



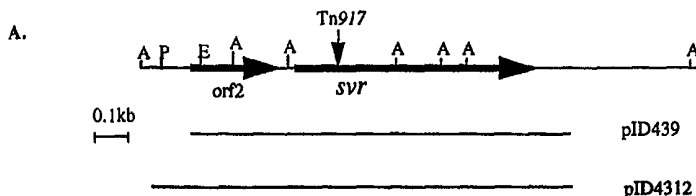
INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

<p>(51) International Patent Classification <sup>6</sup> : C07K 14/31, C12Q 1/18, A61K 39/085</p>	<p>A3</p>	<p>(11) International Publication Number: <b>WO 99/01473</b> (43) International Publication Date: 14 January 1999 (14.01.99)</p>
<p>(21) International Application Number: PCT/GB98/01974 (22) International Filing Date: 3 July 1998 (03.07.98) (30) Priority Data: 887,534 3 July 1997 (03.07.97) US (71) Applicant (for all designated States except US): IMPERIAL COLLEGE INNOVATIONS LIMITED [GB/GB]; Sherfield Building, London SW7 2AZ (GB). (72) Inventor; and (75) Inventor/Applicant (for US only): HOLDEN, David, William [GB/GB]; Imperial College of Science, Technology and Medicine, Hammersmith Hospital, Dept. of Infectious Diseases, Du Cane Road, London W12 0NN (GB). (74) Agent: BASSETT, Richard; Eric Potter Clarkson, Park View House, 58 The Ropewalk, Nottingham NG1 5DD (GB).</p>	<p>(81) Designated States: AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GE, GH, GM, GW, HR, HU, ID, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZW, ARIPO patent (GH, GM, KE, LS, MW, SD, SZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG).</p> <p><b>Published</b> With international search report. Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.</p> <p>(88) Date of publication of the international search report: 29 April 1999 (29.04.99)</p>	

(54) Title: ANTI-BACTERIAL METHODS AND MATERIALS

(57) Abstract

*Staphylococcus aureus* virulence genes are identified, thereby allowing the identification of novel anti-bacterial agents that target these virulence genes and their products, and the provision of novel *S. aureus* mutants useful in vaccines.

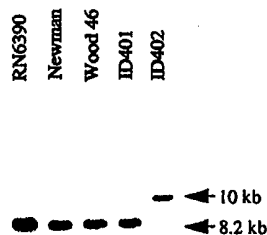


B.

```

MKDEQLYYFEKSPVFKAMMHFSLPMMIGTLLSVIYGILNYIFIGFSEESH 50
MISAIISLTLPVFAILMGLNLFVGAGTYISRLLGARDYSKSKFEVSSFSI 100
YGGIALGLIVLVTLPFSDQSQQF* 124
    
```

C.



**FOR THE PURPOSES OF INFORMATION ONLY**

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

<b>AL</b>	Albania	<b>ES</b>	Spain	<b>LS</b>	Lesotho	<b>SI</b>	Slovenia
<b>AM</b>	Armenia	<b>FI</b>	Finland	<b>LT</b>	Lithuania	<b>SK</b>	Slovakia
<b>AT</b>	Austria	<b>FR</b>	France	<b>LU</b>	Luxembourg	<b>SN</b>	Senegal
<b>AU</b>	Australia	<b>GA</b>	Gabon	<b>LV</b>	Latvia	<b>SZ</b>	Swaziland
<b>AZ</b>	Azerbaijan	<b>GB</b>	United Kingdom	<b>MC</b>	Monaco	<b>TD</b>	Chad
<b>BA</b>	Bosnia and Herzegovina	<b>GE</b>	Georgia	<b>MD</b>	Republic of Moldova	<b>TG</b>	Togo
<b>BB</b>	Barbados	<b>GH</b>	Ghana	<b>MG</b>	Madagascar	<b>TJ</b>	Tajikistan
<b>BE</b>	Belgium	<b>GN</b>	Guinea	<b>MK</b>	The former Yugoslav Republic of Macedonia	<b>TM</b>	Turkmenistan
<b>BF</b>	Burkina Faso	<b>GR</b>	Greece			<b>TR</b>	Turkey
<b>BG</b>	Bulgaria	<b>HU</b>	Hungary	<b>ML</b>	Mali	<b>TT</b>	Trinidad and Tobago
<b>BJ</b>	Benin	<b>IE</b>	Ireland	<b>MN</b>	Mongolia	<b>UA</b>	Ukraine
<b>BR</b>	Brazil	<b>IL</b>	Israel	<b>MR</b>	Mauritania	<b>UG</b>	Uganda
<b>BY</b>	Belarus	<b>IS</b>	Iceland	<b>MW</b>	Malawi	<b>US</b>	United States of America
<b>CA</b>	Canada	<b>IT</b>	Italy	<b>MX</b>	Mexico	<b>UZ</b>	Uzbekistan
<b>CF</b>	Central African Republic	<b>JP</b>	Japan	<b>NE</b>	Niger	<b>VN</b>	Viet Nam
<b>CG</b>	Congo	<b>KE</b>	Kenya	<b>NL</b>	Netherlands	<b>YU</b>	Yugoslavia
<b>CH</b>	Switzerland	<b>KG</b>	Kyrgyzstan	<b>NO</b>	Norway	<b>ZW</b>	Zimbabwe
<b>CI</b>	Côte d'Ivoire	<b>KP</b>	Democratic People's Republic of Korea	<b>NZ</b>	New Zealand		
<b>CM</b>	Cameroon	<b>KR</b>	Republic of Korea	<b>PL</b>	Poland		
<b>CN</b>	China	<b>KZ</b>	Kazakstan	<b>PT</b>	Portugal		
<b>CU</b>	Cuba	<b>LC</b>	Saint Lucia	<b>RO</b>	Romania		
<b>CZ</b>	Czech Republic	<b>LI</b>	Liechtenstein	<b>RU</b>	Russian Federation		
<b>DE</b>	Germany	<b>LK</b>	Sri Lanka	<b>SD</b>	Sudan		
<b>DK</b>	Denmark	<b>LR</b>	Liberia	<b>SE</b>	Sweden		
<b>EE</b>	Estonia			<b>SG</b>	Singapore		

# INTERNATIONAL SEARCH REPORT

Inte. n. Application No

PCT/GB 98/01974

**A. CLASSIFICATION OF SUBJECT MATTER**  
 IPC 6 C07K14/31 C12Q1/18 A61K39/085

According to International Patent Classification (IPC) or to both national classification and IPC

**B. FIELDS SEARCHED**

Minimum documentation searched (classification system followed by classification symbols)  
 IPC 6 C07K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

**C. DOCUMENTS CONSIDERED TO BE RELEVANT**

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	EP 0 625 575 A (LILLY CO ELI) 23 November 1994 See page 12, line 34-40 see the whole document ---	1-3,8-14
X	WO 96 17951 A (RPMS TECHNOLOGY LTD ;HOLDEN DAVID WILLIAM (GB)) 13 June 1996 see the whole document ---	1-3,8-14
Y	WO 97 11690 A (MICROCIDE PHARMACEUTICALS INC) 3 April 1997 see the whole document ---	1-3,8-14
	-/--	

Further documents are listed in the continuation of box C.

Patent family members are listed in annex.

° Special categories of cited documents :

- "A" document defining the general state of the art which is not considered to be of particular relevance
- "E" earlier document but published on or after the international filing date
- "L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
- "O" document referring to an oral disclosure, use, exhibition or other means
- "P" document published prior to the international filing date but later than the priority date claimed

- "T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
- "X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
- "Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.
- "&" document member of the same patent family

Date of the actual completion of the international search

8 February 1999

Date of mailing of the international search report

03. 03. 1999

Name and mailing address of the ISA

European Patent Office, P.B. 5818 Patentlaan 2  
 NL - 2280 HV Rijswijk  
 Tel. (+31-70) 340-2040, Tx. 31 651 epo nl,  
 Fax: (+31-70) 340-3016

