 220  
Pro Lys Arg Leu Asn Thr Arg Met Arg Ile Ala Ala Val Gln Pro Ser  
225 230 235 240  
Ser Thr Glu Ala Lys Asn Val Asn Asp Leu Ile Thr Ser Asn Thr Thr  
245 250 255  
Leu Thr Val Val Asp Ala Asp Lys Asn Asn Lys Ile Val Pro Ala Gln  
260 265 270  
Asp Tyr Leu Ser Leu Lys Ser Gln Ile Thr Val Asp Asp Lys Val Lys  
275 280 285  
Ser Gly Asp Tyr Phe Thr Ile Lys Tyr Ser Asp Thr Val Gln Val Tyr  
290 295 300  
Gly Leu Asn Pro Glu Asp Ile Lys Asn Ile Gly Asp Ile Lys Asp Pro  
305 310 315 320  
Asn Asn Gly Glu Thr Ile Ala Thr Ala Lys His Asp Thr Ala Asn Asn  
325 330 335  
Leu Ile Thr Tyr Thr Phe Thr Asp Tyr Val Asp Arg Phe Asn Ser Val  
340 345 350  
Gln Met Gly Ile Asn Tyr Ser Ile Tyr Met Asp Ala Asp Thr Ile Pro  
355 360 365  
Val Ser Lys Asn Asp Val Glu Phe Asn Val Thr Ile Gly Asn Thr Thr  
370 375 380  
Thr Lys Thr Thr Ala Asn Ile Gln Tyr Pro Asp Tyr Val Val Asn Glu  
385 390 395 400  
Lys Asn Ser Ile Gly Ser Ala Phe Thr Glu Thr Val Ser His Val Gly  
405 410 415

-continued

---

Asn Lys Glu Asn Pro Gly Tyr Tyr Lys Gln Thr Ile Tyr Val Asn Pro  
 420 425 430  
 Ser Glu Asn Ser Leu Thr Asn Ala Lys Leu Lys Val Gln Ala Tyr His  
 435 440 445  
 Ser Ser Tyr Pro Asn Asn Ile Gly Gln Ile Asn Lys Asp Val Thr Asp  
 450 455 460  
 Ile Lys Ile Tyr Gln Val Pro Lys Gly Tyr Thr Leu Asn Lys Gly Tyr  
 465 470 475 480  
 Asp Val Asn Thr Lys Glu Leu Thr Asp Val Thr Asn Gln Tyr Leu Gln  
 485 490 495  
 Lys Ile Thr Tyr Gly Asp Asn Asn Ser Ala Val Ile Asp Phe Gly Asn  
 500 505 510  
 Ala Asp Ser Ala Tyr Val Val Met Val Asn Thr Lys Phe Gln Tyr Thr  
 515 520 525  
 Asn Ser Glu Ser Pro Thr Leu Val Gln Met Ala Thr Leu Ser Ser Thr  
 530 535 540  
 Gly Asn Lys Ser Val Ser Thr Gly Asn Ala Leu Gly Phe Thr Asn Asn  
 545 550 555 560  
 Gln Ser Gly Gly Ala Gly Gln Glu Val Tyr Lys Ile Gly Asn Tyr Val  
 565 570 575  
 Trp Glu Asp Thr Asn Lys Asn Gly Val Gln Glu Leu Gly Glu Lys Gly  
 580 585 590  
 Val Gly Asn Val Thr Val Thr Val Phe Asp Asn Asn Thr Asn Thr Lys  
 595 600 605  
 Val Gly Glu Ala Val Thr Lys Glu Asp Gly Ser Tyr Leu Ile Pro Asn  
 610 615 620  
 Leu Pro Asn Gly Asp Tyr Arg Val Glu Phe Ser Asn Leu Pro Lys Gly  
 625 630 635 640  
 Tyr Glu Val Thr Pro Ser Lys Gln Gly Asn Asn Glu Glu Leu Asp Ser  
 645 650 655  
 Asn Gly Leu Ser Ser Val Ile Thr Val Asn Gly Lys Asp Asn Leu Ser  
 660 665 670  
 Ala Asp Leu Gly Ile Tyr Lys Pro Lys Tyr Asn Leu Gly Asp Tyr Val  
 675 680 685  
 Trp Glu Asp Thr Asn Lys Asn Gly Ile Gln Asp Gln Asp Glu Lys Gly  
 690 695 700  
 Ile Ser Gly Val Thr Val Thr Leu Lys Asp Glu Asn Gly Asn Val Leu  
 705 710 715 720  
 Lys Thr Val Thr Thr Asp Ala Asp Gly Lys Tyr Lys Phe Thr Asp Leu  
 725 730 735  
 Asp Asn Gly Asn Tyr Lys Val Glu Phe Thr Thr Pro Glu Gly Tyr Thr  
 740 745 750  
 Pro Thr Thr Val Thr Ser Gly Ser Asp Ile Glu Lys Asp Ser Asn Gly  
 755 760 765  
 Leu Thr Thr Thr Gly Val Ile Asn Gly Ala Asp Asn Met Thr Leu Asp  
 770 775 780  
 Ser Gly Phe Tyr Lys Thr Pro Lys Tyr Asn Leu Gly Asn Tyr Val Trp  
 785 790 795 800  
 Glu Asp Thr Asn Lys Asp Gly Lys Gln Asp Ser Thr Glu Lys Gly Ile  
 805 810 815  
 Ser Gly Val Thr Val Thr Leu Lys Asn Glu Asn Gly Glu Val Leu Gln  
 820 825 830

