Purdue Extension
2018 Annual Accomplishment Report to USDA NIFA
Impact Statements by Planned Program

The Purdue Extension and Research outcomes and impact statements are organized into these seven Planned Programs:

- Childhood Obesity
- Climate Change
- Food Safety
- Global Food Security and Hunger
- Human, Family, and Community, Health and Well-being
- Natural Resources and the Environment
- Sustainable Energy

Outcomes provide the metrics that our Educators and Specialists report across research and Extension program efforts. The impact statements shared highlight a program or project addressing the outcomes and provide narrative on the issue, what has been done, and the results. There are impact statements for research projects, for Extension programs, and for integrated research and Extension activities.

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Food Safety

Title
Retail Food Safety in Indiana

Extension
Rhonda Taylor, Vickie Hadley, Nancy Manuel, Jennifer Stefancik, Marcia Parcell, Teri Hornberger, Janice Dougan, Janet Steffens, Nancy Hudson, Amanda Deering, Annette Lawler, Atina Rozhon, Jaclyn Franks, Jane Horner, Lindsey Pedigo, Linda Curley, Brittney Schori, Christina Ferroli, Karen Richey, Abigail Creigh, Megan Peterson, Peggy Ehlers, Meagan Brothers, Amber Noll, Joni Muchler, Alicia Criswell, Molly Hoag, Cindy Barnett, Tonya Short, Shannon Chipman, Marilyn Sink

Outcome
5373 - FS 3.2 - # Food handlers receiving food safety training and education in safe food handling practices

Issue
Foodborne illnesses are common, costly, yet preventable, public health problems. CDC estimates that 1 in 6 Americans gets sick from contaminated foods or beverages and 3,000 die each year. The USDA estimates that food borne illnesses cost $15.6 billion each year. Reducing foodborne illness by 10% would keep 5 million American from getting sick each year. Preventing a single fatal case of E. coli infection would save an estimated $7 million.

What has been done
Purdue Extension Health and Human Science (HHS) Educators have become certified instructors and proctors for the ServSafe curriculum. Purdue Extension partners with the National Restaurant Association, Indiana Restaurant and Lodging, ServSafe, State and Local Boards of Health, and Ivy Tech community college to provide one- and two-day trainings, recertification training, proctoring of exams, and the Food Handler training for those in the food service industry needing certification to meet Indiana Food Code requirements. Other partnerships are with local area restaurants, hospitals, country clubs, schools, senior living facilities, food trucks and Illinois business owners.

Results
27 Health and Human Sciences educators and one specialist have become certified as dual instructors and proctors for the ServSafe curriculum for Manager Certification. In 2017-2018, 44 one-day, 12 two-day, 10 Recertification trainings

and 278 Exam-Only sessions were taught and proctored across Indiana. There were 688 participants in the one- or two-day trainings with 602 (88%) achieving certification on the exam (average score 84%). 88 individuals participated in the recertification training with 77 (89%) receiving certification (average score 85%). 278 took the exam either online or on paper with 218 (78%) receiving certification (average score 81%). A total of 1,052 attempted the exam either with direct training and 897 (85%) received certification. The Food Handler program was offered 4 times with 36 participants and 29 (80%) received a certificate of completion (average score 78%). A total of 123 participants completed the end of session surveys: 1) 74% found the program valuable, 2) 95% felt the objectives were realistic, 3) 93% thought they learned more than reading on their own, 4) 93% felt the resource people were well prepared, and 5) 93% found the materials were presented at the proper level of difficulty. As a result of the training: 1) 74% were washing hands more frequently during food preparation of and service, 2) 80% were checking temperature of food to make sure it had cooked to a safe temperature, 3) 84% were taking temperatures of food to make sure it cooled quickly to a safe temperature, 4) 81% were keeping raw foods separate from ready-to-eat foods, and 5) 79% were making sure all work surfaces, equipment and utensils were cleaned and sanitized before preparing and serving foods. Having workers trained, certified, and adopting safe practices in food preparation will help contribute to reducing risk of food borne illnesses in Indiana.

Title
Colloidal Phenomena in Food and Bioprocessing

Research
Ganesan Narsimhan, Agricultural and Biological Engineering

Outcome
6 - FS 1.2 # Viable prevention, control and intervention strategies for all food production scales for foodborne threats along the food production continuum

