Final Progress Report for Research Projects Funded by Health Research Grants

Instructions: Please complete all of the items as instructed. Do not delete instructions. Do not leave any items blank; responses must be provided for all items. If your response to an item is "None", please specify "None" as your response. "Not applicable" is not an acceptable response for any of the items. There is no limit to the length of your response to any question. Responses should be single-spaced, no smaller than 12-point type. The report **must be completed using MS Word**. Submitted reports must be Word documents; they should not be converted to pdf format. Questions? Contact Health Research Program staff at 717-783-2548.

1. **Grantee Institution:** Temple University of the Commonwealth System of Higher Education

2. **Reporting Period (start and end date of grant award period):** 01/01/2009 – 12/31/2012

3. **Grant Contact Person (First Name, M.I., Last Name, Degrees):** Germaine A Calicat, MLA

4. **Grant Contact Person's Telephone Number:** 215.204.7655

5. **Grant SAP Number:** 4100047651

6. **Project Number and Title of Research Project:** 21 - *The Role of Mucin in Promoting Survival of CA-MRSA and Streptococcus mutans*

7. **Start and End Date of Research Project:** 9/15/2009 – 11/30/2010

8. **Name of Principal Investigator for the Research Project:** Bettina A Buttaro, PhD

9. **Research Project Expenses**.

   9(A) Please provide the total amount of health research grant funds spent on this project for the entire duration of the grant, including indirect costs and any interest earned that was spent:

   $ 30,000

   9(B) Provide the last names (include first initial if multiple individuals with the same last name are listed) of **all** persons who worked on this research project and were supported with health research funds. Include position titles (Principal Investigator, Graduate Assistant, Post-doctoral Fellow, etc.), percent of effort on project and total health research funds expended for the position. For multiple year projects, if percent of effort varied from year to year, report in the % of Effort column the effort by year 1, 2, 3, etc. of the project (x% Yr 1; z% Yr 2-3).

Last Name	Position Title	% of Effort on Project	Cost
Ranglin, Philip	Graduate Assistant	100%	$30,000

9(C) Provide the names of **all** persons who worked on this research project, but who *were not* supported with health research funds. Include position titles (Research Assistant, Administrative Assistant, etc.) and percent of effort on project. For multiple year projects, if percent of effort varied from year to year, report in the % of Effort column the effort by year 1, 2, 3, etc. of the project (x% Yr 1; z% Yr 2-3).

Last Name	Position Title	% of Effort on Project
Oxenford, Megan	MS Candidate Main Campus	100%
Buttaro, Bettina	Principle Investigator	< 1%

9(D) Provide a list of **all** scientific equipment purchased as part of this research grant, a short description of the value (benefit) derived by the institution from this equipment, and the cost of the equipment.

Type of Scientific Equipment	Value Derived	Cost
None		

10. Co-funding of Research Project during Health Research Grant Award Period. Did this research project receive funding from any other source during the project period when it was supported by the health research grant?

Yes_____ No____X_____

If yes, please indicate the source and amount of other funds:

11. Leveraging of Additional Funds

11(A) As a result of the health research funds provided for this research project, were you able to apply for and/or obtain funding from other sources to continue or expand the research?

Yes_____ No____X_____

If yes, please list the applications submitted (column A), the funding agency (National Institutes of Health—NIH, or other source in column B), the month and year when the application was submitted (column C), and the amount of funds requested (column D). If you have received a notice that the grant will be funded, please indicate the amount of funds to be awarded (column E). If the grant was not funded, insert “not funded” in column E.

Do not include funding from your own institution or from CURE (tobacco settlement funds). Do not include grants submitted prior to the start date of the grant as shown in Question 2. If you list grants submitted within 1-6 months of the start date of this grant, add a statement below the table indicating how the data/results from this project were used to secure that grant.

A. Title of research project on grant application	B. Funding agency (check those that apply)	C. Month and Year Submitted	D. Amount of funds requested:	E. Amount of funds to be awarded:
None	☐NIH ☐ Other federal (specify:_____) ☐ Nonfederal source (specify:_)		$	$

11(B) Are you planning to apply for additional funding in the future to continue or expand the research?

Yes___X_____ No_____

If yes, please describe your plans:

MRSA: Together with Kevin Waninger (TUSM, Bethlehem Campus) we will seek funding from Sports Medicine Associations (e.g. American College of Sports Medicine Foundation Grants, National Football League, American Medical Society for Sports Medicine) for further investigation of MRSA survival on artificial turf as a risk factor for athletes.

***S. mutans*:** A collaborative project between William Wuest (Department of Chemistry, Temple University), Patrick Piggot and Bettina Buttaro to further investigate the molecular mechanism behind the induction of survival by mucin is currently underway. An NIH RO1 grant application to NIDCR is planned after preliminary data is obtained and manuscripts are accepted for publication.

12. Future of Research Project. What are the future plans for this research project?

MRSA: Experiments are underway to (1) examine the survival of CA-MRSA on natural grass fields where soil bacteria provide a source of competition and (2) to expand the observations that oral secretions can provide a source of CA-MRSA contamination if players are colonized, in collaboration with East Stroudsburg University.

S. mutans: The role of mucin in survival will be part of a larger study designed to examine the molecular mechanism by which two subpopulations of *S. mutans* arise during survival in stationary phase. This research will be a collaboration combining chemical (William Wuest, Department of Chemistry, Temple University) and mutant library screens (Patrick Piggot, Department of Microbiology, TUSM) to examine the molecular mechanisms that lead to the

development of two *S. mutans* subpopulations during survival in mucin. One of the subpopulations expresses *pdh* and promotes the survival of a second subpopulation, which grows in response to sugar pulses.

13. **New Investigator Training and Development**. Did students participate in project supported internships or graduate or post-graduate training for at least one semester or one summer?

