

**ASMCUE 2010 Microbrew Session**  
**“Microbial Friends Where New Students Least Expect Them: The Microbiology of Water Bottles.”**  
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With this MicroBrew, there is not much time to present! So I hope that this handout answers some questions and perhaps raises a few more---I find that this exercise is one that new microbiology students enjoy, ponder, and can be taken many places. Please contact me with questions, or to share your own findings. In fact, I have some thoughts on the latter, which I will share at the end of this handout!

- **The goal:** to remind students of the ubiquity of microbes in their everyday lives, using something that most students have in their possession: **their reusable water bottles!** Students observe for themselves that their water bottles possess a variety of interesting organisms, either from streak plates or via molecular analysis. Connections can be drawn to microbiology in everyday life, standard techniques in microbiology, simple bioinformatics, prokaryotic taxonomy, and relationships between biofilm formation, oligotrophy, and so forth. This is a very flexible exercise.
- **The audience:** this activity can be “pitched” on several levels, ranging from very introductory to quite advanced (from simple microbiological techniques and observations of colonial morphology, to bioinformatic and phylogenetic analysis of 16s rRNA data). This depends on the course level and the available resources. I could easily see this exercise explored at a wide variety of institutions.
- **The approach:** have students bring their water bottles to lab. Most students have them; all students know someone with one. Have the students write in their lab notebooks all they know about their water bottles: how often they clean them, in what manner are they cleaned, type of water bottle, etc. Provide the students with sterile swabs and two types of nutrient plates: high nutrient level and low nutrient level (simplest idea: Nutrient Broth and 0.1X or even 0.01X Nutrient Broth plates). The students first swab the plates in the upper quadrant, and then streak the “swabate” as if for single colonies. The plates are parafilm and then incubated at room temperature. The students observe the plates over the next week or so, recording and describing what they see (digital cameras work very well; my inexpensive digital camera has a “macro mode” that provided the images in this handout). For a bioinformatic analysis, a Copan-style sampling swab is used, eDNA isolated, 16s rRNA amplicons generated (using 27F and 1492R “universal” 16s rRNA primers and standard PCR conditions; please contact me if you need advice), and a “mini-library” of those amplicons generated by cloning and transformation (I used the Invitrogen TA cloning kit with good results). Students are given overnight cultures made from individual transformants and plasmid DNA isolated; the DNA is then submitted for sequencing. Both BLAST and RDP “Classifier” programs were used to assign possible identities to the clones.

Some thoughts after trying this out in my microbiology courses over the past two years.

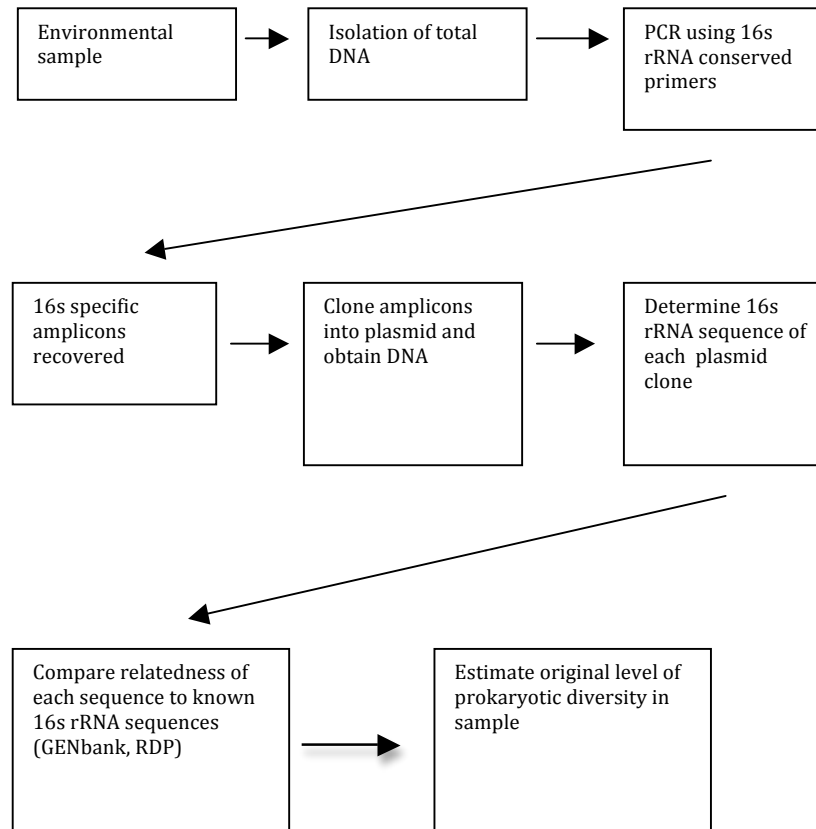
1. Students LOVE this exercise. It both horrifies and fascinates them! I am always surprised by the students who sheepishly admit that they never clean their water bottles---just rinse them (which, when you think about it, is a form of selection!).
2. I don’t think it is likely that horrific pathogens could be isolated, but I parafilm plates and recommend caution to students. It certainly seems little different from some of the high school “swab an environment” exercises I see from Carolina Biological Supply. Still, I recommend caution and oversight.
3. A very nice “portfolio” of students and their plates can be generated (I include some examples at the end of this handout, in color). Students often ask me for a copy of the class portfolio! I also point out to the

