

(19) World Intellectual Property Organization  
International Bureau



(43) International Publication Date  
27 April 2006 (27.04.2006)

PCT

(10) International Publication Number  
**WO 2006/043182 A3**

(51) International Patent Classification:

*C12N 15/31* (2006.01) *A61K 39/40* (2006.01)  
*G01N 33/569* (2006.01) *A61K 31/7105* (2006.01)  
*C12Q 1/68* (2006.01) *C12N 15/11* (2006.01)  
*C12Q 1/02* (2006.01) *C07K 14/31* (2006.01)  
*C12N 15/74* (2006.01) *A61P 31/00* (2006.01)

(21) International Application Number:

PCT/IB2005/004081

(22) International Filing Date:

8 September 2005 (08.09.2005)

(25) Filing Language:

English

(26) Publication Language:

English

(30) Priority Data:

60/607,896 8 September 2004 (08.09.2004) US

(71) Applicant (for all designated States except US): **THE UNIVERSITY OF WESTERN ONTARIO** [CA/CA];

Industry Liaison, 1151 Richmond Street North, Stevenson-Lawson Building, Room 319, London, Ontario N6A 5B8 (CA).

(72) Inventors; and

(75) Inventors/Applicants (for US only): **HEINRICHS, David, E.** [CA/CA]; 923 Maitland Street, London, Ontario N5Y 2X2 (CA). **DALE, Suzanne** [US/US]; 40-5 Lilac Drive, Rochester, NY 14620 (US).

(74) Agents: **SCHUMACHER, Lynn, C.** et al.; Hill & Schumacher, 87 Falcon Street, Toronto, Ontario M4S 2P4 (CA).

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

(84) Designated States (unless otherwise indicated, for every kind of regional protection available): ARIPO (BW, GH, GM, KE, LS, MW, MZ, NA, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European (AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HU, IE, IS, IT, LT, LU, LV, MC, NL, PL, PT, RO, SE, SI, SK, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

Published:

— with international search report

(88) Date of publication of the international search report:

21 September 2006

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

(54) Title: SCREENING ASSAYS FOR INHIBITORS OF A STAPHYLOCOCCUS AUREUS SIDEROPHORE

(57) Abstract: Isolation of an iron regulated, nme-gene operon (designated sbn) from Staphylococcus aureus (RN6390), responsible for the biosynthesis of staphylobactm, a novel S. aureus siderophore Methods for treating or preventing a disease or condition caused by S. aureus infection, as well as methods for identifying agents that inhibit the biosynthesis of staphylobactm or inhibit the expression of genes in said sbn operon are further disclosed.



WO 2006/043182 A3

# INTERNATIONAL SEARCH REPORT

International application No.  
PCT/IB2005/004081

## A. CLASSIFICATION OF SUBJECT MATTER

IPC: *C12N 15/31* (2006.01), *G01N 33/569* (2006.01), *C12Q 1/68* (2006.01), *C12Q 1/02* (2006.01), *C12N 15/74* (2006.01), *A61K 39/40* (2006.01), *A61K 31/7105* (2006.01), *C12N 15/11* (2006.01), *C07K 14/31* (2006.01), *A61P 31/00* (2006.01)

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: *C12N 15/31* (2006.01), *G01N 33/569* (2006.01), *C12Q 1/68* (2006.01), *C12Q 1/02* (2006.01), *C12N 15/74* (2006.01), *A61K 39/40* (2006.01), *A61K 31/7105* (2006.01), *C12N 15/11* (2006.01), *C07K 14/31* (2006.01), *A61P 31/00* (2006.01)

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

Electronic database(s) consulted during the international search (name of database(s) and, where practicable, search terms used)

STN (Medline, CAPLus, BIOSIS), PubMed, Delphion, Canadian Patent Database, Espacenet, GenomeQuest

Keywords: Siderophore, sbn, Staphylobactin, Staphylococcus aureus, Heinrichs, D.E., iron-regulated

