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A Method of Culturing Air Bacteria for Protozoa Media

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Abstract. An infusion-filtrate method for a continuous supply of air-borne bacteria is described, using either hay, soil, rice, or alfalfa for the infusion-filtrate bacterial cultures. Four cc of autoclaved infusion filtrate provided 1 cc of available bacteria each day. Three experiments each for hay, rice, soil, and alfalfa filtrate for periods of 31 and 60 days are described.

This experiment was designed to provide a continuous supply of bacteria, at a time when desired, for research with cultures of holozoic protozoa. The bacteria were supplied in concentrated and even distribution, with a continuous overlapping of the growth factors for all the bacteria over a period of time.

A method for controlling the hydrogen ion condition for amoeba cultures was described by Hopkins (1926). Hopkins and Johnson (1928) described buffer salts to adapt the amoeba to the increasing salt concentration so that a constant pH value was obtained. Needham (1937) reviewed the use of fresh sterilized hay whereby the acid condition opposes the alkaline tendancy of the culture.

Alfalfa, rice, soil, and hay-infusion filtrate were the basis for the bacterial culture media of this experiment which consisted of the following:

1. Preparation of the infusion.
2. Obtaining air-borne bacteria.
3. Preparation of the autoclaved filtrate.
4. Use of the autoclaved filtrate as a basis for the bacterial media.
5. Inoculation of the autoclaved filtrate medium with air-borne bacteria.
6. Daily requirements to maintain the bacterial cultures.

Methods and Materials

The hay, alfalfa, soil, and rice infusions were prepared differently and are described as follows:

Alfalfa infusion: Into a 1000-cc beaker, 21 grams of alfalfa with 850 cc of distilled water were added. The alfalfa contained mostly alfalfa stalks 2-3 inches in length with a few alfalfa leaves. This was boiled for 15 minutes. After boiling, the alfalfa stalks were removed and the infusion was placed in two one-pint Mason jars with approximately 250 cc in each. The infusion culture jars were left open and air-exposed for 48 hours.

Hay infusion: Placed in a 1000-cc beaker were 15 grams of hay (oat straw) and 850 cc of distilled water. This mixture was boiled for 15 minutes and then filtered through No. 40 fine filter paper. The filtrate was placed in two one-pint Mason jars, and left air-exposed for 48 hours.

Rice infusion: One-half gram of rice (29 large rice particles) and 500 cc of distilled water were boiled for five minutes. No filtering was necessary. The starch water without the rice particles was placed in two one-pint Mason jars, and left air-exposed for 48 hours.

Soil infusion: Two hundred grams of dry or damp loam top soil were used. The 200 grams of top soil with 800 cc of distilled water were placed in a large air-exposed surface jar, and agitated until the soil particles were in suspension. This was allowed to stand for 13 hours air exposed.

At 13 hours the numbers of soil infusion bacteria were quite adequate and the infusion was less likely to have small flagellates present. If the infusion was left for a longer period of time, the flagellates became a nuisance and the chance of contamination from the small flagellates increased.

After standing air-exposed these infusions were filtered and the following were made from each of the hay, alfalfa, rice, and soil infusions: (1) 10 cc of each of the infusion filtrate were placed in an open test tube to be used as air-borne bacterial inoculate, and this was not autoclaved; (2) 15 cc of each infusion filtrate—soil, hay, alfalfa, and rice—were placed in four different one-pint Mason jars.. Regular one-pint Mason jar lids and metal bands were then placed on the jars but not tightened; (3) Very small test tubes with 4 cc each of the hay, soil, alfalfa, and rice infusion-filtrates were autoclaved for 20 minutes at 15 pounds pressure. The 10 cc to be used as the bacterial inoculate were set aside.

After autoclaving, to each of the one-pint Mason jars containing the 15 cc of filtrate respectively of hay, rice, alfalfa, and soil was added one particle of rice that had been boiled for 5 minutes. The metal bands and Mason lids were left on so that

no jar was sealed after autoclaving. When the filtrates had cooled, they were ready for inoculation with bacteria.

