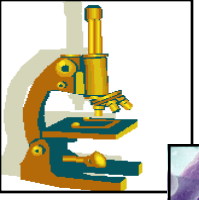
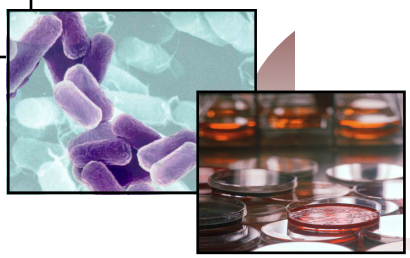
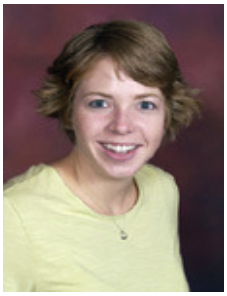




IBASM NEWSLETTER

Volume 14, Issue 1
August, 2011

Greetings from the President: Jennifer Metzler



I hope this edition of the newsletter finds you all well and enjoying a bit of relaxation as the summer is quickly slipping away from us and we ready ourselves for back to school time.

Our spring meeting this year at Brown County State Park in Nashville was very successful and much thanks to all who attended and worked so hard in the planning and execution of the event. Special thanks go to our President-Elect Rebecca Sparks-Thissen for her planning efforts for this meeting. The student posters and presentations were fantastic, so please encourage your students to attend next year, as it is such a great environment to present their work and get some really useful feedback and ideas for their research. Plus, we had some representatives from schools who have not attended the meeting recently, so that was very exciting for our organization. We are already working hard on putting together our Spring 2012 meeting, which will be held at the campus of Wabash College in Crawfordsville. Look for details on this meeting in our next edition. Some of our planning efforts involve working on a mechanism to do online registration and payment for the meeting, setting up some networking time with various companies and government organizations in Indiana for our student members to explore internship and networking opportunities, and also organizing a panel discussion with individuals using their microbiological training in varied careers. So, if you have any ideas for any of these efforts please do not hesitate to contact me at jametzler@bsu.edu.

I am happy to announce we will be continuing our grant opportunity for both undergraduate and graduate /professional students in good standing with IBASM to apply for research grants of up to $1000. One award will be made in each student category. The awards are intended to offset costs to purchase laboratory/field supplies, to support travel, and provide other items required to conduct novel scientific research. A stipulation of getting a grant is that you must present an oral presentation at the spring meeting within two (2) calendar years of the award being made. Please take a look at the program announcement, which contains all the important application details, in this edition of the newsletter.

I would also like to send out a call for nominations for President-Elect, self-nominations are welcome. Our current President-Elect will be assuming the presidency in May of 2012, so we will be electing her replacement at the spring 2012 meeting. In the next edition of our newsletter I would like to have biographies of all nominees presented, so that our members can make an informed decision when they vote at the spring meeting. Please send all nominations to me at jametzler@bsu.edu. I would ask that I have all of these nominations by November 1, 2011 so as to give me time to ask the individuals if they accept their nomination and then give them time to write their biographical sketch. For those who are unaware, the primary job of the President-Elect is to handle the planning and registration efforts for the annual meeting. As always, if you have any suggestions

In this issue:

or ideas, I would love to hear them, or if you have any concerns or ideas for IBASM in general feel free to contact me at jametzler@bsu.edu.

Message from the President– Elect — Becky Sparks-Thissen

I hope you've had a productive and relaxing summer! I enjoyed meeting many of your at our meeting in April and look forward to seeing you again for next year's meeting. Congratulations to all who gave a poster or a talk! I continue to be impressed with the level of your enthusiasm and quality of the science you present.

Keep our annual meeting in mind as you plan out your schedules for the upcoming academic year. It will be held at Wabash College, April 20-21, 2012. As most of you already know, the annual meeting is a good place for undergraduate students, graduate students and postdocs to present their research. It has been particularly useful for my students, coming from a small institution where they are the only students working in microbiology, to get to know other students who have similar interests. We hope to add a panel on potential careers in microbiology to the schedule to give students a chance to interact with individuals who have chosen different careers paths using their microbiology degrees.

Best wishes for the upcoming fall semester.

Message from the Student Representative — Lindsey Steiner

I hope everyone enjoyed the 2011 IBASM Spring Meeting! I was thrilled to see the number of students who attended the meeting, and even more excited about the increase in the number of student presenters this year! I want to continue this forward momentum into the following year by providing students with even more opportunities to be active within the organization.

First, I am looking for student feedback on the types of professionals you would like to see in a panel focused on answering questions about career opportunities. One idea I had was to include professionals with different degree levels to learn about the types of careers available or not available to them based on their qualifications within the field of microbiology. I would like to include someone each with a bachelor's degree, MD, Ph.D, and MD-Ph.D, and may also include someone from both academia and the industry. I would like some feedback on whether this is something you would be interested in, or if there is a different type of comparison you would like to see within the panel. I am very open to new ideas, and I would be glad to begin putting this together!

Additionally, our IBASM Awards Committee is now looking for nominations for the 2012 IBASM Academic Teaching Award. Please take this opportunity to recognize a professor that has made an impact on your education and/or career aspirations. The professor does not have to me an IBASM member initially, but may become one prior to the next spring meeting. However, the student nominator does need to be a member. If you have any questions, please contact Dr. Dominique Galli at dgalli@iupui.edu.

