



Biology 331 - General Microbiology

River Proteobacteria and Waterborne Diseases



Enteric Proteobacteria (12.11)

- Non-sporulating rods, oxidase (-) facultative aerobes
- Chemoheterotrophs - simple monomers
- Coliforms = lac (+) "feces indicators"
- BUT there are lac (-), some pathogens too!

You will each use phenotypic tests and tables to identify 1 river coliform unknown and 1 control Enteric.

Pseudomonas (12.7)

- Non-sporulating rods, lac (-), oxidase (+) aerobes
- Chemoheterotrophs - monomers and polymers
- Legendary pollution tolerance and bioremediation
- Some pathogens, but typically not as diarrhea agents

Neisseria, which causes gonorrhea and some forms of meningitis, also oxidase (+) - common test.

Diarrhea/Enteritis-Causing Proteobacteria

#1 *Campylobacter jejuni* (12.14 and 29.9)

- Spirilla found in poultry GI flora, feces
- 1 week - intestine pain, 103+ fever, blood/pus in stool
- Secreted toxin - ATP to cAMP, salt and fluid loss
- LPS toxins - HUGE inflammation, MS-like symptoms
- Sterility, spontaneous abortion in cattle and sheep

Won't grow on MacConkey as it has complex nutritional requirements and low oxygen tolerance.

#2 *Salmonella*, over 2000 strains (12.11 and 29.7)

- Lac (-) Enteric pathogen in human/mammal GI/feces
- Dose HIGH - 10,000 to 1 million bacteria

- 8-24 hours - headache, vomiting, diarrhea, low fever
- Ruffle membrane - penetrate, inflame GI mucosa
- Variable pili, flagella - many alleles confuse defenses

Typhoid fever by S. typhi - invades blood, fever for weeks, 15% fatal - developing nations, Typhoid Mary.

Salmonella Aside - Largest BW Event on US Soil

- LONG history (500 B.C.) - feces and water
- 1984 - Rajneesh Cult in Antelope, OR
- Goal was to take over elected seats in county
- typhimurium obtained by nurse posing as health lab
- Sprayed on salad bars, Dalles - 751 cases, no deaths

#3 *Shigella*, 4 major species (12.11 and 29.8)

- Lac variable Enteric pathogen, close relative of E. coli
- Human feces ONLY, LOW dose - 1-10 bacteria
- POWERFUL secreted Shiga toxin halts translation
- 1 day - HUGE diarrhea (20 BM/day) with blood/pus
- 40% of all victims' family catches - THINK ABOUT
- 5% develop HUS - septicemia, anemia, kidney failure

#4 Enterohemorrhagic *E. coli* O157:H7

- True coliform Enteric pathogen - 1 of 200 strains
- Undercooked ground beef, feces-tainted food/water
- 2-4 days moderate bloody diarrhea, 10% HUS
- Shiga-like Vero toxin inhibits protein synthesis
- 60,000 cases/year and 50 deaths

First emerged 1980 Jack in the Box; Odwalla raw apple juice, unpasteurized milk cases in OR/WA - 2005

ACTIVITIES

Session One - Collection and Plating

At least 2 students from different teams will accompany me to Independence Park at 7:30 a.m. There, we will gather river water and mud (for the phototroph lab) from Willamette and Gentle.

Site Analysis and Collection

On Site: Collect 10 ml water; assess temperature, pH, N-compounds (possibly) - photodocument

In Lab: Gently swirl or invert river water before removing every sample to avoid settling

Each person will spread 0.2 ml onto each of 2 MacConkey plates - i.e. 2 replicates/person

Keep track of YOUR plates as grading is individual from here on out; incubate 37°C

Session Two - Individual Sub-Culture and Assessment

Lac+/True Coliforms - INDIVIDUAL

Select 2 different COLIFORM colonies; perfectly streak onto MacConkey plates; incubate 37°C

Check plate within 24 hours; Good/store in refrigerator; Bad/repeat until perfect by NEXT LAB!!

