Preparation of culture medium for microaerobic magnetotatic bacteria in JCM (medium no. 915)

https://www.jcm.riken.jp/cgi-bin/jcm/jcm_grmd?GRMD=915

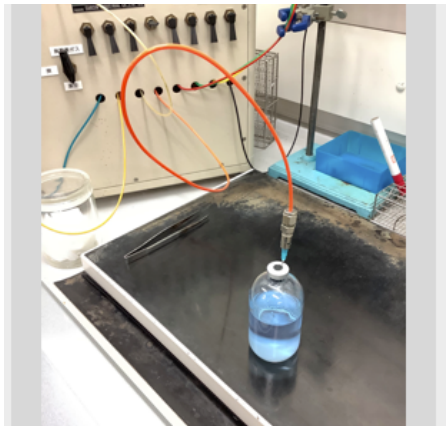
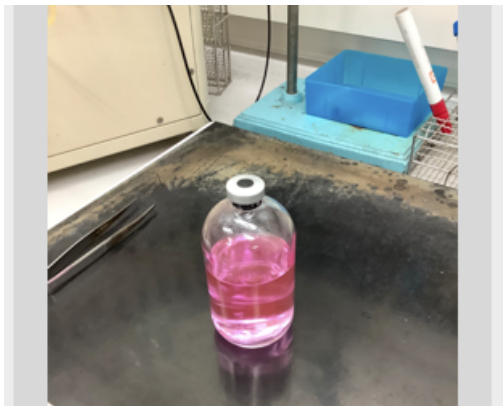
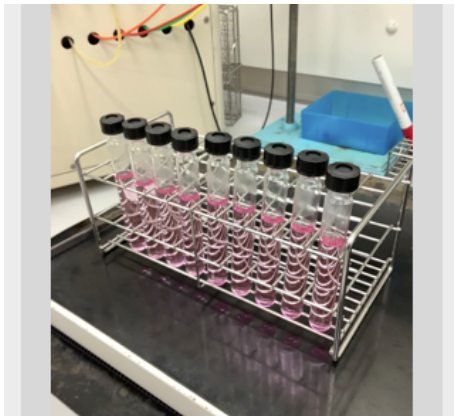
JCM holds a variety of microaerophilic bacteria. In this page, we show how to prepare the culture medium (medium no. 915) for cultivation of microaerophilic magnetotactic bacteria. The applicable bacteria for this medium are as follows.

・JCM 17883 *Magnetococcus marinus*

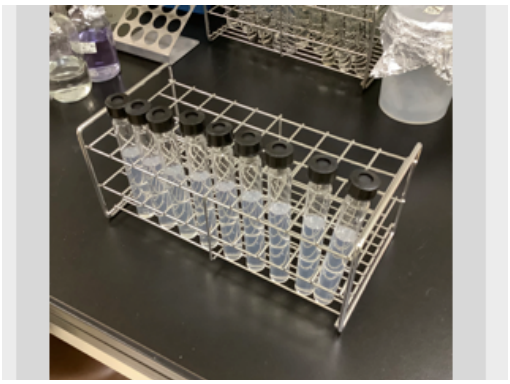
・JCM 17960 *Magnetospira thiophila*

・JCM 17961 *Endothiovibrio diazotrophicus* (non-magnetotactic bacteria)

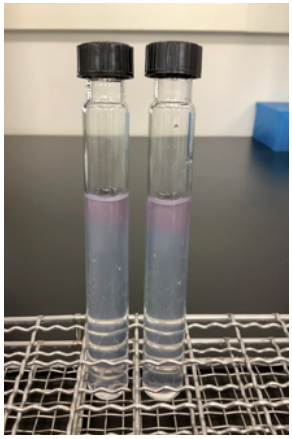
(Note) The vessel used for incubation, the volume of medium, and the gas displacement method for the gas phase should be changed according to the user's facilities as appropriate. Nitrogen gas and air used after autoclaving should be sterile through a sterilization filter.

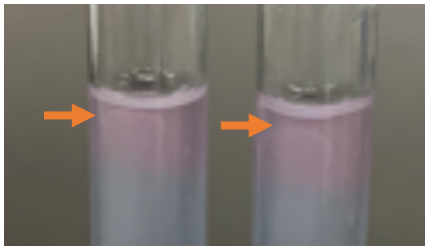
1. Dissolve the components (NaCl to Resazurin in the recipe) in 100 mL of ultrapure water in a beaker, and adjust the pH to 7.0 with 0.5N NaOH. Transfer the solution to a glass vial and add agarose. Put a butyl rubber stopper, seal with a aluminum cap, and replace the gas phase with N2 gas (see photo).	
2. Autoclave the vial containing the culture medium (photo: the color of the redox indicator Resazurin turns pink after autoclaving). Add the remaining medium components (phosphate buffer and others) anaerobically and aseptically. After all medium components have been added, check the pH. Adjust pH to 7.0-7.2 by adding drops of sterile HCl or NaOH as needed.	
3. Open the vial bottle on a clean bench (under the atmospheric conditions), dispense into sterile glass tubes (for ø16 x 125 mm size Hungate tubes, dispense 11 mL each), put a butyl rubber stopper and attach a cap.	

4. Replace the gas phase with N2 gas. After a few hours, the agarose will solidify. The oxygen will be removed by the reducing regents and the color of the Resazurin becomes transparent (see picture). The medium in this state can be stored at 4ºC for a few months.



5. A syringe and a long syringe needle (e.g. 22Gx70mm) are used for inoculation. In a clean bench, collect the inoculum from the source of inoculation using a syringe with a needle attached. Before inoculation, sterilize the upper surface of the butyl rubber stopper as appropriate. With the cap closed, stick the needle into the solidified medium (as far as it can reach). Withdraw the needle while pushing the inoculum solution into the medium.
6. Take air (2 mL for the Hungate tube) with a syringe and add it to the gas phase through a 0.2 µm pore size filter. Place the inoculated tubes in an incubator set at incubation temperature.
7. After a few days, the color of the resazurin at the top of the medium will change to pink as oxygen is dissolved into the medium. A convex dome-shaped layer of microbial cells can be seen visually in the area where the oxygen has dissolved (arrow).





8. The layer of microbial cells (arrows) will migrate to the vicinity just below the surface of the medium in about 1 week.
9. A syringe and a long syringe needle can be used to collect the cell concentrated layer for microscopic observation and in vitro culture.