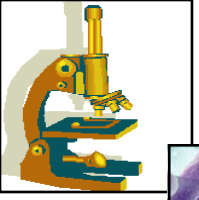
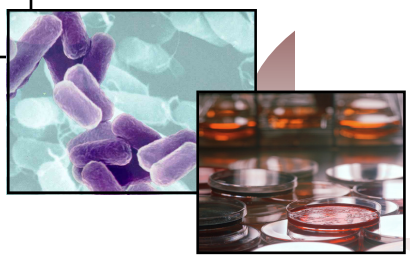
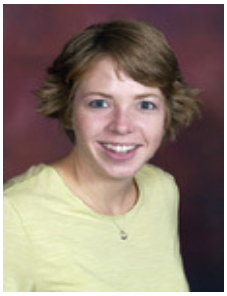




IBASM NEWSLETTER

Volume 13, Issue 1
August, 2010

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 spring semester is finally over and we transition into summer.

It is also a time of transition for us here at IBASM. I am moving into the office of the Presidency, taking the reins from our very capable former President, John McKillip. During his leadership, IBASM did some great things, including introducing the grant program I will talk about here shortly. I am also happy to announce our newly elected President-elect Rebecca Sparks-Thissen from Wabash College. She will continue planning our 2011 meeting to be held at Brown County State Park on April 15-16, so mark your calendars now. Also, please read her article in this newsletter, where she introduces herself and gives some more details about the 2011 meeting. I would also like to introduce our new student representative Lindsey Steiner from Ball State University. Be sure to check out her article in the newsletter as well. IBASM also will be revamping its website, so look for these changes to be announced in an upcoming edition of the newsletter. Much thanks goes to Melody Bernot of Ball State University for taking on the role of our new webmaster. We so appreciate the work Glenn Merkel has done for us in this role in the past.

Our spring meeting this year at University of Southern Indiana in Evansville was quite successful and much thanks to all who attended and worked so hard in the planning and execution of the event. Special thanks go to Cindy DeLoney-Marino for all the on- and presentation were fantastic, so please encourage your students to attend next year, as it is such a great environment to present their work.

IBASM would also like to congratulate our very hardworking secretary/treasurer Christian Chauret on his receipt of the Powell Service Award. Since he was not able to attend the spring meeting, the award was presented at the Executive Committee meeting on May 12. We are all continually grateful for Christian's service to our organization.

I am happy to announce a grant opportunity for both undergraduate and graduate students (M.S., Ph.D., or Ed.D.) in good standing with IBASM to apply for research grants of up to $500. Two awards 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 make you aware of several fellowship opportunities through national ASM. There are several undergraduate fellowships available to support summer research. A three year fellowship is also available for graduate students. Awardees must conduct their research with an ASM member. The Raymond W. Surber Awards recognize students at the

In this issue:

undergraduate and pre-doctoral levels for research excellence as well as potential. Please check out these opportunities at www.asm .org.

I am very much looking forward to my new role within IBASM. One of my main areas of focus will be to try and increase representation from other universities and colleges within our state, both at the full and student membership level. So, if you have any suggestions 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

First, thank you for the opportunity to work with all of you! I am an Assistant Professor of Biology at Wabash College and am beginning my fifth year at the college. I received my Ph.D. in 2000 from Princeton University in molecular biology and did postdoctoral research at Washington University in St Louis. My lab is interested in understanding the contribution of host genes to viral replication. We are currently focused on understanding which *E. coli* genes are required for bacteriophage replication. I also teach undergraduate courses in cell biology, immunology and microbiology as well as introductory biology and courses for non-majors.

Keep our annual meeting in mind as you plan out your schedules for the upcoming academic year. It will be held at Brown County state park April 15-16, 2011. 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.

I look forward to working along with Jennifer Metzler, our president and outgoing president John McKillip to plan an interesting and exciting meeting. Please let me know if you have any suggestions.

Best wishes for a relaxing and productive summer.

Message from the Student Representative — Lindsey Steiner

I am very pleased to have been chosen to be the student representative for IBASM. I am a junior biology and pre-medical student at Ball State University. My career plans include becoming an Infectious disease doctor and periodically working in Africa to provide free aid to the people. I am an active member of both IBASM and BSUASM.

As the new student representative, I plan to work with professional members to increase attendance and participation at IBASM meetings. IBASM provides excellent opportunities for students to engage with professionals in microbiology and assert themselves as strong candidates within the field. I will address how IBASM information is marketed in order to maximize its distribution and appeal to students. Also, I want to emphasize student involvement in both poster presentations and oral presentations. At the last meeting in Evansville, students passed up the opportunity for personal growth and exposure through an oral presentation. I consider taking advantage of these opportunities to be key in student development and networking. I hope to increase student participation by clarifying what is expected for an oral presentation and selling the benefits. Between those two efforts, I hope that the next IBASM meeting will have a higher attendance and will have more student driven lectures.

If you have any questions or suggestions, please contact me at lmsteiner@bsu.edu.

Special Thanks to All Judges!

