Food Microbiology Projects: Student Ownership from Topic Selection and Experimentation to Presentation in 10 Weeks

Resource Type: Curriculum: Laboratory

Publication Date: 2/2/2009

Authors

Joy G. Waite
Department of Food Science and Technology
The Ohio State University
Columbus, OH 43210
Email: waite.47@osu.edu

Ahmed E. Yousef
Department of Food Science and Technology
The Ohio State University
Columbus, OH 43210
Email: yousef.1@osu.edu

Abstract

Inquiry-based, student-centered projects were designed and implemented in a food microbiology laboratory setting. Students work in groups (three to five people) to ask a researchable question, design and complete experiments, analyze data, prepare a scientific poster, and defend their research in a public poster presentation. Groups are organized based on topic interest and guided to complete successful projects through a series of assignments and structured and open discussions during and outside of class time. Instructors guide students through the scientific method. Inclusion of projects in curriculum has increased students' interest in the food microbiology course and in this area of research.

Activity

Invitation for User Feedback. If you have used the activity and would like to provide feedback, please send an e-mail to MicrobeLibrary@asmusa.org. Feedback can include ideas which complement the activity and new approaches for implementing the activity. Your comments will be added to the activity under a separate section labeled "Feedback." Comments may be edited.

Learning Objectives.

Upon completion of this project, students will be able to…

1) locate relevant scholarly journal articles related to food microbiology topics,
2) ask questions that can be answered via experimentation in a microbiology laboratory,
3) effectively cooperate with group members to brainstorm experimental questions and formulate hypotheses, both in person and via a virtual chat,
4) design experiments (with a duration of four to six laboratory sessions over 2 weeks; for approximately 12 hours total) to answer experimental questions, including selection of appropriate methodology, growth media, and microorganisms of interest,
5) perform experiments using appropriate sampling methods, aseptic technique, and sound protocols,
6) analyze data collected from experimentation and draw conclusions based on these data,
7) design a presentation-ready poster,
8) publicly present and defend the project design and outcome, and
9) critically evaluate their own and other students' research projects.

Background.

For effective participation in these projects, students should have completed at least one general microbiology course with a laboratory component. Skills needed include familiarity with general microbial culturing techniques and use of selective and differential media, understanding dilution series and plate counting procedures, knowledge of proper handling and disposal of microbial cultures, and awareness of other safety considerations specific to the laboratory environment. In concert with these projects, students gain formal training in microbiological analysis of food during the course. This project is performed in a teaching laboratory with one professor and four teaching assistants, these individuals will be referred to generically as instructors throughout this manuscript. However, one of the instructors is designated as the "projects coordinator." The typical teaching assistant to student ratio is 1:8, with the project-coordinating instructor providing general guidance and oversight.

PROCEDURE

Materials.

Materials vary with the project and these will be determined by student groups. It is common that student groups request food products, specific microbiological cultures, and general supplies (media, dilution tubes, etc). Students typically choose materials (media, etc.) and protocols from those used previously in the course (5, 6); however, depending on the experiment, they may need to select different materials to maximize selection and differentiation of specific microorganisms. Students should first be directed to the literature to determine suitable media for experiments. These may include the course textbooks (5, 6) or one of the many reference books available on the subject (e.g., 1, 3, 4). Students should discuss their findings with the instructors to determine the most suitable medium based on cost, availability, and previous experiences. The authors commonly purchase supplies, including media, from Fisher Scientific, Weber Scientific, and VWR. Students submit a preliminary and final draft of the supply list 2 weeks prior to experimentation to allow ample time for media preparation and purchasing of supplies.

The planning stage of the project includes using the “Survey” and “Chat” features of an online learning management system (e.g., Carmen, which is implemented at The Ohio State University). Similar systems should be available through other online course programs (e.g., Blackboard, WebCT). If online programs are unavailable, surveys can be distributed via email and chat sessions can be created via instant messaging software. Alternatively, class time could be dedicated to performing these tasks.

Student Version.

Provided as a series of handouts (see Handout 1).

Instructor Version.

The goal of this project is to guide the students while investigating a researchable question using the scientific method within 10 weeks. An organizational flow chart of the project is shown in Fig. 1.

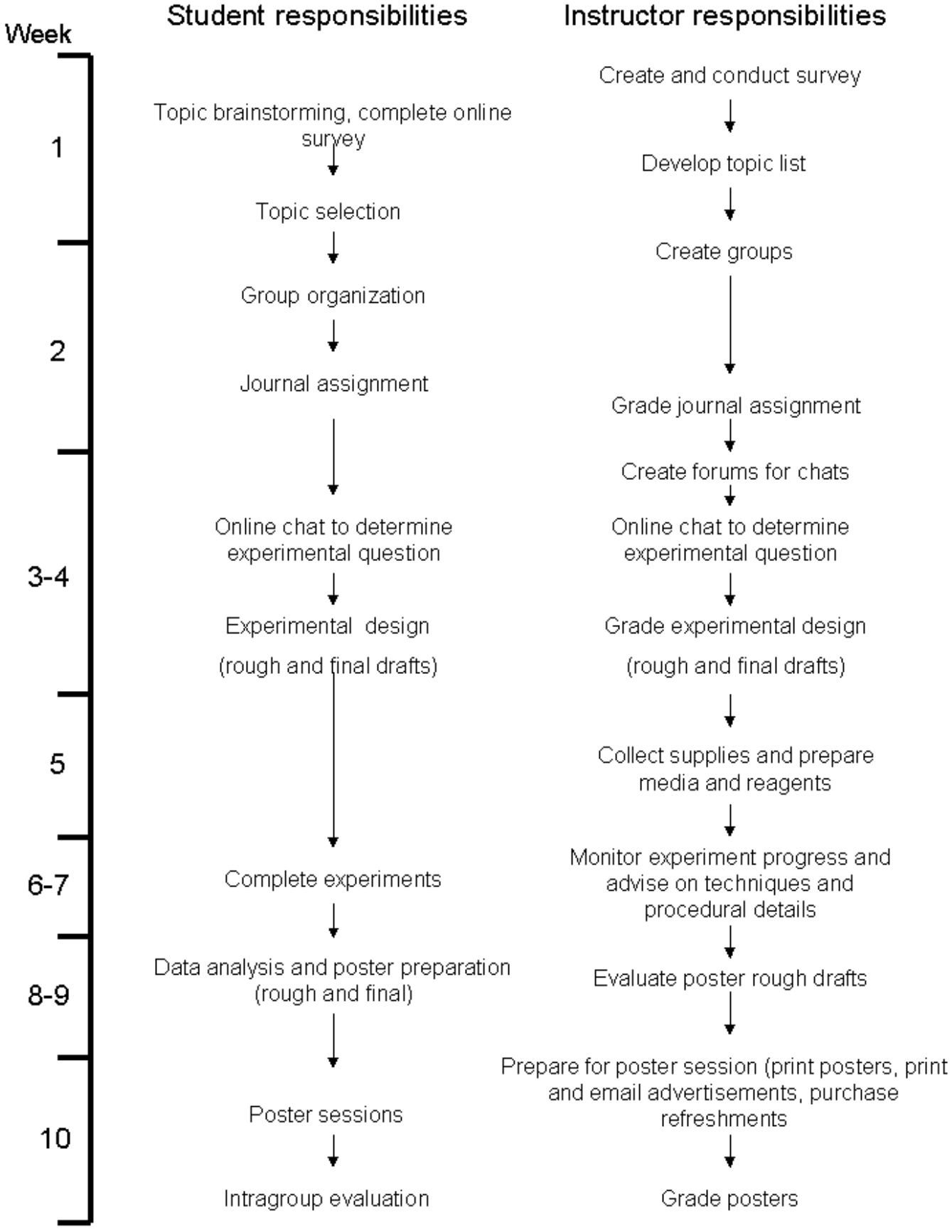


FIG. 1. Overall structure of food microbiology term projects including student and instructor responsibilities. Sidebar indicates typical weekly organization during a 10-week quarter.

Idea inception and compiling a topics list

Preparation for this project begins the first day of the quarter. Students are asked to provide at least two topics or concepts related to food microbiology that they would be interested in learning more about (see Project Inception). The students are asked to complete a general survey on the web-based course system within the first two class sessions. The culminating question of the survey requests these topics. Topics are judged for testability in the laboratory setting (biosafety level, access to equipment, past experience with the topic, etc.) The suitable topics are compiled in a list (an example is shown in Handout 2) for the students to rank their choices.

The list would ideally contain topics that can be reasonably researched using the facilities available at the university and within the time frame assigned. Depending on the availability of facilities and enthusiasm for the project, some students may desire to use resources outside the teaching laboratory; these students should be encouraged to do so if other researchers are willing to share resources and provide a safe research environment. For example, our course is offered in the Department of Microbiology but the students may have access and permission to use certain equipment in other departments (e.g., Food Science or Animal Sciences). In previous quarters, students have used ozone equipment, the meat processing facility, the irradiation facility, and the dairy pilot plant, which all were in external departments.

Success of projects depends largely on students' motivation and perseverance; however, some topics are harder to investigate than others. Additionally, some topics lead to projects that are more prone to fail than others. Therefore, it is critical that instructors use their own judgment and experience to determine topics appropriate for execution. Students are often curious about topics related to alcoholic beverages, including beer and wine; however there are limited applications for these topics within the 2 weeks allocated for project execution. Generally, the project duration is not long enough to test the contribution of different ingredients to the microbiological quality of end products, particularly when the finished products are typically low in microbial load. There is also a great deal of curiosity about antimicrobial ingredients in food, but many of these ingredients have been previously investigated and the goal is for students to test a new hypothesis and perform a unique experiment. Therefore, these topics are usually excluded from the complied list of potential projects.

Introduction of project

The project coordinator verbally presents the basics of the project and clearly explains the project details as shown in Handout 1. Examples of different assignments are provided in this same handout. These examples help students have realistic expectations of various assignments throughout the project. Examples of previously completed projects are mentioned and related posters are shown to the students so that they can visualize the project deliverables. These posters may be made available throughout the course for students to examine.

Topic selection and grouping students

Students are given Handout 2 with the list of topics that have been approved by the instructors. Students are instructed to choose five topics of interest and designate their top three choices. Students are advised to make topic selections independently, and instructors should emphasize that each student brings unique experiences to the project. Projects are more likely to succeed when students are genuinely interested in a topic than when their only motivation is to work with friends within the group. Experience has shown that close friends often do a poor job working effectively as a group and rarely do they equitably share the workload; the result is often a substandard project on many levels. Once the students have chosen topics, the instructors will regroup the students based on topic selection. Grouping students by topics is often a challenge, and it takes time to make sure that everyone gets to work on a topic in their top five selections. A course with 32 to 36 students enrolled produces eight or nine groups of three to five students. After topics are chosen, the students are physically reorganized in the laboratory so that members of each group are seated in proximity. When organizing groups, the instructors should make a good effort to include both graduate and undergraduate students in each group; it is also wise to avoid placing more than two graduate students in a group. Each group is assigned an "instructor-in-charge," with the project coordinator providing overall guidance on potential pitfalls.

Journal assignment

The first task that will be graded in the project is the journal assignment, which is completed by individual students. The objectives of this assignment are to (i) search the scientific literature for a topic of interest, (ii) obtain a relevant published article, (iii) correctly cite the article using a commonly-used style formatting, e.g., a format similar to that commonly used in the publications of the American Society for Microbiology, (iv) gain an understanding of the researchable topic, and (v) formulate potential experimental questions for the projects, guided by the published articles. Students will read the chosen article to become familiar with the topic and think about what constitutes a testable question related to this topic. A primary research article is ideal for this assignment; however a review article may also be acceptable. The authors accept both types of manuscripts for this assignment due to the general unfamiliarity of students with scientific articles. Book chapters are not accepted, but are recommended as supplemental reading for understanding the topic. Questions do not need to relate directly to the article but must relate to the topic and be microbiological questions.

Students may be unfamiliar with scientific literature searches, thus Handout 3 is provided to help them locate an appropriate reference for this assignment. Due to the time constraints of a quarter system, this assignment is graded and handed back to the students by the subsequent class period. Instructors give feedback on those experimental questions that seem most interesting and feasible, given facility availability and time constraints.

Virtual chat

After reviewing the graded journal assignment, members of each group are asked to schedule a virtual chat with all group members, their group's instructor-in-charge, and the project coordinator. The project coordinator will set up a private chat via the web-based course system. The objectives of the chat are to (i) brainstorm about the selected topic and discuss various testable questions, (ii) decide on a final experimental question of common interest to group members, and (iii) begin designing the experiment, including schedule and supplies. The instructor and project coordinator should guide the group towards consensus on the final question and assist in brainstorming ways to approach answering the question via the experimental design. Students should avoid repeating a published study or retesting a well-established phenomenon. These projects are unlikely to produce ground-breaking research findings; however the design and outcomes should be new to most students in the class. The students should rely on techniques practiced in the course, the work of researchers from other laboratories, or published research papers to

determine methods to use and supplies needed (e.g., appropriate growth media). Chat sessions are scheduled for approximately 1 hour, but the time necessary to achieve the goal varies among groups. The chat session was originally included in the project because of the difficulty in coordinating student schedules outside of class sessions. Most students have internet access at home; therefore, many of the chats occur later in the evening when everyone is at home. The virtual chat encourages all members to contribute to the discussion, regardless of their personality type. Those with a more introverted personality, who tend to not share ideas in face-to-face meetings or in classroom settings, are more forthcoming with questions and ideas on the virtual chat platform. Points are assigned based on participation during the chat session.