Authorized officer

Hagenmaier, S

## INTERNATIONAL SEARCH REPORT

Inter national Application No

PCT/GB 98/01974

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT		
Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	<p>BERGER-BÄCHI ET AL.: "FemA A HOST MEDIATED FACTOR ESSENTIAL FOR METHICILLIN RESISTANCE IN STAPHYLOCOCCUS AUREUS: MOLECULAR CLONING AND CHARACTERIZATION" MOL.GEN.GENET., vol. 219, 1989, pages 263-269, XP002081494 &amp; DATABASE EMPRO AC:X17688, 100 % identity in 1260 bp overlap with Seq.ID 1 &amp; DATABASE PIR2 AC/ID:S06784, 100 % identity in 419 aa overlap with Seq. ID 2 see abstract</p>	1-3,8-14
Y	<p>--- JÜNGST UND ZUMFT: "INTERDEPENDENCE OF RESPIRATORY NO REDUCTION AND NITRITE REDUCTION REVEALED BY MUTAGENESIS OF nirQ, A NOVEL GENE IN THE DENITRIFICATION GENE CLUSTER OF PSEUDOMONAS STUTZERI" FEBS, vol. 314, no. 3, 1992, pages 308-314, XP002092571 see the whole document &amp; DATABASE TRPRO AC/ID: Q02441, 1996 99.6 % identity in 275 aa overlap with Seq.ID 12 see abstract</p>	1,2,8-14
Y	<p>--- KAMATA ET AL.: "PRIMARY STRUCTURE OF THE ALANINE CARRIER PROTEIN OF THERMOPHILIC BACTERIUM PS3" J.BIOL.CHEM., vol. 267, no. 30, 1992, pages 21650-21665, XP002092572 see the whole document &amp; DATABASE SWISSPROT AC: P30145, 1993 100.0 % identity in 445 aa overlap with Seq.ID 61 see abstract</p> <p>--- -/--</p>	1,2,8-14

## INTERNATIONAL SEARCH REPORT

International Application No

PCT/GB 98/01974

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT		
Category	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	<p>EBBOLE ET AL.: "CLONING AND CHARACTERIZATION OF A 12-GENE CLUSTER FROM BACILLUS SUBTILIS ENCODING NINE ENZYMES FOR DE NOVO PURINE NUCLEOTIDE SYNTHESIS" J.BIOL.CHEM., vol. 262, no. 17, 1987, pages 8274-8287, XP002092573 &amp; DATABASE SWISSPROT AC: P12042, 1989 54.3 % identity in 738 aa overlap with Seq.ID 30 see abstract</p> <p style="text-align: center;">---</p>	1,2,8-14
A	<p>SAMUELSSON: "A MYCOPLASMA PROTEIN HOMOLOGOUS TO MAMMALIAN SRP54 RECOGNIZES A HIGHLY CONSERVED DOMAIN OF SRP RNA" NUCLEIC ACIDS RESEARCH, vol. 20, no. 21, 1992, pages 5763-5770, XP002092574 &amp; DATABASE SWISSPROT AC: Q01444, 1993 40.7% identity in 427 aa overlap with Seq.ID 74 see abstract</p> <p style="text-align: center;">---</p>	1,2,8-14
A	<p>KAPPES ET AL.: "THREE TRANSPORT SYSTEMS FOR THE OSMOPROTECTANT GLYCINE BETAINE OPERATE IN BACILLUS SUBTILIS: CHARACTERIZATION OF OpuD" J.BACTERIOL., vol. 178, no. 17, 1996, pages 5071-5079, XP002092575 &amp; DATABASE SWISSPROT AC: P54417, "GLYCIN BETAINE TRANSPORTER OPUD" 53.8 % identity in 366 aa overlap with Seq. ID 80 see abstract</p> <p style="text-align: center;">---</p>	1,2,8-14
A	<p>RUDNER ET AL.: "THE spoOK LOCUS OF BACILLUS SUBTILIS IS HOMOLOGOUS TO THE OLIGOPEPTIDE PERMEASE LOCUS AND IS REQUIRED FOR SPORULATION AND COMPETENCE" J.BACTERIOL., vol. 173, no. 4, 1991, pages 1388-1398, XP002092576 &amp; DATABASE SWISSPROT AC:P24136, RUDNER ET AL.: 31.2 % identity in 253 aa overlap with Seq. ID 18 see abstract</p> <p style="text-align: center;">---</p> <p style="text-align: center;">-/--</p>	1,2,8-14

