desiccator. The use of a graduated cylinder for the O_2 has been previously described by Johnstone[2], but was developed independently by the authors. These changes greatly facilitate the measurement of the O_2 and the changing of the KOH solution and make possible more accurate determinations.

To determine the respiration rate the material to be studied is weighed into a round wire mesh basket that fits into the desiccator. This basket has a tubulature through the center so that when it is placed in the desiccator the stem of the upper funnel can extend down to the lower funnel. After sealing the lid on the desiccator, the upper funnel is fitted into the lid and a solution of twice normal KOH (usually 25 ml) is drained into the lower funnel through the upper funnel and rinsed down with a small quantity (10 ml) of water. The O_2 cylinder is connected to the beaker "B" and the clamp at "1" opened. The desiccator is then connected to the O_2 cylinder and the clamp at "2" opened. The lid must be sealed airtight to the desiccator and all connections must be airtight.

As the material respires, CO_2 is given off and O_2 absorbed. The CO_2 given off is absorbed by the KOH solution in the lower funnel and the O_2 used is replaced by O_2 from the cylinder C. The O_2 withdrawn from C is replaced by water from B. Thus the system remains at atmospheric pressure and the concentration of O_2 and CO_2 in the atmosphere in the respiration chamber remains practically unchanged.

At the end of a run the clamp at "2" is closed and the KOH solution in the lower funnel drained into a flask. The lower funnel is then rinsed into the flask by adding water through the upper funnel. Fresh KOH is added through the upper funnel, the amount of water in the cylinder recorded, the clamp at "2" reopened and the respiration determined continued. The KOH is titrated against standard 2 normal H_2SO_4 to the phenolthalein end point and then to the methyl orange end point and the amount of CO_2 computed from the difference between the two end points. The volume of water drawn into the cylinder represents the amount of O_2 consumed by the material after it has been corrected for changes in barometric pressure during the run, for O_2 and N_2 dissolved in the water and has been converted to standard conditions of pressure and temperature.

The humidity in the respiration chamber may be controlled by placing different concentrations of H_2SO_4 in the bottom of the desiccator around the lower funnel. It is desirable that the size of the sample be so regulated that about 200 to 300 cc of O_2 are used and about a third of the KOH neutralized by the CO_2 evolved during a run. When this is done there is no appreciable accumulation of CO_2 in the respiratory chambers. If about half or more of the KOH is neutralized the phenolthalein end point in the titration becomes indistinct and there is likely to be an accumulation of CO_2 in the respiratory chambers due to the decreased efficiency of the KOH solution. On the other hand, if less than 100 cc of O_2 are used during a 24 hour period, a lowering of the atmospheric pressure of more than .3 inch may cause the gas in the desiccator to expand more than 100 cc and result in a flow of gas from the respiration chamber to the oxygen cylinder.

[2] G. R. Johnstone, "Physiological Study of Two Varieties of *Ipomoea batata*," *Bot. Gaz.*, 80: 145–167, 1925.

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VEGETABLE PEPTONE AGARS FOR QUANTITATIVE WORK WITH LACTOBACILLUS ACIDOPHILUS[1]

LACTOBACILLUS ACIDOPHILUS does not grow well on plain nutrient agar. To meet this difficulty, various special media have been suggested from time to time. The most widely used of these are: the whey agar proposed by Rettger and Cheplin,[2] 1912; the galactose agar of Rettger and Kulp,[3] 1922; the casein digest galactose agar of Kulp and Rettger,[4] 1924; the whey peptone galactose agar of Kulp,[5] 1926, and the tomato peptone agar of Kulp,[6] 1927. The third and fourth named are made in the dehydrated form by the Difco Laboratories, Detroit, Mich.

In connection with work on another problem in which we used acidophilus milks made with the "Scav" strain, we amassed considerable quantitative data consisting of a series of parallel counts on galactose whey agar and a cabbage agar which proved very satisfactory.

[1] Read at the Baltimore meetings of the Society of American Bacteriologists, Dec., 1931.

[2] L. F. Rettger and H. A. Cheplin. "The Transformation of the Intestinal Flora with Special Reference to the Implantation of *Bacillus acidophilus*," Yale University Press, 1921.

[3] L. F. Rettger and Walter L. Kulp, "A Note on the Choice of Culture Media for the Study of Lactobacillus with Special Reference to the Carbohydrate Employed," *Abs. Bact.* 6, 24, 1922.

[4] W. L. Kulp and L. F. Rettger, "A Comparative Study of *L. acidophilus* and *L. bulgaricus*," *Jr. Bact.*, 9, 357–394, 1924.

