STUDIES ON SARCINA VENTRICULI¹
I. Stock Culture Method

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Study of Sarcina ventriculi (Zymosarcina ventriculi) has been encumbered by the absence of a satisfactory stock culture method. Smit (1930), as well as Milhau et al. (1956) reported that transfers of the organism at 1- or 2-day intervals were necessary to keep viable cultures. In this paper a method is described in which a viable stock culture may be maintained for a period of 2 months. A brief report of some of these findings has appeared (Canale-Parola and Wolfe, 1959).

EXPERIMENTAL METHODS

Isolation. The organism was isolated using a method similar to that described by Beijerinck (1905) and Smit (1930). About 2 g of mud from a local polluted stream were inoculated into a 60-ml glass-stoppered bottle which had been completely filled with a liquid medium (medium A) consisting of malt extract (Difco), 10 g; maltose (Pfanstiel, technical grade), 2 g; and deionized water, 100 ml. Prior to inoculation the pH was adjusted to 2.3 with sulfuric acid, and the medium was heated to near boiling temperature and then rapidly cooled. The inoculated bottles were incubated at 37 C. After 16 to 20 hr or when violent gassing was visible in the bottles, about 1 ml of the sediment which contained packets of cells was transferred to freshly prepared bottles of the same medium. After a similar incubation period, a third transfer of the enrichment culture was made using the same technique. From the third enrichment bottle a serial dilution of an aliquot of the sediment was made at 45 C in tubes containing a melted agar medium (medium B) consisting of malt extract, 10 g; maltose, 2 g; agar, 1.5 g; and deionized water, 100 ml. The pH of this medium was not adjusted. The contents of each test tube was poured into a separate petri dish, and after solidification, about 10 ml of the same medium were added to each plate to form a thin layer over the surface. After incubation at 37 C, a typical, whitish-brown, multilenticular colony was picked and inoculated into a 16 by 150 mm standard test tube containing 10 ml of a liquid medium (medium C) of the following composition: glucose, 2 g; yeast extract (Difco), 2 g; and deionized water, 100 ml.

Stock culturing. Two methods of maintaining stock cultures were used:

1. Growing the cells in a standard test tube containing 10 ml of a growth-limiting liquid medium (medium D) consisting of glucose, 2 g; yeast extract, 0.2 g; and deionized water, 100 ml. The cultures were incubated at 30 or 37 C.

2. About 300 ml of a medium (medium E) consisting of yeast extract, 1 g; glucose, 2 g; agar, 1.5 g; and deionized water, 100 ml, were added to a 750-ml Erlenmeyer flask. The flask was stoppered with a cotton plug and the entire mouth of the flask was covered with several layers of cheesecloth. After sterilization the flask was allowed to stand at room temperature for approximately 1 week or until the medium was free of surface water. Then, by means of a sterile 10-ml pipette, the tip of which had been heated in a flame, a depression about 8 mm deep and 8 mm in diameter was melted in the center of the medium in the flask. By means of a sterile pipette, a drop, containing cells from a culture which was actively gassing, was placed in the depression. The stock culture was then incubated at 30 or 37 C.

Chemical. Products from the fermentation of glucose were detected by use of a fermentation train and specific analytical methods as described by Neish (1952). The fermentation was carried out in medium C under a nitrogen atmosphere.

RESULTS

Identification of the organism. The organism which was isolated formed packets of eight or more cells (figure 1); these packets were often

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irregularly shaped. The diameter of the individual cells varied from 3 to 4 μ as measured with a calibrated ocular micrometer. Growth at 37 C occurred at a faster rate than at 30 C. The organism exhibited a negative catalase reaction and positive cellulose reaction as described by Smit (1930).

Strictly anaerobic conditions were not necessary for growth; the organism was grown in test tubes containing broth, without special anaerobic precautions. In liquid media the cells formed a white sediment on the bottom of the tube; the liquid remained clear and, during fermentation, small gas bubbles rose upwards from the sediment, producing foam on the surface of the liquid.

The analysis of fermentation products showed that, per mole of glucose fermented, the organism produced the following moles of products: H₂, 1.4; CO₂, 1.9; ethanol, 1.0; volatile acids (calculated as acetic acid), 0.6; lactic acid, 0.1; and a trace of acetoin. These results are in good agreement with those presented by Milhaud et al. (1956).

Viability in stock culture. When *S. ventriculi* was grown in the growth-limiting broth (medium D) and kept at 30 or 37 C, the organism remained viable for approximately 7 days. If, at the end of this period of time, the cells were transferred to medium C, growth occurred within 24 hr. Viability was similarly prolonged if glucose was the limiting component of the medium.

The agar depression technique gave better results, and it is currently used to maintain viable cultures. The organism, when grown in Erlenmeyer flasks in the manner described (figure 2), was viable for at least 2 months if kept at 30 or 37 C. Viability could be maintained indefinitely, if a 2-month-old culture was transferred to a test tube containing medium C and then, after overnight growth, transferred again to a sterile agar depression. It is important that the surface of the solid medium in the flask be dry; the success of the stock culture depends upon growth occurring only in the depression and, if present, moisture may carry some of the cells to the sides and to the bottom of the flask, where they rapidly multiply. When this happens, the organism soon loses its viability. The function of the depression is to prevent the cells from spreading over the surface of the medium; in addition, growth is initiated more readily if the organism is inoculated in the depression than if it is inoculated.
on the flat surface. Generally growth occurs on the bottom and sides of the depression but sometimes appears on the surface immediately around the depression.

**DISCUSSION**

The morphological and physiological characteristics, together with the fermentation products establish that the organism isolated is a strain of *S. ventriculi*.

The ability of the organism to survive under the conditions provided by the two stock culturing methods suggests that a product (or products) of metabolism may be toxic to the organism. If a growth-limiting medium is used, the amount of toxic product formed may not be sufficient to cause rapid death, and when the depression technique is employed the toxic product may be diluted throughout the relatively large amount of solid medium.

The pH of the medium is probably not involved in the rapid loss of viability; both the growth-limiting medium (D) in which the cells remain viable for approximately 7 days and the regular growth medium (C) in which the organism loses its viability after 2 days, have roughly the same pH of 4.2 to 4.5 after inoculation and 48 hr of incubation at 37 C. Sensitivity of the organism to oxygen is apparently not involved in rapid loss of viability. If the two methods of stock culturing are combined by using a growth-limiting medium in the agar depression technique, the viability of the cultures is not appreciably prolonged.

**SUMMARY**

A technique by which viable stock cultures of *Sarcina ventriculi* can be kept for at least 2 months was developed. It is suggested that a metabolic product toxic to the organism may be responsible for the rapid loss of viability which is characteristic of cultures of this species.

**REFERENCES**


