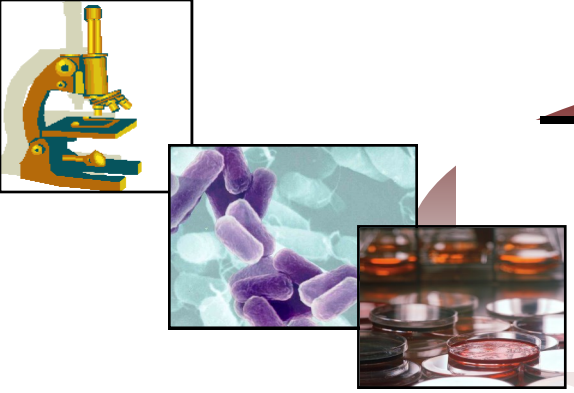


IBASM NEWSLETTER

Volume 19, Issue 2
February, 2017

Greetings from the President: Nancy Magill

Hi everyone! Hope you are all having a productive time in the lab. We've had fairly nice weather lately so I hope also that you will have some research to present at the next branch meeting. This next meeting will be on March 31 and April 1.

It will feel good to be back again at Turkey Run State Park in Marshall, Indiana. It proved to be an excellent venue last time we were there, plenty of room for posters and such. Faculty, please encourage your students to present their research this year. We encourage oral presentations as well as posters.

After a wonderful meeting last year at Fort Wayne, our President –elect, Tanya Soule, has put together another panel of talks that I will find very interesting. I hope that you do too!

Our keynote speaker on Friday night will be Dr. Vincent Young, from the University of Michigan. On Saturday, Dr. Lynn Enquist from Princeton University will be speaking followed by a research award recipient, Dr. Frank Yang, from the Indiana University School of Medicine. There is plenty of room that day for student talks. We will then close the meeting with student awards.

I am hoping that we continue to have good weather, in particular for our meeting. I look forward to seeing you then.

In this issue:

Message from the President-Elect - Tanya Soule

I am excited that our spring meeting will be March 31-April 1, 2017 at Turkey Run Inn in Marshall, IN. This year we are pleased to announce that ASM Past-President Dr. Lynn Enquist from Princeton University will be speaking on mechanisms of herpesvirus pathogenesis and our ASM Branch Lecturer, Dr. Vincent Young from the University of Michigan, will be speaking about how the human microbiome can influence a variety of health issues and disease conditions. We are also excited to host our research award recipient, Dr. Frank Yang from the Indiana University School of Medicine, who will share some of his research on spirochetes.

The IBASM meeting is a wonderful opportunity for both graduate and undergraduate students to present their work. As a graduate student I attended branch meetings annually and was excited to see that Indiana offered the same opportunities. I encourage you to all submit abstracts for either a poster or oral presentation. For those of you working on a thesis, dissertation, senior, or honor's project this meeting is a great opportunity to present your research to microbiologists for valuable feedback.

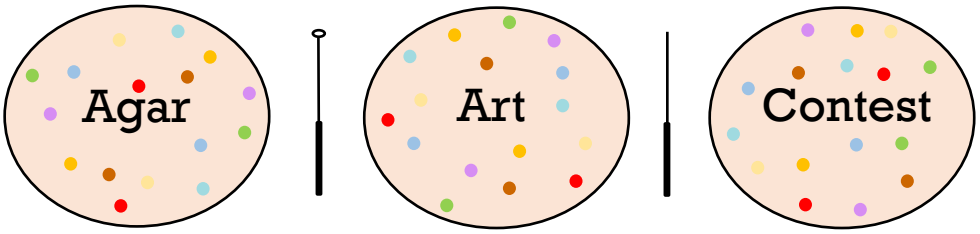
I would like to thank Dr. Becky Sparks-Thissen for her years of service as President of IBASM and Dr. Nancy Magill, who is serving as our current President. Furthermore, Dr. Christian Chauret, the IBASM Secretary, has been extremely helpful. I could not have put this meeting together without their assistance. Thank you also to our student representatives, Jamison Law (IPFW) and Grace Gomez (IUPUI Dental School) for their work in organizing our first ever Agar Art Contest (see below for details)!

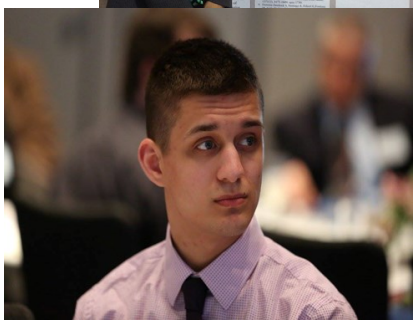
Finally, I would like to remind you that abstracts are due **March 8** while the meeting registration is due **March 1** to align with the deadline for reserving rooms. A block of rooms has been reserved at Turkey Run Inn. To reserve your room you can call 877-563-4371 or go online at www.indianainns.com and use the **group code 0331MB** to pull from the rooms in our meeting block. More information on rooms and dining can be found below on the Meeting Registration Form.

Thank you and I look forward to an educational, fun, and successful meeting!

Indiana Branch of the American Society for Microbiology

PRESENTS





An agar art contest will be held during the 2017 IBASM meeting at Turkey Run State Park in Marshall, Indiana. All submitted works will be projected for display and judged by participating faculty. First and second place winners will receive a prize and, in addition, the first place winner's agar art will be featured on the back cover of the summer issue of *Fine Focus*! All questions concerning the contest can be directed to Jamison Law (lawjd02@ipfw.edu) or Grace Gomez (gfelixgo@iu.edu).

Submissions for the contest must meet the following criteria:

- The art can be worked on individually or in a group, but the names of everyone involved must be listed.
- Any microorganism and media can be used, but everything used must be listed.
- A theme or motto must be provided (in a separate document) that is suitable and concisely describes the art.
- Two photos must be submitted, one of the agar art being held by the individual(s) involved in making
- the agar art and one of just the agar art.
- Images must be no less than 8"x10" and have a resolution of at least 300 dpi.
- Editing of any kind that changes the appearance of the agar art is prohibited, no photoshop!
- Everything must be emailed to Jamison Law or Grace Gomez by March 24, 2017.
- This document must be signed and dated by all participants of the contest; entries will otherwise be
- considered invalid. Forms can be scanned and emailed to Jamison Law or Grace Gomez or signed at the meeting.

By signing I am agreeing that my submission meets all the above requirements:

Name: _____ Date: _____

IBASM 2017 Call for Abstracts
Student Poster Competition

The abstract submission form is included here but will be distributed by email separately as a word document. We will be utilizing 4x4 sq.ft. tri-fold styrofoam poster boards and each student is limited to one board. Push pins will be supplied but it wouldn't hurt to bring some extras in case we run short. You may participate in both oral (limited # of slots available) and poster sessions but you will only be judged for an award in the poster session. Awards will be presented in the following divisions: Undergraduate, MS graduate and Ph.D. graduate. Post-Doctoral Fellows are welcome to participate in either session but are not eligible for the award competition.