On behalf of all of the students in the poster competition, we 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 = *Shivi Selvaratnam (IDEM) and Rich Gregory (IUSD)*

Team #2: Undergraduate division = *Cindy DeLoney-Marino (USI) and Carolyn Vann (BSU)*

Team #3: MS division = *Dominique Galli (IUSD), Jeanne Barnet (USI) and Becky Sparks-Thissen (WC)*

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

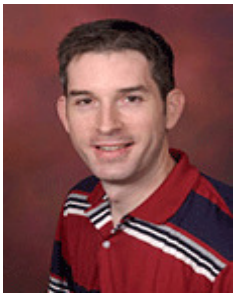
Students contributed a total of 13 posters and 1 oral presentation at the IBASM meeting. The quality of all the presentations was awesome and it was very informative for me to see the range of different research areas. Elizabeth Listman (BSU) and Mallory Wilson (IUSD) won first and second place respectively in the undergraduate division.

Jennafer Whybrew (IUPUI) received the *Leland S. McClung* award for 1st place in MS division and Allison Veach (BSU) received 2nd place. **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.

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 received a certificate and a monetary gift when a short paper is published in the IBASM newsletter.

Message from the Past President—
John McKillip



As I transition from IBASM President to the Councilor position, please let me say that it has been a great pleasure to serve for the last two years. My time as President was enjoyable and rewarding, in large part due to the commitment and hard work shown by our other officers and members. I would like to especially thank Dominique Galli, who helped get me off to a smooth start by answering my many questions early on, and to Christian Chauret for being such a loyal steward in the Secretary/Treasurer position (and for which he was awarded the H.M. Powell IBASM Service Award during the Executive Committee Planning Meeting in May). I also would like to thank Shivi Selvaratnam, our Newsletter Editor, for her long-standing quality work with this informative and professional outlet for IBASM news and general interest stories.

Our new President, Jennifer Metzler, has already done a terrific job of planning our last two branch meetings, and will serve her new post with the same energy and positive spirit that has made such a difference recently in recruiting new student members to IBASM. Without enthusiastic new leaders bringing in fresh ideas, IBASM would not be the respected and active Chapter that it is today. I look forward to seeing this momentum continue in the coming years.

As most of you may recall, we have recently amended our IBASM By-Laws with respect to the Teaching and Service Award criteria, which I believe is a change for the better because the descriptions for these two awards are now more consistent with each other and perhaps more clear to those reading them when considering a worthy nominee. IBASM is also pleased to announce a research grant program aimed at undergraduate and graduate students who wish to hone their grantsmanship abilities. This issue of the newsletter includes the official announcement about this, which was proposed and approved at our recent Executive Planning Meeting.

Lastly, I'd like to announce that we have a new IBASM student representative. Lindsey M. Steiner, who is a junior at Ball State University majoring in microbiology/pre-med. Lindsey is currently an officer in the BSU Chapter of IBASM, and has served as a student peer tutor/mentor in numerous capacities while at Ball State. Lindsey is currently engaged in undergraduate research, and has been active in the Society for Industrial Microbiology (SIM), Southern Great Lakes Chapter regional meeting last Fall. Lindsey's dynamic personality and great ideas will be perfect timing as we look ahead to our next Spring meeting, and how to actively recruit additional students to attend and present. I know she will be a terrific liaison between our Branch and potential student members. At this writing, Lindsey is on an immersive learning field study course in South Africa, so if there is not an introductory article from her included herein, that is why.

All the best to everyone for a great summer! Thank you again for letting me serve as your President.

An Exciting Opportunity

I. SUMMARY & ANNOUNCEMENT

The Indiana Branch of ASM announces an opportunity for undergraduate or graduate students (M.S., Ph.D., or Ed.D.) in current good standing with IBASM to apply for research grants of up to $500. Two awards will be made for undergraduates and two for the graduate 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 September 15, 2010 (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, M.S. students, or doctoral 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.

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 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 by Wed., September 15, 2010** and must arrive within three days of these deadlines applications. 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 (listed on pg. 1 of this announcement)
- ✓ Abstract of 300 words or less
- ✓ Project description that follows general guidelines on pg. 2
- ✓ Statement of compliance approval
- ✓ Preliminary data, if applicable
- ✓ Budget of $500.00 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 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 below. **Deadline: September 15, 2010**

Name Research Advisor

Address

Phone Fax E-mail

Please indicate your student status:

☐ Undergraduate
☐ M.S./M.A.
☐ Ph.D./Ed.D.

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

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

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

Random and Site-Directed Mutagenesis of *ERG25* in *Saccharomyces cerevisiae*

Jennafer M. Whybrew and Martin Bard
Department of Biology, Indiana University-Purdue University Indianapolis
Indianapolis, IN

Introduction

The *ERG25* gene encodes a sterol C-4 methyloxidase essential for sterol biosynthesis in plants, animals, and yeast. This gene functions in turn with *ERG26*, a sterol C-3 dehydrogenase, and ERG27, a sterol C-3 keto reductase, to remove two methyl groups at the C-4 position on the sterol A ring (Figures 1 & 2). In *Saccharomyces cerevisiae*, *ERG25* has four putative histidine clusters which bind non-heme iron (2) and a C-terminal KKXX motif which is a Golgi to ER retrieval motif.

