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| Table of  Contents  [Introduction](http://docs.google.com/title.html)  [Prediction/](http://docs.google.com/pred-hypo.html)  [Hypothesis](http://docs.google.com/pred-hypo.html)  [Procedure](http://docs.google.com/procedure.html)  [Data](http://docs.google.com/data.html)  [Conclusion](http://docs.google.com/conclusion.html)  [Daily Log](http://docs.google.com/dailylog.html)  [Bibliography](http://docs.google.com/biblio.html) | Emails With Bonnie Bassler  1/7/00  To: Bonnie Bassler  From: Shelley Doljack and Jasper LaBelle   While searching on the internet for a certain bioluminescent bacteria called Vibrio harveyi, we came across your name. Reading the article titled Intercellular Communication and Quorum Sensing in Bacteria, we became excited about contacting you. We are both high school students at Amador Valley High School in Pleasanton, California. Being enrolled in Advanced Placement Biology, we have a research project to do. Our research project is called Lighting It Up: Using Bioluminescence to Reveal Pollution. The bioluminescent bacteria we want to use is the species Vibrio harveyi. What we want to do exactly is to take ordinary tap water and creek water, expose the bioluminescent bacteria to these media and use a light sensor to measure the amount of light emitted over time until the bacteria stops glowing. Both of us cannot wait to get started and do the experiment. However, we have some problems that we still need to figure out.   One of our problems is that we do not know how long to grow the bacteria in a growth medium of 40mL. We have a procedure that says to put the Vibrio harveyi in a growth medium of 40mL into a 250mL flask and place this flask in a gyratory shaker at 25ºC to 27ºC at a shaking rate of 190rpm. We were wondering if you could tell us how long the bacteria will grow and if we shake it for 3 days, will that be enough or too much? Also, once taken out of the water bath shaker, how long will the bacteria stay alive before they start to die off?  These are just technicalities; our main problem lies in obtaining Vibrio harveyi. We have tried a number of sources but have been unable to locate any. Do you know of any suppliers of this bacteria?       Thanks,  Shelley Doljack and Jasper LaBelle  1/12/00  Reply to: RE: Vibrio harveyi  Dear Shelley and Jasper  I would be glad to send you the wild type strain of V. harveyi.  If you give me an address I can send it today.  You should grow the bacteria shaking at 30 degrees Celsius overnight, about 14 hrs should be fine.  They will stay alive for several days thereafter, but the light will begin to fade after a few hours.  They need oxygen to produce light, so you can give them a quick shake and they will make light again.  Still, I only use the cultures about one day, then they run out of the substrate for making light, so even if they have oxygen they get dimmer and dimmer.  Let me know where to send the strain and good luck on your project.  Bonnie Bassler  1/13/00  To: Bonnie Bassler  From: Shelley Doljack and Jasper LaBelle   Thank you so much. Here is the address to send the bacteria strain to:      Shelley Doljack and Jasper LaBelle      Teacher: Mr. Thiel      C/O Amador Valley High School      1155 Santa Rita Road      Pleasanton, CA 94566  Would you please send us enough to make four cultures and maybe some brief instructions on how to raise the bacteria? We have never been sent bacteria before and do not know what to expect. Thanks again for all the help. We really appreciate it.  1/14/00  Reply to: RE: Vibrio harveyi  Dear Shelly  I will send you the bacteria on a petri plate.  Simply take a sterile applicator stick or toothpick and touch it to one of the single colonies.  Then shake the stick or toothpick into your liquid growth media.  The bacteria should be grown up by the next day.  The petri plate will have lots of single colonies on it, so there should be plenty of colonies to start cultures.  You can streak the bacteria fresh onto new plates if you need more.  Be sure to go in the dark room and look at your cultures to see them glow!  Good luck,  Bonnie Bassler  2/1/00  Dear Bonnie,   We have received the bacteria you sent us on Thursday, Jan. 27th. Thank you. On that day we also prepared our growth medium, a photobacterium growth medium that was in a powder form that we rehydrated and heated to help it dissolve. We left this in our classroom's refridgerator over the weekend and innoculated it on Monday. When we took out the petri dish that you sent us, from a drawer that we stored it in at room temperature, we noticed that it did not look as if it grew at all during the weekend. We thought that it would be a little fuzzy around the lines that you put on the dish, indicating a little growth, but there were none. Is it supposed to grow a little bit or is it supposed to stay exactly how you sent it? Did we store the petri dish in the wrong environment? We put the flask containing innoculated grwoth medium in the water bath shaker at 30 degrees Celsius and 150 rpm and left it overnight. On Tuesday, we took it out and it did not grow. We believe that the growth medium we used was the wrong one. There was a group that attempted this project in this course last year and we were following what they said to use as the growth medium, but we have decided that they were wrong because the growth medium is not a liquid, but a gel. We were wondering if you could send us the procedure you use for preparing the growth medium for Vibrio harveyi and the temperature and rpm you use, since the information we have is inaccurate.  Thanks,  Jasper LaBelle and Shelley Doljack  2/2/00          Reply to:   RE: growth of bacteria  Dear Shelley  The plate should look the same as when I sent it.  Here is a recipe for a medium that will work:  20 g NaCl  10 g Bacto-tryptone  5 g Bacto-yeast extract  Dissolve in 1 L of water.  Aliquot the appropriate amount into different flasks, and then autoclave 20 min and cool completely.  Grow the bacteria shaking at 200 rpm 30 degrees overnight.  Bonnie Bassler  2/8/00  Thank you very much for your assistance thus far. We followed the protocol that you e-mailed us. We made one change in that we prepared only 100ml instead of 1L and we were forced to boil rather than autoclave the medium. After subjecting the medium to water bath shaking for 24 hours no luminescence was observed. We checked the contents in a completely dark room and using a photo sensor in a lightproof test tube.  Our first thought was that there were no bacteria present. However when  the medium was viewed under the microscope we observed many rod-shaped, highly motile organisms swimming about. We photographed these organisms at 1000x using our teacher�s digital flexcam and attached the image for you to use if you would like.  We have no desire to become an inconvenience to you and will cease writing if we are becoming bothersome. We have become very excited about this research and would love to see some results so that we may demonstrate to our teacher that this project deserves continued attention.  If you have any suggestions that may assist us further we would be greatly appreciative!!  Sincerely,  Shelley Doljack and Jasper LaBelle  [[Top]](#gjdgxs) |