Finally, I am planning to include a student meeting during the 2012 Spring Meeting, prior to the general business meeting next year so all of the students active in the organization can get together and share ideas. I will be looking for someone interested in taking over my position, so this is a good opportunity to assert yourself as a strong and involved candidate. During the meeting, we can discuss the role students play within the organization currently, and how we might want to change that for the future. If you have anything in particular you would like to discuss, please let me know and I will put together a discussion outline!

If you have any suggestions or questions, don't hesitate to contact me at lmsteiner@bsu.edu.

Special Thanks to All Judges!

On behalf of all of the students in the poster competition I would like to express sincere appreciation to all of the members who volunteered their time to judge at the meeting. Students were evaluated in 5 different categories: professional appearance, scientific thought, creativity, thoroughness and presentation (abstract, oral and poster). This was no easy task! Next time you see any of these persons please thank them for sweating through a very difficult challenge:

Team #1: Undergraduate division = *Dominique Galli (IUSD) and Jeffrey Hughes (Millikin Univ.)*

Team #2: Undergraduate division = *Jennifer Metzler (BSU) and Jim Tiedje (Michigan State Univ.)*

Team #3: Undergraduate division = *Carolyn Vann (BSU) and John McKillip (BSU)*

Team #4: Undergraduate & MS divisions = *Becky Sparks-Thissen (WC) and Kenneth Noll (Univ. Connecticut)*

Team #5 MS division = *Rich Gregory (IUSD) and Dina Leech (DePauw Univ.)*

Team #6 Ph.D. division = *Douglas Stemke (UI) and Jim Mitchell (BSU)*

From the Desk of Jim Mitchell-...Educational Representative

Students contributed a total of 32 posters and 5 oral presentations at the IBASM meeting. The quality of all the presentations was awesome and it was very informative for me to see the range of different research areas. Jennifer Yu (IUB) and Grace Walworth (Millikin Univ.) won first and second place, respectively, in the undergraduate division. Pierre Nimmer (BSU) received the *Leland S. McClung* award for 1st place in MS division and Leslie O'Neill (BSU) received 2nd place. Anirban Chakraborty (IUB) received the *Leland S. McClung* award for 1st place and Kyle Hetrick (IUB) for 2nd place in the Ph.D. division.

Congratulations !!! The socializing which occurred during the open poster session was almost deafening at times, but a great opportunity for students to visit with each other and also interact with professionals who can provide valuable ideas and advice for future education and employment. All of us who viewed the poster segment and attended oral presentations look forward to even a greater number of participants next year, and I hope to possibly see students compete in the high school division. Winners receive a certificate and a monetary gift when a short paper is published in the IBASM newsletter.

IBASM Academic Teaching Award 2012

Do you have a great professor who deserves a teaching award? We call on all student IBASM members to nominate their favorite lecturer/instructor for the IBASM Academic Teaching Award 2012. Your nomination letter should explain why you think your teacher deserves the award. Please provide as many details as possible. Also, your letter will carry more weight if you can get some of your peers to co-sign it.

The IBASM Awards Committee consisting of Dominique M Galli (IU School of Dentistry), John McKillip (Ball State University), and Doug Stemke (University of Indianapolis) will select the awardee based on your letter and additional information obtained from the nominee's departmental chair. The award will be presented at the IBASM Annual Spring Meeting in 2012 where the awardee will be expected to give an oral presentation. Note, that the awardee must be a member of the IBASM at the time the award is received. Please send your nominations to dgalli@iupui.edu on or before November 1, 2011.

An Exciting Opportunity

I. SUMMARY & ANNOUNCEMENT

The Indiana Branch of ASM announces an opportunity for undergraduate or graduate/professional students in current good standing with IBASM to apply for research grants of up to $1000. One award will be made for undergraduates and one for the graduate/professional student category. The awards are intended to offset costs to purchase laboratory/field supplies, to support travel, and provide other items required to conduct novel scientific research. The deadline for the electronic submission of the proposal to the IBASM President is November 1, 2011 (see below for additional details). Awardees are expected to deliver an oral presentation of their findings at the Annual Spring IBASM meeting within two (2) calendar years of the award being made.

III. ELIGIBILITY

Student applicants who meet any of the following criteria are eligible to apply for research grants:

- Current student members of IBASM in good standing during the preceding year and at the time of the application who are undergraduates, graduate students (masters or doctoral level), or professional students with a G.P.A. of 3.25 or higher (on a 4.0 scale).

Awards will be made to the academic institution through the relevant grants/sponsored programs office, not to an individual. Therefore, each application must be signed by the organization's official with the authority to approve the request (e.g., President, Chief Academic Officer, College or University Research Officer, etc.) and commit the institution to the conditions of the award. This information is to be included on the mini-grant cover page and, if awarded, will necessitate an account be set up for the student use.

IV. APPLICATION PROCEDURE

Submit the grant application to the President of IBASM (Dr. Jennifer Metzler, Ball State University, jametzler@bsu.edu). Each research proposal should be brief but complete and must include the following information arranged in the following order, with each section starting on a new page:

1. A COVER PAGE FORM (see attached) giving the name, mailing and e-mail addresses, and affiliation of the student investigator and of the faculty mentor, together with a descriptive title for the proposed project. The cover page should also include the date of submission; the level of funding requested; the name of the person authorized to make grant-related commitments on behalf of the applicant organization; and, the name and address of the institutional official to whom the check should be sent (again, note that award checks are made out to the institution,

not to any individual). Student applicants are also asked to include a statement as to whether the requested funding is for a 'new' project, or an 'ongoing' one with current funding. Also include status of compliance.

