TRANSLATION NO. 661

DATE: July 1968

DDC AVAILABILITY NOTICE

This document is subject to special export controls and each transmittal to foreign governments or foreign nationals may be made only with prior approval of Commanding Officer, Fort Detrick, ATTN: SMUFD-AE-T, Frederick, Md. 21701

DEPARTMENT OF THE ARMY
Fort Detrick
Frederick, Maryland

JPRS: 12547

CSO: 6533-D

SIMPLE METHOD FOR THE CONTINUOUS CULTURE OF MICRO-ORGANISMS UNDER STATIONARY CONDITIONS

- USSR -

(Following is a translation of an article by V. A. Kordyum in the Ukrainian-language periodical Mikrobiologichniy Zhurnal (Microbiological Journal) Vol. XXIII, No. 2, Kiev, 1961, pages 73-75)

Nearly all basic methods for sustaining microorganisms in a culture create so many sharp differences from the conditions of existence in natural substrates that the majority of the bacteria, fungi and even actinomycetes, when they are kept in the laboratory, sharply alter their physiology and morphology, degenerate, and, in a number of cases, die. This is explainable by the unnatural composition of the feeding medium and stagnation. The cause of the latter condition is the extenuation of the feeding medium and accumulation of waste products.

Stagnation phenomena can be eliminated only by continuous culturing. Continuous, or flow, culturing was first developed by the Soviet investigator, Ut'onkov. A distinct feature of this method is the constant renewal of the medium in the vessels where the growth of the microorganisms takes place. Simultaneous with the addition of the fresh substrate, a portion of the old culture fluid and a certain amount of cells are poured out. This ensures the continuous development of the

population or a fresh medium without a considerable amount of waste products.

Continuous cultures are now widely used in microbiology. However this method has some substantial defects: the complexity and cumbersomeness of the apparatus and the necessity for the systematic inspection of its operation. In addition to this, the possibility of contamination increases.

The simplest apparatus for continuous culture is made up of three vessels, mounted on any type of support. Three or four or even more compact apparatuses could not be placed in a thermostat, while ten of them would occupy the entire volume of the thermostat chamber. As regards the construction of the apparatus, one apparatus can occupy a whole room and would require two or three service personnel units.

All of this leads to the fact that even laboratories using the continuous culture method have only several sets of apparatus. Parallel repetitive tests are absent; reports on research variations are at a minimum.

For just such reasons we undertook the task of changing the continuous culture technique so that the operation by this method would not be any more complex than the work with Petri dishes. The theory of the continuous method is this: microorganisms in a compact feeding medium, upon being sown at one point, grow locally and create colonies. In a diluted medium growth occurs over the entire area and it is too rapid. In second case rapid propagation of the cells occurs due to streams of liquid, in the first part of which propagation is not observable. In addition to this, rapid culture in a diluted medium can

move about actively; in a compact one, only individual varieties are capable of moving about on the surface (at low concentrations of agar-agar).

Between the compact and diluted media there is an unnoticeable scale of movements through the semi-liquid substrate. The growth of organisms in semi-liquid media upon sowing in one place takes a spherical shape since the growth process goes downward without an increase of cells by streams of liquid. In this case the individuals continuously multiply in the volume of the medium and grow in a new portion of the fresh substrate. However, when a large biomass has grown, divisions of the fresh medium nearest it are saturated with waste products.

The picture changes when the shape of the vessel is changed. If the semi-liquid agar feed overflows along the tube placed horizontally, then the effect of the biomasses on the neighboring divisions of the medium will be weaker (Fig. 1). This is explained by the fact that surface growth occurs more rapidly than growth downward. As a result, the substrate which has germinated will have a mowed-down appearance, while the cells nearest the edge of growth find themselves under the lesser effect of the waste products of the biomass than in the previous case.

The diffusion of metabolites can be even more significantly lessened. A dividing wall should be placed across the tube to accomplish this, but in such a way that they do not touch the surface of the substrate (Fig. 2).

The young growth divisions (in the form of wedge) will be

almost completely isolated from the basic biomass. But since diffusion is directly proportional to the area through which it occurs, then a thin film of the medium which passes over the dividing walls practically completely excludes the effect of the waste products on the youngest cells. As a result we can contain the continuous propagation of cells in a fresh medium without the complex flow system. Whereas in the classical scheme the fresh medium is brought to the cells which are propagating, then in the proposed variation the cells themselves grow towards the urdistrube feeding medium.

