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14. ABSTRACT

This project aims to "Unlock[ing] the potential of bacterial ParE toxins: developing a blueprint for co-opting molecular time bombs that impact bacterial cell survival". The central problem addressed by the aims is of treating bacterial infections with an outcome of making existing antibiotics work better, and in understanding a fundamental bacterial mechanism that may help bacteria become resistant. When successful, this will provide an innovative new way to control bacterial growth, including antibacterial resistant strains.

During this reporting period we have constructed reagents needed to determine which of the ParE proteins in two pathogenic bacteria have the greatest impact on bacterial cell survival. These assays revealed that of two different ParE toxins in each bacteria tested to date, only one of them is potently toxic and likely to

15. SUBJECT TERMS

Toxin-antitoxin systems, antibacterial applications, toxicity, mutations

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Introduction

The objective of this project is to provide proof-of-concept for a new way to control bacterial growth, including genetically resistant as well as phenotypically persistent bacteria. This approach will manipulate chromosomally-encoded bacterial "time bombs" called toxin-antitoxin (TA) systems.

As emerging infections and increases in resistance make the need for antibacterials more pressing, it is also increasingly evident that our homeostatic balance and health also depend on bacteria. This revelation then further challenges antibacterial approaches to minimize impact on beneficial "good" bacteria. **Incorporation of narrow-spectrum antibacterial treatment approaches are highly desirable to minimize disruption of the host microbiome.** Antibacterial discovery has long relied on directed serendipity via screening of natural products and libraries to identify inhibitors and their corresponding bacterial targets. Currently the most fruitful approaches are dominated by derivatization of existing antibacterials; these activities are absolutely required for short-term defenses against infection. However, **longer-term approaches that rely on new and unique strategies are badly needed, especially as emerging resistance is outpacing antibacterial development.**

Body

TA systems are a non-secreted component of a bacterial cell's intrinsic physiologic response. These are protein pairs used to tailor bacterial physiology towards either death (a "time bomb") or survival, depending on the cellular target of the toxin, in effect acting as resiliency factors. **We propose to co-opt TA systems for health purposes, but this is currently unfeasible because of a lack of fundamental knowledge on how to leverage TA systems as tools.** The current study is focused on ParE toxin subtypes, as we propose these are uniquely useful for an antibacterial approach: in their ability to mediate detrimental DNA degradation to the expressing bacterial cells, and their widespread presence in different Gram-negative bacteria of concern. **Our long-term aim is to co-opt these ParE toxins to directly cause death to only the specific type of bacteria in which they are found, an advantageous narrow-spectrum approach. These types of ParE toxins are present in bacteria of significant concern to human health and are the focus of our investigations: *P. aeruginosa*, *V. cholera, M. tuberculosis*, and *Burkholderia* sp.** Of specific interest to the funder, these pathogens have a directly negative impact on military personnel in field environments and when dealing with wounds, including biofilm formation, that can occur in non-optimal treatment conditions.

Hypothesis: That the presence of ParE toxins within a bacterial cell imparts (1) an increased mutagenic potential that at a native concentrations contributes to emerging antibiotic resistance, and that (2) increasing ParE toxin activity can significantly weaken the bacterial cell's ability to survive, and this effect will be additive or synergistic with existing antibiotic regimens. To assess this hypothesis the following specific studies are in progress:

Specific Aims:
(1) Determine the spectrum of ParE activity in native hosts by measuring viability, accumulation of mutations, and antibiotic susceptibility as a function of induced ParE toxin expression.
(2) Increase ParE availability *in vivo* as proof-of-concept of a therapeutic approach by engineering each targeted species' ParD antitoxin degradation model system in an *E. coli* host.

The outcomes of this project will be (1) identifying a fundamental mechanism potentially contributing to rise of resistance, providing a window for potential intervention, and (2) demonstrating proof-of-concept of co- opting this mechanism into a novel treatment that by definition will be specific for a given bacterial species. This idea is directly responsive to the "Area of Encouragement" identified as "Antimicrobial Resistance", for the "Development of novel and/or innovative interventions to prevent the spread of or treat infections from multi-drug-resistant organisms, focused on hardware-associated infections and biofilms."

The short-term impact will demonstrate for the first time a usable approach for co-opting TA systems, and will provide insight into a potential fundamental mechanism of genetic resistance through error-prone repair after low dose toxin-induced DNA damage. ***This study will provide the proof-of-concept badly needed to allow further development into an applied product.*** The long-term potential therapeutic applications will offer very high specificity to a single pathogen, and versatility in providing a means to potentiate current treatments including those with developing resistance.

Keywords

Toxin-antitoxin systems, antibacterial applications, toxicity, mutations

Research Accomplishments

Specific Aim 1: Assessing viability and antibiotic susceptibility as a function of induced ParE toxin expression, and ParE-induced mutations *via* fluctuation analysis.
Four subtasks were identified to achieve Aim 1, culminating in three Milestone Achievements.