Students will be judged in 5 categories:

Professional Appearance: Jeans and sweat pants are unacceptable; torn, dirty, or frayed clothing is unacceptable. Business casual dress is the standard dress code. (20 points)

Scientific Thought: Is there a clear hypothesis? Are the goals of the study defined? Were data correctly analyzed? Were statistical analyses performed? Did a logical conclusion result? (20 points)

Creativity: Was the topic original? Is there anything new in the approach to answering the question? Were new methods developed? (20 points)

Thoroughness: Was the study as complete as possible? Does the student understand the background material? Were subject headings (e.g. Introduction, Materials & Methods, etc.) presented? Is the student aware of the drawbacks of the study? (20 points)

----------*CALL FOR MANUSCRIPT SUBMISSIONS*----------

We are an international journal dedicated to showcasing undergraduate research in all fields of microbiology. Fine Focus is managed entirely by undergraduate students from production to print, but utilizes an external Editorial Board of experts for double-blind peer review of manuscripts.

MISSION We publish original research by undergraduate students in microbiology. This includes works in all microbiological specialties and microbiology education.

ABOUT US *Fine Focus* is a peer-reviewed academic journal with a mission to publish findings of international undergraduate microbiology research in both print and electronic platforms.

As an international journal, *Fine Focus* accepts article submissions from undergraduates, and works with more than 70 Editorial Board members from around the world. We use a double-blind review system to ensure fair & objective review.

WHY WE ARE UNIQUE We are the first international undergraduate journal specifically in microbiology. *Fine Focus* allows interested students the opportunity to see their research efforts through to fruition *via* publication while learning about the scientific peer review process at the same time. **By offering an opportunity for undergraduate students to publish early in their training, they become the authors of, and authorities on, their own education and professional development.**

DONATE We are a non-profit journal, and we rely on donations that able us to continue our work with undergraduates and get also their word heard in the field of microbiology. To donate, please visit: http://finefocus.org/donate

WANT TO GET INVOLVED? Undergraduate students can submit microbiology research by going to finefocussubmissions.org. Microbiology professors and other professionals can get involved with *Fine Focus* as editors or reviewers. Let us know if you would like *Fine Focus* in your college or university library.

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Fine Focus at a glance:

- Free - no page charges or subscription costs
- Partnering with the American Society for Microbiology (ASM) & National Institutes of Health (NIH)
- Published articles searchable and indexed *via* EBSCO Host and Cardinal Scholar
- Full text free digital access: https://cardinalscholar.bsu.edu/handle/123456789/199

ABSTRACT FORM FOR THE 2017 IBASM ANNUAL MEETING

Complete all appropriate boxes of this form (downloadable version http://ibasm.iweb.bsu.edu/) and email it on or by **March 8th 5:00 pm** to Dr. John McKillip at jlmckillip@bsu.edu. **Late submissions will not be accepted**. Abstracts should be prepared according to the National ASM guidelines (see below). All abstracts should include the title, author(s), and institutional address. Accepted abstracts will be published in the meeting program if submitted by the deadline. Limited funding will be available to subsidize lodging and food for student presenters when requested in the registration form.

Presenting author information:

Name:	Phone:
Address:	Fax:
Subject Category:	Email:

Are you a student presenter? ☐ Yes or ☐ No (check one)

☐ Oral and/or ☐ Poster presentation (check applicable boxes)

If you are not selected for an oral presentation, are you willing to present a poster?

☐ Yes ☐ No ☐ Does not apply

Check if presenting author is a student competing for: ☐ Undergraduate ☐ MS Graduate or ☐ Ph.D. Graduate Student Award (a short paper is required from award winners). If competing for an award the student must present a poster. (If left blank, the student will not be judged in the competition).

Check if presenting student will also be presenting at the 2017 ASM General Meeting: ☐

Are you competing for the national travel award to the 2017 ASM General Meeting? ☐ Yes ☐ No

ABSTRACT

Keywords:

From the Desk of John McKillip...Educational Representative

ABSTRACT GUIDELINES BASED ON AMERICAN SOCIETY FOR MICROBIOLOGY (ASM) REQUIREMENTS

SUMMARY OF SUBMISSION STEPS

Step One: Submit the 2017 IBASM Membership Application/Renewal and Registration Forms.

Step Two: Affirmations

- Names of all the authors (primary and co-authors) will appear on the abstract.
- The submitted abstract representing your research has not been accepted for publication in a journal or in an international scientific journal based on the date submitted.
- Upon acceptance of the abstract, the prepared poster (see guidelines) will be placed on the scheduled day and time for viewing and only be removed after the scheduled time.
- Any changes in contact information should be corresponded with IBASM.

Step Three: Title

Please use a short and concise title that indicates the content of the abstract.

- Capitalize the first letter of each word except prepositions, articles and species names.
- Title is not included in the total character count of 2000.
- Do not place a period at the end of your title.
- Do not place hard returns in your title.
- Italicize scientific names (example: "Staphylococcus aureus" will appear as *Staphylococcus aureus*). For therapeutic agents, only generic names may be used (NO trade names are permitted in abstract titles).
- A title of 10 words will be appropriate.

- **Examples of a properly formatted title:**
  - *Cryptococcus neoformans* in Black, White and Color
  - Real Time PCR Assay for *G. destructans* Identification in Fungal Cultures and in Bat Tissues.
  - *In Vitro and In Vivo* Characterization of Orf6, a Putative Thioesterase

Step Four: ***Primary Presenting Author*****, Co-Authors, Affiliation**

- Authors, groups and institutions and spaces are not included in the 2000-character limit.
- Author's names will be displayed using first initial(s) and full last name. Presenting author will be printed with an asterisk (e.g. J. Smith*, W. S. Brown, and R. A. Jones).
- Each institution and author will be referenced with superscript numbers and include the institution's city, two letter state/province abbreviation and country.
- Please note that IBASM will correspond with the presenting author only. Changes in the presenting author must be communicated to IBASM. It is the responsibility of the presenting author to contact all co-authors with the disposition and scheduling of the abstract. The complete address of the presenting author is required in order to assure that correspondence arrives promptly and easily.

Step Five: ***Keywords***

- Keywords are completely independent of each other and should be able to stand alone in the index.
- Words should be lowercase, except for genus names and proper nouns. For Greek characters, please spell out names.
- Organisms will be italicized in final publications.
- Enter up to three keywords.

Step Six: **Abstract Text** *(included in the 2000-character limit - spaces are not counted)*

- Your abstract may have up to 2000 characters, which does not include the title, authors, affiliations, and keywords. Spaces are not counted. Do NOT include abstract title, authors or keywords in the abstract text.
- Abstract text may be submitted using either of the following methods: Copy/paste or direct entry keystrokes.
- Your abstract may be written without the use of the following bolded headers **(Background, Materials, Results, Conclusion)**. However, your abstract should include a one to two sentence introduction, the description of the methods used, the results obtained, and a conclusion with inclusion of its significance.

POSTER GUIDELINES FOR THE SPRING 2017 IBASM ANNUAL MEETING

1. ABSTRACT
    - Including an abstract on your poster is optional, although I would discourage it simply because the poster itself is already an abbreviated form of your research so having an abstract is like abbreviating the abbreviation. Other than being a tad redundant, abstracts take up precious space that could be used for including other information that one would otherwise not have the room for.
2. INTRODUCTION
    - The introduction should give the reader a solid foundation on which to base their understanding of the rest of the poster. It should convey: the importance of the research, the problem you are trying to solve, why the research is necessary, your approach to the problem and the logic behind it, and your hypothesis.
    - Be to-the-point and avoid being wordy. Nobody wants to read a novel! Instead of having one big clump of text try using bullet points to make things appear less word-heavy.
3. METHODS
    - Give enough detail to where the reader understands what was done, but do not explain every detail! For example: instead of writing the entire protocol for running an SDS-PAGE, simply write, “We ran an SDS-PAGE on our protein.” Assume basic knowledge.
    - Present methods in a way that is easy to follow.
4. RESULTS
    - Some people (albeit very few) are colorblind and, as a result, do not derive the same information from figures because they cannot perceive the colors that the data is portrayed in- this leaves them at a great disadvantage! Therefore, try to make your figures, if possible, in black and white or colors that are colorblind friendly.
    - Make sure to adjust the resolution on all of your figures, otherwise you may find that some of your images are pixelated, which can be quite embarrassing and frustrating for those trying to interpret your data!
    - DO NOT DISCUSS your results in the Results section. You should merely state the observed results.
5. DISCUSSION
    - This is where you interpret your results and discuss them.
    - Refer to all of your figures when discussing the corresponding data!
    - State your conclusions and future directions.

6. REFERENCES

- References are an important thing to include on a poster, especially if someone is curious about where a particular protocol, figure, or fact came from. If it is not an original work of yours- cite it!
- Space is limited so you should only include your top 3 most important references! You will have different formatting styles (THAT IS OKAY!), but make sure to be CONSISTENT!
- Author(s) last name and author(s) initial of first name. *Title*. Journal Name, Month and Year. **Volume #**(Issue #): pages. Digital Object Identifier (DOI).
- Law J and Gomez G. *Poster guide for the 2016 IBASM annual meeting*. Journal of Exampleology, January 2016. **10**(1): p. 103-107. doi: 10.1007/s02253-072-1135-4

7. ACKNOWLEDGMENTS

- It is always important to recognize those who helped you analyze data, conduct some of the research, your advisor/mentor who supervised the research, and especially those who provided you with funding, supplies, or organisms.
- Do not thank someone if they happen to be a coauthor of the poster!

8. GENERAL NOTES

- Have poster dimensions set at 40 inches by 24 inches.
- Avoid having font smaller than 24 in Times New Roman (for example). If you can read your poster on an 8x11 print-off then you should be good!
- Make sure the formatting scheme is consistent!
- The poster should stand alone- it should tell the story of your research without you having to be there.
- Poster sections should be well organized and have a natural flow to them.
- All figures and sections should be properly titled.
- Make sure all edges line up properly.
- Give at least a 1 inch page margin on all sides or you may find your print off eating into your texts and figures!

2017 IBASM Spring Meeting

March 31-April 1, 2017 at Turkey Run Inn

8102 E. Park Road, Marshall, IN 47859

877-500-6151 http://www.in.gov/dnr/parklake/inns/turkeyrun/

Friday March 31, 2017

5:00-7:00 PM	Registration
6:00-7:00 PM	Dinner (Dining Hall)
7:00-8:00 PM	**ASM Branch Lecture**- Vincent Young, MD/Ph.D. University of Michigan, Ann Arbor
8:00-10:00	Welcome Reception with Poster Viewing

Saturday April 1, 2017

7:30-8:30	Breakfast (Dining Hall)
8:30-10:30	Poster Judging and Viewing
10:30-11:30	**ASM Past President**- Lynn W. Enquist, Ph.D. Princeton University
11:30- 12:00	Student Oral Presentations
12:00-12:30	IBASM Business Meeting
12:30-1:30	Lunch (Dining Hall)
1:30-2:30	Student Oral Presentations
2:30-3:30	**IBASM Research Award Recipient Lecture**– Frank Yang, Ph.D. Indiana University School of Medicine
3:30-4:00	Announcement of Student Award Winners Closing Remarks

IBASM Annual Meeting Registration and Meal Reservation Form

March 31-April 1, 2017 at Turkey Run Inn
8102 E. Park Road, Marshall, IN 47859
877-500-6151 http://www.in.gov/dnr/parklake/inns/turkeyrun/

Please use this form (downloadable version http://ibasm.iweb.bsu.edu/) to register for the IBASM meeting and to reserve your room. The meeting registration fee is $30 for regular members and $7 for student members. You must be an IBASM member to participate in the meeting. Family members are encouraged to attend and do not have to pay registration fees. Upon completion, email (or mail) this form to Tanya Soule (soulet@ipfw.edu) no later than **March 1, 2017**. If necessary, forms may also be mailed to Tanya Soule at the address given on the Membership Form below. Payment can be in the mail with a check payable to IBASM (do not send cash) or provided at the meeting (cash or check). Registrations received after March 1 will be subject to a $7.00 late fee (regular members) or a $4.00 late fee (student members). Please feel free to contact Tanya Soule at the email address provided above if you have any questions. **Remember, meeting abstracts are due March 8, 2017**.

Please fill in the requested information.

Name:	#Adults:	#Children:
Address:		
Institution: Phone:	 Fax:	 Email:

If you are not a member, you will need to become a member and include your dues with your payment for the meeting.
2017 IBASM member: ☐ Yes ☐ No

Please indicate which sessions you plan to attend:

☐ Friday evening session ☐ Saturday morning session ☐ Saturday afternoon session

If you are a student presenter, do you request travel assistance? ☐ Yes ☐ No

Lodging and Meals: A block of rooms has been reserved at Turkey Run Inn. To reserve your room you can call 877-563-4371 or go online at www.indianainns.com and use the **group code 0331MB** to pull from the rooms in our meeting block. The first night must be provided as a deposit at the time of making the reservation. **Reservations must be made by March 1, 2017 in order to be guaranteed.** Meals will be served buffet style in the dining hall. Breakfast is $9.99, lunch is $10.99, and dinner is $14.99. **Please be prepared to pay individually (cash or card) for each meal.**

Payment:

Registration: ☐ Member ($30) ☐ Student ($7) $_____

Dues (if applicable; see following page): ☐ Non-student ($15) ☐ Student ($5) $_____

Late fees (if applicable) $_____

Total Enclosed (mail or pay on-site) $_____

2017 Membership Application/Renewal

If you have not done so already, it is time to pay your IBASM dues for 2017. You can do it either online when you pay your dues to the ASM National Organization (www.asm.org) or by using this form (downloadable version http://ibasm.iweb.bsu.edu/). Dues are $15.00 for non-students and $5.00 for students (per year). Please return the completed form with payment to either the IBASM meeting (cash or check) or by mail with a check (do not send cash), payable to IBASM, to:

Dr. Tanya Soule
Department of Biology
2101 E Coliseum Blvd
Indiana University-Purdue University, Fort Wayne
Fort Wayne, IN 46805
Phone: (260) 481-0229; e-mail: soulet@ipfw.edu

Please check: □ New Member Application or □ Renewal for 2017

Please check: □ Student Member in 2017 ($5) or □ Full Member in 2017 ($15)

Please check: ☐ Dues paid to IBASM directly or ☐ Dues paid online at www.asm.org

Name:

Current Position & Title:

Institution:

Mailing Address (new address Yes / No?):

Phone:

Email:

Fax:

National ASM Member # (if applicable):

Background

Highest Degree:

Institution:

Professional Interests:

Second Place Graduate (Ph.D. Division) Winner

Targeting Intracellular Pathogenic Bacteria with Pepkan (Kanamycin Antibiotic Peptide Conjugate)

Mohamed F. Mohamed[a], Anna Brezden[b], Jean Chmielewski[b] and Mohamed N. Seleem,[a]
[a] Department of Comparative Pathobiology and [b] Department of Chemistry
Purdue University

Abstract:

Treatment of infections due to intracellular pathogens represents unresolved medical challenge. Bacteria such as *Salmonella*, *Brucella* and *Mycobacterium* reside inside host cells and most antibiotics do not pass through cellular membranes effectively. To address this significant problem, we conjugated the antibiotic kanamycin with a novel cell penetrating antimicrobial peptide (P14). The kanamycin peptide conjugate (Pepkan) showed enhanced antimicrobial activity against Gram-positive and Gram-negative pathogens. Flow cytometry and confocal images demonstrated high cell penetration of the kanamycin peptide conjugate (Pepkan) inside macrophage cells. Moreover, the conjugate effectively killed intracellular pathogenic *Salmonella*, *Brucella* within macrophages. Notably, significant reduction of *Mycobacterium tuberculosis* within macrophages was observed with the dual antibiotic conjugate. Additionally, the conjugate was found to exhibit excellent activity *in vivo* in a *Caenorhabditis elegans Salmonella* infected whole animal model. Taken together, this strategy has proven to be very effective tool in combating intracellular bacteria.

Introduction:

A large number of antibiotics, especially those of the aminoglycoside family, effectively permeate prokaryotic cells. However, due to their inability to traverse eukaryotic membranes, their therapeutic value as a method to fight against IB is limited. Antimicrobial peptides (AMPs) are evolutionary conserved defenses but are hampered by cytotoxicity. Proline-rich class of AMPs are non-membrane lytic and non-toxic, but are also incapable of traversing mammalian cells. We have conjugated the aminoglycoside drug, kanamycin, with an unnatural proline rich peptide P-14. Briefly, this peptide is known to have non-membrane lytic antimicrobial activity and mammalian cell penetrating capability. Using such a conjugate (Pepkan) would allow for kanamycin to enter the mammalian cells, thereby increasing its therapeutic value. Additionally, we chose to link the peptide and kanamycin using a disulfide tether. We hypothesize that upon internalization in the reductive environment of the cytosol, the linker will be cleaved to release free kanamycin. Through dual delivery of these two antibacterial agents, we expect to clear the intracellular pathogens without compromising the integrity of the host cellular membrane.

Results:

The intracellular penetration of pepkan-FL was investigated by flow cytometry and confocal microscopy. Flow cytometry proved intracellular penetration of **Pepkan-FL** (10 mM) and P-14 within J774A.1 cells.

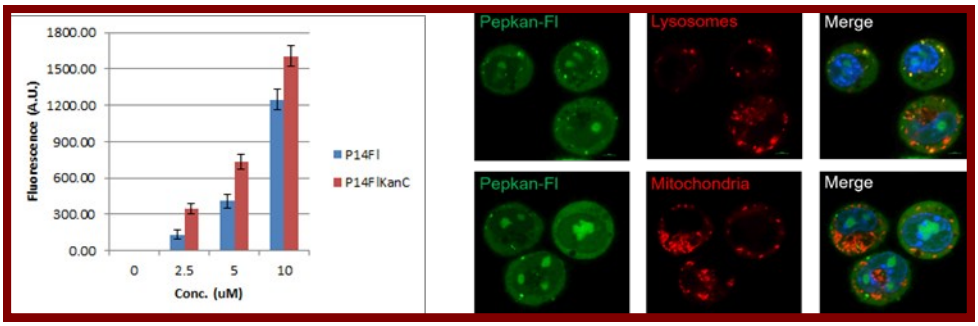

Figure 1. Flow cytometry proved intracellular penetration of **Pepkan-FL** (10 mM) and P-14 within J774A.1 cells. Confocal microscopy images of co-localization studies using **Pepkan-FL** (10 mM) and lysotracker or mitotracker within J774A.1 cells. Nucleus stained blue with Hoechst stain.

Antibacterial potency of the conjugate was explored by performing broth micro dilution assay. Minimum inhibitory concentration was determined for *Salmonella enteritidis* and *Brucella abortus, Mycobacterium smegmatis and Mycobacterium tuberculosis.* Pepkan displayed potent antibacterial activity against a broad spectrum of pathogenic bacteria.

Compound	The minimum inhibitory concentration (MIC) (μM)								
	S. Enteritidis	*B. abortus*	MRSA	*Biofilm S. aureus*	*Biofilm S. epidermidis*	*P. aeruginosa*	*A. baumanii*	*M. smegmatis*	*M. tuberculosis*
P-14	32	16	64	16	32	64	32	8	16
Kanamycin	16	4	4	4	>256	>256	128	8	2
Pepkan	2	0.12	1	1	1	1	1	1	1
Fold enhancement	8	32	4	4	>256	>256	128	8	1

Intracellular antibacterial activity

We investigated the ability of the peptide conjugate to clear intracellular pathogens *Salmonella enteritidis* and *Brucella abortus* within J774a.1 macrophage cells with an *in vitro* bacterial assay. Treatment with Pepkan, showed significant reduction of intracellular *Salmonella, Brucella, Staphylococcus, Pseudomonas and Mycobacterium* more than treatment with kanamycin or peptide alone.

