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|  | Methods:  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. Shown in picture:  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. (Dr. Cory 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  4. After you weigh out all the compounds and materials you add distilled water and bring the final volume up to1 liter.  5. 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.  6. 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: |

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

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