Experimental design—preliminary and final drafts

After the chat, the students need to layout the experimental design and supply list, in preparation for submitting a preliminary draft. Students should meet at their convenience to discuss these matters. The students are given an example of an experimental design in Handout 1. The objectives of the experimental design assignment are to design an experiment that will answer the chosen question and create a supply list so that the instructors can procure project supplies. It is likely that many students have no previous experience in designing an experiment, particularly in the area of microbiology. Students and instructors can review Barnard et al. (2) for assistance with the experimental design. After the chat and before the preliminary draft is due, the instructors need to follow up with each group to answer any questions and encourage discussion among the group members. For grading the experimental design assignments, the preliminary is worth three times as many points as the final draft. The assumption is that the students will spend the majority of their time and energy on the preliminary draft and that the final draft will only require limited changes. Preliminary drafts need to be carefully reviewed by instructors to make sure that the correct amount of supplies (e.g., plates, dilution blanks, food products) have been requested. This is also a good opportunity for the project coordinator to compare the designs of the different projects and determine if projects need to be modified to fit within the allotted time or to adjust the degree of complexity of project designs. The project coordinator will use the final draft to construct an electronic spreadsheet containing all supplies needed and determine which instructor is responsible for procuring those supplies. The spreadsheet is distributed to all instructors and associated staff members who are involved in this process.

Experimentation

Students will complete predesigned experiments during class time over a period of 2 weeks (three sessions a week, 2 hours per session). Some groups will need additional attention from instructors on the first day of experimentation, especially those groups that are inoculating food products with specific organisms. Challenging food with microorganisms is a new experimental approach to most students and they need guidance to complete this task safely and in a timely manner. Group members often want to start earlier or leave later than the scheduled class session time in order to complete required bench work; instructors should be able to accommodate these needs. Students should not run laboratory work unsupervised. Additionally, some projects may require access to equipment in other buildings; therefore, students and instructors need to arrange for permission to use the equipment and access to these buildings.

Poster preparation

Students work together outside of class time to compile a scientific poster using Microsoft PowerPoint or other appropriate software. Written guidelines are given to the students about what should be included in the poster (Handout 4). The objectives of poster preparation are to analyze and determine an effective way to present data and work as a team to produce a poster that effectively presents their experiment to people unfamiliar with their work. A template is not provided to the students to avoid similarity between the posters. However, some of the students may choose to acquire templates of posters presented in previous course offerings. The students have approximately a week between the culmination of experimentation and the due date for the preliminary draft of the poster. Students are highly encouraged to ask for advice from instructors as they prepare the poster and proofread their poster multiple times to catch grammar mistakes. Each student group is asked to deliver an electronic copy of their poster. There are no points associated with the preliminary draft of the poster. The preliminary drafts are reviewed and proofread over a weekend by a single instructor (often, the project coordinator); this ensures that the other instructors have unbiased views while evaluating the finished products of all groups. The project coordinator meets individually with each group during the subsequent class period to provide advice to improve the overall quality and appearance of the poster.

Poster printing

The final draft of the poster is due by the end of the ninth week of the quarter. Immediately after the electronic file of the poster is delivered, the project coordinator (or a designated member of the group) prints the posters. Two weeks prior to printing, the project coordinator should identify a suitable nearby poster printing facility (e.g., Kinko's, Staples, or similar office supply stores). Some departments have a dedicated poster printer; in this case, the project coordinator should ensure the functionality of the printer and check that there is enough ink or toner to print all posters.

Poster presentations

During the poster presentations, students are expected to effectively explain their research to people unfamiliar with their experiment. The posters are presented by the students on two separate days in two different, but related, locations on campus (for the course offered at The Ohio State University, poster presentations are held at the Department of Microbiology and the Department of Food Science and Technology). In preparation for this occasion, the project coordinator should reserve rooms or lobbies and poster stands several weeks in advance. The poster presentation sessions are designed to mimic poster sessions at scientific conferences. Students should dress professionally and interact with attendees from diverse backgrounds. For a group of four students, two students present their work on the first day and the other two on the second day. For groups of three members only, one student presents on one day and the other two on the second day. For groups of five, three students present on one day and the remaining two on the second day. The students decide among themselves who will present on each day. On the day they are not presenting, these group members will be evaluating the presentations of other groups in the class. This is the first opportunity for the students to collectively learn about all work done in the class. The poster sessions should be open to the public, faculty members, and students from other classes; these diverse attendees ask questions and evaluate the presentations. Advertisements for the poster presentations are emailed to the departmental listserve and are posted around the departments to encourage attendance. A festive environment during poster presentations improves students' morale. Efforts to create this environment include inviting administrators and popular individuals to evaluate the posters and providing refreshments (e.g., bottled water, soda, and cookies) for the visitors and the presenters. Students who are presenting are asked to wear a name tag (provided by instructors). Due to constraints of scheduled class times, instructors mount the posters prior to class and remove them when the session is completed. Students are provided with forms to write feedback about other groups' posters (Handout 5). Specially-marked forms are given to attendees from outside the class. Instructors should complete their own detailed forms.

Grading the work of groupmates

After the completion of the poster session, but prior to the release of grades for poster presentations, students are asked to evaluate their individual group members using numerical scores (50 points maximum). Each student is asked to email to the project coordinator a grade for each student within the group. No explanation is required for the scores; however students who have a strong opinion about a particular issue may choose to include a justification of the score. Some students may be reluctant to evaluate other students; these students should be reminded (e.g., by e-mail) to complete their evaluations. Students who contribute positively to the team work and are punctual for meetings outside of class time usually earn high scores from their classmates. Instructors cannot evaluate these matters because they do not usually witness details of group dynamics. Therefore, these scores provide insight to the instructors about the inner workings of individual groups and provide anonymous feedback to individuals on how their work ethic and cooperation is perceived by their peers.

Feedback from students

After the completion of the poster session, students are asked to fill out a questionnaire about the projects as a learning tool (Handout 6). This is an opportunity to gather feedback about the projects and learn how the projects could be improved in future offerings. The students can complete the questionnaire anonymously, if they choose. This questionnaire was particularly useful when designing and modifying the project assignments and deadlines. Suggestions from students encouraged instructors of this course to modify the culminating work product from a written laboratory report and oral presentation to the scientific poster and poster presentation.

Safety Issues.

Projects give students some freedom to choose a topic, design an experiment, and work independently while executing the study. However, this freedom should be met with a sense of responsibility on the part of the students to ensure their own safety and the safety of their classmates. Ideally, these projects are executed with the least intrusion of instructors. However, instructors should interfere immediately if they sense that safety rules are overlooked. Safety becomes a significant issue when the project involves the use of pathogenic microorganisms.

Instructors and students must be aware of the risks associated with using pathogenic or potentially pathogenic cultures, and the laboratory work must be conducted with the necessary precautions based on risk classification of the test organism. Handout 7 is included to address specific issues and general guidelines related to setting up and maintaining a safe teaching laboratory that meets Biosafety Level 2 (BSL-2) guidelines. Readers in the USA should be aware that infectious waste regulations are governed at the state level. Therefore, readers should review the infectious waste regulations for their state, including what is considered infectious waste and how it should properly be disposed. In the food microbiology laboratory course, students receive formal training and gain experience relevant to safe handling of pathogenic organisms. This training occurs during the structured laboratory exercises, before students begin working on these projects. A formal description of the safety guidelines and rules for the laboratory is communicated to the students at the beginning of the quarter and a "safety contract" is signed by the students indicating their willingness to adhere to these guidelines (Handout 8). As the quarter progresses, specific safety issues are discussed in the context of specific laboratory exercises and projects.

Additional safety issues arise if students choose to work in a pilot plant to produce their own products using food processing equipment. Students who include food processing in their project often have worked with that particular processing equipment in a previous class or during their own research. Based on our experience, both students and instructors favor projects with no major safety concerns.

ML Safety Statement Regarding Environmental Isolates.

The Curriculum Resources Committee recognizes that isolated organisms can be a powerful learning tool as well as a potential biological hazard. We strongly recommend that:

- Environmental enrichment laboratories should only be performed in classes in which students have been trained to work at a BSL2.
- Direct environmental samples (eg. soil, water) which are known to contain infectious organisms should be handled according to the biosafety level of that infectious agent.
- Cultures of enriched microorganisms, derived from environmental samples, should be handled using Biosafety Level 2 precautions.
- Mixed, enriched or pure cultures of microorganisms from environmental samples with a significant probability of containing infectious agents should be manipulated in a biosafety cabinet if available.
- Where possible, media used for the enrichment of environmental isolates should contain an appropriate anti-fungal agent.
- Instructors should be aware if they are teaching in regions with endemic fungi capable of causing systemic infections, and should avoid environmental isolations.

Suggestions for Determining Student Learning.

Student effort is assessed on an individual and group basis for a number of assignments for this project. Successful completion of objectives by the students is judged by a variety of methods including written assignments and verbal communication. Below are the list of objectives and how each is evaluated.

Objective 1. Locate relevant scholarly journal articles related to food microbiology topics.

Assessment: Evaluation of written journal assignment. The assignment is graded on delivering a full-text copy of the article (5 points) and correctly adhering to the agreed-upon style in citing a reference (10 points).

Objective 2. Ask questions that can be answered via experimentation in a microbiology laboratory.

Assessment: Evaluation of written journal assignment. This is evaluated on whether the question is related to food microbiology, appropriateness of the topic, and testability of the questions (10 points).

Objective 3. Effectively cooperate with group members to brainstorm experimental questions and formulate hypotheses, both in

person and via a virtual chat.

Assessment: Observation and evaluation of students during virtual chat and in-class discussion times. Students are evaluated based on participation during the chat, asking questions, and cooperatively making decisions. Most students receive full credit on the chat assignment unless instructors felt that they were not participating or the student was not available during the chat.

Objective 4. Design an experiment (given four to six laboratory periods over 2 weeks, approximately 10 hours) to answer the testable question, including selection of appropriate methodology, growth media, and microorganisms of interest.

Assessment: a) Observation and evaluation of students during virtual chat and class discussions, and b) evaluation of preliminary and final drafts of experimental design. Students are evaluated on whether their experimental design actually addresses the testable question and if they have requested the correct supplies to complete the experiment. The rough draft of the experimental design is worth a total of 75 points. This is the main opportunity for the instructors to provide feedback to the students on their design and to correct any issues with the experimental approach. Points are deducted from groups that need to further modify their design and/or their supply list. Groups that provide drafts requiring substantial modifications to the experimental design and supply list are penalized 10 to 15 points. Groups needing to modify their supply list usually lose 5 to 10 points. The final draft of the experimental design is worth 25 points. The groups' scores for the final draft are determined upon completion of the experimentation when instructors can document if students planned appropriately and ordered all necessary supplies. Minor issues are forgiven; however substantial modification (i.e., last-minute request for twice the original number of dilution blanks) will result in a deduction of 5 to 10 points. In practice, full points for the final draft are commonly awarded because missteps in the design and supply list may have been overlooked by the project coordinator, the instructors, and the students.

Objective 5. Perform experiments, with particular attention to sampling methods and aseptic technique.

Assessment: Visual observation of students doing experimentation during class time. There is no formal assessment of this objective (i.e., no points are given by instructors).

Objective 6. Analyze data collected from experimentation.

Assessment: Evaluation of data as presented on the final poster. Additionally, raw data, calculations, and figures may be reported to the instructors for assessment before the poster session. Assessment of this objective is included with the assessment of the poster.

Objective 7. Design a scientific poster.

Assessment: Evaluation of final poster. Posters are graded using a ranking system. The instructors share among themselves their opinions about each poster. Each instructor then ranks each poster relative to the other posters in the class. Overall ranks are determined and the instructors agree on a high score and a low score, out of 100 points, based on the poster receiving the highest and lowest overall ranking. The remaining ranks are converted to grades using a regression line. Scores usually range between 70 and 100 points, out of 100 total points. Alternatively, the poster evaluation rubric could be used for determining scores (Handout 9).

Objective 8. Publicly present and defend the project.

Assessment: Discussion with students during poster session. Poster presentation scores are determined in a similar fashion as the poster scoring mentioned earlier. Scores generally range between 75 and 100 points, out of 100 total points. Alternatively, the presentation evaluation rubric could be used for determining scores (Handout 10).

Objective 9. Students critically evaluate their own and other students' projects.

Assessment: a) Observe students as they visit other posters, and b) evaluate written comments of students on the poster evaluation forms (Handout 5). There is no formal assessment of this objective; however assessment could be included on the student comment evaluation form.

Field Testing.

The concept of this project was first discussed in the autumn of 2004. It was first included in the curriculum in spring quarter of 2005 in the food microbiology laboratory course (MICRO/FD SC&T 636.02) at The Ohio State University. The class size is approximately 36 students (nine groups of four). Students in this course are undergraduates in microbiology, biology, and food science, and technology majors and graduate students in food science or veterinary preventive medicine. The project has been included in the curriculum during the past seven quarters. Several permutations of the project were necessary before this exercise could be completed smoothly with appropriate scheduling, guidance, and communication of clear expectations. Attendance at the poster sessions usually consists of the students in the class (18 presenting and 18 visiting posters), the instructors (five), several outside faculty members (five to six), and students not taking the course (approximately 12).

The project, as described in this manuscript, has been very successful and popular with the students and poster session attendees. The following is a list of responses to the questionnaire given at the end of the course (Handout 6):

"This was the first time I did anything like this and I think it helps a lot. It is more like the real world and teaches you how to work with people and talk to people as well as design experiments."

"I have not done similar projects, it was very valuable."

"I have never done this before, but it was a good experience and I would recommend this class to others because of it."

"I learned to design experiments based on searches from papers, to work together, and discuss, and resolve some questions, and to write down experimental notes at the time."

"This was the best and most helpful part of the class."

"I have never done it before. This food micro class has given me such precious opportunity. I really liked it."

"I feel much more capable of doing scientific research."

"This was my first time doing a poster presentation. It was fun."