-continued

Thr	Thr	Lys	Thr	Asp	Lys	Asp	Gly	Lys	Tyr	Gln	Phe	Thr	Gly	Leu	Glu
		835					840						845		
Asn	Gly	Thr	Tyr	Lys	Val	Glu	Phe	Glu	Thr	Pro	Ser	Gly	Tyr	Thr	Pro
	850					855					860				
Thr	Gln	Val	Gly	Ser	Gly	Thr	Asp	Glu	Gly	Ile	Asp	Ser	Asn	Gly	Thr
865					870					875					880
Ser	Thr	Thr	Gly	Val	Ile	Lys	Asp	Lys	Asp	Asn	Asp	Thr	Ile	Asp	Ser
			885						890					895	
Gly	Phe	Tyr	Lys	Pro	Thr	Tyr	Asn	Leu	Gly	Asp	Tyr	Val	Trp	Glu	Asp
			900					905					910		
Thr	Asn	Lys	Asn	Gly	Val	Gln	Asp	Lys	Asp	Glu	Lys	Gly	Ile	Ser	Gly
		915					920					925			
Val	Thr	Val	Thr	Leu	Lys	Asp	Glu	Asn	Asp	Lys	Val	Leu	Lys	Thr	Val
	930					935					940				
Thr	Thr	Asp	Glu	Asn	Gly	Lys	Tyr	Gln	Phe	Thr	Asp	Leu	Asn	Asn	Gly
945					950						955				960
Thr	Tyr	Lys	Val	Glu	Phe	Glu	Thr	Pro	Ser	Gly	Tyr	Thr	Pro	Thr	Ser
			965						970						975
Val	Thr	Ser	Gly	Asn	Asp	Thr	Glu	Lys	Asp	Ser	Asn	Gly	Leu	Thr	Thr
			980					985					990		
Thr	Gly	Val	Ile	Lys	Asp	Ala	Asp	Asn	Met	Thr	Leu	Asp	Ser	Gly	Phe
		995					1000						1005		
Tyr	Lys	Thr	Pro	Lys	Tyr	Ser	Leu	Gly	Asp	Tyr	Val	Trp	Tyr	Asp	Ser
	1010					1015					1020				
Asn	Lys	Asp	Gly	Lys	Gln	Asp	Ser	Thr	Glu	Lys	Gly	Ile	Lys	Asp	Val
1025					1030						1035				1040
Lys	Val	Thr	Leu	Leu	Asn	Glu	Lys	Gly	Glu	Val	Ile	Gly	Thr	Thr	Lys
			1045						1050						1055
Thr	Asp	Glu	Asn	Gly	Lys	Tyr	Cys	Phe	Asp	Asn	Leu	Asp	Ser	Gly	Lys
		1060						1065						1070	
Tyr	Lys	Val	Ile	Phe	Glu	Lys	Pro	Ala	Gly	Leu	Thr	Gln	Thr	Gly	Thr
		1075					1080						1085		
Asn	Thr	Thr	Glu	Asp	Asp	Lys	Asp	Ala	Asp	Gly	Gly	Glu	Val	Asp	Val
	1090					1095						1100			
Thr	Ile	Thr	Asp	His	Asp	Asp	Phe	Thr	Leu	Asp	Asn	Gly	Tyr	Tyr	Glu
1105					1110					1115					1120
Glu	Glu	Thr	Ser	Asp	Ser	Asp	Ser	Asp	Ser	Asp	Ser	Asp	Ser	Asp	Ser
			1125						1130						1135
Asp	Arg	Asp	Ser	Asp	Ser	Asp	Ser	Asp	Ser	Asp	Ser	Asp	Ser	Asp	Ser
		1140						1145						1150	
Asp	Ser	Asp	Ser	Asp	Ser	Asp	Ser	Asp	Ser	Asp	Ser	Asp	Ser	Asp	Arg
		1155						1160					1165		
Asp	Ser	Asp	Ser	Asp	Ser	Asp	Ser	Asp	Ser	Asp	Ser	Asp	Ser	Asp	Ser
		1170				1175						1180			
Asp	Ser	Asp	Ser	Asp	Ser	Asp	Ser	Asp	Ser	Asp	Ser	Asp	Ser	Asp	Ser
1185					1190					1195					1200
Asp	Ser	Asp	Ser	Asp	Ser	Asp	Ser	Asp	Ser	Asp	Ser	Asp	Ser	Asp	Ser
			1205						1210						1215
Asp	Ser	Asp	Ser	Asp	Ser	Asp	Ser	Asp	Ser	Asp	Ser	Asp	Ser	Asp	Ser
			1220					1225						1230	
Asp	Ser	Asp	Ser	Asp	Ser	Asp	Ser	Asp	Ser	Asp	Ser	Asp	Ser	Asp	Ser
		1235					1240						1245		
Asp	Ser	Asp	Ser	Asp	Ser	Asp	Ala	Gly	Lys	His	Thr	Pro	Val	Lys	Pro



