

Lab. 6. Identification of Microbes from Natural Habitats

The aim of this investigation is to obtain isolated strains from selected natural habitats and determine their identity, at the level of genus and possibly species. Two approaches are used:

(1) PCR amplification of their 16s RNA (rDNA) gene sequence. This method was pioneered by Carl Woese and Norm Pace (Hugenholtz et al, 1998). In this paper, the authors use selected pairs of primers to amplify organisms obtained from Yellowstone hydrothermal habitats. The same primers can be used to identify microbes from any terrestrial habitat.

(2) Physiological characterization. If an organism's rDNA fails to amplify, it can be characterized based on metabolism and environmental requirements.

Isolation and DNA analysis.

March 20. Each group of 3 or 4 students selects a habitat to investigate. Examples might include: a dorm location; soil from forest or wetland; human body; animal body. This year, a new opportunity is the digestive flora of a pitcher plant such as *Nepenthes* sp.

Obtain microbial samples using a sterile cotton swab. If the source is dry, moisten the swab with sterile saline solution. Streak on four TSA plates, and on four MacConkey plates, to isolate individual colonies. Decide appropriate temperature (37°C or room temperature?) Store streaked plates in the cold room.

Before March 27. Restreak 15 colonies. Make slant cultures. Select 10 isolated colonies from different original colonies. Inoculate a colony of each into a tube of Tryptic Soytone Broth (TSB). Grow overnight at appropriate temperature, with rotation or shaking.

March 27. DNA purification, using Epicentre kit. Also purify *E. coli* control.

April 3. PCR amplification, using a pair of primers from Hugenholtz: 533F and 1492R. Next day, load 3- μ l sample of each DNA prep onto a large agarose gel. Observe bands; select samples for DNA sequence analysis.

April 4. Purify PCR samples that produced a 1-kb band, using Qiagen spin column. After purification, samples will be sent to the Ohio State lab for sequence analysis.

April 10, 17. DNA analysis and physiology tests.

Sequence results are analyzed.

Is the organism known previously? Confirmation tests are performed.

For isolates in which DNA failed to amplify: This could be a newly discovered species distantly related from those known previously (or its DNA could just be tough to amplify.)

Characterize your new isolate by physiological tests.

Physiological tests

Restreak each of your isolates on TSA agar, and grow overnight at the optimal temperature. Observe morphology and test physiology. These standard tests and observations can narrow down the identification to a small number of species, in some cases the organism family. Conduct for each strain:

- Record colony shape, color and texture on agar plates.
- Wet mount microscopy. Is the organism motile or non-motile? Eukaryotic or prokaryotic? (Best hypothesis, based on cell size.)
- Gram Stain. Include positive and negative controls. Note cell shape and size.
- Lactose MacConkey agar. Include +/- controls. Lactose fermenters produce red colonies. Gram + strains fail to grow (inhibited by bile salts).
- Salt plates: TSA plus 5%, 10%, 15% salt
- Growth temperature: Plate at 45°C.
- Anaerobic growth (GasPak-Plus)
- Enterotube and Enteric.xls (Gram-negative only)
- Other tests as needed.

Use *Bergey's Manual* and on-line resources to discuss how you narrowed down your identification. Each group will present a group lab report as a Powerpoint presentation.

Each student turns in an individual written lab report.

Questions to answer in the lab report:

1. What genera and/or species were determined? What are the significance and limitations of Genbank statistical analysis of rDNA sequences?
2. How did your investigation compare with that of Hugenholtz et al, 1998? How were your approach and results similar, and how did they differ?
3. Compare the results of your physiological confirmation testing. Were these results consistent with your sequence data?
4. What conclusions, if any, can you suggest regarding the natural flora of the habitat investigated by your group?
5. What is the composition of TSA agar? Why was this a useful medium for general isolation of diverse organisms? Nevertheless, what are the major limitations of observing only organisms grown in colonies on TSA plates? How else could the experiment be designed to overcome these limitations?

References

Holt, J. G. et al, 1994. *Bergey's Manual of Determinative Bacteriology (shortened)* 9E. Lippincott Williams & Wilkins, Philadelphia.

Hugenholtz, P., C. Pitulle, K. L. Hershberger, and N. R. Pace. 1998. Novel division level bacterial diversity in a Yellowstone hot spring. *J. Bacteriol.* **180**:366-376.