"It is very valuable. We got to succeed or fail (in the experimental sense). It was very good."

"I have done projects before, but this was by far the most in-depth, hands-on, and interesting project. It was exciting."

"No, I have never done a similar project and really wish I could have as an undergrad. It's kind of scary to do but I think it makes everyone more confident and knowledgeable about the research process."

Student Data.

The following have been included as appendices: example of the online chat session, example of a final draft of experimental design, examples of two finished posters, and two pictures from a poster session.

SUPPLEMENTARY MATERIALS

Possible Modifications.

When this project was first implemented, a laboratory report and an oral presentation were required by each group in lieu of the poster and its presentation. Both of these formats have a similar aim and could deliver suitable end products. Choosing the correct final product is crucial for project success and positive student experience at the culmination of these projects. Based on the authors' experiences, written laboratory reports should be chosen as the final work product only if students have ample experience in writing scientific reports. If oral presentations are chosen, students should be given practice time, preferably with feedback from at least one instructor, prior to the final class presentation.

The amount of time dedicated to the experimentation portion of the project may be modified as necessary to fit the course schedule. In-class time for experimentation has been successfully implemented with four to six class periods devoted exclusively to project experiments. In a semester system, a longer time could be allocated to project experiments. It is inadvisable to execute these projects in a period shorter than 2 weeks.

This inquiry-based project could be modified in a number of ways. The success of these types of projects depends largely on balancing structure of the project with the flexibility to allow the students to own the project. This type of project, using the guidelines presented, could be implemented in many microbiology laboratory courses.

References.

1. **Atlas, R. M.** 2004. Handbook of microbiological media, 3rd ed. CRC Press, Boca Raton, FL.

2. **Barnard, C. J., F. S. Gilbert, and P. K. McGregor.** 2007. Asking questions in biology: a guide to hypothesis testing, experimental design and presentation in practical work and research projects, 3rd ed. Benjamin Cummings, New York, NY.

3. **Downey, F. P., and K. Ito (ed.).** 2001. Compendium of methods for the microbiological examination of foods, 4th ed. American Public Health Association, Washington, DC.

4. **Jackson, G. J., R. I. Merker, and R. Bandler (ed.).** 2001. Bacteriological analytical manual. http://www.cfsan.fda.gov/~ebam/bam-toc.html. U.S. Food and Drug Administration, Washington, DC.

5. **Yousef, A. E., and C. C. Carlstrom.** 2003. Food microbiology: a laboratory manual. John Wiley & Sons, Hoboken, NJ.

6. **Yousef, A. E., J. G. Waite, and C. C. Carlstrom.** Analytical food microbiology: a laboratory manual, 2nd ed., in press. John Wiley & Sons, Hoboken, NJ.

Acknowledgments.

The authors would like to thank the students who have completed these projects; their patience and interest have made these projects a successful experience, and their feedback has made the projects improve over time. The authors express extreme gratitude to Matt Mezydlo of The Ohio State University, Department of Microbiology, for his hard work and patience in preparing and organizing supplies for these projects. The authors would like to thank Dr. Sujaya Rao and Devora Shamah of Oregon State University for essential training in inquiry-based curriculum design. The authors would also like to thank Dr. L. H. Newcomb, The Ohio State University, and his Methods and Techniques in Agricultural and Extension Education students (AGR EDUC 735N, winter 2007) for ideas and techniques to enhance student-centered learning. The authors also express gratitude to Tina Bognac, biosafety officer at The Ohio State University, for her time and assistance with biosafety guidelines.

Appendices.

- Project inception
- Handout 1: Food microbiology project details
- Handout 2: Project topics
- Handout 3: Hints for finding and locating journal articles
- Handout 4: Tips for poster preparation and presentation
- Handout 5: Class poster evaluation
- Handout 6: Project questionnaire
- Handout 7: Biological safety guidelines for instructors

- Handout 8: Biological safety guidelines for students
- Handout 9: Poster evaluation rubric
- Handout 10: Presentation evaluation rubric
- Example chat session
- Poster 1: An example of a poster presentation
- Poster 2: An example of a poster presentation
- Picture 1: Poster presentation day
- Picture 2: Poster presentation day
- Picture 3: Laboratory session

Project inception survey question (online via Carmen)

In this course, you will work with a group of students on a term project that you will design, conduct, and share by presenting your findings. We need to develop a list of topics that are interesting to students. Please list several topics related to food microbiology (at least two) that you would like to learn more about. Some broad topics to get you started would be: a specific food product, food processing applications (e.g., thermal, acidification, ultrahigh pressure), fermentations, specific microorganisms, different types of media for recovery of food microbiota, etc.

1. Food Microbiology Project Details

General information

The goal of this project is for students to gain insight and experience in designing and implementing a short term research project related to food microbiology. During the early exercises of the course, students will gain knowledge and experience with basic microbiological techniques. Upon completion of the first stage of the course, students will have the tools and knowledge to address questions about food microbiology.

These projects will be completed in groups of four students. The projects are student-designed with the help of a planning advisor (instructor-in-charge). Each group will have a planning instructor, who provides guidance on their project and with whom they will discuss their plans. This instructor will not design experiments or provide key information to the group. It is the group's responsibility to research the necessary components of their project and to become familiar and knowledgeable about their research topic.

Project timeline

Date	Subject	Points
Week 1, Day 1	Provide at least two potential topics via survey	
Week 2, Day 1	Rank topics that you would like to work on	
Week 2, Day 3	Receive group assignment and instructor-in-charge , rearrange seat assignments	
Week 2, Day 5	Turn in journal assignment: journal review	25
Week 3	Discuss and work towards decision of final question Discuss and work on experimental design using online chat	25
Week 3, Day 5	Turn in experimental design and supply list rough draft (group)	75
Week 4, Day 3	Turn in experimental design and supply list final draft (group)	25
Weeks 6 and 7	Complete experiments	
Week 8, Day 5	Turn in project poster draft	
Week 9, Day 5	Turn in final project poster	100
Week 10	Give poster presentations (group)	100
Week 10	Score your groupmates (individual)	50
	Total	400

Considerations

Group projects are usually a challenge for a number of students. All students are pressed for time, but we believe that this will be a wonderful opportunity for each student to research a topic of interest. Students are asked to be respectful of their teammates and their time.

Group personnel will be assigned based on the individual's topic preferences. In general, enthusiasm of a group of students for a topic enhances work ethic and teamwork. Students' backgrounds are taken into account when forming groups. Due to the integrated nature of these projects, groups comprising undergraduate and graduate students, as well as containing some food science majors and some microbiology majors would be optimal. The group dynamic obtained from such a combination of students is seen as optimal, but this is unattainable for all groups; some groups will likely not have any graduate students and some may be entirely microbiology students.

Due to the importance that we place on this research and design opportunity, this term project will be a significant portion (25%) of each student's final grade. Students will note that there are several steps at which no points are associated with a specific requirement. These tasks are expected to be completed appropriately and reported on the assigned due date.

General topics

The instructors have compiled a list of general topics based on the results from the student survey completed earlier in the quarter. Each student ranks the top five topics of greatest interest. Students will then be grouped based on their interests.

Following is a list of the topics from previous course offerings:

1. Coliform counts in chicken Caesar salads from various fast food restaurants and variation with extended storage
2. Efficacy of different sanitizers on inactivation of *Salmonella* and *Escherichia coli* inoculated on fresh vegetables
3. Changes in microbiota of ground versus whole muscle meat products with extended storage
4. Growth of *Escherichia coli* and native microbiota on fresh spinach
5. Effect of salt concentration on the fermentation process of sauerkraut
6. Does fat content affect the microbial spoilage of milk?
7. Growth and survival of pathogens inoculated in organically- versus traditionally-produced food products
8. Does flavor additive affect the ability of *Listeria* to grow and survive in commercial yogurt products?

Journal assignment (25 points)—journal review and five questions or ideas

Each student is required to locate and copy a journal article related to their topic that will be a potential reference for their poster. Each student turns in a copy of their journal article, a list of five questions or ideas that they have for a short term research project (these need to be

topic questions, not experimental design questions), and their journal article reference formatted in ASM style (see style guidelines on website). This initial report will familiarize you with searching for journal articles, reading journal articles, practicing ASM-style reference formatting, and brainstorming questions.

Students may seek instructors' help if they are unfamiliar with databases for finding journal references (i.e., PubMed, SciFinder Scholar, Google Scholar, Agricola, etc.).

We encourage students to work on this portion of the project as individuals. It will help if everyone attempts to learn background information about their topic on an individual basis so that everyone can contribute to the overall brainstorming and understanding of the experiment. Groups will then have an opportunity to discuss their options and decide on a final question or idea for their experiment.

An example, Project Report—draft 1, is attached to these instructions.

Virtual chat for experimental design (25 points), schedule for week 3

Due to the diversity of the project groups, it may be difficult to schedule meetings for planning the experiments and discussing options. Therefore, a virtual chat is scheduled amongst the participants, their instructor, and the project coordinator to decide on an approach for the experiment and design considerations. The chat should be scheduled by week 2 for chat time during week 3. Students are graded on their participation in the chat.

Experimental design and supply list (rough draft, 75 points; final draft, 25 points)

Students of each group submit a short report identifying the final question or idea chosen for the research topic, objectives, experimental design, and a supply list separated by day of experiment. This is the most crucial component of the project; miscalculation of supplies will result in frustration and/or change of experimental procedures. Students need to make sure this report is very clear, with no room for incorrect interpretation. This is crucial to avoid burdening the media preparation facility and staff. A rough version of the final report is submitted by the end of the third week and reviewed by instructors to improve the accuracy of the finalized report.

An example, Experimental Design and Supply List—final draft, is attached to these instructions.

Experiments (weeks 6 and 7)

Each group organizes and performs necessary experiments to answer their question. In order to not hamper students' creativity, we have not included strict requirements for this portion. Each group's project is independent of the others; therefore all necessary experiments should be thought out and performed in a reasonable time frame with the participation of all of the group members. The students should consider whether their results will be qualitative or quantitative.

Poster preparation and presentation (draft due week 8, final project due week 9, presentation week 10)

Upon completion of the experiments, each group will prepare a scientific poster using Microsoft PowerPoint software and present this poster during two sessions. The poster session on Wednesday will be held in Venue A. The poster session on Friday will be held in Venue B. Students will be assessed on the clarity and content of the poster, as well as their interaction with and presentation of the poster to visitors. The poster session will be open to the public in both buildings (venues) and feedback on the posters will be sought from all attendees. Students will also critique the other groups' posters. Instructors will collect written comments from the students and attendees and feedback will be provided to the presenters. Everyone in the group must equally participate in the group presentation (two members should be in charge of presenting the poster and answering questions for each session, during the alternate session these members will critique the other posters).

It is understandable that many students have not prepared a scientific poster prior to this class. These students are encouraged to ask instructors for assistance with any questions regarding design of the poster. This is an important and common way of presenting scientific information at scientific conferences. PowerPoint is the program most commonly used for this application.

Students are graded on the content and design of the poster (100 points) and on their presentation and ability to answer questions during the poster presentation sessions (100 points). A draft of the poster is due during week 8 so that instructors can make any final comments and corrections before the final posters are due in week 9. The project coordinator will print posters for the students and deliver them to Venue A for the first poster presentation.

Intragroup evaluation (50 points, at the discretion of group members)

Occasionally, there are members who fail to contribute effectively to the group. Therefore, each group member will score the other group members based on their performance throughout the course of the project (up to 50 points). These scores should be submitted via email to the project coordinator by the end of week 10.

Jane Doe

Project Report—draft 1: journal review and five questions or ideas

Journal article

1. **Smittle, R. B., J. A. Downing, and T. F. Spaulding.** 2000. Microbiological safety of mayonnaise, salad dressings, and sauces produced in the United States. J. Food Protect. **63:**1144–1153.

Five questions or ideas

1. Can pathogens survive in ranch salad dressing stored at room temperature?
2. What organisms live in refrigerated ranch salad dressings?
3. Do the components of salad dressing protect microorganisms from lethal processing conditions (heat, high pressure, etc.)?
4. What media result in the greatest recovery for enumerating spoilage organisms from salad dressings?
5. What are the components of or processes involved in making ranch salad dressing that inhibit microbial growth?

Remember to staple a copy of your article to this one-page report when you hand it in. The copy will be given back to you. Don't worry if there is writing on the copy of the article.

Sam Johnson, Jim Fox, Janet Brown, and Tracy Smith

Experimental Design and Supply List—final draft

Final question or idea: Does extended refrigeration of ground meat promote microbial growth?

Objectives

- Observe relative quality of prepackaged, refrigerated ground meat
- Compare the total microbial load of three ground meats during extended refrigeration
- Compare most probable number of coliforms in three ground meats during extended refrigeration
- Compare number of *Pseudomonads* in three ground meats during extended refrigeration

Experimental design

Three different types of ground meat (with approximately similar sell-by dates) will be obtained from a local grocery store and refrigerated for a variable number of days. Meats will be aseptically sampled on determined days, appropriate dilutions will be carried out for each test, and media will be inoculated. All inoculated agar media will be incubated at 37ºC for 72 hours prior to enumeration of microbiota.

Materials needed

Day 1 (Date)

Three meat samples from store
Three 99-ml bottles of peptone water
18 9-ml test tubes of peptone water
18 trypticase soy agar plates
21 lauryl sulfate tryptose broth tubes
21 *Pseudomonas* Isolation Agar plates
Eschericia coli control culture
Pseudomonas sp. control culture

Day 2 (Date)

Same as above, except meat samples

Day 3 (Date)

Same as above, except meat samples

Day 4 (Date)

Same as above, except meat samples

Day 5 (Date)

No supplies are needed. We will be counting the plates.

Name: _____

2. Project topics

Here is a large list of topics that all of the students proposed via the online survey on the first day of class. If you don't see an idea that you submitted, it is likely due to our limitations regarding equipment and supplies.

