

A Modified Morse-Kopeloff's Anaerobic Culture Method.

By

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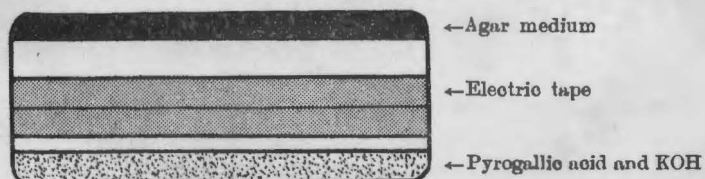
In the course of our investigation on the thermophilic bacteria, it was necessary to find an anaerobic culture method which stands against high temperature and also resists the evaporation. Although several such methods have been reported previously, most of them were not available for our use owing to such factors as high cost, complexity of method or inability to stand for high temperature as 65°C. The most promising method was the one reported by TETRAULT¹⁾ who modified MORSE-KOPELOFF'S²⁾ anaerobic plate culture method. However it was necessary to be modified in order to meet our purpose, and it will be reported as follows.

Description of Method :

As in MORSE-KOPELOFF'S method²⁾, two Petrie dishes of the same diameter were put their faces together and sterilized as usual, and into one of them, the culture medium with the organism is poured and on the solidification, the dish is turned up side down. Then in the lower dish, a piece of absorbent cotton is placed to which 0.5—1.0 g. pyrogalllic acid and 10—15 cc. of 10% KOH are added. Around the joint, Arabian gum is painted over which an electric tape was wound three—four times. The plate thus prepared can be incubated at high temperature viz. 65°C. for a considerable time without losing water. The finished plate is shown in Fig. I.

Fig. I.

Modified Morse-Kopeloff's Anaerobic Culture Dish.



Experimental :

In order to test the efficacy of the method, the following chemical and biological tests were carried out ;

I. A test for the anaerobiosis was carried out by using 10 cc. of 2% glucose agar which contained 0.1 cc. of 1% aqueous methylene blue and the reaction was kept at P_H 7.8, was poured and the dish was treated as described above. No oxidation of the dye took place which indicates the absence of oxygen.

II. *Clostridium pastorianum* was cultured by this method and succeeded in getting the good growth of colonies as shown in Plate XXIV.

Discussions :

In this proposed method, the pre-existing methods were modified in the following two aspects ;

1. The electric tape was substituted for other materials to wind around the joint.

2. Much less quantity of pyrogallol acid and potassium hydroxide were used.

The use of electric tape is recommended as it is readily available at low cost and serves the purpose admirably.

The amount of pyrogallol acid and the alkali indicated herein, is sufficient according to KOVACS-ZORKOCZY³⁾ whose investigation indicates that two gram-molecules of pyrogallol acid are necessary to absorb one gram of oxygen. Since the inside volume of the Petrie dish chamber is about 270—300 cc. of air space which contains ordinarily 0.0325 g. of oxygen, theoretically 0.26 g. pyrogallol is all necessary. However to insure the complete absorption, 0.5—1.0 g. pyrogallol is recommended.

Again as it has been stated previously, the absence of oxygen was experimentally proven chemically as well as biologically.

Summary.

The method reported here serves satisfactorily for the anaerobic plate culture of bacteria at different temperature especially at high temperature.

References :

- 1) P. A. TETRAUT, *Jour. Lab. and Clin. Medicine*, 15: 177, 1929.
- 2) S. MORSE and N. KOPELOFF, *Jour. of Public Health*, 12: 119, 1922.
- 3) E. VON KOVACS-ZORKOCZY, *Biochem. Zeitsch.*, 162: 161, 1925.

PLATE XXIV.

Colony of *Cl. pastorianum*, 3 days old culture
on glucose agar, at 28°C.

(2/3 original size.)