We have conducted Site-Directed and Random mutagenesis in the *S. cerevisiae* wild-type strain SCY876. Site-Directed mutagenesis focused on the four histidine clusters, the KKXX C-terminal motif and other conserved amino acids among species including: *Mus musculus, Homo sapien, Arabidopsis thaliana, Candida albicans, and Saccharomyces cerevisiae*. Random mutagenesis was used in an effort to find novel changes in enzyme function outside of the parameters utilized for site-directed mutagenesis. Plasmids carrying specific *ERG25* mutations were screened for complementation, verified by DNA sequencing, and transformed back into yeast for GC/MS analysis. Strains that do not complement should have sterol profiles indicating the inability to synthesize ergosterol and an accumulation of 4,4-dimethylzymosterol. The four putative histidine clusters are expected to be essential for gene function by acting as non-heme iron binding ligands bringing in the oxygen required for the oxidation-reduction in the C-4 demethylation reaction.

Experimental Methods

ERG25 Disruption: The ERG25 coding sequence of *Saccharomyces cerevisiae* wildtype strain SCY876 was disrupted using a PCR product containing the HIS3 selectable marker from pRS303 and 60 base pairs of homology with ERG25 on each end by homologous recombination during a yeast lithium acetate transformation (Figure 3). Transformants grown anaerobically and aerobically were screened for complementation on dropout media with and without ergosterol. GC and GC/MS analysis confirmed disruptions viable anaerobically with sterol supplementation. Strains containing an ERG25 disruption show a sterol accumulation profile of 4,4-dimethylzymosterol and no ergosterol production.

Site-Directed Mutagenesis: Site-Directed mutants were created using the Stratagene QuikChangeÒ Lightening Site-Directed Mutagenesis kit. Amino acid changes were chosen in the four histidine clusters, the KKXX C-terminal motif and other areas of consensus among species including: *Mus musculus*, *Homo sapien*, *Arabidopsis taliana*, *Candida albicans*, and *Saccharomyces cerevisiae* (Figure 4). Amino acid residues were changed to alanine, expect tyrosine residues were changed to phenylalanine.

Random mutagenesis: Random mutants were created by gap repair. A PCR product of the ERG25 gene was created with EcoRI and SalI restriction sites along with a putative mutation generated with the Go Taq Polymerase, which estimates a mutation rate of approximately 1/1000 base pairs. This PCR product, referred to as the insert, was co-transformed with a linearized vector containing homologous 60 base pair ends into the disrupted ERG25 strain (Figure 5). Mutations were first screened for complementation with and without ergosterol anaerobically and then aerobically. Plasmids were extracted from mutant yeast colonies that failed to complement and then digested with EcoRI and SalI to screen for a 1kb insert. If an insert was noted, the DNA was sequenced. To verify random mutants, plasmids with putative mutations were retransformed into erg 25.

Results

All site-directed and random mutants were verified by DNA sequencing and analyzed for complementation in yeast with and without sterol supplementation. Strains positive for complementation could grow aerobically without the presence of sterol suggesting the amino acid residue change does not affect gene function. Mutant strains that do not complement only grow anaerobically in the presence of sterol suggesting these amino acid residue changes do affect gene function. Sterol uptake in yeast occurs under anaerobic conditions.

The sterol profile for each mutant was analyzed via gas chromatography grown anaerobically in the presence of cholesterol (Tables 1 & 2); complementing strains were also grown aerobically without sterol (data not shown); for two days in liquid media and saponified. GC data is listed in tables 1 and 2 as a percentage of sterol accumulation. (EP includes the ergosterol precursors zymosterol, fecosterol, episterol, and other ergosta-diene sterols). The key points to the sterol profile are an accumulation or lack of accumulation of ergosterol (Erg), 4-methylfecosterol (4-Methyl), lanosterol (Lano), and 4,4-dimethylfecosterol (4,4). Complementing strains show a wild-type or nearly wildtype ergosterol profile on GC, whereas non-complementing mutant strains show an accumulation of 4,4-dimethylzymosterol.

Table 1: Complementing strains grown anaerobically

Sample	Squ	Chol	Erg	EP	4-methyl	Lano	4,4
RM213	35.2	60.5	1.8	0.5	0	0.7	2.0
F67A	33.4	49.4	1.9	7.7	1.8	3.4	2.4
Q88A	33.6	51.0	1.2	8.2	0.4	2.8	2.8
Q98A	51.7	37.5	0.5	4.6	1.7	1.8	2.2
C101A	36.6	30.0	7.2	11.2	1.7	6.7	6.7
L102A	41.3	27.4	7.9	8.8	1.7	8.5	4.4
I115A	52.1	40.3	0	3.6	1.5	1.0	1.5
Y169F	19.2	47.4	12	12.1	1.1	6.8	1.4
P182A	34.8	51.0	1.1	4.5	1.7	2.2	4.7
E188A	9.6	73.5	2.9	9.9	0.4	3.3	0.4
H191A	32.4	41.8	7.5	7.6	2.5	2.2	6.0
R228A	42.1	43.8	0	6.3	1.6	1.0	5.2
H236A	23.8	63.3	4.5	4.7	0	1.9	1.8
Y239A	10.1	9.7	52.7	16.9	4	1.2	5.4

