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: Allegheny-Singer Research Institute**

2. **Reporting Period (start and end date of grant award period): 01/01/2010 – 06/30/2011**

3. **Grant Contact Person (First Name, M.I., Last Name, Degrees): Rebecca E. Pfeifer**

4. **Grant Contact Person's Telephone Number: 412-359-3137**

5. **Grant SAP Number: 4100050887**

6. **Project Number and Title of Research Project:**

   1. 16S FISH-based FACS Purification of Unculturable Bacteria for Whole Genome Amplification and Sequencing

7. **Start and End Date of Research Project: 01/01/2010 – 06/30/2011**

8. **Name of Principal Investigator for the Research Project: Garth D. Ehrlich, PhD**

9. **Research Project Expenses**.

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

   $ 187,893.38

   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
Ehrlich	Director	4.00%	$9,845.10
Hu	Assistant Professor	20.00%	$12,212.53
Eutsey	Lab Manager	15.00%	$6,538.94
Bennett	Research Fellow	86.00%	$28,608.84
Earl	Assistant Professor	13.30%	$6,414.65
Janto	Research Fellow	47.81%	$22,761.69
Kreft	Research Fellow	6.70%	$5,604.48
Powell	Research Assistant	12.76%	$3,350.10

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
None		

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:
	☐NIH ☐ Other federal (specify:_____ _____) ☐ Nonfederal source (specify: _____)		$	$
	☐NIH ☐ Other federal (specify:_____ _____) ☐ Nonfederal source (specify: _____)		$	$
	☐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:

We will search for an appropriate funding sponsor to continue the work.

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

We plan to apply for external funding.

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	
Unknown				
Total			**2**	

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

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

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

In the future we will be able to use the technology we developed as well as hire additional personnel once we have secured additional funding.

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_____ No____X_____

If yes, please describe the collaborations:

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_____ No____X_____

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

17. Progress in Achieving Research Goals, Objectives and Aims.
List the project goals, objectives and specific aims (as contained in the grant application's strategic plan). Summarize the progress made in achieving these goals, objectives and aims <u>for the entire grant award period.</u> 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.

PROJECT SUMMARY

Our goal for this project was to conduct 16S FISH-based FACS purification of unculturable bacteria for whole genome amplification and sequencing. To this end novel unculturable bacterial species of interest were: (a) identified based on prevalence of 16S ribosomal sequences obtained from IBIS technology or 454-based deep 16S amplicon sequencing of complex medical and environmental microbiomes; (b) purified using 16S FISH-based FACS; (c) whole genome amplified by multiple displacement amplification; and (d) whole genome sequences using 454-based shotgun sequencing. The following is a detailed report of the research activities accomplished, and data produced for the reporting period of 1/1/10 to 6/30/11.

A. PROOF OF CONCEPT:

Our first objective was to test this experimental pipeline for optimization and proof of concept. We took human adenoid and orthopedic tissue samples that had been shown via IBIS technology and confocal microscopy to contain *Staphylococcus aureus.* Next we isolated the bacteria from the tissue and labeled the bacteria with a *S. aureus* specific 16S rRNA FISH probe allowing us to FACS sort the specific bacterial population.

I. Confirmation of *Staphylococcus aureus:* DNA was isolated from joint tissue and adenoid tissue samples. These DNAs were run on both the IBIS T-5000 universal biosensor and the 454 Life Sciences pyrosequencer to confirm the presence of *S. aureus* DNA. *S. aureus* DNA was detected in the joint tissues and the adenoid tissues. Confocal FISH microscopy was used to confirm the IBIS and 454 results for both types of tissues (**FIGURE 1**). Based on the results, samples were selected for sorting.

II. Bacterial isolation from the tissue: Single cell bacterial suspensions were prepared by bead beating the tissues with large [~1 mM] diameter beads (these will not lyse the bacteria). These lysates were then sequentially filtered through a 50 micron filter and then a 10 micron filter. Any remaining host cells and debris were pelleted by low speed centrifugation.

