# The American Biology Teacher

Vol. 2 JANUARY, 1940 No. 4

# An Introductory Series of Problems in Practical Bacteriology for the High School Biology Course

B. BERNARR VANCE
Daniel Kiser High School, Dayton, Ohio

The following three problems are intended to introduce the pupil to elementary routine work in bacteriology and to suggest some of the basic techniques employed. We use them as special group exercises for pupils in College Prep or Pre-nursing courses, and for others working beyond the minimum requirements of the course of study for tenth year biology. These problems require simple apparatus and materials, and can be completed with a minimum amount of teacher direction.

PROBLEM 1. To prepare a standard culture medium for growing bacteria.

Instructor's OK ——

*Materials:* Beef extract or bouillon cubes; agar-agar or flake gelatin; balances; beaker or small cooking vessel; roll cotton; test tubes; steam pressure cooker; sterilizer, autoclave, or large vessel for boiling water; flask or bottle; glass funnel; rubber tubing; pinch clamp; burner; test tube rack; distilled water.

*Method:* Measure out 100 cc. of clear or distilled water into a beaker or small cooking vessel, and add ½ g. of beef extract or bouillon cube. Mark on the side of the vessel the level of the contents. Heat until all solid materials are dissolved. Add to this beef broth 1½ g. of agar-agar (flake gelatin may be substituted). Boil until dissolved, adding enough water to replace that boiled away. Line the funnel with a layer of cotton, and filter the solution into a flask or bottle.

Now rinse the funnel and fit it with a short length of rubber tubing and a pinch clamp. Pour the hot solution back into the funnel, and draw off into test tubes enough to fill each tube about ¼

full. Roll cotton plugs, fitting them into the tubes to a depth of about ¾ in.

Put the prepared tubes in a steam pressure cooker, sterilizer, or autoclave for about ½ hr. at about 15 lbs. pressure, or boil in water for an hour, to kill all bacteria. Take out, tilt one tube against a book or other support to make an "agar slant" tube, and set the remainder upright in the rack to cool. Store in a dry place.

To make larger quantities of the medium, use proportionately larger amounts of all materials.

Submit the culture tubes to the instructor for criticism. Be prepared to answer the following questions:

1. What purpose does beef extract serve in the culture medium? The agar-agar?
2. What is the source of agar-agar? State another commercial use of agar-agar.
3. Why are cotton plugs used in the culture tubes instead of cork stoppers?
4. How does steam under pressure kill bacteria more effectively than boiling water?

PROBLEM 2. To grow and transfer bacteria. Instructor's OK ——

*Materials:* Culture tubes prepared in preceding exercise; 5 petri dishes; straight inoculating needle; burner; vessel for boiling water; glass plate; incubator or darkened box equipped with light bulb or other warming device; wax pencil; gentian violet stain or ink; slide; microscope; glass rod.

*Method:* Sterilize 5 petri dishes in a hot dry oven for three hours. Set the upright tubes of culture medium from the preceding exercise in a vessel of hot water until contents are melted. Taking one of the tubes between two fingers of one hand, remove the cotton plug with the other hand. Hold the neck of the tube in a hot flame for a few seconds. Using the hand holding the cotton plug, raise the lid of a sterile petri dish enough to admit the lip of the tube and pour the melted medium into the dish. Allow the lid to drop back into place immediately. Repeat for each of the other petri dishes. Mark the dishes A, B, C, D, and E respectively, and set them aside on a glass plate to cool.

When they are cool, carry Dish A outdoors, remove the lid entirely and expose it to the air for five seconds; replace the lid. Expose Dish B in the same manner in the school hallway while classes are passing. Raise the lid of Dish C slightly at one side and allow a fly or other insect to walk across the surface of the medium. Rub the finger tips across a dusty table top or window sill and press against the surface of the medium in Dish D. Catch a drop of drinking water on a glass rod. Introduce into Dish E, allowing the drop to run across the surface of the medium. If you are unable to expose dishes exactly as directed above, work out similar exposures for yourself, and note any changes made in the directions.

Place all dishes *upside down* in an incubator at a temperature between 30 and 35 degrees Centigrade (86 to 95 degrees Fahrenheit), or in a darkened box equipped with a light bulb or other warming device, for about three days. Take out and examine carefully. Note general observations of respective dishes.

Different kinds of bacteria can be roughly distinguished by color and manner of branching over the surface of the medium. Select and mark one of the more interesting colonies. Clean a microscope slide, place a small drop of water on it, and set it aside on the table. Holding the handle of the inoculating

needle with one hand, thrust the needle into a hot flame until it glows along its entire length. This is called "flaming" the needle. Cool quickly in the air, raise the lid of the dish containing the colony selected, and touch the needle to the surface of the colony. Remove and wash the needle tip back and forth in the drop of water on the slide until the bit of colony is distributed throughout the water. Again "flame" the needle and lay it aside on the table.

