Food Science 324
Food Microbiology Lab
Fall 2016

Course Description:
Food Science 324 is a laboratory course where students conduct exercises dealing with food preservation, spoilage, and food poisoning. Basic techniques for the isolation, identification and quantification of specific microbes occurring in foods are assayed. Students will also learn molecular techniques (PCR and 16S sequencing) to identify bacteria in food. Also, some food fermentations mediated by bacteria and yeast will be conducted during the semester.

Meeting Times and Locations:

Laboratory  Monday 1:20 – 4:20 pm  2145 Microbial Sciences Building
            Wednesday

Instructor

Jan Peter van Pijkeren (JP)
A203B Babcock Hall
Office hours: by appointment
Email: vanpijkeren@wisc.edu

Teaching Assistants

Neil Gandhi  Bingquin Cai
227 Babcock Hall  Office hours: by appointment
Office Hours: by appointment
Email: gandhi@wisc.edu  Email: bcai2@wisc.edu
General Course Information

Welcome to the Food Microbiology laboratory course at the University of Wisconsin – Madison. This laboratory will complement the information provided in the lecture course by giving “hands-on” experience working with food and microorganisms important in the food industry. There are several broad teaching goals of this course. First, we want to introduce you to the basic techniques and tools used in the food industry. The experiments conducted in this course will give you an understanding of the theory and basis for a wide range of approaches from classical methods to molecular methods, including ELISA and 16S sequencing for detection and determination. Secondly, you will be introduced to food-related, important organisms as well as try your hand at food production using microorganisms. The third and most important goal is to give you even more skills and experience you need to become a good scientist. This includes writing a lab report, understanding the importance of proper controls and what makes a control appropriate, to become more comfortable in a lab setting, and overall to better learn the scientific process. Throughout the course we will emphasize mathematic problems (dilutions, molar concentrations etcetera) and we will pay close attention to scientific writing skills.

To accomplish these goals you will perform a series of experiments in the areas of basic microbiology, food safety and food production. The experiments mimic industrial situations and when possible will not have predetermined, artificial results. There are a few simple things that you can do to assure success in this class. First, come to lab and do the experiments. To learn about experimental science you have to do it in-person. It is impossible to make-up missed lab periods and difficult to take quizzes later. Second, read the experiments, and view the relevant video material(s) before you come to class. Well-prepared students not only understand why they are doing what they are doing (which makes the lab more enriching) but they also finish in a more timely fashion (they get out of lab on time). Finally, take careful lab notes during the experiment. This will serve you well when it comes to writing the lab reports.

Collecting notes during class

During the semester you will be expected to maintain a laboratory notebook and to keep it up-to-date. During lab sessions, we refer to these as raw notes. After lab, you use the raw notes to outline, categorize, and process your findings in a succinct electronic lab notebook (ELN). For collecting the raw notes, the following points should be kept in mind as you collect data:

1. Do not collect data on scraps of paper or paper toweling! It will still look like a scrap of paper or a towel and you may toss it into the garbage can in a moment of extreme tidiness!

2. Date and identify all entries when you collect raw notes.
3. Good data collection is a function of preparedness. If you set up an assay that needs daily monitoring, set up a chart that has spaces for each day’s observation. This way the information is all in one place and if you have missed an observation period, it will be immediately obvious to you. A few minutes of preparation before coming to lab can save you hours of searching and frustration later!

Writing the lab report: ELN

In both the lab manual as well as in an instructional video it is outlined in detail how to make your electronic lab notebook (ELN). We will not repeat that here. You are provided with a blank template for your ELN that you can download. Also, for you available to download are two example ELNs: one which is considered a poorly written/organized ELN whereas a second example illustrates a well laid-out and written ELN. Collectively, you should be provided with all the tools to be successful in delivery quality ELNs.

Due dates ELN

Please refer to the road map at the end of this document to identify when the ELNs should be received. Students attending labs on Monday will have to send in the ELN on Monday (at the latest), students attending labs on Wednesday will have to send in the ELN on Wednesday (at the latest) in the week that the ELN is due. LATE REPORTS ARE NOT ACCEPTED AND AUTOMATICALLY ASSIGNED A SCORE OF ZERO (0) unless the student has a valid reason that is cleared with the instructor.

Lab Safety

Microbiology is a fascinating science and many different procedures are used to study it. Often these protocols involve the use of hazardous equipment or dangerous chemicals. It is crucial for the success of experiments and the safety of the experimenter that certain rules be followed in the laboratory. Below is a list of general protocols, which will ensure your personal safety. Please read them and make sure you understand them completely.