2. An ABSTRACT of no more than 300 words.
3. A NARRATIVE of *no more than three pages* (single or double spaced with double spaces between paragraphs, 10-12 point font, 1" margins). This section must

   - describe the problem to be investigated and justify the proposed study,
   - provide a review of the published relevant literature,
   - state the research objectives, hypothesis, scope of the problem, and methodology to be followed,
   - describe previous research, if applicable,
   - include 1-2 sentences on the timeline of the proposed activities, and
   - a brief statement on how the research results will be disseminated.
   - If applicable, include approx. ½ page of preliminary data

   This is an important part of the proposal and will be scrutinized by the reviewers. Applications with a narrative exceeding three pages may be returned without review.
4. A REFERENCES CITED page listing all literature cited in the application (ASM style, not included in 3 page limit).
5. A *detailed* BUDGET. The budget may include

   - supplies (defined as items that by their nature will be consumed during the course of the research),
   - vehicular travel to field sites or related; travel outside of Indiana is not supported

   The budget *may not* include funds to

   - attend meetings,
   - pay publication costs,
   - support institutional administrative or overhead/indirect costs,
   - pay salaries to applicants or sponsors,
   - sponsor pedagogical research, or
   - purchase computers or computer time.

   The budget must also describe the expected or actual source of funding for resources essential for the project not included in the request to IBASM.

   It is essential to understand that enough detail must be provided to allow members of the IBASM Executive Committee to evaluate the appropriateness of each element of the budget. The Committee may delete items from the request that it feels are not justified as long as it believes the project would not be unduly impaired; such a conclusion usually results in the application's rejection. Consequently, it is essential that each item be explained and justified for the request to receive full funding.

6. For work involving Human Subjects, Animal Care & Use, infectious agents, and/or recombinant DNA, a statement needs to be included indicating how and when compliance approval will be obtained from the sponsor institution. Formal compliance approval is not needed for grant awards to be made, but is required prior to the commencement of any research work.
7. Current transcript(s)
8. Support letter from faculty mentor

V. REVIEW PROCESS

The IBASM Executive Committee will review every application. The Executive Committee members represent a wide range of subdisciplines within the field of microbiology, and the results of their discussions determine which requests will be awarded and for what elements of the request. The IBASM President will inform applicants by email of the Committee's final decision and will contact the Branch Secretary/Treasurer who will then mail award checks to the official identified in the application. The review process is normally completed within six weeks of the application deadline. Names of awardees will be published in the IBASM newsletter. The decision of the Committee is final and all questions concerning awards and the decision process should be directed to the IBASM President.

In reviewing proposals for possible support, the IBASM Executive Committee will consider the following criteria:

- Significance of project
- Soundness of scientific idea
- Appropriateness of methodological approach
- Feasibility of study within given timeframe and environment
- Quality of preliminary data (if applicable)
- Appropriateness of budget
- Completeness and clarity of proposal

No grant proposal, however worthy, may request more than $500; proposals requesting more than this amount will not be reviewed.

VI. GENERAL POLICIES

Grants will normally be made for investigative periods of one year beginning on the date of the award letter unless otherwise requested in the grant application, approved by the Committee, and specifically mentioned in the award letter.

Unexpended funds are to be returned to the IBASM Secreatry/Treasurer.

Oral presentation of research results at the Annual Spring IBASM meeting expected.

Publications or presentations resulting from projects supported by IBASM mini-grant funds must acknowledge Branch support.

VII. REQUESTS FOR CHANGES IN FUNDED REQUESTS

Requests for extensions, without additional funds, or requests for redirection of funds, will be made on a case-by-case basis through individual requests to the IBASM President.

VIII. APPLICATION DEADLINES AND SUBMISSION DETAILS

Applications for IBASM Student Research Mini-Grants **must be postmarked or received by Tuesday, November 1, 2011** and must arrive within three days of this deadline. Receipt of application will be acknowledged by electronic mail within a week of the final deadline date. Grant announcements will normally be made by the IBASM President within six weeks of the deadline date. Applicants whose requests are denied will be notified on or soon after the corresponding award announcement dates.

CHECKLIST

- ✓ Cover sheet with all requested information (included with this announcement)
- ✓ Abstract of 300 words or less
- ✓ Project description that follows general guidelines provided in this announcement
- ✓ Statement of compliance approval
- ✓ Preliminary data, if applicable
- ✓ Budget of $1000.00 or less with justification(s)
- ✓ Transcripts (e-mail electronic copies or post hard copies)
- ✓ Faculty mentor support letter (e-mail electronic copies or post hard copies)

Applications should be mailed or e-mailed to:

Dr. Jennifer Metzler
IBASM President
Ball State University
Department of Biology
2000 W. University Ave.
Muncie, IN 47306
jametzler@bsu.edu

IBASM Student Research Grant Cover Page

Complete the following information below and save/send this cover page with the other proposal components to the contact provided in guidelines. ***Deadline:*** November 1, 2011

Name: **Institution:**

Address:

Phone: **Fax:** **email:**

Please indicate your student status:
__ **Undergraduate**
__ **Graduate (M.S/M.A. or Ph.D./Ed.D.)**
__ **Professional**

Research advisor: **Institution:**

Address:

Phone: **Fax:** **email:**

What is the title of your proposed project?

