The Effect of Hands-On Experience with Microbial Identification Technologies on Student Understanding of These Methodologies.

Jeffrey D. Newman, Lycoming College, Williamsport PA 17701

Abstract

For several years, as part of our environmental unknown microbe identification laboratory exercise, the lab report instructions have included the following prompt in the background section,

"What are the different ways that one can identify microbes (e.g. API tests, Biolog, MIDI/FAME, 16S rRNA)? What is the relative value of each, advantages, disadvantages. (you will need to research this!!)"

Recent grant funding has allowed us to purchase Biolog and MIDI/FAME instruments, and these technologies have been incorporated into our Spring 2010 Microbiology (Bio321W) course being taken by 26 mostly sophomore and junior level students. It is hypothesized that hands-on experience with these techniques will improve student understanding and ability to effectively describe the techniques in their lab reports. This improvement will be measured using a detailed rubric to score the level of understanding demonstrated in student lab reports and comparing scores from 2009 (before incorporation) and 2010 (after incorporation). The description of API tests will serve as a negative control, because we still do not use these tests. The description of 16S rRNA sequencing, which was used in both years, will serve as a positive control. The description of Biolog and MIDI/FAME are the experimental. It is expected that there will be a significant increase in the quality of the technique descriptions and the number of related concepts that are articulated in the lab reports.

Figure 1. Promotional Brochure for Biolog GenIII Metabolic Characterization /Microbial Identification Technology

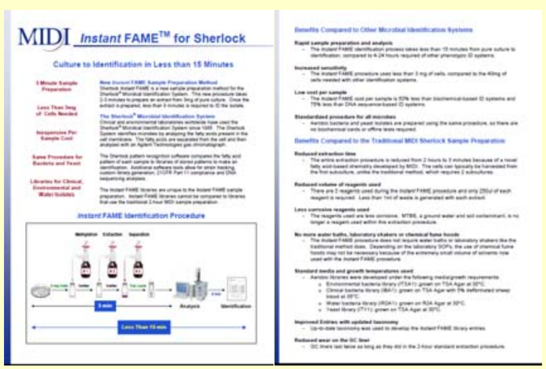
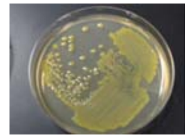


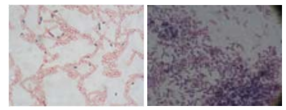
Figure 2. Promotional Literature for MIDI Inc.'s Sherlock Microbial Identification System based on Gas Chromatographic Fatty Acid Methyl Ester Analysis

Figure 3. The LycoMicro Unknown Microbe Lab

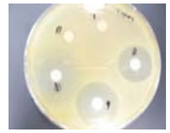
Week 1 - Aseptic technique/Inoculation
- pipetting sterile media
- selection of knowns and unknowns
- streak plates, inoculation of liquid
- preparation of frozen permanents



Week 2 – Staining & Microscopy
- Gram stain
- Endospore stain
- Wet mount



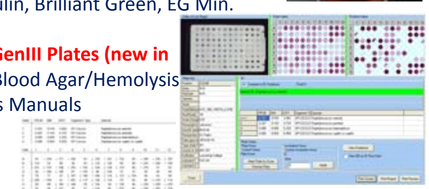
Week 3 – Antibiotic sensitivity, pH, [NaCl], Temperature, Oxygen requirements
- Kirby-Bauer Disk Diffusion assay (10)
- oxidase, catalase, Gas-Pak Jar



Week 4 – Carbohydrate & Nitrogen Metabolism
- MRVP, citrate, phenol red, TSI
- urease, nitrate reduction, SIM, decarboxylases

Week 5 – Exoenzymes, Differential/Selective media
- caseinase, lipase, amylase, DNase,
- EMB, HEA, MSA, PhenylEthanol,
- Bile Esculin, Brilliant Green, EG Min.

