Microbiology Experiment – Biolog GEN III plates

Background

Over the past 6 weeks, we have examined many our organism's traits such as Gram stain reaction, carbon metabolism, nitrogen metabolism, the production of exoenzymes, inhibition by antibiotics, temperature, oxygen requirements and now are beginning to use Bergey's Manual of Determinative Bacteriology to identify our unknown organism. While this approach has been useful to learn about our organism and principles of microbiology, it would take far too long if decisions about a patient's diagnosis and medical treatment were dependent on this information. In clinical microbiology, more rapid tests are done to provide an identification as quickly as possible. These tests include API test strips, Enterotubes, Vitek "cards", and finally, Biolog GEN III plates, which we will use here. The principle by which these methods work is that a variety of tests are conducted simultaneously, in a convenient, standardized format (that can be distributed commercially), and the results are compared to a database to identify the organism.

The Biolog GEN III test plate contains different nutrients or inhibitory substances dried into the bottom of each well of a 96 well plate (Figure 1). Cells grown on a plate of Biolog Universal Growth medium + 5% sheep's blood (BUG+Blood) are suspended in an inoculating fluid at a specific cell density and added to the wells. The plates are incubated at the desired temperature in the OmniLog instrument. The inoculating fluid contains a redox dye that turns purple if the organism is actively metabolizing in the well. In columns 1-9, the carbon source is varied (A1 is the negative control) so that if the organism is able to utilize the particular compound present, a purple color will result. In columns 10-12, a carbon source is available in all wells, as well as specific potentially inhibitory substances (A10 is the positive control and lacks inhibitory substances). Inhibition of growth will decrease the intensity of purple color that will develop in the well. The response in each well is determined by scanning the plate every 15 minutes, on the 15 minute mark. The pattern of respiration is compared to a database to identify the organism. We will use the "Full Data Logger" option in order to collect data over a 36 hr period.

	1	2	3	4	5	6	7	8	9	10	11	12
A	negative control	**Dextrin** amylose glu-α1-4, α1-6 poly	**D-maltose** glu-α1-4-glu	**D-trehalose** glu-α1-α1-glu	**D-cellobiose** glu-β1-4-glu	**gentiobiose** glu-β1-6-glu	**Sucrose** glu-α1-β2-fru	**D-turanose** glu-α1-α3-fru	**Stachyose** gal(α1→6) gal(α1→6) glc(α1↔2β) fru	positive control	pH 6	pH 5
B	**D-raffinose** gal-α1-6-glu-α1-β2-fru	**α-D-lactose** gal-β1-4-glu (α)	**D-melibiose** gal-α1-6-glu	β-methyl-D-glucoside	**D-salicin** aspirin-β1-glu	N-acetyl-D-glucos-amine	N-acetyl-β-D-mannos-amine	N-acetyl-D-galactos-amine	N-acetyl neuraminic acid	1% NaCl	4% NaCl	8% NaCl
C	α-D-glucose	D-mannose	D-fructose	D-galactose	3-methyl glucose	D-fucose	L-fucose	L-rhamnose	inosine	1% Na-lactate	fusidic acid	D-serine
D	D-sorbitol	D-mannitol	D-arabitol	myo-inositol	glycerol	D-glucose-6-PO4	D-fructose-6-PO4	D-aspartic acid	D-serine	Troleando-mycin	rifamycinSV	minocycline
E	gelatin	glycyl-L-proline	L-alanine	L-arginine	L-aspartic acid	L-glutamic acid	L-histidine	L-pyro-glutamic acid	L-serine	lincomycin	guanidine HCl	niaproof 4
F	pectin	D-galact-uronic acid	L-galact-uronic acid lactone	D-gluconic acid	D-glucuronic acid	Glucuron-amide	mucic acid	quinic acid	D-saccharic acid	vancomycin	tetrazolium violet	tetrazolium blue
G	p-hydroxy-phenyl-acetic acid	methyl pyruvate	D-lactic acid methyl ester	L-lactic acid	citric acid	α-keto-glutaric acid	D-malic acid	L-malic acid	bromo-succinic acid	nalidixic acid	LiCl	K-tellurite
H	tween-40	γ-amino-butyric acid	α-hydroxy-butyric acid	β-hydroxy-D,L-butyric acid	α-keto-butyric acid	acetoacetic acid	propionic acid	acetic acid	formic acid	aztreonam	Na-butyrate	Na bromate