students that the vast majority of environmental microbes are not easily cultivable on standard laboratory media: so the rare student who doesn't get much growth on her or his plates should not feel smug!

4. Students find great visible diversity in terms of colonial morphology, "type," and prevalence. Some students did not even find any cultivable microbes from their water bottles (but not this year!).

5. Different nutrient levels seem to reveal different organisms---it isn't as simple as more growth with more nutrients. But a water bottle environment should enrich for oligotrophs? However, the majority of microbes are not cultivable in the laboratory, again. So....

6. This year I sampled two water bottles and carried out the following flow chart to provide students with clones representing the 16s rRNA genes of water bottle denizens (photos at the end of this handout):

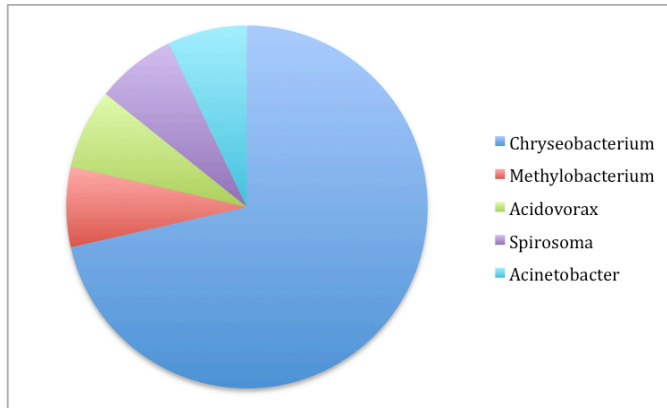


The 16s rRNA data was analyzed by using BLAST (<http://blast.ncbi.nlm.nih.gov/Blast.cgi>, set for the nr/nt nucleotide collection) and the RDP "Classifier" program (<http://rdp.cme.msu.edu/>). I give the students a specific set of examples of how to analyze and think about the use of each program (and the limitations and cautions for each), and how to relate the results to possible identity (I try to focus on ecophysiology as much as possible). I often find that students think that, if 10/20 clones is a particular phylotype, that the result suggests 50% prevalence. Not so, of course! The limitation with this kind of technique is the assumption that all microbes yield the same amount of DNA targets, that each target is equally amplifiable, and that each amplicons is equally clonable. None of these things are necessarily true. This leads to a great discussion of similar "microbial census" journal articles, and how to interpret them.

I should also point out that it is certainly possible to do colony PCR with individual isolates and either clone or directly sequence the amplicons. That is, a possible identity for a given colony could be achievable. I did not do so in this laboratory exercise, instead choosing to focus on a culture-independent approach. The sample size, naturally, is modest, and is budget based. I would love to collaborate with some deep pockets on this kind of thing!

Here is what we found this semester, depicted graphically and then with summaries of the data:

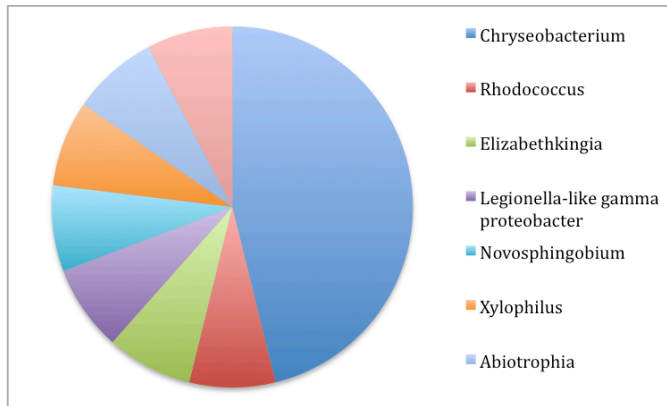
**Franny's water bottle data:**



*Chryseobacterium*: 10/14  
*Methylobacterium*: 1/14  
*Acidovorax*: 1/14  
*Spirosoma*: 1/14  
*Acinetobacter*: 1/14