Subtask 1: Cloning ParD and ParDE genes into appropriate vectors, bacterial propagation, and transformation of constructs.

The proposed study focused on six unique ParDE TA systems from four bacterial pathogens (*Pseudomonas aeruginosa,* Pa; *Burkholderia cenocepacia*, Bc; *Vibrio cholera,* Vc; *Mycobacterium tuberculosis*, (smegmatis model, Mt)).

All cloning is now complete with the exception of modifications to the *Mycobacterial* pMind vector, which has poor induction using the original plasmid promoter; this is being modified to instead use an arabinose-responsive promoter. Subsequent addition of affinity tags for probing of protein expression by Western blotting is included in constructs. The Bc-specific expression vector pMLBad was found to have poor induction control; subsequent cloning and testing has utilized only pSCrha2 vector constructs.

An additional ParDE system (PaDE2) was included for Pa, as it has subsequently been published as active and involved in prophage activation in approx. one-third of clinical isolates. A putative ParDE system (BcDE2) was included for evaluation in Bc. We have now demonstrated that BcDE2 is not a gyrase-inhibiting toxin and instead belongs to the structurally related HigB class of toxins with RNase activity; further testing has been discontinued as per the objectives of this project.

Constructs for TA systems from Pa, Bc, and Vc have been transformed into their native hosts for further study, and transformation of Mt clones are in progress.

Table 1. TA systems targeted for study
(Subtask 1, completed pending *ara* promoter insertion in pMind)

Source Bacteria	Native Operon(s)	Successful Cloning (vector, construct, notes)
P. aeruginosa	PaDE1, PaDE2	pHerd20T constructs for toxin, antitoxin, operon; *Additional constructs include His or Strep affinity tags*
B. cenocepacia	BcDE1, BcDE2*	pSCrha2 constructs for toxin, antitoxin, operon *Additional constructs include Strep affinity tags*
M. tuberculosis	MtDE1, MtDE2	pMind constructs for toxin, antitoxin, operon *Additional constructs include Strep affinity tags* ***all pending ara promoter insertion*
V. cholera	VcDE1, VcDE2	pBAD33 constructs for toxin, antitoxin, operon *Additional constructs include Strep affinity tags*

*studies on the BcDE2 system have been discontinued due to mis-annotation (this is not a ParDE system)

Subtask 2: Viability assays to assess toxicity versus induction strength

These studies are complete for:
BcD1, E1, DE1 expressed in *B. cenocepacia* strain LMG 16656
VcD1, E1, DE1 expressed in *V. cholerae* El Tor strain 16961
VcD2, E2, DE2 expressed in *V. cholerae* El Tor strain 16961
PaD1, E1, DE1 expressed in *P. aeruginosa* strains PAO1 and PA14
PaD2, E2, DE2 expressed in *E. coli* strain MG1655

Remaining tasks:
PaD2, E2, DE2 expressed in Pa strains PAO1 and PA14
MtD1, E1, DE1 expressed in *M. smegmatis* strain mc(2)155
MtD2, E2, DE2 expressed in *M. smegmatis* strain mc(2)155
Clones are prepared with modified promoters allowing induction with arabinose, as the original pMind construct was unable to drive protein expression with the tet induction system.

Table 2. Summary of results for the viability assays of ParE toxins

	Max. change in CFU/mL vs vector control	Time, %induction at max. change	Approx. doubling time of cultures
BcE1 in Bc	4.8-log reduction	15 hrs, 0.002% rha	4 hrs
VcE1 in Vc	4.0-log reduction	8 hrs, 0.2% ara	18 min
VcE2 in Vc	5.4-log reduction	8 hrs, 0.2% ara	18 min
PaE1 in Ec MG1655	0.2-log reduction	8 hrs, 2% ara	20 min
PaE1 in Pa PAO1	0.05-log gain	8 hrs, 2% ara	25 min
PaE1 in Pa PA14	0.9-log reduction	8 hrs, 2% ara	25 min
PaE1 in Ec MG1655	5.5-log reduction	4 hrs, 0.2% ara	20 min

Methods for these studies have relied on spot dilution assays to measure the colony forming units (CFU) as a function of time, and using multiple cultures induced at different concentrations. Control samples are the same vectors with no inserted genes, the antitoxin, and/or the operonic antitoxin-toxin units. Growth curve results for each sample are provided in the Appendix, and summarized in Table 2.

Cultures are propagated in M9 defined media from a frozen glycerol stock for 16-20 hrs with supplemented glucose to repress leaky expression. These overnight growths are diluted at a one to twenty ratio in fresh M9 media supplemented with increasing concentrations of the inducing sugar. At given time points and aliquot is removed and serially diluted for determination of CFU/mL by spot assays. Typically these are completed for at least three biological replicates.

