AWARD NUMBER: **W81XWH-19-1-0679**

TITLE: **Microfluidic Assembly of Synthetic Ecologies for Drug Discovery (MASEDD)**

PRINCIPAL INVESTIGATOR: **Yousif Shamoo**

CONTRACTING ORGANIZATION: **William Marsh Rice University, 6100 Main St., Houston, TX 77005**

REPORT DATE: October 2020

TYPE OF REPORT: **Annual**

PREPARED FOR: U.S. Army Medical Research and Development Command
Fort Detrick, Maryland 21702-5012

DISTRIBUTION STATEMENT: Statement A Approved for Public Release;
Distribution Unlimited

The views, opinions and/or findings contained in this report are those of the author(s) and should not be construed as an official Department of the Army position, policy or decision unless so designated by other documentation.

REPORT DOCUMENTATION PAGE		*Form Approved* *OMB No. 0704-0188*
Public reporting burden for this collection of information is estimated to average 1 hour per response, including the time for reviewing instructions, searching existing data sources, gathering and maintaining the data needed, and completing and reviewing this collection of information. Send comments regarding this burden estimate or any other aspect of this collection of information, including suggestions for reducing this burden to Department of Defense, Washington Headquarters Services, Directorate for Information Operations and Reports (0704-0188), 1215 Jefferson Davis Highway, Suite 1204, Arlington, VA 22202-4302. Respondents should be aware that notwithstanding any other provision of law, no person shall be subject to any penalty for failing to comply with a collection of information if it does not display a currently valid OMB control number. **PLEASE DO NOT RETURN YOUR FORM TO THE ABOVE ADDRESS.**		
1. REPORT DATE October 2020	**2. REPORT TYPE** Annual	**3. DATES COVERED** **1SEPT2019 - 31AUG2020**
4. TITLE AND SUBTITLE Microfluidic Assembly of Synthetic Ecologies for Drug Discovery (MASEDD)		**5a. CONTRACT NUMBER** W81XWH-19-1-0679
		5b. GRANT NUMBER **W81XWH**-19-1-0679
		5c. PROGRAM ELEMENT NUMBER
6. AUTHOR(S) Yousif Shamoo E-Mail: shamoo@rice.edu		**5d. PROJECT NUMBER**
		5e. TASK NUMBER
		5f. WORK UNIT NUMBER
7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES) WILLIAM MARSH RICE UNIVERSITY RICE UNIVERSITY 6100 MAIN ST HOUSTON TX 77005-1827		**8. PERFORMING ORGANIZATION REPORT**
9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES) U.S. Army Medical Research and Development Command Fort Detrick, Maryland 21702-5012		**10. SPONSOR/MONITOR'S ACRONYM(S)**
		11. SPONSOR/MONITOR'S REPORT NUMBER(S)
12. DISTRIBUTION / AVAILABILITY STATEMENT Approved for Public Release; Distribution Unlimited		
13. SUPPLEMENTARY NOTES		

14. ABSTRACT

We have used our expertise in experimental evolution and adaptation to develop **Microfluidic Assembly of Synthetic Ecologies for Drug Discovery** or **MASEDD** as a novel approach to the discovery of new antimicrobial leads and antimicrobial strategies. *MASEDD drives Streptomyces toward production of new antimicrobials by linking Streptomyces reproductive success to the ability to kill clinically relevant pathogens.* We have built and optimized microfluidic devices for the rapid generation of water-in-oil droplets to co-encapsulate two strains: a Predator and Prey. The Predator strain (*S. venezuelae)* has been chromosomally labeled with a gene encoding red fluorescent protein (RFP) and chemically mutagenized to increase diversity. The Prey strain (*Pseudomonas aeruginosa)* is labelled with a green fluorescent protein (GFP) encoding plasmid. Growth conditions have been optimized to achieve comparable growth rates of both strains. Following co-encapsulation, we use Fluorescence Assisted Droplet Sorting (FADS) to enrich for successful *S. venezuelae* lineages that have the ability to inhibit the Prey (pathogen). In addition to the expensive microscope-based FADS device, we have constructed a bench-top device which is cost effective, allowing us to have multiple FADS devices running simultaneously to allow sorting of multiple replicate populations. Following droplet sorting, the challenge was to separate the predator strain from the prey cells for the next round of encapsulation. We have been able to devise a simple solution for this problem by taking advantage of the extensive branching morphology of Streptomycetes. We find that using a 20µm pore size filter allows effective separation of the homogenously growing planktonic Prey cells from the Predator. The recovered Predators are then improved through additional iterations of MASEDD. During the COVID 19 period when experimental work was not allowed on campus, we developed a computationally simulated Predator-Prey MASEDD model to fit experimental data. In this quarter, we have initiated the initial rounds of the Predator-Prey “cage matches” between *Pseudomonas aeruginosa* and *S. venezuelae* (Predator). We have also begun testing biochemical strategies for characterization of the killer Streptomyces strain and antibiotic lead molecules.