10/14 Flavobacteria  
 1/14 Alpha proteobacteria  
 1/14 Beta proteobacteria  
 1/14 Gamma proteobacteria  
 1/14 Bacterioidetes

**Hillary's water bottle data:**



*Chryseobacterium*: 6/13  
*Rhodococcus*: 1/13  
*Elizabethkingia*: 1/13  
*Legionella*-like proteobacters: 1/13  
*Novosphingobium*/  
*Sphingomonas*: 2/13  
*Xylophilus*: 1/13  
*Abiotrophia*: 1/13

6/13 Flavobacteria  
 4/13 Alpha proteobacteria  
 1/13 Beta proteobacteria  
 1/13 Gamma proteobacteria  
 1/13 Firmicutes/Bacilli

**Thoughts on what was found in this modest data set:**

- Again, a modest sample size, but interesting!
- Mostly Flavobacteria, with a smattering of Firmicutes and Proteobacters.
- Relatives of *Chryseobacterium* appear to predominate in both samples, though there was no connection between the two women, and the water bottles were composed of different materials.
- My guess is that the yellowish colonies on the plates are *Chryseobacterium*. But I cannot be sure without doing colony PCR.
- Some of these genera HAVE been associated with pathogens, but also with many environmental and water-associated isolates. It is great to have the students really think about Classifier (and how that data is evaluated), as well as chasing down various BLAST data and associated PUBMED references (the top hits, by the way, are usually uncultured microbes).
- In this sample, not much to suggest oral microbiota (which surprised me).
- None of this takes into account Archaea or Eukarya (different primers in different experiments?).
- It would be interesting to really explore this at a number of institutions? How about a “**Water Bottle Microbial Census Initiative**”? We could do this as a consortium of individuals (or Norm Pace might do it...wouldn't it be nice if coauthored with several of us?).

**Details of Franny's water bottle data:**

F-1

BLAST hit: [AM232813.1](#) Chryseobacterium indologenes (Score: 1628; 98% coverage)  
 Classifier: unknown - Root[100%] Bacteria[100%] "Bacteroidetes"[100%] Flavobacteria[100%]  
 "Flavobacteriales"[100%] Flavobacteriaceae[100%] Chryseobacterium[100%]

F-2

BLAST hit: [AM232813.1](#) Chryseobacterium indologenes (Score: 1657; 96% coverage)  
 Classifier: unknown - Root[100%] Bacteria[100%] "Bacteroidetes"[100%] Flavobacteria[100%]  
 "Flavobacteriales"[100%] Flavobacteriaceae[100%] Chryseobacterium[100%]

F-3

BLAST hit: [AM232813.1](#) Chryseobacterium indologenes (Score 1646; 96% coverage).  
 Classifier: unknown - Root[100%] Bacteria[100%] "Bacteroidetes"[100%] Flavobacteria[100%]  
 "Flavobacteriales"[100%] Flavobacteriaceae[100%] Chryseobacterium[100%]

F-4

BLAST hit: [AJ785572.1](#) Methylobacterium aquaticum (Score 1600; 95% coverage)  
 Classifier: unknown Root[100%] Bacteria[100%] "Proteobacteria"[100%] Alphaproteobacteria[100%]  
 Rhizobiales[100%] Methylobacteriaceae[100%] Methylobacterium[100%]

F-5

BLAST hit: [FJ870662.1](#) Chryseobacterium sp. pp2f (Score: 1598; 97% coverage)  
 Classifier: unknown Root[100%] Bacteria[100%] "Bacteroidetes"[100%] Flavobacteria[100%]  
 "Flavobacteriales"[100%] Flavobacteriaceae[100%] Chryseobacterium[100%]

F-6

BLAST hit: [AJ012071.1](#) Acidovorax sp. (Score: 1639; 96% coverage).  
 Classifier: unknown Root[100%] Bacteria[100%] "Proteobacteria"[100%] Betaproteobacteria[100%]  
 Burkholderiales[100%] Comamonadaceae[100%] Acidovorax[100%]

F-7

BLAST hit: [AM232813.1](#) Chryseobacterium indologenes (Score: 1661; 96% coverage).  
 Classifier: unknown - Root[100%] Bacteria[100%] "Bacteroidetes"[100%] Flavobacteria[100%]  
 "Flavobacteriales"[100%] Flavobacteriaceae[100%] Chryseobacterium[100%]

