Temperature Extremes

Objective: To determine the temperature limits at which thermophilic organisms can live, students will monitor activity in samples from a natural hydrothermal environment that are exposed to increasingly warm temperatures.

This activity is intended to be a "quick and dirty" field analysis; it does not follow standard microbiological procedures.

There are many variations on this activity that can be attempted in the classroom. A version of this activity was attempted with some success during the Life at the Limits: Earth, Mars, and Beyond 2005 field experience. Feedback is welcome!

Materials

For each group of 4 students:
- ~100 ml of sample from a hydrothermal environment (living, mobile microorganisms present)
- 1 150 ml beaker (or test tube)
- stove-proof container for immersing the beaker or test tube
- laboratory grade hot plate with low setting; cooking hot plate can be used, but temperature will be hard to maintain
- thermometer
- pH paper (optional)
- refractometer, hydrometer (optional)
- microscope slides with wells
- pipettes
- lab notebook
- writing utensils

For each student:
- Protective eyewear
- Lab gloves

Time

2 50 minute class periods: one for experiment, one for observations and discussions

National Science Education Standards

Science as inquiry. Content Standard A
Students will ask questions, plan and conduct an experiment, employ simple equipment and tools to gather data, analyze and interpret results to construct a reasonable explanation, communicate investigations and explanations.

K-4. Life Science. Content Standard B.
Organisms have basic needs. Behavior of individual organisms is influenced by internal and external queues. An organism's patterns of behavior are related to the nature of that organism's environment.

5-8. Life Science. Content Standard B.
All organisms must be able to obtain and use resources, grow, reproduce, and maintain stable internal conditions while living in a constantly changing environment. Biological adaptations include changes in structures, behaviors, or physiology that enhance survival and reproduction in a particular environment.

9-12. Life Science. Content Standard B. Most cell functions involve chemical reactions. Cell functions are regulated. Organisms can both cooperate and compete in ecosystems.

Procedure

Examine the sample by placing a few drops in the well of a microscope slide and observing the sample under the microscope.

> *Are there microorganisms present?*
>
> *In what abundance?*
>
> *Are they mobile?*

Have the students sketch some of the microbes for later possible identification.

> *What are the physical characteristics of the field sample (salinity, temperature at time of sample collection, pH)?*
>
> *Do the conditions change across the field site and/or during the year? In what way? How might the organisms respond to such changes?*
>
> *What special mechanisms might organisms that live in a hydrothermal environment have?*

Organisms living in high temperature environments are called “thermophiles” (heat loving). One organism, *Pyrolobus fumarii*, has been found at temperatures of 113° C (235° F) – that’s hotter than the boiling point of water (100° C / 212° F)! Most thermophiles live at temperatures between 60 and 80° C. Thermophiles belong to the Bacteria and Archaea domaines. These organisms are capable of growing, carrying out metabolic processes, and reproducing at these extreme temperatures.

Thermophiles have special enzymes - protein molecules that conduct functions like photosynthesis and digestion within a cell - that can work at these extreme temperatures. Enzymes are tightly packed, 3-D structures held together by chemical links. They commonly unfold and the links break apart at temperatures above 47° C / 116° F. But enzymes in thermophiles are packed very tightly and held by especially strong links. DNA of thermophiles is adapted to life at high temperatures as well. The DNA more tightly coiled than DNA in microbes that live at lower temperatures. Protein coatings encase the DNA, making it even more thermally stable. Thermophilic microbes also have differences in their cell membranes – the walls around the cells. Typically cell walls are made of a double layer, but thermophiles have “glued” the double layer together, making a single, thicker wall.

What are the temperature limits to life as we know it?
Above 125° C protein breaks down completely.
Below freezing, chemical reactions (metabolism) are too slow to support life.

Early Earth was a very hot, volcanically active planet (heat from impacts, reorganization of its interior into layers, and decay of radioactive materials). Some scientists believe that the earliest life forms were thermophiles and that they evolved on the hot young Earth. The most primitive – and heat tolerant - organisms on Earth today are thermophiles in the domain Archaea.

Invite the students to prepare their experiment.

> *How might they test the limits of temperatures at which these organisms can live for the short term?* (organisms living and mobile before the temperature is altered are living and mobile after the alteration; their survival over the short term indicates they are <u>tolerant</u> of the increased temperature)
>
> *Long term?*(maintaining the higher temperature for a long-period of time [hours to days] and observing an increase in the population of organisms at the higher temperatures)

Have each group collect their lab materials. They should use gloves and protective eyewear.

Have the students fill the larger container with water (tap water is fine) and place it on the hot plate. This will be the "bath."

Have the student groups carefully immerse the sample in its beaker in the water bath, not allowing the water from the bath to mix with the sample.

Begin by recording the temperature of the hydrothermal sample. Very slowly heat the water bath and monitor the temperature of the sample.

Have the students pipette a small amount of the sample into the well of a microscope slide. Invite the students to inspect the samples.

> *Are there living microorganisms present?*
> *In what abundance?*

Repeat the process for every 5 degree temperature increase. Note when the sample reaches the temperature of the natural environment from which it was collected. Continue to increase the temperature and sample at 5 degree intervals.

<u>Alternative</u>: Assign each group a different temperature at which to collect a sample. This is a faster method of experimentation, but it does not allow multiple data points for each temperature / verification of reproducible results.

> *Are the same types of microorganisms present in each of the samples, or have some not adjusted to the change in temperature?*
>
> *Is there a notable change in abundance in types of living organisms as the temperature increases?*
>
> *What do the observations say about the upper limits of temperature at which these organisms can adapt?* Note that this experiment allows only an assessment of the temperatures at which organisms can survive short exposure;

the data do not provide information about temperatures at which the organisms can thrive and reproduce.

Is there a difference between tolerating the high temperatures and being able to live at these temperatures? How should the experiment be altered to address this question?

Why might some of the organisms be able to live in the beakers at the lower temperatures, but not thrive in another natural environment of similar conditions with this lesser temperature? (competition)

How might understanding how thermophiles survive extreme heat help the food or clothing industry?

What might the findings from this experiment tell us about the possibility of life on other planets at present or in the past?

Thermophiles are capable of withstanding high temperatures because they have modified enzymes – enzymes that remain active in very hot conditions without breaking down. This attribute has allowed the food, clothing, and paper industries – to name a few – to create better, safer, and/or more environmentally friendly products. The industry is using thermophilic enzymes in processes that used to be limited in the temperature at which they could occur. Food preparation can occur at higher temperatures, reducing the risk of contamination. Alkaliphilic enzymes can withstand the high alkalinity of detergents and are able to better break down proteins and fats in stains at hot washing temperatures. Thermophilic enzymes have helped in DNA fingerprinting; *Taq* polymerase can operate at high temperatures without breaking down, replicating DNA pieces rapidly and allowing crime labs to process trace amounts of DNA quickly. *Taq* polymerase was first obtained from the hot spring thermophile *Thermus aquaticus* in Yellowstone National Park.

Extensions

Have the students design an experiment to test the cooler extremes at which the organisms can survive using cold water / ice water baths / refrigeration at different, cooler temperatures.

Extend the time for which the organisms are exposed to higher temperatures to assess the temperatures at which they can thrive and reproduce. Maintain sample temperatures over several days using the water bath (lower temperatures with water level monitored) or ovens. Samples will have to be held in containers that will not be affected by the heat. Container tops will need to prevent evaporation and introduction of air-borne particles, but permit gas exchange (e.g., Nalgene bottles with lids partially screwed in place).