-continued

1250	1255	1260	
Met Ser Thr Thr Lys Asp His His Asn Lys Ala Lys Ala Leu Pro Glu			
1265	1270	1275	1280
Thr Gly Asn Glu Asn Ser Gly Ser Asn Asn Ala Thr Leu Phe Gly Gly			
	1285	1290	1295
Leu Phe Ala Ala Leu Gly Ser Leu Leu Leu Phe Gly Arg Arg Lys Lys			
	1300	1305	1310
Gln Asn Lys			
	1315		
<210> SEQ ID NO 6			
<211> LENGTH: 3945			
<212> TYPE: DNA			
<213> ORGANISM: Staphylococcus aureus			
<400> SEQUENCE: 6			
atgctaaaca gagaaaataa aacggcaata acaaggaaag gcatggtatc caatcgatta			60
aataaatttt cgattagaaa gtacacagtg ggaacagcat caattttagt aggtacaaca			120
ttaatttttg gtctggggaa ccaagaagca aaggctgcag aaagtactaa taaagaattg			180
aacgaagcga caacttcagc aagtgataat caatcgagtg ataaagttga tatgcagcaa			240
ctaaatcaag aagacaatac taaaaatgat aatcaaaaag aaatggtatc atctcaaggt			300
aatgaaacga cttcaaatgg gaataaatta atagaaaag aaagtgtaca atctaccact			360
ggaaataaag ttgaagtttc aactgccaaa tcagatgagc aagcttcacc aaaatctacg			420
aatgaagatt taaacactaa acaactata agtaatcaag aagcgttaca acctgatttg			480
caagagaata aatcagtggt aaatgttcaa ccaactaatg aggaaaacaa aaaggtagat			540
gccaaaactg aatcaactac attaaatggt aaaagtgatg ctatcaagag taatgatgaa			600
actctgttg atacaatag taattcaaat aatgaaaata atgcagatat cattttgcca			660
aaaagtacag cacctaaacg tttgaataca agaatgcgta tagcagcagt acagccatca			720
tcaacagagg ctaaaaatgt taatgattta atcacatcaa atacaacatt aactgtcgtt			780
gatgcagata aaaacaataa aatcgtacca gcccaagatt atttatcatt aaaatcacia			840
attacagttg atgacaaagt taaatcaggt gattatttca caattaata ctcagatata			900
gtacaagtat atggattgaa tccggaagat attaaaaata ttggtgatat taaagatcca			960
aataatggtg aaacaattgc gactgcaaaa catgatactg caaataattt aattacatat			1020
acatttacag attatgttga tcgatttaat tctgtacaaa tgggaattaa ttattcaatt			1080
tatatggatg ctgatacaat tctgttagt aaaaacgatg ttgagtttaa tgttacgata			1140
ggtaatacta caacaaaaac aactgctaac attcaatatc cagattatgt tgtaaatgag			1200
aaaaattcaa ttggatcagc gttcactgaa acagtttcac atggttgaaa taaagaaaat			1260
ccagggtact ataacaacac gatttatgta aatccatcgg aaaattcttt aacaaatgcc			1320
aaactaaaag ttcaagctta ccaactcaag tatcctaata atacgggca aataaataaa			1380
gatgtaacag atataaaaat atatcaagtt cctaagggtt atacattaaa taaaggatac			1440
gatgtgaata ctaagagct tacagatgta acaaatcaat acttgacaaa aattacatat			1500
ggcgacaaca atagcgtgtg tattgatttt ggaaatgcag attctgctta tgttgaatg			1560
gttaatacaa aattccaata tacaaatagc gaaagcccaa cacttgttca aatggctact			1620
ttatcttcaa caggaataaa atccgtttct actggcaatg ctttaggatt tactaataac			1680
caaagtggcg gagctggtca agaagtatat aaaattggtg actacgtatg ggaagatact			1740

