

**BIOL 3702L:  
Bacterial  
Growth  
Responses**




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

1

### Purpose of This Week's Labs

- 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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2

### Purpose of This Week's Labs (cont.)

- 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  $\text{NH}_2\text{OH}$ )

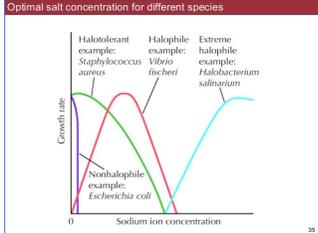
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3

### Purpose of This Week's Labs (cont.)

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



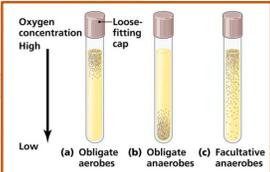
https://www.siteshare.net/jrh/qg-enomics/bi2c-lecture-11-microbial-growth-and-functions

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### Purpose of This Week's Labs (cont.)

- 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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### Today's Exercises

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

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6

### 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.

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7

### 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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8

### 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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9

### Today's Exercises (cont.)



*E. coli* on EMB agar



*Salmonella* on EMB agar

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10

### 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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11

### 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 will not grow on MSA
    - *Streptococcus pyogenes*
    - *Micrococcus luteus*

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12

### 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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14

### Today's Exercises (cont.)




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

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15

### 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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16

### Today's Exercises (cont.)




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

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17

### 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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18

### Today's Exercises (cont.)

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



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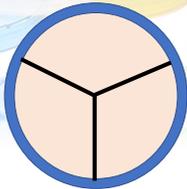
    graph TD
      A[Nitrate Broth, 37°C, 48 hours] --> B[Red Color]
      A --> C[Colorless]
      B --> D[Nitrate Reduction Positive]
      C --> E[Presumptive Nitrate Reduction Negative]
      E --> F[Add zinc dust]
      F --> G[Red Color]
      F --> H[Colorless]
      G --> I[Nitrate Reduction Negative]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

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19

### 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

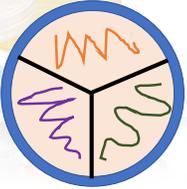


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20

### 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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21

### 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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22

### 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 (+O<sub>2</sub>) 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 (-O<sub>2</sub>) incubation condition.

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23

### Today's Exercises (cont.)

- After 36-48 hours, perform the following:
  - Remove the plates from the +O<sub>2</sub> 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 -O<sub>2</sub> 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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24

### 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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25

### 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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26



https://www.huffpost.com/barbara-bacchi/asking-questions-is-really-hard\_3\_7052722.html

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27