Microbiology 20 Biochemical Unknown – Spring 2009
(due May 13th)

You should be prepared to turn in your notebook with your biochemical unknown identification completed after lab on Wednesday May 13th. 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-positive)
- acid fast stain (only if Gram-positive 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>
</tr>
</thead>
<tbody>
<tr>
<td><em>Aeromonas hydrophila</em></td>
<td><em>Bacillus cereus</em></td>
</tr>
<tr>
<td><em>Alcaligenes faecalis</em></td>
<td><em>Bacillus megaterium</em></td>
</tr>
<tr>
<td><em>Citrobacter freundii</em></td>
<td><em>Bacillus subtilis</em></td>
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<tr>
<td><em>Enterobacter aerogenes</em></td>
<td><em>Enterococcus faecalis</em></td>
</tr>
<tr>
<td><em>Enterobacter cloacae</em></td>
<td><em>Lactococcus lactis</em></td>
</tr>
<tr>
<td><em>Escherichia coli</em></td>
<td><em>Micrococcus luteus</em></td>
</tr>
<tr>
<td><em>Klebsiella pneumoniae</em></td>
<td><em>Micrococcus roseus</em></td>
</tr>
<tr>
<td><em>Proteus mirabilis</em></td>
<td><em>Mycobacterium phlei</em></td>
</tr>
<tr>
<td><em>Proteus vulgaris</em></td>
<td><em>Sporosarcina ureae</em></td>
</tr>
<tr>
<td><em>Pseudomonas aeruginosa</em></td>
<td><em>Staphylococcus aureus</em></td>
</tr>
<tr>
<td><em>Pseudomonas fluorescens</em></td>
<td><em>Staphylococcus epidermidis</em></td>
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<tr>
<td><em>Serratia marcescens</em></td>
<td><em>Streptococcus pyogenes</em></td>
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<tr>
<td><em>Shigella flexneri</em></td>
<td><em>Streptococcus salivarius</em></td>
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</tbody>
</table>