The mutations created in the three putative histidine clusters thus far including: H160A, H173A, H176A, H258A, and H263A are all negative for complementation and require sterol supplementation anaerobically for growth. However, mutants created in the fourth histidine cluster suggested by Kaplan (2) including H236A and Y239F complement 2% of the time or all of the time respectively. Site-directed mutants E152A and D153A, which are adjacent to the first histidine cluster are also negative for complementation. All other site-directed mutations accumulated thus far are complementing strains. The KKXX C-terminal motif data is still pending

Table 2: Non-complementing strains grown anaerobically

Sample	Squ	Chol	Erg	EP	4-methyl	Lano	4,4
E152A	33.2	52.0	0	8.6	0.4	0.5	5.3
D153A	51.6	40.9	0	0	0	0.4	7.1
Y157F	25.0	33.5	0.8	2.8	0	0	3.9
H160A	49.5	36.6	0	5.6	4.7	0	3.6
K170A	29.1	59.6	0	6.5	0	0.2	4.6
H176A	46.7	40.5	0	7.4	0.4	1.6	3.4
H258A	35.4	59.6	0	2.0	0	0	3.0
H236A	24.0	63.5	0	8.3	0.7	1.4	2.2
W276R	33.0	57.0	0	6.1	0	0.7	3.2
R274G	34.0	50.5	0	4.4	0.8	5.2	5.1
S45G	47.9	36.7	0	3.2	0.2	0	12
H263L	37.1	43.4	0	2.4	0	0.3	16.8
RM51	41.8	49.5	0	5.3	0.3	0.6	2.5

The four random mutations analyzed thus far including: S45G, H263L, R274G, and W276R, were negative for complementation and appear just before the proposed transmembrane domain and in or adjacent to the last conserved histidine cluster. RM213 is a wild-type control strain, which was also created during random mutagenesis.

Conclusions

Mutations created thus far in and adjacent to the histidine boxes suggested by Shanklin et al. have been negative for complementation and show a mutant sterol profile by GC analysis indicating that as expected these amino acid residues are essential for the *ERG25* gene to function. However, mutations created thus far in the fourth histidine cluster suggested by Kaplan complement and show a wild-type profile by GC analysis indicating this putative histidine cluster is not essential for *ERG25* gene function. Several other conserved amino acids complement and do not require supplementation with sterol displaying a wild-type GC profile indicating these residues are also not essential for gene function.
Further analysis of all of these mutations by western blot will indicate whether the amino acid change results in the loss of function of the gene product or if it is causing a decrease in stability of the Erg25 protein.

References

1) Shanklin *et al* (1994) *Biochemistry* 33, 12787-12794.
2) Kaplan et al (1996) *J. Biol. Chem.* 271(28), 16937-16933.

Award papers from Mallory Wilson and Allison Veach will be published in the next issue of the newsletter.

First Place Undergraduate Winner

Effects of Antibiotics on Microbial Activity in CAFO and Urban Influenced Streams

Elizabeth A. Listman and Melody J. Bernot
Ball State University, Department of Biology, Muncie, IN

Abstract

Recently, increasing attention has been given to the fate of antibiotic compounds in the environment. Antibiotics used for human and animal applications can enter aquatic ecosystems via wastewater or sewer effluent (in the case of humans) or through manure runoff from Confined Animal Feeding Operations (CAFO) that administer veterinary antibiotics. This study tested the hypothesis that chronic exposure to human and veterinary antibiotics may influence the development of resistant strains of microorganisms in receiving stream ecosystems. Eleven sites with varying land use were selected for measurement of microbial response to antibiotics; five sites were located downstream of a CAFO, five were located downstream of a combined sewer overflow (CSO), and one was a reference site located in Mounds State Park, Indiana. Sediment and water collected from all sites was randomly assigned to three antibiotic treatment groups: control (no antibiotic addition), erythromycin, or tetracycline addition. Sediment respiration was measured using dehydrogenase activity assays to quantify changes in microbial respiration as a measure of antibiotic resistance. Microbial respiration was highest when exposed to the antibiotic tetracycline suggesting resistance to this compound. Treatment with erythromycin antibiotic did not influence respiration relative to controls. There was a significant land use by treatment interaction ($P<0.001$) indicating that the influence of antibiotics on sediment respiration varied depending on the surrounding land use in the sub-watershed. Specifically, in streams receiving CSO input, microbial respiration decreased in response to erythromycin but increased in response to tetracycline relative to controls. Neither antibiotic significantly influenced microbial respiration in CAFO influenced. These data indicate that stream microbial communities may adapt to antibiotics in the surrounding ecosystem.