Directions: Circle your top five choices. Please indicate the top three preferences with numbers or some identifying feature.

Spoilage organisms
Growth rates in foods
Organic versus conventional foods
Certain foods supporting certain organisms (i.e., *Salmonella* in chicken, *Escherichia coli* in beef)
Environments causing spoilage
Ozone
Acidity to control microbes in foods
Acid tolerant yeast
Shelf-stable dairy products
Raw seafood products
Spices
Lactic acid bacteria as a biopreservative
Biofilms
Fermentation
Pasteurization
Meat
Yogurt
Berries
Thermal processing
Sanitation
Apples
Milk (chocolate milk)
Bread
Ice cream
Alcohol
Produce
Meat spoilage
Expiration dates
Cheese
Fruit
Escherichia coli
Fermented foods
Probiotics
Spinach

3. Hints for finding and locating journal articles

Google Scholar**,** located at www.scholar.google.com, is a free search engine for scholarly publications (journal articles, books, patents, etc). Just type in the keywords and a list of related articles appears.

Sometimes, when you click on the title of the article the search engine displays the entire article (yeah!) and you can print it. If you are on a campus computer, there may be a link next to the journal article title that says "Find it with….". Clicking on this may lead you to a PDF version of the file through the library service. Often, neither of these will work and clicking on the article title may only bring the abstract from another site (e.g., PubMed). This means that you have to go to the library website to see if the university has a web subscription. Search for the journal title that you are looking for and follow the links and print the article. Alternatively, you can find the print holding for the article on the library website, go to the appropriate library, and make copies.

There are numerous other search engines available for searching scholarly publications:

PubMed at www.pubmed.org
SciFinder Scholar
Link on the library homepage for "Research databases"

For this assignment, a complete scientific journal article is needed; abstracts only will not be accepted. A book chapter or patent will not be accepted. Likewise, articles from mainstream magazines will not be accepted.

4. Tips for poster preparation and presentation

Turn in electronic copies of the rough and final drafts of the poster on a CD with your names on it.

POSTER PREPARATION

The poster should be prepared in Microsoft PowerPoint or other appropriate software program. Page setup in the program should be changed from its default value to a suitable poster presentation size. It is recommended that the page be set at 46 inches by 36 inches (landscape). We will not be providing template files in order to avoid obvious similarities between the poster designs. The following sections should be included in the poster.

Title

The title is located at the top center of the poster. It must be descriptive and indicative of the basic premise of the project. The title font size should be ≥50 point. The names of authors of the poster and their department affiliation should be listed under the title. The font size for author text should be 40 to 50 point.

Abstract

The poster should contain a 200 to 250 word abstract. The abstract should summarize concisely various project components (introduction, objectives, methods, results, discussion, and conclusion). Visitors to the poster presentation should be able to read the abstract and get a clear idea about the experiment. When properly prepared, the abstract invites the participants to read the presentation details.

Introduction

The introduction section should contain pertinent background information that is important for understanding the goal of the project. This section contains factual information from references that should be cited correctly. Use the agreed upon reference citation format. Ideally, students will use some of the references from their journal assignment. The introduction should lead to, and conclude with, project objectives.

Materials and Methods

This section is a concise description of supplies, equipment, experimental design, and methods used in the project. Instead of text, this section may contain flow charts, pictures, and clip arts; the more visual, the better. Include any product information, media type, and microbial cultures, as needed.

Results

Results should be presented as figures, tables, or pictures, as necessary. It is rare that results are reported as text only. Include detailed titles for all presented data; remember that a figure legend goes below the figure and a table title goes above the table. Feel free to include additional arrows and text to highlight certain specific information of importance.

Discussion

The discussion section should concisely highlight and explain the findings of the experiment. Each data series or data cell may be explained in one or two sentences. Bullet each point and refer to the figure or table that the statement is discussing. A meaningful conclusion (one or two sentences) should be provided at the end of the discussion section.

References

References cited in various sections of the poster should be listed. Follow the style formatting used in ASM publications.

Acknowledgments (optional)

This section may be included to acknowledge any persons, outside of the group, who helped with the experiment or poster. Acknowledgment becomes necessary if the group received external help or when members are allowed to share equipment that was not available in the food microbiology teaching laboratory.

Cautionary notes

- The poster should be constructed with the assumption that students of the class are the intended patrons. For example, one can assume that items learned throughout this course are known by all classmates.

- In general, font size should be a minimum of 18 point, preferably 24 point.

- Posters look the most professional with four columns. These columns are not necessarily identical in width. However, some segments or boxes may span more than one column (e.g., two columns wide).

- Use pictures where appropriate, but don't use them if they are obnoxious or unrelated to your project.

PREPARING FOR PRESENTATION

Students are evaluated on their presentation skills; this includes ability to explain the experiment or address questions from their peers, instructors, and visitors. The poster session will be open to the public in both venues (buildings) and instructors will ask for feedback on the posters from all attendees. Students will also critique the other groups' posters. Everyone in the group must equally participate in the group presentation. Two members will present the poster in Venue A, while the other two members critique the posters of the other groups. Group members will switch responsibilities when the presentations are held in Venue B. The poster session on Wednesday is held in Venue A, whereas the session on Friday is held in Venue B.

GENERAL TIPS

- Students should dress in business casual attire on the day they are presenting. No denim.

- Each member of the group should be able to quickly explain (5 to 10 minutes) their experiment and findings to poster visitors. Each poster will likely have more than one visitor at a time; try not to seclude anyone. Poster attendees are generally very interactive and every visitor has a different expectation. Some visitors like to view poster contents before asking questions of the presenter, while others prefer the project is explained to them first before they ask questions. Presenters should be accommodating to both types of visitors.

- The presenter should refer to the poster contents while answering questions. Point to the steps in the method that you are describing and point to the results that you are summarizing. Do not point to the text that you are reading! You should be relaxed and having a conversation about your work, not reading to the visitor what you wrote.

Name: _____

5. Class poster evaluations

Please comment on the following posters and presentations (positive and negative feedback is helpful). Do not evaluate your own poster. Please circle the poster title that you were the most interested in (best topic) and put a star next to the poster that was the best presented (particularly that was easy to understand).

Biofilm formation and removal of *Listeria* innocua on various cutting surfaces
Comments:

The growth of *Listeria* in selected cheeses
Comments:

Expiration dates as predictive indicators of changes in microbial load
Comments:

Penicillin and tetracycline resistances in various organic and conventional fruits, vegetables, meats, and dairy
Comments:

Effectiveness of citric acid and lactic acid against *Salmonella typhimurium*- and *Escherichia coli*-contaminated chicken breast surface
Comments:

Examination of ozonated water treatments on fresh salmon and tuna to improve refrigeration shelf life
Comments:

Antimicrobial activity of garlic, ginger, habanero pepper, and rosemary against *Staphylococcus aureus* and *Escherichia coli* in green beans and turkey baby food
Comments:

Inhibitory effect of *Enterobacter asburiae* on persistence of *Escherichia coli* O157:H7 in minimally-processed spinach leaves
Comments:

Name (optional):_____

6. Project questionnaire

Please answer the following questions about the laboratory project. You can submit these anonymously if you prefer. These will not be graded, nor will the comments be taken personally, however your name often helps us put your comments in context with the experiences that you discuss in your responses. These comments are taken seriously and we do use your comments to guide us while planning the course in the following quarters.

1. Did you enjoy your research topic? Did you have enough direction to come up with research questions?

2. Did you feel that all of the assignments (journal assignment, online chat, project rough and final drafts, poster rough and final drafts, presentation, group grading) and due dates were worthwhile and related to your project? Was the online chat effective and useful?

3. How did you work with your group members? Did you feel that everyone contributed equally? Were there any major conflicts?

4. Did you have adequate access to the instructor who was assigned to your group? Did you need more help or direction than was given?

5. How did you feel about your poster and presentation? Where did you need more instruction or advice?

6. Did you enjoy the poster presentations? Did you feel that you learned anything from the other groups' posters?

7. This project is an inquiry exercise to give students experience and opportunity to ask questions, design experiments, perform experiments, and draw conclusions. Do you feel that this process is valuable?

8. What did you learn from your project? (I am not talking about the conclusion of the experiment; rather did you learn something that you can carry to other classes or projects, e.g., time management, using research journal articles, working with group members, etc.)

7. Biological safety guidelines for instructors

Based on the authors' experience and risk assessment, most projects require either a Biosafety Level 1 or 2 (BSL-1 or BSL-2) facility. According to a biosafety officer at The Ohio State University, teaching laboratories are exempt from formal requirements for Biosafety Level classification (i.e., regular inspection, paperwork, and inventory); however the safety precautions for research laboratories should also be applied to teaching laboratories. Each institution and laboratory may handle biological material a little differently; therefore instructors should refer to their academic institution's "Institutional Laboratory Biosafety Manual" to provide definitions and guidelines for determining and maintaining appropriate biosafety status for each laboratory. The Ohio State University Institutional Laboratory Biosafety Manual is available online at http://www.ehs.ohio-state.edu/docs/biosafe/ibsm/BSM0708.pdf. The "Institutional Biosafety Level 2 Checklist for Inspection Procedures" provides a concise list of necessary equipment and procedures for adequate safety. The Ohio State University BSL-2 Checklist is available online at http://www.ehs.ohio-state.edu/docs/biosafe/BSL-2Checklist2007.pdf.

Communication with the institutional biosafety officer is the most efficient way to evaluate the safety status of a laboratory. The following is a list of issues specific to the BSL-2 teaching laboratory that should be addressed by the instructors. Specific examples are included to illustrate how materials are handled in the authors' teaching laboratory; however requirements may differ at other institutions.

Safety guidelines to be stressed to students. The following guidelines should be followed for all students during all laboratory activities in a BSL-2 teaching laboratory. Instructors often remind the students about specific risks associated with a given laboratory exercise.

- **Personal protective equipment.** Students should wear laboratory coats, long pants, close-toed shoes, and disposable gloves whenever working in the laboratory.
- **Hygienic practices.** Students should sanitize the laboratory bench before and after working with an appropriate disinfectant (bleach, ethanol, quaternary ammonium, etc). Students should wash their hands with soap and water when entering the laboratory and before leaving the laboratory.
- **Restricted access.** Access to the laboratory should be restricted during active experimentation (i.e., closed doors). Likewise, all experimental work should be performed under the supervision of at least one instructor.

Guidelines for waste disposal. Waste disposal in the teaching classroom can become complicated due to the various types of waste generated during the laboratory period. Providing specific examples to the students helps them to understand and properly dispose of items throughout the course. Review waste disposal guidelines regularly; this is especially important if a new type of waste is generated on a given day.

- **Specific trash receptacles for infectious versus noninfectious solid waste.** Students should be able to distinguish infectious from noninfectious solid waste, and disposal of these items should be separated and clearly identified. For example, all disposable gloves and disposable petri dishes should be discarded in an infectious waste container (i.e., biohazard bag and box for incineration). Paper towels used for disinfection of the laboratory bench and empty pipette wrappers should be discarded in the regular trash can.
- **Specific receptacles for infectious versus noninfectious liquid waste.** Students should be able to distinguish infectious from noninfectious liquid waste, and disposal receptacles should be separated and clearly identified. For example, liquid infectious waste (e.g., flasks of cultured media or bottles of enrichment broth) should be thoroughly autoclaved. Alternatively, the infectious liquid can be collected in an infectious waste jug (i.e., biohazard carboy for incineration) and the empty container can be autoclaved separately. Noninfectious liquid waste (i.e., Gram staining reagents) should be collected for proper chemical disposal or dumped in the sink as deemed appropriate by the instructor.
- **Handling infectious waste after completion of exercises.** After students have completed the exercise and properly disposed of infectious items, instructors (or other trained laboratory personnel) must treat and/or dispose of these items. Depending on institutional guidelines, these items may be sterilized (via autoclaving) or sent to a secondary facility for incineration. Communication with the institutional biosafety officer is necessary to determine the most appropriate way to handle both solid and liquid biological waste.

Guidelines for labeling and paperwork. The following is a list of guidelines that are considered ideal for the teaching laboratory. Many of these are required for the research laboratory. Inclusion of these items in the classroom setting provides a formal opportunity for students to learn and understand the record keeping requirements associated with the research setting.

- **Chemical hygiene plan and material safety data sheets.** The chemical hygiene plan (CHP) includes all the information necessary for record keeping and risk assessment of the chemical components in the laboratory. Material safety data sheets should be included along with an updated chemical inventory for the students to review and access, as necessary. Standard operating procedures for chemical procedures should be included in the CHP (e.g., Gram staining, ethidium bromide use, fume hood operation). A hard copy of the CHP and material safety data sheets should be accessible to the students at all times in the laboratory.
- **Biological safety manual and training records.** The biological safety manual includes all information necessary for record keeping and risk assessment of the biological components in the laboratory. Copies of the students' safety contracts should be maintained as well as any training records pertinent to the laboratory. Standard operating procedures for biological procedures should be included in the biological safety manual (e.g., biological spill clean up). A hard copy of the

manual and associated records should be accessible to the students at all times in the laboratory.

- **Exposure records and reporting.** Any adverse student exposure to chemicals or biological material should be reported to the student health center and records should be maintained in the CHP and/or biological safety manual as directed by the institution.
- **"Biohazard" signage.** The door to the laboratory should have a BSL-2 sign which includes any special procedures for entering and exiting the laboratory and an emergency contact and phone number. Biohazard labels should be affixed to all equipment (e.g., incubators, freezers) and containers (e.g., waste containers) that hold infectious biological material.
- **Spill clean-up procedures.** Specific spill clean-up procedures should be posted inside the laboratory, and spill kits should be available at several locations within the laboratory. Spill kits should include gloves, goggles, absorbent pads, bleach, and a container to dilute the bleach. Spills containing infectious waste must be treated with 10% bleach for 30 minutes before clean-up.