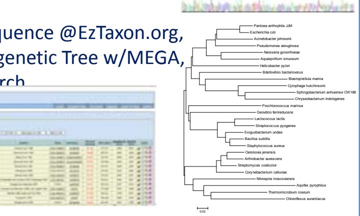
Week 6 – **Biolog GenIII Plates (new in 2010),** Blood Agar/Hemolysis Bergey's Manuals



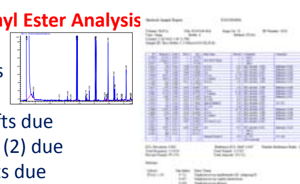
Week 7 – PCR of 16S rDNA, gel, send out for sequencing ($2.50/rxn)

Spring Break!

Week 8 – Analyze DNA sequence @EzTaxon.org, Construct Phylogenetic Tree w/MEGA, Literature Research



Week 9 – **MIDI Fatty Acid Methyl Ester Analysis (FAME)(new in 2010)** Repeat other tests as necessary



Week 11 (Fri) – Lab Report Drafts due
Week 13 (Mon) – Peer Reviews (2) due
Week 14 (Fri) – Final Lab reports due

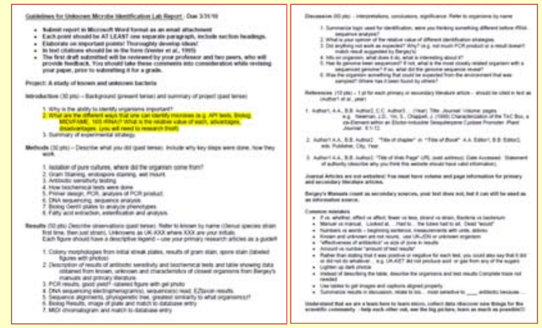


Figure 4. Lab Report Guidelines Provided to Students

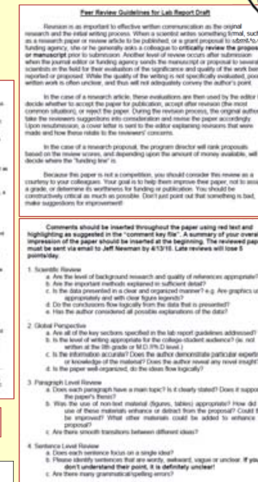


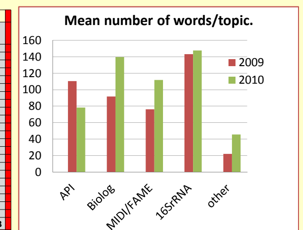
Figure 5. Peer Review Guidelines Provided to Students

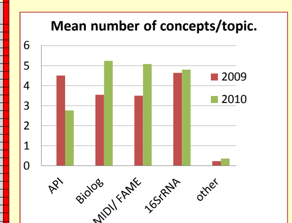
API test	Biolog	MIDI/FAME	16S rRNA
P = Biochemical Phenotypes	P = Biochemical Phenotypes	P = Biochemical Phenotypes	G=phylogenetic
S = 20 tests in strips;	W = 96 well plates	F = Fatty Acids	S = Sequence based
M = Method details – types of tests	M = Method details – color change during growth	M = Method details - GC	M = Method details – PCR, primers
DB = Database	DB = Database	DB = Database	DB = Database
U = Uses/Applications	U = Uses/Applications	U = Uses/Applications	U = Uses/Applications
A= advantages	A= advantages	A= advantages	A= advantages
D=disadvantages	D=disadvantages	D=disadvantages	D=disadvantages