    Yes___X____ No_____

    If yes, how many students? Please specify in the tables below:

	Undergraduate	Masters	Pre-doc	Post-doc
Male			1	
Female		1	1	
Unknown				
Total		**1**	**2**	

	Undergraduate	Masters	Pre-doc	Post-doc
Hispanic				
Non-Hispanic				
Unknown		1	2	
Total		**1**	**2**	

	Undergraduate	Masters	Pre-doc	Post-doc
White		1		
Black			1	
Asian				
Other			1	
Unknown				
Total		**1**	**2**	

14. **Recruitment of Out-of–State Researchers**. Did you bring researchers into Pennsylvania to carry out this research project?

    Yes_____ No_____X____

    If yes, please list the name and degree of each researcher and his/her previous affiliation:

15. **Impact on Research Capacity and Quality**. Did the health research project enhance the quality and/or capacity of research at your institution?

Yes_____ No____X_____

If yes, describe how improvements in infrastructure, the addition of new investigators, and other resources have led to more and better research.

16. Collaboration, business and community involvement.

16(A) Did the health research funds lead to collaboration with research partners outside of your institution (e.g., entire university, entire hospital system)?

Yes____X____ No_____

If yes, please describe the collaborations:

MRSA: Keith Vanic from East Stroudsburg University has been recruited as a collaborator for the MRSA project to study the ability of *S. aureus* colonized players to secrete MRSA onto the artificial turf. The studies will include determining if the nasal secretions contain planktonic cells or biofilms, since our studies show this has implications for the amount of time the MRSA can survive on the artificial turf.

***S. mutans*:** We have recruited a chemist from Main Campus Temple University, William Wuest, as a collaborator for the *S. mutans* project to characterize the chemical signals allowing for survival of *S. mutans* in the presence of mucin.

16(B) Did the research project result in commercial development of any research products?

Yes_____ No___X_____

If yes, please describe commercial development activities that resulted from the research project:

16(C) Did the research lead to new involvement with the community?

Yes____X____ No_____

If yes, please describe involvement with community groups that resulted from the research project:

Recently, we have worked with a Temple University School of Medicine Biomedical Sciences Masters Student to create an awareness video about CA-MRSA for elementary- and high-school students and their parents.

17. Progress in Achieving Research Goals, Objectives and Aims.

List the project goals, objectives and specific aims (as contained in the grant agreement). Summarize the progress made in achieving these goals, objectives and aims for the period that the project was funded (i.e., from project start date through end date). Indicate whether or not each goal/objective/aim was achieved; if something was not achieved, note the reasons why. Describe the methods used. If changes were made to the research goals/objectives/aims, methods, design or timeline since the original grant application was submitted, please describe the changes. Provide detailed results of the project. Include evidence of the data that was generated and analyzed, and provide tables, graphs, and figures of the data. List published abstracts, poster presentations and scientific meeting presentations at the end of the summary of progress; peer-reviewed publications should be listed under item 20.

This response should be a DETAILED report of the methods and findings. It is not sufficient to state that the work was completed. Insufficient information may result in an unfavorable performance review, which may jeopardize future funding. If research findings are pending publication you must still include enough detail for the expert peer reviewers to evaluate the progress during the course of the project.

Health research grants funded under the Tobacco Settlement Act will be evaluated via a performance review by an expert panel of researchers and clinicians who will assess project work using this Final Progress Report, all project Annual Reports and the project's strategic plan. After the final performance review of each project is complete, approximately 12-16 months after the end of the grant, this Final Progress Report, as well as the Final Performance Review Report containing the comments of the expert review panel, and the grantee's written response to the Final Performance Review Report, will be posted on the CURE Web site.

There is no limit to the length of your response. Responses must be single-spaced below, no smaller than 12-point type. If you cut and paste text from a publication, be sure symbols print properly, e.g., the Greek symbol for alpha (α) and beta (ß) should not print as boxes (□) and include the appropriate citation(s). DO NOT DELETE THESE INSTRUCTIONS.

Summary of Research Completed

Specific Aim 1. Degradation of mucin during survival of *S. mutans* and CA-MRSA.

It was not feasible to examine degradation of mucin in the medium. Therefore, this Aim was replaced with determining the effects of sunlight and temperature on MRSA survival on astroturf in the laboratory and experiments to develop methods to detect MRSA and *Staphylococcus aureus* on football playing fields where players of deposited oral secretions, which represent a natural source of bacteria in mucin.

S. aureus could be detected on areas of a football playing field where players often deposited oral secretions (Fig. 1). Since *S. aureus* has almost identical survival as MRSA, the methods developed can be applied to epidemiologic studies. However, *S. aureus* were detected only when temperatures on the field dropped to less than 37°C. Consistent with these findings high temperatures (44°C) and sunlight (ultraviolet) was found to significantly reduce survival even in the presence of mucin (Fig. 2A and 2B). Interestingly, MRSA grown in biofilms survived longer than 30 days at 44°C in sunlight in three independent experiments, suggesting there is a difference in the environmental survival of planktonic (free-living) MRSA and biofilms. A manuscript is being prepared describing these results. For continuation of the project, we are collaborating with K. Vanic at East Stroudsburg University to determine whether human carriers of *S. aureus* secrete planktonic or biofilm cells in nasal secretions.

Specific Aim 2. Mutation of genes in the mannose and galactose utilization pathways.