Issue
Cell death resulting from pore formation in cell membranes by peptides has important consequences in biology. An important function of pore forming peptides is in the defense of plants and animals against invading microbes. These antimicrobial peptides could also be very useful for combating drug resistant microbes. The exact mechanism of pore formation and cell death is not well understood. Elucidation of this mechanism can be applied to arrive at guidelines for design of antimicrobial peptides to replace antibiotics. Much lower intensity ultrasound can deactivate a microorganism by thinning the cell membranes as a result of regular oscillations of bubbles produced by cavitation. Ultrasound has found recent applications in sterilization of food when used in combination with heating or high pressure (or both) by increasing the efficiency of the process. It is of interest to lower processing cost of food products to improve quality and safety at lower cost. For conventional sterilization processes, energy input required to produce products with impeccable safety is very high. It is believed that pore formation by antimicrobial peptides (AMP) will be assisted by thinning of the cell membranes that are caused by low intensity ultrasound. Combined use of antimicrobial peptides with low intensity ultrasound is expected to be more energy efficient than conventional processes with minimum loss of quality. Pathogenic bacteria are known to develop resistance to antibiotic which is its biggest limitation. As compared to the traditional antibiotics, AMPs kill bacteria rapidly and can involve multiple targets. Naturally occurring AMPs are expensive and are of limited availability. Currently, there are no rational methodology for design of synthetic peptides (SP) with antimicrobial activity. Selection of SP for specific activity would require screening of large number of potential candidates. Such a screening by experimental investigation of kill rates of microorganism is currently empirical and is therefore not feasible. For example, synthesis of one gram of an AMP can cost up to $400, whereas for a conventional antibiotic, this price can be under $1. Thus, commercial-scale production platforms to synthesize AMPs are urgently needed.

What has been done
Curcumin (CUR) is a natural food ingredient with known ability of targeting microbial cell membrane. In this study, the interaction of CUR with different types of model lipid bilayer (POPE, POPG, POPC, DOPC, and DPPE), mixture of model lipid bilayer (POPE/POPG), and biological membrane mimics (E. coli and yeast) were investigated by all atom explicit solvent molecular dynamics (MD) simulation. CUR readily inserts into different types of model lipid bilayer systems in liquid crystalline state, staying in the lipid tails region near the interface of lipid head and lipid tail. Parallel orientation to

the membrane surface is found to be more probable than perpendicular for CUR as indicated by the tilt angle distribution. This orientation preference is less significant as the fraction of POPE is increased in the system, likely due to the better water solvation of perpendicular orientation in POPE bilayer. In E. coli and yeast bilayers, tilt angle distributions were similar to that for POPE/POPG mixed bilayer with water hydration number around CUR for the former being higher. Insertion of CUR resulted in membrane thinning.

Results

The results from these simulations can provide insights into the possible differences in membrane disrupting activity of CUR against different types of microorganisms. Recent studies have shown that both low frequency (20-100 kHz) ultrasonication and antimicrobial peptides (AMPs) treatment processes have a significant advantage in inactivating bacterial cells than the conventional heat treatment due to higher food texture quality of the final product. However, the effect of the combined process has not been fully investigated in complex matrices such as food. In this study, deactivation of Escherichia coli in different concentrations of milk and orange juice were performed using three different treatments: low frequency ultrasonication (20 kHz) at different power levels, antimicrobial peptide Cecropin P1 at different concentrations, and combination of both. The results of all samples showed that the combined treatment is more efficient, reducing the cell density of E. coli up to four orders of magnitude, compared to individual treatments. However, the milk concentration results in lower synergistic effect. This is believed to be due to complexation of milk proteins with Cecropin P1 thus resulting in less availability of the latter for antimicrobial action. This dependence was not observed in orange juice samples.

Title

Characterization of the lettuce microbial community by metagenomic sequencing and optical light scattering

Research

Bob Pruitt, Botany and Plant Pathology

Outcome

3 - FS 4.1 # Projects focused on increased understanding of the ecology of fecal indicators and pathogens

Issue

There are major gaps in our understanding of how well human pathogenic bacteria persist as members of plant-associated microbial communities and what factors influence their survival. Fungal metagenomic data for fresh produce are lacking, as are datasets that combine both fungal and bacterial communities from leafy greens. Analysis of the community will allow us to identify changes that are associated with the presence of human pathogens. In addition, the fresh produce industry currently does not have a method to rapidly screen samples for spoilage and/or pathogenic microorganisms that may be associated with the product. Creating a database of BEAM scatter patterns and DNA barcodes from microbes that are associated with lettuce will provide the industry with a means to identify and prevent the distribution of contaminated product to consumers. Research is needed to generate baseline data for the bacterial and fungal communities that are present on lettuce and how this influences the entry and persistence of human pathogenic bacteria into the plant phylloplane (leaf surface habitat for microorganisms). This research may also identify species that increase or decrease when pathogens are present. These indicator organisms may then be used to predict the presence of human pathogenic bacteria, even if the number of the pathogens is low. The work described here will extend the technology to characterize an entire bacterial community through the production of an extensive library backed by DNA sequence based taxonomic identification.