## INTERNATIONAL SEARCH REPORT

International Application No

PCT/GB 98/01974

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT		
Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	US 5 587 288 A (CHEUNG AMBROSE ET AL) 24 December 1996 see the whole document ----	1,2,8-14
A	WO 96 03647 A (ZENECA LTD ;GARMAN ANDREW JOHN (GB); HOLLAND JANET DORA (GB)) 8 February 1996 see the whole document ----	8-12
A	US 5 283 173 A (FIELDS STANLEY ET AL) 1 February 1994 see the whole document ----	9-11
A	US 4 973 554 A (LUONG JOHN H ET AL) 27 November 1990 see the whole document ----	12
P,Y	DATABASE MEDLINE MOL. MICROBIOLOGY, vol. 26, no. 2, pp 399-407, October 1997 MEI ET AL.: "IDENTIFICATION OF STAPHYLOCOCCUS AUREUS VIRULENCE GENES IN A MURINE MODEL OF BACTERAEMIA USING SIGNATURE-TAGGED MUTAGENESIS" XP002092578 see abstract ----	1-3,8-14
P,Y	DATABASE GCG_GENESEQ_P AC: W28288, BLACK ET AL.: "NOVEL POLYPEPTIDE FROM STAPHYLOCOCCUS AUREUS, ABC TRANSPORTER PROTEIN" XP002092610 94.5 % identity in 181 aa overlap with Seq.ID 20 ----	1,2,8-14
P,A	HOLDEN: "DISCOVERING NEW TARGETS FOR ANTI-BACTERIAL AGENTS BY IDENTIFICATION OF GENES ESSENTIAL FOR PATHOGENICITY" BRITISH JOURNAL OF PHARMACOLOGY, vol. 123, March 1998, page 349P XP002092577 see the whole document ----	1-3,8-14
P,A	DATABASE PIR2 AC: F69884, 5 December 1997 KUNST ET AL.: "CONSERVED HYPOTHETICAL PROTEIN ymdA-BACILLUS SUBTILIS" XP002092579 68.5 % in 517 aa overlap with Seq.ID 74 see abstract -----	1,2,8-14

# INTERNATIONAL SEARCH REPORT

International application No.  
PCT/GB 98/01974

## Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1.  Claims Nos.:  
because they relate to subject matter not required to be searched by this Authority, namely:
  
2.  Claims Nos.:  
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:
  
3.  Claims Nos.:  
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

## Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

see additional sheet

1.  As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.
  
2.  As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
  
3.  As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:  
  
1-3,8-14 (all partially). A complete search was done for Seq.ID  
1,2,9,10,12,18,20,30,61,66,74 and 80
  
4.  No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

### Remark on Protest

- The additional search fees were accompanied by the applicant's protest.
- No protest accompanied the payment of additional search fees.

## FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

This International Searching Authority found multiple (groups of) inventions in this international application, as follows:

## 1. Claims: 1-3,8-14 (all partially)

## Inventions 1-4:

Method of identifying an anti-bacterial agent comprising the steps of assaying potential agents for the ability to interfere with the expression of the virulence gene with Seq. ID 1 or the function of the encoded protein, wherein the protein is involved in cell surface metabolism and a *Staphylococcus aureus* organism containing a functional mutation in the gene represented by Seq. ID 1 as well as a vaccine composition comprising that mutated *S.aureus*.

...ibidem for each of sequences represented by Seq. ID 6,60,65.

