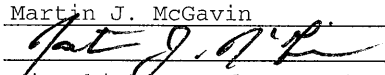
THE UNIVERSITY OF WESTERN ONTARIO
BIOHAZARDOUS AGENTS REGISTRY FORM
Approved Biohazards Subcommittee: March 27, 2009
Biosafety Website: www.uwo.ca/humanresources/biosafety/

This form must be completed by each Principal Investigator holding a grant administered by the University of Western Ontario or in charge of a laboratory/facility where the use of Level 1, 2 or 3 biohazardous agents is described in the laboratory or animal work proposed. The form must also be completed if any work is proposed involving animals carrying zoonotic agents infectious to humans or involving plants, fungi, or insects that require Public Health Agency of Canada (PHAC) or Canadian Food Inspection Agency (CFIA) permits.

This form must ~~also~~ be updated at least every 3 years or when there are changes to the biohazards being used.

Containment Levels will be established in accordance with Laboratory Biosafety Guidelines, 3rd edition, 2004, Public Health Agency of Canada (PHAC) or Containment Standards for Veterinary Facilities, 1st edition 1996, Canadian Food Inspection Agency (CFIA).

Completed forms are to be returned to Occupational Health and Safety, (OHS), (Support Services Building, Room 4190) for distribution to the Biohazard Subcommittee. For questions regarding this form, please contact the Biosafety Officer at extension 81135 or biosafety@uwo.ca. If there are changes to the information on this form (excluding grant title and funding agencies), contact Occupational Health and Safety for a modification form. See website: www.uwo.ca/humanresources/biosafety

PRINCIPAL INVESTIGATOR	Martin J. McGavin
SIGNATURE	
DEPARTMENT	Microbiology and Immunology
ADDRESS	Schulich School of Medicine and Dentistry,3014 Dental Sciences
PHONE NUMBER	519-850-2458
EMERGENCY PHONE NUMBER(S)	Not available yet
EMAIL	mmcgavin@uwo.ca

Location of experimental work to be carried out: Building(s) Siebens Drake Room(s) 126, 122, 123,119

*For work being performed at Institutions affiliated with the University of Western Ontario, the Safety Officer for the Institution where experiments will take place must sign the form prior to its being sent to the University of Western Ontario Biosafety Officer (See Section 12.0, Approvals).

FUNDING AGENCY/AGENCIES: 1, CIHR; 2, Canadian Dermatology Foundation; 3, Sanofi-Aventis
GRANT TITLE(S): 1, Role of S. aureus proteases in tissue invasion and immune evasion;
2, Clonal types and characterization of S. aureus associated with atopic dermatitis
3, Evaluation of PrsA prolyl isomerase as a vaccine antigen for S. aureus

PLEASE ATTACH A BRIEF DESCRIPTION OF YOUR WORK THAT EXPLAINS THE BIOHAZARDS USED AND HOW THEY WILL BE USED. PROJECTS SUBMITTED WITHOUT A SUMMARY WILL NOT BE REVIEWED.

Names of all personnel working under Principal Investigators supervision in this location:

1. Daniel Thompsen Passos (Postdoc)
2. Julienne Kaiser (Summer student)

*** DESCRIPTION MUST BE ATTACHED TO THIS FORM OR PROJECT WILL NOT BE REVIEWED***

CIHR

McGavin, MJ Modulation of inflammation and invasion by *S. aureus* proteases $125,794 **Summary**

Staphylococcus aureus is a major cause of infectious morbidity and mortality. As a leading cause of bacteremia, its ability to thrive in the bloodstream is reflected by coagulase and staphylokinase; proteins that bind and subvert plasma serine protease precursors prothrombin and plasminogen, which participate in proteolytic cascade pathways to control coagulation. **We hypothesize** that another integral component of the niche-adaptation paradigm defined by subversion of plasma serine proteases, is the Staphylococcal Proteolytic Cascade pathway (SPC), consisting of the metalloprotease Aureolysin, the serine protease SspA, and the cysteine protease SspB (Staphopain B), which together with Staphopain A (ScpA), promote tissue invasion and immune evasion. In this proposal, we will:

1. Quantify virulence of clinical isolates that differ in protease expression, including deletion mutants defective in specific proteases.
2. Evaluate *in vivo* protease expression by *S. aureus*, and of host-derived cathepsins that contribute to inflammation.
3. Challenge neutrophils and macrophages with *S. aureus* and purified proteases, to evaluate how proteases affect immune cell function.
4. Use *in vivo* and *ex vivo* techniques to define how proteases promote toxin dissemination, and signaling through protease activated receptors (PARs).

Aim 1 evaluates a prototypic infection strategy defined by growth in contained abscesses, where proteases are expressed only in response to *in vivo* conditions, in relation to strains that have altered the prototype by dysregulated protease expression. Strain Newman, does not exhibit *in vitro* protease expression and defines the prototypic strategy in a murine model of kidney abscess infection, but exhibits invasive growth in response to a *sar*A mutation that inactivates a repressor of protease expression. We will compare this phenotype to hyper-virulent community-aquired MRSA (CA-MRSA) that do exhibit *in vitro* protease expression, and will inactivate specific protease genes in Newman *sar*A and CA-MRSA to determine if there is reversion to contained abscesses. Quantitative parameters include weight loss; assay of kidney homogenates for microbial cfu, cytokines and chemokines, and myeloperoxidase activity; and pathology and immuno-histology of detect bacteria, IL-1β, the neutrophil specific marker Ly6G, and secreted microbial proteases in tissue sections. **Aim 2** will determine if mice challenged with strain Newman develop antibodies to specific proteases, and will compare antibody titres to mice challenged with Newan *sar*A and hyper-virulent CA-MRSA, to provide a measure of the *in vivo* expression of proteases. We will also develop the use of an activity-based probe that covalently targets the papain family of cysteine proteases, including Staphopains and lysosomal Cathepsins. This will allow affinity purification of tagged proteases from kidney homogenates, from which we hope to identify via mass spectrometry the microbial Staphopains, and evaluate how the profile of cathepsins is altered by strains with different virulence traits. **Aim 3** assesses how neutrophils and macrophages respond to treatment with proteases, or *S. aureus* strains with different proteolytic capacities. Neutrophils will be treated with proteases alone or in combination prior to addition of opsonized *S. aureus* Newman, and quantification of phagocytic killing over a 6h span, to determine if proteases impair this process. Chemokine IL-8 will be treated with purified proteases, followed by assay for its ability to stimulate neutrophils. Macrophages will be challenged with live opsonized bacteria, including Newman, and isogenic *sar*A or protease defective mutants, as well as CA-MRSA, followed by (i), evaluation of phagocytosis and killing; (ii), cathepsin profiling of cell lysates; and (iii), cytokine microarray analyses. **Aim 4** assesses how proteases may facilitate delivery of toxins into the blood stream, or modify proteases activated receptors (PARs). An *ex vivo* assay with porcine vaginal mucosa will determine if proteases promote passage of toxic shock syndrome toxin (TSST) across an epithelial layer, and this will be combined with use of transgenic HLA-DR4 mice that respond to systemic superantigens, to determine if there is greater sensitivity to TSST in mice challenged with wild type or protease deficient *S. aureus* expressing TSST. Using cell lines that express different PARs, which when activated promote an inflammatory response, we will determine if *S. aureus* proteases promote inflammation by activating PARs, or down-regulate inflammation by disarming PARs.