-continued

---

```

aataaaaaacg gtgttcaaga attaggagaa aaaggcgttg gcaatgtaac tgtaactgta 1800
tttgataata atacaaatac aaaagtagga gaagcagtta ctaaagaaga tgggtcatac 1860
ttgattccaa acttacctaa tggagattac cgtgtagaat tttcaaactt accaaaaggt 1920
tatgaagtaa ccccttcaaa acaaggtaat aacgaagaat tagattcaaa cggcttatct 1980
tcagttatta cagttaatgg caaagataac ttatctgcag acttaggtat ttacaaacct 2040
aaatacaact taggtgacta tgtctgggaa gatacaata aaaatgggat ccaagaccaa 2100
gatgaaaaag gtatatctgg cgtaacggta acattaaaag atgaaaacgg taacgtgtta 2160
aaaacagtta caacagacgc tgatggcaaa tataaattta ctgattttaga taatggtaat 2220
tataaagttg aatttactac accagaaggc tatacaccca ctacagtaac atctggtagc 2280
gacattgaaa aagactctaa tggtttaaca acaacaggtg ttattaatgg tgctgataac 2340
atgacattag atagtggatt ctacaaaaca ccaaaatata atttaggtaa ttatgtatgg 2400
gaagatacaa ataaagatgg taagcaggat tcaactgaaa aaggatattc aggcgtaaca 2460
gttacattga aaaatgaaaa cggtgaaagt ttacaaacaa ctaaacaga taaagatgg 2520
aaatatcaat ttactggatt agaaaatgga acttataaag tggaattcga aacaccatca 2580
ggttacacac caacacaagt aggttcagga actgatgaag gtatagattc aaatggtaca 2640
tcaacaacag gtgtcattaa agataaagat aacgatacta ttgactctgg tttctacaaa 2700
ccgacttaca acttaggtga ctatgtatgg gaagatacaa ataaaaacgg tgttcaagat 2760
aaagatgaaa agggcatttc aggtgtaaca gttacgttaa aagatgaaa cgacaaagt 2820
ttaaaaacag ttacaacaga tgaatgggt aaatatcaat tcaactgatt aaacaatgga 2880
acttataaag tggaattcga gacaccatca ggttatacac caacttcagt aacttctgga 2940
aatgatactg aaaaagattc taatggttta acaacaacag gtgtcattaa agatgcagat 3000
aacatgacat tagacagtg tttctataaa acaccaaat atagtttagg tgattatggt 3060
tggtagcaca gtaataaaga cggcaaacaa gattcaactg aaaaaggtat caaagatggt 3120
aaagttactt tattaaatga aaaaggcgaa gtaattggaa caactaaaac agatgaaaat 3180
ggtaaaact gctttgataa tttagatagc ggtaaataca aagttatttt tgaaaagcct 3240
gctggcttaa cacaaacagg tacaataca actgaagatg ataaagatgc agatggtggc 3300
gaagttgacg taacaattac ggatcatgat gatttcacac ttgataatgg ctactacgaa 3360
gaagaaacat cagatagcga ctcagattcg gacagcgact cagattcaga cagagactca 3420
gactcagata gtgattcaga ctcggatagc gattcagatt cagacagcga ttcagattca 3480
gatagcgatt cagattcaga cagagactca gatagtgatt cagactcaga tagcgactca 3540
gattcagaca gcgactcaga ttcagacagc gactcagact cagatagtga ttcagactca 3600
gatagcgact cagattcaga tagcgactca gattcagaca gcgactcaga ctcggatagt 3660
gattcagact cagatagcga ctcagactca gatagcgatt cagattcaga tagcgactca 3720
gactcagaca gcgattcaga ctcagacagc gactcagact cagatgcagg taagcacaca 3780
cctgttaaac caatgagtag tactaaagac catcacata aagcaaaagc attaccagaa 3840
acaggtaatg aaaatagcgg ctcaaataac gcaacgttat ttggcggatt attcgcagca 3900
ttaggatcat tattgttatt cggctcgtcgt aaaaaacaaa ataaa 3945

```

```

<210> SEQ ID NO 7
<211> LENGTH: 1166
<212> TYPE: PRT

```

-continued

&lt;213&gt; ORGANISM: Staphylococcus aureus

&lt;400&gt; SEQUENCE: 7

```

Met Ile Asn Arg Asp Asn Lys Lys Ala Ile Thr Lys Lys Gly Met Ile
  1           5           10           15
Ser Asn Arg Leu Asn Lys Phe Ser Ile Arg Lys Tyr Thr Val Gly Thr
  20           25           30
Ala Ser Ile Leu Val Gly Thr Thr Leu Ile Phe Gly Leu Gly Asn Gln
  35           40           45
Glu Ala Lys Ala Ala Glu Asn Thr Ser Thr Glu Asn Ala Lys Gln Asp
  50           55           60
Asp Ala Thr Thr Ser Asp Asn Lys Glu Val Val Ser Glu Thr Glu Asn
  65           70           75           80
Asn Ser Thr Thr Glu Asn Asn Ser Thr Asn Pro Ile Lys Lys Glu Thr
  85           90           95
Asn Thr Asp Ser Gln Pro Glu Ala Lys Lys Glu Ser Thr Ser Ser Ser
 100           105           110
Thr Gln Lys Gln Gln Asn Asn Val Thr Ala Thr Thr Glu Thr Lys Pro
 115           120           125
Gln Asn Ile Glu Lys Glu Asn Val Lys Pro Ser Thr Asp Lys Thr Ala
 130           135           140
Thr Glu Asp Thr Ser Val Ile Leu Glu Glu Lys Lys Ala Pro Asn Asn
 145           150           155           160
Thr Asn Asn Asp Val Thr Thr Lys Pro Ser Thr Ser Glu Pro Ser Thr
 165           170           175
Ser Glu Ile Gln Thr Lys Pro Thr Thr Pro Gln Glu Ser Thr Asn Ile
 180           185           190
Glu Asn Ser Gln Pro Gln Pro Thr Pro Ser Lys Val Asp Asn Gln Val
 195           200           205
Thr Asp Ala Thr Asn Pro Lys Glu Pro Val Asn Val Ser Lys Glu Glu
 210           215           220
Leu Lys Asn Asn Pro Glu Lys Leu Lys Glu Leu Val Arg Asn Asp Ser
 225           230           235           240
Asn Thr Asp His Ser Thr Lys Pro Val Ala Thr Ala Pro Thr Ser Val
 245           250           255
Ala Pro Lys Arg Val Asn Ala Lys Met Arg Phe Ala Val Ala Gln Pro
 260           265           270
Ala Ala Val Ala Ser Asn Asn Val Asn Asp Leu Ile Lys Val Thr Lys
 275           280           285
Gln Thr Ile Lys Val Gly Asp Gly Lys Asp Asn Val Ala Ala Ala His
 290           295           300
Asp Gly Lys Asp Ile Glu Tyr Asp Thr Glu Phe Thr Ile Asp Asn Lys
 305           310           315           320
Val Lys Lys Gly Asp Thr Met Thr Ile Asn Tyr Asp Lys Asn Val Ile
 325           330           335
Pro Ser Asp Leu Thr Asp Lys Asn Asp Pro Ile Asp Ile Thr Asp Pro
 340           345           350
Ser Gly Glu Val Ile Ala Lys Gly Thr Phe Asp Lys Ala Thr Lys Gln
 355           360           365
Ile Thr Tyr Thr Phe Thr Asp Tyr Val Asp Lys Tyr Glu Asp Ile Lys
 370           375           380
Ser Arg Leu Thr Leu Tyr Ser Tyr Ile Asp Lys Lys Thr Val Pro Asn
 385           390           395           400