Procedure

Day 1 - Obtain a Biolog Universal Growth + Blood (BUG+B) plate. Divide and label the plate for you and your lab partner, then inoculate your unknown organisms onto half the plate, streaking for single colonies. Place the plates onto the front bench and they will be stored at 4C, then placed in the incubator on the day before the GEN III plates are to be set up. Sign up on the front bench for a time to come with with your partner to set up the Biolog plate.

Day 2 - At your scheduled time, retrieve your BUG+B streak plate from the incubator and proceed to the labeling station.

A. Labeling Station - Carefully unwrap a GEN III plate from the packaging. Be sure not to touch the bottom of the plate below the wells. Use a dark, extra-fine point Sharpie to label the GEN III plate on the short side of the plate, opposite the Biolog Logo (label the plate, not the lid). Label the plate with the designation UK-XXX for the unknown. Proceed to the inoculation station!

B. Inoculation Station

1. Zero the Turbidimeter - Wipe the sides of an inoculating fluid (IF-A) tube with a Kimwipe and place it into the turbidimeter. Use the adjustment dial to set the % transmittance to 100%.

2. Inoculate the Tube of IF-A - Estimate the colony size on the BUG+B culture. Use a sterile "Inoculatorz" swab (does not release cotton fibers) to pick up a single colony if they are large, several medium size colonies or a small amount of confluent growth if colonies are extremely small. Remove the IF-A tube from the turbidimeter and vigorously inoculate the IF-A, being sure that the bottom of the swab contacts the bottom of the IF-A tube to dislodge the bacteria.

3. Check the Turbidity - Place the inoculated IF-A tube back in the Turbidimeter and wait for the % transmittance to stabilize between 90 and 98%T. If the % transmittance is above 98%, reinoculate the tube to add more bacteria. If the % transmittance is below 90%, inoculate a fresh tube of IF-A, using a smaller inoculum.

4. Fill the Pipettor - Pour the IF-A cell suspension into a sterile plastic reservoir. Turn on the multi-channel pipettor by pressing the center button - the display should show "Multi - 100uL". Push the pipettor tip holders onto the pipette tips until it clicks. Hold the pipettor so that the tips can draw from the bottom of the reservoir, then click the top button to draw up a sufficient amount of sample for the entire GEN III plate.

5. Fill the GEN III Plate - Lift the lid from the GEN III plate, align the tips to the 8 rows of column 1 such that the tips are contacting the far wall of the well (this will prevent the formation of bubbles). Press the dispense button to dispense 100 uL from each tip, then move the pipettor to align the tips with column 2 and dispense. After dispensing into column 12, the pipettor should beep twice to indicate that the plate is finished. Place the tips over the reservoir and press the dispense button again to "purge" the

tips. Then press the eject button to eject the tips into the autoclave bag. *If you missed a well or two when pipetting, use a standard single channel pipettor to inoculate the missed wells with 100 ul of the IF-A cell suspension.* Replace the lid on the plate. Dispose of the reservoir and swab in the autoclave bag. Then proceed to the data entry station!