Hold the slide well above the flame until all the water evaporates, leaving the bacteria fixed to the surface. Cover this area with ink or gentian violet stain and allow to stand for three minutes. Wash off all extra stain, dry over the flame, and place under the highest power of the microscope. No cover glass is needed. Use the oil immersion lens, if available. Consult the instructor if you have not previously used this lens. Focus carefully, using as little light as possible. Observe individual bacteria. What shape are they?

Now take an agar slant tube between two fingers of one hand. Take up the inoculating needle with the other hand and flame it. Using the hand holding the needle, remove the cotton plug from the tube, grasping it between two other fingers. Bring the neck of the tube into the hot flame for a few seconds, and again touch the tip of the needle to the original colony in the petri dish. Insert the needle to the bottom of the culture tube, dragging the tip over the surface of the culture medium as it is removed. Be careful not to break or scratch the surface of the medium with the needle or to touch the sides of the culture tube. Again heat the neck of the tube and replace the cotton plug.

Incubate for three days. Remove from incubator, and carefully examine the colonies in both tubes comparing their spread and color with the original colony in the petri dish. Remove a bit of the colony from one tube to a microscope slide, stain, and observe under the microscope as before.

Be prepared to answer the following questions:

1. An isolated colony growing in a petri dish is usually considered a pure culture of bacteria. Why?

2. Are more bacteria present in outdoor air than in the air of a crowded hallway? Why should this be true?

3. What may be a reason for heating the neck of the culture tube as the cotton plug is withdrawn or replaced?

4. What advantage for growing bacteria does the agar slant tube have over a "stab" tube (here the medium is allowed to harden as the tube stands in an upright position)?

5. What qualities should the metal of an inoculating needle possess?

PROBLEM 3. To count the bacteria in milk. Instructor's OK ——

*Materials:* Four sterile petri dishes; 1 cc. and 10 cc. pipettes (these may be made up from measured and marked lengths of glass tubing); reading glass or hand lens; wax pencil; three wide-mouthed bottles; sample of fresh milk; four tubes of agar-agar culture medium; glass plate; graduated cylinder.

*Method:* Measure 99 cc. of distilled water into each of two wide-mouthed bottles, marked A and B respectively, with the wax pencil. Put 90 cc. of water into a third bottle marked C. Plug all with loosely-rolled cotton. Sterilize as for culture media in the preceding exercise. Sterilize the petri dishes and pipettes in a dry oven for several hours.

Secure a fresh sample of milk, shake

it well, and pipette 1 cc. into bottle A. Shake this mixture thoroughly, and then pipette 1 cc. of it into bottle B. (Use a fresh sterilized pipette for each separate transfer.) Bottle B now contains a 1/10,000 dilution of the original milk sample. Now pipette 10 cc. of the solution in bottle A into bottle C. Bottle C now contains a 1/1000 dilution of the original sample. After shaking it thoroughly, carefully transfer 1 cc. of the solution in bottle C into each of the two sterile petri dishes, introducing only the tip of the pipette into the dish by raising the lid slightly at one side. Mark these dishes No. 1 and No. 2 respectively. Now pipette 1 cc. from bottle B into each of two sterile petri dishes, in the same manner. Mark No. 3 and No. 4 respectively.

Melt the sterile culture medium by placing the tube in hot water. Cool slightly, and pour the contents of one tube into petri dish No. 1, raising the lid carefully at one side only enough to allow the liquid to be poured. Repeat for the remaining three dishes. Rotate each dish several times to distribute the culture medium evenly, and set aside on a glass plate to harden. Mark the bottom of each dish with the dilution figure, date, a mark to identify the milk sample, and your initials. Invert the dishes in an incubator or warm chamber at a temperature of 30 to 35 degrees Centigrade for three days. (The dishes may be placed in a warm dark room, if an incubator is not available, or a light bulb may be hung in a box and the temperature checked over a period. Different sizes of bulbs may be used to secure the temperature desired.)

After three days remove from the incubator, and with the wax pencil mark off the bottom of each dish into eight equal sectors. Holding each dish against a dark surface and using the magnifier, count the number of colonies growing on the surface of the culture medium. Ask the instructor to help you distinguish bacteria colonies from flecks of mineral or impurities in the culture medium. It is supposed that each colony started from a single bacterium. Hence, the number of colonies in one dish, multiplied by the dilution figure, represent the approximate number of bacteria in 1 cc. of the original milk sample. Too many colonies may be present in dishes No. 1 and No. 2 for accurate counting. If so, these two may be disregarded. However, all four dishes should be counted if possible, and the results from all four averaged to secure the count for final report.

# Exchange

The Kiamichi Club, a Natural History and Museum Club of the Idabel High School, Idabel, Oklahoma, is anxious to exchange natural history specimens with similar groups throughout the United States.

The Kiamichi Club has to offer herbarium specimens, reptiles, amphibians, fossils, tree fruits, stems and leaves. The club is interested in receiving marine material, amphibians, reptiles and mollusca.

If you or your school are interested won't you please communicate with our club.

Special collections will be made if desired.

Willis W. Collins
*Idabel, Oklahoma*