```

-continued

Glu	Thr	Ser	Leu	Asn	Leu	Thr	Phe	Ala	Thr	Ala	Gly	Lys	Glu	Thr	Ser
				405					410					415	
Gln	Asn	Val	Thr	Val	Asp	Tyr	Gln	Asp	Pro	Met	Val	His	Gly	Asp	Ser
			420					425					430		
Asn	Ile	Gln	Ser	Ile	Phe	Thr	Lys	Leu	Asp	Glu	Asp	Lys	Gln	Thr	Ile
		435					440					445			
Glu	Gln	Gln	Ile	Tyr	Val	Asn	Pro	Leu	Lys	Lys	Ser	Ala	Thr	Asn	Thr
	450					455					460				
Lys	Val	Asp	Ile	Ala	Gly	Ser	Gln	Val	Asp	Asp	Tyr	Gly	Asn	Ile	Lys
465					470					475					480
Leu	Gly	Asn	Gly	Ser	Thr	Ile	Ile	Asp	Gln	Asn	Thr	Glu	Ile	Lys	Val
				485					490					495	
Tyr	Lys	Val	Asn	Ser	Asp	Gln	Gln	Leu	Pro	Gln	Ser	Asn	Arg	Ile	Tyr
			500					505						510	
Asp	Phe	Ser	Gln	Tyr	Glu	Asp	Val	Thr	Ser	Gln	Phe	Asp	Asn	Lys	Lys
		515						520					525		
Ser	Phe	Ser	Asn	Asn	Val	Ala	Thr	Leu	Asp	Phe	Gly	Asp	Ile	Asn	Ser
		530				535					540				
Ala	Tyr	Ile	Ile	Lys	Val	Val	Ser	Lys	Tyr	Thr	Pro	Thr	Ser	Asp	Gly
545					550						555				560
Glu	Leu	Asp	Ile	Ala	Gln	Gly	Thr	Ser	Met	Arg	Thr	Thr	Asp	Lys	Tyr
				565					570					575	
Gly	Tyr	Tyr	Asn	Tyr	Ala	Gly	Tyr	Ser	Asn	Phe	Ile	Val	Thr	Ser	Asn
			580						585					590	
Asp	Thr	Gly	Gly	Gly	Asp	Gly	Thr	Val	Lys	Pro	Glu	Glu	Lys	Leu	Tyr
		595						600					605		
Lys	Ile	Gly	Asp	Tyr	Val	Trp	Glu	Asp	Val	Asp	Lys	Asp	Gly	Val	Gln
	610					615					620				
Gly	Thr	Asp	Ser	Lys	Glu	Lys	Pro	Met	Ala	Asn	Val	Leu	Val	Thr	Leu
625					630					635					640
Thr	Tyr	Pro	Asp	Gly	Thr	Thr	Lys	Ser	Val	Arg	Thr	Asp	Ala	Asn	Gly
				645					650					655	
His	Tyr	Glu	Phe	Gly	Gly	Leu	Lys	Asp	Gly	Glu	Thr	Tyr	Thr	Val	Lys
			660					665						670	
Phe	Glu	Thr	Pro	Thr	Gly	Tyr	Leu	Pro	Thr	Lys	Val	Asn	Gly	Thr	Thr
			675					680					685		
Asp	Gly	Glu	Lys	Asp	Ser	Asn	Gly	Ser	Ser	Val	Thr	Val	Lys	Ile	Asn
	690					695					700				
Gly	Lys	Asp	Asp	Met	Ser	Leu	Asp	Thr	Gly	Phe	Tyr	Lys	Glu	Pro	Lys
705					710					715					720
Tyr	Asn	Leu	Gly	Asp	Tyr	Val	Trp	Glu	Asp	Thr	Asn	Lys	Asp	Gly	Ile
			725						730					735	
Gln	Asp	Ala	Asn	Glu	Pro	Gly	Ile	Lys	Asp	Val	Lys	Val	Thr	Leu	Lys
			740					745					750		
Asp	Ser	Thr	Gly	Lys	Val	Ile	Gly	Thr	Thr	Thr	Thr	Asp	Ala	Ser	Gly
		755					760						765		
Lys	Tyr	Lys	Phe	Thr	Asp	Leu	Asp	Asn	Gly	Asn	Tyr	Thr	Val	Glu	Phe
	770					775					780				
Glu	Thr	Pro	Ala	Gly	Tyr	Thr	Pro	Thr	Val	Lys	Asn	Thr	Thr	Ala	Asp
785					790					795					800
Asp	Lys	Asp	Ser	Asn	Gly	Leu	Thr	Thr	Thr	Gly	Val	Ile	Lys	Asp	Ala
				805					810					815	
Asp	Asn	Met	Thr	Leu	Asp	Arg	Gly	Phe	Tyr	Lys	Thr	Pro	Lys	Tyr	Ser