## C. DOCUMENTS CONSIDERED TO BE RELEVANT

| Category* | Citation of document, with indication, where appropriate, of the relevant passages                                                                                                                                                                                                    | Relevant to claim No.         |
|-----------|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-------------------------------|
| X         | DALE, S.E. <i>et al.</i> Role of Siderophore Biosynthesis in Virulence of <i>Staphylococcus aureus</i> : Identification and Characterization of Genes Involved in Production of a Siderophore. Infection and Immunity. January 2004, Vol. 72, No. 1, pages 29-37. see whole document. | 1, 10 and 19-32               |
| X         | US 2004/0029129 A1 (WANG, L. <i>et al.</i> ) February 12, 2004. see whole document.                                                                                                                                                                                                   | 1, 10 and 19, 21-23 and 25-26 |
| X         | AOKI, K. GenBank Accession Number BAB93954. May 25, 2002.                                                                                                                                                                                                                             | 1 and 10                      |
| X         | HOLDEN, M.T.G. <i>et al.</i> GenBank Accession Number CAG41857. June 24, 2004.                                                                                                                                                                                                        | 1 and 10                      |

☐ Further documents are listed in the continuation of Box C.

☒ See patent family annex.

|                                                                                                                                                                         |                                                                                                                                                                                                                                                  |
|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| * Special categories of cited documents :                                                                                                                               | "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                                              |
| "A" document defining the general state of the art which is not considered to be of particular relevance                                                                | "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                                                                     |
| "B" earlier application or patent but published on or after the international filing date                                                                               | "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 |
| "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) | "&" document member of the same patent family                                                                                                                                                                                                    |
| "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                                                                  |                                                                                                                                                                                                                                                  |

|                                                                                                                                                                                                                       |                                                                                 |
|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|---------------------------------------------------------------------------------|
| Date of the actual completion of the international search<br>24 May 2006 (24-05-2006)                                                                                                                                 | Date of mailing of the international search report<br>29 June 2006 (29-06-2006) |
| Name and mailing address of the ISA/CA<br>Canadian Intellectual Property Office<br>Place du Portage I, C114 - 1st Floor, Box PCT<br>50 Victoria Street<br>Gatineau, Quebec K1A 0C9<br>Facsimile No.: 001(819)953-2476 | Authorized officer<br><br>Robin Green (819) 997-3077                            |

**INTERNATIONAL SEARCH REPORT**  
Information on patent family members

International application No.  
**PCT/IB2005/004081**

| Patent Document<br>Cited in Search Report | Publication<br>Date | Patent Family<br>Member(s) | Publication<br>Date |
|-------------------------------------------|---------------------|----------------------------|---------------------|
| US2004029129 A1                           | 12-02-2004          | AU4934501 A                | 03-10-2001          |
|                                           |                     | EP1268774 A2               | 02-01-2003          |
|                                           |                     | CA2404260 A1               | 27-09-2001          |
|                                           |                     | JP2004516805T T            | 10-06-2004          |
|                                           |                     | US2002061569 A1            | 23-05-2002          |
|                                           |                     | WO0170955 A2               | 27-09-2001          |
|                                           |                     | WO02077183 A2              | 03-10-2002          |

**INTERNATIONAL SEARCH REPORT**International application No.  
PCT/IB2005/004081**Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of the 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. ☒ Claim Nos. : 23-26  
because they relate to subject matter not required to be searched by this Authority, namely :  
  
they are directed to a method for treatment of the human or animal body by surgery or therapy which the International Searching Authority is not required to search under Rule 39.1 (iv) of the PCT. Regardless, a search has been carried out based on the alleged effects of the composition in claims 23-26.
2. ☐ Claim Nos. :  
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically :
3. ☐ Claim Nos. :  
because they are dependant claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

**Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)**

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

See extra sheet (Continuation of Box. No. III)

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 additional fees, this Authority did not invite payment of additional fees.
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 claim Nos. :
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 claim Nos. :  
  
1 and 10 (in whole) and 19-32 (in part) as they relate to SbnA, defined by SEQ ID NO:2 and 4.