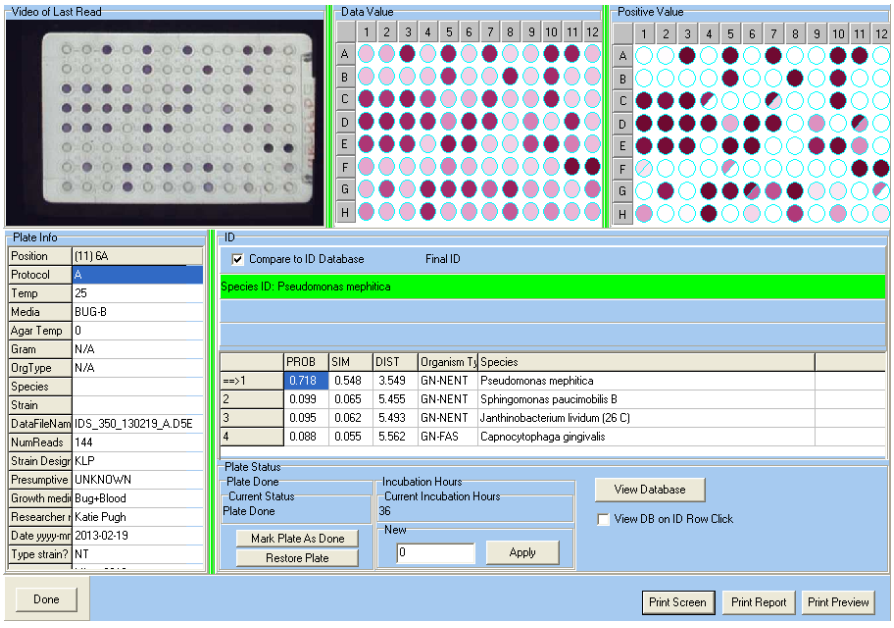
C. Data entry station using the OmniLog Data Collection Software

1. Choose the lowest stack # and position available (not locked). Enter the data requested for each field in the right-side panel
   - Strain Designation = XXX (your initials)
   - Presumptive species = unknown
   - Growth medium = BUG+Blood
   - Researcher Name = your name
   - Date = today's date (e.g. 2013-02-16)
   - Type strain = NT
   - Course or research = Bio321
   - Institution = Lycoming College
   - Comment 1 = UK-XXX
   - Comment 2 = 25C
   - Click "Save and Next"

2. Place the plate onto a tray with your lab partner, place the tray into the appropriate slot in the OmniLog.

D. Analysis of Biolog Results

1. Download the pdf files corresponding to the “print screen” (UK-XXX PS.pdf) and report (UK-XXX report.pdf) options on the OmnilLog software.



2. Use the Print screen document to address the following questions regarding the **IDENTIFICATION** in your lab report.

   a) Was a positive identification made?

   b) What was the best match?

   c) How does this compare to the FAME and 16S-based identifications?

   d) Are any of the other Biolog matches the same as your 16S sequence?

   e) Examine your 16S rRNA EZtaxon.org results to determine how similar your sequence was to the Biolog best-match organism.

   f) Examine the Biolog Database list to determine if the 16S rRNA best match organism is in the Biolog GEN III database.

3. Go to the class website and open the "Class Biolog Data" excel file. This file contains the exported data normalized to a range of 1-100. Copy the data from the column corresponding to your unknown organism to the appropriate location on the known and unknown microbe data sheet (see next page). For our purposes, a value below 25 is considered negative (-), 25-50 is weak positive (w), while a value above 50 is positive (+). Address the following questions regarding the **METABOLIC PROFILE** in your lab report.

   a) Did the negative control (A1) give you a negative result? If not, what does this mean? What is a possible explanation? How does this affect your interpretation of the remaining results for columns 1-9?