8. Biological safety guidelines for students

Living microorganisms have the potential to cause human diseases. The safety of each student in the laboratory depends on observing and obeying the following rules.

Personal protective equipment

1. It is mandatory that a laboratory coat or laboratory apron be worn as a protective covering during each laboratory period. This covering must have either buttons or a zipper (i.e., not a style that has to be removed over the head), be labeled with the student's name, and be kept completely buttoned or zipped for the duration of the laboratory period. Students who are not wearing laboratory coats or aprons will be asked to leave the room and will not be allowed to return until they are wearing the appropriate protective covering. Laboratory coats will remain in the laboratory and can be stored in one of the storage cabinets provided.
2. In order to prevent hair from accidentally catching on fire, hair that is shoulder length or longer must be pinned or tied back so that none falls from the shoulders. In addition, hats with brims may not be worn in the laboratory unless they are worn with the brim facing backwards.
3. Shoes must be worn during each laboratory period. No sandals, open-toed shoes, or bare feet are permitted.

Hygienic practices

1. Hands are to be washed at the appropriate sinks before starting any laboratory exercises and before leaving the laboratory.
2. Students must wipe off the laboratory bench top at the beginning and the end of each laboratory period using disinfectant and paper towels. These paper towels are disposed of in the regular trash.
3. The application of any cosmetics, including lotion, is not permitted. The insertion of contact lenses is not permitted.
4. Smoking and eating are not permitted in the laboratory. This includes chewing gum or tobacco as well as drinking coffee or soft drinks.
5. Do not place any objects (pencils, pens, gum, fingers, lozenges, etc.) in mouth while in the laboratory.
6. Keep the laboratory as neat as possible at all times. At the end of each laboratory period, check and arrange all materials neatly. Return all materials to their proper places when the work is finished.

Restricted access

1. Even if they arrive early, students are not allowed to begin work on any exercise until the instructor indicates that it is appropriate to do so. This generally includes waiting until after the instructor's presentation on the day's activities.
2. Never remove equipment, media, or microbial cultures from the laboratory.

Safety equipment

1. Pipettes can be hazardous if not used properly. Mouth pipetting is not permitted. The laboratory is equipped with propipette bulbs and manual pipette aids for students to use.
2. During many laboratories, burners will be used. It is therefore important that students

know both the locations of the safety equipment and how to handle accidents involving fire if they occur.

a. A fire blanket is located at the back of the room. Additionally, two fire extinguishers located by each door are to be used by the instructors only. Inform an instructor immediately if an uncontrolled fire should start.
b. If anyone's hair or clothing should catch on fire, pull down the handle on the bottom of the fire blanket case, pull out the fire blanket, wrap the person in the blanket, and have them roll on the floor to extinguish the flames.
c. Burning alcohol jars are extinguished by smothering the flames with the lid of the jar.
d. If a major fire occurs, proceed to the nearest exit. Do not use the elevators!

3. For safety, students should be constantly aware of any burners near them. Keep burners away from staining bottles. Always use the burner within the assigned personal work area. Do not reach across the bench to use someone else's burner.
4. The safety shower is located in the hallway. To operate it, simply pull down the handle.
5. The safety eyewash station is located by the handwashing sink. To operate the eyewash, remove the red protective covers, pull the hose over the sink, and squeeze the handle (it will remain on once the handle has been depressed). Wash the eyes thoroughly for 10 to 15 minutes. To turn the eyewash off, squeeze the handle again and lift up the button on top of the sprayer.
6. A first aid kit is located at the front of the room, next to the blackboard. It contains the following: gauze bandages, adhesive bandages, bandage tape, sterile swabs, burn cream, antiseptic wipes, and hydrogen peroxide. Instructor's assistance should be sought before using the first aid kit.
7. Report any personal injury to the instructor immediately. If an accident does occur, an accident report form must be filled out as soon as possible.
8. If there is a tornado warning, proceed to the middle of the building.

Hazard identification, waste disposal, and supply disposal

1. Label all materials properly for easy identification. For example, all tubes are labeled using white labeling tape and a marker. Do not label tubes by writing directly on the cap or the body of the tube. Petri plates are labeled on the bottom with student's name, plated organism, microbiological medium, incubation temperature, and date.
2. The red plastic biohazard containers on the bench tops are to be used for the disposal of small items such as used slides, toothpicks, pipettes, microcentrifuge tubes, and oxidase test cards, unless otherwise stated.
3. All paper, paper towels, gauze, and extinguished matches are to be discarded in the regular trash cans at the front and back of the classroom. Do not discard these items in the sink or in the biohazard containers.
4. There is a container in each laboratory designated for broken glassware. All decontaminated broken glassware should be placed in this container.
5. If a slant or liquid culture is spilled or dropped, cover the area with paper towels soaked in disinfectant (10% bleach) and let stand for 30 minutes before wiping up. Dispose of the paper towels in one of the large biohazard waste cans on the disposal cart. Broken glassware should be cleaned up with tongs or a broom and dust pan and placed in the broken glassware container. Wash your hands immediately afterwards.

Inform your instructor of the incident as soon as possible.

6. If syringes are used for a laboratory exercise, these should be disposed of in containers designated by the instructor.
7. Liquid contaminated waste in reusable containers must be disposed of in the plastic biohazard carboys. Pour liquid cultures into the carboy and place glassware on the cart so that it can be decontaminated and washed.
8. Place used glass culture tubes in specially-marked racks (e.g., blue coated racks) after removing all of the tape from the tubes and caps. Do not return used culture tubes to the laboratory exercise set-up. All tubes must be placed in the marked racks, according to size, on the disposal cart.
9. Plastic disposable tubes are discarded in the large biohazard waste cans.
10. Place used petri dishes that are no longer needed and contaminated soft trash (paper towels, gloves, etc.) in the large biohazard waste cans on the discard cart.
11. Place large contaminated glassware on the lower shelves, so it can be decontaminated and washed. This includes flasks, beakers, cylinders, etc.

9. Poster evaluation rubric

Criteria	Excellent	Good	Poor
Poster in general	Met all requirements; was presented in correct format; communicated information effectively; had no spelling and grammatical errors; was aesthetically pleasing.	Followed directions; was presented in correct format; communicated information, but not in most effective manner; had no spelling and grammatical errors; was aesthetically pleasing.	Did not adhere to directions; was presented in incorrect format; communicated incorrect information; contained spelling and grammatical errors; was not aesthetically pleasing.
Title	Clearly indicates the topic of research.	Is appropriate but could be improved to clarify research question.	Is misleading or does not address main topic of research. Contains grammatical or spelling errors.
Abstract	Includes introduction, objectives, materials and methods, results, discussion, and conclusion. Is 200 to 250 words in length.	Is difficult to understand, missing a section, and/or slightly over or under the specified word count.	Is missing sections, is significantly too long or too short, contains grammatical or spelling errors.
Introduction	Includes basic background information and project justification. Information is presented in a logical fashion, is easy to read and understand, and includes appropriate reference citations.	Includes basic background information and project justification. Information is not presented in a logical order or is difficult to read. Includes appropriate reference citations.	Does not pertain to the research topic. Does not include references. Contains grammatical or spelling errors.
Objectives	Are clearly worded, presented in logical order, easy to locate on the poster.	Are clearly worded, but not presented in a logical order or are difficult to locate.	Are not presented or do not pertain to the research question.
Materials and methods	Clear, predominantly visual, presentation of the general procedure. Includes foods and media used, as well as incubation temperatures. Focus is on the main variables.	Predominantly visual presentation of procedure. Includes most of the necessary details. Gives details of some of the procedure while ommiting other components.	Missing critical information. Steps of the general procedure are presented in an incorrect order. No illustrations are used to present the procedure.

Results	Presented in a logical order that matches the order of the objectives. Appropriate types of graphs are used. Legends of figures clearly indicate purpose of figure. Good use of additional labels to make figures easy to understand.	Good presentation using well-selected figures, but may not be in the best order. Figures could have been labeled differently for a better presentation of the results. Axes difficult to read.	Missing key results. Inappropriate types of graphs. Awkward choice of x- and y-axes. Over emphasis on minor components of experiments.
Discussion	Contains concise, bulleted statements that refer to figures in the order they are presented. Includes other pertinent discussion points not otherwise indicated in the results section.	Contains concise bulleted statements. Statements not presented in the correct order.	Is missing or difficult to read and comprehend. Poor interpretation of results.
Conclusion	Is clear, concise, and pertinent; easily located on poster.	Is awkwardly worded or difficult to understand.	Is missing or difficult to locate; contradicts results.
References	Are formatted correctly, using the style followed in the publications of the American Society for Microbiology. Sufficient number of references are included and cited correctly in the text.	Are correctly cited but not properly formatted. Sufficient number of references are included.	Are insufficient or not included. Use incorrect citation format.
Acknowledgments	Briefly acknowledge individuals or groups who assisted on projects with name and reason for acknowledgment.	Include names only, without reference to reason for acknowledgment.	Are absent or inappropriate (e.g., including names of instructors). Contain spelling and grammatical errors.
Overall	Aesthetically pleasing. Easy to follow the flow of the information. Limited whitespace. Appropriate font selections and size.	Aesthetically pleasing. Easy to read. Some unacceptable formatting issues.	Not aesthetically pleasing. Flow of information is disjointed. Excessive white space. Difficult to read.

10. Presentation evaluation rubric

Criteria	Excellent	Good	Poor
Telling the story	Can effectively utilize the poster to briefly tell the story of the project in a logical fashion.	Story is effective but disjointed; could have been presented in a more logical order.	Stumbles on components of the poster or skips main parts of the story.
Knowledgeable on subject matter	Knowledgeable on background material, familiar with products and supplies being used beyond the variable being tested. Uses knowledge base to introduce objectives and variables of interest.	Knowledge base is limited but refers to key points related to explaining the project and variable.	Not familiar with background or introductory material.
Awareness of the pitfalls	Can identify problems with the experimental design or execution. Explains what could be done differently if experiment is repeated.	Can identify problems but may be unsure how to address them.	Cannot identify problems in the design or execution.
Extending findings to new ideas	Uses conclusions from project to ask new or improved questions about the general topic. Can formulate new objectives about general topic.	Uses conclusions to ask additional questions but has difficulty formulating new objectives.	Cannot formulate new objectives or ask additional questions. Simply testing more samples or products is the only suggestion.
Communication skills	Good eye contact with visitors. Absence of slang, jargon, and profanity during discussion. Calm and composed, interested in sharing findings and discussing project.	Good eye contact. Some slang or jargon is used. Nervousness is obvious. Interested in sharing findings.	Lack of eye contact. Uncomfortable speaking to visitors. Disinterested in talking with visitors.
Equality among presenters	All presenters contribute significantly to the presentation, interact effectively with visitors, and answer questions posed by visitors.	Some members of the group are obviously not as knowledgeable as others, but have made a conscious effort to learn the material and contribute to the presentation.	At least one presenter does not contribute to the presentation or obviously does not understand project or layout of poster.
Professional appearance	Dressed professionally and appropriately (i.e., business casual). Wearing name tag.	Name tag missing or holes in business casual attire. Athletic shoes.	Dressed too casually (e.g., denim).

Example chat session
Topic: 5-second rule (1 hour, 25 minutes)

Session started.
INSTRUCTOR #1: have you guys talked about any bright ideas in class?
INSTRUCTOR #2: You all asked about different surfaces on your assignment.
Student #2: well we chatted a little bit about it
Student #3: yeah that's kinda what we talked about in class
Student #1: I think maybe we should all put forward what question we thought was most interesting in our articles and go from there
INSTRUCTOR #1: good idea
Student #3: ok well I like the different food surfaces and how they affect the amount of microbes that can be found after 5 seconds
Student #1: what kind of surfaces are you thinking of testing?
Student #2: Find if there are microbial contamination increases/decreases in dropping processed foods on surfaces made of different materials or in different locations
Student #2: that was my best one I think
Student #1: my question was "Does the 5 second rule affect hot and cold foods differently?"
INSTRUCTOR #1: interesting....
INSTRUCTOR #2: I also liked that one Student #1.
INSTRUCTOR #1: are there specific foods or surfaces that you think would be interesting?
Student #2: dining room floors, kitchen floors, fast food restaurant floors
Student #3: um for the floors I was thinking possibly tile, carpet, concrete outside.
INSTRUCTOR #1: Student #3's ideas (other than concrete) will be easier to test in class
Student #3: yeah maybe wood floors as another
INSTRUCTOR #1: Do you want to look at "real" floor contamination? or do you want to inoculate the surfaces to mimic contamination?
Student #2: probably mimic it cause most of the floors in the micro building are sanitized on a regular basis
INSTRUCTOR #1: right...
Student #1: I agree with Student #2
Student #3: yeah that makes sense
INSTRUCTOR #2: Instructor #1 and I both thought it might be difficult to get enough contamination to recover if you don't inoculate.
Student #2: well we can do one that has a surface that has sat out all day and then one that was inoculated to see the difference
Student #1: So, we are all in agreement for going in some direction with contamination on different surfaces
INSTRUCTOR #1: I would probably encourage you to inoculate, but you may want to do a couple of "real" samples to compare it to the real situation
Student #2: I agree
INSTRUCTOR #1: you can look at a couple of different variables
Student #3: yeah that would probably make it easiest
Student #1: mentioned hot and cold foods and Student #2 mentioned processed foods—any specific ideas for either
Student #2: In a lot of studies I have read of they do different foods like bologna and bread
Student #1: Yah just a sec. write out my experimental idea. I'm a slow typer....
Student #2: I would rather do something that is fully cooked and ready to eat so there would be nothing on the food when we dropped it
INSTRUCTOR #1: Student #1, I agree, good idea
INSTRUCTOR #2: Agree.

