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|  | **Doing the Experiment**  **1. Source of Bioluminescent Bacteria:**  We received a culture of Vibrio harveyi from Dr. Anatol Eberhard, Department of Chemistry at Ithaca College in New York. When the bacteria is sent, it is in a test tube on an agar culture. The growth medium, containing agar (to help it gel as it cools), is poured into the tube and the tube angled on its side with the lid off until the agar solidifies. The bacteria are then streaked onto the agar and the tube is tightly-capped.  The Bacteria in the culture looks a lot like this:  These agar slants can contain either 3% NaCl complex or Sea Water Complex (SWC) medium (7 ml medium per 16 x 125 mm screw cap tube). Before use the bacteria are sub-cultured (single-cloned) onto agar growth medium poured into petri dishes and luminescence checked for bright clones. If it is not possible to make agar paths to first getreally bright colonies you can go directly from the slant to the growth medium the risk is tha thte culture amy notbe bright. The bacteria are then transferred from the slants or dishes into liquid culture by steril techniques for us. These cultures are grown on a gyrotary shaker also know as a water bath shaker, at 25 degrees Celcius to 27 degrees Celcius at a shaking rate of about 190 rpm until the culture is in log phase growth (Waters).  **2. Growth Medium for the Bacteria:**  1. The growth medium for V. harveyi is 3% NaCl Complex. For every liter of this complex medium you use:  30 g NaCl  7 g Na2HPO4 7H2O  1 g KH2PO4  0.5 g (NH4)2PO4  3 ml glycerol  3 g yeast extract  5 g bactotryptone  2. After you weigh out all the compounds and materials you add distilled water and bring the final volume up to1 liter.  3. Then you autoclave (which is a heavy vessel for conducting chemical reactions under high pressure) separately a 10% solution of MgSO4. To do this you use 10g of MgSO4 brought up to 100ml distilled water. The autoclave functions like a pressure of sorts to steralize the growth medium.  4. When the growth medium has cooled, add 1 ml of the 10% solution of MgSO4 and mix well. This avoids the formation of precipitates which normally result when MgSO4 is autoclaved along with the other components in the growth medium. This is the standard recipe for growth medium.  The compounds used to grow the medium can be all be ordered through scietific supply catalogues. We had all the compounds in our lab except yeast extract and bactotryptone. These two compounds can be ordered through catalogues such as Carolina (# 1-800-334-5551) or Difco (# 1-800-521-0851).  **3. Equipment Needed for Experiment:**  Equipment is also a big part of the job. You will need:  a. Gyrotary shaker also known as a water bath shaker: When growing bioluminescent bacteria they need oxygen which can easily be provided when a water bath shakeris used to keep the cultures oxygenated. This piece of equipment can be hard to find. You may find it in larger labs in your city.  b. 300-ml Erlenmeyer Flask: This flask is what you will use to contain the medium and the growing bacteria. Erlenmeyer Flasks can be found in most labs.  c. Pipettes: These are used to take amounts of one thing and add it another thing. These can be found in most labs.  d. Inoculating loop: Used to make the first inoculum and streak onto plates or into the growth medium.  e. Spectrophotometer: Used to measure the turbidity (cloudyness) of the culture to get an idea of how well the bacteria are growing;very turbid cultures have a lot of bacteria.  f. Weighing Scale: Used to measure or weigh the amount of compounds used and added.  g. Bunsen burner: Used to flame steralize the inoculating loop and the tops of flasks when they are opened/closed.  h. Timer: To time how long it takes for the Vibrio Harvyi to dim their lights to the maximum.  i. Photographic paper: Used to measure the amount of light being emitted from the bioluminescence. You can get these at any photo lab.  **4. Making the First Inoculum:**  To make the first inoculum you will need:  1. Steralize transfer bacteria from the slant, using an inoculating loop.  2. This is added to a 300-ml Erlenmeyer flask containing 40-ml of fresh growth medium.  3. The flask is capped loosely and put in a water bath shaker. The water bath is set at a shake rate of 190 rpm at 25 degrees Celcius to 27 degrees Celcius and turned on.  4. This culture is grown until a sample of it (taken out sterilely) is needed. This sample of culture needs to be at an A660>0.5 on the Spectrophotometer. A culture this dense with bacteria is called a ìlate-log phaseî culture.  **5. Making the Controls For Our Experiment:**  The control for our experiments is done by testing different chemicals which are known to affect the light of Vibrio harveyi. These tests prove that Vibrio Harvyi can be used to test the effects of pollution. We used Kenneth Thomulkaís work as a guide for our tests. He used both Vibrio harveyi and Photobacterium phosphoreum to detect biohazardous materials in water. He used a lot of chemicals and found their potential toxicity as you can see on his chart:     |  |  | | --- | --- | | Compound Exaluated | EC 50 values (ppm) | | Mercuric chloride | 0.002 | | Silver nitrate | 0.038 | | Cobaltous chloride | 3.8 | | Zinc chloride | >100 | | Tributyl tin | 0.3 | | Cadmium chloride | 0.38 | | Dibutyl tin | 0.24 | | Cupric sulfate | >100 | | Sodium hypochlorite | 0.061 | | Calcium hypochlorite | 6.1 | | Hydrogen peroxide | 1.8 | | Potassium dichromate | 60 | | Sodium azide | >100 | | Potassium nitrate | >100 | | Sodium fluoride | >100 | | Methanol | 25000 | | Ethanol | 9000 | | 2-Propanol | 7700 | | Phenol | 12.5 | | Sodium dodecylsulfate | 37 | | Triton-X-100 | >100 | | Formalin | 25 | | Novobiocin | >100 | | Tetracycline | >100 | | Chloramphenicol | >100 | | Nalidixic acid | >100 | | Streptomycin | >100 | | Ampicillin | >100 |   On this graph the first column is the chemicals Thomulka tested for biohazardous effects using the bioluminescent bacterium Photobacterium phosphoreum. The first group of chemicals are metals which reduce the luminescence (Thomulka et al. 1993). The next group of chemicals were "strong oxidizing agents that were very effective in reducing the bioluminescence" (Ibid). The third group of chemicals were not inhibited the bioluminescent. The fourth group were alcohols and had little to no effect on the bioluminescent. The last group was made up of many compounds including many antibiotics. These compounds also had little effect when it came to reducing the bioluminescence.  