Immunocompromised individuals

If you are immunocompromised for any reason (chemo, immunodeficiency diseases, pregnant or other reasons), please inform the instructor immediately. You will be discouraged from attending (without consequence) labs that would present even the slightest risk to you.
Biologically Contaminated Materials

A biologically contaminated material is anything that comes in contact with a living culture (slides, tubes, flasks, petri dishes). Treat these materials with special care to prevent transmission of microbes (a potential source of disease) to unsuspecting individuals.

1. Treat all microorganisms and biologically contaminated materials as potential sources of disease. We will be enriching for organisms from food, and they are pathogens, albeit weakened ones. Please be careful.
2. Discard all biologically contaminated materials as outlined in the Lab Clean-Up section (see below).
3. Never remove any cultures from the laboratory.

Hazardous Chemical

Hazardous chemicals may sometimes be necessary for various experiments. These rules will help you handle them correctly.

Dress

1. Lab coats will be provided and required at all time; safety glasses will be required when deemed appropriate.
2. If you have long hair, take the necessary steps to prevent it from dangling near lit burners or cultures.
3. Wear gloves when handling hazardous materials. The instructor will inform you when necessary. For more information, please watch the instructional video ‘The importance of gloves’.

Chemicals

1. Know your chemicals. Understand their hazards (is it explosive? poisonous? caustic? blistering? etc.). This information will help you know how to treat them when handled or spilled.
2. There will be no mouth pipetting. Many of the chemicals we use in this lab are dangerous. Cultures and chemicals should never be mouth pipetted. will be ingested accidentally.
3. Keep volatile, flammable liquids (i.e. ethanol) away from flames.
4. If something is accidentally swallowed, follow these general guidelines. Identify the chemical ingested. If the person is unconscious do not administer liquids. If the person is conscious and the ingested chemical is known, perform the following treatment.
   • Acids - Do not induce vomiting, do not administer bicarbonate, administer
milk of magnesia in large amounts followed by milk.

- Bases - Do not induce vomiting; administer 1% acetic acid followed by milk.
- Other liquids (except petroleum products) - Administer 2-4 glasses of water and then induce vomiting with finger or water containing 1 tsp. of NaCl per glass. Continue vomiting until vomit fluid is clear.
- Gases - Remove the afflicted person from the area while avoiding inhaling the gas yourself. If the individual is not breathing, begin mouth-to-mouth resuscitation and continue until the victim is breathing normally or a physician orders a halt.
- Burns - Immerse in cold H2O for 15 minutes.

In all cases call a physician and tell them what the chemical is and what steps have been taken.

**To get help**

If you witness a trauma, go directly to any phone and dial 911. Make sure you know the seriousness of the situation and tell the dispatcher. If you say it is a life threatening situation, the ambulance will arrive in a few minutes, otherwise it could be 15 minutes. The nearest phone is a ways away; please use your/or somebody’s cell phone.

**Glassware**

1. Treat all glassware as if it were fine china. Much of the equipment used in this laboratory is expensive. Please be careful!
2. Rinse out all non-biologically contaminated glassware with water and place in the appropriate discard area.
3. Treat pipettes with care when inserting them into a propipette.
4. If you break something, report it and then clean it up, taking into consideration any chemical that might have been spilled. There is no charge in this lab for glassware breakage, but please, no bowling for beakers or Erlenmeyer volleyball.
5. Know where the extinguishers, fire blankets and other fire fighting gear are in the laboratory. Also know the appropriate exit in case of fire in the building. Your instructor will give you the specifics on this during the introductory lab period.

**Lab Clean-Up**

Here are a few rules to follow to keep your lab bench clean and help prevent contamination problems.

**Lab Bench**

1. **Disinfect the work area** on the bench with disinfectant and a paper towel. Do this before and after each lab period.
2. Remove all materials from the lab bench when finished with an experiment. Leave the work area in the same state it was when the period began.

3. Do not place backpacks, coats, purses, and other items on the bench. There are lockers in the hallway to store these items during lab. Cell phones should not be on the bench. CELL PHONES SHOULD NEVER BE HANDLED WITH GLOVES

Microscopes

1. Put the 10X lens above the stage.
2. Center the stage on the microscope.
3. Clean oil off the lens using lens paper. Do not use xylol on any lens. Oil should only contact the 100X lens. Never use oil on non-oil immersion lenses. Do not even put these lenses in place on a slide containing oil.
4. Place the power cord and the microscope carefully in the cabinet under your lab bench.