Significance: At the end of these initial rounds of MASEDD, we will characterize Predators with the ability to inhibit or kill the pathogen of interest and begin isolation of potential antimicrobial leads that could be of clinical relevance. If successful, MASEDD has the ability to produce new molecules on demand with the potential to be developed as therapeutics for combating antimicrobial resistance.

15. SUBJECT TERMS

NONE LISTED

16. SECURITY CLASSIFICATION OF:			**17. LIMITATION OF ABSTRACT**	**18. NUMBER OF PAGES**	**19a. NAME OF RESPONSIBLE PERSON** USAMRMC
a. REPORT Unclassified	**b. ABSTRACT** Unclassified	**c. THIS PAGE** Unclassified	Unclassified	20	**19b. TELEPHONE NUMBER** *(include area code)*

Standard Form 298 (Rev. 8-98)
Prescribed by ANSI Std. Z39.18

TABLE OF CONTENTS

	Page
1. Introduction	4
2. Keywords	4
3. Accomplishments	4
4. Impact	12
5. Changes/Problems	13
6. Products	15
7. Participants & Other Collaborating Organizations	17
8. Special Reporting Requirements	20
9. Appendices	20

1. **INTRODUCTION:** *Narrative that briefly (one paragraph) describes the subject, purpose and scope of the research.*

Antimicrobial resistance is a global crisis that undermines both military and civilian medicine. Infections caused by Multidrug resistant (MDR) pathogens continue to increase and threaten to make our current inventory of antibiotics obsolete. The purpose of this research is to solve this problem by discovering new antimicrobial agents using a novel methodology: **Microfluidic Assembly of Synthetic Ecologies for Drug Discovery or MASEDD**. The scope of this research is to test, evaluate and deliver MASEDD as a scalable method for the discovery of novel antibiotic leads and their production within industry-ready *Streptomyces* spp. strains.

2. **KEYWORDS:** *Provide a brief list of keywords (limit to 20 words).*

MASEDD, Streptomyces, experimental evolution, cryptic pathways, antimicrobial resistance, antibiotic discovery, microfluidics.

3. **ACCOMPLISHMENTS:** *The PI is reminded that the recipient organization is required to obtain prior written approval from the awarding agency grants official whenever there are significant changes in the project or its direction.*

What were the major goals of the project?
List the major goals of the project as stated in the approved SOW. If the application listed milestones/target dates for important activities or phases of the project, identify these dates and show actual completion dates or the percentage of completion.

Year 1:
Specific aim 1: We will innovate a two-species, microfluidic-based emulsion selection system that uses Predator-Prey dynamics to identify new strategies for killing a panel of DOD relevant pathogens including enterococci, *A. baumannii*, and *P. aeruginosa* using MASEDD.

Major task 1: Use the MASEDD system for directed evolution of *Streptomyces roseosporus* NRRL 11379 (Predator) to kill different pathogens that will serve as Prey (Predator: Prey Cage Matches).
Year 1 completion percentage: 70% Technology and platform development completed. (*P. aeruginosa* and *S. venezuelae* competition experiment has been initiated).

Major task 2: Characterization of evolved *S. roseosporus* killer strains.
Year 1 completion percentage: 20%