The BcParE1 protein is exceptionally toxic, and the selected vector appears able to readily induce protein expression. Follow-up studies utilized a top-down proteomic approach to detect expression of BcE1and confirmed that a lack of toxicity was accompanied by an absence of toxin protein. This allows us to conclude that very few molecules of this toxin protein are needed to have a deleterious effect on the total cell numbers. We sought to establish if the noted toxicity was at a maximal level by continuing to increase rhamnose; no significant gain of toxicity is observed at 0.2%, 1%, or 2% induction and yielding a 4.5 to 5 log reduction as compared to the empty vector at the same induction level. This likely represents a saturation of the rhamnose induction system.

The VcParE2 and PaParE1 proteins are also exceptionally toxic. However, the PaParE1 toxin protein exhibits essentially no toxicity, consistent with other studies demonstrating a functional *recA* and DNA repair pathway can protect cells from this toxin. We confirmed that the PaParE1 construct does express soluble protein; therefore, something about this toxin sequence is different and prevents the potent toxicity of the other ParE proteins.

Cultures exhibiting toxicity appear to regain growth at later time points, indicative of (a) escape mutants via genetic mutations, (b) phenotypic adaptation, or (c) consumption of the induction sugar. Continuing studies are discriminating among these possibilities by re-culturing longer time point samples and subsequent re-induction, and by supplementation of inducing sugars at later time points. *This is an important component to evaluate, as our translational application will be optimum with sustained toxicity. It is also expected to be most useful for bacteria harboring ParE toxins that exert the largest reduction in CFU counts with the fewest number of toxin molecules. Of the three bacteria examined, each harbor at least one very toxic ParE protein.*

Subtask 3: Fluctuation assays to determine if ParE toxins increase native mutagenic capacity

These studies are complete for:
PaE1 expressed in *P. aeruginosa* strains PAO1, PA14

Pilot completed for:
BcE1 expressed in *B. cenocepacia* strain LMG 16656

Remaining tasks:
VcE1 and VcE2 expressed in *V. cholerae* El Tor strain 16961

PaE2 expressed in *P. aeruginosa* strains PAO1, PA14
MtE1 and MtE2 expressed in *M. smegmatis* strain mc(2)155

The rate of spontaneous mutations was expected to correlate with repair of the DNA breaks arising from the inhibition of DNA gyrase. We were expecting to find the less toxic levels of ParE expression potentially generating higher mutation levels; however, the data to date are not consistent with that hypothesis. When combined with results from subtask 2 (viability assays), it appears that overall increased levels of mutations contribute to loss of viable cells. *This is an ideal outcome for the application of our translational approach, with the caveat that it should not contribute to the antibiotic resistance (tested in Subtask 4).*

Analysis of the rate of mutations relies on selection using antibiotics that require a single nucleotide change to generate resistance: trimethoprim, rifampicin, or a fluoroquinolone such as ciprofloxacin. Specific induction levels and times are selected based on the viability data (subtask 2) and typically are compared to the gyrase inhibiting ciprofloxacin (CIP), for which the ParE toxins share a mechanistic basis resulting in DNA damage.

Cultures are propagated in M9 defined media from a frozen glycerol stock for 16-20 hrs with supplemented glucose to repress leaky expression. These overnight growths are diluted at a one to one-thousand or ten-thousand ratio in fresh M9 media, are grown to an optical density of 0.2 (very early exponential phase), and supplemented with a defined concentration of the inducing sugar. At given time points and aliquot is removed and serially diluted for determination of CFU/mL, and the remaining culture is plated on a 3-5x MIC concentration of selection media (either trimethoprim or rifampicin). Colony enumeration is quantified for non-mutation selecting plates as above for viability assays, while the high antibiotic concentrations used to select for mutations typically require two-times longer for adequate growth before counting. Calculations of mutation rate follow standard procedures and are reported as the ratio of colony units on mutation selecting media divided by the total colonies in the culture.

While preliminary, a trend is emerging of increased mutation rates only for concentrations of ParE toxin that reduce colony forming units in the viability assay. The PaParE1 toxin is essentially non-toxic in the host *P. aeruginosa*, consistent with previous observations of a reliance on the *recA* recombination repair pathway (intact in native *P. aeruginosa* strains). PaParE1 does not appear to increase the rates of mutation above background. For the potently toxic BcParE1 protein, the higher concentration of inductant leads to a dramatic increase in the rate of mutations by almost 10-fold, and essentially equivalent to the positive control CIP samples. Lower induction concentrations that produce only a modest 1.1-log decrease in CFU counts in our viability assays have no significant changes in mutation rate. Pending replicates will establish the statistical level of these results.