Figure 6. Conceptual Understanding Rubric

	Number of words						Conceptual Understanding Rubric									
Student Initials, year	API	Biolog	MIDI/ FAME	16SrRNA	other	total	API		Biolog		MIDI/FAME		16SrRNA		other	
RDR 2009	86	109	191	137	402	925	P,DB	2	P,W,DB,U,A	5	P,F,DB,A,D,M,U	7	G,S,M,DB,A,D	6	D,R,MLEE,PFGE	4
KAS 2009	95	103	62	138	0	398	P,M,S,A,D,DB	6	W,A,D,DB	4	D,A,DB	3	G,A,M,D	4		0
KET 2009	60	57	62	82	0	261	S,M	2	P,W	2	M,A,DB	3	S,U,M,A	4		0
ANA 2009	98	52	46	45	0	241	S,A,M,DB	4	P	1	F,A,M,DB	4	G	1		0
SSX 2009	64	133	49	123	0	369	S,P,A,D,M,DB	6	P,W,M,U,A,D,DB	7	F,M,A,D	4	G,S,M,U,A,D,DB	7		0
TMG 2009	141	47	64	139	0	391	S,M,A,D,U	5	P,A,D	3	F,M,A,D	4	G,S,M,U,A,D,DB	7		0
MAC 2009	166	93	81	103	0	443	S,P,M,A,D	5	P,W,M,U,A,D,DB	7	F,M,A,D,DB	5	S,M,A,DB	4		0
KMH 2009	169	157	160	332	0	818	S,P,M,U,A,D,DB	7	P,W,A,D,DB	4	A,D,DB	3	G,S,M,U,A,D,DB	7		0
JAC 2009	209	102	79	181	0	571	S,P,M,U,A,D,DB	7	W,M,DB,D	4	P,F,DB	3	S,M,U,A,D,DB	6		0
BAR 2009	135	118	139	232	0	624	S,P,A,D,DB	5	P,W,A,D	4	P,F,DB,A,D,M,U	7	G,S,M,U,A,D,DB	7		0
JJY 2009	135	220	195	240	0	790	S,P,D,DB	4	P,W,M,A,D	5	P,F,DB,A,D,M	6	S,M,U,A,D,DB	6		0
KMH2 2009	101	108	87	106	0	402	S,P,A,D,DB	5	P,W,A,D,DB	5	F,DB,A,D,M,U	6	G,S,M,A,D,DB	6		0
AMF 2009	99	112	0	290	0	501	S,P,M,U,A	5	P,W,M,A,D,DB	6		0	S,M,DB	3		0
MAT 2009	85	73	88	36	0	282	S,P,M,DB	4	P,U,A,DB	4	F,DB,A,D	4	M,A	2		0
AMW 2009	112	129	63	93	0	397	S,M,A,D	4	P,U,A,DB	4	F,A,M,U	4	G,S,M,A,D,DB	6		0
WFS 2009	173	93	0	140	0	406	S,P,U,A,D	5	P,W,U,	3		0	S,M,A,D,DB	5		0
ANP 2009	0	0	0	416	0	416		0		0		0	G,S,M,U,A,D,DB	7		0
KGD 2009	112	109	112	109	0	442	S,M,A,D	4	P,D	2	F,DB,A,D	4	S,DB,U	3		0
JMJ 2009	90	82	56	105	0	333	S,P,U,A,D	5	P,W,A,D	4	F,A,M	3	G,S,U,A,D	5		0
BAM 2009	116	0	86	43	86	331	S,P,M,U,A,DB	6		0	F,M,U	3	G,S	2	R	1
RGR 2009	103	71	31	57	0	262	S,P,M,U	4	U,DB	2	F	1	S,M,U,DB	4		0
AMT 2009	79	49	26	0	0	154	S,P,M,U	4	P,W	2	F,DB,M	3		0		0
mean	110.4	91.7	76.2	143.0	22.2	443.5		4.50		3.55		3.50		4.64		0.23
	Number of words						Conceptual Understanding Rubric									
Student Initials, year	API	Biolog	MIDI/ FAME	16SrRNA	other	total	API		Biolog		MIDI/FAME		16SrRNA		other	
KMH 2010	160	163	200	283	0	806	S,P,M,U,A,D,DB	7	P,W,M,U,A,D,DB	7	P,F,DB,A,D,M,U	7	G,S,M,U,A,D,DB	7		0
JJM 2010	64	174	110	210	0	558	S,P,M,U,A,D,DB	7	P,W,M,U,A,D,DB	7	F,DB,A,D,M,U	6	G,S,M,U,A,D,DB	7		0
DNM 2010	176	208	52	208	149	793	S,P,M,U,A,DB	6	P,W,M,U,A,D,DB	7	F,M	2	G,S,M,U,A,D,DB	7	B	1