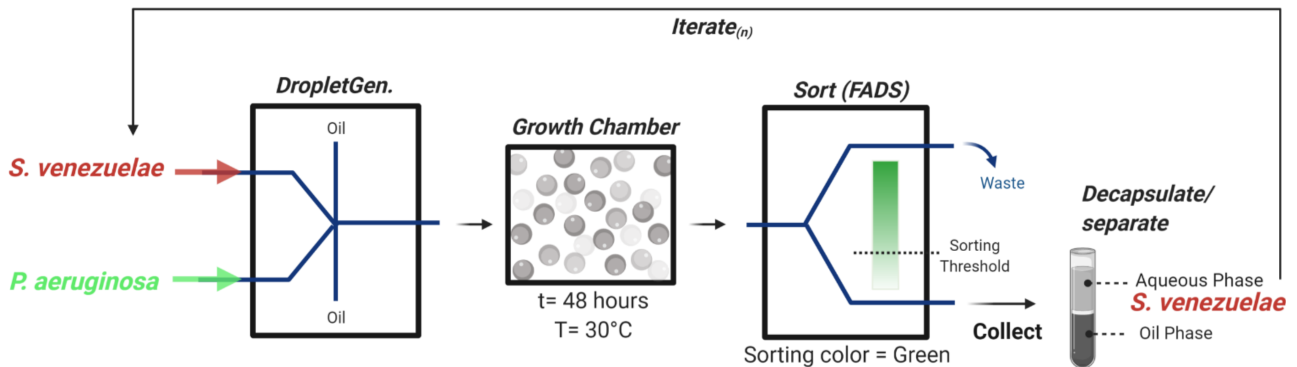
Year 2:
Specific aim 2: We will complete the identification of a new antibiotic lead as a complete delivery cycle demonstrating the capability of MASEDD for scalable development (In collaboration with Dr. K. C. Nicolaou). Just underway (10%)

Year 1 completion percentage: 0% (Not scheduled to begin yet)

What was accomplished under these goals?

For this reporting period describe: 1) major activities; 2) specific objectives; 3) significant results or key outcomes, including major findings, developments, or conclusions (both positive and negative); and/or 4) other achievements. Include a discussion of stated goals not met. Description shall include pertinent data and graphs in sufficient detail to explain any significant results achieved. A succinct description of the methodology used shall be provided. As the project progresses to completion, the emphasis in reporting in this section should shift from reporting activities to reporting accomplishments.

1) Major activities: Progress and Current Outcomes



Schematic of the MASEDD selection for Aim 1. Initially, we mutagenize *S. venezuelae* (Predator candidates) to increase mutation supply and then co-encapsulate with *P. aeruginosa*^{GFP} (Prey). We then select and enrich for variants of *S. venezuelae* that inhibit growth of *P. aeruginosa*^{GFP} more strongly than the ancestor. In the FADS step, we gate to collect the microdroplets with weaker GFP signal as a proxy for improved *S. venezuelae* ability to inhibit *P. aeruginosa*^{GFP}. The recaptured *S. venezuelae* will be used for the next iteration with fresh *P. aeruginosa*^{GFP}.

Predator-prey cage match between *S. venezuelae* and *P. aeruginosa:*

RFP labeled *S. venezuelae* was chemically mutagenized by treatment with 1.5 mg/ml of the mutagen NTG ((*N*-methyl-*N′*-nitro-*N*-nitrosoguanidine) for 2 hours, followed by growth on non-selective CSM media. Chemically mutagenized RFP labeled *S. venezuelae* (Predator) and GFP labeled *P. aeruginosa* PAO1 (Prey) were co-encapsulated in 70 µm droplets. Bacteria were cultured in CSM medium (tryptic soy broth supplemented with yeast extract, magnesium sulfate, glucose, and maltose) in shaking flasks at 30 °C (S. *venezuelae*) or 37 °C (PAO1). Cultures were diluted to reach 5.8e6 cfu/ml (λ = 1) for *S. venezuelae* and 1.13e8 cfu/ml (λ = 20) for PAO1 and co-encapsuled in water-in-oil emulsion droplets. The average number of S. *venezuelae* cells per droplets was determined to be 1.054 (Poisson distribution). The fraction of droplets containing no cells, 1 cell and ≥2 cells are 34.9%, 36.7%, and 28.4%, respectively. To avoid droplets containing

only *S. venezuelae* without PAO1, a relatively higher λ value was used for PAO1. The average number of PAO1 cells per droplets was 20.284. The fluorescence microscopy images in Fig. 1 show the baseline fluorescence intensity immediately after co-encapsulation.

Following co-encapsulation, droplets were incubated overnight for 2 days at 30 °C (optimized growth condition to obtain comparable growth rate for predator and prey). Although all droplets initially contained an average of 20 cells of PAO1, the GFP signal intensity after the growth period was not uniform across droplets (fluorescence microscopy images in Fig. 2). It is evident that GFP labelled PAO1 outcompeted RFP labelled *S. venezuelae* in some droplets (high green fluorescence and low red fluorescence), whereas in some droplets, *S. venezuelae* was able to outgrow PAO1 (high red and low green fluorescence). We are interested in the droplets with lower green fluorescence as these are the droplet that are most likely to contain *S. venezuelae* cells that have begun to produce molecules capable of inhibiting growth of PAO1.

