Creek Sediments as a Rich Source of BioSafety Level-1 Organisms for Use as Unknowns in an Undergraduate Microbiology Laboratory.

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Abstract

Most general Microbiology courses include some type of project in which students must identify an organism that is unknown to them. Often, the identity of students' "unknowns" are known by the instructor, putting pressure on the student to get the right answer and making the activity more like a test than investigative science. In this study, microbes were obtained from creek sediments and used as unknowns in a Microbiology course to test the hypothesis that this real research experience will engage students and increase the likelihood that they will continue in Microbiology.

Samples were obtained from a variety of sites along the Loyalsock Creek in PA and spread on TSA plates. Isolated colonies were then purified and used as unknown organisms in a sophomore-junior level Microbiology course. During the eight weeks of unknown microbe characterization, students performed microscopic analysis, biochemical, physiological and antibiotic sensitivity tests and 16S rRNA gene sequence analysis to identify their organisms. During 2004 and 2005, when students themselves chose colonies from the spread plates, many duplicate unknowns were obtained. Only 23 different species were identified by the 91 students. Nearly 60% of students had members of the genus *Bacillus* and another 25% had one of four *Serratia* species. In 2006 and 2007, morphologically distinguishable colonies were chosen from spread plates and streaked by the instructor to maximize the diversity of unknowns. Of the 89 unknown organisms identified in 2006 and 2007, 66 different species were obtained, from 30 genera and 20 families. Nine of these were novel species based on having <97% sequence identity to type strains, and only 5 were known BSL-2 organisms.

Student learning was assessed using research style lab reports sent to two other class members for anonymous peer-review and revised before submission for grading. Course evaluation surveys have been very positive. Several students have chosen to follow-up on the novel organisms as part of Independent Studies, Honor's Projects, or the Cell & Molecular Biology Research Methods course and will present their work at the 2007 ASM General Meeting.

Unknown Microbe Identification Experiment

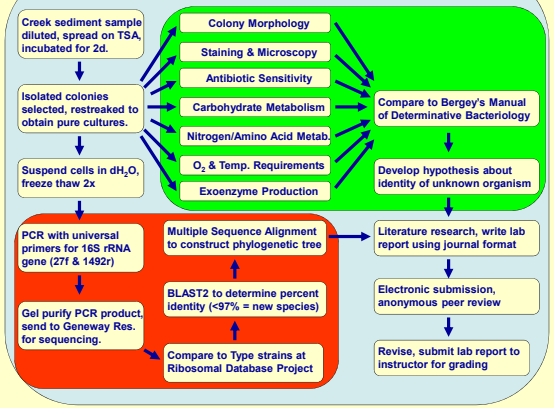
Project Objectives:

- 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

Methods (2007)



2004 & 2005

Table 1. Unknown Organisms Identified by the Spring, '04 & '05 Microbiology Classes

isolates 2004	isolates 2005	BSL	species	16S rRNA % ID
	1	-	Arthrobacter nitroguaiacolicus	98
	3	1	Flexibacteriaceae gen nov. sp. nov.	~91
	1	-	Bacillus aquamarinus	97.5
	2	-	Bacillus arvi	99.5-99.8
	4	1	Bacillus flexus	97.5-99.3
2		1	Bacillus licheniformis	99.5-99.8
1	1	-	Bacillus muralis	99-100
12	11	1	Bacillus mycoides	97.2-100
1	3	-	Bacillus odysseyi	98.3-99.0
6	2	1	Bacillus pumilus	99.3-100
5		1	Bacillus subtilis	98.8-100
1		-	Bacillus sylvestris	98.8
	1	1	Paenibacillus amyloliyticus	99.5
1		1,2	Staphylococcus epidermidis	100
1		2	Staphylococcus haemolyticus	99.5
4	10	1	Serratia fonticola	97.5-100
3		1	Serratia grimesii/proteomaculans	99.0-99.5
	1	-	Serratia novum	96
5		1	Serratia plymuthica	99.5-100
	5	2	Yersinia intermedia	97.5-99.8
1		-	Pseudomonas koreensis	99.5
3		-	Pseudomonas libanensis	98.5
1		-	Pseudomonas mediterranea	99

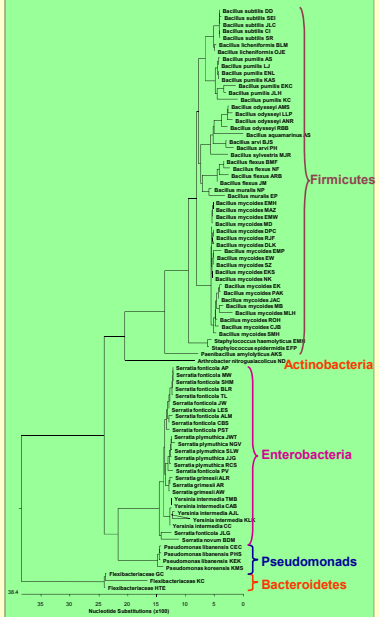


Figure 1. Phylogenetic tree resulting from Clustal W alignment of 2004 & 2005 unknown microbe 16S rRNA gene sequences.

2004-2005 Lessons Learned

- Students tend to choose the same types of colonies, resulting in 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 Bacilli
- High [salt] is worse than low [DNA] for sequencing. Don't concentrate DNA by drying.
- International Journal of Systematic and Evolutionary Microbiology (IJSEM) does not make free full text available until 2 years after publication! Ask library to purchase subscription.