Date: **Total funding amount requested:**

Indicate whether this project is:
__ **New project/no current funding**
__ **Ongoing project/current funding**

Compliance status:

Name & address of the institutional official to whom the check should be sent, if funded:

Name & address of the person authorized to make grant-related commitments on behalf of the organization:

McClung First Place Graduate (M.S. Division) Winner

Effect of Carvacrol on *hblC* and *nheA* Gene Expression in *Bacillus cereus* for the Treatment of Endophthalmitis

Pierre Nimmer and John McKillip
Ball State University, Department of Biology, Muncie, IN

Introduction

Endophthalmitis is a rare but very serious eye condition where the bacterium (often *Bacillus cereus*) enters the eye through blunt trauma, intraocular contamination through surgery (1), or a systemic infection as a result of the breakdown of the blood-ocular barrier (2). The Hbl protein from *B. cereus* can cause irreversible tissue damage to the photoreceptors of the retina in less than 24 hours causing the patient to go blind in the affected eye (3). In most instances of *B. cereus* induced endophthalmitis, vision loss occurs regardless of the type of therapeutic or surgical intervention utilized (4).

Currently there is no suitable agent to decrease the inflammatory response specifically for the eye's tissue architecture. Carvacrol (key extract in oil of oregano) has previously been shown to kill *B. cereus* (5, 6) along with containing anti-inflammatory properties (acts as a COX-2 inhibitor) (7). Since the human eye contains the proinflammatory COX-2 enzyme in the cornea, iris, ciliary body, and retina (8), we hypothesize that carvacrol will kill *B. cereus*, neutralize its toxin production, along with decrease the inflammatory response in the surrounding ocular tissues thus protecting the retina. We tested various doses of carvacrol on *B. cereus in vitro* in order to assess its effects on HblC and NheA toxin production and to see which carvacrol dose would be most clinically relevant.

Experimental Methods

Bacterial Growth Curves: determine *B. cereus* ATCC14579 growth kinetics in order to know what volume to seed SIC tubes with 100 CFU of bacteria per tube.

Subinhibitory Concentration Study (SIC): determine level of carvacrol to sublethally injure *B. cereus.*

Minimum Bactericidal Concentration (MBC) study: determine level of carvacrol needed to completely kill *B. cereus.*

RNA standard curves: used to standardize the RNA samples in this study.

real-time Reverse Transcriptase Polymerase Chain Reaction (real-time RT-PCR): target mRNA for *hblC* and *nheA* genes.

Immunoassays: target HblC and NheA toxin proteins.

Transmission electron microscopy: assess cell membrane damage to *B. cereus* in response to carvacrol.

Flow cytometry: assess number of intact *B. cereus* cells left after carvacrol exposure.

Results

Results suggest that SIC levels (1mM) of carvacrol downregulates mRNA expression of *hblC* 12.9% (14.96 ± 0.46 control vs 16.89 ± 0.81 carvacrol-treated) and the positive control *gyrB* 30.7% (15.46 ± 0.94 control vs 20.21 ± 1.9 carvacrol-treated) (Table 1). Enzyme-linked immunosorbent assay (ELISA) analysis revealed a 46.8% increase in NheA protein toxin (0.231 ± 0.014 control vs 0.339 ± 0.019 carvacrol-treated) while reverse passive latex agglutination (RPLA) analysis revealed a 50% increase in HblC protein toxin expression in response to SIC levels of carvacrol (Table 2). However, a 2 mM (MIC) or 8 mM concentration of carvacrol inhibited any detectable level of either the HblC or NheA proteins. Minimum bactericidal concentration (MBC) studies revealed that 11mM of carvacrol is needed to completely kill *B. cereus* (not shown). Transmission electron microscopy (TEM) revealed cell membrane damage to *B. cereus* in response to carvacrol (Fig. 1). Flow cytometry revealed a decrease in intact *B. cereus* cells (2.2% control vs 0.1% carvacrol-treated) exposed to the following doses of carvacrol: 1mM (SIC), 2mM (MIC), 8mM, 64mM, and 128mM (not shown).

Table 1: 1mM carvacrol (SIC) exposure causes mRNA downregulation to the following genes: gyrB, hblC, and upregulation of the nheA**.**

Genes	***B. cereus* control average C_T value**	**Carvacrol treated *B. cereus* average C_T value**	**mRNA expression levels in response to carvacrol**	**P value**	**Statistically Significant**
gyrB (positive control)	15.46 ± 0.94 (n=14)	20.21 ± 1.9 (n=14)	30.7% decrease	0.035	Yes
hblC	14.96 ± 0.46 (n=36)	16.89 ± 0.81 (n=36)	12.9% decrease	0.04	Yes
nheA	14.56 ± 0.62 (n=17)	13.85 ± 0.50 (n= 17)	4.9% increase	0.381	No

***B. cereus* enterotoxin tested**	**Method**	**Type of test**	***B. cereus* control sample toxin expression level**	**1 mM carvacrol (SIC) treated *B. cereus* toxin expression level**	**2 mM carvacrol (MIC) treated *B. cereus* toxin expression level**	**8 mM carvacrol treated *B. cereus* toxin expression level**	**Percent increase in toxin expression at 1 mM carvacrol (SIC) compared to controls**
HblC	RPLA	Qualitative	titer 32	titer 64	0	0	~ 50%
NheA	ELISA	Quantitative	0.231±0.014 (n=3) absorbance (490 nm)	0.339±0.019 (n=3) absorbance (490 nm)	0	Not tested	46.8%