F-8

BLAST hit: [AM232813.1](#) Chryseobacterium indologenes (Score: 1670; 96% coverage).  
 Classifier: unknown - Root[100%] Bacteria[100%] "Bacteroidetes"[100%] Flavobacteria[100%]  
 "Flavobacteriales"[100%] Flavobacteriaceae[100%] Chryseobacterium[100%]

F-9

BLAST hit: [AM159535.1](#) Chryseobacterium sp. MN13.3d (Score: 1596; 96% coverage)  
 Classifier: unknown Root[100%] Bacteria[100%] "Bacteroidetes"[100%] Flavobacteria[100%]  
 "Flavobacteriales"[100%] Flavobacteriaceae[100%] Chryseobacterium[100%]

F-10

BLAST hit: [EU370956.1](#) Spirosoma panaciterrae strain Gsoil 1519 (Score: 1410; 95% coverage).  
 Classifier: unknown - Root[100%] Bacteria[100%] "Bacteroidetes"[100%] "Sphingobacteria"[100%]  
 "Sphingobacteriales"[100%] Cytophagaceae[100%] Spirosoma[100%]

F-11

BLAST hit: [EU337120.1](#) Acinetobacter sp. 1B3 (Score: 1559; 95% coverage).  
 Classifier: unknown - Root[100%] Bacteria[100%] "Proteobacteria"[100%] Gammaproteobacteria[100%]  
 Pseudomonadales[100%] Moraxellaceae[100%] Acinetobacter[100%]

F-12

BLAST hit: [AM159535.1](#) Chryseobacterium sp. MN13.3d (Score: 1618; 96% coverage).  
 Classifier: unknown Root[100%] Bacteria[100%] "Bacteroidetes"[100%] Flavobacteria[100%]  
 "Flavobacteriales"[100%] Flavobacteriaceae[100%] Chryseobacterium[100%]

F-13

BLAST hit: [FJ870662.1](#) Chryseobacterium sp. pp2f (Score: 1598; 97% coverage).  
 Classifier: unknown Root[100%] Bacteria[100%] "Bacteroidetes"[100%] Flavobacteria[100%]  
 "Flavobacteriales"[100%] Flavobacteriaceae[100%] Chryseobacterium[100%]

F-14

BLAST hit: [AM232813.1](#) Chryseobacterium indologenes (Score: 1644; 96% coverage).  
 Classifier: unknown - Root[100%] Bacteria[100%] "Bacteroidetes"[100%] Flavobacteria[100%]  
 "Flavobacteriales"[100%] Flavobacteriaceae[100%] Chryseobacterium[100%]