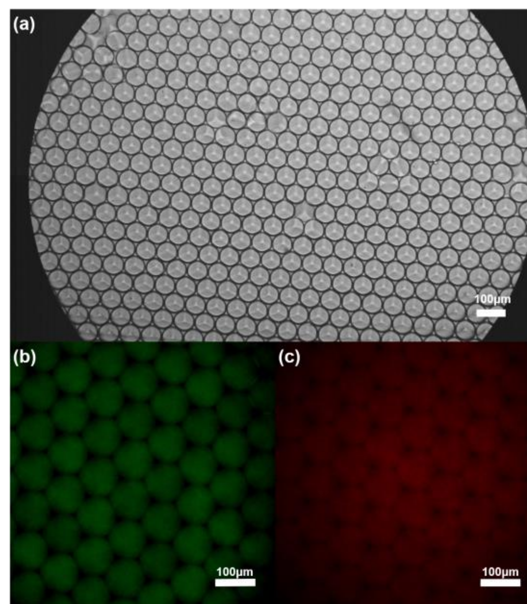


Fig. 1. Visualization of droplets immediately after co-encapsulation containing RFP labeled S. venezuelae (predator) and GFP labeled P. aeruginosa (prey) under (a) visible light (b) blue light for the baseline signal of green fluorescence, and (c) orange light for the baseline signal of red fluorescence.

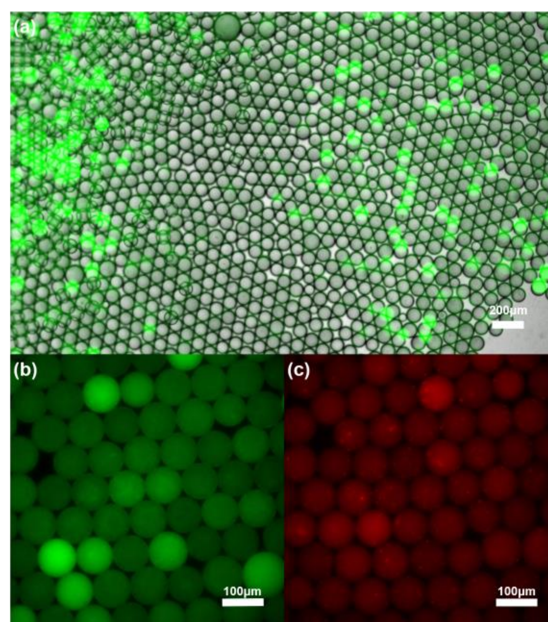


Fig. 2. Visualization of droplets after 2 days of incubation containing RFP labeled *S. venezuelae* (predator) and GFP labeled *P. aeruginosa* (prey) by (a) merging the signals of green fluorescence and the visible light image through 4x objective lens, (b) under blue light for the signal of green fluorescence, and (c) Orange light for the signal of red fluorescence through 10x objective lens.

Using Fluorescence Activated Droplet Sorting (FADS) we will sort the population to recover droplets with lower green fluorescence (less than 80%) as that is consistent with inhibition by Streptomyces variants. Recovered Streptomyces variant populations will be subjected to additional rounds of enrichment by MASEDD. The sorting threshold was determined using a control group. For the control group, droplets containing only PAO1 were injected into the FADS chip in order to determine the maximum intensity of droplets and identify the threshold voltage. When the droplets in the FADS chip were irradiated by a 488-nm laser, the photomultiplier tube (PMT) generated a signal of 1 V (0.5 gain setting). To collect droplets with less than 80% of average green fluorescence for the next round of experimental evolution, the threshold was determined to be 0.8 V.

After determining the threshold, incubated droplets were injected into the FADS chip (Fig. 3). 1 μL/min of droplet flow rate and 10 μL/min of oil flow rate was used to appropriately space out the droplets with oil while they moved across the sorting junction to avoid potential detection errors. Droplets having less than 80% green fluorescence were diverted to the collection channel and the rest of the droplets were sent to the waste channel.

The collected droplets were mixed with 1H,1H,2H,2H-perfluorooctanol to break the emulsion. Then, cultures containing enriched *S. venezuelae* cells mixed with *P. aeruginosa* were washed three times with CSM medium and were filtered at 20-μm to preferentially recover Streptomyces. The fraction retained by the filters were the branching *S. venezuelae* cells. The recovered cells were then re-suspended in fresh CSM medium. After that, the culture was diluted to obtain the pre-determined λ value of 1 and was mixed with freshly prepared PAO1 cultures for the next iteration of MASEDD.

We will continue this cycle of incubation, sorting and re-encapsulation until we have majority of droplets enriched for the predators that can outcompete the wild type PAO1 cells.