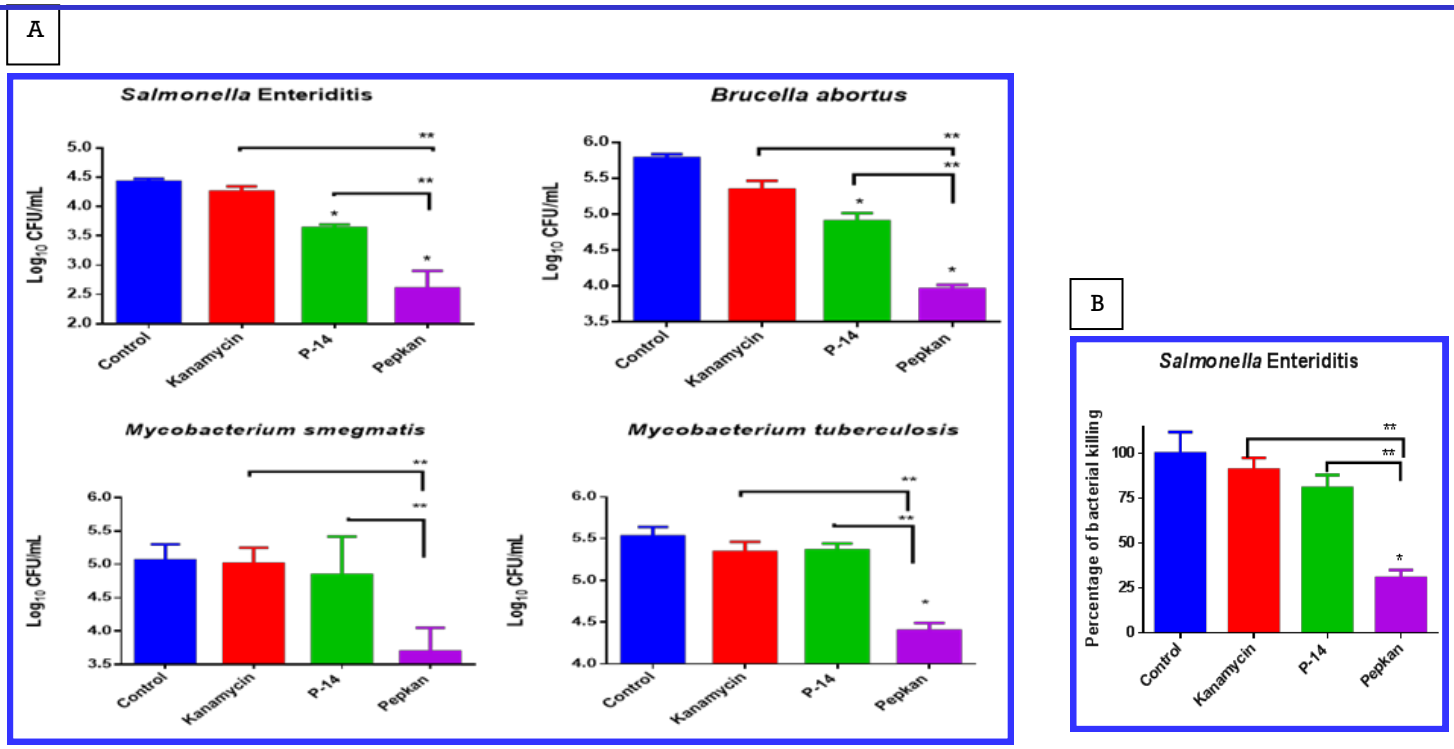

Figure 2. A: Intracellular antibacterial activity and toxicity of P-14 (10 μM), Kanamycin (10 μM), Pepkan (10 μM), in J774A.1 cells infected with pathogenic bacteria. B: Treatment of infected *Caenorhabditis elegans* infected with *Salmonella* Enteritidis with P-14 (50 μM), Kanamycin (50 μM) and Pepkan (50 μM) for 24 h.

Conclusion

Pepkan demonstrated a broad spectrum antimicrobial activity against Gram-positive and Gram-negative bacteria as well as intracellular bacteria residing within mammalian cells. Moreover, Pepkan construct also demonstrated 100% survival of whole animal model of bacterial infection and *Salmonella* counts were significantly reduced in an in vivo *C. elegans* model as compared to the individual antibiotics. Taken together, this strategy has proven to be very effective tool in combating intracellular bacteria.

References:

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Zumla, A., Nahid, P. & Cole, S. T. Advances in the development of new tuberculosis drugs and treatment regimens. *Nature reviews. Drug discovery* **12**, 388-404, doi:10.1038/nrd4001 (2013).

Carryn, S. *et al.* Intracellular pharmacodynamics of antibiotics. *Infectious disease clinics of North America* **17**, 615-634 (2003).

Second Place Graduate (M.S. Division) Winner

Biochemical Characterization of a Mycobacterial Glycerol-3-Phosphate Acyltransferase

Jamison Law and Jaiyanth Daniel
Department of Biology, Indiana University-Purdue University Fort Wayne, Fort Wayne, IN

Introduction

Tuberculosis (TB) is a disease caused by *Mycobacterium tuberculosis* (*Mtb*) that affects 2 billion people and is responsible for 1.5 million deaths and 9.6 million new diagnoses in 2014 with 480,000 of those new diagnoses having multiple and extensively drug-resistant tuberculosis (MDR/XDR-TB). Drug-susceptible TB treatment lasts 6 months with an 85% success rate while MDR/XDR-TB treatments last about 20 months and use more expensive and toxic drugs that yield much lower success rates [1]. After infecting alveoli, the immune system isolates *Mtb* within a hypoxic granuloma and causes it to acquire antibiotic resistance by entering dormancy where it survives by accumulating triacylglycerol (TAG) by esterifying fatty acids, derived from host lipids, to a glycerol backbone [2]. Therefore, the biosynthetic pathway of TAG is probably essential for the survival of *Mtb* during dormancy. Multiple sequence alignment (**Fig. 1**) of Rv1551, an *Mtb* gene, suggests that Rv1551 encodes a glycerol-3-phosphate acyltransferase (mGPAT), which is the first enzyme involved in TAG synthesis. If the enzymatic activity of mGPAT is blocked, *Mtb* cannot synthesize TAG that it needs to survive during dormancy, which means it will either die or be forced out of dormancy where it will once again be susceptible to current antibiotics. Thus our research focused on biochemically characterizing mGPAT.

