Food Microbiology Lab.

BACTERIOLOGICAL ANALYSIS OF MEAT

All foods contain a resident microflora and in their processing they usually become further contaminated. Although many bacteria on food are harmless, some may be potentially pathogenic. One aspect of food microbiology is the detection and identification of pathogenic bacteria on food as well as assessing the general microbial load of the food which relates to the shelf-life and spoilage potential of the food.

In some countries meat is eaten raw. In this case, the meat must be fresh because meat is a nutrient rich food. Raw meat is usually eaten as ground meat (Steak Tartar). The grinding process makes more nutrient available to the microbes as well as mixing the microbial flora with the interior of the meat as well as adding bacteria from the meat processing equipment. Most meat is cooked before consumption. But, some cooking methods (particularly time of cooking) leave large portions of the meat in almost a raw state while cooking the surface of the meat. Consequently undercooked meat can possess microbes and in some instances has transmitted disease. In the USA, the most common meat transmitted diseases are caused by *Salmonella* (diarrheas) from poultry and hemolytic uremic syndrome (from beef) caused by a particular strain of *Escherichia coli*: *E. coli* O157:H7.

In this experiment, we will determine the microbiological load of some meat obtained from local markets and determine whether pathogens or meat spoilage organisms are present. These bacteria will be isolated and determination of species identification will use some diagnostic tests commercially supplied as strips (API20e) and plates (Biolog). In addition, we will test for fecal contamination and generally assess the sanitary quality of the meat using total counts and possible coliform contamination.

Coliforms are an indicator of food and water quality utilizing the presence of *Escherichia coli* to indicate the presence of fecal contamination. USPH water quality standards for drinking water are less than one fecal coliform/liter of water.

Materials

Lab Week One
Materials/Sample

1. Blender and sterile blender jars.
2. 450 ml sterile dilution fluid.
3. 5 9 ml dilution blanks with sterile dilution fluid.
4. 15/meat sample - 10 ml Lauryl SO_4 Lactose broth tubes, all 1X.
5. 12 EMB plates
6. 12 Mannitol Salt Agar (MSA) plates.
7. 20 TGY Agar plates (Tryptone-Glucose-Yeast Extract).
8. 5 Rappaport-Vassiliadis (RV) broth and 5 Tetrathionate (TT) broth, 10 ml/16 x 150 ml tubes.
9. 5 Bismuth Sulfite Agar plates, 5 Xylose-Lysine-Desoxycholate (XLD) Agar plates and 5 Hektoen-Enteric (HE) Agar plates.

10. 5 Triple Sugar Iron (TSI) deeps, 5 Lysine-Iron-Agar (LIA) deeps.
11. Sterile pipettes, micropipetters.
12. Glass spreaders, beakers 1/3 full alcohol.
13. Meat samples: one each of ground beef, ground turkey and ground chicken. If there are more students, then add in ground pork.

Lab Week Two

1. TGY plates.
2. Skim Milk Agar.
3. Catalase and Oxidase test reagents.
4. Gram staining kits.

Lab Week Three

1. API 20e diagnostic strips (10 to 20).
2. Sterile Pasteur pipets and 10-20 tubes of sterile mineral oil (3 ml/tube)
3. Biolog GN and GP plates (10-20).
4. Sterile 20 x 250 mm screw cap tubes with 25 ml of sterile saline.
5. Sterile pipetting reserviors and tips for Biolog multichannel pipetter.
6. API and Biolog Databases.

Procedure

***Week One* - Lab Day One**

1. Weigh 50 grams of ground meat and place into a sterile blender jar.

2. Add 450 of the sterile dilution fluid and blend for at least one minute until the meat is thoroughly blended. This is a 1:10 dilution of the meat.

3. Inoculate 3 serial 1:10 dilutions with the 9 ml dilution tubes from the first 1:10 dilution (blender jar).

4. Starting with the highest dilution, inoculate duplicate 50 µl amounts onto each of EMB, MSA, and TGY plates. Spread each inoculum with an alcohol sterilized glass spreader.

5. From each of the lowest dilutions, inoculate 1 ml into each of three Lauryl SO_4 Lactose broth tubes.

6. Incubate the TGY plates at 30°C and the EMB and MSA plates as well as the Lauryl SO_4 Lactose broth tubes at 37°C. Incubate for 48 hours.