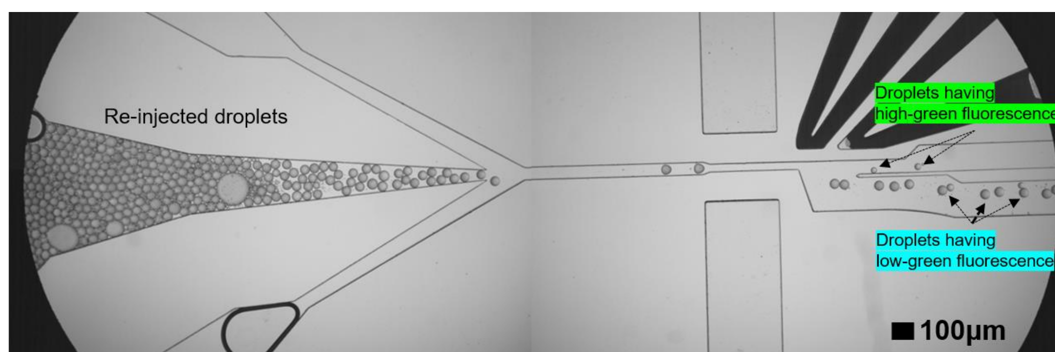


Fig. 3. Representative optical microscopy image of droplets sorting after 2-day incubation containing RFP labeled *S. venezuelae* (Predator) and GFP labeled *P. aeruginosa* (Prey).

<u>Lead characterization:</u>

<u>Discovery of wild Streptomyces strain producing a bioactive molecule that has potent activity against Gram positive bacteria:</u> As part of this project we have continued to add to our collection of potential Streptomyces predator strains from wild isolates located in Texas. By serendipity, we found that one of these strains (T4-11) already produced an antibiotic lead that was highly effective at inhibiting the growth of Gram positive but not Gram negative bacteria. We have advanced this strain into our lead characterization pipeline. Using a conditioned media assay, we have demonstrated that the antimicrobial candidate is secreted into the media and can be reintroduced in this unpurified form to demonstrate strong inhibition of Gram positives including several that are already multi-drug resistant (Fig 4).

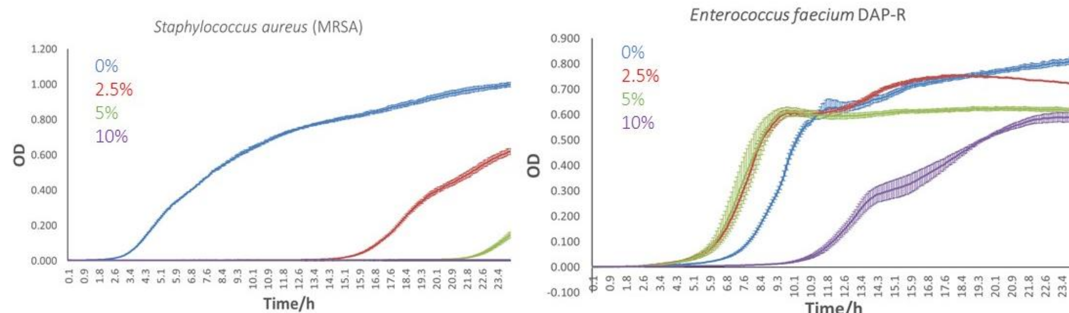


Fig. 4. T4-11 antimicrobial candidate (unpurified) strongly inhibits multidrug resistant pathogen MRSA and partially inhibits vancomycin-daptomycin resistant *E. faecium*. The percentage in the legend refers to the percentage of T4-11 conditioned media used to inhibit growth as the lead has not been purified or characterized as yet.

2) Specific objectives:

Specific objectives of year 1 included successfully testing the MASEDD system for identification of a Streptomyces strain evolved to kill an *ESKAPE* pathogen and to characterize the evolved killer strain.

3) Significant results:

In spite of the shutdown of research activities due to COVID-19, we have been able to catch up and initiate one round of the Predator-Prey cage match. We have improved our technology to create more efficient devices and sorting machinery as well as developed simple strategies to overcome some challenges of working with aggregating Streptomyces strains.

We have also designed methods for lead characterization and will be using them to characterize the evolved killer cells from this experiment. We have advanced one potential lead molecule into our purification and characterization pipeline.

