



**BIOL 3702L:
Bacterial
Growth
Responses**

<http://www.aviano.af.mil/News/Article-Display/Article/724769/mids-airmen-ensure-success-through-support/>

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Purpose of This Week's Labs

- 1) To assess the growth of various microbes on selective and/or differential media
 - Selective media permit the growth of a particular type of microbe
 - Differential media permit the distinction among different types of microbes
 - Selective and differential media perform both of the above functions simultaneously

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Purpose of This Week's Labs (cont.)

- 2) To assess the metabolic abilities of microbes to utilize nitrate
 - Some microbes use nitrate as a final electron acceptor producing nitrite ($\text{NO}_3 \Rightarrow \text{NO}_2$)
 - Others reduce nitrate to molecular nitrogen gas ($\text{NO}_3 \Rightarrow \text{N}_2$)
 - Still others reduce nitrate to ammonia or hydroxylamine ($\text{NO}_3 \Rightarrow \text{NH}_4$ or NH_2OH)

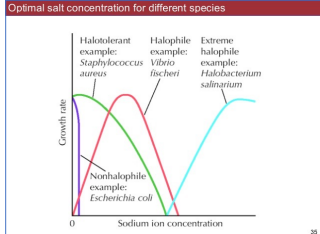
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Purpose of This Week's Labs (cont.)

- 3) To assess the growth response of various microbes to different osmotic pressures



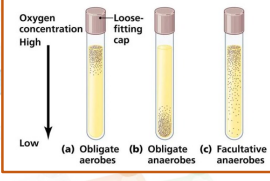
<https://www.slideshare.net/jrhlogeomics/bi2c-lecture-11-microbial-growth-and-functions>

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Purpose of This Week's Labs (cont.)

- 4) To assess the oxygen requirements of various bacteria
 - Some microbes are obligate aerobes
 - Others are obligate anaerobes
 - Still others are facultative anaerobes



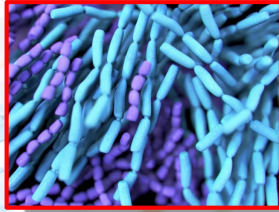
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Purpose of This Week's Labs (cont.)

- 5) To assess the various bacteria found in yogurt
 - Others are obligate anaerobes
 - Still others are facultative anaerobes



<https://www.medicalnewstoday.com/articles/295714#benefits>

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Today's Exercises

- Today's exercises will be done in Groups.
- There are five different exercises to be initiated today.
 - Selective/Differential Media
 - Nitrate reduction
 - Growth response to varying osmotic conditions
 - Growth under aerobic and anaerobic conditions
 - Growth and characterization of yogurt bacteria

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Today's Exercises

- Suggestion: Assign 1-2 persons per group to be responsible for a particular exercise.
 - *However*, each group member must see/share the results of each exercise.
 - *In addition*, 1) each person in a group needs to record their own results, and 2) share real-time observations when possible.
 - **SPECIAL PERMISSION TO USE MOBILE PHONE CAMERA TO RECORD RESULTS FOR SHARING**

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Today's Exercises (cont.)

- To be successful in today's exercise:
 - Students must work collegially and effectively
 - Students must participate – *no lazy slackers!*
 - Share results, but **DO NOT PLAGIARIZE!**
- The following slides contain a BRIEF synopsis of what students are to do in each exercise; for details, *follow the instructions* given in the exercise handouts.

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Today's Exercises (cont.)

- Selective and Differential Media
 - Eosin Methylene Blue (EMB) agar plates
 - **Selective** – only Gram-negative bacteria grow on this plate (rare exceptions)
 - **Differential**
 - Lactose-fermenting bacteria appear brown to blue-black in color
 - Non-lactose-fermenting colonies appear transparent and colorless
 - Colonies of *E. coli*, a lactose-fermenting microbe, typically appear with a green-metallic sheen


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Today's Exercises (cont.)



E. coli on EMB agar



Salmonella on EMB agar

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Today's Exercises (cont.)


- Selective and Differential Media (cont.)
 - Mannitol Salt Agar (MSA) plates
 - **Selective** – only Gram-positive, salt-tolerant (6.5%) bacteria grow on this plate, but not Gram-negative bacteria
 - **Differential** – Mannitol fermentation turns medium yellow due to acid production

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Today's Exercises (cont.)

- Mannitol Salt Agar (MSA) plates (cont.)
 - *Staphylococcus aureus* – grows on MSA and turns medium yellow
 - *Staphylococcus epidermidis* – grows on MSA, but does not turn medium yellow
 - Salt intolerant Gram-positive cocci that that will not grow on MSA
 - *Streptococcus pyogenes*
 - *Micrococcus luteus*


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Today's Exercises (cont.)

Salt Tolerant Mannitol Fermentation	Salt Tolerant No Mannitol Fermentation	Salt Intolerant No Growth
		
<i>Staphylococcus aureus</i>	<i>Staphylococcus epidermidis</i>	<i>Micrococcus luteus</i>


MSA (Mannitol Salt Agar)

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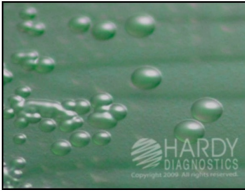

Today's Exercises (cont.)