Table 2: ***ELISA analysis illustrates a 46.8% increase in NheA protein toxin in response to 1 mM carvacrol (SIC) exposure while RPLA analysis illustrates a 50% increase in HblC protein toxin expression in response to SIC levels of carvacrol. However, a 2 mM (MIC) or 8 mM concentration of carvacrol inhibits any detectable level of either HblC or NheA proteins.***

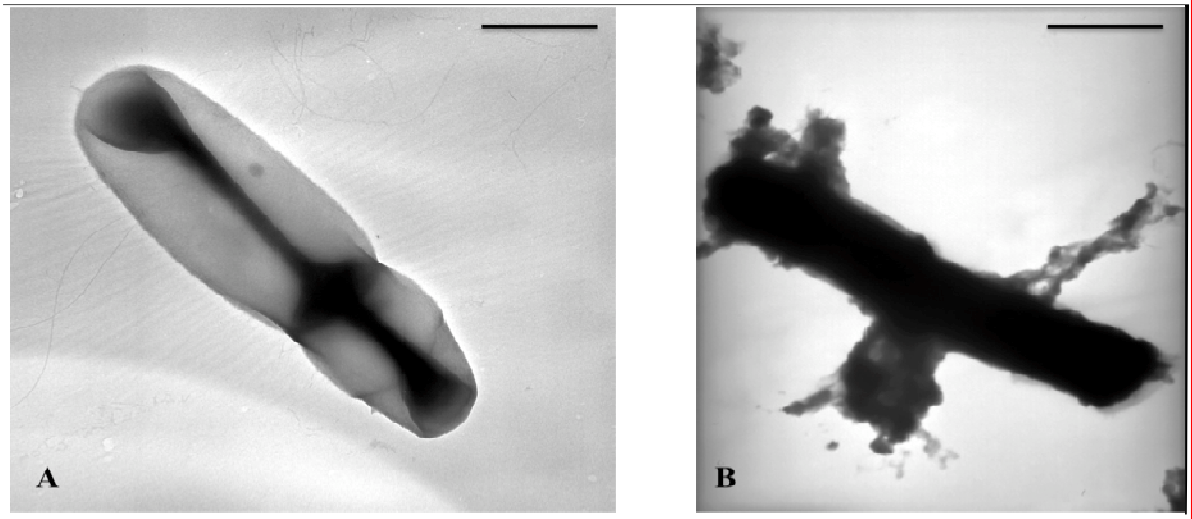


Figure 1: ***SIC levels of carvacrol (1mM) cause cell membrane lysis to* B. cereus *(Panel B) compared to* B. cereus *controls (Panel A).*** *B. cereus* was grown in Mueller-Hinton broth, exposed to carvacrol for 30 min, centrifuged, and washed 10 times with cacodylate buffer. The bacteria were then negatively stained with uranyl acetate. Image was acquired with a Hitachi H-600 transmission electron microscope operating at 75 kV for accelerating voltage. Image A was taken at a magnification of 12,000 and image B was at 20,000 magnification. Scale, 2.5 µm.

Discussion

In conclusion, these data suggest that an SIC concentration of 1 mM carvacrol increases production of both the HblC and NheA toxins while a 2 mM MIC concentration of carvacrol inhibits both the HblC and NheA protein toxin expression. This *in vitro* data would imply that treating patients with a MBC level of carvacrol (11mM) or above would be necessary to decrease the degree of *B. cereus* infection. The implications of this study may further support better treatment options for this serious eye condition.

References

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2. **Romero, C.F., M.K. Rai, C.Y. Lowder, and K.A. Adal.** 1999. Endogenous endophthalmitis: a case report and brief review. Am. Fam. Phys. **60:**510-514.
3. **Callegan, M.C., B.D. Jett, L.E. Hancock, and M.S. Gilmore.** 1999. Role of hemolysin BL in the pathogenesis of extraintestinal *Bacillus cereus* infection assessed in an endophthalmitis model. Infect. Immun. **67:**3357-3366.
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8. **Wang, J., Y. Wu, S. Heegaard, and M. Kolko.** 2009. Cyclooxygenase-2 expression in the normal human eye and its expression in selected tumours. Acta. Ophthalmol. 1-5.

Award papers from other winners will be published in the next issue of the newsletter.

Second Place Undergraduate Winner

Possible Relationship Between *in vivo* S-adenosyl-*L*-methionine hydrolase Activity and Quorum Sensing in *Escherichia coli*

Grace Walworth and Jeff Hughes
Department of Biology, Millikin University, Decatur, IL

Abstract

Recent literature has documented inter-bacterial communication through a class of N-actyl homoserinc lactone "autoinducer" molecules that mediate quorum sensing. In part, this process directs bacterial aggregation to produce biofilms in which collected cells enmeshed in an extracellular matrix accomplish more than could any individual. In previous work with S- adenosyl-L- methionine (SAM) and SAM hydrolase (SAMase), cultures of cells transformed with SAMase expression plasmids demonstrated cell "clumps." It is conceivable that SAMase activity could produce large amounts of homoserine lactone, a SAMase reaction product, that could then be exported into the media and used as an autoinducer to encourage "clumping." This mostly qualitative research suggests that SAMase expression is correlated with observed cell aggregation in a mechanism that does not involve entangled filamentous cells, excess polysaccharide glue, or other visible consequences of SAMase expression.