Introduction

Antibiotics are one of the most commonly used pharmaceuticals in the treatment of both human and animal diseases. As such, the prevalence of antibiotic compounds in the natural environment from human and animal wastewater pollution is beginning to gain more attention. Of particular concern is the effect of increasing antibiotics in the environment on fostering bacterial resistance. Such research stems from the idea that after administration of antibiotics, the compounds usually pass through human and animal systems incompletely metabolized. From the excrement, the antibiotic can then enter the aquatic ecosystem by way of sewer effluent or through manure runoff from Concentrated Animal Feeding Operations (CAFO) that administer veterinary antibiotics (Kümmerer, 2008). This study tested the hypothesis that exposure to pharmaceutical antibiotics through human and animal waste will increase antibiotic resistance in stream sediment microbial communities.

Methods

Eleven central Indiana stream sites were selected for measurement of sediment respiration. Sites were all located in the Upper White River watershed but differed by land use in the surrounding sub-watershed that would potentially influence antibiotic exposure. Five sites were influenced by combined sewer overflow (CSO) input; five sites were influenced by animal feeding operations (CAFO); and one site did not have animal or human waste input. All streams were < 10 meters in width and intersected a public road where sediment and water was collected for laboratory assays.

At each site, 500 mL of sediment (~5-10 cm top sediment) and 1 L of unfiltered water were collected and returned to the laboratory on ice. Physiochemical parameters including flow, pH, temperature, conductivity, dissolved oxygen, and turbidity were also measured at each site using a Hydrolab© and Marsh-McBirney flow meter. In the laboratory, collected sediment was homogenized through a no. 7 USGS sieve to remove debris and macroinvertebrates. Homogenized sediment was then placed into individual 15 mL falcon tubes for measurement of microbial respiration using the dehydrogenase activity assay. Microbial activity was measured as total respiration under the assumption that if antibiotic resistance is present, microbial respiration will not be influenced by antibiotic treatments. Microbial respiration was measured in response to three treatments applied to sediment from each site: control (no antibiotic addition), erythromycin, or tetracycline addition (0.0024 g of appropriate antibiotic into respective falcon tubes). Tetracycline and erythromycin were selected as target antibiotics for this study as they are prevalently used to treat human illness and continue to be widely used in animal treatment. Antibiotic treatment concentrations were selected to represent environmental concentrations experienced by sediment microbes *in situ* based on previous studies in freshwaters.

Filtered water samples (GF/F, 0.7 mm pore size) were also collected every two weeks for analysis of anions (Cl^-, Fl^-, Br^-, NO_2^-, NO_3^-, PO_4^{3-}) and cations (NH_4^+, Li^+, Ca^+, K^+, Na^+) using a Dionex Ion Chromatograph. Site physiochemical and water chemistry data was used to establish the underlying relationship between water column nutrients, land use, and sediment respiration which may influence the antibiotic interaction.

Pearson correlation statistics were used to assess the relationship between stream physiochemical characteristics and sediment microbial activity in control (no antibiotic treatment) samples. Analysis of Variation (ANOVA) statistics were used to compare the effects of antibiotics on sediment respiration

Results

Overall, mean sediment respiration in central Indiana streams ranged from <1-7.8 mgO_2/L (Fig. 1). Sediment respiration in streams was related to water column nutrient concentrations with sulfate and phosphate concentrations being positively correlated with sediment respiration and nitrate concentrations being negatively correlated with sediment respiration. Microbial respiration without antibiotic treatment was highest in streams receiving CSO input and lowest in CAFO -bordered sites as well as reference streams not influenced by CSOs or animal feeding operations (Fig. 1).

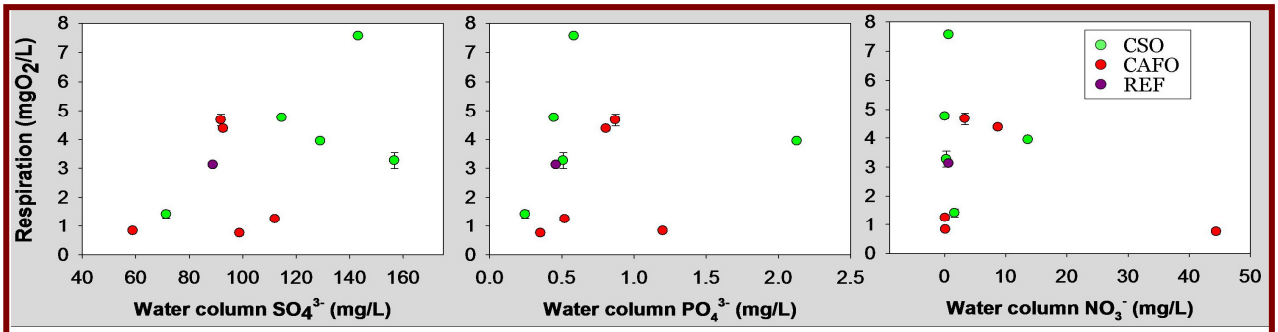


Fig. 1: Relationship between water column nutrients and mean sediment microbial respiration (N=5) without antibiotic treatment. Water column sulfate (SO_4^{3-}) and phosphate (PO_4^{3-}) concentrations were positively correlated with sediment respiration ($P<0.01$); whereas, water column nitrate (NO_3^-) concentrations were negatively correlated with sediment respiration ($P<0.01$). Predominant land use in the surrounding sub-watershed for each site noted in legend.