Disposal of Biological Materials

1. In general, remove all tape and markings on the items and keep caps and plugs in place to prevent contamination of the environment.
2. Tubes containing cultures should be placed in the slanted metal trays on the lower level of the disposal cart.
3. Flasks are placed in the trays on the top of the disposal cart.
4. Petri plates and other plastic items should be placed into the small garbage cans near the disposal cart. Do not confuse these cans with the regular garbage cans.
5. Slides from wet mounts must be placed in the labeled container containing disinfectant at the front of the room. The slides can be retrieved in subsequent lab periods, cleaned and reused. Stained specimens on slides can be thrown in the regular glass trash-Red Buckets. Staining kills the bacteria.

Disposal of Non-Biologicals

Other items used in the lab that do not come in contact with cultures or dangerous chemicals can be rinsed out with water and placed on the disposal cart (if glassware or reusable) or thrown away (if disposable).

Pipettes

Used pipettes must be placed into the aluminum trays containing disinfectant on your lab bench. Do not place test tubes, plastic tips, propipettes, your lab partner or anything else into the trays.
Grading

Your grade in the course is based on lab notebook checks, quizzes, and exams. The weight of the grades is distributed as follows:

- ELNs: 25%;
- Quizzes: 10%;
- Mid-term exams: 30%;
- Final exam: 35%

Grading the ELN

When we are grading the lab reports, the grades are not directly linked to having obtained the expected result. Someone who did not obtain the expected result may get a higher grade than an individual who obtained all the expected results. It is much more about how you organized the lab rapport, and whether you are able to convey your findings in a logical and structured manner with proper use (and labelling) of tables and figures. A common misconception is that students often re-iterate the data in the discussion. Please don’t. Of course, you can refer back to the main findings (little detail in the discussion) but the main goal of the discussion is two-fold. Firstly, in the discussion you can describe why you think you obtained unexpected results (i.e. what is your hypothesis?), and what would you do to improve the experiment if you were to repeat the experiment (i.e., how would you test the hypothesis). Secondly, the discussion provides you with the opportunity to place your (expected) findings in a larger context (i.e. what is the impact?). What could your findings mean to the field of Food Microbiology or Food Industry? I.e., what could the larger impact be? Being concise is key!

We will provide you with two examples of a lab report (same data) that you can compare. Note the differences. One version is a relative poor report, while the second version is of outstanding quality. Also, for the first two reports we will provide you with in-depth information how to improve your report writing skills. Combined, you should be in an excellent position to deliver quality reports. Keep in mind, writing reports is time-consuming. Don’t expect to deliver quality when writing last-minute. It is recommended to outline the report on the same day after you collected the final results, after which you start drafting your report soon after. Keep in mind: being concise in your description is key.

Overview of the point distribution when grading lab reports (100 points maximum).

1. Adherence to the template layout (5)
   a. Any topic lacking / insufficient needs deduction;
2. M&M: are the differences described that were applied in class compared to the manual; (10)
3. Results (52.5). Description of the results in an excessive descriptive manner is strongly discouraged. Use tables/figures to make the data clear;
   a. Are the calculations correct (20);
   b. Is the rationale for using data to process correct (5);
   c. Is raw data reported (2.5);
   d. Is it clear which experiment(s) they have been assigned (7.5);
   e. Is there a logic flow of description of the results directly followed by the processing of the data (5).
   f. Are bacterial names spelled correctly and italicized (2.5)
   g. If more than two topics are discussed in a single report, is this clearly separated (2.5)?
   h. Figures/legends should be grouped. If this is not the case, labels ‘float’ in the text, making it unappealing (2.5)

4. Discussion / conclusion (27.5)
   a. Results should not be directly REPEATED. It should focus on the discussion of the results.
      i. Was it different as expected,
      ii. how was it different,
      iii. can you think of reasons why it may be different?
      iv. how would you perform the experiment different?
      v. Do you think the appropriate controls were included?
      vi. can you explain the controls (relevance)?
      vii. Is the discussion/ conclusion structured?
      viii. Did the student place the findings in a larger context?

Other (5)
Are figures / tables legible?
Are figures / tables labeled?

Quizzes

For each experiment a quiz is uploaded that will only be accessible until the week in which the ELN pertinent to quiz material is due. Students can take the quizzes as often as they like, and the last obtained score counts. Participating in these quizzes will help you assess whether you grasp the topic and have the problem solving abilities that are an indicator for being successful in this course.
Exams

Exams are typical and are multiple choice and/or a mixture of open questions and multiple choice.

You are responsible for keeping track of your own progress in the course-check Learn@UW and notify us if there are discrepancies. The grades in this class loosely follow the standard curve as listed below, but keep in mind this is the “worst-case scenario”. (This way if everyone gets >90%, everyone will get As.) More likely, however, the curve will be tweaked to ensure that the average grade is no lower than a B/C.