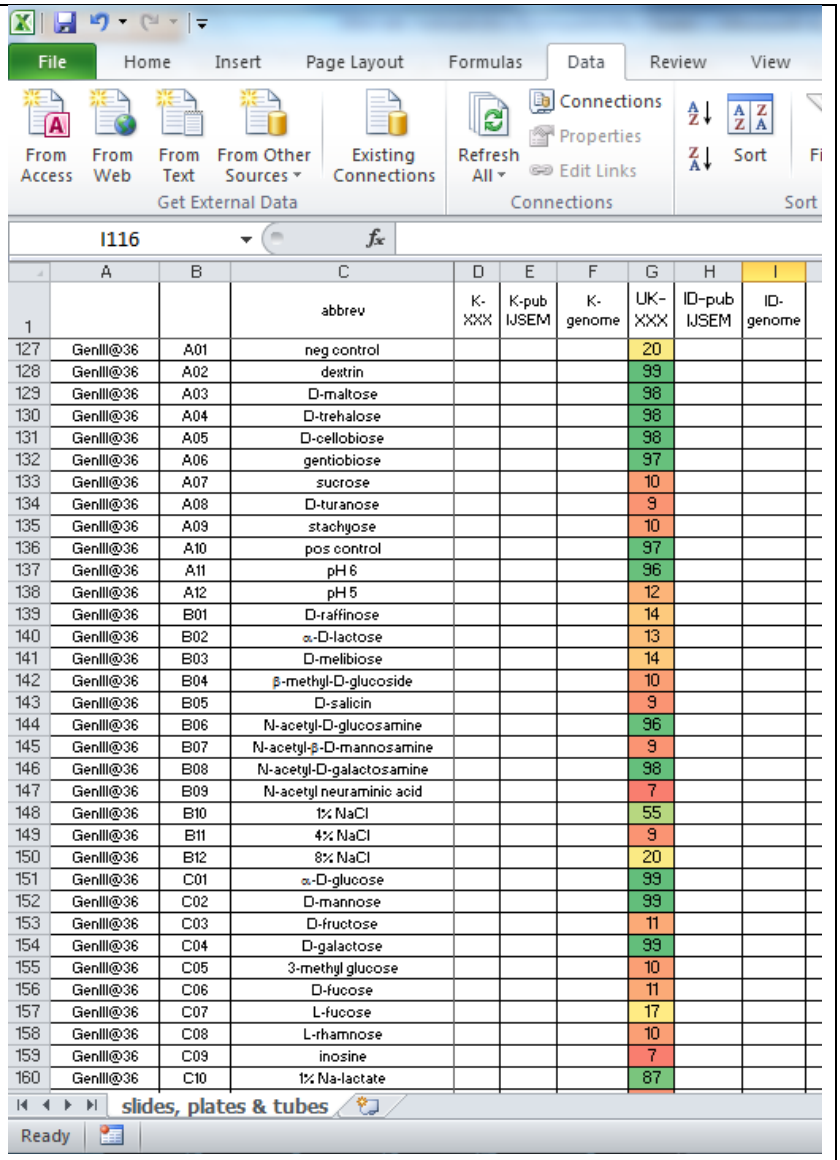
   b) Did the positive control (A10) give you a positive result? If not, what does this mean? What is a possible explanation? How does this affect your interpretation of the remaining results for columns 10-12?

   c) Which wells correspond to other tests we've done (e.g. phenol red fermentation, Kirby-Bauer, exoenzymes)? Are the results consistent? How do wells that do correspond to other tests differ… i.e. what is being measured in each?

   d) How do the results compare to those in Bergey's Manual or the IJSEM paper(s) corresponding to the species identified by 16S rRNA sequencing?

   e) How do the results compare to gene content of the most closely related genome in DOE-JGI-IMG or RAST.