Across all the streams, microbial respiration was highest when exposed to the antibiotic tetracycline. Treatment with erythromycin antibiotic did not influence respiration relative to controls (Fig. 2). However, there was a significant land use by treatment interaction ($P<0.001$) indicating that the influence of antibiotics on sediment respiration varied depending on the surrounding land use in the sub-watershed (Fig. 3). Specifically, in streams receiving CSO input, microbial respiration decreased in response to erythromycin but increased in response to tetracycline relative to controls (Fig. 3). However, neither antibiotic significantly influenced microbial respiration in CAFO influenced (Fig. 3).

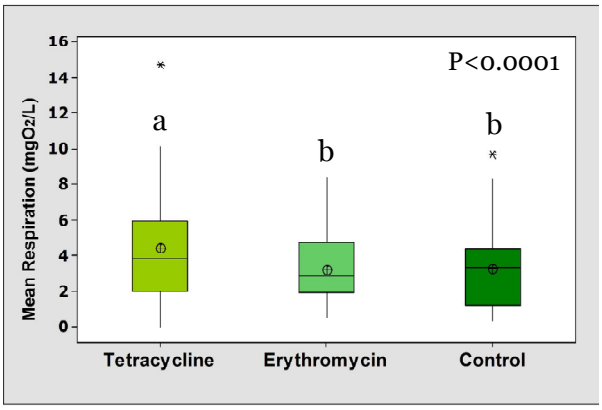


Fig. 2: Mean sediment microbial respiration (N=5) in streams and overall response to antibiotics. Different letters denote significant differences (ANOVA, p<0.1).

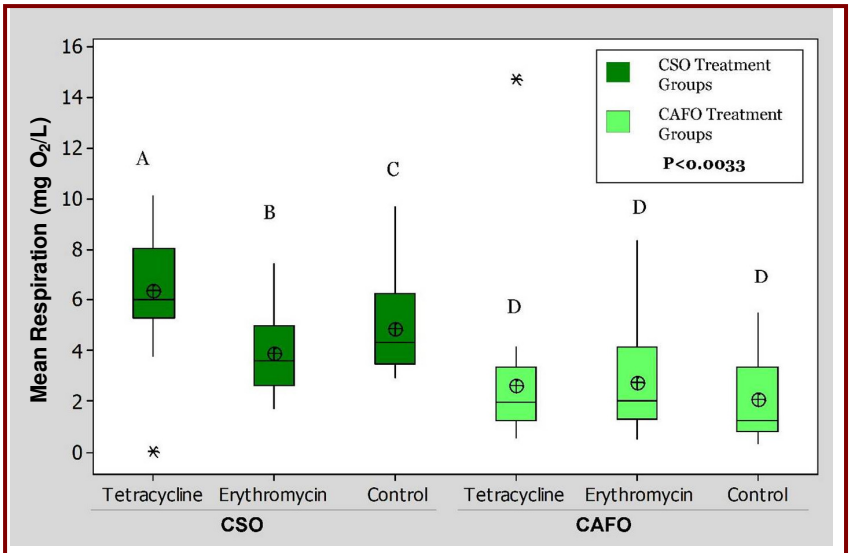


Fig. 3: Mean respiration demonstrating interaction between surrounding land use and antibiotic treatment (N=5). Different letters denote significant differences (ANOVA, p<0.1).

Discussion

These data indicate that freshwater sediment microbial communities can be influenced by antibiotics even at trace concentrations regularly detected in natural waters. However, sediment microbial community response to antibiotics varied with land use and antibiotic compound.

Surrounding land use is likely an indicator of previous exposure to antibiotics and subsequent adaptation. The lack of a respiration response to antibiotics in CAFO-influenced sites may be due to antibiotic concentrations being below effective dosages. However, because sediment microbes responded to antibiotics in CSO-influenced sites; the lack of a response may also indicate antibiotic resistance. Tetracycline and erythromycin are predominantly used in human applications; thus, increased resistance was hypothesized to occur in streams exposed to human waste (CSO). Consistent with this hypothesis, treatment with tetracycline in CSO-influenced streams yielded higher microbial respiration suggesting microbes may have adapted to the prevalence of this compound. Additionally, the fact that tetracycline causes an increase in respiration relative to the controls (no antibiotic exposure) in CSO sites likely suggests that microbes have adapted to the prevalence of the tetracycline compound and may now use it as a nutrient source.

Microbial respiration was not influenced by erythromycin at either CSO or CAFO influenced sites. Variation in response to erythromycin relative to tetracycline may be due to differences in compounds or to microbial resistance across sites. Tetracycline sorbs strongly to sediment relative to erythromycin and, therefore, may more directly influence sediment microbial communities in freshwater ecosystems. Erythromycin may be transported in the water column with little interaction with benthic microbes. Further, erythromycin is slightly more metabolized by the body than tetracycline (20% and <20% respectively) which may explain the lesser impact on microbial respiration in CSO sites: due to lower concentrations entering the freshwater ecosystem (Kümmerer, 2008).