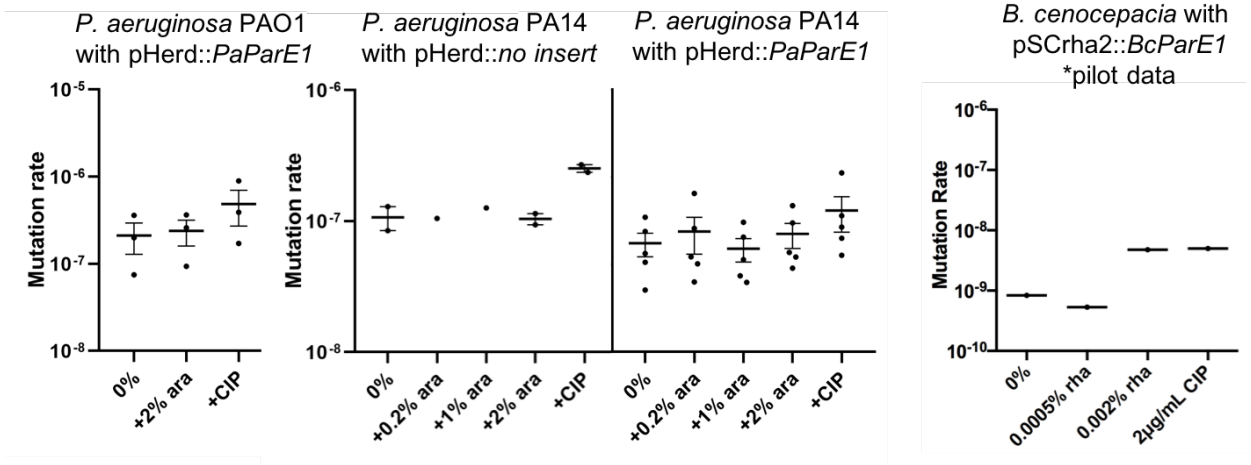


Figure 1. Measurement of mutation rates for the PaParE1 toxin and the BcParE1 toxin, in their native hosts, and with different induction levels. Note that this response is expected to be highly dependent on the bacterial cell's DNA repair pathways, and thus may vary between strains. CIP, gyrase-inhibiting antibiotic ciprofloxacin, used at 0.5X the MIC value.

Subtask 4: Antibiotic susceptibility assays as a function of ParE toxin expression

Pilot studies are complete for:
PaE1 expressed in *P. aeruginosa* strains PAO1, PA14
BcE1 expressed in *B. cenocepacia* strain LMG 16656

Remaining tasks:
VcE1 and VcE2 expressed in *V. cholerae* El Tor strain 16961
PaE2 expressed in *P. aeruginosa* strains PAO1, PA14
MtE1 and MtE2 expressed in *M. smegmatis* strain mc(2)155

The generation of mutations, as measured in Subtask 3, could impart a higher rate of antibacterial resistance. Our hypothesis is that lower amounts of ParE toxin, such as at bacteriostatic levels, may increase the number of cells surviving and thus containing repaired DNA. These repair pathways are intrinsically error-prone, and thus may contribute to an undesired increased resistance. Subtask 3, however, is highlighting a lack of increased mutagenic capacity at non-toxic (bacteriostatic) ParE induction levels. This would be ideal for our approach; however, we wanted to assess the potential for antibiotic tolerance to impact our translational goal.

Cultures were prepared as for Subtask 3 and swabbed onto agar growth media. Variations have included including inductant in these media to maintain expression of the ParE toxin, or an absence of induction. Antibiotic strips (sourced largely from the supplier bioMérieux) are placed on the agar medium and the bacterial are allowed to grow for 24-48 hrs. The resulting size of the halo of no growth around the strip indicates the level of susceptibility to that antibiotic.

Pilot studies have identified essentially no change in antibiotic susceptibility as a function of ParE toxin expression for both the non-toxic PaParE1 and the potently toxic BcParE1. *This is encouraging for our approach of manipulating these TA systems to selectively halt the growth of bacterial cells.*

Specific Aim 2: Increase ParE availability *in vivo* as proof-of-concept of a therapeutic approach by engineering each targeted species' ParD antitoxin degradation model system in an *E. coli* host. Three subtasks were identified for Aim 2, resulting in three Milestone Achievements.

Subtasks 1: Build constructs for the inducible-degradation system

This subtask is in progress. It has been initiated with the identification of needed reagents, requests for bacterial strains, and development of the cloning strategy.

The system requires a vector for inducible expression of the SspB adaptor protein to mediate ClpP degradation, and a compatible vector for inducible expression of the TA system. These vector constructs must encode unique origins of replication for co-expression, and unique antibiotic selection markers and inducible promoter systems. These must be transformed into a strain of *E. coli* with the native *sspB* gene deleted to ensure controlled degradation.