What opportunities for training and professional development has the project provided?
If the project was not intended to provide training and professional development opportunities or there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe opportunities for training and professional development provided to anyone who worked on the project or anyone who was involved in the activities supported by the project. "Training" activities are those in which individuals with advanced professional skills and experience assist others in attaining greater proficiency. Training activities may include, for example, courses or one-on-one work with a mentor. "Professional development" activities result in increased knowledge or skill in one's area of expertise and may include workshops, conferences, seminars, study groups, and individual study. Include participation in conferences, workshops, and seminars not listed under major activities.

New graduate student, David Ibarra is being trained by post-doc, Dr. Seokju Seo and graduate student, Xinhao Song to design, construct and use microfluidic devices. He will be carrying out the next round of MASEDD using *A. baumannii* as the prey.
Professional development for Dr. Seo and Mr. Song have been virtual workshops (ex. ASM Micro) and conferences this year due to COVID 19.

How were the results disseminated to communities of interest?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe how the results were disseminated to communities of interest. Include any outreach activities that were undertaken to reach members of communities who are not usually aware of these project activities, for the purpose of enhancing public understanding and increasing interest in learning and careers in science, technology, and the humanities.

The PI as an active infectious diseases researcher has provided press interviews and outreach lectures to the general public in the Glasscock School of Continuing Studies on the rise of infectious diseases.

https://news.rice.edu/2020/04/27/rices-glasscock-school-hosts-conversation-with-infectious-disease-expert-yousif-shamoo/
https://communityimpact.com/houston/bellaire-meyerland-west-university/coronavirus/2020/03/20/if-the-italians-can-be-overwhelmed-then-we-can-be-overwhelmed-houston-expert-shares-insights-on-coronavirus-response/
https://www.victoriaadvocate.com/premium/texas-health-departments-data-changes-cause-confusion/article_1b6a4580-d381-11ea-b367-43ebbf41b1f7.html

What do you plan to do during the next reporting period to accomplish the goals?
If this is the final report, state "Nothing to Report."

Describe briefly what you plan to do during the next reporting period to accomplish the goals and objectives.

Following completion of the *P. aeruginosa: S. venezuelae* cage match, we will obtain a specialized killer that will be tested biochemically for characterization. Similarly, *A. baumannii* will be co-encapsulated with a *Streptomyces* predator to obtain another killer strain which will be similarly characterized. Lead molecules will be identified in collaboration with Dr. K. C. Nicolaou.

4. **IMPACT:** D*escribe distinctive contributions, major accomplishments, innovations, successes, or any change in practice or behavior that has come about as a result of the project relative to:*

What was the impact on the development of the principal discipline(s) of the project?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe how findings, results, techniques that were developed or extended, or other products from the project made an impact or are likely to make an impact on the base of knowledge, theory, and research in the principal disciplinary field(s) of the project. Summarize using language that an intelligent lay audience can understand (Scientific American style).

The majority of antimicrobials are discovered from natural sources especially soil bacteria like *Streptomyces*. MASEDD uses the principles of Natural Selection to consistently evolve *Streptomyces* strains to kill DOD relevant pathogens. Rather than searching for new antimicrobial producing strains we essentially "breed" them for that purpose. We can select for broad specificity or highly targeted killing. Since these evolved killer strains are *Streptomyces* that can be industrially fermented, we move immediately to production and faster deployment. We believe this would a transformative change to how we currently identify and scale-up new antimicrobial leads.

What was the impact on other disciplines?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe how the findings, results, or techniques that were developed or improved, or other products from the project made an impact or are likely to make an impact on other disciplines.

Upon publication, our improvements in microfluidics technology like rapid emulsion production and encapsulation technology as well as the bench-top FADS system will be made available to the community.

What was the impact on technology transfer?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe ways in which the project made an impact, or is likely to make an impact, on commercial technology or public use, including:

- *transfer of results to entities in government or industry;*
- *instances where the research has led to the initiation of a start-up company; or*
- *adoption of new practices.*

We are in discussion with the Rice Office of Technology Transfer about how to best commercialize MASEDD via provisional patent and subsequent licensing of antibiotic production strains. We may decide to spin out this technology as a start up.

What was the impact on society beyond science and technology?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe how results from the project made an impact, or are likely to make an impact, beyond the bounds of science, engineering, and the academic world on areas such as:

- *improving public knowledge, attitudes, skills, and abilities;*
- *changing behavior, practices, decision making, policies (including regulatory policies), or social actions; or*
- *improving social, economic, civic, or environmental conditions.*

Nothing to report

5. **CHANGES/PROBLEMS:** *The PD/PI is reminded that the recipient organization is required to obtain prior written approval from the awarding agency grants official whenever there are significant changes in the project or its direction. If not previously reported in writing, provide the following additional information or state, "Nothing to Report," if applicable:*

Nothing to report

Actual or anticipated problems or delays and actions or plans to resolve them

Describe problems or delays encountered during the reporting period and actions or plans to resolve them.