Canadian Dermatology Foundation

SUMMARY

Atopic dermatitis is an inflammatory, chronically relapsing skin condition, characterized by susceptibility to secondary infection of the skin. Typically, patients with severe AD exhibit a high rate of colonization by *Staphylococcus aureus*, and a role for *S. aureus* in exacerbating or promoting the inflammation that is characteristic of severe AD has long been suspected; superantigen toxins (*seb*, *tst*) in particular have been strongly implicated. In the currently funded proposal, we proposed that the skin of AD patients is colonized by specific clonal types of *S. aureus* that are optimized to the altered environment of the AD skin. We also proposed that AD patients are at risk for colonization by hyper-virulent strains of community acquired methicillin resistant *S. aureus* (MRSA), and the large AD population will serve as a a nidus for transmission of these strains in the community. We employed advanced genotyping methods to assess this hypothesis with *S. aureus* recovered from n=43 patients visiting the Dermatitis Clinic at Sunnybrook. Our findings to date indicate that (i), clonal types corresponding to hyper-virulent community acquired strains do not yet colonize AD patients; (ii) clonal types that are most commonly associated with asymptomatic nasal carriage, or complicated nosocomial bacteremia are infrequently isolated; (iii) 40% of patients are colonized by isolates corresponding to ST45 and ST15 clonal types; (iv), 70% of patients are colonized by isolates that potentially have novel or absent νSaβ elements which may carry important determinants of colonization, and (v), the distribution of superantigen genes was lower than expected. Our objectives for a second year of funding will be to:

1. Conduct study, of exclusively pediatric patients from Sick Childrens hospital.
2. Conduct follow-up studies at Sunnybrook for selected patients from which isolates were obtained in the past funding period, to determine if they have become re-colonized by similar or different genetic backgrounds after therapy.
3. Determine the structure of genomic DNA corresponding to the region that should contain νSaβ, in strains that have novel or absent νSaβ elements.
4. Screen for a novel pathogenicity island that carries ET-D and EDIN toxins.

Aim 1 will follow from our finding that most isolates we assessed did not carry the superantigen toxins that have typically been implicated in severe atopic dermatitis, and also that hyper-virulent community acquired strains were rare (n=1 patient). As both of these traits may more important in a pediatric population, we will work in collaboration with Dr. Elena Pope at Sick Childrens Hospital, to conduct detailed genotyping of isolates from pediatric patients, and compare the data with our study at Sunnybrook. **Aim 2** will conduct follow-up studies with patients from the Sunnybrook clinic, from which we isolated strains that did not possess superantigens. We will determine if after treatment, the patients are re-colonized by the same or other non-superantigen producing strains. Reciprocal experiments will be performed with patients that were first colonized by superantigen producing strains. **Aims 3 and 4** will determine the structure and/or presence of genes in a variable *S. aureus* genomic island, νSaβ. There are three variations of this element in major human disease causing strains, which may carry either enterotoxins, proteases that are structurally similar to exfoliative toxin, hyaluronate lyase enzyme, and bacteriocin synthesis. These elements may be important determinants of adaptation to ecological niches, and our typing data suggested that many of the isolates recovered from AD skin harbored novel variants of this element. Notably, a νSaβ-like structure described in Aim 4 carries an ADP-ribosylating epidermal differentiation inhibitor toxin. Therefore, we will identify and characterize νSaβ elements that may be unique to strains isolated from AD skin.

Sanofi-Aventis

HYPOTHESIS: We hypothesize that cell-surface PrsA in Gram-positive bacteria, and the periplasmic SurA of Gram-negative bacteria are structurally and functionally related. As with SurA, we anticipate that the N-terminal domain of PrsA provides a chaperone function that promotes the folding of one or more proteins required for cell viability. The same domain could also promote folding of secreted virulence factors. Alternatively, the prolyl-isomerase domain may promote the folding of secreted virulence factors, while the N-terminal domain acts upon other proteins that are essential for viability. The experimental stratagem will distinguish between these possibilities. As PrsA is localized to the cell surface, it is well situated as a target for vaccination strategies, and drugs that inhibit its function. Such therapeutic modalities would simultaneously attenuate virulence and promote reduced cell viability, representing a feasible stand-alone therapy, or valuable adjunct to existing antimicrobial agents.

SPECIFIC AIMS:

1. Develop a conditional-lethal gene-knockout system to assess the essential function of *prs*A in Gram-positive pathogens.
2. Conduct biochemical analysis of PrsA function in S. aureus, and identify protein substrates for PrsA.
3. Determine the 3D-structure of *S. aureus* PrsA by X-Ray crystallography, and compare to the SurA protein.
4. Assess the efficacy of PrsA as a vaccine component, and determine the virulence phenotype of PrsA deficient mutants in appropriate infection models.

1.0 Microorganisms

1.1 Does your work involve the use of microorganisms or biological agents of plant or animal origin (including but not limited to viruses, prions, parasites, bacteria)? O YES ● NO
If no, please proceed to Section 2.0

Do you use microorganisms that require a permit from the CFIA? O YES ● NO
If YES, please give the name of the species. _____
What is the origin of the microorganism(s)? _____
Please describe the risk (if any) of escape and how this will be mitigated:

_____
_____
_____

Please attach the CFIA permit.
Please describe any CFIA permit conditions:

_____
_____
_____

1.2 Please complete the table below:

Name of Biological agent(s)*	Is it known to be a human pathogen? YES/NO	Is it known to be an animal pathogen? YES/NO	Is it known to be a zoonotic agent? YES/NO	Maximum quantity to be cultured at one time? (in Litres)	Source/ Supplier	PHAC or CFIA Containment Level
S. aureus	● Yes O No	● Yes O No	● Yes O No	< 2 liters	Existing freezer inventory	O 1 ● 2 O 3
E. coli lab strains	O Yes ● No	O Yes ● No	O Yes ● No	< 2 liters	Existing freezer inventory	● 1 O 2 O 3
	O Yes O No	O Yes O No	O Yes O No			O 1 O 2 O 3
	O Yes O No	O Yes O No	O Yes O No			O 1 O 2 O 3

*Please attach a Material Safety Data Sheet or equivalent from the supplier.

2.0 Cell Culture

2.1 Does your work involve the use of cell cultures? ● YES O NO
If no, please proceed to Section 3.0

2.2 Please indicate the type of primary cells (i.e. derived from fresh tissue) that will be grown in culture in the table below

Cell Type	Is this cell type used in your work?	Source of Primary Cell Culture Tissue	AUS Protocol Number
Human	O Yes O No	Peripheral blood	Not applicable
Rodent	O Yes O No	Blood; kidney;	In Progress
Non-human primate	O Yes O No		
Other (specify)	O Yes O No		

*** DESCRIPTION MUST BE ATTACHED TO THIS FORM OR PROJECT WILL NOT BE REVIEWED***

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Product Description

Before submitting an order you will be asked to read and accept the terms and conditions of ATCC's Material Transfer Agreement or, in certain cases, an MTA specified by the depositing institution.

Customers in Europe, Australia, Canada, China, Hong Kong, India, Japan, Korea, Macau, Mexico, New Zealand, Singapore, and Taiwan, R.O.C. must contact a local distributor for pricing information and to place an order for ATCC cultures and products.

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Cell Biology

ATCC® Number: TIB-202™ Order this Item
Price: $264.00
Designations: THP-1
Depositors: S Tsuchiya
Biosafety Level: 1
Shipped: frozen
Medium & Serum: See Propagation
Growth Properties: suspension
Organism: *Homo sapiens* (human)
Morphology: monocyte
PHOTO

Source:
Organ: peripheral blood
Disease: acute monocytic leukemia
Cell Type: monocyte;

Cellular Products: lysozyme [58053]

Permits/Forms: In addition to the MTA mentioned above, other ATCC and/or regulatory permits may be required for the transfer of this ATCC material. Anyone purchasing ATCC material is ultimately responsible for obtaining the permits. Please click here for information regarding the specific requirements for shipment to your location.