CAH 2010	52	65	31	102	68	318	S,P	2	D,DB	2	F	1	S,M	2	B	1
DJV 2010	201	322	232	328	0	1083	U,A,D	3	U,A,D,DB	4	P,F,DB,A,D,M,U	7	G,S,M,U,A,D,DB	7		0
ERS 2010	160	130	102	147	0	539	P,U,A,D	4	P,W,M,U,A,D,DB	7	P,F,DB,A,D,M,U	7	G,S,U,A,D,DB	6	microscopy	1
ANN 2010	90	149	114	147	86	586	S,M,A	3	P,W,M,U,A,D,DB	7	P,F,DB,A,D,M,U	7	G,S,U,A,D,DB	6		0
JMW 2010	80	110	87	87	0	364	S,D,DB	3	P,W,A,D,DB	5	P,F,DB,A,D,M	6	G,S,M,A,D	5		0
TML 2010	0	122	44	98	90	354		0	P,W,M,A,D,DB	6	F,DB,A,D	4	G,S,M,A,D,DB	6		0
JLW 2010	0	180	233	259	102	774		0	P,W,M,U,A,D,DB	7	P,F,DB,A,D,M,U	7	G,S,M,U,A,D,DB	7	B	1
RJD 2010	46	151	51	107	0	355	S,P,DB	3	P,W,M,D,DB	5	F,DB,A	3	S,M	2		0
DFL 2010	94	84	124	108	0	410	S,P,M,A,D	5	M,U,A,D,DB	5	P,F,DB,A,D,M,U	7	G,S,U,A,D	5		0
KCF 2010	81	76	100	75	126	458	S,P,M	3	P,W,DB	3	F,DB,M	3	S,DB	2	genome, MLST	2
AMD 2010	0	113	54	120	0	287		0	P,W,M	3	F	1	S,M,A,D	4		0
BMM 2010	32	31	31	0	0	94	A,D	2	P,A,D,DB	4	F,DB,A,D	4		0		0
LMB 2010	0	286	224	224	177	911		0	P,W,M,U,A,D,DB	7	P,F,DB,A,D,U	6	S,M,U,A,D,DB	6	B	1
TDH 2010	0	136	174	191	0	501		0	P,W,M,U,A,D,DB	7	P,F,DB,A,D,M,U	7	G,S,M,U,A,D,DB	7		0
KEC 2010	305	159	199	205	0	868	S,P,M,U,A,D,DB	7	P,W,M,U,A,D,DB	7	P,F,DB,A,D,M,U	7	G,S,M,U,A,D,DB	7		0
AMS 2010	83	108	67	73	0	331	S,P,M,U	4	P,W,M,A,D,DB	6	F,DB,A,D,M	5	S,M,DB	3		0
GMS 2010	0	117	255	161	0	533		0	P,W,U,A,D,DB	6	P,F,DB,A,D,M,U	7	G,S,M,A,D,DB	6		0
BAB 2010	0	280	117	0	0	397		0	P,W,M,U,A,D,DB	7	P,F,DB,A,D,U	6		0		0
KLH 2010	213	184	86	165	138	786	S,P,M,U,A,D,DB	7	P,W,M,U,A,D,DB	7	P,F,DB,A,D,M	6	G,S,M,A,D,DB	6	B	1
SCW 2010	0	79	52	0	0	131		0	U,A	2	F,DB,D,M	4		0		0
ELP 2010	119	62	56	86	0	323	A,D,DB	3	A,D,DB	3	DB,A,D	7	S,M,U,A,D	5		0
ART 2010	0	0	0	305	205	510		0		0		0	G,S,M,U,A,D,DB	7	B	1
mean	78.2	139.6	111.8	147.6	45.6	522.8		2.76		5.24		5.08		4.80		0.4

Table 1. Assessment scores of Individual Lab Reports





Conclusions:

1. **Hands on experience with Biolog and MIDI/FAME Microbial Identification Technologies did increase student understanding of these methods.**
2. **Increases work on new techniques decreases student effort in researching API tests.**

Acknowledgements

The Biolog and MIDI/FAME instruments were purchased with funding from NSF award DBI-0960114 to JDN, **"MRI-R2: Acquisition of Instrumentation for Novel Microbe Characterization by Undergraduate Researchers."**