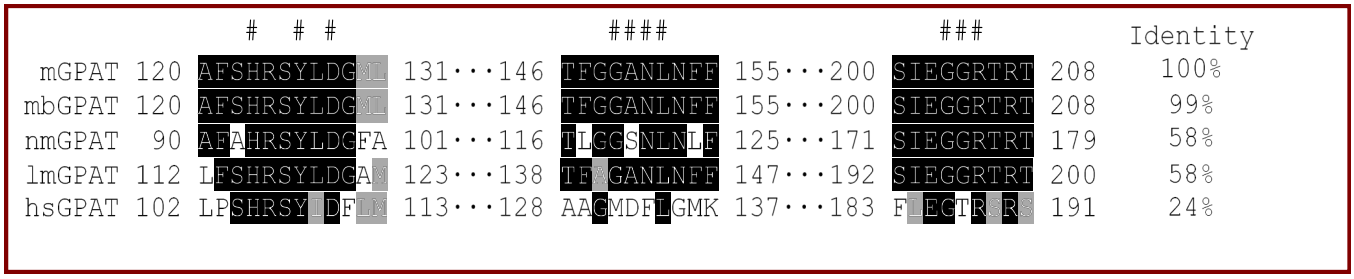

Figure 1: Sequence alignment of mGPAT with GPAT homologues from *Mycobacterium bovis* (mb), *Nakamurella multipartita* (nm), *Luteipulveratus mongoliensis* (lm), and *Homo sapiens* (hs). Identical (black) and similar (gray) amino acids are shaded and hashtags denote components of acyl-acceptor binding pocket. Identity refers to homology between related proteins and the entire mGPAT enzyme.

Method

In order to determine whether *mGPAT* made a difference in endogenous or exogenous fatty acid incorporation into phospholipids, we grew *E. coli* with and without *mGPAT* in LB media to an OD of 0.6-0.8 where we induced the cultures with IPTG. Aliquots were taken from those cultures and incubated with ^{14}C-palmitate and ^{14}C-acetate, lipid products were extracted with chloroform-methanol and chloroform, and then resolved on thin layer chromatography (TLC) plate. The TLC plate was exposed to an X-ray film at -80°C for one week, and radioactivity in the phosphatidylethanolamine (PE) and phosphatidylglycerol (PG) bands were measured by scintillation counting. Finally, to see if *mGPAT* encoded an active enzyme an assay was performed in a similar fashion to the previous assay except that after being induced, the cells were lysed and incubated with a reaction mix containing ^{14}C-glycerol-3-phosphate and palmitoyl-CoA, and radioactivity in the lysophosphatidic acid/phosphatidic acid (LPA/PA) band was measured.

Results

Metabolic incorporation of fatty acids showed that *E. coli* cells transformed with *mGPAT* formed more PE and PG derived from ^{14}C-acetate during stationary phase, but the presence of the gene made no difference in PE and PG formation when supplied with ^{14}C-palmitate (**Fig. 2**).The autoradiogram revealed that there was greater LPA/PA product formation for cell lysates containing mGPAT (**Fig. 3a**), and cell lysates containing mGPAT displayed nearly twice the enzymatic activity of lysates without mGPAT (**Fig. 3b**).

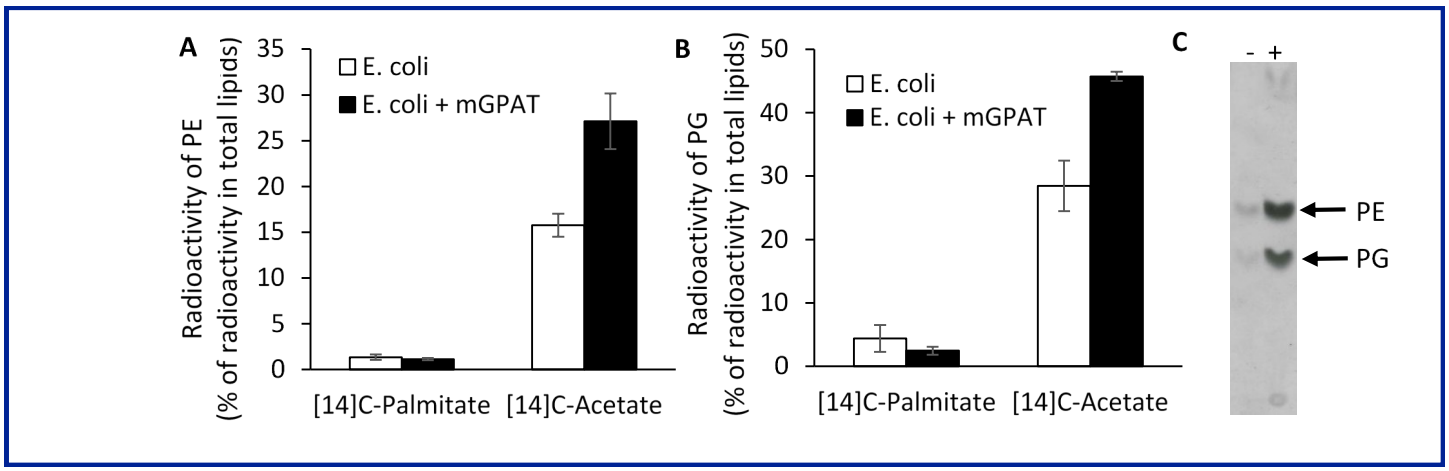

Figure 2: Radioactivity of A) PE and B) PG formed by *E. coli* (-) and E. coli + *mGPAT* (+) grown in Luria-Bertani broth during stationary phase. Average + standard deviation. C) Autoradiogram of TLC plate showing formation of PE and PG radiolabeled with ^{14}C-acetate.

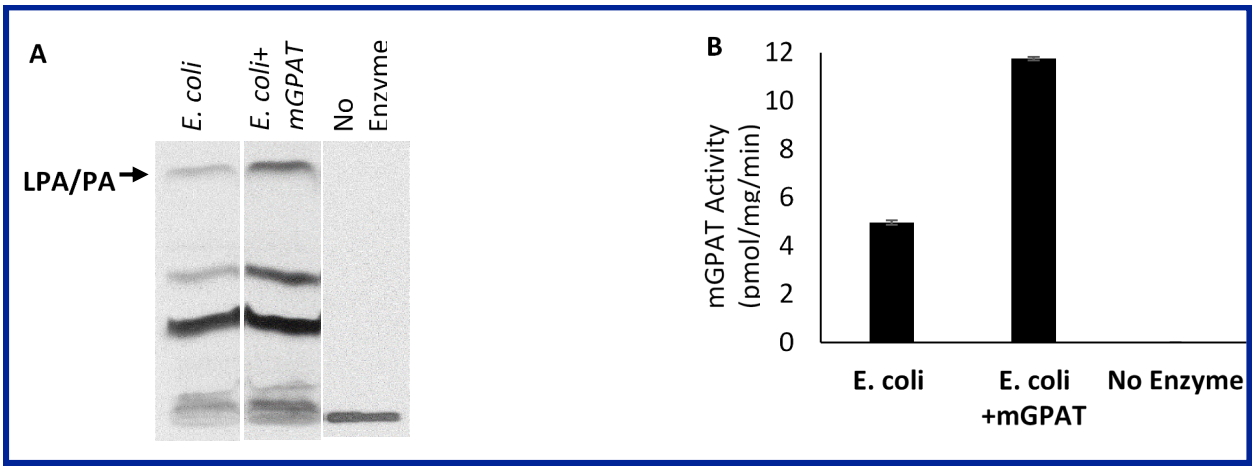

Figure 3: A) Autoradiogram of TLC plate showing formation of radiolabeled LPA/PA product. B) Specific activity of *E. coli* + mGPAT from forming radioactive LPA/PA product from [14C]-G3P. Average + standard deviation.