Student #4: OK that's a good idea
Student #3: yeah that would make it easier
INSTRUCTOR #1: you could break up each of the days in lab to test a different variable
INSTRUCTOR #1: maybe one day could be processed foods, one day hot versus cold
INSTRUCTOR #2: Or contact time.
INSTRUCTOR #1: good call Instructor #2
Student #1: as far as hot and cold foods. I was thinking of having some food, say strawberries, and we have them frozen, at room temperature, and heated. We would take a sample from each and inoculate it. And then we would roll the heated, room temperature, and frozen strawberries on an inoculated table and take a sample from them and inoculate that. We could compare the effect of temperature on food's ability to pick up contamination as well
INSTRUCTOR #1: to give you an idea, each of you can easily handle four samples per day
Student #4: Yes and that way we could test various foods and contact time simultaneously
INSTRUCTOR #1: I would recommend inoculating the floor surface only (not the food)
Student #3: so like one day we could all test like cold versus hot food then one day could be contact length and the third could be different flooring
Student #4: That sounds good
INSTRUCTOR #1: you might be able to do different floorings for the other variables
Student #4: I like the idea of different floorings
Student #1: Instructor #1, I described that wrong. The samples that were hot, room temperature, and cold would be stomached, diluted, and plated. One set of samples that did not touch a surface and one that was exposed for 5 seconds
INSTRUCTOR #1: sorry, that was confusing. Day 1, four different floorings (say, Student #4 does carpet, Student #1 does tile, Student #2 does laminate, Student #3 does hardwood) and you can test a variety of foods on each
Student #2: so we could have the same surface choices (e.g., tile, wood, carpet) each day and then each of us could do a different food, then the first day we could test room temp, second day hot, third day cold and then each of us could have two different contact times on each day
INSTRUCTOR #1: I would compare the same foods on the same days (that way temp is your only variable)
Student #1: Student #2, would we use the same surface every time?
Student #4: That seems like a really good plan
INSTRUCTOR #1: give me a sec to type this out
Student #2: no like we each have food and drop it onto like the different surfaces was what I was going for
Student #1: I understand that, but say you have wood on Day 1 and I have carpet, then we both test at room temperature; the next day you would still have wood and I'd still have carpet but we both would test at cold temperature.
Student #1: I think we're on the same page
INSTRUCTOR #1: Day 1 variable hot versus cold—inoculate surface with desired bug (surfaces assigned as previously described), pick four foods and three different temperatures (refrigerated, room temperature, hot) and drop on all surfaces for 5 seconds; Day 2, same surface and organisms, but pick only two foods and change contact times; etc
Student #1: ok I'm on board
INSTRUCTOR #1: I don't like the idea of doing different temperatures on different days, because you may have significant differences in your inoculum from day to day
Student #3: sounds like a good plan to me
Student #4: I like it
Student #1: Instructor #1, should our experiment span a certain amount of days in the lab?
INSTRUCTOR #1: you have 3 full days in lab and you can count on the 4th day
Student #1: ok

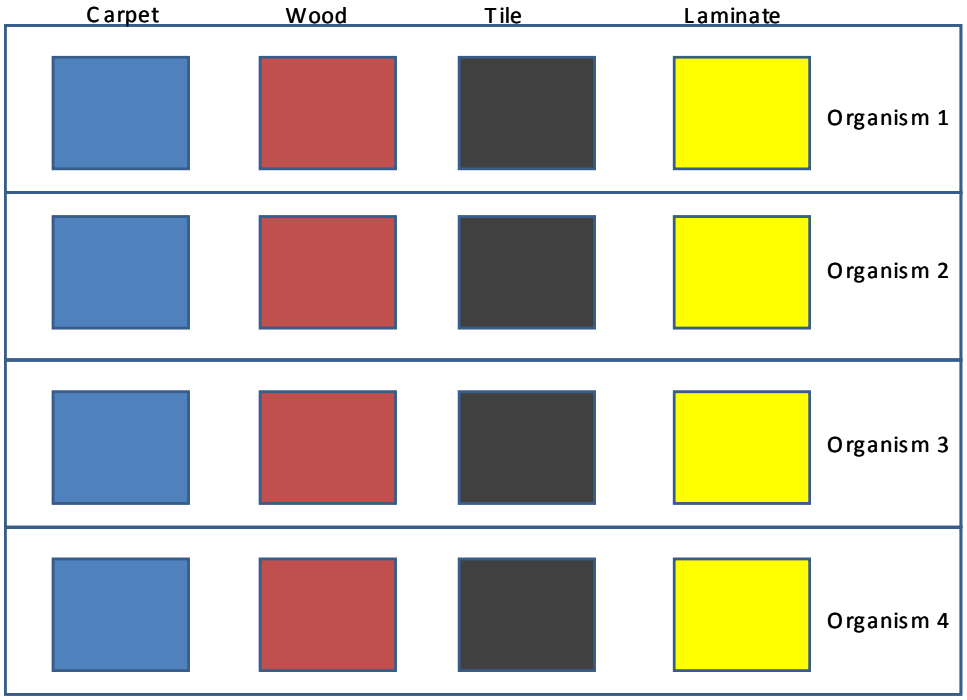
INSTRUCTOR #2: So that would give you three variables if you do it this way.
Student #2: sounds good to me
Student #1: Day 1 - hot vs. cold. Day 2 - contact time. Day 3 - different surfaces
INSTRUCTOR #2: Which three do you think would be most interesting?
Student #3: so should we figure out the different times and stuff today or can we do that more so in class
INSTRUCTOR #2: The way Instructor #1 described it, you would each use a different surface all three days.
Student #2: yeah I like the different surfaces all three days
Student #3: I think Day 1 is hot vs. cold on different surfaces, then Day 2 is different contact times on different surfaces
Student #4: me too
Student #2: exactly
Student #1: ok thanks Student #3
INSTRUCTOR #2: right, then one more variable for Day 3.
Student #4: maybe different foods?
Student #1: maybe after surfaces were washed with different disinfectants
Student #3: well I think for each day we have different foods
Student #2: or different pathogens
Student #4: ok
Student #4: I'm with you
INSTRUCTOR #1: Okay, any ideas for the organisms of interest
Student #3: or what if we somehow had the flooring at different temperatures I don't know how we would do that but that might work.
Student #2: well a coliform would be good
INSTRUCTOR #1: I think food temp would be more appropriate
INSTRUCTOR #2: For coliforms, *E. coli* is easy to recover on selective media.
Student #1: *Pseudomonas* would work, especially if we incubate over a larger range of temperatures
Student #3: yeah that would probably make more sense
INSTRUCTOR #2: You can choose more than one and use different selective plates.
Student #2: yeah that would be more realistic if we had multiple organisms on the surfaces
Student #4: I agree
Student #3: yeah that would make it more realistic
INSTRUCTOR #1: this is going to complicate things
Student #1: I agree but how many should we look at?
INSTRUCTOR #1: you may want to use selective plates for the detection, particularly if you use nonsterile food products
Student #1: I think with only three periods we should choose one microorganism
Student #2: I agree because selective plating would take away from the time to work with different variables
INSTRUCTOR #1: selective plating will not cause any increase in the amount of time necessary for your experiment
Student #3: yeah that would probably be easier if we use just one then maybe on the third day try 5 seconds on several different surfaces with different foods.
INSTRUCTOR #1: here is another option—you could repeat the same experiment (same foods, same temperatures) on Days 1, 2, and 3 but just use a different organism to inoculate the surfaces
Student #1: I like that
INSTRUCTOR #1: I don't know; I kind of feel like testing different times is kind of crucial
Student #4: That sounds reasonable
Student #4: Different times is the basis of the experiment so we need that definitely

Student #2: well if we had the same food, different temps, different surfaces, then in each day a person could be assigned to different contact times, then the next day we could inoculate with the different organism
Student #3: yeah well would it be too much if we each had a different food then we tested it on different surfaces and different temperatures like room temp, cold, and hot
Student #2: well I was saying we all have the same food and the only variable between people would be contact times
Student #3: yeah that would work too
Student #1: how about we each have one organism. We test a food (we all have the same food) with that organism on different temps (Day 1), different contact time, and some third variable. Our experiment would examine these different organisms' abilities to contaminate our food at these variables
Student #2: like we choose cooked chicken and each of us would drop (for a certain time) a cold, room temperature, and hot piece onto plastic, wood, and carpet inoculated on that day by a certain organism.
Student #3: then the third day could be floor surfaces
INSTRUCTOR #2: You all need to have one thing that stays constant over three days. Then choose three things that you want to vary, one per day.
Student #1: ok well let's use one organism and one food.
Student #1: and we will change temperature on Day 1
Student #1: contact time on Day 2
Student #1: and surface on Day 3
INSTRUCTOR #2: So will you all use different foods or organisms?
Student #3: organisms I think is what Student #1 is saying
INSTRUCTOR #2: And just choose one constant food sample for the whole experiment?
Student #1: we all use the same food but each of us has a different organism
Student #2: that could work
Student #3: yeah would it be possible to use two constant food types through
out or would we only have enough time to do one food type
Student #1: and we each run the same experiment hot versus cold, contact time, etc., but with different organisms
INSTRUCTOR #2: What food do you think you might want to use?
Student #1: but keeping the same food
Student #4: A food that is good at picking up bacteria
Student #1: how about ham (sliced for sandwiches)
Student #4: Some things like jelly beans would not pick up hardly any bacteria
Student #4: sliced ham would be good
Student #2: sliced ham could have *Listeria* on it
Student #1: so we need something sterile to start with eh
Student #1: but other than that are we set?
INSTRUCTOR #2: Hold on a second, Instructor #1 has a visual that she is uploading to Carmen for you.
Student #2: agreed
INSTRUCTOR #2: It is a suggestion for the design.
Student #3: ok
INSTRUCTOR #2: It will be under term projects in like 2 seconds.
INSTRUCTOR #2: Now. [See attached]
INSTRUCTOR #1: So slide 1 is Day 1—testing different organisms. From the results you will use the best organism to inoculate on Day 2. On Day 2 you will test a variety of foods, from the results pick the best food to look at the effect of temperature on Day 3
Student #3: that looks really good to me

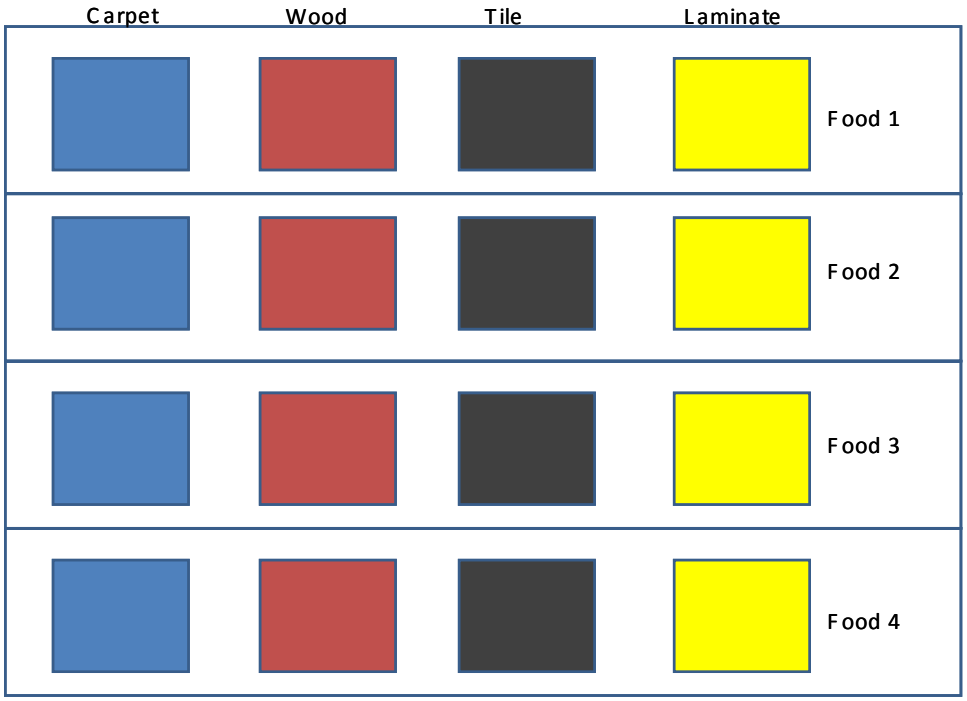
Student #2: sounds good
Student #4: That does sound like a good plan Instructor #1
Student #4: OK
INSTRUCTOR #2: If you like this design, you can decide on the specific organisms, foods, etc. later.
Student #4: I like it
Student #3: ok sounds good to me
Student #2: I second that
Student #1: I'm in
INSTRUCTOR #2: Okay, then you're free. You can look into what you might want to do and discuss specifics in class.
Session ended.

