

McPherson College
**Natural
Science
Research
Forum**
Division of Science and Technology

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McPherson College

The Effects of Amikacin, Doxycycline, Erythromycin, Penicillin, and Sulfamethoxazole with Trimethoprim on Tylosin Resistant *E. coli*

Lezli Warkentin

Antimicrobial resistance has been an issue since Alexander Fleming discovered Penicillin in 1928. In today's society it is considered a health crisis. Not only are there more resistant strains of bacteria, but there are bacteria that are becoming multidrug resistant. There are many reasons for the increase of antibiotic resistance, and at least four mechanisms bacteria can use to become resistant. They have found that bacteria can be resistant to a whole class of antimicrobials, or even many classes. Resistance not only affects humans but also the agricultural industry. It is important to discover the extent of resistance and how it can affect animals and humans. An *Escherichia coli* strain was isolated from a feed lot that showed resistance to Tylosin. Using the Agar Disk Diffusion Method, I tested this strain against Amikacin, Doxycycline, Erythromycin, Sulfamethoxazole with Trimethoprim, and Penicillin to see if resistance to Tylosin affected its resistance to other antimicrobials. I chose to use these antimicrobials because of the families, or classes, they were in. Erythromycin is a macrolide like Tylosin, but the other four antimicrobials come from different families with different mechanisms. I found that this strain of *E. coli* showed more resistance when compared to the control strain for Amikacin, Doxycycline, Erythromycin, and Penicillin, and less resistance to Sulfamethoxazole with Trimethoprim. However there were some problems with my Penicillin control which make the results unreliable.

Keywords: Antibiotic Resistance, *E. coli*, Amikacin, Doxycycline, Erythromycin, Sulfamethoxazole with Trimethoprim, Tylosin.

The 21st Annual Science Research Forum

- 2:00 – 2:05** Welcome and Introductions : **Dr. Jonathan Frye**
- 2:05 – 2:20** **Dr. Nepali Rajapakse:** Photophysical properties of metallo-tetraphenyltetraabenzoporphyrins: insights from experimental and theoretical studies
- 2:20 – 2:35** **Adam Horinek:** Antibiotic Resistance to Oxytetracycline HCL in Kansas Department of Wildlife Fish Hatchery of Pratt, KS
- 2:35 – 2:50** **James Brandon M. Luter:** The Effects of Flood Irrigation on Groundwater *E. coli*/coliform Contamination
- 2:50 – 3:05** **Sean B. McCrae:** Nutrient Composition of Livestock Feed and Its Impact on Farm Productivity
- 3:05 – 3:20** **Break for Refreshments**
- 3:20 – 3:35** **Jacob C. Simon:** The Effects of Temperature Increase and Presence of Light on Methane Production and Emission of *Alocasia x amazonica*
- 3:35 – 3:50** **Erikka Viehman:** The Effect of Temperature on Aerobic Methane Production by the Fiddle Leaf Fig and the Norfolk Island Pine
- 3:50 – 4:05** **Dr. Allan Ayella:** Infectivity of *Macrophomina phaseolina* on Mature Plant Tissues of Alfalfa

Antibiotic Resistance to Oxytetracycline HCL in Kansas Department of Wildlife Fish Hatchery of Pratt, KS

Adam Horinek

The overuse of antibiotics in today's society has caused reduced susceptibility in many species of bacteria. Antibiotics are used to treat humans, as well as animals including domestic, livestock and aquatic animals. Antibiotics are administered to aquatic animals by food mixture. There are two FDA approved antibiotics used in treatment of aquatic animals, oxytetracycline HCL and sulphadimethoxine. The objective of this study was to determine if there were bacteria with reduced susceptibility to oxytetracycline HCL in Kansas Department of Wildlife Fish Hatchery in Pratt, KS where oxytetracycline has been used. Samples were taken from channel catfish (*Ictalurus punctatus*), soil, and water that was exposed to oxytetracycline HCL in the summer of 2008. This study found evidence of three individual species resistant to oxytetracycline HCL. Bacteria were isolated by use of nutrient agar plates amended with oxytetracycline HCL in the recommended concentration of 105mg/1L. Three species of bacteria were isolated. Bacteria with reduced susceptibility isolated from (*Ictalurus punctatus*) were found to be *Stenotrophomonas maltophilia*.