We have identified a colleague who will supply the needed *E. coli* knock-out strain(s), including the isogenic wild-type. A vector encoding the *sspB* gene with a maltose-binding protein affinity tag under control of a lactose promoter is available from the Addgene repository. Finally, we are adapting an existing pET-Duet dual multiple cloning site vector for our needs by replacing the native T7 promoters with arabinose-inducible promoters. This construct already contains a His affinity tag for one site, which we typically use for the antitoxin, and a Strep affinity tag for the second site, which we typically used for the toxin. Once modified as needed, we will sub-clone antitoxin and toxin genes into this construct.

This design allows for co-housing of the needed constructs, controlled expression of each piece, and unique affinity tags for identification by Western blotting, as needed.

Subtasks 2: Determining extent of ParD antitoxin degradation with and without the cognate ParE toxin present

This subtask is pending completion of subtask 1, to be initiated with the systems from Pa.

Subtasks 3: Assessing the gain of phenotypes expected for gyrase inhibition as a function of ParD loss

This subtask is pending completion of subtask 1, to be initiated with the systems from Pa.

Timeline to Milestones

Milestones Achieved:	*Proposed*	*Actual*
Aim 1		
1. Determine dose-dependence of individual ParE toxicity	6	*Completed for Pa, Vc, Bc* *M. smegmatis pending* Expect fully completed by May 2022
2. Determine impact of ParE expression on mutation accumulation	12	*Partially completed* Expect fully completed by July 2022
3. Determine impact of ParE expression on antibiotic susceptibility	12 - 13	*Pilot studies available* Expect fully completed by July 2022
Aim 2		
1. SspB induced degradation experimental test system build complete	17	*In progress* Expect fully completed by Nov 2022
2. Determine the extent of individual ParD antitoxin degradation in response to SspB induction	20	*Pending* Expect fully completed by Jan 2022
3. Determine the impact of degrading individual ParD antitoxins on viability and morphology	24	*Pending* Expect fully completed by Feb 2022

Conclusion

Despite initial delays and subsequent Covid-19 impacts, we have completed approx. two-thirds of Aim 1, and have initiated the Aim 2. Our approach has been to tackle these experiments per the host bacteria, such that one student is working on the *P. aeruginosa* ParDE systems, another on *V. cholerae*, another on *B. cenocepacia*, and then on the fourth organism, *M. smegmatis*. This has resulted in a staggered completion across the Milestones.

For Aim 1, Milestone 1 is completed for three of the four test systems, Milestone 2 is completed for two of the four test systems, and Milestone 3 has pilot data for two of the four testing systems. Of note, Milestone 3 is essentially a component of the Milestone 2 experiments, so it is expected that these will be completed together over the next few months.

We are now focusing more on Aim 2, with the longest Milestone being construction of the needed expression vectors and in the correct strains. While this will take some additional time, the subsequent testing of these for Milestone 2 is anticipated to move more quickly. Milestone 3 will build from the experiments needed for Milestone 2, so it is expected to be completed concurrently.

This project is directly responsive to the pressing need for alternative antibacterial strategies. The demonstration that ParE toxins can be co-opted will be transformative in multiple fields, including microbial physiology, therapeutic development, and the wider TA community. The outcomes have the potential to offer (1) very high specificity to a single pathogen, (2) versatility in providing a means to re-sensitize “tolerant” metabolic states to current treatments in a potentiating approach, (3) will provide insight into a potential fundamental mechanism of genetic resistance through error-prone repair, now recognized as likely only after such high doses of toxin-induced DNA damage that the cells are not viable, and (4) targeting of the antitoxin is predicted to be less prone to resistance because of the need to maintain a productive pairing between cognate toxins and antitoxins.

Overall Outcomes: The successful completion of this project will (1) increase understanding of a fundamental mechanism of ParE toxin-mediated inhibition of DNA gyrase and subsequent impacts on bacterial cell physiology, and (2) will demonstrate the selective *in vivo* degradation of ParD from the ParDE complex. ***This study will provide the proof-of-concept badly needed to allow further development into an applied product that co-opts this mechanism into a novel treatment with strong potential to potentiate existing antibiotics, and that by definition will be a narrow-spectrum approach thus sparing the normal microbiota.***

Impact

(reporting period 2/14/21 – 3/15/22)

We have identified that six of the seven tested ParE toxin proteins reduce bacterial host cell viability in a dose-dependent manner. Lower concentrations of ParE induction lead to bacteriostatic effects, while up to a 5-log reduction can be achieved in actively growing cultures. *This is promising for our application of manipulation of these systems to control bacterial growth.*

Further studies are indicating that loss of viability is due to DNA damage, evident by increased rates of mutation, but that these are not causing overt changes in the MIC values for different classes of antibiotics. *This supports our application of potentiating antibiotic treatments by manipulating these specific TA systems.*

Valuable reagents have been generated, and in the process essential training of graduate students has taken place.