Related Cell Culture Products

Applications: transfection host (technology from amaxa Roche FuGENE® Transfection Reagents)

Receptors: complement (C3), expressed [58053]
Fc, expressed

Antigen Expression: HLA A2, A9, B5, DRw1, DRw2 [58053]

DNA Profile (STR):
Amelogenin: X,Y
CSF1PO: 11,13
D13S317: 13
D16S539: 11,12
D5S818: 11,12
D7S820: 10
THO1: 8,9.3
TPOX: 8,11
vWA: 16

Age: 1 year infant

Gender: male

Comments: The cells are phagocytic (for both latex beads and sensitized erythrocytes) and lack surface and cytoplasmic immunoglobulin. [58053]
Monocytic differentiation can be induced with the phorbol ester 12-O-tetradecanoylphorbol-13-acetate (TPA). [22193]

Propagation:
ATCC complete growth medium: The base medium for this cell line is ATCC-formulated RPMI-1640 Medium, Catalog No. 30-2001. To make the complete growth medium, add the following components to the base medium: 2-mercaptoethanol to a final concentration of 0.05 mM; fetal bovine serum to a final concentration of 10%.
Atmosphere: air, 95%; carbon dioxide (CO2), 5%
Temperature: 37.0°C

2.3 Please indicate the type of established cells that will be grown in culture in the table below.

Cell Type	Is this cell type used in your work?	Specific cell line(s)*	Supplier / Source
Human	O Yes O No	THP-1 Monocytic	Sung Kim Lab; SDRI-119
Rodent	O Yes O No	RAW1-Macrophage	Sung Kim Lab; SDRI-119
Non-human primate	O Yes O No		
Other (specify)	O Yes O No		

*Please attach a Material Safety Data Sheet or equivalent from the supplier. (For more information, see www.atcc.org)

2.4 For above named cell types(s) indicate PHAC or CFIA containment level required O 1 O 2 O 3

3.0 Use of Human Source Materials

3.1 Does your work involve the use of human source materials? ● YES O NO
If no, please proceed to Section 4.0

3.2 Indicate in the table below the Human Source Material to be used.

Human Source Material	**Source/Supplier /Company Name**	**Is Human Source Material Known to Be Infected With An Infectious Agent? YES/NO**	**Name of Infectious Agent (If applicable)**	**PHAC or CFIA Containment Level (Select one)**
Human Blood (whole) or other Body Fluid	Volunteers	O Yes ● No		O 1 ● 2 O 3
Human Blood (fraction) or other Body Fluid		O Yes O No		O 1 O 2 O 3
Human Organs or Tissues (unpreserved)		O Yes O No		O 1 O 2 O 3
Human Organs or Tissues (preserved)		O Yes O No		O 1 O 2 O 3

4.0 Genetically Modified Organisms and Cell lines

4.1 Will genetic modifications be made to the microorganisms, biological agents, or cells described in Sections 1.0 and 2.0? ● YES O NO If no, please proceed to Section 5.0

4.2 Will genetic modification(s) involving plasmids be done? O YES, complete table below O NO

Bacteria Used for Cloning *	Plasmid(s) *	Source of Plasmid	Gene Transfected	Describe the change that results
See attached				

* Please attach a Material Data Sheet or equivalent if available.

*** DESCRIPTION MUST BE ATTACHED TO THIS FORM OR PROJECT WILL NOT BE REVIEWED***

4.3 Will genetic modification(s) involving viral vectors be done? O YES, complete table below ● NO

Virus Used for Transduction *	Vector(s) *	Source of Vector	Gene Transfected	Describe the change that results

* Please attach a Material Safety Data Sheet or equivalent.

4.4 Will genetic sequences from the following be involved?

- HIV O YES, please specify _____ ● NO
- HTLV 1 or 2 or genes from any Level 1 or Level 2 pathogens O YES, specify _____ ● NO
- SV 40 Large T antigen O YES ● NO
- E1A oncogene O YES ● NO
- Known oncogenes O YES, please specify _____ ● NO
- Other human or animal pathogen and or their toxins O YES, please specify _____ ● NO

4.5 Will virus be replication defective? N/A O YES O NO

4.6 Will virus be infectious to humans or animals? N/A O YES O NO

4.7 Will this be expected to increase the containment level required? O YES O NO N/A

5.0 Human Gene Therapy Trials

5.1 Will human clinical trials be conducted using the viral vector in 4.0? O YES ● NO
If no, please proceed to Section 6.0 If YES attach a full description of the make-up of the virus.

5.2 Will virus be able to replicate in the host? O YES O NO

5.3 How will the virus be administered? _____

5.4 Please give the Health Care Facility where the clinical trial will be conducted:_____

5.5 Has human ethics approval been obtained? O YES, number: _____ O NO O PENDING

6.0 Animal Experiments

6.1 Will live animals be used? ● YES O NO If no, please proceed to section 7.0

6.2 Name of animal species to be used Mouse (Balb/c; C57b; Swiss-Webster)

6.3 AUS protocol # In progress

6.4 Will any of the agents listed be used in live animals ● YES, specify: _____ O NO
Murine bacteremia and subcutaneous abscess infection models.

*** DESCRIPTION MUST BE ATTACHED TO THIS FORM OR PROJECT WILL NOT BE REVIEWED***

7.0 Use of Animal species with Zoonotic Hazards

7.1 Will any of the following animals or their organs, tissues, lavages or other body fluids including blood be used?

- Pound source dogs O YES ● NO
- Pound source cats O YES ● NO
- Cattle, sheep or goats O YES ● NO
- Non-human primates O YES, please specify species _____ ● NO
- Wild caught animals O YES, please specify species & colony # _____ ● NO
- Birds O YES ● NO
- Others (wild or domestic) O YES, please specify _____ ● NO

8.0 Biological Toxins

8.1 Will toxins of biological origin be used? O YES ● NO If no, please proceed to Section 9.0

8.2 If YES, please name the toxin(s)_____
Please attach information, such as a Material Safety Data Sheet, for the toxin(s) used.

8.3 What is the LD_{50} (specify species) of the toxin_____

8.4 How much of the toxin is handled at one time*? _____

8.5 How much of the toxin is stored*? _____

*For information on biosecurity requirements, please see:
http://www.uwo.ca/humanresources/docandform/docs/healthandsafety/biosafety/Biosecurity_Requirements.pdf

9.0 Insects Requiring CFIA Permits

9.1 Do you use insects that require a permit from the CFIA? O YES ● NO
If no, please proceed to Section 10.0

9.2 If YES, please give the name of the species. _____

9.3 What is the origin of the insect? _____

9.4 What is the lifestage of the insect? _____

9.5 What is your intention? O Initiate and maintain colony, give location: _____
O "One-off" use, give location: _____

9.6 Please describe the risk (if any) of escape and how this will be mitigated:
_____
_____
_____

9.7 Please attach the CFIA permit.

9.8 Please describe any CFIA permit conditions:
_____
_____
_____

*** DESCRIPTION MUST BE ATTACHED TO THIS FORM OR PROJECT WILL NOT BE REVIEWED***

10.0 Plants Requiring CFIA Permits

10.1 Do you use plants that require a permit from the CFIA? O YES ● NO
If no, please proceed to Section 11.0

10.2 If YES, please give the name of the species. _____

10.3 What is the origin of the plant? _____

10.4 What is the form of the plant (seed, seedling, plant, tree…)? _____

10.5 What is your intention? O Grow and maintain a crop O "One-time" use

10.6 Do you do any modifications to the plant? O YES O NO
If yes, please describe: _____
_____

10.7 Please describe the risk (if any) of loss of the material from the lab and how this will be mitigated:
_____
_____
_____

10.8 Is the CFIA permit attached? O YES O NO

10.9 Please describe any CFIA permit conditions:
_____
_____
_____

11.0 Import Requirements

11.1 Will any of the above agents be imported? O YES, please give country of origin_____
If no, please proceed to Section 10.0 ● NO

11.2 Has an Import Permit been obtained from HC for human pathogens? O YES ● NO

11.3 Has an import permit been obtained from CFIA for animal or plant pathogens? O YES ● NO

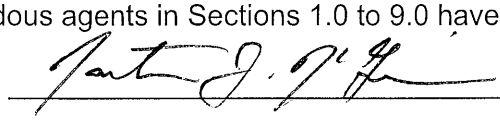
11.4 Has the import permit been sent to OHS? O YES, please provide permit # _____ O NO

12.0 Training Requirements for Personnel Named on Form

All personnel named on the above form who will be using any of the above named agents are required to attend the following training courses given by OHS:

- Biosafety
- Laboratory and Environmental/Waste Management Safety
- WHMIS (Western or equivalent)
- Employee Health and Safety Orientation

As the Principal Investigator, I have ensured that all of the personnel named on the form who will be using any of the biohazardous agents in Sections 1.0 to 9.0 have been trained.