III. Fluorescence in situ hybridization (FISH). FISH was performed on the selected tissues. Briefly, 4% paraformaldehyde (PFA) fixed tissues were sectioned into approximately 0.25-0.5 cm thick sections with a sterile surgical scalpel, tissues were then incubated in an ethanol series of 80% and 100% for 3 minutes each and FISH was performed with both the eubacterial (EU338) and species-specific *S. aureus* 16s rRNA fluorescent probes (Integrated DNA Technologies Inc, Coralville, Iowa), conjugated with Cy3 or Cy5 dyes. Each section was incubated with probe-specific formamide and salt concentrations and then immersed in washing buffer with the probe specific salt concentration. Samples were rinsed in sterile MQ water and observed with confocal scanning laser microscopy (CLSM).

IV. FACS: Sorting was done at the University of Pittsburgh on the MoFlo™ XDP sorter from Beckman Coulter. Our results (**FIGURE 2**) showed successful sorting of both the orthopedic and adenoid tissues. An unstained control was used to set the gates for sorting. Bacterial cells were sorted and collected for downstream applications. These results show that we were able to stain for specific bacteria and sort the bacteria in very small numbers (one sort we collected only 300 cells) (**FIGURE 2b**).

B. USE OF FACS TO OBTAIN PURIFIED POPULATIONS OF TARGET HIGH VALUE, NOVEL, UNCULTURABLE BACTERIA:

Our second objective was to FISH/SORT true unknown bacteria found living symbiotically with the tunicate species *Etcteinascidia turbinata*. The ultimate goal was to identify the bacterium responsible for the production of a medically relevant chemical (similar in structure to saframycin) that can be extracted from *E. turbinata*. The following is a detailed description of how the 16S probes for the candidate unknown bacteria were designed for FISH.

I. 454 Sequencing and assembly of the hologenome: Hologenomic DNA was extracted from frozen *E. turbinata* samples using a DNeasy Tissue kit (Qiagen). DNA was used to prepare: 1) a 16S targeted 454 amplicon library and 2) random shotgun 454 libraries. Sequencing was performed on a Roche/454 Lifesciences FLX Sequencer and later with the 454 Titanium upgrade. Raw reads from the shotgun libraries were assembled using the 454 Newbler assembler. Between two shotgun sequencing runs 839,923 reads were generated with an average size of 332 bp yielding 77,754 total contigs and 15,097 contigs larger than 500bp.

II. Biosynthetic pathway discovery: Raw and assembled data was initially filtered using relatedness of the translated protein sequences to saframycin and safracin non-ribosomal peptide synthases (NRPSs) characterized in *M. xanthus* (U24657), *S. lavendulae* (DQ838002) and *P. fluorescens* (AY061859) using BLASTx and tBLASTn searches. Once candidate sequences were selected, primers were designed off the ends of each filtered read or contig, and PCR reactions were performed based on the location and orientation of the BLAST hit on the reference sequences. Sequencing of positive reactions were performed to link multiple reads or contigs. This approach yielded six contigs of high interest that contained putative non-ribosomal peptide synthase (NRPS) domains expected to be present for biosynthesis of ET-743.

III. Determination of the bacterial populations present in the metagenome:

a) Phylogenetic classification of both the raw reads and of the total assembly was performed using the Metagenomic Rapid Annotations with Subsystems pipeline (MG-RAST). Results from raw reads and assembly were consistent, indicating ~40% of the classified sequences were of eukaryotic origin (mostly identified as *Ciona* [sea squirt/tunicate]). The remaining 60% was dominated by proteobacterial sequence (>90%) and of these two major populations stood out: α-proteobacterial (largely *Rhodobacteraceae*) making up 78-85% of the proteobacterial reads and γ-proteobacterial making up 10-17% of the proteobacterial reads.