Training

During this reporting period, three graduate students continued training in BSL2 procedures. Additional gains were realized through the development of standardized image acquisition and data processing, including automated colony counting using ImageJ, which were implemented for the currently reported results. Significant pilot studies were necessary to capture the mutation rates (Aim 1 subtask 3) because rather than treating cultures with an exogenous compound, we are assaying the impact of intracellular protein expression. Further, the organisms under study are very drug resistant, necessitating additional screening to assess mutation selection conditions.

Changes and Problems

A one year no-cost extension was granted on Oct. 20, 2021. This was requested based on delays early in the project initiation, including a complete laboratory closure (March 24 – May 22, 2020) with limited access until July 1, 2020. Further limitations were experienced with procuring supplies, and a hiring freeze and issues with visa processing preventing hiring of a postdoctoral fellow as was originally planned.

During the current reporting year, no additional delays or changes were experienced outside of the normal supply chain issues (for example, pipette tips are routinely backordered for four months).

Reportable Outcomes / Products

The support of graduate students as part of their training is appreciated and very valuable to their development as well as progress of the project.

We have now built all needed constructs for expression of these toxic systems. This includes modification of the pMind vector to replace the *tet* inducible promoter with that from a pBAD vector, allowing *ara* induction. This will be made available to other researchers upon request, and may be deposited with the Addgene repository after publication. Transformed strains may also be available upon request upon demonstration of BSL2 ready facilities and necessary shipping documentation.

We have identified a putative link between high toxicity of ParE proteins with increased rates of mutagenesis. This important result builds into a refined hypothesis of ParE toxin mediated loss of cell viability as a function of accumulated DNA damage. Importantly, we demonstrate that this does not increase antibacterial resistance for multiple classes, nor is phenotypic tolerance noted. We anticipate a manuscript submission this calendar year describing these results.

We have initiated additional studies that build directly from Aim 1 subtask 2 to carry out top-down proteomic experiments to correlate qualitative numbers of ParE toxin molecules per noted toxicity. This is anticipated to result in a manuscript submission this calendar year.

Participants and other collaborating organizations

The project is carried out solely by members of the Bourne laboratory. To date, this has included graduate students (Chih-Han Tu, Shengfeng Ruan, Michelle Holt, and Kevin Snead), and myself (Christina R. Bourne, PI).

Current and Recently Completed Funding

US Department of Defense W81XWH-20-1-0121 2/15/20 – 2/13/23
"Unlocking the Potential of Bacterial ParE Toxins: Developing a Blueprint for Co-Opting Molecular Time Bombs that Impact Bacterial Cell Survival"

PI: Christina R. Bourne

OCRID CoBRE P20GM103648 10/20/20 – 6/30/21
"A Screening Platform for Pan-Coronavirus Assembly Modulators"

PI: Lin Liu; Pilot Project Leader: Christina R. Bourne

Oklahoma Center for the Advancement of Science and Technology HR17-099 7/1/17 – 3/1/21
"Targeting bacterial cell metabolism by manipulating toxin-antitoxin systems"

PI: Christina R. Bourne

National Institutes of Health (R15) 3/2/21 - 2/29/24

"Development of Allosteric Dihydrofolate Reductase Inhibitors: Exploration of a Novel Inhibitory Mechanism for a Validated Antibiotic Target"

PI: Matthew O'Reilly (Villanova); Consultant: Christina R. Bourne

Special Reporting Requirements

Not applicable.