Keywords: Aquatic Animals, Antibiotic Resistance, Channel Catfish (*Ictalurus punctatus*), Fish Hatchery, *Stenotrophomonas maltophilia*

The Effect of Temperature on Aerobic Methane Production by the Fiddle Leaf Fig and the Norfolk Island Pine

Erikka Viehman

Due to the growing concern of global warming, the emissions of gases are closely monitored. Methane is known to be produced from anaerobic bacterial process from energy production, biomass burning, coal mining, landfills, wetlands, ruminant animals and rice paddies. In 2005 Frankenberg and his colleagues observed a surprisingly high amount of methane over tropical forest regions during the dry seasons using SCIAMACHY (Scanning Imaging Absorption Spectrometer for Atmospheric Chartography). In 2006 Keppeler and his colleagues followed this discovery with the findings that certain plants are a source of aerobic methane production. Because methane is a greenhouse gas, these studies may be important for the prevention of global warming. I studied methane emissions from the leaf of the Fiddle Leaf Fig (*Ficus lyrata*) a plant native to western Africa, and the Norfolk Island Pine (*Araucaria heterophylla*) indigenous to a small island between New Zealand and Australia known as Norfolk Island, using gas chromatography with a flame-ionization detector. Leaves of both plants were incubated in 22 mL glass vials in the dark at 20°C, 30°C, and 40°C. Gas was extracted from the vials and methane was measured at T=0 and T=24 hours. The experiments were analyzed using the paired t-test. The results imply that there is not a statically significant increase in methane in the Fiddle Leaf Fig at 20°C and 30°C or in the Norfolk Island Pine at 20°C, 30°C, and 40°C. Results did show a statistically significant increase of methane emissions in the Fiddle Leaf Fig at 40°C. My results suggest that aerobic methane production is not common in all plants and at all temperatures.

Keywords: methane, greenhouse gas, global methane budget, *Ficus lyrata*, *Araucaria heterophylla*

The Effects of Temperature Increase and Presence of Light on Methane Production and Emission of *Alocasia x amazonica*

Jacob C. Simon

Previous research has been inconclusive about the sources of methane in our atmosphere. Recent studies have indicated that plants may play a larger role than expected as a source of methane emissions due to aerobic methanogenesis. Studies done by Frank Keppler and his colleagues introduced this idea when they discovered that certain plants are a source of aerobic methane production. This was followed by a study by Z.P. Wang, who discovered that there may be many variables that affect each plant's ability to produce methane aerobically. Recently, Jamie Rodriguez discovered that light has a significant effect on the production of methane by *Musa acuminata*. Although the predicted annual methane emissions due to plants is now thought to be much lower than previously, methane is still an important greenhouse gas due to its long half-life and its effects on global warming. Using a flame-ionizing gas chromatography detector, I studied methane emission from the leaves of *Alocasia x amazonica*, a common household tropical plant known as an Elephant-ear, and one that is prevalent in tropical regions of southeast Asia. Leaf samples were incubated in 30 air-tight glass vials in darkness, at temperatures of 25°C, 35°C, and 45°C, with 10 samples at each temperature. Leaf samples were also incubated in 30 air-tight glass vials in the presence of 300 μ Einsteins, with 10 samples at each of the previously mentioned temperatures. Methane readings were taken at times of T=0 and T=24 hours. Using the paired t-test, the data from the experiments were analyzed. The results indicated that the *Alocasia x amazonica* plant is a significant source of aerobic methane production, but the variable affecting its methane production could not be determined. It was found that incubation in 300 μ Einsteins showed no direct effect on the aerobic methane production of the tested samples, where only one set of samples, those incubated at 35°C, showed a statistically significant increase in methane concentration. While the other two sets of samples, analyzed in 300 μ Einsteins, at 25°C and 45°C, showed that the change that occurred with the incubation could not exclude the possibility that it was due to chance. This was also the case for the set of samples incubated in darkness at 45°C. However both sets of samples tested in darkness at 25°C and 35°C showed a statistically significant change in methane concentrations. These results, along with ones from other scientists, give more reason to find the mechanism by which plants produce methane and the issue of eliminating the large output of global greenhouse gases.