SIGNATURE 

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13.0 Containment Levels

11.1 For the work described in sections 1.0 to 9.0, please indicate the highest HC or CFIA Containment Level required. O 1 ● 2 O 3

13.2 Has the facility been certified by OHS for this level of containment?

O YES, permit # if on-campus_____
● NO, please certify
O NOT REQUIRED for Level 1 containment

14.0 Procedures to be Followed

14.1 As the Principal Investigator, I will ensure that this project will follow the Western Biosafety Guidelines and Procedures Manual for Containment Level 1 & 2 Laboratories (and the Level 3 Facilities Manual for Level 3 projects). I will ensure that UWO faculty, staff and students working in my laboratory have an up-to-date Hazard Communication Form, found at http://www.wph.uwo.ca/

SIGNATURE _____ Date: April 15, 2009

15.0 Approvals

UWO Biohazard Subcommittee: SIGNATURE: _____
Date: _____

Safety Officer for Institution where experiments will take place: SIGNATURE: _____
Date: _____

Safety Officer for University of Western Ontario (if different from above): SIGNATURE: _____
Date: _____

Approval Number: _____ Expiry Date (3 years from Approval): _____

Special Conditions of Approval:

*** DESCRIPTION MUST BE ATTACHED TO THIS FORM OR PROJECT WILL NOT BE REVIEWED***

Section 1.2

Public Health Agency of Canada | Agence de la santé publique du Canada

Canada

Staphylococcus aureus - Material Safety Data Sheets (MSDS)

MATERIAL SAFETY DATA SHEET - INFECTIOUS SUBSTANCES

SECTION I - INFECTIOUS AGENT

NAME: ***Staphylococcus aureus***

SYNONYM OR CROSS REFERENCE: Staphylococcal diseases, impetigo, toxic shock syndrome, food poisoning, intoxication

CHARACTERISTICS: Gram positive cocci, usually in clusters; coagulase positive; non-spore forming; non-motile; many strains produce exotoxins including staphylococcal enterotoxins A,B,C,D,E, toxic shock syndrome toxin (TSST-1) and exfoliative toxins A, and B

SECTION II - HEALTH HAZARD

PATHOGENICITY: Opportunistic pathogen, normal flora; produces a variety of syndromes with a range of clinical manifestations; clinically different in general community, newborns, menstruating women, and hospitalized patients; food intoxication is characterized by abrupt/violent onset, severe nausea, cramps, vomiting, and diarrhea using lasting 1-2days; animal bites can result in localized infections; may cause surface or deep/system infections in both community and hospital settings; surface infections include impetigo, folliculitis, abscesses, boils, infected lacerations; deep infections include endocarditis, meningitis, septic arthritis, pneumonia, osteomyelitis; systemic infection may cause fever, headache malaise, myalgia; newborns are susceptible to scalded skin syndrome (SSS) caused by exfoliative toxins; my be colonized during delivery resulting in sepsis meningitis; toxic shock syndrome is an acute multi-system illness caused by TSST-1 a super antigen; characterized by sudden onset, high fever, vomiting, profuse watery diarrhea, myalgia, hypotension erythematous rash

EPIDEMIOLOGY: Occurs worldwide; particularly in areas where personal hygiene is suboptimal; in hospitals by development of antibiotic-resistant strains

HOST RANGE: Humans; to a lesser extent, warm-blooded animals

INFECTIOUS DOSE: Virulence of strains varies greatly

MODE OF TRANSMISSION: Contact with nasal carriers (30-40% of population); from draining lesions or purulent discharges; spread person-to-person; ingestion of food containing staphylococcal enterotoxin (food may be contaminated by food handlers hands); from mother to neonate during delivery

INCUBATION PERIOD: Variable and indefinite, commonly 4-10 days; disease may not occur until several months after colonization; interval between eating food and onset of symptoms is usually 2-4 hours (30 min to 8 hours)

COMMUNICABILITY: As long as purulent lesions continue to drain or carrier state persists;

auto-infection may continue for the period of nasal colonization or duration of active lesions

SECTION III - DISSEMINATION

RESERVOIR: Human; patients with indwelling catheters or IVs act as reservoirs for nosocomial infections; food borne - occasionally cows with infected udders

ZOONOSIS: Yes - direct or indirect contact with infected animals

VECTORS: None

SECTION IV - VIABILITY

DRUG SUSCEPTIBILITY: Many strains are multi-resistant to antibiotics and are of increasing importance; methicillin resistant (MRSA) strains have caused major outbreaks world-wide; Vancomycin resistant (VRSA) are being increasingly isolated; sensitivity must be determined for each strain

SUSCEPTIBILITY TO DISINFECTANTS: Susceptible to many disinfectants - 1% sodium hypochlorite, iodine/alcohol solutions, glutaraldehyde, formaldehyde

PHYSICAL INACTIVATION: Organisms are destroyed by heat (moist heat - 121° C for at least 15 min, dry heat - 160-170° C for at least 1 hour; enterotoxins are heat resistant, stable at boiling temperature

SURVIVAL OUTSIDE HOST: Carcass and organs - up to 42 days; floor - less than 7 days; glass - 46 hours; sunlight - 17 hours; UV - 7 hours; meat products - 60 days; coins - up to 7 days; skin from 30 min to 38 days

SECTION V - MEDICAL

SURVEILLANCE: Monitor for skin inflammation if wounded by a sharp instrument; isolation of organism from wound or blood, CSF, urine; isolation of> 10^5 organisms or enterotoxin from suspected food

FIRST AID/TREATMENT: Fluid replacement for food poisoning; in localized skin infections, drain abscesses; antibiotic therapy for severe infections

IMMUNIZATION: None

PROPHYLAXIS: None

SECTION VI - LABORATORY HAZARDS

LABORATORY-ACQUIRED INFECTIONS: 29 reported cases up to 1973 with 1 death

SOURCES/SPECIMENS: Clinical specimens - blood, abcesses, lesion exudates, CSF, respiratory specimens, feces, urine

PRIMARY HAZARDS: Injuries from contaminated sharp instruments; ingestion; aerosols

SPECIAL HAZARDS: Direct contact with open cuts and lesions of skin

SECTION VII - RECOMMENDED PRECAUTIONS

CONTAINMENT REQUIREMENTS: Biosafety level 2 practices, containment equipment and facilities for activities with cultures or potentially infectious clinical materials

PROTECTIVE CLOTHING: Laboratory coat: gloves when skin contact is unavoidable

OTHER PRECAUTIONS: Thorough handwashing before leaving the laboratory and after handling infectious materials

SECTION VIII - HANDLING INFORMATION

SPILLS: Allow aerosols to settle; wear protective clothing; gently cover spill with paper towel and apply 1% sodium hypochlorite, starting at perimeter and working towards the centre; allow sufficient contact time (30 min) before clean up

DISPOSAL: Decontaminate before disposal; steam sterilization, chemical disinfection

STORAGE: In sealed containers that are appropriately labelled

SECTION IX - MISCELLANEOUS INFORMATION

Date prepared: March, 2001

Prepared by: Office of Laboratory Security, PHAC

Although the information, opinions and recommendations contained in this Material Safety Data Sheet are compiled from sources believed to be reliable, we accept no responsibility for the accuracy, sufficiency, or reliability or for any loss or injury resulting from the use of the information. Newly discovered hazards are frequent and this information may not be completely up to date.

Date Modified: 2001-04-23

Section 2.3

SIGMA-ALDRICH

MATERIAL SAFETY DATA SHEET

Date Printed: 04/15/2009
Date Updated: 02/04/2006
Version 1.2

Section 1 - Product and Company Information

Product Name	THP 1, HUMAN MONOCYTIC LEUKAEMIA CELLS
Product Number	88081201
Brand	SIGMA
Company	Sigma-Aldrich Canada, Ltd
Address	2149 Winston Park Drive Oakville ON L6H 6J8 CA
Technical Phone:	9058299500
Fax:	9058299292
Emergency Phone:	800-424-9300

Section 2 - Composition/Information on Ingredient

Substance Name	CAS #		SARA 313
EUROPEAN COLLECTION OF CELL CULTURES, HUMAN SOURCE	None		No

Ingredient Name	CAS #	Percent	SARA 313
The hazards identified with this product are those associated with the following component(s):	None		
DIMETHYL SULFOXIDE	67-68-5	10	No
CELLS, HUMAN ORIGIN	None	<= 1	No

Chemical Family Human source material.