Introduction

Escherichia coli transformed with plasmids that direct expression of the coliphage T3 S-adenosylmethionine (SAM) hydrolase (SAMase) gene demonstrate a pleiomorphic phenotype (Hughes, et al., 1987; Macintyre, 2001; LaMonte and Hughes, 2006;). An interesting but never studied consequence of *in vivo* expression of the cloned SAMase gene is cell aggregation or "clumping." Advances in understanding biofilm formation suggest that bacteria release and perceive chemical messengers (autoinducers) through the process of quorum sensing that trigger cell aggregation during biofilm formation (Xavier and Bassler, 2005). These autoinducers include a variety of acyl-homoserine lactones, among other chemicals (Truchado, *et al*., 2009).

Interestingly, SAMase expression in *E. coli* produces large quantities and export of homoserine, a product of the hydrolysis of SAM (Hughes, 2006). This suggested three hypotheses to explain SAMase-related cell aggregation:

- Exported homoserine may adopt the lactone structure and serve as an autoinducer that promotes cell aggregation; i.e., quorum sensing lead to clump formation,
- SAMase-induced capsule production may trap cells in polysaccharide "glue"; i.e. clumps form because newly divided cells are enmeshed in a large capsule layer, or
- SAMase-induced cell filamentation causes cells to become entangled; i.e., physical tangling forms a "log jam" that has nothing to do with quorum sensing.

Clumps produced in cultures of *E. coli* transformed with SAMase expression vectors raised in M9 minimal salts media were observed for filamentation and capsule production. There were few filaments and no enhanced capsule despite the presence of clumps. Assuming cell aggregation may interfere with cell motility, cells were assayed for altered chemotaxis. These results were inconclusive, and the potential for impaired chemotaxis or other SAMase-related cell stress suggests that a different testing method must be used in the future.

Materials & Methods

E. coli K12 strains JM107, K12 (wild type), and BW545 were used. Each strain was transformed with a control (no SAMase gene) plasmid (either pBR332 or pUC18) or a SAMase-containing plasmid (pHBBR2). Plasmids were transformed with incubation in $CaCl_2$ followed by heat and cold shocks; these and all bacterial manipulation procedures were

performed with standard procedures (Miller, 1972).

The chemotaxis tests were performed on soft agar (0.4%) plates using three media: M9 minimal salt media (Miller, 1972), Motility Test Agar (MTA; Accu-Media #7247a), and MTA + 0.2% glucose. Each strain was stabbed into the center of five plates of each type of media, producing 90 plates total. All plates were scanned on a flat bed scanner and resulting .jpg file images were analyzed for colony area using the ImageJ program (NIH).

Cell filamentaion was assessed qualitatively by light microscopy followed by photography.

The capsule size was determined by staining a smear of cells dried onto a microscope slide with 10% nigrosin followed by photography. Capsules are indicated by clear zones around the cell.

Results

The chemotaxis/motility test showed significance differences between colony sizes of JM107 and K12 samples in MTA + 0.2% glucose media. Oddly, the strains produced contradictory growth patterns (Fig. 1). There were no statistical differences between any other cells.

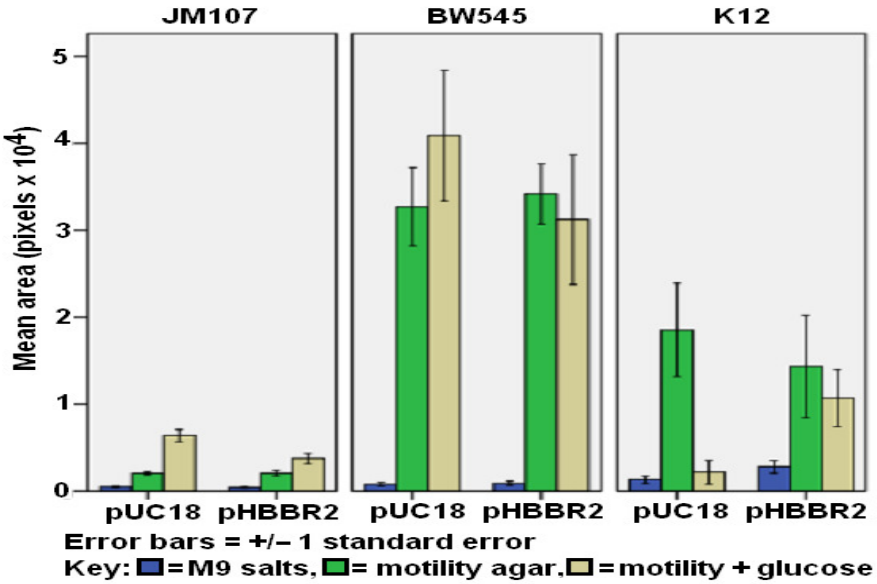

Figure 1: Motility of bacteria in three media. Five chemotaxis plates of each transformant type were scanned, analyzed with ImageJ, and averaged. Results were either insignificantly different or contradictory in ways that require further testing.

As demonstrated in Fig. 2, SAMase-containing cells in clumps were not filamented, though filamented cells were common in the culture. Cultures without in vivo SAMase expression did not have clumps and had only occasional filamented cells.