7. Isolation of *Salmonella*:
    a. Inoculate 0.1 ml of blended meat into 10 ml each of Rappaport-Vassiliadis (RV) medium and tetrathionate (TT) medium. Incubate in a water bath set to 42°C for

24 hours.

b. Streak a loopful (~ 10 µl) for isolation of each enrichment onto Bismuth Sulfite Agar (BS), xylose-lysine-desoxycholate (XLD) agar and Hektoen-enteric agar (HE).

c. Incubate the plates at 37°C for 24 to 48 hours. Typical *Salmonella* colony morphology on these plates are:

   1. BS agar: gray-brown or black colonies +/- metallic sheen. Surrounding medium may turn brown to black
   2. XLD agar: pink colonies +/- black centers. Some strains will produce colonies with large, glossy black centers or be almost completely black.
   3. HE agar: Blue-green to blue colonies +/- black centers. Some strains will produce colonies with large, glossy black centers or be almost completely black.

d. Touch positive colonies with an inoculating needle and streak surface+stab both a triple sugar iron agar (TSI) deep and a lysine-iron agar (LIA) deep. Inoculate at least one positive colony from each selective (BS, XLD and HE) agar plate.

8. Remove TGY, EMB, MSA from their incubators and refrigerate. At 48 hours, read the Lauryl-SO_4-lactose tubes and inoculate some positive tubes to EMB (place the plates at 37°C for 48 hours.

Lab Week Two - Day One

1. Observe the TGY plates, count the colonies to determine the bacterial titer.

2. Describe the representative types of colonies and Gram stain each. Streak each type onto TGY plates for pure culture isolation.

3. Perform the oxidase and catalase test on these colonies.

4. Using Skim Milk Agar, patch on each representative colony from TGY plate count to determine proteolytic potentia.

5. Observe the EMB and MSA plates, describe the colonies and Gram stain. Perform the oxidase and catalase test on these colonies. If EMB plates have green-sheen colonies, streak one of these for isolation onto TGY. If the MSA plates have yellow colonies, streak one of these for isolation on to TGY.

6. Observe the BS, XLD and HE plates for positive *Salmonella* colonies. Gram stain positive ones, and streak positive ones onto TGY for isolation of pure cultures. Describe the TSI and LIA reactions for the *Salmonella* isolates.

Day Two or Three

1. Check for growth on the TGY plates, refrigerate if the restreaks are pure cultures. Otherwise, if not, restreak again to get a pure culture. Please notify the TA of the plates because these bacteria will have to be inoculated onto fresh media the day before Lab Week Three day one.

Day Before Third Week Lab

1. If the restreaks are pure cultures, inoculate a fresh Tryticase Soy Agar plate: primary streak should cover 1/3 to 1/2 of plate. Secondary and tertiary streaks to see isolated colonies (if the culture is pure). Incubate the plates at 30°C or 37°C.

***Lab Week 3* - Day One**

Preparation of Cell Suspensions for API-20e and Biolog Plates.

1. For each culture, first blank a sterile saline solution in a 25mm screw cap tube in the modified Spectronic 20.

2. Make a suspension of each culture using a sterile cotton swab by gently rolling the swab over the surface of growth in the primary or secondary streak area into the sterile saline solution in a 25mm screw cap tube. Adjust the cell density to have an optical density at 660nm of 0.25 +/- 0.01.

3. Use this suspension to inoculate an API strip using a sterile Pasteur pipette. Add mineral oil to the Arg, Lys, Orn decarboxylase, H_2S and Urease tests. Make sure that each test cup is inoculated to the right level (most are half full, but the Citrate, Voges Proskauer and Gelatin cups must be totally full).

4. Use the rest of the suspension to inoculate a Biolog GN or GP plate using the multichannel pipetter in the sterile hood. Make sure each well gets 125 to 150 µl of cell suspension. GP plates are for Gram negative bacteria, GN plates are for Gram negative bacteria.

5. Incubate the API strips and Biolog GN plates for 24 hours at 30°C (or 37°C for coliform and *Salmonella* poisitive isolates). After incubation add necessary reagents to the API strips to obtain the results. Sign up with the TA to use the databases on Monday or Tuesday: Day Two.

Day Two-to-Before Next Lab

6. Record results of your isolates: API 20e data directly onto the API 20e form; Biolog plate on to the xeroxed box form. Refer to color charts. It is sometimes taxing to tell faint colors

and score reactions as positive or negative. Refer to the product literature for color interpretation. For the Biology system there are only two colors - purple or colorless, but as you will see sometimes the purple gets precipitated and other times not score doubtful wells as +/-. Be careful.