Attached file of Proposed Design by Instructor #1

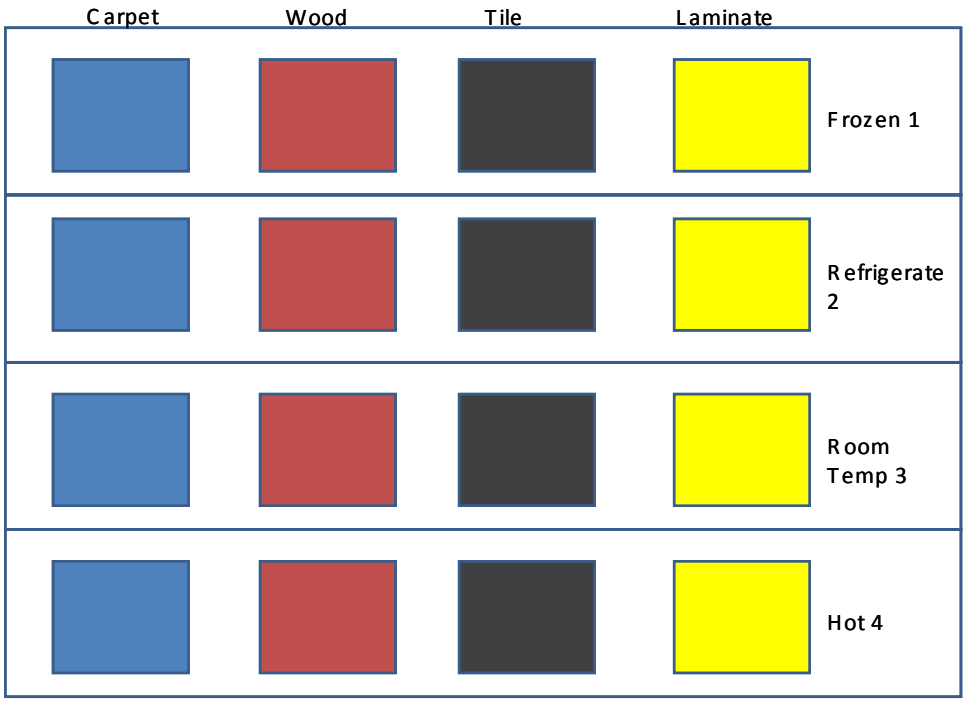
Day 1 Layout



Day 2 Layout



Day 3 Layout


Carpet
Wood
Tile
Laminate
Frozen 1
Refrigerate 2
Room Temp 3
Hot 4

Penicillin and tetracycline resistances in various organic and conventional fruits, vegetables, meats, and dairy

Student names

[1]Department of Food Science & Technology [2]Department of Animal Sciences [3]Department of Biology

Abstract

Organic and conventional foods are grown and processed differently. These differences may contribute to different antibiotic resistance in food samples. The antibiotic resistance of conventional and organic food was tested and compared. This was performed by using Tryptic soy agar (TSA), TSA with penicillin, and TSA with tetracycline to determine any microbial antibiotic resistance that may be present in the products. Standard dilution procedures were followed and plated on agar, which was then incubated. Resulting colony growth was then counted, recorded, and compared to other foods used in the experiment. Organic products did not show a significant difference in resistance to antibiotics over their conventional counterparts. For vegetable growth, more antibiotic resistance was observed on penicillin than on the tetracycline. In meat products, overall antibiotic growth was consistent with normal TSA growth, telling of high antibiotic resistance compared to the other foods.

Introduction

Organic and conventional products must be grown and raised on different farms because each requires different standards (2). Standards for organic products are declared by the Organic Foods Production Act (OFPA). Regulations are set for all aspects of organic production and handling (2).

Organic claims are separated by crop and livestock. Crops can be claimed organic only if no prohibited substances are applied within at least the past three years (2). Livestock (including meat, milk, and eggs) can be claimed organic only if livestock is not given any hormones or antibiotics and raised under organic management (2).

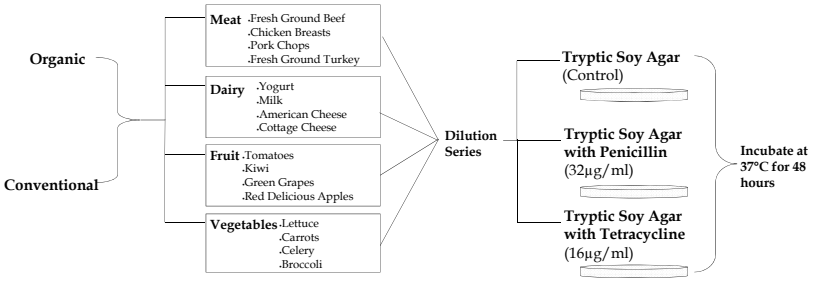
Unlike organic livestock, antibiotics are very heavily used in conventional agriculture for the purpose of preventing sickness and promoting growth of animals (5). There are at least 17 different approved antibiotics that can be used (1). Many of these antibiotics are also closely related to those used on humans (7). Unfortunately, the abundant use of antibiotics has led to microbial resistance in certain bacterial strains (5). This is an increasing threat to conventional agriculture because it relies heavily on antibiotic treatments (6). Cross contamination of these resistant microorganisms to other farms or other foods are questionable considerations and problematic.

Different antibiotic resistant rates are expected between different types of food and organic and conventional foods due to different agricultural practices. The organic approach may lead to lower antibiotic resistant rates, but may not be prevalent due to runoff. Research has shown no significant difference between organic and conventional antibiotic resistance (4).

Objective

Compare antibiotic resistance of various organic and conventional foods.

Materials and Methods



Results

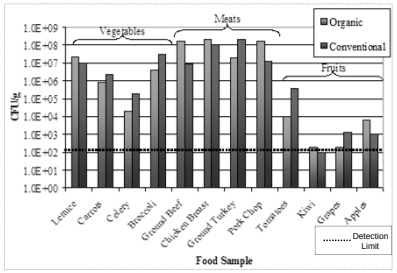


Figure 1: Organic vs. Conventional CFU/g on TSA with Penicillin

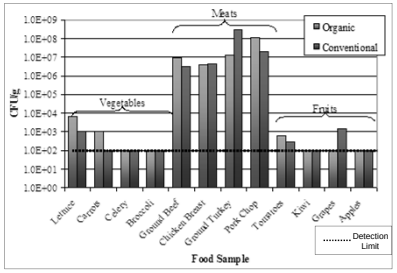


Figure 2: Organic vs. Conventional CFU/g on TSA with Tetracycline

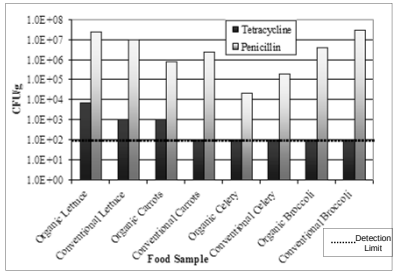


Figure 3: Difference in CFU/g on TSA with Penicillin and TSA with Tetracycline in Vegetables

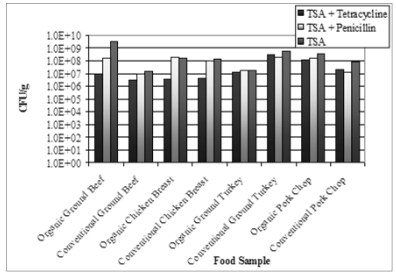


Figure 4: CFU/g of Meat Samples on TSA, TSA with Penicillin, & TSA with Tetracycline

Discussion

- Fruit and vegetable microorganisms were more susceptible to the antibiotics than the meat samples (Figure 1, 2).
- Organic and conventional vegetables (romaine lettuce, carrots, celery, broccoli) presented more microbial growth on penicillin than tetracycline (Figure 3).
- For the meat samples used, antimicrobial growth was similar in magnitude to that of normal TSA growth (Figure 4).
- Total plate counts of dairy products on all media were insignificant which suggests that the normal microflora of dairy products require a more fastidious media to allow growth (data not shown).

Conclusions

- The data suggests that microorganisms from organic foods actually have similar, and in some cases higher resistance to antibiotics compared to conventional foods.
- Microorganisms show more growth on penicillin than tetracycline, possibly due to different concentrations used.

References

1. **Larson, E.** 2007. Community factors in the development of antibiotic resistance. Annu. Rev. Public Health. **28:** 435-447
2. October 2002. The national organic program. USDA: Organic production and handling standards. [Online] http://www.ams.usda.gov/nop/factsheets/prodhande.html. Accessed 12 November 2007
3. **Rosenquist, H., L. Smidt, S. R. Andersen, G. B. Jensen, and A. Wilcks.** 2005. Occurrence and significance of *Bacillus cereus* and *Bacillus thuringiensis* in ready-to-eat food. FEMS Microbiology Letters. **250:** 129-136.
4. **Sato, K., P. C. Bartlett, J. B. Kaneene, and F. P. Downes.** 2003. Comparison of prevalence and antimicrobial susceptibilities of *Campylobacter* spp. isolates from organic and conventional dairy herds in Wisconsin. Applied and Environmental Microbiology. **70:** 1442-1447.
5. **Teuber, M.** 1999. Spread of antibiotic resistance with food-borne pathogens. Cell. Mol. Life Sci. **56:** 755-763.
6. **Tifofsky, L. L., J. W. Barlow, C. Santisteban, and Y. H. Schukken.** 2003. A comparison of antimicrobial susceptibility patterns for *Staphylococcus aureus* in organic and conventional dairy herds. Microbial Drug Resistance. **9:** 39-45.
7. **Woteki, C. E., and B. D. Kineman.** 2003. Challenges and approaches to reducing foodborne illness. Annu. Rev. Nutr. **23:** 315-344.

Acknowledgments

We greatly appreciate the wonderful suggestions and contributions advised by Joy Waite and Richard Nist. We thank The Ohio State University for the provided supplies and laboratory space.

Washing methods to remove pathogens from fresh vegetables

Student names

Food Science & Technology 636.02, The Ohio State University, Columbus, OH

ABSTRACT

To address the risk of foodborne diseases associated with fresh fruits and vegetables, several home washing solutions were tested against *Escherichia coli* and *Salmonella* Typhimurium. Broccoli, spinach, tomatoes, and cucumbers were inoculated with bacteria and each was washed with distilled water, 10% lemon juice solution, 200 ppm chlorine solution, or Fit ™ fruit and vegetable wash. Remaining bacteria were recovered in PBS and plated onto XLD to determine *Salmonella* remaining and onto TBX to determine *E. coli* remaining. Reduction of the two bacteria species ranged from 1 to 2 log CFU/200 ml PBS compared to an unwashed control. There were greater reductions in bacteria on tomatoes and cucumbers than on broccoli or spinach. In most cases, washing with water was as effective at removing *E. coli* and *Salmonella* Typhimurium as 10% lemon juice or Fit fruit and vegetable wash. Chlorine solution was the most effective at reducing pathogen levels in this experiment, but it is not recommended for home use.

INTRODUCTION

The ability to effectively wash produce has become more important as consumers increase their intake of fruits and vegetables . The heightened consumption of fruits and vegetables has been caused by interest in healthy diets and lifestyles (5).

Salmonella and *Escherichia coli* have each been responsible for at least one vegetable related food-born disease outbreak every year between 1998 and 2002 (10), and the infectious dose for each pathogen may be less than 100 cells (11).

Vegetables are the vehicles for many types of food born pathogens, including *Salmonella* spp. *Escherichia coli* O157:H7, *Shigella* spp., and *Listeria monocytogenes* (5) . It is recommended that fruits and vegetables be washed underneath tap water (6), but other washing methods have also been proposed and marketed. Vinegar or acetic acid solutions have been shown to reduce *E. coli* O157:H7 (13) and *Salmonella* (12) from fresh fruits, and l3% lemon juice was effective against *Listeria inoccua* on vegetables (9). Since *E. coli* O157:H7 cannot survive below pH 4.5 and *Salmonella* spp. not below 4.05 (8), lemon juice should also reduce their numbers. Several studies have found chlorine to reduce microbial contamination of fruits and vegetables (2,3,12) and 200 ppm free chlorine is recommended for tomato packing houses (14). Fit ™ fruit and vegetable wash, made from generally recognized as safe (GRAS) ingredients, has also been found to be effective against *L. monocytogenes* and *Salmonella* spp. on tomatoes (3).

OBJECTIVES

- Measure the efficacy of different wash solutions for removing *Escherichia coli* and *Salmonella* spp. from fresh vegetables.
- Compare pathogen removal from vegetables that are easy to wash (tomatoes, cucumbers) with that from vegetables that are difficult to wash (broccoli, spinach).

METHODS

Vegetables Used and Wash Preparations

Ripe plum tomatoes, heads of broccoli, cucumbers, and bunch spinach were purchased at a local grocery store and stored at 4°C until inoculation. Tomatoes and cucumbers were left whole, broccoli was cut to heads 165 ± 5 grams, and spinach was weighed out to 50-gram bunches.

Two-liter wash solutions were made using distilled water, Fit ™ fruit and vegetable wash, lemon juice from concentrate, and chlorine bleach. The lemon juice wash was prepared as a 10% solution (pH 2.74), the fruit and vegetable wash according to the recommendation of the manufacturer, and the chlorine wash as a 200 parts per million (ppm) sodium hypochlorite solution. Unwashed vegetables served as controls.

Table 1. Inoculation solutions prepared using sterile water and *Escherichia coli* broth and *Salmonella* Typhimurium broth.

Vegetable	Sterile Water (ml)	*Escherichia coli* broth (ml)	Salmonella typhimuria in broth (ml)	Total Volume (ml)
Tomatoes	700 ml	75 ml	75 ml	850 ml
Spinach	1400 ml	250 ml	250 ml	1900 ml
Broccoli	1400 ml	150 ml	150 ml	1700 ml
Cucumbers	1400 ml	150 ml	150 ml	1700 ml

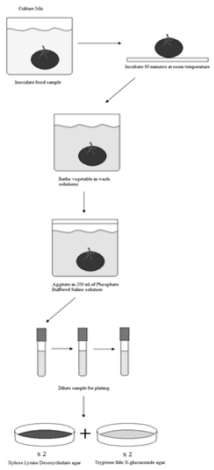


Table 2. Dilution scheme for plating onto xylose lysine desoxycholate (XLD) agar and tryptone bile X-glucuronide (TBX) agar.