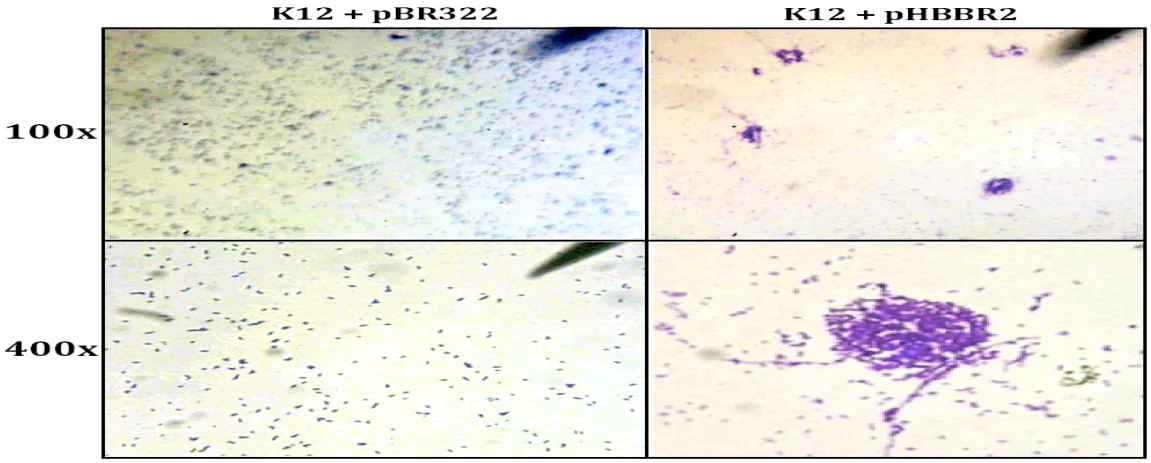

Figure 2: Cell appearance after growth in M9 minimal salts medium. pBR322 (No SAMase) transformed cells were evenly distributed in the medium while pHBBR2 (SAMase) cultures showed many cell clumps in addition to individual cells. Neither culture had many cell filaments.

The nigrosin capsule stain revealed capsules surrounding cells, but clumped cells did not share a capsule and were not apparently bound to each other in a single capsule (below).

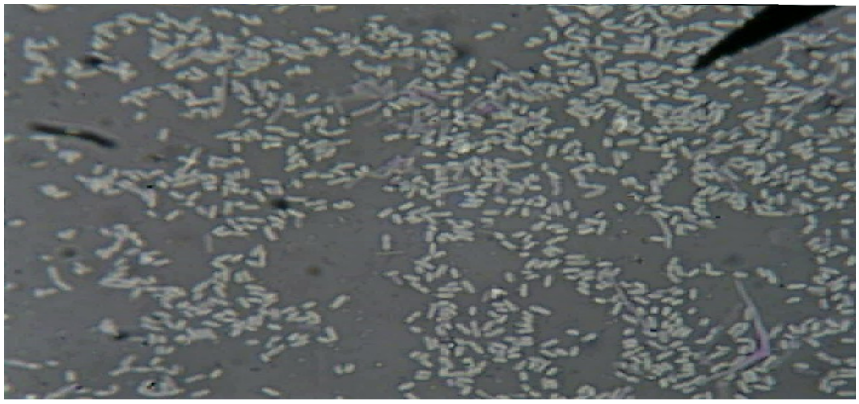

Figure 5: Nigrosin capsule stain of *E. coli* cells. Cells grown overnight in rich LB liquid medium were transferred to M9 medium with 2% glucose and 100 μg/mL ampicillin, grown with shaking to early log phase at 37°C, stained with crystal violet and nigrosin capsule stain, and then observed under the microscope at 400x.

Discussion

The motility tests results were inconclusive, but the other tests eliminated our alternative hypotheses. Clumped cells were not necessarily filamentous, so physical entanglement cannot explain these cell aggregations. Furthermore, capsules did not surround entire clumps, so it is difficult to explain aggregation as simple cell entrapment were being stuck together in polysaccharide glue from expanded capsules.

Alternate hypotheses for the clumping remain. For example, hypomethylation of chemotaxis receptor proteins inside the cell may inhibit chemotaxis, SAM deficiencies could interfere with flagellar activity, or some other SAMase-mediated disturbance of cell function could induce clumping. However, these preliminary experiments give us no reason to doubt that SAMase activity could produce enhanced autoinducer production, causing the cells to aggregate through a quorum sensing mechanism.

Understanding SAMase-induced cell clumping will require further research. We plan to test growth media for the SAMase-associated presence and quantity of homoserine lactone to verify its potential for directing aggregation. We must also grow cells lacking in vivo SAMase activity in the presence of increasing levels of N-actyl homoserine lactone and observe cell motility and aggregation. Ideally, the results of these experiments will help us better understand not only the mechanism behind cell clumping but also lead to strategies to interfere with biofilm formation, especially among pathogenic microbes for which biofilms provide protection against antibiotics and agents of the immune system.

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Photos from the 2011 Annual Meeting

Leslie O'Neill receiving her award from IBASM President **Dr. Jennifer Metzler**

Jennifer Yu receiving her award from IBASM President **Dr. Jennifer Metzler**

Pierre Nimmer receiving his award from IBASM President **Dr. Jennifer Metzler**

IBASM appreciates Dr. John McKillip's photography skills and thanks him for his continued contributions.

Highlights from the Journals of the ASM, July 2011 (from ASM Tipsheet)

How Flu Virus Spreads To College Community: Major Implications for Control

Many different strains of the H1N1 influenza virus were represented among 57 students at the University of California, San Diego (UCSD) who were infected during the epidemic in the fall of 2009, according to a paper in the July *Journal of Virology*. The findings have major implications in the controversy over how best to reduce the virus' spread.

The investigators had planned the study in the spring of 2009, after a new strain of H1N1 was identified in San Diego, and spread rapidly around the world, says coauthor Robert T. Schooley of UCSD. "We reasoned that the epidemic would resume in the fall and that the college-age population would be particularly at risk since people under age 50 had lower levels of immunity to the new strain."

