SUBSTITUTION OF BROM-THYMOL-BLUE FOR LITMUS IN ROUTINE LABORATORY WORK

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Received for publication December 10, 1921

One of the common methods for the qualitative determination of acid or alkali production by bacteria is to inoculate nutrient extract broth containing various carbohydrates with litmus as an indicator.

Litmus possesses the disadvantage of being reduced by many organisms to a colorless compound, thus rendering it useless as an indicator. Clark and Lubs (1917) mention that the sulphonphthalin indicators are much more resistant to bacterial action than indicators like methyl red or litmus. They suggest, because of certain preliminary tests, that indicators like brom-thymol-blue and brom-cresol-purple might be used to advantage in replacing other indicators which are now used in making indicator media.

With this suggestion in mind, an experiment was undertaken to find a method of preparing media to determine qualitatively acid or alkali production by bacteria, which would be easy to prepare, and more sensitive than litmus; and one in which the reaction could be quickly determined at any time during the incubation period.

In this experiment, sugar free broth was used, which was prepared as follows:

One pound of ground lean meat was digested for two hours with 1 liter of distilled water. After cooking, the broth was filtered through absorbent cotton into a flask and sterilized in the autoclave at 18 pounds pressure for twenty minutes. When cold the broth was inoculated with a culture of *Bact. saccharolyte* (Rivas)

and incubated at 37°C. for forty-eight hours to render the medium sugar-free. Then the medium was sterilized in the Arnold for twenty minutes; 10 grams of peptone and 5 grams of sodium chloride were added; the reaction was adjusted to pH 7.0 with brom-thymol-blue; the medium was again steamed for twenty minutes, the reaction readjusted, and the medium filtered.

To determine the amount of brom-thymol-blue which would inhibit acid production by microorganisms, fifty cubic centimeters of sugar free broth were placed in each of fifteen flasks.

TABLE 1

The influence of brom-thymol-blue upon acid production by Bact. coli-communis. Using 1 per cent glucose broth, initial pH 7.0, increasing amounts of a 0.2 per cent alcoholic solution of brom-thymol-blue inoculated with 0.1 cc. of an eighteen hour broth culture

LENGTH OF INCUBATION	DILUTIONS OF BROM-THYMOL-BLUE													
	0	1:125,000	1:62,500	1:41,666	1:31,250	1:25,000	1:20,750	1:17,810	1:15,610	1:13,900	1:12,500	1:11,360	1:10,420	1:9,650	1:8,875
hours															
2	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
2½	+	+	+	+	+	+	+	+	+	+	+	+	+	−	−
3	++	++	++	+	+	+	+	+	+	+	+	+	+	+	+
4	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++

− = No acid production; + = moderate acid production; ++ = strong acid production.

To each flask was added sufficient 0.2 per cent alcoholic solution of brom-thymol-blue to give the concentrations as shown in the tables. Equal amounts of the media were placed in five test tubes of similar size, and the tubed media were sterilized in the autoclave at 18 pounds pressure for twenty minutes.

When the tubes were cold, one cubic centimeter of a sterile 20 per cent glucose solution was added, under aseptic conditions, to each tube. The tubes were incubated at 37°C. for forty-eight hours, in order to make certain that none of them became contaminated during the process of adding the glucose solution.

TABLE 2

The influence of brom-thymol-blue upon acid production by Bact. typhosum, using media and dilutions as in table 1

LENGTH OF INCUBATION	DILUTIONS OF BROM-THYMOL-BLUE													
	0	1:125,000	1:62,500	1:41,666	1:31,250	1:25,000	1:20,750	1:17,810	1:15,610	1:13,900	1:12,500	1:11,360	1:10,420	1:9,650	1:8,875
hours															
2	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
4	+	+	+	+	+	+	−	−	−	−	−	−	−	−	−
5	+	+	+	+	+	+	+	+	+	+	+	−	−	−	−
6	++	++	++	++	++	++	+	+	+	+	+	+	+	+	+

One series of the tubes was inoculated with 0.1 cubic centimeter of an eighteen-hour culture of *Bact. coli-communis;* the second set was inoculated with the same amount of *Bact. typhosum;* the third with *Staphylococcus albus;* the fourth with *Bacillus subtilis;* and the fifth series was left as a check. All five sets of tubes were incubated at 37°C.