Discussion

Sequence alignment suggests that the Rv1551 encodes a GPAT, metabolic labeling suggests that the presence of mGPAT enhances the ability of *E. coli* cells to incorporate ^{14}C-acetate into PE and PG, and the autoradiogram and enzymatic activity assay suggest that mGPAT is an active enzyme. Future studies will involve purification and biochemical characterization of mGPAT and generating a gene-knockout mutant in *Mycobacterium smegmatis* to study the effects of the loss of the gene.

References

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Korb VC, Chuturgoon AA, & Moodley D (2016) Mycobacterium tuberculosis: manipulator of protective immunity. International Journal of Molecular Sciences 17(3):131

Second Place Undergraduate Division Winner

Mycobacterial Protein mEttA and its Role in Resuscitation from Stationary Phase

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Introduction

Tuberculosis (TB) is a leading infectious disease killer, caused by the infectious agent *Mycobacterium tuberculosis* (*Mtb*). Although the active disease is curable and preventable, the disease is very widespread. Approximately one-third of the world population harbors the dormant form of *Mtb*, which causes the multidrug resistant, long-lived latent infection of TB. People harboring this latent infection do not have the active disease and cannot transmit the disease, but may develop the disease later on in life (WHO 2014).

A mycobacterial gene *Rv2477c* has been found to be highly homologous to a gene encoding protein EttA (energy translational throttle A) in *Escherichia coli* (*E. coli*). EttA is part of the bacterially ubiquitous ABC-F (ATP-binding cassette F) family of which no protein has been fully functionally characterized as of yet. It is thought that EttA in particular is a translation factor involved with resuscitation from stationary phase, and could further be involved in dormancy. It functions to suppress protein synthesis by the ribosomal regulation of entry into the elongation cycle after the formation of the first peptide bond in energy depleted cells with a lack of ATP in the surrounding environment. When ATP levels are restored, hydrolysis occurs, allowing EttA to be disengaged from the ribosome and enabling protein synthesis to proceed into the elongation cycle of translation (Böel 2014).

In this project, the product of *Rv2477c*, termed as mEttA, is expressed in strains of *E. coli* retaining and lacking their native EttA in order to investigate its possible role in cell regrowth after residency in long term stationary phases. Growth is quantified as CFUs/ml (colony forming units per milliliter). We hypothesize that mEttA will positively affect the resuscitative capabilities of the *E. coli* cells because of its high similarity and homology with proposed translation factor EttA.

Methods

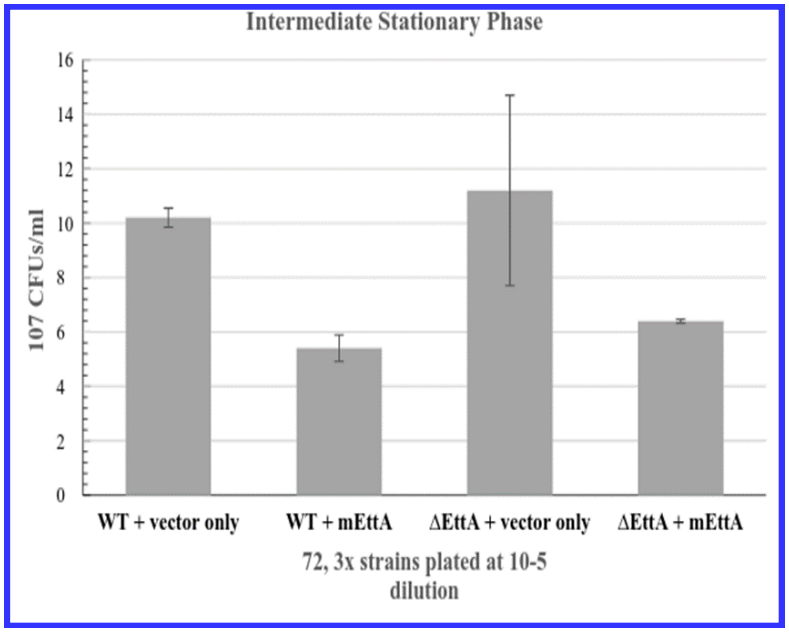
The host strain used in this project was MG1655. Four strains of *E. coli* were used in this project, specifically the WT (wild-type) strain expressing mEttA, the WT strain lacking mEttA, the ΔEttA mutant strain expressing mEttA, and the ΔEttA mutant strain lacking mEttA. The expression of mEttA in the ΔEttA strains and in the pBAD vector was confirmed in SDS-PAGE and PCR, respectively, in preliminary work.

Freshly transformed overnight cultures of these strains were diluted 100-fold into fresh LB media. These original stationary-phase cultures were grown for 72 h and 144 h for their respective time interval cultures. The 72 h interval served as the intermediate stationary phase and the 144 h interval served as the prolonged stationary phase. 1000-fold dilutions into fresh LB media were executed every 72 h for the 72 h culture and every 144 h for the 144 h culture for each resuscitation. Three resuscitations were performed for the 72 h culture while two resuscitations were performed for the 144 h culture. The 144 h culture had a special regrowth condition where an additional 1000-fold dilution into fresh LB media was conducted after the second resuscitation, further allowed to grow overnight. All cultures were selected for with 50mg/ml antibiotic carbenicillin and induced with 20% L-arabinose at all times of growth in accordance with the requirements of the pBAD vector.

E. coli cells of the third resuscitation of the 72 h culture and the special condition second resuscitation of the 144 h culture were serial diluted and plated onto LB agar plates and incubated at 37 °C overnight. Growth was then enumerated as CFUs/ml. Duplicates of the plates were prepared, from which averages and standard deviations were calculated.

Results

The insertion of mEttA (*Rv2477c*) suppressed cell regrowth after residency in both the intermediate and prolonged stationary phases for the WT and ΔEttA strains as exhibited by the plates prepared at 10^{-5} dilutions. Two additional repeats were conducted where the same effect was seen [data not shown]. Across the repeats, variable growth levels were seen between the experimental and control WT and ΔEttA strains, but the primary effect seen was the suppressed resuscitative abilities of those with the mEttA insert (Figures 1 and 2).