Vegetable	Dilutions plated		
Roma Tomatoes	undiluted	10^{-1}	10^{-2}
Spinach	10^{-2}	10^{-3}	10^{-4}*
Broccoli	10^{-2}	10^{-3}	10^{-4}
Cucumbers	10^{-2}	10^{-3}	10^{-4}

* Control dilution was 10^{-3}, instead of 10^{-4}

RESULTS

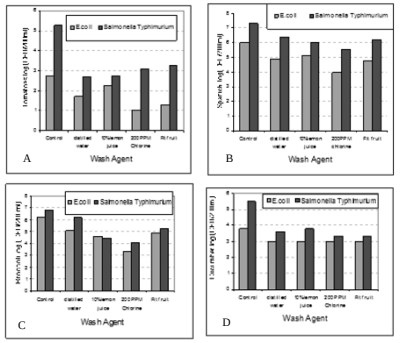


Figure 1. Comparative *E.coli* and *Salmonella* Typhimurium counts recovered from 200 ml PBS solution after washing tomatoes (A), spinach (B), broccoli (C), and cucumbers (D). Treatments included an unwashed control and wash solutions of distilled water, 10% lemon juice, 200 ppm chlorine, and Fit fruit and vegetable wash.

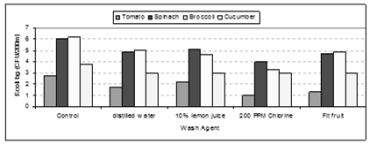


Figure 2. Comparative *E.coli* counts recovered from 200 ml PBS solution after washing tomatoes, spinach, broccoli, and cucumbers. Treatments included an unwashed control and wash solutions of distilled water, 10% lemon juice, 200 ppm chlorine, and Fit fruit and vegetable wash.

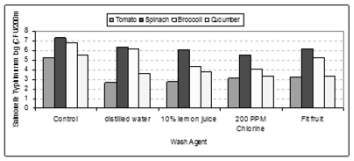


Figure 3. Comparative *Salmonella* Typhimurium counts recovered from 200 ml PBS solution after washing tomatoes, spinach, broccoli, and cucumbers. Treatments included an unwashed control and wash solutions of distilled water, 10% lemon juice, 200 ppm chlorine, and Fit fruit and vegetable wash.

- When washing tomatoes, all of the treatments resulted in approximately a 2 log CFU/200 ml reduction in *Salmonella* Typhimurium compared to the unwashed control (Fig. 1A). The 200 ppm chlorine solution and Fit detergent was most effective against *E. coli*, reducing it by about 2 log. Distilled water reduced *E. coli* by about 1 log, while 10% lemon juice reduced the pathogen by less than 1 log compared to the control.
- All treatments also reduced *Salmonella* by about 1 log CFU/200 ml on spinach (Fig. 1B). The chlorine wash was most effective at reducing *E. coli*, resulting in levels 2 log CFU/200 ml lower than the control. Water, lemon juice, and Fit reduced *E. coli* by about 1 CFU/200 ml on spinach.
- Chlorine solution was the most effective treatment against *Salmonella* Typhimurium and *E. coli* on broccoli, resulting in a 3-log reduction of both pathogens (Fig 1C). Lemon juice solution reduced *Salmonella* by over 2 log and *E. coli* by over 1 log CFU/200 ml. Fit reduced *Salmonella* by almost 2 log, but reduced *E. coli* by only 1 log. Water was least effective at washing broccoli, but still reduced *E. coli* by 1 log CFU/200 ml compared to the unwashed control.
- There was little difference between the washing treatments for removing pathogens from cucumbers (Fig. 1D). All reduced *Salmonella* Typhimurium by about 2 log and *E. coli* by about 1 log CFU/200 ml.
- The level of pathogen reduction achieved through washing varied between vegetables, with tomatoes and broccoli the most conducive to washing and spinach and broccoli the least conducive (Fig. 3 and 4).

DISCUSSION and CONCLUSIONS

•Chlorine solution (200 ppm) was the most effective at reducing pathogen levels in this experiment, but it is not recommended for home use because consumers might ingest unsafe levels of chlorine (6). Only 4 ppm chlorine is allowed in drinking water (4).

•In most cases, washing with water was as effective at removing *E. coli* and *Salmonella* Typhimurium as 10% lemon juice or Fit fruit and vegetable wash. However, none of the washing treatments achieved the 5-log reduction in pathogens recommended for sanitizers (8).

•In this experiment, vegetables were inoculated with pathogens only 90 minutes prior to washing. These results might not be representative of measurements made to remove native microflora from vegetables. Given time, *E. coli* O157:H7 can move into stomata or other small openings on plant surfaces, or be protected by the biofilms of other bacteria (1). Salmonella can form aggregates with other bacteria as a way to protect itself (1).

•Cross-contamination by wash water was not addressed in this experiment. Even if a washing method such as water is effective at removing pathogens, the wash water might contaminate the next vegetable to be washed.

•This experiment was directed at washing fresh vegetables at home. Washing methods for commercial processing must not only address pathogen removal, but also pathogen survival since wash water is commonly recycled. Pathogen levels, biological oxygen demand, and chemical oxygen demand must be considered for commercial wash and waste water.

REFERENCES

1. **Arnaouyage, D., K. Lee, S. Miller, and J. T. LeJeune.** 2006. Interactions affecting the proliferation and control of human pathogens on edible plants. J. Food Sci. 71:R83-R89.
2. **Beuchat, L. R., B.V Nail, B.B Adler, and M.R.S. Clavero.** 1998. Efficacy of spray applicatio nof chlorinated water in killing pathogenic bacteria on raw apples, tomatoes, and lettuce. J. Food Prot. 61: 1305-1311.
3. **Beuchat, L. R., L.J. Harris, T.E. Ward, and T.M. Kaja.** 2001. Development of a proposed standard method for assessing the efficacy of fresh produce sanitizers. J. Food Prot. 64:1103-1109.
4. **City of Columbus, OH.** 2006. Consumer Confidence Report. Primary Drinking Water Standards. Available online: http://utilities.columbus.gov/WaterPDFs/CCR2006.pdf. Accessed 10 May 2007.
5. **DeReuver, C.** 1998. Microbiological safety evaluations and recommendations on fresh produce. Food Control. 9:321-347.
6. **Food Safety and Inspection Service.** 2006. Washing food: does it promote food safety. United States Department of Agriculture. Available online: http://www.fsis.usda.gov/Fact_Sheets/Does_Washing_Food_Promote_Food_Safety/index.asp. Accessed 19 May 2007.
7. **Guo, X., J. Chen, R.E. Brackett, and L.R. Beuchat.** 2001. Survival of salmonellae on and in tomato plants from the time of inoculation at flowering and early stages of fruit development through fruit ripening. Appl. Environ Microbiol. 67: 4760-4764.
8. **Jay, J. M.** 2000. Modern food microbiology, 6th ed. Aspen Publishers, Inc., Gaithersburg, MD.
9. **Kilonzo-Nthenge, A., C. Fur-Chi, and S.L. Godwin.** 2006. Efficacy of home washing methods in controlling surface microbial contamination on fresh produce. J Food Prot. 69:330-334.
10. **Lynch, M. J. Painter, R. Woodruff, and C. Braden.** 2006. Surveillance for Foodborne-Disease Outbreaks - United States, 1998-2002. MMWR: SS: 1-34.
11. **Montville, T.J., and K.R. Matthews.** 2005. Food microbiology, an introduction. ASM Press, Washington, D.C. 96, 122.
12. **Parnell, T.L. and L.J. Harris.** 2003. Reducing *Salmonella* on apples with wash practices commonly used by consumers. J Food Prot. 66: 741-747.
13. **Yu, K., M.C. Newman, D.D. Archbold, and T.R Hamilton-Kemp.** Survival of *Escherichia coli* O157:H7 on strawberry fruit and reduction of the pathogen population by chemical agents. J. Food Prot. 64:1334-1340.
14. **Zhuang, R.Y., L.R. Beuchat, and F.J. Angulo.** 1995. Fate of *Salmonella montevideo* on and in raw tomatoes as affected by temperature and treatment with chlorine. Appl. Environ. Microb. 61: 2127-2131.

OHIO STATE
Results
Conclusion
Future Work
References
Acknowledgement
END

Microbial Profile and Changes in pH of Sauerkraut Juice at Varying Salt Concentrations and Temperatures

Jena M. Hurst, Annegret Männig, Chanun Somboonvechakarn, Jenny Wu

Abstract

Despite the control of harmful microbiota in foods today, many microorganisms are still used in the production of foods, such as the fermentation of sauerkraut. The goal of the experiment was to compare the microbial load of batches of fermenting cabbage using temperature and salt concentration as variants. The shredded cabbage was separated into eight large beakers with different salt concentrations (0%, 1%, 2.5%, and 5%). Each beaker with designated salt concentration was then incubated in either room temperature (25°C) or 37°C. Over the course of seven days, samples were taken from shredded cabbage, the pH was taken, and each sample was diluted and then spread plated onto violet red bile glucose agar (VRBG) for *Enterobacteriaceae*, tryptic soy agar (TSA) for total plate count, and *Lactobacillus* agar (MRS). Overall, the pH by the end of the experiment had lowered in all of the samples, but began to increase towards the end for some samples. All bacterial counts were significantly higher at the end of the experiment than the beginning on each agar, with no obvious correlation between salt concentration or temperature. The sauerkraut made with the conventional conditions (2.5% salt, room temperature), however, consistently showed lower counts than the other samples.

Introduction

For centuries, the fermentation of vegetables has been a widely adopted food processing technique for the purpose of food storage [illegible] different parts of the world and even still used today. S[illegible]ement was of the most well-known fermented vegetable in the w[illegible] usually made with the aid of naturally present lactic [illegible]um as *Leuconostoc mesenteroides*, *Leuconostoc fallax*, *Pediococcus pentosaceus* and *Lactobacillus plan[illegible]* is traditionally made with the sole presenc[illegible] involvement of a starter microbiological cultur[illegible] of health concern, there is a trend of using de[illegible] the production of sauerkraut. With the decrea[illegible] time is required during the process of fermenta[illegible] risk of exposure to foodborne path[illegible]s for [illegible] those who check the taste dur[illegible] who are pregnant (1). L[illegible] relationship betwe[illegible] different salt cond[illegible] that there is little [illegible] of different salt [illegible] Further investig[illegible] with varied sa[illegible] fermentation is h[illegible]

Objective

Monitor the pH, total [illegible] lactic acid bacteria co[illegible] temperature vs. 37°C a[illegible]

Materials and Methods

Cabbage

2 kg of chopped cabbage was placed in each of 8 beakers.

Salt was added at 0, 1, 2.5 and 5 % of cabbage weight in duplicates.

Sauerkraut was incubated at 25°C and 37°C in anaerobic condition.

[illegible] plate counts were

Results

Figure 1. Final pH of sauerkraut juice after 7 days of fermentation at 25°C vs 37°C with 0%, 1%, 2.5% or 5% salt

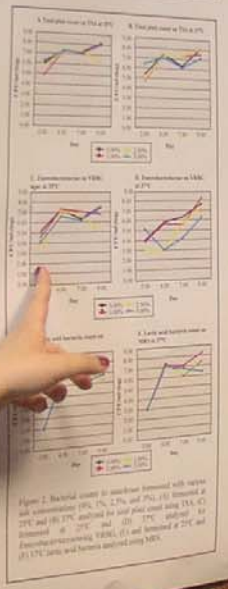


Figure 2. Bacterial counts in sauerkraut fermented with various salt concentrations (0%, 1%, 2.5%, and 5%). (A) fermented at 25°C and (B) 37°C analyzed for total plate count using TSA. (C) fermented at 25°C and (D) 37°C analyzed for *Enterobacteriaceae* using VRBG. (E) and fermented at 25°C and (F) 37°C lactic acid bacteria analyzed using MRS.

Discussion

The pH (Figure 1) of the sauerkraut was most affected by the salt concentration. The higher the salt concentration, the lower the pH. The samples with high salt concentrations (2.5%, 5%) had a final pH below 4.6. Temperature affected the pH to a lesser extent. The lower the temperature, the higher the pH within each salt concentration. The samples at room temperature, 0% salt even had an increase in pH.

The total plate counts on TSA agar (Figure 2) indicated that generally, there was an increase of bacteria load over time in the sauerkraut samples, independent of salt concentration or temperature. A notable exception was the batch made using the conventional conditions (2.5% salt, room temperature), which showed a decrease in CFU/mL.

The *Enterobacteriaceae* count in the sauerkraut assessed using VRBG agar (Figure 3) indicated that over time their counts increased. The batch made using the conventional conditions (2.5% salt, room temperature) showed a decrease in CFU/mL.

The lactic acid bacteria count of sauerkraut assessed using MRS agar (figure 4) yielded similar results as the previous counts. At first no lactic acid bacteria were detected, then the colony counts increased rapidly for all samples. However, samples treated with 1% acid 2.5% salt at room temperature, or 5% salt at 37°C had fewer final counts than the other samples.

Conclusion

While temperature and salt concentration do have an effect onto sauerkraut fermentation, the conventional method for making sauerkraut (2.5% salt, room temperature) had the lowest bacterial counts for everything tested.

Future Work

- Determine the effect of salt concentration and temperature over a longer time period
- Compare the quality of the products
- Determine the effect of an added starter culture onto the microflora of sauerkraut

References

1. **Nikin, M., S.E. Seibold, J.S. Bickon, G.F. Mendonca, J.J. Kozizkowski, and J.L. Ellegood.** 2005. Survival of *Listeria monocytogenes* and *Escherichia coli* O157:H7 during sauerkraut fermentation. J. Food Protect. **68**: 1367-1374.

2. **Vander, R., M. Miki, and A. Pabu.** 2005. Impact of low salt concentration, salt purity on initial high-salt sauerkraut fermentation. J. Food Microbio. **20**: 391-395.

Acknowledgement

The authors would like to thank Richard Nisi, Jennifer Perry, Joy Waite, Mustafa Varma, and Ahmed Yousef for making this project possible.