Experiments were completed examining the role of the *pdh* operon on survival in *S. mutans* in the presence of mucin. Previous data has shown the *pdh* operon has a significant effect on survival of *S. mutans* during carbohydrate starvation in the presence of mucin. In recent studies, using a P*pdh*-GFP fusion, we have found that *pdh* is expressed in only a subpopulation of cells (0.1-1.0% of the cells). Early in starvation (~24 hrs), this subpopulation of cells is present as single cells or in short chains. Later in starvation, these cells produce long chains, suggesting the *pdh* subpopulation is surviving and replicating (Busuioc, Buttaro and Piggot *et al.*, J. Bacteriol., 192:4395-4402, 2010). These studies were done in medium containing amino acids and a hexose carbohydrate source that was metabolized to various triose endproducts by the end of exponential growth. Both the amino acids and the sugar endproducts could be used as alternate carbon sources after the primary sugar had been depleted. Therefore, the effect of mucin on survival in CDM lacking amino acids (MCDM) and lacking spent medium was determined. Under these conditions, mucin significantly increased survival of the cells and the survival was dependent on the *pdh* operon (Busuioc, Buttaro and Piggot *et al.*, J. Bacteriol., 192:4395-4402, 2010). Mucin is a glycoprotein that could provide both a fermentable sugar source (e.g. galactose) and amino acids, however, the presence of excess free amino acids alone in CDM did not prolong survival, suggesting the contribution to survival may be the sugar residues of mucin. Galactose is the only sugar known to be present in mucin and be metabolized by *S. mutans*. *S. mutans* has two glucose utilization pathways; the Leloir pathway and the Tagatose pathway (Fig. 3). However, an enzyme for the conversion of galactose-6-P to galactose has not been identified in *S. mutans*, so the pathway is incomplete. The tagatose pathway produces glycolytic intermediates that could enter glycolysis and allow for the production of pyruvate. The genes encoding one of the galactose transporters and some of the enzymes in the tagatose pathway were found to be up-regulated in response to mucin in preliminary microarray experiments (*lacFE*, *lacAB*, and *lacD*; Fig. 3). We have found that mucin compensated for the absence of amino acids to promote exponential growth and biofilm formation in minimal medium supplemented with glucose (Fig. 4). Mucin extended survival in conditions where there was no net growth provided the operon encoding the pyruvate dehydrogenase complex was intact (Fig. 5A). Mucin extended survival in conditions of amino acid sufficiency provided the tagatose pathway for galactose utilization was intact, suggesting that *S. mutans* can scavenge sufficient galactose from mucin to enhance survival, though not to serve as a primary carbon and energy source (Fig. 5B). The results indicate that mucin may have a metabolic role in

promoting survival of *S. mutans.* A manuscript has been submitted to FEMS Microbiology describing these results.

Given the difficulty in interpreting these data in a bacterium with a well-defined metabolism, these experiments were not completed with MRSA and the MRSA mucin research was focused on establishing techniques for the epidemiologic studies.

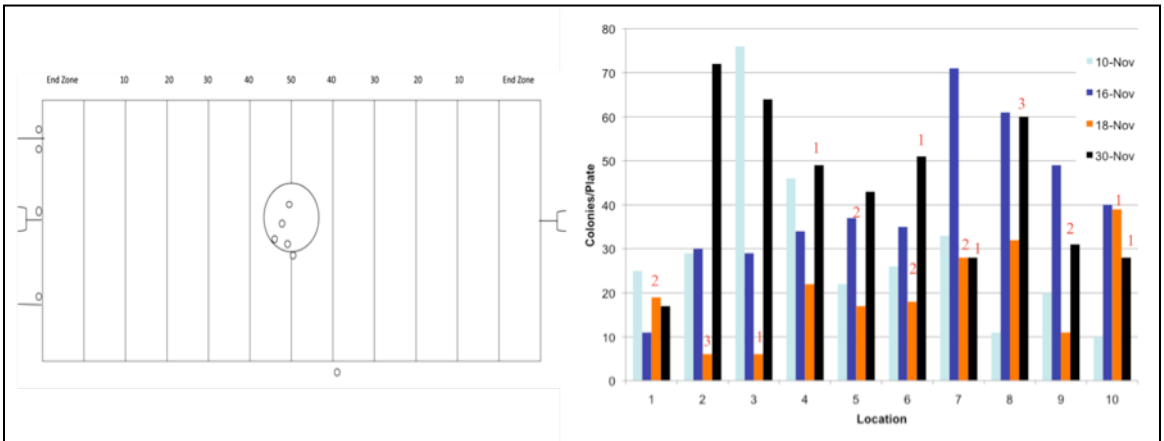

Fig. 1 Detection of CA-MRSA on artificial turf. To determine if *S. aureus* and by extension CA-MRSA can be detected on an outdoor artificial turf playing field, football practices were observed; samples were taken from 10 areas where players had deposited oral expectorations (circles on football field diagram). Chromagar *Staphylococcus aureus* plates were pressed on the field within the 10 areas. The presence of *S. aureus* was detected by formation of colonies of the correct color on the Chromagar *Staphylococcus aureus* plates. Results are reported as total numbers of bacteria detected and the red numbers above each bar are the number of *S. aureus* isolates.