b) Analysis of the 16S amplicon sequencing run was performed by assembling the raw 16S reads with an identity threshold of 95%. The assembled contigs were then submitted to the Ribosomal Database Project (RDP) for classification. Analysis of our 16S rRNA gene amplicon sequencing run identified 30 variants but only three significant ones (making up >1% of the total reads). The largest population of 16S rRNA gene reads are classified as *Rhodobacteraceae* (~78%), consistent with the classification of total reads by MG-RAST. The second most abundant 16S rRNA gene variant is an unclassified γ-proteobacteria (~19%). A third small population of 16S rRNA gene reads (~1%) is observed. These three variants account for >97% of the total number of 16S rRNA gene sequencing reads therefore it is likely the NRPS module identified in II is present one of these three organisms.

IV. Use of FACS to obtain purified populations of target bacteria: FISH probes were designed based on the three assembled 16S sequence variants. These probes were used in steps II-III in the proof of concept section. As shown in Figure 2D, we were able to FISH 60,000 specific bacteria from the tunicate tissue using the facilities at the University of Pittsburgh. Since then, the CGS has aquired a BD Influx™ Cell Sorter (#646500). Training on the machine was conducted both off and onsite and completed in May of 2011. Testing is ongoing as we prepare the machine for high-throughput sorting of low amounts of bacterial cells directly into plates in which lysing and whole genome amplification can be carried out.

V. Whole Genome Amplification (WGA) of genomic DNA isolated from FACS sorted bacteria: Our initial attempts to amplify genomic DNA from the bacteria sorted in IV failed. We believe this was due to the fixation process required for FACS staining and sorting of the cells. The fixation causes the cells to become much harder to lyse and as a result we were not able to liberate genomic DNA from the cells for amplification using standard protocols. We have done significant work to optimize lysing of bacterial cells and WGA. We have been able to successfully amplify bacterial genomic DNA from as low as 100 cfu based on a revised method. This method involves diluting a bacterial stock to the correct serial dilutions. After the dilutions have been prepared, they are fixed in PFA. Fixation in PFA replicates the condition of the bacterial cells after they have been sorted using the FISH - FACS technology. The PFA-fixed bacterial dilutions are then amplified using a modified Qiagen REPLI-g FFPE kit protocol. The cells are spun down at 13,000 rpm for 10 minutes to pellet the cells on the bottom of the tube and the supernatant is removed. 100 uL of FFPE lysis solution is added. The sample is heated at 95°C for 10 minutes and then cooled to room temperature. 20uL of proteinase K are then added and the sample is incubated for 2 hours at 60°C and then a further 10 minutes at 95°C. After the lysis incubation 50 uL of glass beads are added to the sample and the sample is homogenized for 10 minutes at 25 mHz. At this point the rest of the Repli-G FFPE protocol is followed according to the manufacturer's instructions starting at step 8. After the WGA is completed the sample is tested using eubacterial 16S primers and species-specific primers to verify the presence of the correct bacteria. As stated previously we have been able to optimize this protocol to work at 100 cfu of bacteria. In **FIGURE 3** the results of our optimization can be visualized. Serial dilutions of *Haemophilus influenzae* were prepared as processed according to the protocol outlined above. The gel shows that *H. influenzae* was able to be detected at 100 cfu with both the 16S primer pair and the *H. influenzae*-specific GAPDH primer pair. In the experiment there were also two positive and two negative controls. The positive control was *H. influenzae* genomic DNA (isolated from a standard non-fixed culture) and the negative control was nuclease-free water. One set of controls are for the WGA reaction to prove that the WGA worked properly. The other set of controls are for the PCR reaction to confirm the primers are working correctly.