Section 3 - Hazards Identification

EMERGENCY OVERVIEW

Biohazard.
Handle as if capable of transmitting infectious agents. Readily absorbed through skin. Target organ(s): Eyes. Skin.

HMIS RATING

HEALTH: 0*
FLAMMABILITY: 0
REACTIVITY: 0
SPECIAL HAZARD(S): Human Source

NFPA RATING

HEALTH: 0
FLAMMABILITY: 0
REACTIVITY: 0
SPECIAL HAZARD(S): Human Source

*additional chronic hazards present.

For additional information on toxicity, please refer to Section 11.

Section 4 - First Aid Measures

ORAL EXPOSURE
If swallowed, wash out mouth with water provided person is conscious. Call a physician immediately.

INHALATION EXPOSURE
If inhaled, remove to fresh air. If not breathing give artificial respiration. If breathing is difficult, give oxygen.

DERMAL EXPOSURE
In case of skin contact, flush with copious amounts of water for at least 15 minutes. Remove contaminated clothing and shoes. Call a physician.

EYE EXPOSURE
In case of contact with eyes, flush with copious amounts of water for at least 15 minutes. Assure adequate flushing by separating the eyelids with fingers. Call a physician.

Section 5 - Fire Fighting Measures

FLASH POINT
N/A

AUTOIGNITION TEMP
N/A

FLAMMABILITY
N/A

EXTINGUISHING MEDIA
Suitable: Use extinguishing media appropriate to surrounding fire conditions.

FIREFIGHTING
Protective Equipment: Wear self-contained breathing apparatus and protective clothing to prevent contact with skin and eyes.
Specific Hazard(s): Emits toxic fumes under fire conditions.

Section 6 - Accidental Release Measures

PROCEDURE(S) OF PERSONAL PRECAUTION(S)
Wear self-contained breathing apparatus, rubber boots, and heavy rubber gloves.

METHODS FOR CLEANING UP
Sweep up, place in a bag and hold for waste disposal. Avoid raising dust. Wash spill site with 10% bleach and ventilate area after material pickup is complete.

ENVIRONMENTAL PRECAUTION(S)
Avoid contaminating sewers and waterways with this material.

Section 7 - Handling and Storage

HANDLING
User Exposure: Do not breathe vapor. Avoid prolonged or repeated exposure. Avoid contact with DMSO solutions containing toxic materials or materials with unknown toxicological properties. Dimethyl sulfoxide is readily absorbed through skin and may carry such materials into the body.

STORAGE

Suitable: Keep tightly closed.

Section 8 - Exposure Controls / PPE

ENGINEERING CONTROLS

Safety shower and eye bath. Mechanical exhaust required.

PERSONAL PROTECTIVE EQUIPMENT

Respiratory: Use respirators and components tested and approved under appropriate government standards such as NIOSH (US) or CEN (EU). Where risk assessment shows air-purifying respirators are appropriate use a dust mask type N95 (US) or type P1 (EN 143) respirator.
Hand: Compatible chemical-resistant gloves.
Eye: Chemical safety goggles.

GENERAL HYGIENE MEASURES

Wash thoroughly after handling.

Section 9 - Physical/Chemical Properties

Appearance Physical State: Liquid

Property	Value	At Temperature or Pressure
pH	N/A	
BP/BP Range	N/A	
MP/MP Range	N/A	
Freezing Point	N/A	
Vapor Pressure	N/A	
Vapor Density	N/A	
Saturated Vapor Conc.	N/A	
SG/Density	N/A	
Bulk Density	N/A	
Odor Threshold	N/A	
Volatile%	N/A	
VOC Content	N/A	
Water Content	N/A	
Solvent Content	N/A	
Evaporation Rate	N/A	
Viscosity	N/A	
Surface Tension	N/A	
Partition Coefficient	N/A	
Decomposition Temp.	N/A	
Flash Point	N/A	
Explosion Limits	N/A	
Flammability	N/A	
Autoignition Temp	N/A	
Refractive Index	N/A	
Optical Rotation	N/A	
Miscellaneous Data	N/A	
Solubility	N/A	

N/A = not available

Section 10 - Stability and Reactivity

STABILITY

Stable: Stable.
Materials to Avoid: Strong oxidizing agents.

HAZARDOUS DECOMPOSITION PRODUCTS

Hazardous Decomposition Products: Nature of decomposition products not known.

HAZARDOUS EXOTHERMIC REACTIONS

Hazardous Exothermic Reactions: Methyl sulfoxide (DMSO) undergoes a violent exothermic reaction on mixing with copper wool and trichloroacetic acid. On mixing with potassium permanganate it will flash instantaneously. It reacts violently with: acid halides, cyanuric chloride, silicon tetrachloride, phosphorus trichloride and trioxide, thionyl chloride, magnesium perchlorate, silver fluoride, methyl bromide, iodine pentafluoride, nitrogen periodate, diborane, sodium hydride, and perchloric and periodic acids. When heated above its boiling point methyl sulfoxide degrades giving off formaldehyde, methyl mercaptan, and sulfur dioxide.

HAZARDOUS POLYMERIZATION

Hazardous Polymerization: Will not occur

Section 11 - Toxicological Information

ROUTE OF EXPOSURE

Skin Contact: May cause skin irritation.
Skin Absorption: Readily absorbed through skin. May be harmful if absorbed through the skin.
Eye Contact: May cause eye irritation.
Inhalation: May be harmful if inhaled. Material may be irritating to mucous membranes and upper respiratory tract.
Ingestion: May be harmful if swallowed.

TARGET ORGAN(S) OR SYSTEM(S)

Eyes. Skin.

SIGNS AND SYMPTOMS OF EXPOSURE

Potentially biohazardous material.

Section 12 - Ecological Information

No data available.

Section 13 - Disposal Considerations

APPROPRIATE METHOD OF DISPOSAL OF SUBSTANCE OR PREPARATION

Contact a licensed professional waste disposal service to dispose of this material. Disposal should be made in accordance with existing disposal practices employed for infectious waste at your institution. Observe all federal, state, and local environmental regulations.

Section 14 - Transport Information

DOT

Proper Shipping Name: None
Non-Hazardous for Transport: This substance is considered to be non-hazardous for transport.

IATA

Non-Hazardous for Air Transport: Non-hazardous for air transport.

Section 15 - Regulatory Information

EU ADDITIONAL CLASSIFICATION
Symbol of Danger: B
Indication of Danger: Biohazard.

US CLASSIFICATION AND LABEL TEXT
Indication of Danger: Biohazard.
US Statements: Handle as if capable of transmitting infectious agents. Readily absorbed through skin. Target organ(s): Eyes. Skin.

UNITED STATES REGULATORY INFORMATION
SARA LISTED: No

CANADA REGULATORY INFORMATION
WHMIS Classification: This product has been classified in accordance with the hazard criteria of the CPR, and the MSDS contains all the information required by the CPR.
DSL: No
NDSL: No

Section 16 - Other Information

DISCLAIMER
For R&D use only. Not for drug, household or other uses.

WARRANTY
The above information is believed to be correct but does not purport to be all inclusive and shall be used only as a guide. The information in this document is based on the present state of our knowledge and is applicable to the product with regard to appropriate safety precautions. It does not represent any guarantee of the properties of the product. Sigma-Aldrich Inc., shall not be held liable for any damage resulting from handling or from contact with the above product. See reverse side of invoice or packing slip for additional terms and conditions of sale.

