TECHNICAL NOTES

DIRECT ANALYSIS OF LACTOSE IN MILK AND SERUM[1]

Barnett and Tawab (1) showed that the colorimetric phenol-sulfuric acid method of Dubois *et al.* (2) could be used for the direct determination of lactose in milk and cheese. The authors stated that the amount of phenol added was not critical, provided that the same quantity was used throughout. However, variations which occurred in our replicate analyses of standard lactose solutions were traced to minute differences in the amount of phenol reagent added. No information appeared to be available concerning the effect of sulfuric acid concentration on color development. The effect of both reagents on the determination of lactose was, therefore, studied.

Intensity of the color obtained with various concentrations of phenol (Figure 1-A) was maximum between 5 to 12 mg/ml of total arithmetic volume. In comparison, the concentrations used by Barnett and Tawab (1) and Dubois *et al.* (2) were approximately 18.5 and 15.5 mg/ml, respectively; these levels are outside the optimum range and can cause variation in color intensity if not made very accurately. The concentration of sulfuric acid also influenced the intensity of the color (Figure 1-B). Maximum intensity was obtained when the volume of sulfuric acid comprised from 70 to 75% of the total arithmetic volume, a proportion similar to that recommended by previous authors (1, 2).

On the basis of these investigations, we recommend the following procedure, which introduces 11 mg. of phenol and 0.74 ml. of sulfuric acid per milliliter of total arithmetic volume.

PROCEDURE

Pipette 2.0 ml. of test solution (10-75 μg. of lactose), or standard lactose monohydrate solution, or water (for the blank) into a colorimeter tube, followed by 0.10 ml. of 89% w/v phenol reagent (20 ml. water + 80 g. phenol, dissolved at 50° C.; the reagent is stable for at least 4 mo. at room temperature). Add 6.0 ml. of concentrated sulfuric acid slowly, letting the acid run down the side of the tube. Then, swirl to obtain good mixing, let stand for 10 min. at room temperature, and read at 490 mμ (the 470-mμ filter on an Evelyn colorimeter is equally satisfactory). Color is stable for at least 1 hr.

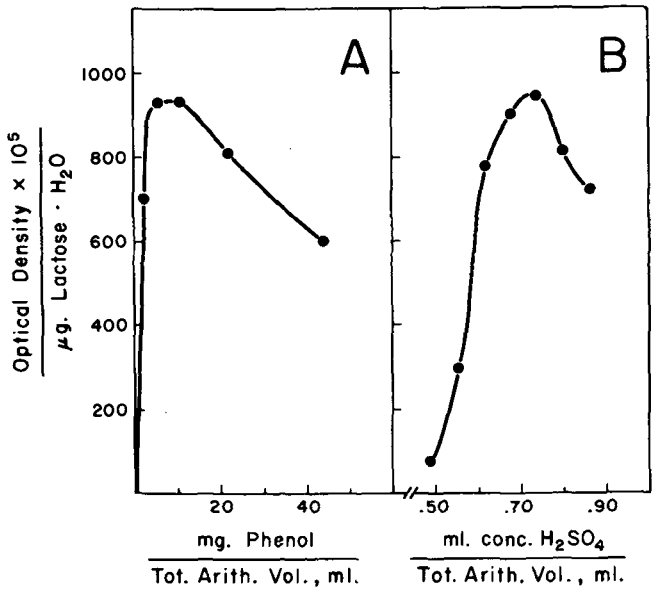

FIG. 1. Effect of concentration of reagents on color intensity. (Total arithmetic volume, 8.1 ml.; readings made at 470 mμ on an Evelyn colorimeter.)

(A) Effect of phenol (0.74 ml. of sulfuric acid/ml).
(B) Effect of sulfuric acid (11 mg. of phenol/ml).

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REFERENCES

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(2) DUBOIS, M., GILLES, K. A., HAMILTON, J. K., REBERS, P. A., AND SMITH, F. Colorimetric Method for Determination of Sugars and Related Substances. *Anal. Chem.*, 28: 350. 1956.