Additional Details for METHODS:

IBIS technology: The IBIS technique is based on the principle that despite the large diversity among microbial organisms, shared sets of common traits are included in their genomes. The extracted nucleic acid from the original tissue sample is aliquoted into the wells of a microtiter

plate which contains broad range primers for PCR. The broad-range primers are designed to amplify a product from a group of organisms from a selected domain of microbial life, for example, all bacteria or specific bacterial divisions. The PCRs produce a mixture of products reflecting the diversity of the original mixture of organisms present in the starting sample. The products from the PCRs are desalted in a 96-well plate format and sequentially electrosprayed into a mass spectrometer. The spectral signals are processed to determine the masses of each of the PCR products present with sufficient accuracy that the base composition of each amplicon can be unambiguously deduced. Using combined base compositions from multiple PCRs, the identities of the pathogens and their relative concentrations in the starting sample can be determined.

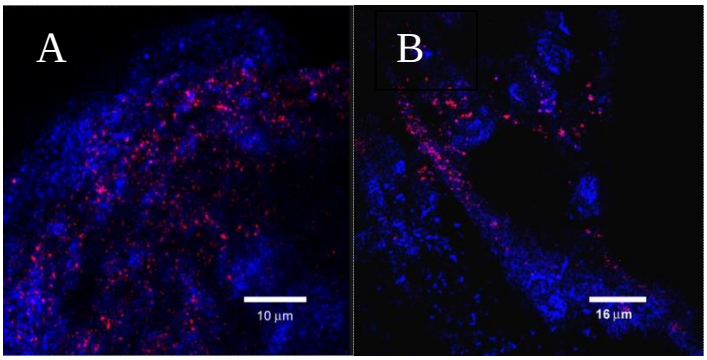

FIGURE 1: Confocal FISH microscopy confirmed the presence of *Staphylococcus aureus* in both the adenoid and orthopedic tissues (only the adenoid tissue is shown here). (A&B) FISH was performed on adenoid tissue with eubacterial (EU338) and with species-specific *S. aureus* 16s rRNA fluorescent probes, conjugated with Cy3 or Cy5. CSLM imaging was performed and the images were collected and analyzed using the Leica LCS software. (blue = live/dead stain; pink = *S. aureus* specific probe)

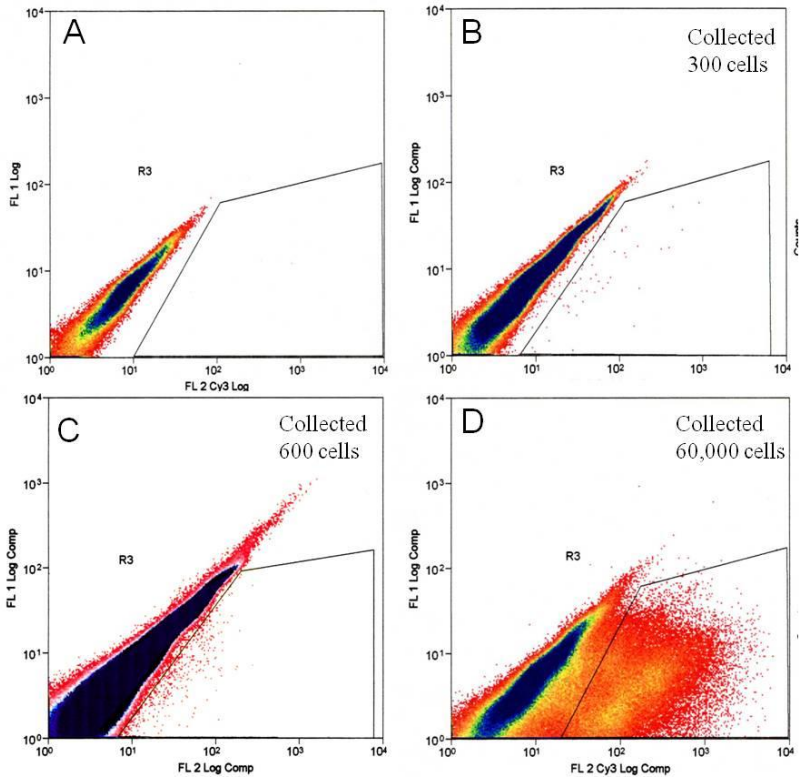

FIGURE 2: Sorting pure populations of bacteria targeted with species specific 16S FISH probes. (A) Unstained control (used to set the proper gates for the sort) (B) Bacteria isolated from orthopedic tissue and stained with specific 16S probe for *S. aureus* (cy3). 300 cells were collected. (C) Bacteria isolated from adenoid tissue and stained with specific 16S probe for *S. aureus* (cy3). 600 cells were collected. (D) Bacteria isolated from Tunicate samples and stained with specific 16S probe for *Type 1-4* (cy3). 60,000 cells were collected.