Lac- Colonies Oxidase Sorting - INDIVIDUAL

Handle oxidase disks with forceps in empty petri dishes to avoid contact with skin, benches, etc.

SORT 5 lac negative colonies into oxidase + or - categories; Why? Significance?

Press colony into disc using toothpick; dark purpling = (+); perform 1 at a time to avoid confusion

DON'T FORGET Waterborne Virus assignment: each person researches/locates pictures of different agent (see web template).

Final Sessions - Individual Identification and Microscopy Practical

Each person will use the following tests to identify 1 river isolate (which may not match anything because it is natural) AND 1 control from the ID table. The latter will be graded all or nothing. When recording data in your notebook, DESCRIBE what you see (colors, cloudiness, etc.) - NOT just +/-.

Protozoa:

(1) Each person will be working with Entamoeba histolytica, Balantidium coli, Cryptosporidium parvum, Giardia lamblia.

(2) Microscopy Drawings: View and draw all four agents under oil immersion using prepared slides. Make certain you can distinguish between the agent and host cells/tissue. It may be useful to review your text and/or the CDC website before tackling the slides as some are tricky.

(4) Using your text and the CDC website, complete all worksheet questions about these agents.

(3) Microscopy Practical: During the final hour of this lab unit (session four), you will be required to physically set up 1 assigned protozoa slide, center a representative protozoa in focus (using oil immersion), and show your instructor. Thus, you have done all previous assignments by this time so you will be able to perform this task.

ID Chart For Enterics/Coliforms - From Text p. 353 and Bergey's Manual

	Sulfur	Indole	Lactose	Glucose-Gas	Citrate
<u>Escherichia coli</u>	-	+	+	+	-
<u>Citrobacter freundii</u>	+	-	+	+	+
<u>Citrobacter intermedius</u>	-	+	+	+	+
<u>Salmonella typhimurium</u>	+	-	-	+	+
<u>Klebsiella pneumoniae</u>	-	-	+	+	+
<u>Proteus mirabilis</u>	+	-	-	+	var
<u>Proteus vulgaris</u>	+	+	-	+	var

Make sure there is growth before interpreting each.

Citrate Utilization Media

MgSO₄: 0.2 g
Ammonium Dihydrogen Phosphate: 1 g
K₂HPO₄: 1 g
Sodium Citrate: 2 g
NaCl: 5 g
Bromothymol Blue: 0.08 g

Prepared as green plates using commercial mix with agar. Inoculate 2 isolates per plate and grow 2 days, looping large amounts as far apart as possible (i.e. keep middle clear). Following growth, did the media turn blue? (yes = citrate utilized to a base).

<u>Glucose Gas Media</u> Phenol Red Broth Base: Peptone #3: 10 g Beef Extract: 1 g NaCl: 5 g Bacto Phenol Red: 0.018 g Dextrose/Glucose: 10 g	Prepared as 7 ml red broth tubes with Durham tubes. Inoculate 1 isolate per tube and grow 2 days. Following growth, yellow = glucose to acid; bubble in Durham = gas.
<u>Lactose Utilization - MacConkey Media</u> <u>Sulfur-Indole-Motility (SIM)</u> Peptone: 30 g Beef Extract: 3 g Peptonized Iron: 0.2 g Sodium Thiosulfate: 0.025 g Agar: 3 g - boil/dissolve BEFORE aliquoting	Review Lab One Prepared as semi-solid 7 ml tubes using a commercial pre-mix. Inoculate 1 isolate per tube by plunging down agar center and grow 2 days. After growth, assess IN ORDER: Sulfur: black? (yes = sulfide); Indole: Add 10-15 drops Kovacs; wait 2 minutes. Red? (yes = indole).