<table>
<thead>
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<th>Grade</th>
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<tbody>
<tr>
<td>90-100</td>
<td>A</td>
</tr>
<tr>
<td>85-89</td>
<td>AB</td>
</tr>
<tr>
<td>80-84</td>
<td>B</td>
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<td>75-79</td>
<td>B/C</td>
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<td>70-74</td>
<td>C</td>
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<tr>
<td>60-69</td>
<td>D</td>
</tr>
<tr>
<td>&lt;60</td>
<td>F</td>
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</tbody>
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Absences

Attendance in a laboratory course is absolutely essential to understanding the material. This does not, however, mean that you need to come to class when you are sick or have some other emergency for which you need to attend. Please contact the instructor (whenever possible) to let them know you will be absent. Any absence without a reasonable explanation will be considered un-excused. You alone are responsible for understanding the material you miss, as it is usually not possible to make up lab work. Two un-excused absences (without valid reasons) is grounds to fail a student for the course.
Food Science 324  
Food Microbiology Lab  
FALL 2016

Laboratory: M, W: 1:20 – 4:20 p.m.  
Location: 2145 Microbial Sciences Building

I. BASIC MICROBIOLOGY & TECHNIQUES
Experiment 1: Microbial Techniques Checkup  
Experiment 2: The Effect of Heat on Microbes  
Experiment 3: Food Spoilage and Preservation  
Experiment 4: Shelf Life of Foods

II. FOOD SAFETY
Experiment 5: Antibiotic Residue Test  
Experiment 6: Detection of Listeria  
Experiment 7: Identification of Bacteria:  
    Part A, the conventional approach  
Experiment 8: Identification of Bacteria:  
    Part B, the molecular approach  
Experiment 9: Detection of S. aureus by Immuno-assay

III. FOOD PRODUCTION
Experiment 10: Bacteriophages in Food  
Experiment 11: Kimchi Production  
Experiment 12: Yoghurt Production
| Exp #1: | Plating / Gram stain  
|        | CFU count  
|        | **ELN due** |
| Exp #2: | Heat on microbes  
|        | CFU count  
|        | **ELN due** |
| Exp #3: | Preservation  
|        | CFU count ctrl  
|        | plate w/ preservative  
|        | CFU counts w/ preser/ctrl  
|        | **ELN due** |
| Exp #4: | Shelf-life study  
|        | CFU count  
|        | **ELN due** |
| Exp #5: | Antibiotic residue test  
|        | Read plates  
|        | **ELN due** |
| Exp #6: | Listeria detection  
|        | Plate PALCAM  
|        | Plate TSAYE  
|        | catalase, blood agar stab  
|        | assess blood plate  
|        | **ELN due** |
| Exp #7: | Bacterial detection, part A  
|        | inoculate LST-MUG  
|        | plate MRS, BHI, MAC, NB  
|        | test UV, plate L-EMB  
|        | col. pur. MRS,BHI,MAC,NB  
|        | MRVP, citrate, TB, NA plate  
|        | test MRVP, indole, citrate  
|        | **ELN due** |
| Exp #8: | Bacterial detection, part B  
|        | colony PCR on pur cfu  
|        | run+visualize DNA gel  
|        | column purify amplicons  
|        | quantify by Qubit  
|        | prepare sequencing rxn  
|        | analyze results  
|        | **ELN due** |
| Exp #9: | Detection of S. aureus  
|        | **ELN due** |
| Exp #10: | Bacteriophage in food  
|        | inoculate milk  
|        | perform plaque assay  
|        | analyze plaques and milk  
|        | **ELN due** |
| Exp #11, 12: | Kimchi & Yoghurt  

**MID-TERM EXAMS**

- **Listeria detection**
- Plate PALCAM
- Plate TSAYE
- Catalase, blood agar stab
- Assess blood plate
- **ELN due**

- **Bacterial detection, part A**
- Inoculate LST-MUG
- Plate MRS, BHI, MAC, NB
- Test UV, plate L-EMB
- Col. pur. MRS, BHI, MAC, NB
- MRVP, citrate, TB, NA plate
- Test MRVP, indole, citrate
- **ELN due**

- **Bacterial detection, part B**
- Colony PCR on pur cfu
- Run+visualize DNA gel
- Column purify amplicons
- Quantify by Qubit
- Prepare sequencing rxn
- Analyze results
- **ELN due**

- **Detection of S. aureus**
- **ELN due**

- **Bacteriophage in food**
- Inoculate milk
- Perform plaque assay
- Analyze plaques and milk
- **ELN due**

- **Kimchi & Yoghurt**

**December 15:** Last day of class, no ELNs needed for EXP#11, #12

**Final Exam:** December 22 2016, 2:45-4:45pm, room TBA

Dr J.P. van Pijkeren - FS324 - Fall 2016