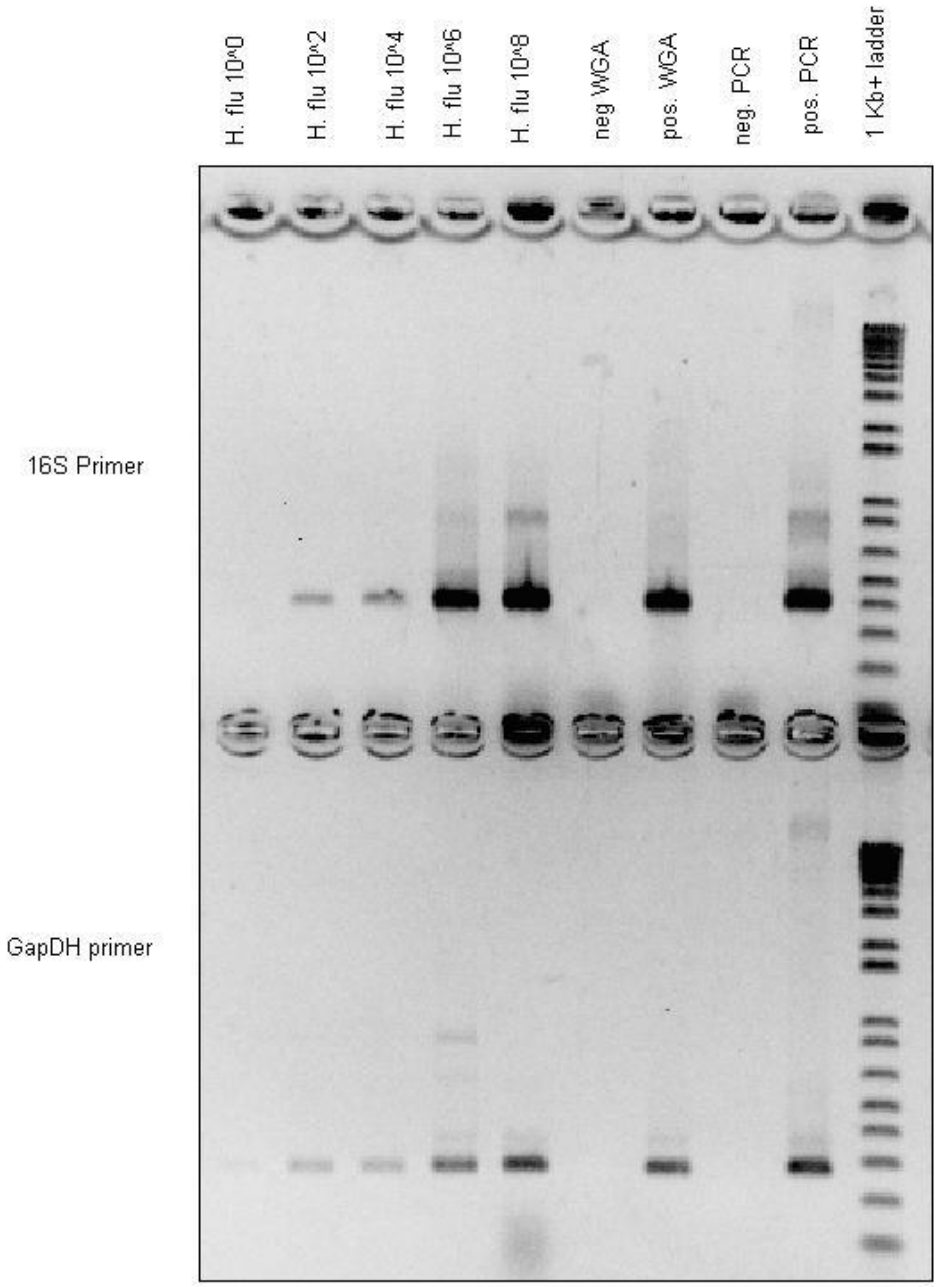

FIGURE 3: PCR of eubacterial and *H. influenzae* specific amplicons from WGA-amplified genomic DNA isolated from a dilution series of PFA-fixed *H. influenzae* cells.

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, listed in the table, in a PDF version 5.0.5 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.				☐Submitted ☐Accepted ☐Published
2.				☐Submitted ☐Accepted ☐Published
3.				☐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:

We will be preparing a methods paper describing the protocols that we developed which provide for whole genome sequencing (WGS) of unculturable organisms using a combination of deep 16S sequencing, 16S FISH probe development for species identified by the deep 16S sequencing; FISH-labeling of disrupted microbiome specimens, FACS separation of the FISH labeled species, WGA and WGS.

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 primary impact of the methods development that we have performed is that it provides a routine and facile means to access the metabolic potential of all micro-organisms whether they are culturable or not. The reason this is so significant is that >99.9% of micro-organisms are not culturable and heretofore there was no means to access their genomic and metabolic potential. Thus, we have for the first time at our disposal the vast "dark matter" of the world's metabolic capabilities.

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.

We have used some of the technology developed herein to pull out the biosynthetic pathway for an approved anti-cancer drug that currently has to be made by a 20+ step synthetic chemical process, costing tens of thousands of dollars to produce enough drug for one patient. This pathway is in the process of being cloned into a genetically tractable organism for production via biofermentive means.

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__X_____ No_____

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:

   A Biosynthetic Pathway for Heterologous Expression of a Nonribosomal Peptide Synthetase Drug and Analogs

b. Name of Inventor(s): David Sherman, Garth Ehrlich, Chris Rath, Benjamin Janto, et al.

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