**Remark on Protest** ☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.

☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.

☐ No protest accompanied the payment of additional search fees.

Continuation of Box No. III

**Group 1:** Claims 1, 10 (in whole) and 19-32 (in part) are directed to an isolated nucleic acid comprising SEQ ID NO:2, the polypeptide comprising SEQ ID NO:4 (SbnA), pharmaceutical compositions comprising either an antibody to SEQ ID NO:4, a fragment of SEQ ID NO:4, nucleic acid antisense to SEQ ID NO:2 or an siRNA molecule comprising SEQ ID NO:2, methods of treating or preventing a disease or condition that is caused by infection of *S. aureus* using said pharmaceutical compositions, as well as methods for identifying an agent that binds to SbnA and inhibits the biosynthesis of a siderophore, as well as an agent that inhibits the expression of *SbnA*;

**Group 2:** Claims 2 and 11 (in whole) and 19-32 (in part) are directed to an isolated nucleic acid comprising SEQ ID NO:5, and the polypeptide comprising SEQ ID NO:7 (SbnB) and uses thereof;

**Group 3:** Claims 3 and 12 (in whole) and 19-32 (in part) are directed to an isolated nucleic acid comprising SEQ ID NO:8, and the polypeptide comprising SEQ ID NO:10 (SbnC) and uses thereof;

**Group 4:** Claims 4 and 13 (in whole) and 19-32 (in part) are directed to an isolated nucleic acid comprising SEQ ID NO:11, and the polypeptide comprising SEQ ID NO:13 (SbnD) and uses thereof;

**Group 5:** Claims 5 and 14 (in whole) and 19-32 (in part) are directed to an isolated nucleic acid comprising SEQ ID NO:14, and the polypeptide comprising SEQ ID NO:16 (SbnE) and uses thereof;

**Group 6:** Claims 6 and 15 (in whole) and 19-32 (in part) are directed to an isolated nucleic acid comprising SEQ ID NO:17, and the polypeptide comprising SEQ ID NO:19 (SbnF) and uses thereof;

**Group 7:** Claims 7 and 16 (in whole) and 19-32 (in part) are directed to an isolated nucleic acid comprising SEQ ID NO:20, and the polypeptide comprising SEQ ID NO:22 (SbnG) and uses thereof;

**Group 8:** Claims 8 and 17 (in whole) and 19-32 (in part) are directed to an isolated nucleic acid comprising SEQ ID NO:23, and the polypeptide comprising SEQ ID NO:25 (SbnH) and uses thereof and;

**Group 9:** Claims 9 and 18 (in whole) and 19-32 (in part) are directed to an isolated nucleic acid comprising SEQ ID NO:26, and the polypeptide comprising SEQ ID NO:28 (SbnI) and uses thereof.

The application is directed to an iron-regulated, nine gene operon involved in the biosynthesis of siderophore in *Staphylococcus aureus*. Each of the genes and corresponding proteins of said operon are not so linked as to form a single general inventive concept and are considered to represent separate inventions. None of the chemical compounds defined by the groups of claims are related by common structure and function and are therefore, regarded as multiple distinct types of chemical compounds that are related only by their combined alleged utility for the production of siderophore.

Moreover, in view of *a posteriori* consideration, Dale, S. *et al.*, (Infection and Immunity, January 2004, Vol. 72, No. 1, pages 29-37) disclose, prior to the claim date of the present application, the isolation and characterisation of an iron regulated, nine-gene operon involved in the biosynthesis of a siderophore in *S. aureus* (RN6390), which is the same strain as used in the present application. Accordingly, the plurality of sequences claimed by the application cannot be considered as representing a single inventive concept.