We have not encountered any scientific setbacks. With the COVID-19 shutdown we lost about 3 months of work due to a shut down and then additional time as the university ramped back up over the summer. We have successfully re-started all our experiments as of August 1, 2020.

Changes that had a significant impact on expenditures

Describe changes during the reporting period that may have had a significant impact on expenditures, for example, delays in hiring staff or favorable developments that enable meeting objectives at less cost than anticipated.

Nothing to report

Significant changes in use or care of human subjects, vertebrate animals, biohazards, and/or select agents

Describe significant deviations, unexpected outcomes, or changes in approved protocols for the use or care of human subjects, vertebrate animals, biohazards, and/or select agents during the reporting period. If required, were these changes approved by the applicable institution committee (or equivalent) and reported to the agency? Also specify the applicable Institutional Review Board/Institutional Animal Care and Use Committee approval dates.

Significant changes in use or care of human subjects

Not applicable.

Significant changes in use or care of vertebrate animals

Not applicable.

Significant changes in use of biohazards and/or select agents

No significant changes
IRBNet Registration ID: 1162601-3

6. **PRODUCTS:** *List any products resulting from the project during the reporting period. If there is nothing to report under a particular item, state "Nothing to Report."*

- **Publications, conference papers, and presentations**
  Report only the major publication(s) resulting from the work under this award.

Journal publications. *List peer-reviewed articles or papers appearing in scientific, technical, or professional journals. Identify for each publication: Author(s); title; journal; volume: year; page numbers; status of publication (published; accepted, awaiting publication; submitted, under review; other); acknowledgement of federal support (yes/no).*

Nothing to report

Books or other non-periodical, one-time publications. *Report any book, monograph, dissertation, abstract, or the like published as or in a separate publication, rather than a periodical or series. Include any significant publication in the proceedings of a one-time conference or in the report of a one-time study, commission, or the like. Identify for each one-time publication: author(s); title; editor; title of collection, if applicable; bibliographic information; year; type of publication (e.g., book, thesis or dissertation); status of publication (published; accepted, awaiting publication; submitted, under review; other); acknowledgement of federal support (yes/no).*

Nothing to report

Other publications, conference papers and presentations. *Identify any other publications, conference papers and/or presentations not reported above. Specify the status of the publication as noted above. List presentations made during the last year*

(international, national, local societies, military meetings, etc.). Use an asterisk (*) if presentation produced a manuscript.

Nothing to report

- **Website(s) or other Internet site(s)**
  List the URL for any Internet site(s) that disseminates the results of the research activities. A short description of each site should be provided. It is not necessary to include the publications already specified above in this section.

Nothing to report

- **Technologies or techniques**
  Identify technologies or techniques that resulted from the research activities. Describe the technologies or techniques were shared.

Improved microfluidic for use in the encapsulation of a wider range of bacterial strains including those that grow as mycelia mats such as Streptomyces.

- **Inventions, patent applications, and/or licenses**
  Identify inventions, patent applications with date, and/or licenses that have resulted from the research. Submission of this information as part of an interim research performance progress report is not a substitute for any other invention reporting required under the terms and conditions of an award.

Nothing to report

- **Other Products**

  Identify any other reportable outcomes that were developed under this project. Reportable outcomes are defined as a research result that is or relates to a product, scientific advance, or research tool that makes a meaningful contribution toward the understanding, prevention, diagnosis, prognosis, treatment and /or rehabilitation of a disease, injury or condition, or to improve the quality of life. Examples include:
  - *data or databases;*
  - *physical collections;*
  - *audio or video products;*
  - *software;*
  - *models;*
  - *educational aids or curricula;*
  - *instruments or equipment;*
  - *research material (e.g., Germplasm; cell lines, DNA probes, animal models);*
  - *clinical interventions;*
  - *new business creation; and*
  - *other.*

Nothing to report

7. PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS

What individuals have worked on the project?

Provide the following information for: (1) PDs/PIs; and (2) each person who has worked at least one person month per year on the project during the reporting period, regardless of the source of compensation (a person month equals approximately 160 hours of effort). If information is unchanged from a previous submission, provide the name only and indicate "no change".