2006

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

BSL	species	16S rRNA % ID	BSL	species	16S rRNA % ID
-	Corynebacterium sp. nov.	94	1	Ochrobactrum tritici	99.5
-	Gordonia amicalis	98.5	1	Comamonas kerstenii	98.5
1	Arthrobacter oxydans	99	1	Janthinobacterium lividum	98
-	Arthrobacter rhombi	99	-	Chromobacterium sp. nov.	96
1	Microbacterium flavescens	98	2	Aeromonas hydrophila	98.5
1	Sanguibacter keddieii	100	1	Buttiauxella agrestis	99
1	Streptomyces avermitis	98.5	1	Buttiauxella izardii	99.3
-	Chryseobacterium sp. nov	94	1	Enterobacter asburiae	99
1	Flavobacterium hibernum	98	1	Serratia fonticola	99
-	Flavobacterium sp. nov.	96.3	1,2	Acinetobacter calcoaceticus	97.6
2	Sphingobacterium faecium	98.8	-	Acinetobacter sp. nov.	95
-	Bacillus arvi	99.3	1	Pseudomonas fragi	99.1
1	Bacillus flexus	99	-	Pseudomonas frederiksbergensis	98.8
1	Bacillus licheniformis	97.5	1	Pseudomonas graminis	98.5
1	Bacillus pumilus	100	-	Pseudomonas kilonensis	99
1	Exiguobacterium oxidotolerans	98.8	-	Pseudomonas libanensis	99
1	Paenibacillus cookii	98	-	Pseudomonas proteolytica	98.5
1	Staphylococcus caprae	99.8	-	Pseudomonas sp.	bad
1	Carnobacterium viridans	98.5	1	Pseudomonas syringae	bad

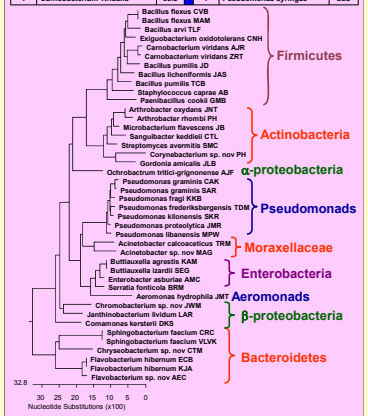


Figure 2. Phylogenetic tree resulting from Clustal W alignment of 2006 unknown microbe 16S rRNA gene sequences.

2006 Lessons Learned

- Growth of initial sediment sample dilution plates at 22ºC or 37ºC yield different types of organisms that can still grow at 30ºC.
  - Bacteroidetes, α-proteobacteria, β-proteobacteria & pseudomonadales are favored at 22ºC.
- 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.

2007

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

BSL	species	16S rRNA % ID	BSL	species	16S rRNA % ID
-	Arthrobacter bergeri	99	1	Janthinobacterium lividum	>99
-	Arthrobacter koreensis	>97	1	Aeromonas salmonicida	99
-	Arthrobacter nitroguaiacolicus	98.5	1	Aeromonas sobria	100
-	Arthrobacter sp.	bad	-	Aeromonas sp.	bad
1	Curtobacterium citreum	98	1	Shewanella putrefaciens	99
1	Microbacterium esteraromaticum	>97	1	Enterobacter asburiae	>97
1	Oerskovia turbata	99	1	Hafnia alvei	>98
1	Flavobacterium hibernum	98	1,2	Kluyvera cryocrescens	98
-	Flavobacterium sp. nov.	96	1	Pantoea agglomerans	98
-	Flavobacterium sp. nov.	95	1	Pantoea ananatis	bad
-	Sejongia sp. nov.	95	1	Pseudomonas veronii	100
1	Bacillus flexus	>97	1	Pseudomonas fulva	>99
-	Bacillus muralis	>98	1	Pseudomonas jessenii	>97
-	Bacillus sp. nov.	96	-	Pseudomonas libanensis	>98
1	Bacillus pumilis	98.5	-	Pseudomonas proteolytica	98
1	Bacillus subtilis	>97	-	Pseudomonas umsongensis	97
-	Exiguobacterium undae	97			
1	Paenibacillus amyloliyticus	98			
2	Staphylococcus pasteuri	99			

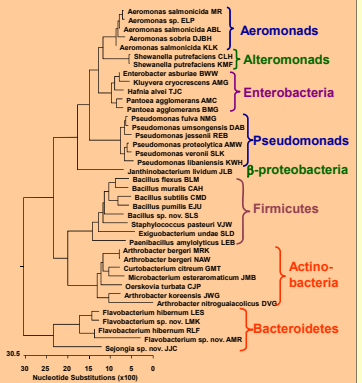


Figure 3. Phylogenetic tree resulting from Clustal W alignment of 2007 unknown microbe 16S rRNA gene sequences.

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.

Diversity Summary

	isolates	species	genera	families	orders	phyla
2004	47	15	4	4	3	2
2005	44	13	6	5	4	4
2004+2005	91	23	8	7	5	4
2006	45	38	24	19	11	4
2007	44	36	18	11	8	4
2006+2007	89	66	30	20	12	4
2004-2007	180	79	33	21	12	4

Outcomes

- Novel organisms attract students to research.
  - 1 Independent study – Fall, 2005
  - 3 Research Methods Projects – Fall, 2006
  - 1 Honors Project – Spring, 2007
  - 3 Research Methods Projects – Fall, 2007
  - 1 ASM Poster #R-049 by Kellie Cicconi.
- Methods have been applied to other projects
  - Evaluation of biofilm community in a wastewater treatment sequencing batch reactor – ASM Poster #Q-079 by Brittane Miller
- Bacterial Strain Collection has expanded dramatically.
- Raw materials for study in other courses - Fatty acid compositions are determined by Biochemistry class.

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