The data in the tables show that brom-thymol-blue did not have a marked effect of inhibition on the acid production by the Gram negative *Bact. coli-communis* or *Bact. typhosum* and inhibited the two Gram positive organisms only in concentrations

TABLE 3

The influence of brom-thymol-blue upon acid production by Staphylococcus albus, using media and dilutions as in table 1

LENGTH OF INCUBATION	DILUTIONS OF BROM-THYMOL-BLUE													
	0	1:125,000	1:62,500	1:41,666	1:31,250	1:25,000	1:20,750	1:17,810	1:15,610	1:13,900	1:12,500	1:11,360	1:10,420	1:9,650	1:8,875
hours															
2	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
4	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
5	+	+	+	+	+	−	−	−	−	−	−	−	−	−	−
6	++	++	+	+	+	+	+	+	+	+	+	+	+	−	−
7	++	++	++	+	+	+	+	+	+	+	+	+	+	+	+

which were much higher than need to be used in actual acid determinations.

Bact. paratyphosum A, *Bact. dysenteriae*, *Bact. sanguinarium*, *Bact. pullorum*, and *Bact. enteritidis* were also tested out in the same dilutions shown in the tables. Results were obtained closely approximating those for *Bact. typhosum.*

It has been found by experience that a 1:41,666 solution of brom-thymol-blue gives the most desirable concentration for colorimetric comparison. The data in the tables show that this concentration can be used in the media without inhibiting the

TABLE 4

The influence of brom-thymol-blue upon acid production by Bacillus subtilis, using media and dilutions as in table 1

LENGTH OF INCUBATION	DILUTIONS OF BROM-THYMOL-BLUE													
	0	1:125,000	1:62,500	1:41,666	1:31,250	1:25,000	1:20,750	1:17,810	1:15,610	1:13,900	1:12,500	1:11,360	1:10,420	1:9,650	1:8,875
hours															
2	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
3	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
4	+	+	+	−	−	−	−	−	−	−	−	−	−	−	−
5	+	+	+	+	+	+	+	+	+	+	+	−	−	−	−
6	++	++	++	+	+	+	+	+	+	+	+	+	+	−	−
7	++	++	++	++	+	+	+	+	+	+	+	+	+	+	+

production of acid. This dilution is easily made by adding 12 cc. of a 0.2 per cent alcoholic solution of the indicator to every liter of sugar free broth before it is put into the fermentation tubes.

Media prepared in this way are now used in this laboratory by the students, and have been found successful for all their qualitative acid production tests.

It has also been used by a member of the department in determining acid production by a certain bacterium, upon about twenty carbohydrates. In this particular instance, readings of the reaction of the cultures were made every day over a period of four weeks of incubation.

The advantages of this medium are:

1. Brom-thymol-blue includes the neutral point in its range of hydrogen-ion concentration, so that the medium can be adjusted to exact neutrality before being inoculated.

2. A medium containing sufficient brom-thymol-blue to act as an indicator will not inhibit acid production.

3. Brom-thymol-blue is not reduced by microbial action.

4. The reaction of a carbohydrate medium containing brom-thymol-blue can be recorded at any time during incubation.

5. Changes in color with slight changes in hydrogen-ion concentration are more marked with brom-thymol-blue than with litmus.

6. Brom-thymol-blue is easier to prepare than litmus.

7. Heat does not affect brom-thymol-blue during sterilization.

8. The reaction of carbohydrate broth containing brom-thymol-blue can be read by artificial light, but this is impossible with litmus.