Example:

Name: *Mary Smith*
Project Role: *Graduate Student*
Researcher Identifier (e.g. ORCID ID): *1234567*
Nearest person month worked: *5*

Contribution to Project: *Ms. Smith has performed work in the area of combined error-control and constrained coding.*
Funding Support: *The Ford Foundation (Complete only if the funding support is provided from other than this award.)*

1. Name: Yousif Shamoo
   Project Role: PI
   Researcher Identifier (e.g. ORCID ID): 0000-0001-9241-8962
   Nearest person month worked: 2

   Contribution to Project: No change
   Funding Support: CDMRP W81XWH1910679, NIH R01 AI0807, DTRA HDTRA-1-15-1-0069

2. Name: Heer Mehta
   Project Role: Research scientist
   Researcher Identifier (e.g. ORCID ID): 0000-0002-5357-2112
   Nearest person month worked: 1

   Contribution to Project: No change
   Funding Support: CDMRP W81XWH1910679, NIH R01 AI0807, DTRA HDTRA-1-15-1-0069

3. Name: Seokju Seo
   Project Role: Postdoctoral Associate
   Researcher Identifier (e.g. ORCID ID): 0000-0003-1699-6271
   Nearest person month worked: 3

   Contribution to Project: Dr. Seo is a microfluidics expert that is responsible for the design and development of our newest generation of microfluidic devices. He carried out the improvements to our sorting machinery. He is carrying out the *P. aeruginosa:S. venezuelae* cage match experiment.
   Funding Support: CDMRP W81XWH1910679, HDTRA-1-15-1-0069

4. Name: David Ibarra
   Project Role: Graduate student
   Researcher Identifier (e.g. ORCID ID): -
   Nearest person month worked: 2

   Contribution to Project: Mr. Ibarra is a new graduate student who is getting trained to use the microfluidics technology and will be performing the cage matches with *Acinteobacter baumannii*. He is also developing biochemical techniques for lead characterization.
   Funding Support: CDMRP W81XWH1910679, NIH R01 AI0807

4.	Name:	Xinhao Song
	Project Role:	Graduate student
	Researcher Identifier (e.g. ORCID ID):	-
	Nearest person month worked:	2
	Contribution to Project:	Mr. Song worked on creating the predator-prey models and identification of the wild Streptomyces strain capable of killing Gram positive bacteria.
	Funding Support:	CDMRP W81XWH1910679, NIH R01 AI0807

Has there been a change in the active other support of the PD/PI(s) or senior/key personnel since the last reporting period?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

If the active support has changed for the PD/PI(s) or senior/key personnel, then describe what the change has been. Changes may occur, for example, if a previously active grant has closed and/or if a previously pending grant is now active. Annotate this information so it is clear what has changed from the previous submission. Submission of other support information is not necessary for pending changes or for changes in the level of effort for active support reported previously. The awarding agency may require prior written approval if a change in active other support significantly impacts the effort on the project that is the subject of the project report.

Nothing to report

What other organizations were involved as partners?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe partner organizations – academic institutions, other nonprofits, industrial or commercial firms, state or local governments, schools or school systems, or other organizations (foreign or

domestic) – that were involved with the project. Partner organizations may have provided financial or in-kind support, supplied facilities or equipment, collaborated in the research, exchanged personnel, or otherwise contributed.

Provide the following information for each partnership:
Organization Name:
Location of Organization: (if foreign location list country)
Partner's contribution to the project (identify one or more)

- *Financial support;*
- *In-kind support (e.g., partner makes software, computers, equipment, etc., available to project staff);*
- *Facilities (e.g., project staff use the partner's facilities for project activities);*
- *Collaboration (e.g., partner's staff work with project staff on the project);*
- *Personnel exchanges (e.g., project staff and/or partner's staff use each other's facilities, work at each other's site); and*
- *Other.*

Nothing to report

8. SPECIAL REPORTING REQUIREMENTS

COLLABORATIVE AWARDS: *For collaborative awards, independent reports are required from BOTH the Initiating Principal Investigator (PI) and the Collaborating/Partnering PI. A duplicative report is acceptable; however, tasks shall be clearly marked with the responsible PI and research site. A report shall be submitted to* ***https://ers.amedd.army.mil*** *for each unique award.*

QUAD CHARTS: *If applicable, the Quad Chart (available on https://www.usamraa.army.mil) should be updated and submitted with attachments.*

9. APPENDICES:

Attach all appendices that contain information that supplements, clarifies or supports the text. Examples include original copies of journal articles, reprints of manuscripts and abstracts, a curriculum vitae, patent applications, study questionnaires, and surveys, etc.