Unknown Microbe Identification Experiment
Project Objectives:
- Engage students in an investigative research experience.
- Minimize the risk of working with pathogenic organisms.
- Integrate lab activities with associated lecture material.
- DISCOVER NOVEL BACTERIAL SPECIES!!!

Student Learning Objectives:
- Phenotypic characterization of microbes, evaluation of advantages & disadvantages.
- Molecular characterization of microbes, evaluation of advantages & disadvantages.
- Application of bioinformatic tools and databases to investigate evolutionary relationships among microbes.
- Finding relevant information in the scientific literature.
- Writing a primary literature-style research paper.
- The use of peer review in scientific publication.

2004 & 2005

- Students choose two of the most similar taxa
- Bergey's Manual of Determinative Bacteriology does not allow one to distinguish among Bacillus species. Avoid obvious Bacillus

2006 Lessons Learned
- Growth of initial sediment sample dilution plates at 22°C or 37°C yield different types of organisms that can still grow at 30°C.
- Bacteroidetes, β-proteobacteria, β-proteobacteria & pseudomonadales are favored at 25°C.
- The NCBI databases contain many sequences for organisms whose names have not been "officially" published. Use Ribosomal Database Project (Cole et al., 2007) to identify Type strain with most similar sequence. Then use BLAST2 (NCBI) to determine percent identity.

2004-2005 Lessons Learned
- Students should choose the same type of colonies, resulting in a poor diversity of unknown organisms.
- Instructor should select colonies from initial dilutions.
- Bergey's Manual of Determinative Bacteriology does not allow one to distinguish among Bacillus species. Avoid obvious Bacillus

Methods (2007)
- PCR with universal primers for 16S rDNA (27F & 1492R)
- Get purified PCR products sent to Genway Biosciences Inc.
- Sequencing libraries: 384 wells, 100 ng DNA per well
- Get primers for sequencing.
- Colony Morphology
- Staining & Microscopy
- Antibiotic Sensitivity
- Carbohydrate Metabolism
- Nitrogen fixation/Acid Metabolism
- O2 & Temp. Requirements
- Exoenzyme Production
- Nucleotide Substitutions (x100)

2007 Lessons Learned
- Universal rRNA Primers 27F & 1492R (Lane, 1991) work as well as rRNA1 (∼356f) & rRNA2 (785r) (Newman, 2000) and yield the nearly full length rRNA gene PCR product.

Table 1. Unknown Organisms Identified by the Spring 2004 & 2005 Microbiology Class

Table 2. Unknown Organisms Identified by the Spring 2006 Microbiology Class

Table 3. Unknown Organisms Identified by the Spring 2007 Microbiology Class

Diversity Summary

<table>
<thead>
<tr>
<th>Year</th>
<th>Actinobacteria</th>
<th>Alphaproteobacteria</th>
<th>Betaproteobacteria</th>
<th>Firmicutes</th>
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</thead>
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<tr>
<td>2004</td>
<td>91</td>
<td>23</td>
<td>8</td>
<td>7</td>
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<tr>
<td>2004-2007</td>
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<td>79</td>
<td>33</td>
<td>21</td>
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Literature references: