GROWING BACTERIA AT HOME

Any amateur can successfully isolate and grow bacteria at home as a hobby. This is a fascinating and rewarding pastime and can result in many rewarding.hours. The basic equipment required iz listed below. All of these materials can be obtained through the lab supply house also listed below.

20x150mm Pyrex test tubes, screw cap (Corning 9825) Test tube racks (2). 25-milliliter pi ets (4) Rubber Pipettor (2) Sterilized cotton Alcohol lamp (1) Plastic pre-sterilized Petri dishes (35 x 10, Falcon) Inoculating loops (2) 3hoculati needles (2) Incubator see below) (1) 500-milliliter Erlemeyer flasks (4) Hydrion PH paper (1 vial) Glass Stirring rods (4) Distilled water (boiled) Polyethylene waA bottles (for distilled water) Magnifying glass (1) Gram balance (available in health food stores) Filtering cloth Rubber gloves Pressure.cooker (1) Kim.Wipe paper towels . 100-milliliter graduated cylinder Centigrade thermometer, marking crayon (Incubator* closed box with door and accurate thermometer. mall (25 watt) lamp is placed inside and turned on and off so that temperature hovers around 37°C.)

CHEMICAL SUPPLIM

Difco Laboratories., Detroit, Michigan 48201
General Biological Supply House,' 8200 T. Hoyne Avenue
Chicago, Illinois 60620
SciChem Company, 45 Williams Street,
Boston,
Mass. 02149

CHEMICAL SUPPLIES

Difco Nutrient Agar (1)
Saboura d Dextrose Agar 1)
Nf10 Sodium hydroxide solution (5 pt)
N/10 Hydrochl ic acid (5 pt)
Lysol Disinfectcint (for cleaning Denatured Ethanol (1 gal)work area)

Shake up to 1 gram with distilled water in a clean test tube. Place a drop of the clear water onto the solidified agar, of one plate and cover it. Use sterilized glass stirring rods. Follow the same pro-cedure with a drop of saliva. Cover the air-exposed dish. Place the three covered Petri dishes back in the incubator and wait several days. Examine the dishes. Colonies of bacteria should have formed (fig. z) one or more species of bacteria have now, begun incubating or growing on the dishes. Similar-looking colonies probably represent a single species.

Examine them with your magnifying glass and make a sketch in your notebook along with all the other pertinent data. Keep dishes covered!

Resterilize the slant test tubes, removing the cotton and using fresh cotton as the tubes cook, (keep the cotton dry!). Using an inoculating needle, open one of the Petri dishes and remove a tiny bit of one of the isolated colonies. Recover the dish and immediately transfer the sample to the agar slant in one of the tubes. Restopper it with the cotton as soon as possible. Place the tube in the incubator. (Remember to label all tubes and dishes as to the type of nutrient. Different bacteria like different nutrients, so your sample may not "take" if nutrients are changed.) After several days examine the slant culture tubes (several can be made each using material from. different dish cultures collected from different sterilized inoculating needles.) Single, pure cultures of the original bacteria found on the original dishes should be found. If contaminated (obvious to the eye), repeat the process using fresh slant tubes and inoculating needles. Once pure cultures have been obtained, remove them from the incubator and store them at room temperature (about 20°C).

This will retard the bacterial growth but it can be renewed at the incubators temperature. After several such experim,=ts, a number of pure cultured bacterial species will have been collected. Don't forget, record everything in your notebook.

Assemble all the materials listed above, (Fig 1). One or 5 gallons of distilled water, usually available inthe yellow pages, is more than sufficient.

The pressure cooker on the kitchen stove is used to sterilize all materials prior to use and. during operations.

Bacteria and fung are everywhere....on your hands, in the air, in water....so remember to always sterilize as follows:

Consulting the instruction book of the pressure cooker, place 6-10 ounces of distilled water in the previously cleaned and dried vessel. Add-the equipment or materials you wish to sterilize. Heat for 15-'30 minutes at at least 15--20 pounds pressure. Sterilize 2 polyethylene wash bottles, fill one with boiled, distilled water.and.the other with-he denatured alcohol, Fill the alcohol lamp with alcohol. Sterilize the pipets, loops, needles, stirring rods and graduated cylinders. Place these all on your clean workbench on fresh Kim Wipe towels. All equipment must be iesterilized after use or before the next experiment.

As stated before, bacteria exist everywhere: air, soil, water, saliva, skin, food. The first experiment will snow you how to prepare nutrient solutions, inoculate them, culture them.and.establiah pure isolated colonies of bacteria.. Bacteria that produce antibiotics are also covered. The third experiment covers isolation of bacteria from different sources.

EXPEPJMENT #1

Weigh out 6 grazes of nutrient agar into a 500-m! flask and add 250-ml of distilled water. Heat to 150°F (just below boiling.) and stir until all the agar is dissolved. Sterilize the mixture as above. Record all steps in your notebook and label the resulting mixture (#1-Date _L/). Weigh out 16 grams Saboura d agar and perform the same steps as above. Label the resulting mixture (#2-Date r,/ 1 /). Both of the mixtures above "Jells" or"set$" to a solid below about 3$°C. While still hot and liquid, pour each. of these mixtures into the petri dishes (i") end test tubes (1"). The test tubes are tilted at about 45° while cooling to form a "slant" with a greater surface area than just the tube itself. The petri dishes are covered and the tubes are plugged with dry, sterile cotton. Place both the test tubes and the petri dishes in the incubator for several days. Examine the tubes and dishes. If everything has been sterilized correctly, no spots or patterns will have appeared. If some microorganism has crept in,,.a "culture" or "cultures" will have formed (See below).

If nothing has appeared on the petri dishes of tubes, remove both from the incubator and let them cool. open one petri dash and leave it open to the air for 1 hour. Go outside and gather a small soil sample.

Bacteria are as diverse in type as mammals, fish or birds. But because they are microscopic animals, the amateur experimenter without a microscope must learn to classify bacteria in terms of the following:

1. TYPE OF COLONY

A. NAKED EYE

1. Smooth Ss) 2. Rough (R) . Color

4. Discrete

Spreading or pin-point 6. Opalescent or. shiny

7. Mucoid (Viscous or slimy)

8. Antibiotic (zone of'inhibition)

E. LOW MAGNIFICATION magnifying glass)

1. Fine structure sketch)

The chta above must be recorded for each colony if it is ever to be identified. The antibiotic feature is evidenced by a culture that has no others near it in a zone, such as in Figure 3.

Such common antibiotic bacteria as Pencillium exhibit this charact-eristic.

Slant cultures, of bacteria can be sent to t.e biology departments of most state universities for identification. Also, common (non-toxic) bacteria can be obtained from the American Type Cuture Collection, 2112-'M Street NW, Washington, D.C. 20036. Many further experiments in bacteriology can be performed. at home using the materials, equipment and techniques described above. A large collection of different bacterial species can be built up. For further reference, consult the biology or bacteriology sections of your local or state library.