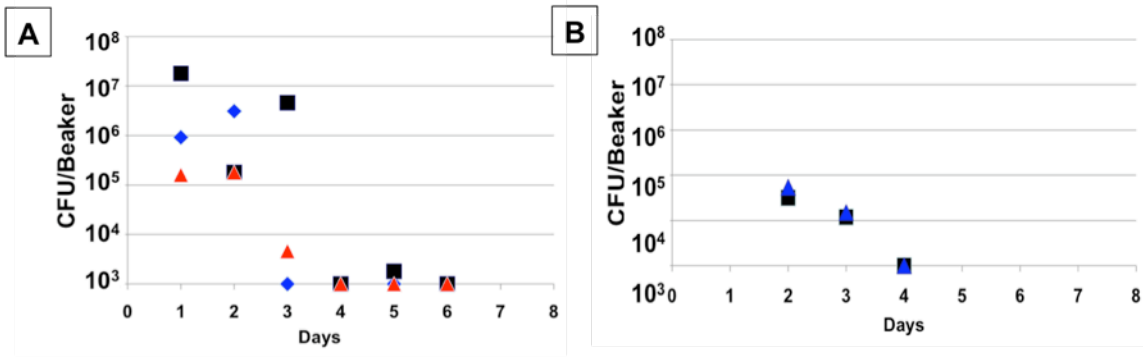

Fig. 2 Survival of MRSA in mucin was decreased by exposure to UV and high temperature. CA-MRSA CDC 167-05 were grown as planktonic cells, resuspended in salivary mucin and used to inoculate beakers containing pieces of artificial turf. (A) Sample beakers were incubated at 37°C with a light source which duplicates sun light, including UV-A and UV-B wavelengths (ReptiSun 5.0 lamp). (B) Sample beakers were incubated at 44°C. Survival was determined by adding 40 ml of 0.9% saline to the beaker, rigoriously pipetting and plating on Mannitol Salt Agar. The number of colony forming units (CFUs) was calculated at various days post-inoculation. The results are presented as changes in CFU over time with survival time being the last time point (day) post-inoculation where colonies were detected (10^3 lower limit of detection). Different color symbols are the CFU obtained in independent experiments.

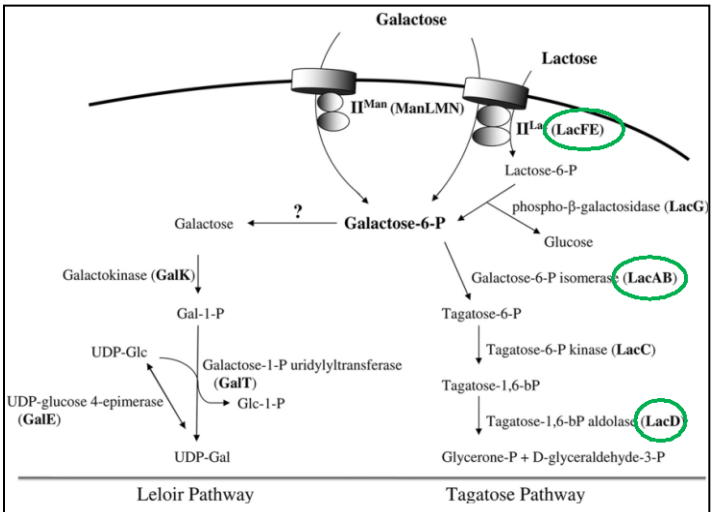

Fig. 3 The galactose utilization pathways in *S. mutans*. Modified from Zeng *et al.* (J. Bacteriol. 2010 192:2434-2444). The question mark denotes an unknown enzyme/gene. The green circles are the enzymes for whose genes show increased transcription in the presence of mucin.

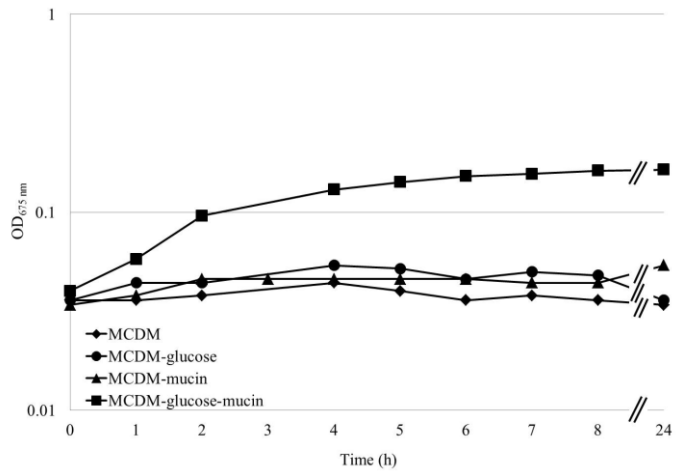

Fig. 4. Growth of *Streptococcus mutans* UA159 in MCDM alone, supplemented with glucose/mucin or with both glucose and mucin.

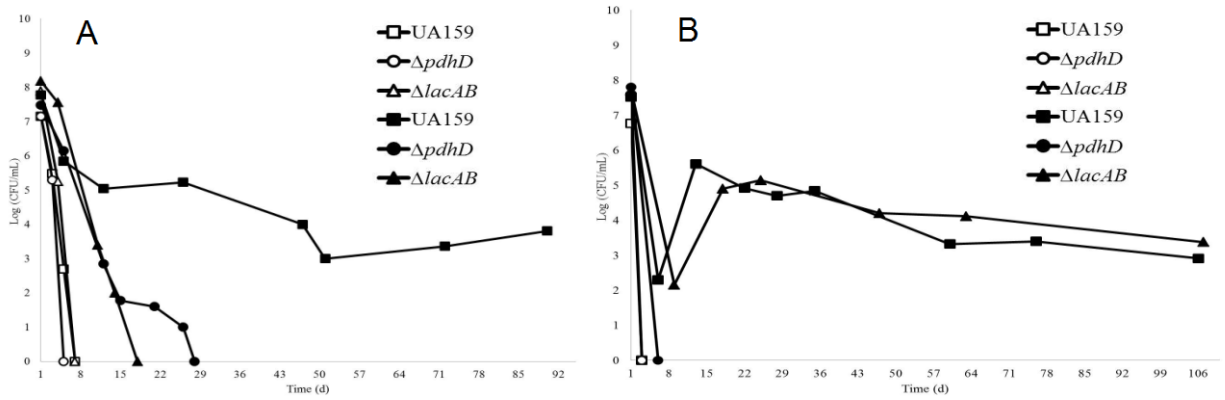

Fig. 5. Survival of batch cultures of *Streptococcus mutans* UA159, Δ*pdhD* and Δ*lacAB* in a)CDM supplemented with mucin (filled symbols) or CDM alone (empty symbols) b) MCDM supplemented with mucin (filled symbols) or CDM alone (empty symbols). The results of a representative experiment of at least three experiments are shown.

18. Extent of Clinical Activities Initiated and Completed. Items 18(A) and 18(B) should be completed for all research projects. If the project was restricted to secondary analysis of clinical data or data analysis of clinical research, then responses to 18(A) and 18(B) should be "No."

18(A) Did you initiate a study that involved the testing of treatment, prevention or diagnostic procedures on human subjects?

_____Yes
__X__No

18(B) Did you complete a study that involved the testing of treatment, prevention or diagnostic procedures on human subjects?

_____Yes
__X__ No

If "Yes" to either 18(A) or 18(B), items 18(C) – (F) must also be completed. (Do NOT complete 18(C-F) if 18(A) and 18(B) are both "No.")

18(C) How many hospital and health care professionals were involved in the research project?

_____Number of hospital and health care professionals involved in the research project

18(D) How many subjects were included in the study compared to targeted goals?

_____Number of subjects originally targeted to be included in the study
_____Number of subjects enrolled in the study

Note: Studies that fall dramatically short on recruitment are encouraged to provide the details of their recruitment efforts in Item 17, Progress in Achieving Research Goals, Objectives and Aims. For example, the number of eligible subjects approached, the number that refused to participate and the reasons for refusal. Without this information it is difficult to discern whether eligibility criteria were too restrictive or the study simply did not appeal to subjects.

18(E) How many subjects were enrolled in the study by gender, ethnicity and race?

Gender:
_____Males
_____Females
_____Unknown

Ethnicity:
_____Latinos or Hispanics
_____Not Latinos or Hispanics
_____Unknown

Race:
_____American Indian or Alaska Native
_____Asian
_____Blacks or African American
_____Native Hawaiian or Other Pacific Islander
_____White
_____Other, specify:_____
_____Unknown

18(F) Where was the research study conducted? (List the county where the research study was conducted. If the treatment, prevention and diagnostic tests were offered in more than one county, list all of the counties where the research study was conducted.)

19. Human Embryonic Stem Cell Research. Item 19(A) should be completed for all research projects. If the research project involved human embryonic stem cells, items 19(B) and 19(C) must also be completed.

19(A) Did this project involve, in any capacity, human embryonic stem cells?
_____Yes
__X__ No

19(B) Were these stem cell lines NIH-approved lines that were derived outside of Pennsylvania?
_____Yes
_____ No

19(C) Please describe how this project involved human embryonic stem cells:

20. Articles Submitted to Peer-Reviewed Publications.

20(A) Identify all publications that resulted from the research performed during the funding period and that have been submitted to peer-reviewed publications. Do not list journal abstracts or presentations at professional meetings; abstract and meeting presentations should be listed at the end of item 17. **Include only those publications that acknowledge the Pennsylvania Department of Health as a funding source** (as required in the grant agreement). List the title of the journal article, the authors, the name of the peer-reviewed publication, the month and year when it was submitted, and the status of publication (submitted for publication, accepted for publication or published.). Submit an electronic copy of each publication or paper submitted for publication, listed in the table, in a PDF version 5.0.5 (or greater) format, 1,200 dpi. Filenames for each publication should include the number of the research project, the last name of the PI, the number of the publication and an abbreviated research project title. For example, if you submit two publications for PI Smith for the "Cognition and MRI in Older Adults" research project (Project 1), and two publications for PI Zhang for the "Lung Cancer" research project (Project 3), the filenames should be:

Project 1 – Smith – Publication 1 – Cognition and MRI
Project 1 – Smith – Publication 2 – Cognition and MRI
Project 3 – Zhang – Publication 1 – Lung Cancer
Project 3 – Zhang – Publication 2 – Lung Cancer

If the publication is not available electronically, provide 5 paper copies of the publication.

Note: The grant agreement requires that recipients acknowledge the Pennsylvania Department of Health funding in all publications. Please ensure that all publications listed acknowledge the Department of Health funding. If a publication does not acknowledge the funding from the Commonwealth, do not list the publication.

Title of Journal Article:	Authors:	Name of Peer-reviewed Publication:	Month and Year Submitted:	Publication Status (check appropriate box below):
1. Pilot Study: Community Associated MRSA Survival on Artificial Turf Substrates.	Waninger, K., Rooney, T.P., Miller, J., Berberian J., Fujimoto, A. and Buttaro, B. A.	Med. Sci. Sports Med.	April 2010	☐Submitted ☐Accepted ☒Published

20(B) Based on this project, are you planning to submit articles to peer-reviewed publications in the future?

Yes___X____ No_____

If yes, please describe your plans:

Oxenford, M.B, B.A. Buttaro, T.P. Rooney and K.N. Waninger. CA-MRSA can survive on artificial turf under environmental conditions. Manuscript in preparation for Med. Sci. Sports Med.

21. Changes in Outcome, Impact and Effectiveness Attributable to the Research Project. Describe the outcome, impact, and effectiveness of the research project by summarizing its impact on the incidence of disease, death from disease, stage of disease at time of diagnosis, or other relevant measures of outcome, impact or effectiveness of the research project. If there were no changes, insert "None"; do not use "Not applicable." Responses must be single-spaced below, and no smaller than 12-point type. DO NOT DELETE THESE INSTRUCTIONS. There is no limit to the length of your response.

The information on the survival of CA-MRSA on artificial turf playing fields demonstrates playing fields may be an additional risk factor for student athletes, particularly football players, who sustain injuries due to direct contact with the artificial turf playing field.

22. Major Discoveries, New Drugs, and New Approaches for Prevention Diagnosis and Treatment. Describe major discoveries, new drugs, and new approaches for prevention, diagnosis and treatment that are attributable to the completed research project. If there were no major discoveries, drugs or approaches, insert "None"; do not use "Not applicable." Responses must be single-spaced below, and no smaller than 12-point type. DO NOT DELETE THESE INSTRUCTIONS. There is no limit to the length of your response.

