Microbiology 20 Biochemical Unknown – Spring 2009
(due May 14th)

You should be prepared to turn in your notebook with your biochemical unknown identification completed after lab on Thursday May 14th. Out of a possible 70 points you will be graded as follows:

Successfully performing the necessary tests to identify your unknown (25 pts):

- Gram stain (verify with instructor)
  - morphology & arrangement
- capsule stain
- spore stain (only if *Gram-negative*)
- acid fast stain (only if *Gram-negative rod*)
- motility (via wet mount)
- colony characteristics on nutrient agar plate (see pg. 70 of lab manual)
  - color (observe color of colonies as well as any changes in color of agar)
  - diameter of typical isolated colony
  - overall appearance
  - margin
  - elevation
- color, growth characteristics on agar slant at 37° C and room temp (pg. 79)
- characteristics of broth culture (*before* mixing culture)
  - pellicle?
  - turbid?
  - flocculent?
  - sediment?
- *oxidation-fermentation/OF test*
- *starch hydrolysis test* (amylase)
- *PR-glucose, PR-lactose, PR-sucrose tests* (fermentation)
- *methyl red/MR test* (fermentation → mixed acids)
- *Vogues-Proskauer/VP test* (fermentation → acetoin)
- *citrate utilization test*
- *gelatin hydrolysis test* (gelatinase)
- *urea hydrolysis test* (urease)
- *phenylalanine deaminase test*
- *H₂S test*
- *motility-indole-ornithine/MIO test*
- *nitrate reduction test*
- *oxidase test* (cytochrome C oxidase)
- *catalase test*

* Perform biochemical tests of unknown as part of Exercises 13 to 17.
Adequately recording the procedures and results of each test (25 pts):

- I (or anyone else) should be able to repeat your work using only your notebook
- your results should be recorded in color and/or sufficiently described and labeled
  - indicate the total magnification for all microscopic images
  - it should be clear on what date each test was done and analyzed
  - procedures already written out in your notebook can be referred to by page
- your notebook entries should be neat, well-organized, and easy to read
  - set aside a section of pages in your notebook to keep records of your unknown

Correctly interpreting your results and identifying your unknown (20 pts):

- correctly indicating what each result means (e.g., “pink” Gram stain = Gram-neg.)
- conclusion summarizing your results and the identity of your unknown
  - can be in chart, table or list form

**Verify identity of unknown using Bergey's Manual of Determinative Bacteriology**

### Possible Unknown Bacterial Species

<table>
<thead>
<tr>
<th>Gram-negative</th>
<th>Gram-positive</th>
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</thead>
<tbody>
<tr>
<td>Aeromonas hydrophila</td>
<td>Bacillus cereus</td>
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<tr>
<td>Alcaligenes faecalis</td>
<td>Bacillus megaterium</td>
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<tr>
<td>Citrobacter freundii</td>
<td>Bacillus subtilis</td>
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<tr>
<td>Enterobacter aerogenes</td>
<td>Enterococcus faecalis</td>
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<td>Enterobacter cloacae</td>
<td>Lactococcus lactis</td>
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<td>Escherichia coli</td>
<td>Micrococcus luteus</td>
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<td>Klebsiella pneumoniae</td>
<td>Micrococcus roseus</td>
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<td>Proteus mirabilis</td>
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<td>Sporosarcina ureae</td>
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<td>Serratia marcescens</td>
<td>Streptococcus pyogenes</td>
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<td>Shigella flexneri</td>
<td>Streptococcus salivarius</td>
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<td>Gram-neg chart</td>
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<td><strong>Morphology</strong></td>
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<td><strong>O/F</strong></td>
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</tbody>
</table>

- FA: facultative anaerobe
- OA: obligate aerobe
- +: 90% or greater positive
- -: 90% or greater negative
- d: 26-75% of strains are positive
- [+] 76-89% positive
- [-] 76-89% negative
## Gram-pos chart

<table>
<thead>
<tr>
<th>Morphology</th>
<th>B cereus</th>
<th>B megat</th>
<th>B subtilis</th>
<th>E faecalis</th>
<th>L lactis</th>
<th>M luteus</th>
<th>M roseus</th>
<th>M phlei</th>
<th>S ureae</th>
<th>S aureus</th>
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