Keywords: methane, aerobic methanogenesis, *Alocasia x amazonica*

Aerobic Methane Production from a Red Oak Tree Leaf

Nicole Laska

Recent studies have shown that there is more methane in the atmosphere than previously thought. After discovering large amounts of methane emission above tropical rainforest area, Frank Keppler and colleagues performed research concerning aerobic methane emission from plants. Keppler concluded that some plants may be capable of aerobic methane production. Methane emissions are an important area of research since methane is one of the most prominent greenhouse gases being released into the atmosphere. Furthermore, for my research I chose to examine the methane emissions from a plant from the ecosystem of the United States, the red oak tree leaf, *Quercus rubra*, using gas chromatography with a flame-ionizing detector. I collected red oak tree leaves and cut them into 0.2g samples using aseptic techniques and incubated 20 samples in the dark at 30°C and 20 samples in the presence of 300 μ mol/s² m² of light at 30°C. At the times T=0 hours and T=24 hours, the concentration of methane was measured by gas chromatography with a flame-ionizing detector. The paired t-test comparing the difference of methane produced by samples incubated in the dark at 30°C found that there was a statistically significant amount of methane produced in the time interval from T=0 hours to T=24 hours. Also, the paired t-test comparing the difference in methane production for the samples incubated in the presence of 300 μ mol/s² m² of light at 30°C showed that there was a statistically significant amount of methane produced from time T=0 hours to T=24 hours. Both of the paired t-tests had a p value < 0.001 showing that the change in production of aerobic methane was not due to chance. Next, a t-test was performed comparing the amount of methane produced from the samples incubated in the dark at 30°C to the amount of methane produced from the samples incubated in the presence of 300 μ mol/s² m² of light at 30°C. The results of this t-test found a p value of 0.715 which showed that there was not a statistical significance between the amounts of aerobic methane produced in the samples incubated in the dark when compared with the sample incubated in the presence of 300 μ mol/s² m² of light indicating that the amount of methane produced was not more active when incubated in the dark or when incubated in the light. The results of this research imply that methane is being produced aerobically and that the aerobic production of methane is not dependent on the presence of light or dark.

Keywords: methane, greenhouse gas, anthropogenic, methanogenesis, aerobic

The Effects of Flood Irrigation on Groundwater *E. coli*/coliform Contamination

James Brandon M. Luter

Recent field observations raise the question of a connection between flood irrigation and groundwater *E. coli* contamination. Previous studies have shown that total coliform and *E. coli* serotype O157:H7 are capable of transmission in a freshwater medium. The most common water sources associated with human outbreak are irrigation, wastewater, recreational, and drinking. Further investigation of water as a growth medium revealed that *E. coli* can survive in sterile water with low carbon concentrations. These studies show only the transmission of *E. coli* in water and the growth of *E. coli* in sterile fresh water. At present no literature can lead one to say that flood irrigation has an impact on domestic water wells. A statistically significant correlation of contamination by total coliform bacteria with irrigation has been found ($P = 0.014$).