	Strain	ABG	AED	AHK	AKS	ANG	ARC	BMS
A01	neg control	20	9	78	84	15	10	13
A02	dextrin	99	98	97	98	97	14	95
A03	D-maltose	98	98	96	98	98	6	97
A04	D-trehalose	98	98	94	97	97	20	97
A05	D-cellobiose	98	98	95	93	98	7	98
A06	gentiobiose	97	98	92	95	99	4	98
A07	sucrose	10	83	90	91	98	6	98
A08	D-turanose	9	99	80	81	98	5	98
A09	stachyose	10	68	83	72	7	7	17
A10	pos control	97	59	82	84	97	91	95
A11	pH 6	96	29	85	82	95	92	94
A12	pH 5	12	1	67	77	82	95	81
B01	D-raffinose	14	83	98	97	7	7	10
B02	α-D-lactose	13	99	70	84	25	5	95
B03	D-melibiose	14	99	92	93	38	3	92
B04	β-methyl-D-glucoside	10	97	95	95	78	6	95
B05	D-salicin	9	62	86	86	97	3	95
B06	N-acetyl-D-glucosamine	96	99	89	86	89	10	96
B07	N-acetyl-β-D-mannosamine	9	29	43	74	9	5	69
B08	N-acetyl-D-galactosamine	98	29	35	56	89	3	90
B09	N-acetyl neuraminic acid	7	99	0	37	2	5	4
B10	1% NaCl	55	7	82	89	95	91	93
B11	4% NaCl	9	6	83	77	58	21	92
B12	8% NaCl	20	6	87	79	18	6	62
C01	α-D-glucose	99	96	94	89	97	79	97
C02	D-mannose	99	98	82	83	94	70	93
C03	D-fructose	11	98	74	88	83	22	92
C04	D-galactose	99	99	96	92	95	84	93
C05	3-methyl glucose	10	15	31	74	43	3	49
C06	D-fucose	11	12	76	70	97	8	31
C07	L-fucose	17	25	43	68	95	6	31
C08	L-rhamnose	10	99	15	51	95	95	33
C09	inosine	7	23	1	51	42	48	81
C10	1% Na-lactate	87	4	77	88	91	95	91
C11	fusidic acid	9	13	4	4	5	92	4
C12	D-serine	10	5	4	4	5	70	4
D01	D-sorbitol	11	17	31	74	7	45	38
D02	D-mannitol	11	20	90	78	91	38	92
D03	D-arabitol	6	19	19	68	8	30	25
D04	myo-inositol	8	17	67	83	5	62	94
D05	glycerol	8	18	76	63	89	42	92
D06	D-glucose-6-PO4	28	46	40	65	25	4	92
D07	D-fructose-6-PO4	36	35	50	61	68	5	92
D08	D-aspartic acid	5	9	53	39	13	4	21
D09	D-serine	5	5	3	0	2	60	1
D10	troleandomycin	9	9	4	4	5	89	4
D11	rifamycin SV	93	11	4	4	5	90	4
D12	minocycline	12	17	4	4	6	60	5
E01	gelatin	97	35	93	88	30	10	21
E02	glycyl-L-proline	95	94	63	49	45	7	28
E03	L-alanine	32	20	50	63	16	60	77
E04	L-arginine	80	97	76	76	13	80	48
E05	L-aspartic acid	96	38	85	81	44	90	82
E06	L-glutamic acid	98	99	86	84	55	97	87
E07	L-histidine	41	29	70	70	3	98	12
E08	L-pyroglutamic acid	14	22	67	63	32	82	22
E09	L-serine	92	10	45	41	13	96	79
E10	lincomycin	91	15	74	71	5	89	4
E11	guanidine HCl	10	11	4	14	67	83	79
E12	niaproof 4	15	3	4	4	5	94	4
F01	pectin	23	46	93	89	77	12	95
F02	D-galacturonic acid	98	32	80	65	64	96	27
F03	L-galacturonic acid lactone	11	13	1	44	2	7	2
F04	D-gluconic acid	17	32	80	81	96	88	95
F05	D-glucuronic acidi	72	99	95	72	44	98	35
F06	glucuronamide	24	30	60	56	41	14	17
F07	mucic acid	15	33	65	49	21	97	15
F08	quinic acid	19	23	67	54	20	100	23
F09	D-saccharic acid	14	30	33	24	2	95	7
F10	vancomycin	95	18	4	4	5	87	4
F11	tetrazolium violet	99	76	4	4	22	98	18
F12	tetrazolium blue	99	49	4	4	19	99	20
G01	p-hydroxy-phenylacetic acid	12	9	11	63	95	12	3
G02	methyl pyruvate	15	18	85	72	3	12	1
G03	D-lactic acid methyl ester	19	24	78	87	11	7	13
G04	L-lactic acid	10	25	80	86	76	77	9
G05	citric acid	31	8	83	84	2	99	97
G06	α-keto-glutaric acid	19	33	76	81	4	36	47
G07	D-malic acid	19	24	65	70	5	7	34
G08	L-malic acid	19	55	85	78	88	94	96
G09	bromo-succinic acid	6	9	45	54	2	14	91
G10	nalidixic acid	13	15	4	4	93	29	86
G11	LiCl	9	4	80	76	93	4	94
G12	K-tellurite	20	9	87	89	95	97	95
H01	tween-40	89	6	92	92	61	20	95
H02	γ-amino-butyric acid	18	17	94	84	8	88	2
H03	α-hydroxy-butyric acid	15	9	93	87	83	12	4
H04	β-hydroxy-D,L-butyric acid	16	11	91	91	10	53	5
H05	α-keto-butyric acid	11	9	74	88	93	15	13
H06	acetoacetic acid	89	12	92	88	95	12	94
H07	propionic acid	9	9	96	86	16	16	55
H08	acetic acid	98	10	86	87	69	78	91
H09	formic acid	10	8	96	86	4	14	3
H10	aztreonam	96	37	6	4	96	86	93
H11	Na-butyrate	14	12	92	92	85	6	88
H12	Na bromate	15	1	21	21	70	12	64