Section 2.3

SIGMA-ALDRICH

MATERIAL SAFETY DATA SHEET

Date Printed: 04/15/2009
Date Updated: 02/04/2006
Version 1.2

Section 1 - Product and Company Information

Product Name	RAW 264.7 CELLS, MOUSE MONOCYTE MACROPHAGE
Product Number	91062702
Brand	SIGMA
Company	Sigma-Aldrich Canada, Ltd
Address	2149 Winston Park Drive Oakville ON L6H 6J8 CA
Technical Phone:	9058299500
Fax:	9058299292
Emergency Phone:	800-424-9300

Section 2 - Composition/Information on Ingredient

Substance Name	CAS #	SARA 313
EUROPEAN COLLECTION OF CELL CULTURES, NON-HUMAN / NON-PRIMATE	None	No

Ingredient Name	CAS #	Percent	SARA 313
The hazards identified with this product are those associated with the following component(s):	None		
DIMETHYL SULFOXIDE	67-68-5	10	No

Section 3 - Hazards Identification

EMERGENCY OVERVIEW

Readily absorbed through skin. Target organ(s): Eyes. Skin.

HMIS RATING

HEALTH: 0*
FLAMMABILITY: 0
REACTIVITY: 0

NFPA RATING

HEALTH: 0
FLAMMABILITY: 0
REACTIVITY: 0

*additional chronic hazards present.

For additional information on toxicity, please refer to Section 11.

Section 4 - First Aid Measures

ORAL EXPOSURE

If swallowed, wash out mouth with water provided person is conscious. Call a physician.

INHALATION EXPOSURE

If inhaled, remove to fresh air. If not breathing give

artificial respiration. If breathing is difficult, give oxygen.

DERMAL EXPOSURE
In case of contact, immediately wash skin with soap and copious amounts of water.

EYE EXPOSURE
In case of contact, immediately flush eyes with copious amounts of water for at least 15 minutes.

Section 5 - Fire Fighting Measures

FLASH POINT
N/A

AUTOIGNITION TEMP
N/A

FLAMMABILITY
N/A

EXTINGUISHING MEDIA
Suitable: Water spray. Carbon dioxide, dry chemical powder, or appropriate foam.

FIREFIGHTING
Protective Equipment: Wear self-contained breathing apparatus and protective clothing to prevent contact with skin and eyes. Specific Hazard(s): Combustible liquid. Emits toxic fumes under fire conditions.

Section 6 - Accidental Release Measures

PROCEDURE TO BE FOLLOWED IN CASE OF LEAK OR SPILL
Evacuate area.

PROCEDURE(S) OF PERSONAL PRECAUTION(S)
Wear respirator, chemical safety goggles, rubber boots, and heavy rubber gloves.

METHODS FOR CLEANING UP
Cover with dry-lime, sand, or soda ash. Place in covered containers using non-sparking tools and transport outdoors. Ventilate area and wash spill site after material pickup is complete.

ENVIRONMENTAL PRECAUTION(S)
Avoid contaminating sewers and waterways with this material.

Section 7 - Handling and Storage

HANDLING
User Exposure: Do not breathe vapor. Avoid contact with DMSO solutions containing toxic materials or materials with unknown toxicological properties. Dimethyl sulfoxide is readily absorbed through skin and may carry such materials into the body. Avoid prolonged or repeated exposure.

STORAGE
Suitable: Keep tightly closed.

Section 8 - Exposure Controls / PPE

ENGINEERING CONTROLS
Safety shower and eye bath. Mechanical exhaust required.

PERSONAL PROTECTIVE EQUIPMENT
Respiratory: Use respirators and components tested and approved under appropriate government standards such as NIOSH (US) or CEN (EU). Where risk assessment shows air-purifying respirators are appropriate use a full-face respirator with multi-purpose combination (US) or type ABEK (EN 14387) respirator cartridges as a backup to engineering controls. If the respirator is the sole means of protection, use a full-face supplied air respirator.
Hand: Compatible chemical-resistant gloves.
Eye: Chemical safety goggles.
Skin-Specific: Chemical resistant apron.

GENERAL HYGIENE MEASURES
Wash contaminated clothing before reuse. Wash thoroughly after handling.

Section 9 - Physical/Chemical Properties

Appearance — Physical State: Liquid

Property	Value	At Temperature or Pressure
pH	N/A	
BP/BP Range	N/A	
MP/MP Range	N/A	
Freezing Point	N/A	
Vapor Pressure	N/A	
Vapor Density	N/A	
Saturated Vapor Conc.	N/A	
SG/Density	N/A	
Bulk Density	N/A	
Odor Threshold	N/A	
Volatile%	N/A	
VOC Content	N/A	
Water Content	N/A	
Solvent Content	N/A	
Evaporation Rate	N/A	
Viscosity	N/A	
Surface Tension	N/A	
Partition Coefficient	N/A	
Decomposition Temp.	N/A	
Flash Point	N/A	
Explosion Limits	N/A	
Flammability	N/A	
Autoignition Temp	N/A	
Refractive Index	N/A	
Optical Rotation	N/A	
Miscellaneous Data	N/A	
Solubility	N/A	

N/A = not available

Section 10 - Stability and Reactivity

STABILITY
Stable: Stable.
Materials to Avoid: Strong oxidizing agents.

HAZARDOUS DECOMPOSITION PRODUCTS

Hazardous Decomposition Products: Nature of decomposition products not known.

HAZARDOUS EXOTHERMIC REACTIONS

Hazardous Exothermic Reactions: Methyl sulfoxide (DMSO) undergoes a violent exothermic reaction on mixing with copper wool and trichloroacetic acid. On mixing with potassium permanganate it will flash instantaneously. It reacts violently with: acid halides, cyanuric chloride, silicon tetrachloride, phosphorus trichloride and trioxide, thionyl chloride, magnesium perchlorate, silver fluoride, methyl bromide, iodine pentafluoride, nitrogen periodate, diborane, sodium hydride, and perchloric and periodic acids. When heated above its boiling point methyl sulfoxide degrades giving off formaldehyde, methyl mercaptan, and sulfur dioxide.

HAZARDOUS POLYMERIZATION

Hazardous Polymerization: Will not occur

Section 11 - Toxicological Information

ROUTE OF EXPOSURE

Skin Contact: May cause skin irritation.
Skin Absorption: May be harmful if absorbed through the skin. Readily absorbed through skin.
Eye Contact: May cause eye irritation.
Inhalation: May be harmful if inhaled. Material may be irritating to mucous membranes and upper respiratory tract.
Ingestion: May be harmful if swallowed.

TARGET ORGAN(S) OR SYSTEM(S)

Eyes. Skin.

Section 12 - Ecological Information

Section 13 - Disposal Considerations

APPROPRIATE METHOD OF DISPOSAL OF SUBSTANCE OR PREPARATION

Contact a licensed professional waste disposal service to dispose of this material. This combustible material may be burned in a chemical incinerator equipped with an afterburner and scrubber. Observe all federal, state, and local environmental regulations.

Section 14 - Transport Information

DOT

Proper Shipping Name: None
Non-Hazardous for Transport: This substance is considered to be non-hazardous for transport.

IATA

Non-Hazardous for Air Transport: Non-hazardous for air transport.

Section 15 - Regulatory Information

US CLASSIFICATION AND LABEL TEXT

US Statements: Readily absorbed through skin. Target organ(s): Eyes. Skin.

UNITED STATES REGULATORY INFORMATION
SARA LISTED: No

CANADA REGULATORY INFORMATION
WHMIS Classification: This product has been classified in accordance with the hazard criteria of the CPR, and the MSDS contains all the information required by the CPR.
DSL: No
NDSL: No

Section 16 - Other Information

DISCLAIMER
For R&D use only. Not for drug, household or other uses.

WARRANTY
The above information is believed to be correct but does not purport to be all inclusive and shall be used only as a guide. The information in this document is based on the present state of our knowledge and is applicable to the product with regard to appropriate safety precautions. It does not represent any guarantee of the properties of the product. Sigma-Aldrich Inc., shall not be held liable for any damage resulting from handling or from contact with the above product. See reverse side of invoice or packing slip for additional terms and conditions of sale.