## 2. Claims: 1,2,8-14 (all partially)

## Inventions 5-67:

Method of identifying an anti-bacterial agent comprising the steps of assaying potential agents for the ability to interfere with the expression of a virulence gene with Seq. ID 2 or the function of the encoded protein and a *Staphylococcus aureus* organism containing a functional mutation in the gene represented by Seq. ID 2 as well as a vaccine composition comprising that mutated *S.aureus*.

...ibidem for each of sequences represented by Seq. ID 3,5,7,9,10,12,14,16,18,20,23,24,26,28,30,32,34,36,38,40,42,45,47-53,55-59,61-64,66-74,76,78,80,81,83,85-94

## 3. Claims: 1,2,4,8-14 (all partiall)

## Inventions 68-78

Method of identifying an anti-bacterial agent comprising the steps of assaying potential agents for the ability to interfere with the expression of the virulence gene with Seq. ID 4 or the function of the encoded protein, wherein the protein is involved in cellular biosynthetic pathway and a *Staphylococcus aureus* organism containing a functional mutation in the gene represented by Seq. ID 4 as well as a vaccine composition comprising that mutated *S.aureus*.

...ibidem for each of sequences represented by Seq. ID 11,13,22,25,27,29,31,33,35,82

## 4. Claims: 1,2,7-14 (all partially)



**FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210**

**Inventions 79-82:**

Method of identifying an anti-bacterial agent comprising the steps of assaying potential agents for the ability to interfere with the expression of the virulence gene with Seq. ID 8 or the function of the encoded protein, wherein the protein is involved in cellular regulatory and repair processes, and a Staphylococcus aureus organism containing a functional mutation in the gene represented by Seq. ID 8 as well as a vaccine composition comprising that mutated S.aureus.

...ibidem for each of sequences represented by Seq. ID 21,46,84

**5. Claims: 1,2,6,8-14 (all partially)**

**Inventions 83-89**

Method of identifying an anti-bacterial agent comprising the steps of assaying potential agents for the ability to interfere with the expression of the virulence gene with Seq. ID 15 or the function of the encoded protein, wherein the protein is an oligopeptide transport protein and a Staphylococcus aureus organism containing a functional mutation in the gene represented by Seq. ID 15 as well as a vaccine composition comprising that mutated S.aureus.

...ibidem for each of sequences represented by Seq. ID 17,19,54,75,77,79

**6. Claims: 1,2,5,8-14 (all partially)**

**Inventions 90-94:**

Method of identifying an anti-bacterial agent comprising the steps of assaying potential agents for the ability to interfere with the expression of the virulence gene with Seq. ID 37 or the function of the encoded protein, wherein the protein is a component of the TCA cycle and a Staphylococcus aureus organism containing a functional mutation in the gene represented by Seq. ID 37 as well as a vaccine composition comprising that mutated S.aureus.

...ibidem for each of sequences represented by Seq. ID 39,41,43,44.

# INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No

PCT/GB 98/01974

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
EP 0625575 A	23-11-1994	AU 6180294 A	03-11-1994
		CA 2122202 A	31-10-1994
		HU 70300 A	28-09-1995
		JP 6319561 A	22-11-1994
		US 5587307 A	24-12-1996
WO 9617951 A	13-06-1996	AT 171477 T	15-10-1998
		AU 4121996 A	26-06-1996
		CA 2206515 A	13-06-1996
		CN 1176662 A	18-03-1998
		CZ 9701755 A	15-10-1997
		DE 69505011 D	29-10-1998
		EP 0796341 A	24-09-1997
		EP 0889120 A	07-01-1999
		FI 972424 A	06-08-1997
		HU 76975 A	28-01-1998
		JP 10509872 T	29-09-1998
		NO 972468 A	08-08-1997
		WO 9711690 A	03-04-1997
US 5587288 A	24-12-1996	NONE	
WO 9603647 A	08-02-1996	AU 2986395 A	22-02-1996
		EP 0774116 A	21-05-1997
		GB 2291708 A,B	31-01-1996
US 5283173 A	01-02-1994	US 5468614 A	21-11-1995
		US 5667973 A	16-09-1997
US 4973554 A	27-11-1990	NONE	