- Hektoen Enteric (HE) agar plates
 - **Selective** – inhibits Gram-positive bacteria and some Gram-negative bacteria
 - **Differential** – distinguishes enteric pathogens from slow lactose fermenters
 - *Salmonella* - blue to blue-green colonies with black centers
 - *Shigella* - blue to blue-green colonies
 - *Escherichia coli* - Partial inhibition; may be slight growth of yellow to salmon colored colonies


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Today's Exercises (cont.)


Shigella (left image) and *Salmonella* (right image) on HE agar

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Today's Exercises (cont.)

- MacConkey (MAC) agar plates
 - **Selective** – inhibits Gram-positive bacteria
 - **Differential**
 - Lactose fermenters form pink colonies surrounded by a zone of bile salt precipitation
 - Non-lactose fermenters develop as transparent, colorless colonies with no precipitated zone


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Today's Exercises (cont.)


Lactose fermenter *Escherichia coli* (left image) and non-lactose fermenter *Salmonella* (right image) on MAC agar

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Today's Exercises (cont.)

- Nitrate Reduction – use the commercially-purchased broth medium provided
 - Heavily inoculate three tubes with *E. coli*, *S. epidermidis*, and *P. fluorescens*
 - In a fourth tube, place a gram of soil
 - Leave a fifth tube uninoculated (control)
 - Incubate for 48 hours at 37°C with lids loosened (but not falling off!)



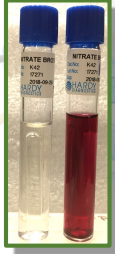
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Today's Exercises (cont.)


- Follow the flow chart in adding Nitrate Reagents A, B, and C



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    graph TD
      A[Nitrate Broth, 37°C, 48 hours] --> B[Add sulfanilic acid and N,N-dimethylnaphthylamine]
      B --> C[Red Color]
      B --> D[Colorless]
      C --> E[Nitrate Reduction Positive]
      D --> F[Presumptive Nitrate Reduction Negative]
      F --> G[Add zinc dust]
      G --> H[Red Color]
      G --> I[Colorless]
      H --> J[Nitrate Reduction Negative]
      I --> K[Complete Nitrate Reduction Positive]
    
```

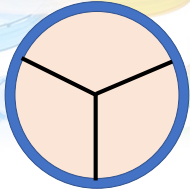
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Today's Exercises (cont.)

- Growth Response to Osmotic Pressure
 - Divide the following Nutrient Agar plates into three areas
 - 0% NaCl
 - 0.5% NaCl
 - 5% NaCl
 - 10% NaCl
 - 20% NaCl
 - 25% NaCl




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Today's Exercises (cont.)

- Streak *E. coli*, *S. aureus*, and *Halobacterium salinarium* onto a separate sector of each plate
- Wrap edges of plates with Parafilm, then incubate at 37°C for 48 hours
- Observe and record the amount of growth on each plate
- **NOTE:** *Halobacterium salinarium* may take up to 10-14 days to show significant growth



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Today's Exercises (cont.)

- Growth Response to Aerobic or Anaerobic Conditions
 - Swab two plates each of TSA with the following organisms
 - *Alkaligenes faecalis*
 - *Escherichia coli*
 - *Clostridium sporogenes*
 - *Clostridium histolyticum*

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Today's Exercises (cont.)

- Incubate one plate of each organism together in a single resealable bag
- Place the remaining four plates in a second resealable bag
- Seal one bag and incubate it at 37°C for 36-48 hours. This will be the aerobic (+O₂) incubation condition.
- Place an anaerobic generating sachet in the second sealable bag, seal it, then incubate it at 37°C for 36-48 hours. This will be the anaerobic (-O₂) incubation condition.

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Today's Exercises (cont.)

- After 36-48 hours, perform the following:
 - Remove the plates from the +O₂ bag, record your observations, return them to the bag and place an anaerobic generating sachet in the bag. Seal the bag and incubate it for 48 hours at 37°C.
 - Remove the plates from the -O₂ bag, record your observations, return them to the bag, but remove anaerobic generating. Seal the bag and incubate it for 48 hours at 37°C.

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Today's Exercises (cont.)

TAKE NOTE: The Tuesday/Thursday lab will allow the second incubation step to continue until Monday!!!

- After the second incubation step for each condition, remove the plates and record your observation.
- Discard all plates and bags in the waste barrel.

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Today's Exercises (cont.)

- Growth and Characterization of Yogurt Bacteria
 - Isolation of yogurt bacteria
 - Use a loop to smear three plates of MRS agar with a sample of Activia yogurt.
 - Incubate one plate in under anaerobic (-O₂) conditions
 - Incubate the second plate in a carbon dioxide (+CO₂) environment
 - Incubate the third plate under aerobic (+O₂) conditions

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Today's Exercises (cont.)

- Isolation of yogurt bacteria (cOnt.)
 - Incubate plates at 37°C for 36-48 hours
 - From these plates, select different colony types for the following tests:
 - Gram Stain
 - Esculin hydrolysis
 - Arginine hydrolysis
 - Direct staining of yogurt
 - Smear a glass slide with small yogurt sample
 - Gram stain slide and make observations

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Lab Report Expectations

- Remember, where appropriate, **YOUR ANSWERS MUST BE CLEAR, CONCISE, AND GRAMMATICALLY SOUND SENTENCES!**
- Retain this lab report. Your laboratory instructor may not call for it at this time, but may do so in the near future.

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