EFFECTS OF PENICILLIN ON THE MORPHOLOGY OF *STREPTOCOCCUS LACTIS*, *STREPTOCOCCUS THERMOPHILUS*, and *LEUCONOSTOC DEXTRANICUM* [1]

Numerous reports have appeared in the literature on the effect of penicillin upon the growth and acid production of lactic acid bacteria. Inhibition of growth or of acid production by penicillin in lactic cultures is the basis for tests that have been developed to detect penicillin and other antibiotics in the milk supply. Reviews by Overby (7), by Stoltz and Hankinson (9), and by Berriidge (3) cover the subject thoroughly.

Werber-Alture *et al.* (10) and Pulvertaft (8) reported that penicillin, in low concentrations, caused morphological changes in *Escherichia coli* cultures. Chain *et al.* (4) observed this same reaction using *Staphylococcus* cultures. Gardner (5) observed morphological changes caused by penicillin in a variety of cultures. Baughman and Nelson (2) reported similar results with some lactic cultures after this study was in progress.

This investigation was made to determine the effect of penicillin on the morphology of certain lactic acid bacteria.

EXPERIMENTAL PROCEDURE

Cultures of *Streptococcus lactis, Streptococcus thermophilus,* and *Leuconostoc dextranicum,* obtained from the Department of Bacteriology, University of Wisconsin, Madison, were used in the study. The cultures were grown in 10% sterile reconstituted nonfat dry milk and transferred in the usual manner. *S. lactis* and *S. thermophilus* were used for inoculum after incubation at 37° C. for 8–10 hr.; *L. dextranicum* after incubation for 16 hr. at 25° C.

Penicillin (G Potassium)[2] was prepared in sterile buffered aqueous solution, dispensed in 1-ml. portions (1,000 units) in sterile test tubes and immediately placed in storage at — 15° C., until needed for the tests.

When removed from storage, the 1-ml. portions of penicillin were thawed at room temperature and diluted 1:1,000 in sterile buffered distilled water just prior to use. The 1:100 and 1:1,000 dilutions were dispensed into sterile test tubes to give final concentrations in a 10-ml. volume of 0.025, 0.05, 0.10, 0.20, and 0.50 units/ml. A control containing no penicillin was prepared in each series of tests. Sufficient sterilized antibiotic-free skimmilk (10% reconstituted NDM) was added to bring the volume of the mixture up to 9.5 ml. in each tube. Next, 0.5 ml. of the test culture was added per tube, bringing the total volume in each tube to 10 ml. Mixing of the contents was accomplished by inverting the tubes three times. Methylene blue stains (1) were made on each test after 30, 45, 60, and 120 min. incubation at 32 and 37° C. for *S. lactis* and *S. thermophilus;* for *L. dextranicum,* stains were made after 4, 8, and 16 hr. incubation at 25° C. These stains were examined with a microscope, using oil immersion, and compared to the control to observe changes in morphology.

Two cows of the Agricultural Experiment Station herd were given, in each quarter, infusions of an antibiotic mixture containing 100,000 units of penicillin G, 250 mg. dihydrostreptomycin, 250 mg. sulfisoxazole, 750 mg. sulfathiazole, and 5 mg. cobalt sulfate suspended in 3% aluminum monostearate.[3] Samples were collected from each cow in sterile containers for six consecutive milkings spaced at 12-hr. intervals.

These samples were tested for effects on the morphology of a *S. thermophilus* test culture. For each sample, tests were performed with the milk as obtained and after dilutions with antibiotic-free raw milk of 1:10, 1:100, 1:1,000, and 1:10,000. This procedure gave an indication of the concentration of antibiotics in milk from the various milkings. In the test, 9.5 ml. of a sample (as drawn or as diluted) were placed in a sterile test tube to which 0.5 ml. of culture was added, tubes were inverted to mix contents, and were placed in a 37° C. bath for 1 hr. Methylene blue stains were prepared for examination and compared with those of a control test with antibiotic-free milk.

[1] Florida Agricultural Experiment Station Journal Series No. 875.

[2] Chas. Pfizer and Company, New York.

[3] Fort Dodge Laboratories, Fort Dodge, Iowa.