None.

23. Inventions, Patents and Commercial Development Opportunities.

23(A) Were any inventions, which may be patentable or otherwise protectable under Title 35 of the United States Code, conceived or first actually reduced to practice in the performance of work under this health research grant? Yes_____ No___X_____

If "Yes" to 23(A), complete items a – g below for each invention. (Do NOT complete items a - g if 23(A) is "No.")

a. Title of Invention:

b. Name of Inventor(s):
c. Technical Description of Invention (describe nature, purpose, operation and physical, chemical, biological or electrical characteristics of the invention):

d. Was a patent filed for the invention conceived or first actually reduced to practice in the performance of work under this health research grant?
Yes_____ No____

If yes, indicate date patent was filed:

e. Was a patent issued for the invention conceived or first actually reduced to practice in the performance of work under this health research grant?
Yes_____ No____
If yes, indicate number of patent, title and date issued:
Patent number:
Title of patent:
Date issued:

f. Were any licenses granted for the patent obtained as a result of work performed under this health research grant? Yes_____ No____

If yes, how many licenses were granted?_____

g. Were any commercial development activities taken to develop the invention into a commercial product or service for manufacture or sale? Yes___ No____

If yes, describe the commercial development activities:

23(B) Based on the results of this project, are you planning to file for any licenses or patents, or undertake any commercial development opportunities in the future?

Yes_____ No____X_____

If yes, please describe your plans:

24. Key Investigator Qualifications. Briefly describe the education, research interests and experience and professional commitments of the Principal Investigator and all other key investigators. In place of narrative you may insert the NIH biosketch form here; however, please limit each biosketch to 1-2 pages. *For Nonformula grants only – include information for only those key investigators whose biosketches were not included in the original grant application.*

BIOGRAPHICAL SKETCH

Provide the following information for the Senior/key personnel and other significant contributors.
Follow this format for each person. **DO NOT EXCEED FOUR PAGES.**

NAME	POSITION TITLE
Bettina A. Buttaro	Associate Professor
eRA COMMONS USER NAME (credential, e.g., agency login) bbuttaro	

EDUCATION/TRAINING *(Begin with baccalaureate or other initial professional education, such as nursing, include postdoctoral training and residency training if applicable.)*

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	MM/YY	FIELD OF STUDY
South Dakota State University	B.S.	1985	Biology, Microbiology
University of Minnesota	Ph.D.	1993	Microbiology
University of Minnesota Medical School	Post Doc.	1993-96	Microbiology
University of Ulm, Germany	Post Doc.	1996-98	Microbiology

A. Personal Statement

Enterococcus faecalis is a major multi-drug resistant opportunistic pathogen, which causes endocarditis, septicemia and urinary tract infections. In addition to causing opportunistic infections, *E. faecalis* is a member of the gastrointestinal tract normal flora. It contains conjugative plasmids, which provide a stable reservoir for antibiotic resistance genes. The conjugative plasmids result in antibiotic resistant Enterococcal infections such as VRE and mediate horizontal gene transfer the resistance determinants to other bacteria such as *Staphylococcus aureus.* One unusual feature of these plasmids is their exceptional stability in the absence of antibiotics. Using pCF10 as model plasmid, we found three features contribute to stability, which have not been previously described to affect replicon activity in any characterized plasmids. These features are (a) interactions of the replication initiation protein PrgW with pre-pheromone peptide (pre-cCF10), (b) disulfide bond formation and (c) protein processing. One focus of ongoing experiments is to determine the novel molecular mechanisms involved in PrgW replication and stability. Since these plasmids play a central role in antibiotic resistant Enterococcal infections and in the dissemination of antibiotic resistance genes to other genera of bacteria, we have initiated a collaborative project with Bill Wuest, Department of Chemistry, Temple University to develop a new therapeutic. We hypothesize, that a pre-cCF10 peptide analog could be synthesized which binds to the replication initiation protein PrgW and inhibits its activity. This could result in complete loss of the plasmid pCF10 from the bacterial population. This peptoid therapeutic would reduce antibiotic resistance in *E. faecalis* and prevent de novo transfer of resistance determinants to other genera and species during antibiotic treatment. Other recent areas of study in the laboratory have included formation of *Streptococcus mutans* subpopulations in biofilms during survival and genomic diversification of *Streptococcus pyogenes* during survival in vitro in eukaryotic cells and in biofilms. Finally, collaborative studies have been completed with clinicians on the tracheostomy tube biofilms, survival of methicillin-resistant *Staphylococcus aureus* (MRSA) on artificial turf, and contamination of suture materials by MRSA.

B. Positions and Honors

Positions and Employment

1985-87 Graduate Student, Department of Microbiology, South Dakota State University, Brookings, SD

1987-88 Graduate Student, Department of Microbiology, University of Minnesota, Minneapolis, MN

1988-93	Predoctoral Fellow, Department of Microbiology, University of Minnesota, Minneapolis, MN. NIH Cancer Training Grant CA09138-16 made to the University of Minnesota.
1993-96	Postdoctoral Fellow, Bioprocess Technology Institute and Department of Microbiology, University of Minnesota, St. Paul/Minneapolis, MN (NIH Individual Award AI08742)
1996-98	Alexander VonHumboldt Scholar, Department of Medical Microbiology and Hygiene, University of Ulm, German (IV-USA/1039266)
1998-04	Assistant Professor, Department of Microbiology and Immunology, Temple University School of Medicine, Philadelphia, PA.
2004-present	Associate Professor, Department of Microbiology and Immunology, Temple University School of Medicine, Philadelphia, PA