Test tubes containing the 10 cc of non-autoclaved infusion filtrate exposed to air were checked by microscope to determine if protozoa were present. Upon determination that only bacteria were present in the infusion, one drop from the 10-cc non-autoclaved infusion was placed into the setrile one-pint filrate jars. This was done for alfalfa, rice, soil, and hay with the autoclaved bacterial filtrate corresponding to the non-autoclaved infusion with respect to hay, soil, alfalfa, and rice. Each day one of the small test tubes containing 4 cc of the autoclaved infusion-filtrate was added to the original 15 cc containing bacteria, the daily autoclaved filtrate corresponding to the bacterial culture whether rice, alfalfa, hay, or soil filtrate.

Autoclaved microscope slides may be used to exclude protozoan contamination from the bacterial sample when determining if protozoa are present. If no protozoa are present, each drop containing the infusion bacteria can be washed with one or two drops of distilled water directly into the autoclaved 15-cc filtrate culture.

All glassware was washed with a good detergent, rinsed eight times, and air dried. All autoclaving was done with 15 pounds of pressure for 20 minutes.

Micropipettes were used to obtain the bacterial sample from the bacterial culture medium. These were 5-6 inches long with a fine opening of one to two millimeters. After each usage these pipettes were autoclaved to avoid contamination before being used again.

To avoid air contamination of the autoclaved bacterial filtrate medium, the Mason jars were opened only sufficiently to allow the daily addition of the 4 cc of autoclaved filtrate and to withdraw bacteria from the medium.

Experiment 1. Three identical experiments for each of the four autoclaved bacterial infusion-filtrate cultures (hay, soil, rice, and alfalfa) were established for a 31-day period. After inoculation with bacteria, the daily 4 cc autoclaved filtrate was added. At 5-7 days, 1 cc of bacteria was withdrawn from each bacterial culture. This daily withdrawal continued throughout the 31 days. A determination of the pH was made every third day.

Experiment 2. Three identical experiments for each of the bacterial filtrate media (hay, soil, rice, and alfalfa) were established for a 60-day period. After inoculation with air-borne bacteria the daily 4-cc autoclaved filtrate was added. A random withdrawal of bacteria was tested throughout this experiment. The pH determination was made at the beginning, and at the

conclusion of the 60-day experiment. Accutint test paper (pH) was used.

Discussion

Throughout this study, dark field of a microscope was used to observe the condition of the bacteria filtrate cultures, The alfalfa bacteria medium provided dense, concentrated numbers of bacteria. The hay medium provided excellent numbers of bacteria evenly distributed throughout the medium. The soil medium provided good numbers with diverse types of bacteria. The rice medium was poor in numbers throughout the experiment.

At the beginning of both 31- and 60-day experiments, the cool, fresh, autoclaved filtrate gave the following results: hay 6.6 pH, alfalfa 5.9 to 6.0 pH, rice 6.3 pH, and soil filtrate 6.3 pH. At conclusion of the 31-day experiment results were as follows: hay 7.9 to 8.0 pH, alfalfa 8.4 to 8.5 pH, rice 6.4 pH, and soil filtrate 6.4 pH.

The termination of the 60-day study gave values higher than those for the 31-day experiment. These values were as follows: hay 8.5 pH, alfalfa 8.8 pH, rice 6.6 pH, and soil 6.6 to 6.8 pH.

The pressure exerted against the 60-day experiment was an actual demand to inoculate protozoan holozoic cultures. Some days no bacteria were used from the bacterial cultures, and other days more than 1 cc were withdrawn during the 60-day experiment.

Subculture can be made directly from the original bacterial filtrate cultures, once started. The danger from molds is reduced with autoclaved micropipettes, and in only one culture did mold develop during this study.

With comparable experimentation the carbon dioxide content of the distilled water, the type of soil, and the condition of the hay and alfalfa may change pH values.

This experiment required little daily maintenance and gave a good source for air-borne bacteria.

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