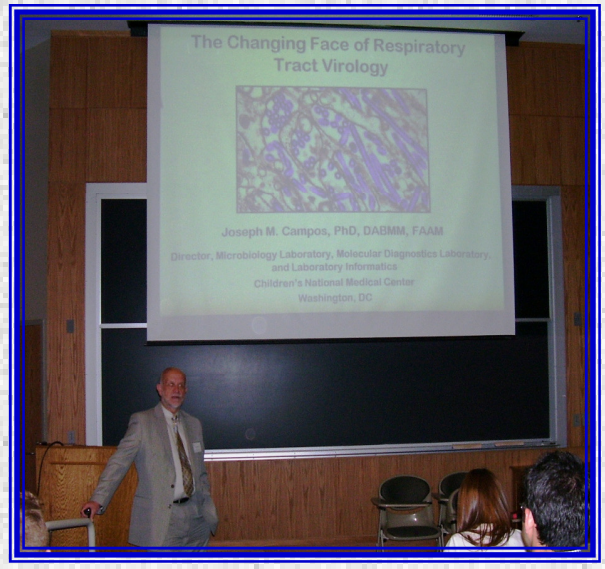
Acknowledgements

We thank the Ball State University *Undergraduate Research in Ecology* program for funding and Matthew L. Johnson for field and laboratory assistance.

Literature Cited

Kümmerer, Klaus. Pharmaceuticals in the environment : sources, fate, effects and risks. Springer: 2008.

Photos from the 2010 Annual Meeting

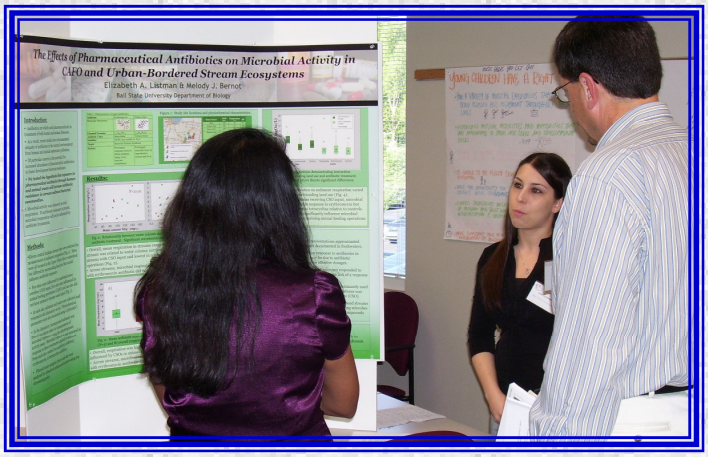


Presentation by ASM Secretary,

Dr. Joseph Campos

Elizabeth Listman receiving her award from IBASM President **Dr. John McKillip**

Jennafer Whybrew receiving her award from IBASM President **Dr. John McKillip**



Drs. Shivi Selvaratnam and **Richard Gregory** are busy at work!

Additional photos will be published in the next issue

Highlights from the Journals of the ASM, July 2010 (from ASM Tipsheet)

Prior Exposure to Seasonal Influenza May Explain the Mildness of the 2009 H1N1 Pandemic

Hong Kong researchers suggest a new theory for why swine flu infections turned out to be so mild. Prior exposure to seasonal influenza A, either infection or vaccination, may induce a cross-reactive immune response against the pandemic virus. They report their findings in the July 2010 issue of the *Journal of Virology*.

Although the outbreak of human H1N1 in 2009 spread to pandemic proportions, the illness was considered mild in most patients compared to seasonal influenza. Currently available seasonal flu vaccines do not offer cross-reactivity to pandemic H1N1 in any age group, suggesting that individuals previously infected or exposed to seasonal influenza A viruses may have memory cell-induced cross-protection to pandemic H1N1.

Prior research showed humans having cross-reactive memory cells to a wide range of H5N1 peptides despite any previous exposure to avian influenza A (H5N1). In this study researchers determined that memory cells established by seasonal influenza viruses can break down pandemic H1N1-infected target cells and ultimately induce cross-protective antibodies.

"Our data suggest that individuals who were infected with seasonal human influenza A viruses previously or who received seasonal human influenza vaccines may derive benefit, at least in part, from the preexisting cross-reactive memory cytotoxic T lymphocytes to reduce the severity of pdmH1N1 infection even without protective antibodies," say the researchers.

(W. Tu, H. Mao, J. Zheng, Y. Liu, S.S. Chiu, G. Qin, P.L. Chan, K.T. Lam, J. Guan, L. Zhang, Y. Guan, K.Y. Yuen, J.S. Malik Peiris, Y.L. Lau. 2010. Cytotoxic T lymphocytes established by seasonal human influenza cross-react against 2009 pandemic H1N1 influenza virus. Journal of Virology, 84. 13: 6527-6535).

Microbicide Containing Engineered Bacteria May Inhibit HIV-1

Researchers from the U.S. and abroad used bacteria inherent to the human vaginal tract to develop a live, topical microbicide that may induce production of HIV-1 protein inhibitors and ultimately prevent transmission of the virus. They detail their findings in the July 2010 issue of the journal *Antimicrobial Agents and Chemotherapy*.