Other Experience and Professional Memberships

1998-	Member American Society for Microbiology
2002-present	AdHoc Reviewer: Infect. Immun., Mol. Microbiol., Proteomics, J. Bacteriol., Microbiology
2005-2007	AHA (American Heart Association) Regional Study Section Member
2007	NIH/CSR Study Section, Bacterial Pathogenesis (BACP), ad hoc reviewer
2007-2009	President, Eastern Pennsylvania Branch of American Society for Microbiology
2008-2011	AHA Study Section, Region 1/2, Microbiology and Microbial Pathogenesis Study Group, Chair

Awards and Honors

1984	Undergraduate Microbiol. Scholarship, Sigma Xi Research Award, South Dakota State Univ.
1988-1992	NIH Cancer Training Grant, CA 09138-16 made to the U of MN
1990	Graduate Student Research Award, North Central Branch ASM
1992	Sigma Xi Research Award, University of Minnesota Chapter
1993-1996	NIH Postdoctoral Individual Fellowship, (1F32AI08742)
1996-1998	Alexander VonHumboldt Fellowship, (IV-USA/1039266)

C. Selected Peer-reviewed Publications (Previously published as Leonard)

Most relevant to the current research activity

1. Leonard, B.A.B., Bensing, B.A., Hedberg, P.J., Ruhfel, R.E., Chung, J.W., Dunny, G.M. Pheromone-inducible gene regulation and signalling for the control of aggregation substance expression in the conjugative plasmid pCF10. Dev. Biol. Stand. 85:27-34, 1995.
2. Leonard, B.A.B., Colwell, A., Podbielski, A., Dunny, G.M. Regulation of endogenous pheromone to prevent self-induction of conjugation and its possible role in plasmid replication. Beijerinck Centennial Symposium: Microbial Physiology and Gene Regulation - Emerging Principles and Applications. Scheffers, W.A., van Dijken, J.P., eds. Delft University Press, p. 28-29, 1995.
3. Leonard, B.A.B., Podbielski, A., Hedberg, P.H., Dunny, G.M. *Enterococcus faecalis* pheromone binding protein, PrgZ, recruits a chromosomal oligopeptide permease system to import sex pheromone cCF10 for induction of conjugation. Proc. Natl. Acad. Sci. USA 93:260-264, 1996.
4. Hedberg, P.H., Ruhfel, R.E., Leonard, B.A.B., Dunny, G.M. Definition of the minimum region necessary for negative regulation of PrgB expression in *Enterococcus faecalis* pheromone-inducible plasmid pCF10. Plasmid 35:46-57, 1996.
5. Leonard, B.A.B., Bensing, B.A., Hedberg, P.J., Ruhfel, R.E., Chung, J.W., Dunny, G.M. Pheromone-inducible gene regulation and signalling for the control of aggregation substance expression in the conjugative plasmid pCF10. Dev. Biol. Stand. 85:27-34, 1995.

6. Leonard, B.A.B., Colwell, A., Podbielski, A., Dunny, G.M. Regulation of endogenous pheromone to prevent self-induction of conjugation and its possible role in plasmid replication. Beijerinck Centennial Symposium: Microbial Physiology and Gene Regulation - Emerging Principles and Applications. Scheffers, W.A., van Dijken, J.P., eds. Delft University Press, p. 28-29, 1995.
7. Leonard, B.A.B., Podbielski, A., Hedberg, P.H., Dunny, G.M. *Enterococcus faecalis* pheromone binding protein, PrgZ, recruits a chromosomal oligopeptide permease system to import sex pheromone cCF10 for induction of conjugation. Proc. Natl. Acad. Sci. USA 93:260-264, 1996.
8. Hedberg, P.H., Ruhfel, R.E., Leonard, B.A.B., Dunny, G.M. Definition of the minimum region necessary for negative regulation of PrgB expression in *Enterococcus faecalis* pheromone-inducible plasmid pCF10. Plasmid 35:46-57, 1996.
9. Leonard, B.A.B., Hirt, H., Dunny, G.M. Regulation of aggregation substance expression by bacterial and host factors. Adv. Exp. Med. Biol. 418:785-787, 1997.
10. Dunny, G.M., Leonard, B.A.B. Cell-cell communication in gram-positive bacteria. Annu. Rev. Microbiol. 51:527-564, 1997.
11. Buttaro, B.A. (Leonard), Dunny, G.M., Antiporta, M. Cell-associated pheromone peptide (cCF10) production and pheromone inhibition in *Enterococcus faecalis*. J. Bacteriol. 182:4926, 2000.
12. Meloni, M.*, Mazeffa, V.*, Manias, D. A., Colwell, A., Dunny, G. M., Buttaro, B. A. Unprocessed pheromone (pre-cCF10) contributes to pCF10 maintenance. J. Bacteriol., revised and submitted.
13. Utter, B. and Buttaro, B.A. Pheromone-interacting replication protein controls enterococcal plasmid pCF10 stability through disulfide bonds. J. Bacteriol., revised and submitted.
14. Meloni, M. and Buttaro, B. A., The replication protein, PrgW, regulates pheromone-signaling machinery. In preparation.
15. Izquierdo, J. S. and Buttaro, B. A., Coupling of the partitioning and replication control systems of the *Enterococcus faecalis* conjugative plasmid pCF10. In preparation.