In our experiment we will use three of these compounds. Cupric sulfate is a metal that we expect not to be effective in reducing the luminescence. To obtain the Cupric sulfate we ordered it through a catalogue. Most catalogues wonít let students order chemicals, so we avoided this by ordering through our school. Sodium hypochlorite and hydrogen peroxide are both strong oxidizing chemicals. We expect the Sodium hypochlorite to reduce the bioluminescence. Sodium hypochlorite is bleach which we bought at the grocery store. The hydrogen peroxide is expected to only slightly reduce the light given off by the bioluminescent bacteria. We also bought hydrogen peroxide at the grocery store.  Kenneth Thomulka only used Photobacterium phosophoreum in his experimants so when we test Vibrio harveyi we may find slightly different test results, because V. harveyi more sensitive for some chemicals.We will also be using this Photobacterium phsophoreum as a control. The P. phosphoreum will be used to compare Kennith Thomulkaís results with ours when we test cupric sulfate, sodium hyperchlorite, and hydriogen peroxide. For the most part the chemicals will have the same effect due to the similarities in the two bacteriaís.  Using these chemicals we expect the bioluminescence to act a certain way. Sodium hypochlorite is a strong oxidizing agent. We believe it will reduce the bioluminescence. Hydrogen peroxide is also an oxidizing agent, so it should reduce the Vibrio harveyi. We believe that cupric sulfate will reduce the bioluminescence at all.  **6. Collecting Arroyo Del Valle Creek water:**  We collected water in seven spots along the creek. We picked spots that were fairly easy to get to and by their flow rate. All of our seven spots varied in different ways. There were different levels of traffic of both foot and cars to all the areas. The different areas had different ecosystems that contained all types of natural and exotic plants and animals, also some spots had more litter than others  **7.Making the Control Groups:**  1. Using an Erlenmeyer flask make a control solution of 0.3g NaCl in 10ml distilled water.  2. Using another Erlenmeyer flask take 10ml of hydrogen peroxide and add 0.3g of NaCl making a solution 10% salinity.  3. Repeat step 2 for the other chmicals that you choose to use as controlls.  **8. Making the Test Cultures for the Arroyo Del Valle:**  For the Arroyo Del Valle sample, we had to add some NaCl first before teating them doing this movement will keep the Vibrio Harvyi from being ìosmotically shokedî and keep the cultures healthy.  1. Use a 300-ml Erlenmeyer flask pour 10 ml of your desired sample. Then add 0.3g of NaCl . Mix well. This makes a solution of 10% salinity.  2. Do this to each creek sample you want to test  3. Take out 2-ml (aliquot) of the medium and distribute it to about seven test tubes depending on the number of water samples you test at that particular time.  **9. Doing the Experiment:**  To test the bioluminescence you first want to test the controls:  1. Find a dark room that you can conduct your experiment in, making sure that it has a red light in it so you can see what you are doing. The light must be red.  2.Set up eleven test tubes that are clean and labled with the chemical or the number of the water sight that you have choosen. Place on a test tube rack  3. Put 0.5ml of your already prepared solutions in the appropriatley labled test tube.  4. Remove the cultured bacteria from the water bath shaker and bring it into the dark room along with the following mateials:  \* prepared and labled test tube with solution in it (should be on a test tube rack).  \* graduated cylinder to measure bacteria  \* piece of paper and pen  5. After entering the room you need to dark adapt for approximatley 20 minuites, but no less that 15 minuites.  6. After dark adapting take one test tube at a time and add 2ml of the bioluminescent bacteria by measuring it in the graduated cylinder.  7. After they are mixed watch it untill it has reached its dimmest or brightest point.  8. Write on you paper under an approprialley labled section if it has gone up or down by using a + or -  9. repeat steps 6-8 for each of the test tubes. Make sure that you both watch each test tube and wait to say your results untill you are completly done with the whole expriment, to prevent any possible biases.  ~ If you want to take any sort of picture of the bacteria use photographic paper. A regular camera will be unable to detect any light.( it is not sensitive enough) ~  We expect to see the light emitted from the Vibrio Harvyi decrease if seriously polluted creek water has been added. This will happen because as Thomulka says "something has poisoned the bacteria." |

*This Web Site is Best viewed with 256 or more colors.*

*For More Information about Creekwatch, please contact Eric Thiel at* [*ethiel@pleasanton.k12.ca.us*](mailto:ethiel@pleasanton.k12.ca.us)