HIV-1 has killed more than 25 million people over three decades and there are currently 33 million people living with the virus worldwide. Although health officials are ultimately striving to develop an effective vaccine, topical anti-HIV-1 microbicides are a promising alternate strategy for minimizing transmission. Live microbicides are of particular interest as they utilize bacteria inherent to the human body to induce natural production of anti-HIV-1 agents.

Lactobacillus spp. are ideal candidates for live microbicide development as they are the predominant bacterial species in the female genital tract. In the study researchers engineered a human vaginal isolate of *Lactobacillus jensenii* capable of generating the anti-HIV-1 proteins RANTES and CIC5 RANTES which oppose the HIV-1 receptor protein, CCR5. Both RANTES variants inhibited HIV-1 infection and demonstrated significant activity against various HIV-1 genetic subtypes.

"Our results provide proof of principle for the efficient secretion of an anti-HIV-1 active CCR5 antagonist by an engineered vaginal commensal bacterium, which represents an important advancement toward realistic, safe, and low-cost prevention of sexual transmission of HIV-1," say the researchers.

(L. Vangelista, M. Secchi, X. Liu, A. Bachi, L. Jia, Q. Xu, P. Lusso. 2010. Engineering of *Lactobacillus jensenii* to secrete RANTES and a CCR5 antagonist analogue as live HIV-1 blockers. Antimicrobial Agents and Chemotherapy, 54. 7: 2994-3001.)

MICROBIOLOGY IN THE NEWS

This won't hurt a bit

Science News, July 18, 2010

Researchers have invented a new vaccine-delivery system that replaces the large single needle with 100 tiny dissolvable ones embedded in a Band-Aid–like patch. The new patch can immunize mice against influenza just as effectively as conventional needle vaccination, its developers report online July 18 in Nature Medicine.

http://www.sciencenews.org/view/generic/id/61241/title/This_wont_hurt_a_bit

Enlist malaria-resistant mosquitoes to stop its spread

New Scientist, July 20, 2010

Michael Riehle at the University of Arizona at Tucson and colleagues put a novel gene into the mosquito species that carries malaria in India. The new gene permanently switched on a set of genes normally affected by insulin and involved in the immune system.

http://www.newscientist.com/article/dn19194-enlist-malariaresistant-mosquitoes-to-stop-its-spread.html

Shanghai researchers develop bacteria-fighting paper

The Engineer, July 22, 2010

A new form of paper with the built-in ability to fight disease-causing bacteria could have applications ranging from anti-bacterial bandages to food packaging that keeps food fresher for longer.

http://www.theengineer.co.uk/shanghai-researchers-develop-bacteria-fighting-paper/1003843.article

Genetic mismatch keeps yeast species distinct

Science Daily, July 22, 2010

Researchers have recently identified genes in three closely-related yeast species that cause sterility, increasing our understanding of how species can remain distinct.

http://www.sciencedaily.com/releases/2010/07/100720212923.htm

Hijacked supplies for pathogens

Science Blog, July 23, 2010

Scientists have now discovered how Legionella reprograms cells to ensure its own survival and to propagate. They examined a protein used by the pathogen to divert the material transport within the cells for its own purposes.

http://scienceblog.com/36872/hijacked-supplies-for-pathogens/

Important Dates

February 2011:	Registration form due for Annual IBASM meeting
March 2011:	Abstract form due for Annual IBASM meeting
April 15-16, 2011:	Annual IBASM meeting at Brown County State Park
May 21-24, 2011:	111th Annual Meeting of the ASM, New Orleans, LA

2010-2011 IBASM OFFICERS

Jennifer Metzler, Ph.D., President. Department of Biology, Ball State University, Muncie, IN 47306. Phone: (765) 285-8848; e-mail: jametzler@bsu.edu

Rebecca Sparks-Thissen, Ph.D., President-Elect. Department of Biology, Wabash College, Crawfordsville, IN 47306. Phone: (765) 361-6100; e-mail: thissenr@wabash.edu

Christian Chauret, Ph.D., Secretary/Treasurer. Department of Biology, Indiana University Kokomo, Kokomo, IN 46904. Phone: (765) 455-9290; e-mail: cchauret@iuk.edu

John McKillip, Ph.D., Councilor. Department of Biology, Ball State University, Muncie, IN 47306. Phone: (765) 285-8830; e-mail: jlmckillip@bsu.edu

Dominique M. Galli, **Ph.D., Alternate Councilor.** Department of Oral Biology, Indiana University School of Dentistry, Indianapolis, IN 46202. Phone: (317) 278-1936; e-mail: dgalli@iupui.edu

Jim Mitchell, Ph.D., Educational Representative. Department of Biology, Ball State University, Muncie, IN 47306. Phone: (765) 285-8820; e-mail: jkmitchell@bsu.edu

Shivi Selvaratnam, Ph.D., Newsletter Editor. Office of Water Quality, Indiana Department of Environmental Management, Indianapolis, IN 46219. Phone: (317) 308-3088; e-mail: sselvara@idem.in.gov