

# Morphological Characteristics of a Purified Thermophilic Cellulose Decomposing Culture

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A culture which will decompose cellulose at 65° C. may be readily obtained from the fecal material of ruminants. However, the isolation of the actual cellulose destroyer has not yet been satisfactorily accomplished. Many claims to success have been made, none of which has been generally accepted. The nearest approach to an acceptable pure culture is the one which is obtained from the clear zones on a plate containing finely divided cellulose. These plates are most satisfactory when incubated aerobically in a moist chamber according to the method described by Murray (1). These cultures have not been considered pure chiefly because no well defined colony may be observed and because of their marked heterogeneous nature. They may at best be termed purified.

The purpose of this work was to obtain information regarding the morphological development of such a culture. The possibilities of a life cycle or of a synergistic relation have been mentioned by previous workers to explain their failure to isolate pure cultures of the organism. A study in the development of a purified culture would possibly be helpful in determining the validity of these ideas. In this paper no attempt will be made to correlate the experimental facts with either of the above.

In brief, the idea of the experiment is this: if identical tubes are inoculated from an old culture and then pasteurized all of the cultures should be the same in that the forms of organisms would all be in a heat resistant stage. This would give a uniform starting point from which the morphological development could be observed.

## Procedure

The experiment was carried out in culture tubes 125 mm. x 15 mm. These tubes were thoroughly cleaned, using chromic acid cleaning solution, and were rinsed in distilled water several times. In each tube 0.15 grams of filter paper was placed. This filter paper had been ground in a hammer mill. Ten milliliters of a peptone mineral salt solution were added to each of the tubes. These were plugged with cotton and autoclaved for 20 minutes at 15 lbs. pressure. After autoclaving the column of liquid is about 75 mm. in height and that of the cellulose which is at the bottom is about 20 mm.

These tubes were inoculated from a culture 9 days old which had completed its activity. The tubes were immersed in boiling water for ten minutes, cooled immediately, capped with tin foil and placed in the incubator at 65° C. After 12 hours two of the tubes were removed and smears were made from the material at the top and from that at the bottom of the tube. This was repeated at twelve-hour intervals until ninety-six hours of incubation had elapsed. Simple stains were made

using glycerated carbol fuchsin. The gram stains were carried out according to the Hucker modification, and a modified Dorner method was used for the spore stains.

### Observations

The fermentations were typical in all of the tubes. At the end of 12 hours a white surface and subsurface growth was visible while the lower portions of the tube were apparently free of organisms. Stains from the upper portion showed large rods of uniform diameter, these rods were found in chains and clusters. Rods of varying length were observed in the same chain. These were gram variable and showed a tendency to be gram positive. The preparations from the lower sections contained a few of the large forms but a filamentous form seemed to predominate. This was very thin and extremely flexible. It attained lengths of 20 microns in some cases. The filaments are definitely gram negative in character.

The surface growth became progressively heavier and at the end of 36 hours had spread throughout the medium. The microscopic pictures

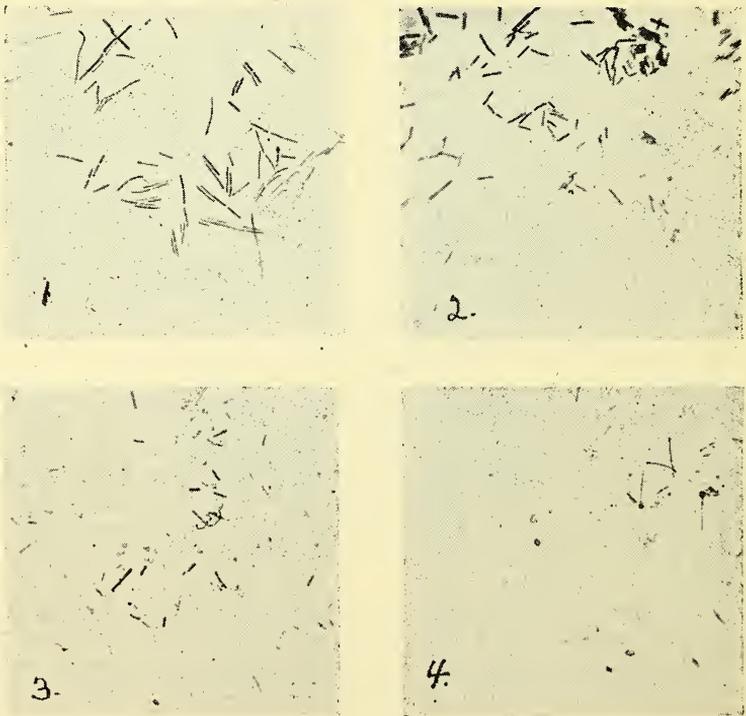


Fig. 1. Progressive development of morphological types found in the fermentation of cellulose by a purified thermophilic culture. 1) Top, 12 hours. 2) Top, 36 hours. 3) Bottom, 36 hours. 4) Bottom, 48 hours.

at the end of 24 and 36 hours were similar. The upper portions contained uniform rods in large numbers. These rods were approximately 8 microns long and 1 micron in width. These were heavily stained with the simple stain and showed a strong tendency to be gram positive. The bottom portions were very heterogeneous. Short filaments, long filaments, and the uniform rods as observed at the surface were all present in considerable numbers. In addition irregular shaped particles of about 1.5 microns in diameter were found. For convenience these have been termed Amorphous cocci. The evolution of gas and the formation of a yellow pigment began some time between 36 and 48 hours. This is the usual evidence of the cellulose digestion. In some cases the cellulose was held at the surface of the media by the rapid evolution of gas. The microscopic picture was that of an actively digesting culture as it is usually observed. The types from the top and the bottom were mixed and the surface no longer gave a uniform appearance. Of the filaments only the shorter ones 3-4 microns seemed to be found in the upper part. The longer filaments seemed to remain in the lower portions of the tube. At the bottom were also found filamentous rods of about ten microns in length and less than .5 microns in diameter. These bore a completely terminal oval spore 2-3 microns in length and 1.5-2.5 microns in diameter. This spore was deeply stained with the simple stain and was variable in its reaction to the gram stain. The spore stain was also very obscure. The spores held the stain only weakly if at all against the action of the nigrosin. With the exception of the spores the culture was gram negative. Little change could be observed in the microscopic picture after 60 hours. The evolution of gas continued vigorously and the cultures remained agitated.

This evolution of gas had stopped after 72 hours of incubation and the cellulose residues settled to the bottom of the tubes. No visible change occurred for the duration of the experiment. The uniform rods seemed to dominate the upper parts again and the bottom is more or less a mixture of the filamentous forms, rods, amorphous cocci, spore bearing filaments and free spores. These spores remained variable to the gram and spore stains even in the older cultures. The Amorphous cocci were numerous and were evenly distributed in the medium. This was the case until the end of the observations.

In the course of the experiment it was readily observed that the organisms could be divided into two groups according to their staining characteristics. The large uniform rod and the oval spore were both stained heavily with the simple stain and were the only organisms which exhibited any tendency to be gram positive. The filamentous forms and the Amorphous cocci were only slightly stained. In fact, the safranin used as a counterstain for the gram reaction had scarcely any effect on the organisms.

### Summary

It seems apparent that two zones of development function in such a tube culture. One of these is near the surface and the other is near the cellulose fibers at the bottom. At the top large uniform rods develop and in the bottom a more filamentous form. "Amorphous cocci" were

observed first in the bottom and later throughout the media. These organisms were mixed thoroughly by the evolution of gas. Filaments bearing a large terminal spore were found after 48 hours. This spore is not typical in its staining reactions. Thus the large oval body cannot properly be termed a spore. The development of the culture has been followed and the evolution of gas accounts for the mixture of types which are usually observed.

#### Reference

1. Murray, H. C. Aerobic Decomposition of Cellulose by Thermophilic Bacteria. *J. Bact.*, **47**:117-122, 1944.