<u>Additional recent publications of importance to the field (in chronological order)</u>

1. Leonard, B.A.B., Lee, P.K., Jenkins, M.K., Schlievert, P.M. Cell and receptor requirements for streptococcal pyrogenic exotoxin T cell mitogenicity. Infect. Immun. 59:1210-1214, 1991.
2. Leonard, B.A.B., Schlievert, P.M. Immune cell lethality induced by streptococcal pyrogenic exotoxin A and endotoxin. Infect. Immun. 60:3747-3755, 1992.
3. Murray, D.L., Shidhar Prasad, G., Earhart, C.A., Leonard, B.A.B., Kreiswirth, B.N., Novick, R., Ohlendorf, D.H., Schlievert, P.M. Immunobiological and biochemical properties of mutants of Toxic Shock Syndrome Toxin-1. J. Immunol. 152:87-95, 1994.
4. Podbielski, A., Pohl, B., Woischnik, M., Krner, C., Schmidt, K.-H., Rozdzinki, E., Leonard, B.A.B. Group A streptococcal (GAS) oligopeptide permease (Opp) affects cysteine protease production. Mol. Microbiol. 21:1087-1099, 1997.
5. Roggiani, M., Stoehr, J.A., Leonard, B.A.B., Schlievert, P.M. Analysis of toxicity of mutants of streptococcal pyrogenic exotoxin A. Infect. Immun. 65:2868-2875, 1997.
6. Leonard (Buttaro), B.A.B., Woischnik, M., Podbielski, A. The production of stabilized virulence factor negative variants by Group A Streptococci during stationary phase. Infect. Immun. 66:3841-3847, 1998.
7. Podbielski, A., Leonard, B.A.B. The group A streptococcal dipeptide permease (Dpp) is involved in uptake of essential amino acids and affects expression of cysteine protease. Mol. Microbiol. 23:1323-1334, 1998.
8. Leonard, B.A.B., Podbielski, A. Emerging density dependent control systems in Gram-positive cocci. In Cell-Cell Communication in Bacteria. Dunny, G.M., Winans, S., eds. ASM Press, Washington, DC, pp. 315-329, 1999.
9. Podbielski, A., Woischnik, M., Leonard, B.A.B., Schmidt, K.-H. Characterization of *nra*, a global negative regulator gene in group A streptococci. Mol. Microbiol. 31:1051-1064, 1999.

10. Podbielski, A., Woischnik, M., Bettenbrock, K., (Leonard) Buttaro, B.A. Cysteine protease SpeB expression in group A streptococci is influenced by the nutritional environment but SpeB does not contribute to obtaining essential nutrients. Med. Microbiol. Immunol. (Berl.) 188:99-109, 1999.
11. Woischnik, M., Buttaro, B.A. (Leonard), Podbielski, A. Inactivation of the cysteine protease SpeB affects hyaluronic acid capsule expression in group A streptococci. Microb. Pathog. 28:221-226, 2000.
12. Kreikemeyer, B., Boyle, M.D.P., Buttaro, B.A. (Leonard), Heinemann, M., Podbielski, A. Molecular characterization of the group A streptococcal *fas* regulon exhibiting functional similarities to the *Staphylococcus aureus agr* operon. Mol. Microbiol. 39:392-406, 2001.
13. Elsner, A., Kreikemeyer, B., Braun-Kiewnick, A., Spellerberg, B., Buttaro, B.A., Podbielski, A. Involvement of Lsp, a member of the LraI-lipoprotein family in Streptococcus pyogenes, in eukaryotic cell adhesion and internalization. Infect. Immun. 70:4859-4869, 2002.
14. Renye, J., Daneo-Moore, L, Piggot, P., Buttaro, B. A. Starvation survival of *Streptococcus mutans* in batch culture and biofilms. Appl. Environ. Microbiol. 70:6181-6187, 2004
15. Isaacson, G. C., Buttaro, B. A., Mazeffa, V., Geming, L. and Frenz, D. Oxymetazoline solutions inhibit middle ear pathogens and are not ototoxic. Ann Otol Rhinol Laryngol. 114:645-51, 2005
16. Wood, D. , Chaussee, M. A., Chaussee, M. S., and Buttaro, B. A. Persistence of *Streptococcus pyogenes* in stationary phase cultures. J. Bacteriol., 187:3319-28, 2005.
17. Soloman, D.H., Wobb J. , Buttaro, B.A., Truant A. and Soliman, A. M. S. Characterization of bacterial biofilms on tracheostromy tubes. Laryngoscope, 119:1633-8, 2009.
18. Wood*, D.N., Weinstein,* K. E., Podbielski, A., Kreikemeyer, B. Gaughan, J.P., Valentine, S., and Buttaro, B. A. Generation of metabolically diverse strains of *Streptococcus pyogenes* during persistence in stationary phase. J. Bacteriol. 191:6242-52, 2009.
19. Busuioc, M., K. Mackiewica, B.A. Buttaro and P. J. Piggot. The role of intracellular polysaccharide in persistence of *Streptococcus mutans*. J. Bacteriol., 191:7315-7322, 2009.
20. Busuioc, M., B.A. Buttaro and P. J. Piggot. The pyruvate dehydrogenase operon is expressed in a subpopulation of stationary-phase bacteria and is important for survival of sugar-starved *Streptococcus mutans.*, J Bacteriol., 192:4395-4402, 2010.
21. Waninger, K., Rooney, T.P., Miller, J., Berberian J., Fujimoto, A. and Buttaro, B. A. Pilot Study: CA-MRSA Survival on Artificial Turf Substrates. Med Sci Sports Exerc, 43:779-784, 2011.
22. Fowler, J. R., Perkins, T. A., Buttaro, B. A. and Torg J.S., Bacteria adhere less to barbed monofilament than braided sutures in a contaminated wound model. Clin Orthop Relat Res, Epub ahead of publication, 2012
23. Weinstein, K. E., Morgan, S. L., Steinberg, G., Runft, D. L., Meissler, J., Eisenstein, T. K., Boyle, M., Neely, M. N. and Buttaro, B. A. Changes in *Streptococcus pyogenes* pyruvate metabolism are selected for under survival conditions and affect eukaryotic cell invasion. J. Bacteriol. submitted.
24. Morgan, S. and Buttaro, B. A. Small colony strain development during intracellular persistence of *Streptococcus pyogenes*. J. Bacteriol., under revision.