[5] W. L. Kulp, "The Determination of Viable *Lactobacillus acidophilus*," SCIENCE, 64, 304–306, 1926.

[6] Walter L. Kulp, "An Agar Medium for Plating *L. acidophilus* and *L. bulgaricus*," SCIENCE, 66, 512–513, 1927.

The cabbage agar was prepared in liter quantities by shredding 250 grams of fresh cabbage, adding one liter of tap water, and boiling for one half hour. The decoction was then decanted off, the loss of water restored, and the liquid filtered through filter paper. To this liquid, ten grams of peptone (Difco), five grams of sodium chloride, two and one half grams of ortho sodium phosphate, and ten grams of Difco agar were added. The mixture was heated in the steamer until the ingredients were in solution. A very clear medium was obtained by sedimentation and removal of detritus by decantation of the clear liquid. This medium was sterilized in the autoclave at fifteen pounds pressure for fifteen minutes. The reaction varied somewhat but the pH was usually about 6.4 which is within the range for the good growth of the Lactobacilli, so that we have not regarded it necessary to adjust the reaction. A few experiments indicate that the sodium orthophosphate may be omitted from the formula without decreasing the value of the medium, and in some other experiments both the phosphate and the peptone have been omitted without decreasing its value as a culture medium.

In the process of plating, the medium was poured directly from bottles or flasks into the Petri dishes, about 16 or 20 cc per plate. When the agar had hardened, the plates were inverted and placed in closed containers and surrounded with an atmosphere of approximately ten per cent. carbon dioxide. The plates were incubated at 40° C. for 48 hours. The excessive amount of water of condensation was absorbed by the use of clay tops or by fitting disks of blotting paper into the tops of the Petri dishes.

A simple microscope, magnifying three and one half times, was used in counting.

The type of colony which appears on cabbage agar shows the rough irregular edge which has been considered characteristic of *L. acidophilus*. On the galactose whey agar there is a tendency for the growth to be compact, while the cabbage agar shows a thinner, somewhat more transparent, and more spreading colony. Although the colonies on the cabbage agar are more transparent, they are easily seen with the naked eye because of their larger size. The first fifty colonies taken at random on each of two plates, which were exact duplicates except for the medium, were measured with an ocular micrometer. The fifty colonies on the whey agar averaged 396 microns in diameter, while the same number on the cabbage agar averaged 531 microns.

Our tabulated quantitative data shows very plainly two interesting trends: first that the cabbage agar counts are higher than the galactose whey agar counts with consistent regularity, and second, that the higher counts on the cabbage agar are especially pronounced on the milks that are from two to three weeks old. Thus in a group of forty-five counts on milks which were from one to seven days old, the ratio of the galactose whey count to the cabbage agar count was as 1:1.55; on milks eight to fourteen days old, the ratio was 1:2.94; on milks sixteen to twenty-one days old, the ratio was 1:2.28, and on milks twenty-two to twenty-eight days old, the ratio was 1:2.17. This may be taken to indicate, it seems to us, that there are substances in the cabbage which stimulate the old bacterial cells in the milk to a vigorous growth, while such substances are lacking in the galactose whey agar. This would seem to be a desirable quality for a medium to possess if it is to be used for controlling a commercial product since it seems fair to assume that similar stimulating substances may exist in the intestinal tract of man. At any rate, the results indicate that many bacteria which are supposedly dead when their viability is tested by seeding in galactose whey agar, are alive and vigorous in our cabbage agar.

The method described for making cabbage agar was used in the preparation of other vegetable agars. Carrots and green beans were used in the same proportions as the cabbage. In tomato and spinach the proportion of the vegetable was doubled. Growth on green bean and spinach agars was very good, yet our data seem to indicate that these two vegetables are slightly less favorable than cabbage. Carrot and tomato agars, on the other hand, gave results very similar to those obtained with cabbage.

Since some milk product has been so generally used in the various media recommended for the growth of *L. acidophilus,* and since we found extracts from various vegetables to be very favorable, it occurred to us that a combination of such materials might be still more favorable. With this in mind, a tryptic casein digest prepared according to the method of Kulp and Rettger,[4] was added to our cabbage agar. With this medium we have obtained on some plates colonies which average twice the size of those on our vegetable media, but in sharpness of definition and in quantitative results, such media appear to offer no advantages over the simple vegetable media.

We believe that our results should recommend the use of a simple vegetable medium for quantitative studies on acidophilus milks because of the ease and simplicity of preparation; because of the characteristic appearance, the sharpness of definition, and size of the colony, and because of the somewhat higher count which is especially marked with the older milks.

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