-continued

820					825					830					
Leu	Gly	Asp	Tyr	Val	Trp	Tyr	Asp	Ser	Asn	Lys	Asp	Gly	Lys	Gln	Asp
		835					840					845			
Ser	Thr	Glu	Lys	Gly	Ile	Lys	Asp	Val	Thr	Val	Thr	Leu	Gln	Asn	Glu
	850					855					860				
Lys	Gly	Glu	Val	Ile	Gly	Thr	Thr	Lys	Thr	Asp	Glu	Asn	Gly	Lys	Tyr
865				870						875					880
Arg	Phe	Asp	Asn	Leu	Asp	Ser	Gly	Lys	Tyr	Lys	Val	Ile	Phe	Glu	Lys
			885						890						895
Pro	Ala	Gly	Leu	Thr	Gln	Thr	Val	Thr	Asn	Thr	Thr	Glu	Asp	Asp	Lys
			900					905					910		
Asp	Ala	Asp	Gly	Gly	Glu	Val	Asp	Val	Thr	Ile	Thr	Asp	His	Asp	Asp
		915					920					925			
Phe	Thr	Leu	Asp	Asn	Gly	Tyr	Phe	Glu	Glu	Asp	Thr	Ser	Asp	Ser	Asp
	930					935					940				
Ser	Asp	Ser	Asp	Ser	Asp	Ser	Asp	Ser	Asp	Ser	Asp	Ser	Asp	Ser	Asp
945				950					955						960
Ser	Asp	Ser	Asp	Ser	Asp	Ser	Asp	Ser	Asp	Ser	Asp	Ser	Asp	Ser	Asp
			965					970						975	
Ser	Asp	Ser	Asp	Ser	Asp	Ser	Asp	Ser	Asp	Ser	Asp	Ser	Asp	Ser	Asp
		980					985					990			
Ser	Asp	Ser	Asp	Ser	Asp	Ser	Asp	Ser	Asp	Ser	Asp	Ser	Asp	Ser	Asp
		995					1000					1005			
Ser	Asp	Ser	Asp	Ser	Asp	Ser	Asp	Ser	Asp	Ser	Asp	Ser	Asp	Ser	Asp
	1010					1015					1020				
Ser	Asp	Ser	Asp	Ser	Asp	Ser	Asp	Ser	Asp	Ser	Asp	Ser	Asp	Ser	Asp
1025				1030					1035						1040
Ser	Asp	Ser	Asp	Ser	Asp	Ser	Asp	Ser	Asp	Ser	Asp	Ser	Asp	Ser	Asp
			1045					1050						1055	
Ser	Asp	Ser	Asp	Ser	Asp	Ser	Asp	Ser	Asp	Ser	Asp	Ser	Asp	Ser	Asp
		1060					1065					1070			
Ser	Asp	Ser	Asp	Ser	Asp	Ser	Asp	Ser	Asp	Ser	Asp	Ser	Asp	Ser	Asp
	1075						1080					1085			
Ser	Asp	Ser	Asp	Ser	Asp	Ser	Asp	Ser	Asp	Ser	Asp	Ser	Asp	Ser	Asp
	1090						1095					1100			
Ser	Asp	Ala	Gly	Lys	His	Thr	Pro	Val	Lys	Pro	Met	Ser	Thr	Thr	Lys
1105					1110					1115					1120
Asp	His	His	Asn	Lys	Ala	Lys	Ala	Leu	Pro	Glu	Thr	Gly	Ser	Glu	Asn
			1125						1130					1135	
Asn	Gly	Ser	Asn	Asn	Ala	Thr	Leu	Phe	Gly	Gly	Leu	Phe	Ala	Ala	Leu
			1140					1145					1150		
Gly	Ser	Leu	Leu	Leu	Phe	Gly	Arg	Arg	Lys	Lys	Gln	Asn	Lys		
		1155					1160						1165		

<210> SEQ ID NO 8  
 <211> LENGTH: 3498  
 <212> TYPE: DNA  
 <213> ORGANISM: Staphylococcus aureus

<400> SEQUENCE: 8

atgattaaca gggataataa aaaggcaata acaaaaaagg gtatgatttc aaatcgctta	60
aacaaatttt cgattagaaa gtatactgta ggaactgcat cgatttttagt aggtacgaca	120
ttgatttttg gtctagggaa ccaagaagct aaagctgctg aaaacactag tacagaaat	180