We have used some of the technology developed herein to pull out the biosynthetic pathway for an approved anti-cancer drug that currently has to be made by a 20+ step synthetic chemical process, costing tens of thousands of dollars to produce enough drug for one patient. This pathway is in the process of being cloned into a genetically tractable organism for production via biofermentive means.

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_____X_____ No_____ (in part, but there were other funding sources involved)

If yes, indicate date patent was filed: April 15, 2010

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 X__
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 X__

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 X__

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____X_____ No_____ __

If yes, please describe your plans: We will attempt to move this biosynthetic pathway for ET743 into a genetically tractable micro-organisms for biofermentive production.

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 in the order listed on Form Page 2.
Follow this format for each person. **DO NOT EXCEED FOUR PAGES.**

NAME EHRLICH, Garth D.	POSITION TITLE Professor, Human Genetics, Otolaryngology and Microbiology & Immunology, Drexel University College of Medicine Executive Director, Center for Genomic Sciences
eRA COMMONS USER NAME (credential, e.g., agency login) GDEHRLICH	

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
Alfred University, Alfred, NY	B.A. *(magna cum laude)*	1977	Biology
Syracuse University, Syracuse, NY	Ph.D.	1987	Molecular Biology
SUNY HSC at Syracuse, Syracuse, NY	Post-Doctoral	1988	Hematology/Oncology

Positions: University of Pittsburgh: 07/90–09/97 Chief, Virology, Microbiology & Inf. Dis. Section, Dept of Pathology; 06/90–09/97 Director, Core PCR Facility, 05/91–09/97 Member, Graduate Faculty, 07/92–09/97; Assistant Professor, Department of Otolaryngology, 01/96–09/97 Associate Professor, Department of Pathology, 01/96–09/97 Associate Professor, Dept. of Otolaryngology, 07/96–09/97 Director, Center for Genomic Sciences; Drexel University: 09/97–Present Professor of Otolaryngology and Executive Director, Center for Genomic Sciences, Allegheny-Singer Research Institute, 2001-Present Professor of Microbiology & Immunology