The vessel for culturing cells can be made easily from glass tubing in which the dividing walls are pressed in the middle of the lower part of the divisions (Fig. 3). The ends of the tube are angled. This offers the possibility of selecting material from any place in the tube with a small spatula. Seeding can be done in the first compartment (on either side), and after a certain period of time the culture grows over to the other end of the tube.

The rate of increase of the microbes will vary, depending on the microorgarism and the compactness of the semi-liquid medium.

Thus, on a must containing 0.5% agar-agar Asp. niger moved 552 mm in 115 days, having an average daily speed of 5 mm. As. chrocooccum on Ashby's culture containing 0.3% agar-agar had a considerably greater speed- - on the average of 23.6 mm per day, and hence it was necessary to resow it more frequently.

Sectional tubes can be used to study the rules of propagation of microorganisms on the surface of a feeding substrate. These investi-

gations have not been completed but already the material obtained compels anew the re-evaluation of this index. Thus in the usual feeding media Bac. mycoides progagates well on the surface, but B. pzodigiosum creates small, strictly local colonies. Upon decreasing the compactness of the substrate the rate of growth of the first batch of culture does not change very much whereas the front of the growth of the second batch moves along with a rate of several centimeters per day.

Other experiments can be conducted in sectional tubes, for example, for adapting the culture to various physical, chemical and biological agents.

In our own experiments the most suitable were the tubes bent at an acute angle (Fig. 4). They are stable and samples can be drawn from them quite easily. However, one of the most valuable elements is the selection of the required concentration of agar-agar. We worked on Chinese agar-agar and used a 0.2-0.5% solution of it. In this connection, greater concentrations were used for fungi and lesser for bacteria.

The simple construction of the device offers the possibility of conducting experiments with many problems in any laboratory, and as a solution for any needed contiruous culture.

Figure Appendix

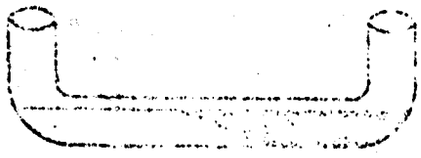

Fig. 1.

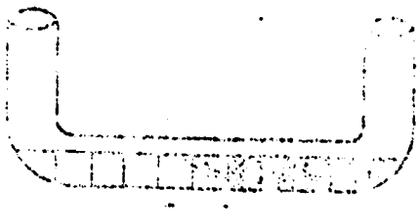

Fig. 2.

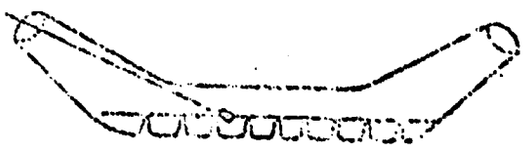

Fig. 3.

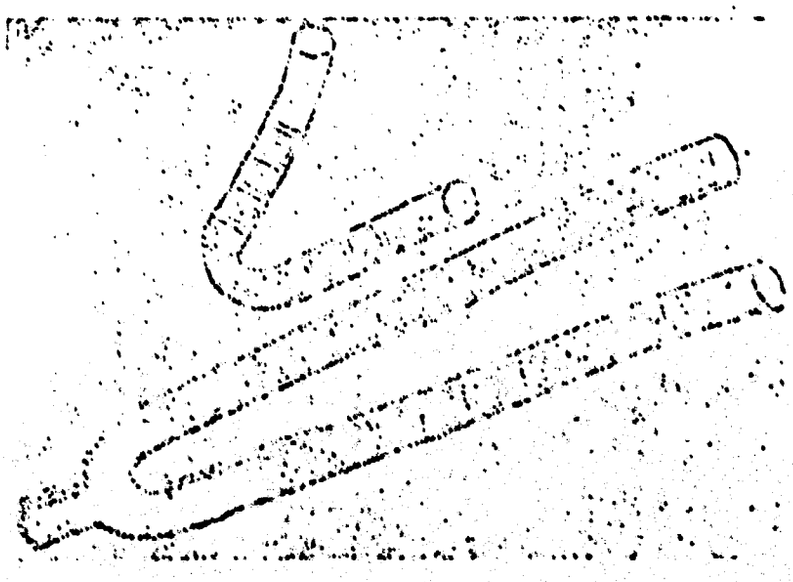

Fig. 4.