The investigators theorized that if they found a single strain, or a very limited number of strains, that would indicate that spread between the campus and the general community might be reduced by quarantines, says Schooley. "But if multiple strains of virus were circulating, that would suggest multiple introductions of the virus into the college community, that would be unlikely to be interdicted by efforts at quarantine." So they set up a prospective study to collect viral isolates from students presenting with influenza-like symptoms "when the epidemic returned in the fall," says Schooley.

The investigators identified at least 21, and possibly as many as 33, different viral strains from among the 57 students. Those results suggested that the virus had been introduced repeatedly into the college population within a very short period of time, suggesting that "quarantine efforts in the college population would have a minimal effect on limiting spread of the newly emerging strain," says Schooley. More generally, he says that quarantine, class cancellation, distribution of respiratory isolation equipment, and other isolation measures "within susceptible socially active populations such as those found on college campuses is unlikely to be effective, and that other approaches such as vaccination, focused use of antiviral drugs among those with underlying illnesses predisposing to more severe illness should be emphasized instead."

(E.C. Holmes, E. Ghedin, R.A. Halpin, et al. Extensive geographical mixing of 2009 human H1N1 influenza A virus in a single university community. J. Virol. 85:6923-6929.)

Protein Boosts Lung Cancer In Smokers, Non-Smokers; Potential Anti-Oncogenic Target

Lung cancer is strongly correlated with smoking, and most lung cancer patients are current or former smokers. But it is not rare in nonsmokers. Now, a team of researchers from the H. Lee Moffitt Cancer Center and Research Institute, Tampa, FL, shows that a protein called ID1 is a key player in lung cancer in both smokers and nonsmokers. The research is published in the July issue of the journal *Molecular and Cellular Biology*.

The investigators were aware that while nicotine does not cause cancer, earlier studies, including their own, had suggested that it might promote growth and metastasis of cancers that had already formed. They exposed cultured cells to nicotine, after which these cells expressed increased levels of a protein called ID1.

"That protein was the first link between lung cancer in smokers and nonsmokers. In non-smokers, who are not exposed to copious nicotine, its expression is induced by a growth promoting protein called epidermal growth factor, which is known to be involved in cancers in non-smokers," says corresponding author Srikumar Chellappan.

The researchers then connected all this to another protein, Src, which was known to be altered in cancers, and in this altered form to promote tumor growth. "Our studies showed that inhibiting Src prevented the induction of ID1," says Chellappan. "Further, removing ID1 protein from cancer cells prevented their growth, as well as their ability to migrate or invade, which are the early steps of metastasis." They removed ID1 from the cancer cells through the use of small-interfering RNAs, which can be designed to block expression of particular proteins.

"Our studies thus show that ID1 might mediate the tumor promoting properties of nicotine, and also facilitate the growth of tumors in response to epidermal growth factor," says Chellappan. "These observations raise the possibility that targeting ID1 might be a viable strategy for combating lung cancer."

(S. Pillai, W. Rizwani, X. Li, B. Rawal, S. Nair, M.J. Schell, G. Bepler, E. Haura, D. Coppola, and S. Chellappan, 2011. ID1 facilitates the growth and metastasis of non-small cell lung cancer in response to nicotinic acetylcholine receptor and epidermal growth factor receptor signaling. Mol. Cell. Bio. 31:3052-3067.)

MICROBIOLOGY IN THE NEWS

Bacteria resists the aroma of caffeine

Sydney Herald, July 13, 2011

Tea and coffee may be linked to reducing antibiotic-resistant bacteria that healthy people carry in their noses

http://www.heraldsun.com.au/news/bacteria-resists-the-aroma-of-caffeine/story-e6frf7jo-1226093362165

Super Gonorrhea: Scientists Discover Antibiotic-Resistant STD

ABC News, July 11, 2011

Scientists have discovered a new strain of gonorrhea-causing bacteria in Japan that is resistant to available treatments.

http://abcnews.go.com/Health/Wellness/super-gonorrhea-scientists-discover-antibiotic-resistant-std/story?id=14027745

Vaccination Ruse Used in Pursuit of Bin Laden

New York Times, July 11, 2011

In the months before Osama bin Laden was killed, the Central Intelligence Agency ran a phony vaccination program in Abbottabad, Pakistan, as a ruse to obtain DNA evidence from members of Bin Laden's family thought to be holed up in an expansive compound there, according to an American official.

http://www.nytimes.com/2011/07/12/world/asia/12dna.html

Happy as a clam? Maybe not.

Virology.ws, July 6, 2011

The expression "Happy as a Clam" comes with new meaning as hepatitis A virus has been detected in clams, mussels, and oysters in markets for human consumption.

http://www.virology.ws/2011/07/06/happy-as-a-clam-maybe-not/

New Salmonella-Based 'Clean Vaccines' Aid the Fight Against Infectious Disease

Science Daily, July 6, 2011

A powerful new class of therapeutics, known as recombinant attenuated Salmonella vaccines (RASV), holds great potential in the fight against fatal diseases including hepatitis B, tuberculosis, cholera, typhoid fever, AIDS and pneumonia.

http://www.sciencedaily.com/releases/2011/06/110629122750.htm

Important Dates

February 2012:	Registration form due for Annual IBASM meeting
March 2012:	Abstract form due for Annual IBASM meeting
April 20-21, 2012:	Annual IBASM meeting at Wabash College
May 21-24, 2012:	111th Annual Meeting of the ASM, San Francisco, CA

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