7. Use the results obtained from the API strips and the Biolog GN plates to get the Index number or Bionumber. Use this number to identify the cultures you have isolated using the API (book) or Biolog (computer) databases.

8. Do both the API data and Biolog data agree? Using your total results - gram stain, oxidase, catalase, API , Biolog characters identify the pure cultures. Turn in your lab report NOW.

Media

Rappaport-Vassiliadis broth.
Broth Base

Tryptone	5g
NaCl	8g
KH_2PO_4	1.6 g
distilled water	1000 ml

Magnesium chloride solution

$MgCl_2 \cdot 6H_2O$	40g
distilled water	100 ml

Malachite Green Oxalate solution.

Malachite Green Oxalate	0.4 g	(Merck)
distilled water	100 ml	

Prep: combine 1000ml broth base with 100 ml $MgCl_2$ solution and 10 ml Malachite Green Oxalate solution. This makes 1110 ml, use less for small classes. Dispense 10 ml in 16 x 150 mm tubes. Autolcave 15 min. Final pH should be 5.5 +/- 0.2.

Tetrathionate broth.
Broth Base

Polypeptone	5g
Bile salts	1 g
$CaCO_3$	10g
$Na_2SSO_3 \cdot 5H_2O$	30g
distilled water	1 liter

Suspend ingredients on day of use. Boil *DO NOT AUTOCLAVE*, cool

Iodine solution

KI	5g
I_2	6g
distilled water	20 ml

Brilliant Green solution

Brilliant Green dye	0.1 g
Distilled water	100ml filter sterilize.

Prep: *ON DAY of USE*: 10 ml of filter sterilized Brilliant Green solution to 1 liter of cool, boiled Broth Base, then when cool add 20 ml iodine solution (needs no sterilization). Aseptically dispense 10 ml into sterile 16 x 150 mm tubes.

Bismuth Sulfite Agar.

Peptone (or polypeptone)	10g
Beef Extract	5g
Glucose	5g
Na_2HPO_4	4g
$FeSO_4$	0.3g
Bismuth sulfite	8g
Brilliant Green	0.025g
Agar	20g
distilled water	1000 ml

Prep: *DO NOT AUTOCLAVE* ! Add all ingredients to distilled water, swirl to dissolve (not all will dissolve). Boil 1 minute. Cool and pour plates. Store in refrigerator in dark.

Xylose-Lysine-Desoxycholate Agar.

Yeast Extract	3g
L-lysine	5g
Xylose	3.75g
Lactose	7.5g
Sucrose	7.5g
Na-Desoxycholate	2.5g
Ferric ammonium citrate	0.8g
Na_2SSO_3	6.8g
NaCl	5g
Agar	15g
Phenol red	0.08g
distilled water	1000ml

Prep: Heat with medium agiatation until just boiling. Immediately cool to 50ºC. *DO NOT AUTOCLAVE*! Pour plates on the day of use. Do not store more than one day.

Hektoen-Enteric Agar.

Peptone	12g
Yeast Extract	3g
Bile Salts No.3	9g
Lactose	12g
Sucrose	12g
Salicin	2g
NaCl	5g
Na_2SSO_3	5g
Ferric ammonium citrate	1.5g
Bromthymol Blue	0.065g
Acid fuchsin	0.1 g
Agar	14g
distillled water	1000ml

Prep: heat to boiling with agiatation. Boil NO LONGER than 1 min. Cool immediately to 45C and pour plates. Do not store more than 1 day.

Triple Sugar Iron Agar

Beef Extract	3g
Yeast Extract	3g
Peptone	15g
Proteose peptone	5g
Glucose	1g
Lactose	10g
Sucrose	10g
$FeSO_4$	0.2g
NaCl	5g
Na_2SSO_3	0.3g
Phenol Red	0.024g
Agar	12g
distilled water	1000ml

Prep: heat to dissolve, dispense in tubes, 10 ml/16x150mm tube. Autoclave. This should be a short slant with a deep.

Lysine Iron Agar

Peptone	5g
Yeast Extract	3g
Glucose	1g
L-lysine HCl	10g
Ferric ammonium citrate	0.5g
Na_2SSO_3	0.04g

Bromcresol purple	0.02g
Agar	15g
distilled water	1 liter

Heat to dissolve, dispense in 4ml/13x100 screw cap tubes. Autoclave. Slant with a long deep and short slant.

Skim Milk Agar

Tryptone	5g
Yeast Extract	2.5g
Glucose	1g
Agar	15g
distilled water	980 ml
Skim Milk	20 ml

Autoclave for 10 minutes.