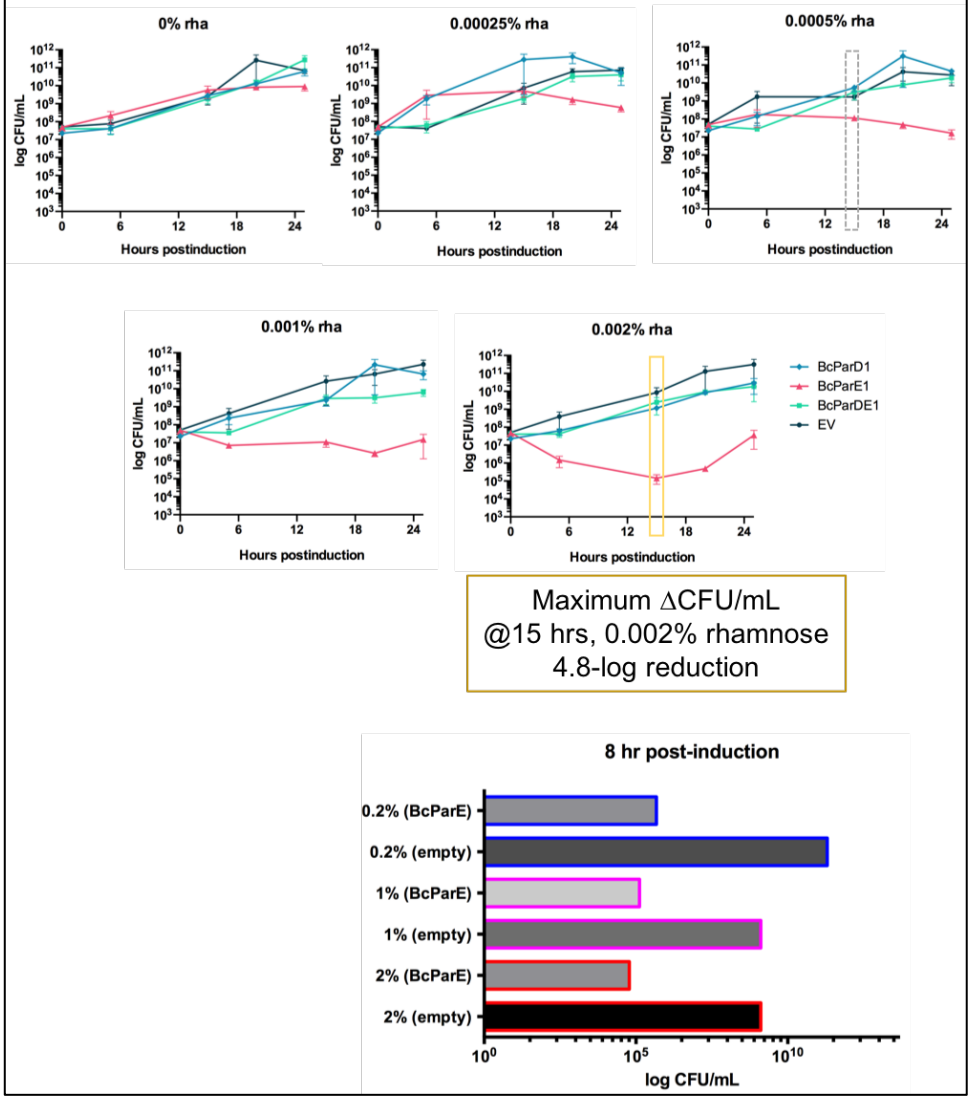
References

Not applicable.

Appendices

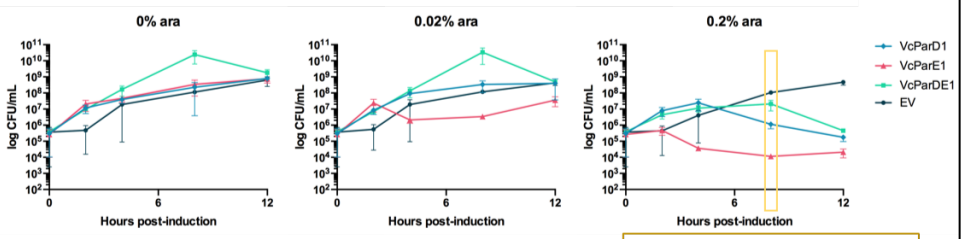
Detailed data, as summarized in the current report, are included as Appendices. These are growth curves used to assess the toxicity of individual ParE toxins (Aim 1 Subtask 2, summarized in Table 2).

BcParDE TA system induced in *B. cenocepacia*



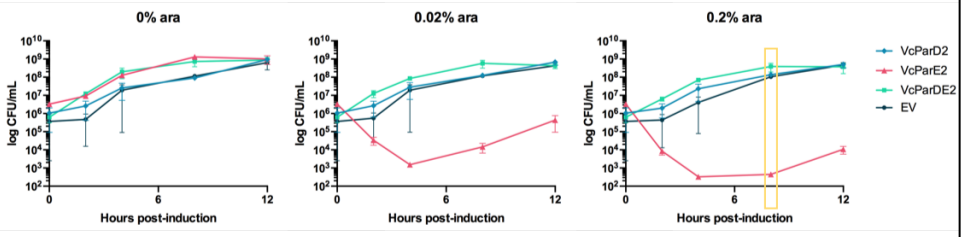
Maximum ΔCFU/mL
@15 hrs, 0.002% rhamnose
4.8-log reduction

VcParDE1 TA system induced in *V. cholerae*



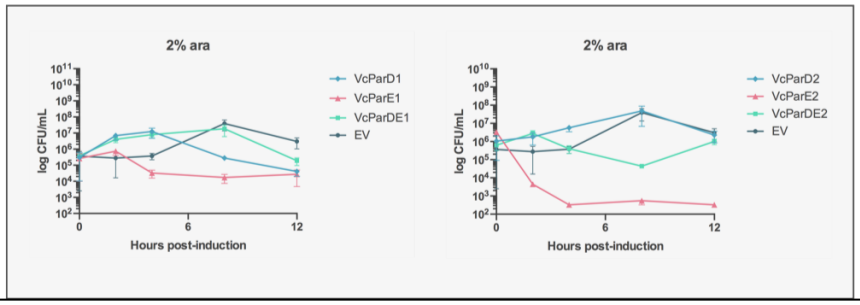
Maximum ΔCFU/mL
@8 hrs, 0.2% arabinose
4.0-log reduction