-continued

---

gcaaaacaag atgatgcaac gactagtgat aataaagaag tagtgtcggg aactgaaaat	240
aattcgacaa cagaaaataa ttcaacaaat ccaattaaga aagaacaaa tactgattca	300
caaccagaag ctaaaaaaga atcaacttca tcaagtactc aaaaacagca aaataacgtt	360
acagctacaa ctgaaactaa gcctcaaac attgaaaaag aaaatgttaa accttcaact	420
gataaaactg cgacagaaga tacatctggt attttagaag agaagaaagc accaaataat	480
acaaataacg atgtaactac aaaacatct acaagtgaac catctacaag tgaattcaa	540
acaaaaccaa ctacacctca agaacttaca aatattgaaa attcacaacc gcaaccaacg	600
ccttcaaaag tagacaatca agttacagat gcaactaatc caaagaacc agtaaatgtg	660
tcaaaagaag aacttaaaaa taatcctgag aaattaaaag aattggttag aaatgatagc	720
aatacagatc attcaactaa accagttgct acagctccaa caagtgttgcc accaaaacgt	780
gtaaaccgca aaatgcgctt tgcagttgca caaccagcag cagttgcttc aaacaatgta	840
aatgatttaa ttaaagtgac gaagcaaaaca atcaaaagttg gcgatggtaa agataatgtg	900
gcagcagcgc atgacggtaa agatattgaa tatgatacag agtttacaat tgacaataaa	960
gtcaaaaaag gcgatacaat gacgattaat tatgataaga atgtaattcc ttcggattta	1020
acagataaaa atgatcctat cgatattact gatccatcag gagaggatcat tgctaaggga	1080
acatttgata aagcaactaa gcaaatcaca tatacattta cagactatgt agataaatat	1140
gaagatataa aatcacgctt aactctatat tcgtatattg ataanaaac agttcctaat	1200
gagacaagtt tgaatttaac atttgctaca gcaggtaaag aaacaagcca aaatgtcact	1260
gttgattatc aagatccaat ggtccatggt gattcaaaaca ttcaatctat ctttcaaaa	1320
ttagatgaag ataagcaaac tattgaacaa caaatttatg ttaaccatt gaaaaatca	1380
gcaaccaaca ctaaagtga tatagctggt agtcaagtag atgattatgg aaatattaaa	1440
ctaggaaatg gtacacccat tattgaccaa aatacagaaa taaaggttta taaagttaac	1500
tctgatcaac aattgcctca aagtaataga atctatgatt ttagtcaata cgaagatgta	1560
acaagtcaat ttgataataa aaaatcattt agtaataatg tagcaacatt ggattttggt	1620
gatattaatt cagcctatat tatcaaagt gttagtaaat atacacctac atcagatggc	1680
gaactagata ttgcccaagg tactagtatg agaacaactg ataaatatgg ttattataat	1740
tatgcaggat attcaaacct catcgtaact tctaatagaca ctggcgggtg cgacggctact	1800
gttaaacctg aagaaaagt atacaaaatt ggtgactatg tatgggaaga cgttgataaa	1860
gacggtgttc aaggtagaca ttcaaaagaa aaaccaatgg caaacgtttt agttacatta	1920
acttaccggc acggtactac aaaatcagta agaacagatg ctaatggta ttatgaattc	1980
ggtggtttga aagacggaga aacttataca gttaaattcg aaacgccaac tggatatctt	2040
ccaacaaaag taaatggaac aactgatggt gaaaaagact caaatggtag ttcggttact	2100
gttaaaatta atggtaaaga tgatattgct ttagatactg gtttttaca agaaccctaa	2160
tacaacttag gtgactatgt atgggaagat actaataaag atggatcca agatgcaaat	2220
gagccaggaa tcaaatggtt taaggttaca ttaaaagata gtactggaaa agttattggt	2280
acaactacta ctgatgcctc gggtaaatat aaatttacag atttagataa tggttaactat	2340
acagtagaat ttgaaacacc agcaggttac acgccaacgg ttaaaaatac tacagctgat	2400
gataaagatt ctaatggttt aacaacaaca ggtgtcatta aagatgcaga taatatgaca	2460
ttagacaggg gtttctataa aacacaaaaa tacagtttag gtgattatgt ttggtacgac	2520
agtaataaag acggcaaaaca agattcaact gaaaaaggt tcaaatggt gacagttaca	2580

-continued

---

```

ttgcaaaacg aaaaggcga agtaattgga acaactaaaa cagatgaaaa tggtaaatat 2640
cgtttcgata atttagatag cggtaaatac aaagttattt ttgaaaagcc tgctggctta 2700
acacaaacag ttacaaatac aactgaagat gataaagatg cagatggtgg cgaagttgac 2760
gtaacaatta cggatcatga tgatttcaca cttgataacg gatacttcga agaagataca 2820
tcagacagcg attcagactc agatagtgac tcagacagcg actcagactc agacagcgac 2880
tcagactcag acagtgattc agattcagac agcgactcag attcagatag cgactcagat 2940
tcggacagcg attcagactc agatagcgac tcagattcag atagcgattc agactcagac 3000
agcgactcag attcagatag cgattcggac tcagacagcg attcagactc agatagcgac 3060
tcagactcag acagcgactc agattcagat agcgattcgg actcagatag cgactcagat 3120
tcagacagcg attcagactc agatagcgac tcagattcag acagcgattc agactcagat 3180
agcgactcag actcagacag tgattcagat tcagacagcg actcagactc agatagcgac 3240
tcagattcag acagcgactc agactcagat agcgactcag actcagacag tgattcagac 3300
agcgattcag actcggatgc aggaaaacat acacctgtta aaccaatgag tactactaaa 3360
gaccatcaca ataaagcaaa agcattacca gaaacaggta gtgaaaataa cggctcaaat 3420
aacgcaacgt tatttgggtg attatttgca gcattagggt cattattggt attcggctcgt 3480
cgcaaaaaac aaaacaaa 3498