	A	B	C	D	E	F	G	H	I
1			abbrev	K-XXX	K-pub IJSEM	K-genome	UK-XXX	ID-pub IJSEM	ID-genome
127	GenIII@36	A01	neg control				20		
128	GenIII@36	A02	dextrin				99		
129	GenIII@36	A03	D-maltose				98		
130	GenIII@36	A04	D-trehalose				98		
131	GenIII@36	A05	D-cellobiose				98		
132	GenIII@36	A06	gentiobiose				97		
133	GenIII@36	A07	sucrose				10		
134	GenIII@36	A08	D-turanose				9		
135	GenIII@36	A09	stachyose				10		
136	GenIII@36	A10	pos control				97		
137	GenIII@36	A11	pH 6				96		
138	GenIII@36	A12	pH 5				12		
139	GenIII@36	B01	D-raffinose				14		
140	GenIII@36	B02	α-D-lactose				13		
141	GenIII@36	B03	D-melibiose				14		
142	GenIII@36	B04	β-methyl-D-glucoside				10		
143	GenIII@36	B05	D-salicin				9		
144	GenIII@36	B06	N-acetyl-D-glucosamine				96		
145	GenIII@36	B07	N-acetyl-β-D-mannosamine				9		
146	GenIII@36	B08	N-acetyl-D-galactosamine				98		
147	GenIII@36	B09	N-acetyl neuraminic acid				7		
148	GenIII@36	B10	1% NaCl				55		
149	GenIII@36	B11	4% NaCl				9		
150	GenIII@36	B12	8% NaCl				20		
151	GenIII@36	C01	α-D-glucose				99		
152	GenIII@36	C02	D-mannose				99		
153	GenIII@36	C03	D-fructose				11		
154	GenIII@36	C04	D-galactose				99		
155	GenIII@36	C05	3-methyl glucose				10		
156	GenIII@36	C06	D-fucose				11		
157	GenIII@36	C07	L-fucose				17		
158	GenIII@36	C08	L-rhamnose				10		
159	GenIII@36	C09	inosine				7		
160	GenIII@36	C10	1% Na-lactate				87		