VcParDE2 TA system induced in *V. cholerae*

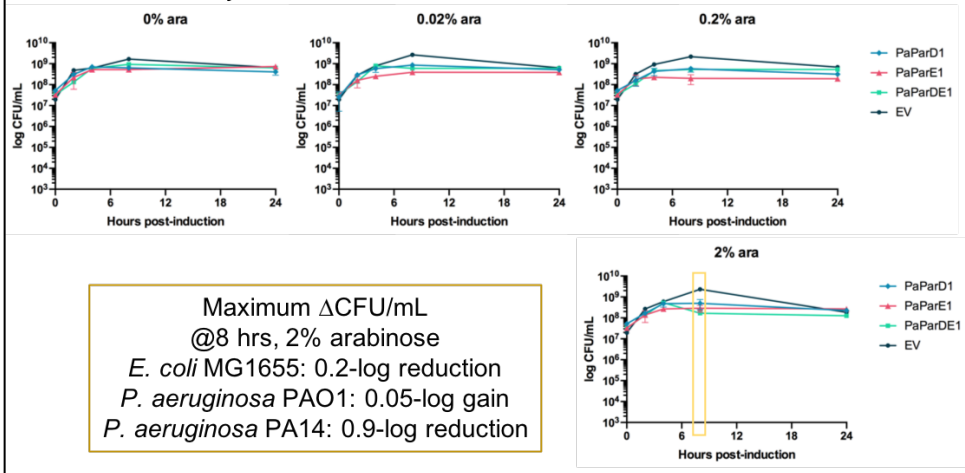


Maximum ΔCFU/mL
@8 hrs, 0.2% arabinose
5.4-log reduction

Arabinose toxicity

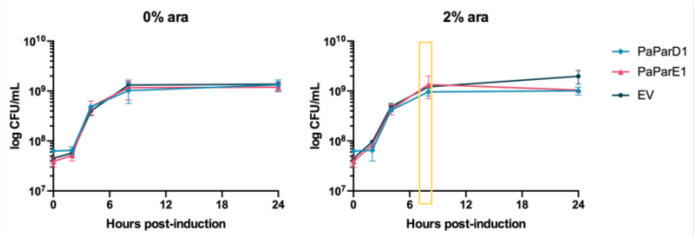


PaParDE1 TA system, induced in *E. coli* MG1655

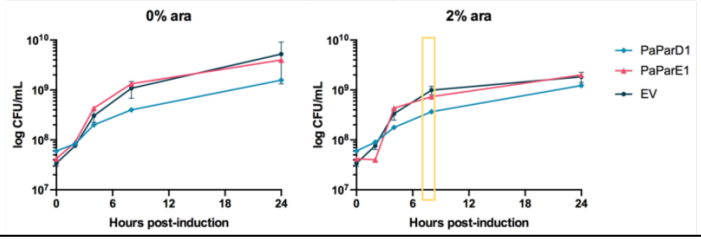


Maximum ΔCFU/mL
@8 hrs, 2% arabinose
E. coli MG1655: 0.2-log reduction
P. aeruginosa PAO1: 0.05-log gain
P. aeruginosa PA14: 0.9-log reduction

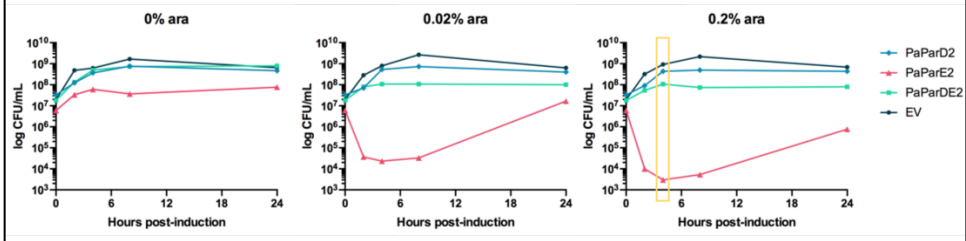
PaParDE1 TA system, induced in *P. aeruginosa* PAO1



PaParDE1 TA system, induced in *P. aeruginosa* PA14



PaParDE2 TA system, induced in *E. coli* MG1655



Maximum ΔCFU/mL
@4 hrs, in *E. coli* MG1655
0.2% ara: 5.5-log reduction
2% ara : 5-log reduction