```

```

<210> SEQ ID NO 9
<211> LENGTH: 18
<212> TYPE: DNA
<213> ORGANISM: Staphylococcus aureus
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (6)..(6)
<223> OTHER INFORMATION: n = (a or c or t or g)
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (12)..(12)
<223> OTHER INFORMATION: n = (a or c or t or g)

```

```

<400> SEQUENCE: 9

```

```

gaytngayt cngayagy

```

18

```

<210> SEQ ID NO 10
<211> LENGTH: 5
<212> TYPE: PRT
<213> ORGANISM: Staphylococcus aureus

```

```

<400> SEQUENCE: 10

```

```

Leu Pro Asp Thr Gly
  1           5

```

```

<210> SEQ ID NO 11
<211> LENGTH: 5
<212> TYPE: PRT
<213> ORGANISM: Staphylococcus aureus

```

```

<400> SEQUENCE: 11

```

```

Asp Tyr Ser Asn Ser
  1           5

```

```

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

```

-continued

---

<400> SEQUENCE: 12

Phe Thr Asp Tyr Val Asn  
 1 5

<210> SEQ ID NO 13

<211> LENGTH: 5

<212> TYPE: PRT

<213> ORGANISM: Staphylococcus aureus

<220> FEATURE:

<221> NAME/KEY: misc\_feature

<222> LOCATION: (2)..(2)

<223> OTHER INFORMATION: Xaa = (Unspecified amino acid)

<220> FEATURE:

<221> NAME/KEY: misc\_feature

<222> LOCATION: (4)..(4)

<223> OTHER INFORMATION: Xaa = (Unspecified amino acid)

<400> SEQUENCE: 13

Asp Xaa Ser Xaa Ser  
 1 5

<210> SEQ ID NO 14

<211> LENGTH: 5

<212> TYPE: PRT

<213> ORGANISM: Staphylococcus aureus

<220> FEATURE:

<221> NAME/KEY: misc\_feature

<222> LOCATION: (3)..(3)

<223> OTHER INFORMATION: Xaa = (Unspecified amino acid)

<400> SEQUENCE: 14

Leu Pro Xaa Thr Gly  
 1 5

<210> SEQ ID NO 15

<211> LENGTH: 33

<212> TYPE: DNA

<213> ORGANISM: Staphylococcus aureus

<400> SEQUENCE: 15

cgaggatcct caggacaatc gaacgataca acg

33

<210> SEQ ID NO 16

<211> LENGTH: 27

<212> TYPE: DNA

<213> ORGANISM: Staphylococcus aureus

<400> SEQUENCE: 16

cgaggtacca ttactgctg aatcacc

27

<210> SEQ ID NO 17

<211> LENGTH: 34

<212> TYPE: DNA

<213> ORGANISM: Staphylococcus aureus

<400> SEQUENCE: 17

cgaaagcttg tcagaacaat cgaacgatac aacg

34

<210> SEQ ID NO 18

<211> LENGTH: 9

<212> TYPE: PRT

<213> ORGANISM: Staphylococcus aureus

<400> SEQUENCE: 18

Thr Tyr Thr Phe Thr Asp Tyr Val Asp  
 1 5



-continued

<210> SEQ ID NO 19  
 <211> LENGTH: 9  
 <212> TYPE: PRT  
 <213> ORGANISM: Staphylococcus aureus  
 <400> SEQUENCE: 19

Thr Tyr Thr Phe Thr Asn Tyr Val Asp  
 1 5

<210> SEQ ID NO 20  
 <211> LENGTH: 9  
 <212> TYPE: PRT  
 <213> ORGANISM: Staphylococcus aureus  
 <400> SEQUENCE: 20

Thr Phe Val Phe Thr Asp Tyr Val Asn  
 1 5

<210> SEQ ID NO 21  
 <211> LENGTH: 9  
 <212> TYPE: PRT  
 <213> ORGANISM: Staphylococcus aureus  
 <400> SEQUENCE: 21

Ile Tyr Thr Phe Thr Asp Tyr Val Asn  
 1 5

<210> SEQ ID NO 22  
 <211> LENGTH: 5  
 <212> TYPE: PRT  
 <213> ORGANISM: Staphylococcus aureus  
 <400> SEQUENCE: 22

Leu Pro Glu Thr Gly  
 1 5

<210> SEQ ID NO 23  
 <211> LENGTH: 14  
 <212> TYPE: PRT  
 <213> ORGANISM: Staphylococcus aureus  
 <400> SEQUENCE: 23

Thr Ile Tyr Phe Thr Val Phe Thr Asp Asn Tyr Val Asp Asn  
 1 5 10

What is claimed is:

1. An isolated protein wherein the protein has an amino acid sequence comprising the sequence of SEQ ID NO: 1.  
 2. An isolated protein encoded by a nucleic acid sequence comprising the sequence of SEQ ID NO:2.  
 3. The protein of claim 2, expressed from a vector in a living organism, wherein the vector contains a nucleic acid sequence comprising the sequence of SEQ ID NO:2.  
 4. The protein of claim 1 in a pharmaceutically acceptable carrier.

50 5. The protein of claim 2 in a pharmaceutically acceptable carrier.  
 6. The protein of claim 1 immobilized on a solid phase.  
 7. The protein of claim 2 immobilized on a solid phase.  
 8. A diagnostic kit comprising the protein according to claim 1 and antibodies binding to said protein.  
 55 9. A diagnostic kit comprising the protein according to claim 2 and antibodies binding to said protein.

\* \* \* \* \*