**Details of Hillary's water bottle data:**

- H-1  
BLAST hit: [GQ161989.1](#) Sphingomonas sp. S8-3 (Score: 1598; 97% coverage).  
Classifier: unknown Root[100%] Bacteria[100%] "Proteobacteria"[100%] Alphaproteobacteria[100%]  
Sphingomonadales[100%] Sphingomonadaceae[100%] Sphingomonas[100%]
- H-2  
BLAST hit: [AB022027.1](#) Abiotrophia para-adiacens (Score: 1676; 97% coverage).  
Classifier: unknown Root[100%] Bacteria[100%] "Firmicutes"[100%] "Bacilli"[100%] "Lactobacillales"[100%]  
"Carnobacteriaceae"[100%] Granulicatella[100%]
- H-3  
BLAST hit: [AB495140.1](#) Xylophilus ampelinus (Score: 1690; 96% coverage).  
Classifier: unknown - Root[100%] Bacteria[100%] "Proteobacteria"[100%] Betaproteobacteria[100%]  
Burkholderiales[100%] Comamonadaceae[100%] Variovorax[84%]
- H-4  
BLAST hit: [FM164632.1](#) Novosphingobium sp. TD IW 02 (Score: 1622; 97% coverage).  
Classifier: unknown Root[100%] Bacteria[100%] "Proteobacteria"[100%] Alphaproteobacteria[100%]  
Sphingomonadales[100%] Sphingomonadaceae[100%] Novosphingobium[100%]
- H-5  
BLAST hit: [AM159535.1](#) Chryseobacterium sp. MN13.3d (Score: 1531; 95% coverage).  
Classifier: unknown Root[100%] Bacteria[100%] "Bacteroidetes"[100%] Flavobacteria[100%]  
"Flavobacteriales"[100%] Flavobacteriaceae[100%] Chryseobacterium[100%]
- H-6  
BLAST hit: [AM232813.1](#) Chryseobacterium indologenes (Score: 1631; 96% coverage).  
Classifier: unknown - Root[100%] Bacteria[100%] "Bacteroidetes"[100%] Flavobacteria[100%]  
"Flavobacteriales"[100%] Flavobacteriaceae[100%] Chryseobacterium[100%]
- H-7  
BLAST hit: [AY741401.1](#) Legionella-like amoebal pathogen HT99 (Score: 1515; 95% coverage).  
Classifier: unknown - Root[100%] Bacteria[100%] "Proteobacteria"[100%] Gammaproteobacteria[93%]  
Gammaproteobacteria\_incertae\_sedis[23%] Sedimenticola[18%]
- H-8  
BLAST hit: [FJ870662.1](#) Chryseobacterium sp. pp2f (Score: 1613; 97% coverage).  
Classifier: Root[100%] Bacteria[100%] "Bacteroidetes"[100%] Flavobacteria[100%] "Flavobacteriales"[100%]  
Flavobacteriaceae[100%] Chryseobacterium[100%]
- H-9  
BLAST hit: No significant similarity (bad sequence?)  
Classifier: unknown - Root[100%] Bacteria[90%] "Nitrospira"[3%] "Nitrospira"[3%] "Nitrospirales"[3%]  
"Nitrospiraceae"[3%] Nitrospira[3%]
- H-10  
BLAST hit: [FJ870662.1](#) Chryseobacterium sp. pp2f (Score: 1507; 94% coverage).  
Classifier: unknown Root[100%] Bacteria[100%] "Bacteroidetes"[100%] Flavobacteria[100%]  
"Flavobacteriales"[100%] Flavobacteriaceae[100%] Chryseobacterium[100%]
- H-11  
BLAST hit: [GU084120.1](#) Elizabethkingia sp. F3 (Score: 1646; 96% coverage).  
Classifier: unknown - Root[100%] Bacteria[100%] "Bacteroidetes"[100%] Flavobacteria[100%]  
"Flavobacteriales"[100%] Flavobacteriaceae[100%] Elizabethkingia[100%]
- H-12  
BLAST hit: [AM159535.1](#) Chryseobacterium sp. MN13.3d (Score: 1592; 96% coverage).  
Classifier: unknown Root[100%] Bacteria[100%] "Bacteroidetes"[100%] Flavobacteria[100%]  
"Flavobacteriales"[100%] Flavobacteriaceae[100%] Chryseobacterium[100%]
- H-13  
BLAST hit: [DQ337546.1](#) Rhodococcus sp. BBCT 63 (Score: 1170; 95% coverage).  
Classifier: unknown - Root[100%] Bacteria[100%] "Proteobacteria"[80%] Alphaproteobacteria[80%]  
Sphingomonadales[79%] Erythrobacteraceae[79%] Croceicoccus[78%]
- H-14  
BLAST hit: [AM159535.1](#) Chryseobacterium sp. MN13.3d (Score: 1611; 96% coverage).  
Classifier: unknown Root[100%] Bacteria[100%] "Bacteroidetes"[100%] Flavobacteria[100%]  
"Flavobacteriales"[100%] Flavobacteriaceae[100%] Chryseobacterium[100%]

**Take Home Lessons and Thoughts:**

- **A conceptually simple and “real life relevant” exercise for students at various levels of expertise and interest, ranging from nonmajors to students with interests in microbial ecology and public health.**
- **Skills taught range from basic (streaking, observation, record keeping) to intermediate (PCR, plasmid isolation), to more advanced (evaluating taxonomy and bioinformatics).**
- **Limitations are set by budget, resources, and student level.**
- **There are different parts of water bottles; I swabbed from the interior surface. What about swabbing the spouts? Or determine what is there “planktonically”?**
- **It might be very interesting to create a central location for this kind of information, to begin to learn if particular phylotypes are most commonly found in water bottles. A website with photographs of cultivable organisms, taxonomic data, etc. Again: “*The Water Bottle Microbiota Initiative*.”?**
- **For more advanced students (or faculty!), isolation and characterization of cultivable organisms and evaluation of biofilm-forming proficiency would seem VERY interesting.**
- **I would love to see people try this, and find out what you and your students uncover.**
- **Don’t tell Nalgene! On the other hand, they might fund it!?!?**
- **Be sure to run your water bottles through the dishwasher often. As one student said to me: “It’s just water!” Not after you take a sip, I reminded her!**