	<h2 style="margin: 0;">Biology 331 - General Microbiology</h2> <h3 style="margin: 0;">River Proteobacteria and Waterborne Diseases</h3>	
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Team Names:

RIVER COLLECTION AND ASSESSMENT

Sample description, temperature, pH, and N-compound information (if class ends up doing)	Picture of River Collection
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TEAM MEMBER 1 - NAME: (cut/paste for as many people as are on your team)

Plate 1 Count - Lactose Positive vs. Negatives	Picture of your river plate 1										
Plate 2 Count - Lactose Positive vs. Negatives	Picture of your river plate 2										
Oxidase Data	Picture of all final oxidase disks										
Isolate 1 Testing Data <table border="1" style="width: 100%; border-collapse: collapse; margin-top: 5px;"> <tr> <td style="padding: 2px;">Sulf</td> <td style="padding: 2px;">Ind</td> <td style="padding: 2px;">Lac</td> <td style="padding: 2px;">Glu-G</td> <td style="padding: 2px;">Cit</td> </tr> <tr> <td style="height: 20px;"></td> <td></td> <td></td> <td></td> <td></td> </tr> </table>	Sulf	Ind	Lac	Glu-G	Cit						Picture of FINAL streak plate (approved)
Sulf	Ind	Lac	Glu-G	Cit							
Provided Control - write number or letter <table border="1" style="width: 100%; border-collapse: collapse; margin-top: 5px;"> <tr> <td style="padding: 2px;">Sulf</td> <td style="padding: 2px;">Ind</td> <td style="padding: 2px;">Lac</td> <td style="padding: 2px;">Glu-G</td> <td style="padding: 2px;">Cit</td> </tr> <tr> <td style="height: 20px;"></td> <td></td> <td></td> <td></td> <td></td> </tr> </table>	Sulf	Ind	Lac	Glu-G	Cit						Picture of Control Plate
Sulf	Ind	Lac	Glu-G	Cit							

Individual Identification:

Identify each of your organisms, explaining your reasoning carefully and thoroughly.

River Calculation and Discussion:

Use combined primary plate data in combination with oxidase results to estimate the AVERAGE number of each of the following classes of organisms per LITER in the original sample.

Coliforms/L =

Pseudomonas/L =

Non-Coliform Enterics/L =

What do the following distributions of Proteobacteria tell you about this site?

Individual Research About Waterborne Viral Pathogens:

All these hardy viruses readily survive outside the body in both food and water - which accounts for most transmission events. Each person completes one (less than 4, take your pick) - individually graded. Folks cannot do the same agent so sort out who takes what before proceeding.

<p style="text-align: center;"><u>Poliovirus</u></p> <p><u>Your Name:</u> <u>Virus Structure:</u> (address protein and genetic material) <u>Key Symptoms/Timecourse:</u> <u>Typical Treatment:</u></p>	<p style="text-align: center;">Insert Digital Image</p> <p style="text-align: center;"><i>Please reduce size to 3 inches.</i></p>
<p style="text-align: center;"><u>Norwalk Agent/Virus</u></p> <p><u>Your Name:</u> <u>Virus Structure:</u> (address protein and genetic material) <u>Key Symptoms/Timecourse:</u> <u>Typical Treatment:</u></p>	<p style="text-align: center;">Insert Digital Image</p> <p style="text-align: center;"><i>Please reduce size to 3 inches.</i></p>
<p style="text-align: center;"><u>Hepatitis A</u></p> <p><u>Your Name:</u> <u>Virus Structure:</u> (address protein and genetic material) <u>Key Symptoms/Timecourse:</u> <u>Typical Treatment:</u></p>	<p style="text-align: center;">Insert Digital Image</p> <p style="text-align: center;"><i>Please reduce size to 3 inches.</i></p>
<p style="text-align: center;"><u>Coxsackievirus or Echovirus</u></p> <p><u>Your Name:</u> <u>Virus Structure:</u> (address protein and genetic material) <u>Key Symptoms/Timecourse:</u> <u>Typical Treatment:</u></p>	<p style="text-align: center;">Insert Digital Image</p> <p style="text-align: center;"><i>Please reduce size to 3 inches.</i></p>