Honors and Awards: Honors in Biology, Phi Kappa Phi; Visiting Professor, Cleveland Clinic, Heritage Foundation Lecturer, Cross Cancer Institute, Alumni Citation Award & Feinstein Lecturer Alfred University, Editorial Boards, *Molecular Diagnosis*, 1996–2000; *Clinical and Diagnostic Laboratory Immunology*, 1996–2004; Invited Lecturer Glaxo Wellcome, Verona, Italy, Invited Participant: NIH Workshop on PCR; NMMS meetings on MS, HTLV-1 and PCR; NCI symposia on HTLV-I,; NIAID symposia on detecting HIV-1 in urogenital fluids; NICHD/NIDCD Symposia on Infectious agents and SIDS; NIDCD Workshop on Otitis Media; Invited speaker NIDCD Council Meetings: 1995 & 2005; Honorary Professor of Medical Genetics, West China University, Chengdu, China; NIDCD Board of Scientific Counselors, 2000; Study Sections for NIAID, NIDCD, NHLBI, NCI, NIDA, NIDDK, DARPA, VA, 1989-2010; Guest Professor of Infectious Disease, Shantou Medical University, Shantou China; International Professor of Microbiology, University of Catania, Italy, 2010-

Selected peer-reviewed publications

1. Ehrlich, G.D., Veeh, R., Wang, X., Costerton, J.W., Hayes, J.D., Hu, F.Z., Daigle, B.J., Ehrlich, M.D., Post, J.C. Mucosal Biofilm Formation on Middle-ear Mucosa in the Chinchilla Model of Otitis Media. *JAMA* 287:1710-1715, 2002.
2. Costerton, J.W., Veeh, R., Shirtliff, M., Pasmore, M., Post, J.C. and Ehrlich, G.D. The application of biofilm science to the study and control of chronic bacterial infections. *Journal of Clinical Investigation* 2003.
3. Hall-Stoodley L, Hu FZ, Stoodley P, Nistico, L., Link, T.R., Burrows, A., Post, J.C. Ehrlich, G.D., and Kerschner, K.E. Direct Detection of Bacterial Biofilms on the Middle-Ear Mucosa of Children With Chronic Otitis Media. *JAMA* 296:202-211, 2006.
4. Kerschner, J.E., Meyers, T., Burrows, A., Ehrlich, G.D. and Post, J.C. Mucin gene cDNA sequence characterization from the chinchilla middle ear epithelium *Intl J. Pediatric Otolaryngol.* 70(8):1449-1456, 2006.