Section 4.2 Details

Bacteria used for cloning	**Plasmid Vector**	**Source of Plasmid**	**Gene Transfected**	**Change that results**
E. coli DH5α/TB1/ M15/XL1, etc	Topo-TA	Invitro Gen	Various PCR products	None; general entry level vector for gene cloning. Genes cloned in this vector are not expressed
As above	pQE30 and derivatives, and pTopoBAD	QiaGen; Invitro Gen	Segments of DNA encoding *S. aureus* cell surface proteins; *S. aureus* protease enzymes, or *S. aureus* chaperone/protease inhibitor proteins	Recombinant proteins are expressed in *E. coli* with N-terminal 6xHis tags for purification by metal affinity chromatography
Staph aureus RN6911, RN6390 and derivatives	pRN5548, pCN51[a] and derivatives	Richard Novick	As above	Plasmids will direct inducible expression/secretion of cloned genes in *S. aureus* host strains
As above	pMAD	Pasteur Institute, France	Segments of *S. aureus* genomic DNA that flank genes that are targeted for disruption/inactivation as above	The vector is designed to promote recombination with the *S. aureus* genome, resulting in disruption or deletion of the target gene. It is anticipated that the modified strains will have reduced capability of causing infections.

[a]Manuscripts describing applications of the pCN and pMAD series of vectors are appended.

APPLIED AND ENVIRONMENTAL MICROBIOLOGY, Oct. 2004, p. 6076–6085 Vol. 70, No. 10
0099-2240/04/$08.00+0 DOI: 10.1128/AEM.70.10.6076–6085.2004

Novel Cassette-Based Shuttle Vector System for Gram-Positive Bacteria

Emmanuelle Charpentier,* Ana I. Anton, Peter Barry, Berenice Alfonso, Yuan Fang, and Richard P. Novick*

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Received 24 November 2003/Accepted 30 April 2004

Our understanding of staphylococcal pathogenesis depends on reliable genetic tools for gene expression analysis and tracing of bacteria. Here, we have developed and evaluated a series of novel versatile *Escherichia coli*-staphylococcal shuttle vectors based on PCR-generated interchangeable cassettes. Advantages of our module system include the use of (i) staphylococcal low-copy-number, high-copy-number, thermosensitive and theta replicons and selectable markers (choice of erythromycin, tetracycline, chloramphenicol, kanamycin, or spectinomycin); (ii) an *E. coli* replicon and selectable marker (ampicillin); and (iii) a staphylococcal phage fragment that allows high-frequency transduction and an SaPI fragment that allows site-specific integration into the *Staphylococcus aureus* chromosome. The staphylococcal cadmium-inducible P_{cad}-*cadC* and constitutive P_{blaZ} promoters were designed and analyzed in transcriptional fusions to the staphylococcal β-lactamase *blaZ*, the *Vibrio fischeri luxAB*, and the *Aequorea victoria* green fluorescent protein reporter genes. The modular design of the vector system provides great flexibility and variety. Questions about gene dosage, complementation, and *cis-trans* effects can now be conveniently addressed, so that this system constitutes an effective tool for studying gene regulation of staphylococci in various ecosystems.

Virulent bacterial strains have developed complex metabolic and regulatory pathways to enable them to thrive in the in vivo environment during infection. Understanding how the regulatory networks operate requires manipulation of many genes and expressing them temporally and spatially at different levels or under separate regulatory controls. In the case of gram-positive bacteria including staphylococci, the introduction of shuttle vector systems has greatly facilitated gene structure-function studies. However, the construction and range of application of the vectors available to date for gram-positive bacteria lack flexibility and variety. Staphylococcal shuttle vectors are mainly based on plasmid chimeras derived from the assemblage of restriction enzyme-generated DNA fragments containing the function of interest. This strategy has several major disadvantages. (i) The cloning of fragments for the backbone structures is limited by the availability of restriction sites in the 5′ and 3′ sequences flanking the region of interest. (ii) The choice of restriction sites available for the cloning of novel fragments is thus restrained. (iii) The fragments are often too big and not optimized. (iv) Interchangeability is limited.

To address these concerns, we have developed a novel series of powerful *Escherichia coli*-staphylococcal shuttle vectors. Our approach is based on PCR-designed cassettes, which can be easily exchanged, providing flexibility. The main elements of the system comprise (i) staphylococcal pT181-based low-copy-number, high-copy-number and thermosensitive replicons (1, 17), (ii) the low-copy-number theta replicon of plasmid pI258 (4, 20), (iii) staphylococcal selectable markers (choice of erythromycin, tetracycline, chloramphenicol, kanamycin, and spectinomycin resistance) (5, 6, 8, 15, 28), (iv) an *Escherichia coli* ColE1-based replicon and selectable marker (ampicillin resistance) (32), (v) a staphylococcal φ11 phage fragment that allows extremely high frequency transduction (14, 19), (vi) a fragment of the staphylococcal pathogenicity island SaPI1 that enables site-specific integration into the *Staphylococcus aureus* chromosome (13, 25), (vii) an expanded multiple cloning site (MCS) derived from pUC19 (32), (viii) inducible and constitutive promoters (2, 22), and (ix) reporter genes (3, 27, 29, 30).

In this paper, we present and describe the structure and properties of the first generation of this novel series of modular shuttle vectors.

MATERIALS AND METHODS

Bacterial strains and cultures. The bacterial strains and plasmids used in this study are listed in Table 1. NCTC8325 is a naturally occurring strain of *S. aureus*, which is lysogenic for three phages, φ11, φ12, and φ13 (16). The restriction-defective *S. aureus* strain RN4220 is a nonlysogenic derivative of NCTC8325 and is an efficient recipient for *E. coli* DNA (11). Routinely, staphylococcal inocula were prepared by overnight growth at 37°C (or at 32°C for plasmids with thermosensitive replication) on GL agar medium with antibiotics as appropriate (18). Broth cultures were grown in CY broth supplemented with glycerol phosphate (0.1 M) at 37°C with vigorous aeration (shaking at 250 rpm) (18). Cell growth was turbidimetrically monitored with a Klett-Summerson colorimeter with a green filter at 540 nm (Klett, Long Island City, N.Y.) or with a THERMOmax microplate reader (Molecular Devices) at 650 nm. *E. coli* DH5α and TOP10 were used as bacterial hosts for plasmid construction (26). All *E. coli* strains were cultured in Luria-Bertani medium either in liquid with shaking (250 rpm) or on agar plates at 37°C. Whenever required, suitable antibiotics were added to the media as follows: chloramphenicol at 10 μg/ml for *S. aureus* (plasmid selection), eryth-

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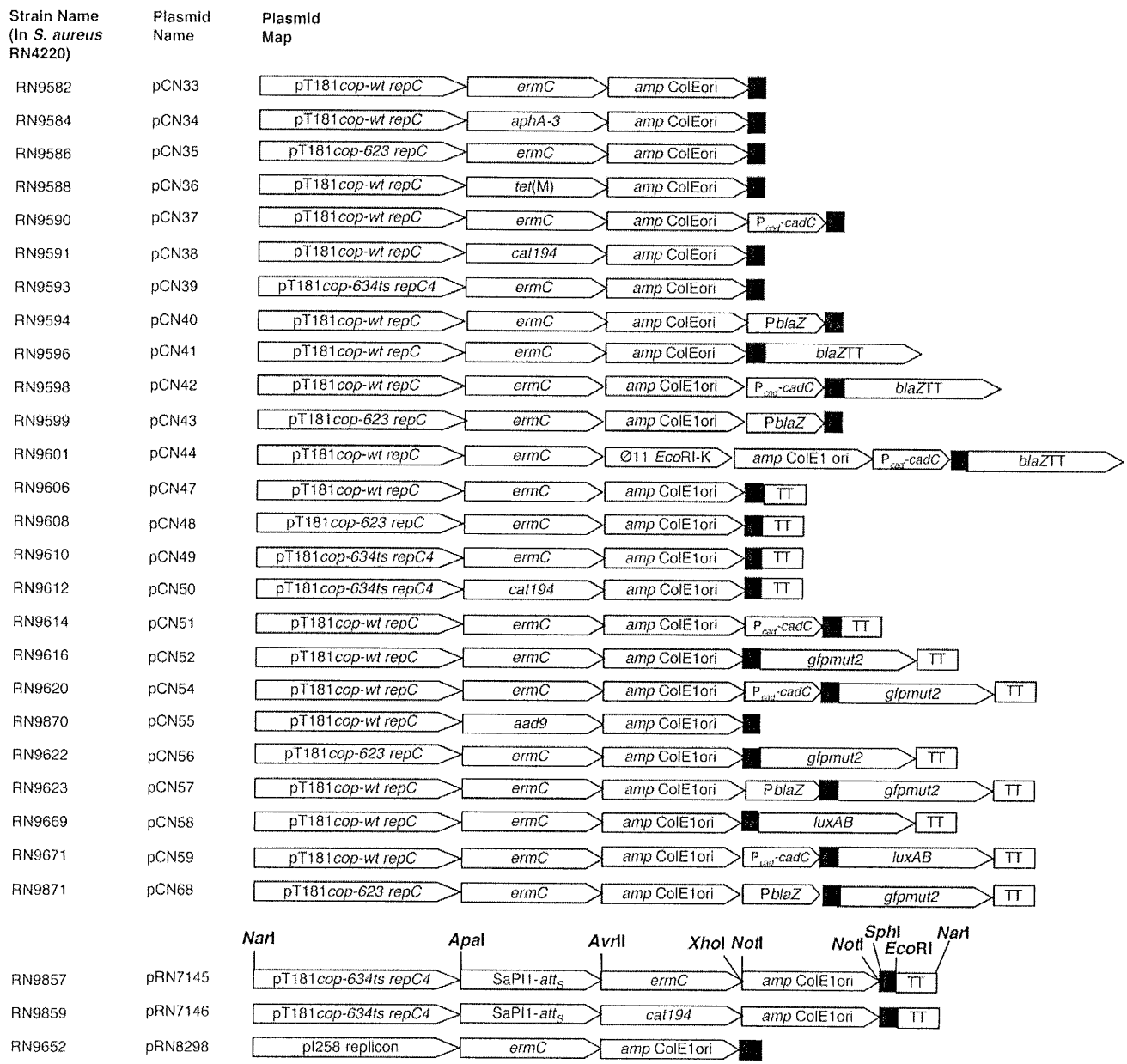