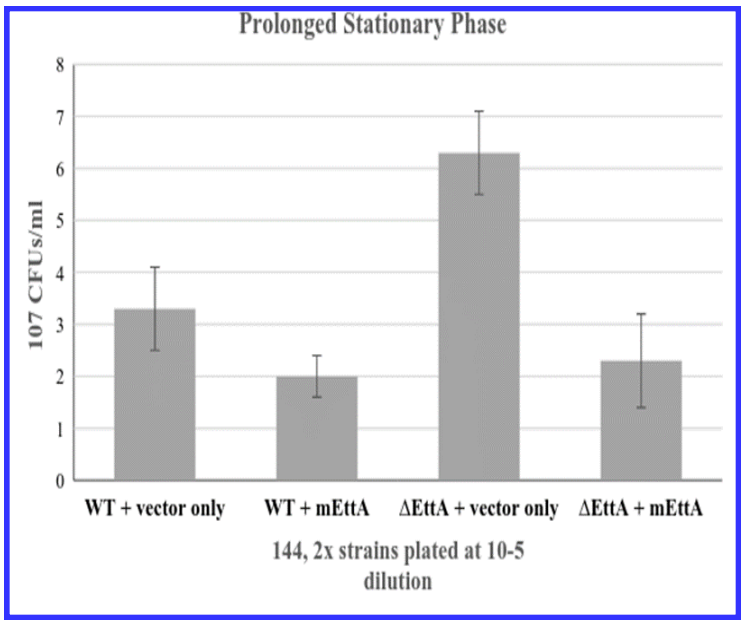


Figure 1. Growth rates of wild-type (WT) and EttA mutant (ΔEttA) *E. coli* in intermediate stationary phase. Expression of mycobacterial mEttA in both wild type and ΔEttA *E. coli* strains suppresses growth rate in resuscitation from intermediate stationary phase, as demonstrated by the third resuscitation of 72 h cultures. CFU = colony forming units. Vector = pBAD. Average ± SD from duplicates is shown.

Figure 2. Growth rates of wild-type (WT) and EttA mutant (ΔEttA) *E. coli* in prolonged stationary phase. Expression of mycobacterial mEttA in both wild type and ΔEttA *E. coli* strains suppresses growth rate in resuscitation from prolonged stationary phase, as demonstrated by the second resuscitation of 144 h cultures. CFU = colony forming units. Vector = pBAD. Average ± SD from duplicates is shown.

Discussion

mEttA appears to have an inhibitory effect on resuscitation in long-term stationary phase for both the wild type and ΔEttA strains, contrary to our hypothesis (Figs. 1 and 2). mEttA could be toxic to the wild-type strain's native EttA, consequently interfering with the intrinsic cellular machinery leading to decreased fitness. Furthermore, mEttA could be detrimental to the ΔEttA strain due to protein-protein specificities in the sense that differences in a few amino acids could greatly affect protein functionality, especially at highly conserved sites.

In conclusion, mycobacterial mEttA appears to impair cell regrowth in *E. coli* cells after residency in long-term stationary phases. In the future, we aim to successfully run an SDS-PAGE confirming protein expression in both the WT and ΔEttA strains and to conduct competition assays of WT and ΔEttA strains with and without the expression of mEttA with growth and genetic presence confirmed by PCR (polymerase chain reaction) analysis. We also intend to isolate the mEttA protein in order to conduct ATPase assays with mEttA. Finally, we plan to study the effects of mEttA in a mycobacterial host.

References

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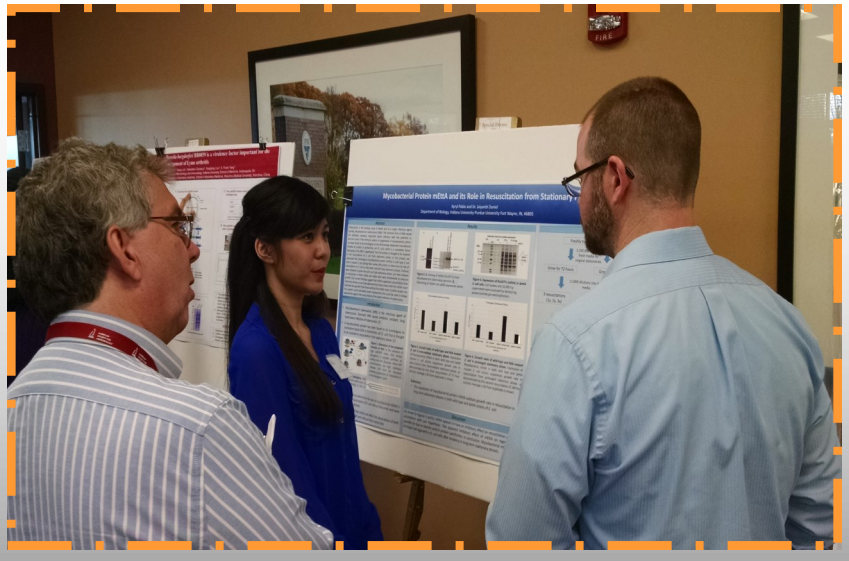
Photos from the 2016 Annual Meeting



MICROBIOLOGY IN THE NEWS

https://www.eurekalert.org/bysubject/index.php?kw=33

Complex bacterium writes new evolutionary story

PLOS ONE

February 1, 2017

A University of Queensland-led international study has discovered a new type of bacterial structure which has previously only been seen in more complex cells. Research team leader UQ School of Chemistry and Molecular Biosciences microbiologist Emeritus Professor John Fuerst said the study had found pore-like structures in a bacterium called *Gemmata obscurglobus*."The pore-like structures appeared embedded into the bacteria's internal membranes, and showed some structural features similar to those in more complex organisms," he said. "This is a remarkable evolutionary finding, since most bacteria do not possess these structures. "Finding nuclear pore-like structures in the bacterial species *Gemmata obscuriglobus* is significant for understanding how the cell nucleus and the pores embedded in its membrane envelope could have evolved - a major unsolved problem in evolutionary cell biology."

Discovery helps explain why only some people develop life-threatening dengue infections

Science

January 31, 2017

For most people who contract it, dengue fever is a relatively mild-mannered disease--at least the first time around. For some, however, a subsequent infection by the virus unleashes a vicious and potentially deadly illness. New research from a team based at The Rockefeller University has begun to reveal why certain people are more vulnerable to these dangerous secondary infections. Their latest findings could lead to better strategies to identify and better treat those most at risk.

Yeast mutants unlock the secrets of aging

Frontiers in Genetics

January 31, 2017

Yeast -- it's more than just a fungus. It can also tell us a lot about growing older. That's because aging in both human and yeast cells is not only the result of passive wear and tear. It's also caused by an active process orchestrated by a distinct set of genes, some of which slow down aging while others speed it up. In two recently published articles, Concordia biology professor Vladimir Titorenko from the Faculty of Arts and Science and a team of fellow researchers take a closer look into what these delaying and accelerating yeast genes might mean for humans.

Important Dates

March 1, 2017:	Registration form due for Annual IBASM meeting
March 8, 2017:	Abstract form due for Annual IBASM meeting
March 31-April 1, 2017:	Annual IBASM meeting, Turkey Run State Park, Marshall, IN
June 1-5, 2017:	117th Annual Meeting of the ASM, New Orleans, LA

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