Keywords: *Escherichia coli*, coliform bacteria

Nutrient Composition of Livestock Feed and Its Impact on Farm Productivity

Sean B. McCrae

Nutrients such as macronutrients and micronutrients are substances that are metabolized by animals to give energy and build tissue. Lack of nutrients, or nutrient deficiency, prevents normal growth of animals. All nutrients play important roles throughout the body and are key to healthy, growing animals. The lack of certain nutrients can cause serious problems to the animals and in the productivity of the farm. In this study, we shall obtain livestock feed from farms that contain fermented feed mixture and non-fermented feed mixture and analyze their nutrient composition. Growth rates will be determined to see if there is any difference between farms in productivity.

Keywords: Nutrients, Livestock Feed, Growth Rates, Nutrient Deficiency, Productivity

Natural Science Research Awards

Two types of awards are bestowed on students who demonstrate excellence in senior research. The highest honor, the **Burkholder Research Award**, honors outstanding achievement. The **Merit Research Award** distinguishes significant achievement. The Natural Sciences of McPherson College and Midwest Oilseeds of Adel, Iowa sponsor both awards. For each award, the faculty of the Natural Sciences selects one or more candidates based on three criteria: 1) selection and design of a senior research project; 2) quality of the research, including technique, observations, and data analysis; and 3) presentation of the research, comprising preparation of a scientific research paper and an oral presentation to students and faculty. A Certificate of Award is granted to each winner. Persons qualifying for the Burkholder Research Award have their name inscribed in a plaque and receive a year membership to the American Association for the Advancement of Science and a subscription to *Science*.

Year	Burkholder Award	Merit Award
2008	Joel Grosbach, Landon Snell, W. Brett Whitenack	Alan Grosbach
2007	Callie Crist	Rhonda Hoffert, Jamie Rodriguez
2006	Travis Allen	Lisa Sader
2005	Joseph Blas	David Cockriel, Jenny Harper, Danielle Lucore
2004	Robert Ullom	
2003	Michelle Schulz	Adeline Cripe
2002	Elizabeth Stover	Renata Lichty
2001	Genelle Wine	Jonas Lichty
2000	Nathan McLaughlin	Jeffrey McPherson
1999	Roy Johnson, Jr.	Jennifer M. Amiot, Janet Bowen, Eric D. Putnam, Anna Katharina Schenk
1998		Rebecca Standafer, Cameron Mahler
1997	Kerri Kobbeman	Rod Samuelson
1996		Stasi Zirkel, Wes Sechler, Chris Owens
1995	Heather Hughbanks, Monica Embers	Erik Harmon
1994	Adam Smith	Paula Worley, Adeola Grillo, Sherry Coopple, Susan Blubaugh
1993	Tyson Burden	Robin Morgan
1992	Pete Hanson	
1991		Shannon Hull, Thomas Champion
1990	James Dechand	David Maxey
1989		Michelle Roesch
1988	David Lehmen	Sandra Ashbaugh, Cynthia Aeschacher
1987	Marla Ullom, David Krehbiel	Jay Nicholson, Marsha Morley, Cassandra Clark

Infectivity of *Macrophomina phaseolina* on Mature Plant Tissues of Alfalfa

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Macrophomina phaseolina is the causative agent for charcoal rot disease in over 500 species of grass and non-grass plants including alfalfa. An isolate of *M. phaseolina* was evaluated for its infectivity through a root-dip and in-vitro plant assay. *M. phaseolina* suspension caused a brown discoloration of whole root network and complete leaf wilting 3 days post inoculation. A 10-fold dilution of this *M. phaseolina* suspension caused plant death 3 weeks post inoculation. Microscopic analysis showed microsclerotia in root tissue 3 days post inoculation. With the in vitro assay, excised whole leaves and leaf discs exhibited yellowing and tissue necrosis 7 days post inoculation with the *M. phaseolina* suspension. The results indicate that infectivity of *M. phaseolina* on mature plants of alfalfa occurs rapidly upon inoculation with $7 \times 10^6/10\text{ml}$ microsclerotia.