5. Buchinsky, F. J., Forbes, M., Hayes, J., Hu, F.Z., Greenberg, P., Post J.C., and Ehrlich, G.D. Phenotypic Plurality among Clincial Strains of nontypeable Haemophilus influenzae determined by symptom severity in the Chinchilla model of Otitis Media. *BMC Microbiology.* 14;7:56. 2007
6. Hogg, J.S., Hu, F.Z., Janto, B., Boissy, R., Gladitz, J., Swierczek, N., Hayes, J., Keefe, R., Yu, S., Post, J.C., and Ehrlich, G.D. Characterization and Modeling of the Haemophilus influenzae Core and Supra-Genome based on the Complete Genomic Sequences of Rd and 12 clinical Nontypeable16 Strains. *Genome Biology*. 8(6)R103 Epub ahead of print, 2007
7. Hiller, N.L., Janto, B., Hogg, J.S., Boissy, R.,, et al and Ehrlich, G.D., and Hu, F.Z. Comparative Genomic Analyses of Seventeen *S. pneumoniae* Strains: Insights into the Pneumococcal Supragenome *J Bacteriol*. 189(22):8186-95, 2007.
8. Forbes, M.L., Horsey, E., Hiller, N.L. Buchinsky, F.J., Hayes, J.D., Compliment, J.M., Hillman, T., Ezzo, S., Shen, K., Keefe, R., Barbadora, K., Post, J.C., Hu, F.Z., and Ehrlich, G.D. Strain-specific Virulence Phenotypes of Streptococcus pneumoniae Assessed Using the Chinchilla laniger Model of Otitis Media. *PLoS One* 3(4): e1969.
9. Wolcott, R.D., and Ehrlich, G.D. Biofilms and Chronic Infections. 2008 *JAMA* Jun 11;299(22):2682-4.
10. Hall-Stoodley, L., Nistico, l., Dice, B., Nguyen, D., Mershon, W.J., Johnson, C., Hayes, J., Hu, F.Z., Post, J.C., Stoodley, P., and Ehrlich, G.D. Characterization of biofilm matrix, degradation by DNase treatment and evidence of capsule downregulation in Streptococcus pneumoniae clinical isolates. *BMC Microbiology* 8(1):173, 2008
11. Kerschner JE, Horsey E, Ahmed A, Erbe C, Khampang P, Cioffi J, Hu FZ, Post JC, Ehrlich GD. Gene expression differences in infected and noninfected middle ear complementary DNA libraries. *Arch Otolaryngol Head Neck Surg 135*(1):33-39, 2009.
12. Kerschner, J.E., Erdos, G., Hu, F.Z., Burrows, A., Cioffi, J., Hayes, J., Keefe, R., Janto, B., Post, J.C., and Ehrlich, G.D. Characterization of normal and Haemophlus influenzae-infected mucosal cDNA libraries in chinchilla middle ear mucosa. *Annals of Otology, Rhinology and Laryngology* 119(4):270-278, 2010.
13. Donati, C., N. L. Hiller, H. Tettelin, A. Muzzi, N. J. Croucher, S. V. Angiuoli, M. Oggioni, D. Riley, A. Covacci, S. D. Bentley, M. Kilian, G. D. Ehrlich, F.Z. Hu, R. Rappuoli, E. R. Moxon and V. Masignani. Structure and dynamics of the pan-genome of Streptococcus pneumoniae and closely related species. *Genome Biol* 2010 Oct 29;11(10):R107
14. Hiller, N.L., Ahmed, A., Powell, E., Eutsey, R.E., Earl, J., Martin, D., Janto, B., Hogg, J.S., Boissy, R., Barbadora, K., Post, J.C., Hu, F.Z., and Ehrlich, G.D. Generation of Genic Diversity among Streptococcus pneumoniae Strains via Horizontal Gene Transfer during a Chronic Polyclonal Pediatric Infection. *PLoS Pathog* 6(9): e1001108. (2010)
15. Nistico, L., Gieseke, A., Kreft, R., Coticchia, J.M., Burrows, A., Khampang, P., Liu, Y., Kerschner, J.E., Post, J.C., Ehrlich, G.D, Stoodley, P, Hall-Stoodley, L. Pathogenic Biofilms in Adenoids: a Reservoir for Persistent Bacteria. *J Clin Micro* 2011
16. Davie, J.J., Earl, J., de Vries, S. P.W., Ahmed, A, Hu, F.Z., Bootsma, H.J., Stol, K., Hermans, P.W.M., Wadowsky, R.M., Ehrlich, G.D., Hays, J., Campagnari, A.A. Comparative Analysis and Supragenome Modeling of Twelve *Moraxella catarrhalis* Clinical Isolates. *BMC Genomics.* 12(1):70. Jan 26, 2011
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18. Nistico, L., Gieseke, A., Kreft, R., Coticchia, J.M., Burrows, A., Khampang, P., Liu, Y., Kerschner, J.E., Post, J.C., Ehrlich, G.D., Stoodley, P., Hall-Stoodley, L. Pathogenic Biofilms in Adenoids: a Reservoir for Persistent Bacteria. J Clin Microbiol. 49(4):1411-20, 2011.