What has been done

The overarching goal of this project is to try and better understand the role that the natural bacterial community associated with romaine lettuce plays in the establishment and persistence of human pathogenic bacteria on commercially grown lettuce. To accomplish this goal we are adapting an existing technology (BEAM optical light scattering) to address a new type of problem: providing taxonomic identification of a wide range of bacterial species in a mixed community isolated from nature. Changes in the microbial communities will be characterized following the addition of bacterial pathogens. This will help establish possible indicator organisms that can be used to detect

contamination if present, as well as establish a library for BEAM that can be used by the leafy green industry to rapidly sample products to determine the spoilage and/or pathogenic organisms present.
This has not been without its challenges, but additional work on both the protocols and the software can most likely meet these challenges. The team is beginning to directly look at how the composition of the native bacterial community affects the growth of the human pathogens on the lettuce leaf. These effects turn out to be highly sensitive to the environment in which the lettuce plants are growing and so additional work will be required to identify the critical factors involved. A photographic library of the colony morphology of the identified bacterial genera associated with romaine lettuce is also being created as a web-based tool to be used as an identification guide for the fresh produce industry.

Results
Objective 1: Develop and test BEAM library of bacterial and fungal genera associated with romaine lettuce and validate BEAM as a tool for making taxonomic assignments at the genus level. Four BEAM libraries of the lettuce microbial community by collecting scatter patterns of 31 bacterial strains representing the 10 most abundant genera of bacteria found on romaine lettuce have been. These strains were divided into 4 libraries based on the optimal incubation time for a well resolved scatter pattern. Training sets were created with these data, and the Positive Predicted Values (PPV) of the cross-validation (CV) matrix were above 90% for all but one of the genera. Validation with pure cultures achieved PPVs of greater than 80%, but some genera showed misclassification rates up to 44% when grown in mixed cultures. Five bacterial genera were identified that produce similar scatter patterns. This issue will be addressed by increasing the number of features differentiating these bacterial genera in order to decrease their misclassification rates as well as by using a two-step classification system that allows clearer distinctions to be made between a smaller number of closely related scatter patterns.
Objective 2: Determine the effect of lettuce bacterial community composition and spatial distribution on the ability of human pathogens to establish themselves in the community and survive treatment with chemical sanitizers. This objective will also identify organisms that are possible indicators for the presence of human pathogens. Inoculation experiments have been carried out using small numbers of human pathogenic bacteria (100-200 cells) on lettuce leaves either removed from the plant and kept in a controlled environment or on intact lettuce plants growing in the greenhouse. Inoculated leaves grown in highly humid environments demonstrated that the pathogens are able to persist and replicate at variable rates under these conditions, with the growth rate observed depending on the age of the lettuce leaf. Pathogenic bacteria inoculated on plants in the greenhouse proved to be eliminated rapidly, probably due to the very low humidity found in this environment. A controlled set of experiments at varying temperatures and humidity's is currently underway in growth chambers.

Title
Food Safety and Good Agricultural Practices for Growers

Extension
Amanda Deering, Scott Monroe, Rhonda Taylor

Outcome
268 - FS 3.1 # Growers, producers, and food workers completing GAPs, GMPs, HACCP, food safety certification and on-farm BMP programs to increase food safety

Issue
The Food Safety Modernization Act (FSMA) was signed into law in 2011. The law shifted the role of the FDA from reaction to prevention. This shift required all states to evaluate their programs and regulations regarding the production and sale of food along the entire food supply chain. The Produce Safety Rule was the newest and one of the more complex rules introduced and little was available with regards to consistent or mandatory training. In response to the Produce Safety Rule, an increased number of wholesale buyers are asking more fruit and vegetable growers to have a third-party audit before they will purchase produce from their farms. The third-party audits are very expensive ($900-$10,000 depending on the size of the farm and the number of different crops grown) and this cost is mostly associated with travel and travel time as there are no Indiana based auditors.

What has been done

The Indiana State Department of Health, in collaboration with Purdue University developed extensive training protocols to meet FDAs new Standards for the Growing, Harvesting, Packing, and Holding of Produce for Human Consumption. To address the possibility of establishing an Indiana based third party audit system, Purdue worked with consultants (New Venture Advisors) to conduct a needs and feasibility study. This study involved growers, regulatory agencies (ISDH, ISDA), buyers, and Purdue Extension and the results indicated that a third-party audit system was feasible in Indiana. By combining several Hatch projects and previously funded USDA research with FDA funding, Indiana was able to administer the regulatory compliance of the Produce Rule of FSMA. Part of this training included an educational component to help growers learn about FSMA and the Produce Rule as well as teaching the Produce Safety Alliance Good Agricultural Practices Training that all growers who are covered by the Produce Rule need to take to be compliant (this replaced the previous GAPs A-Z training that was developed and offered by Purdue Extension).

Results

A total of 22 PSA GAPs trainings with a total of 260 people receiving certificates of completion from the Association of Food and Drug Officials were delivered since November 2016. Although funding has not been received to make a third-party audit system viable in Indiana, Purdue has been offering on-site farm visits and performing mock audits on farms to help them prepare for a third party audit. To date, Purdue has assisted 7 farms (who were doing a third-party audit for the first time) to pass their audit on the first attempt.