FIG. 2. Series of pCN shuttle vectors. The first generation of pCN shuttle vectors is here represented in linear form, opened at the NarI site between the replicon and MCS cassettes. Each of these was assembled using the flanking restriction sites shown in Fig. 1. In cases where two sites are shown, the outer one was used. Note that the replicon cassettes were cloned using their ApaI and SphI sites and that the MCS was subsequently inserted between the SphI and NarI sites at the left end of the cassette. Any cassette can be removed and replaced using these same restriction enzymes. For details of each module, please refer to Fig. 1.

into *S. aureus* RN4220, the functionality of each replication module was analyzed. The three vectors were stably maintained in *S. aureus* RN4220 when cultured at 37°C for pCN33 and pCN35 and at 32°C for the thermosensitive vector pCN39. After approximately 100 generations without selective pressure, 99% of 200 analyzed clones were still erythromycin resistant and exhibited the same copy number as before passage. The plasmid copy number of each of these three vectors corresponded to that of the original plasmid from which the replicons originated (1, 17) (Fig. 3A). The thermosensitivity of the pT181*cop-634 repC4* replicon of pCN39 was assessed by testing for loss of the plasmid at 43°C. After overnight growth at 43°C, none of 200 colonies tested were erythromycin resistant, indicating that pCN39 had retained the replication thermosensitivity of its pT181 parent. As shown in Fig. 3B, loss of pCN39 was further confirmed by whole-cell minilysate analysis.

Low-copy-number theta replicon. Earlier studies have identified a family of relatively large staphylococcal plasmids (25 to 35 kb), responsible for resistance to penicillin and several inorganic ions and referred to as "penicillinase plasmids" (20). These have low copy numbers (~5 copies/cell) and use the theta mode of replication (E. Murphy and R. P. Novick, un-

APPLIED AND ENVIRONMENTAL MICROBIOLOGY, Nov. 2004, p. 6887–6891 Vol. 70, No. 11
0099-2240/04/$08.00+0 DOI: 10.1128/AEM.70.11.6887–6891.2004

New Vector for Efficient Allelic Replacement in Naturally Nontransformable, Low-GC-Content, Gram-Positive Bacteria†

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Unité de Biologie des Bactéries Pathogènes à Gram Positif, Institut Pasteur, Centre National de la Recherche Scientifique, Paris, France

Received 9 April 2004/Accepted 28 June 2004

A shuttle vector designated pMAD was constructed for quickly generating gene inactivation mutants in naturally nontransformable gram-positive bacteria. This vector allows, on X-Gal (5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside) plates, a quick colorimetric blue-white discrimination of bacteria which have lost the plasmid, greatly facilitating clone identification during mutagenesis. The plasmid was used in *Staphylococcus aureus*, *Listeria monocytogenes*, and *Bacillus cereus* to efficiently construct mutants with or without an associated antibiotic resistance gene.

The study of chromosomal genes and the modification of bacterial strains require the development of new strategies and new genetic tools to facilitate the rapid screening of recombinant clones. Molecular genetic analyses require the exchange by homologous recombination of a chromosomal gene by a mutated allele or inactivated copy, and several strategies have been developed for generating gene replacements in bacterial chromosomes. Here, we describe a new plasmid for efficiently accomplishing such constructions in nontransformable bacteria. This plasmid, pMAD, overcomes the limitations of the available vectors, which were largely developed for highly transformable bacteria. For example, a one-step gene inactivation procedure has been developed for *Bacillus subtilis* by using a nonreplicative vector called pMutin (22). This vector carries a *lacZ* reporter gene and an inducible Pspac promoter which can be induced by IPTG (isopropyl-β-D-thiogalactopyranoside). Following chromosomal integration via a single crossover event of a pMutin recombinant vector containing an internal fragment of the target gene, the target gene is inactivated, *lacZ* becomes transcriptionally fused to the gene, and the Pspac promoter controls the transcription of the downstream gene(s) in an IPTG-dependent fashion.

To avoid the introduction of a complete plasmid and duplication of the target sequence in the chromosome, other strategies were described. These two-step strategies proceed by homologous recombination between a target gene and homologous sequences carried on a plasmid which is temperature sensitive for DNA replication. After transformation of the plasmid into the host, integration of the plasmid into the chromosome by a single crossover event is selected during growth at the nonpermissive temperature while maintaining selective pressure. Subsequent growth of the cointegrates at the permissive temperature (30°C) leads to a second recombination event, resulting in their resolution. Screening of clones where an efficient second recombination event has taken place (i.e., where the loss of the vector is concomitant with the deletion of the gene of interest) is rather difficult without positive selection or colorimetric screening of the candidate clones. A general system for generating gene replacement in bacterial chromosomes was developed by using the pORI vector (12). This conditionally replicating vector contains the *Escherichia coli lacZ* gene, which is expressed from a constitutive promoter recognized in gram-positive and gram-negative bacteria. The presence or the absence of the *lacZ* gene enables a simple blue-white screening of recombination events. However, these plasmids lack the *repA* gene encoding the replication initiation protein and are unable to replicate in any bacterial strains unless the RepA protein is provided in *trans*. In consequence, high transformation frequencies are required to obtain integrants and deletions or substitutions are produced after resolution of cointegrants, thus limiting the use of this plasmid vector to naturally competent bacteria with a high efficiency of transformation (12). A system for generating deletions in open reading frames of *E. coli* was developed by using plasmid pK03, which contains a temperature-sensitive origin of replication, a chloramphenicol resistance gene, and a counterselectable *sacB* gene to facilitate allelic exchange. Briefly, the in vitro altered sequences carried on the vector pK03 are integrated into the chromosome by using the sucrose-resistant phenotype, and the sucrose-resistant and chloramphenicol-sensitive colonies are screened for the desired gene replacement (15). In earlier work with the Tn*917* transposon in *B. subtilis* (25), methods of recovering populations of bacteria containing chromosomal insertions of the transposon were based on the use of temperature-sensitive vectors derived from a small *Staphylococcus aureus* plasmid called pE194 (9). However, practical complications arose from the fact that the thermosensitivity of replication of the pE194-derived vectors was incomplete. Replication of the plasmid was impaired at high temperatures but not abolished. New vectors based on the use of pE194^{ts}, a thermosensitive mutant derivative of pE194, were isolated by Alexan-

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