

THE ISOLATION OF ANAEROBIC CELLULOSE-DECOMPOSING BACTERIA FROM VARIOUS SOURCES

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The destruction of cellulose by microbic life has attracted the attention of workers in this field for many years. Cellulose is found in various forms of purity in nature. In its natural form, it is extremely resistant to most chemical and biological action. This fact has made this field of research a difficult one to study and has centered the attention of bacteriologists on methods of attack. Because of the commercial importance of the anaerobic fermentations, much more work has been done along these lines.

This paper deals with the development and growth of crude cultures of cellulose-fermenting organisms from many different sources. The first medium used was modified from that described by Khouvine with the addition of 2 per cent cellulose. To make this medium solid 0.8 per cent agar was added.

Liquid Media

Di-potassium phosphate (K_2HPO_4) 5.0 grams, potassium nitrate (KNO_3) 2.5 grams, peptone 1.0 gram, sodium chloride ($NaCl$) 0.5 gram, cellulose 20.0 grams, calcium carbonate ($CaCO_3$) 10.0 grams, water (H_2O) 1000.0 cc.

Solid Media

Di-potassium phosphate (K_2HPO_4) 5.0 grams, potassium nitrate (KNO_3) 2.5 grams, peptone 1.0 gram, sodium chloride ($NaCl$) 0.5 gram, cellulose 20.0 grams, agar agar 8.0 grams, water (H_2O) 1000.0 cc.

The culture medium was autoclaved for 3 hours at 15 pounds pressure and cooled before inoculation. The liquid medium was placed in litre Erlenmeyer flasks or in 12 inch culture tubes. The tubes were inoculated with the material and covered with liquid paraffine oil to insure anaerobic conditions. The flasks of medium were inoculated and the mouth covered with lead foil to prevent evaporation and help insure anaerobiosis. In the case of the flasks the anaerobic condition was dependent upon the production of carbon dioxide which would exclude the oxygen present.

The presence of anaerobic cellulose fermenting bacteria in muck soil, feces, rotten wood, and the stomach of certain insects is easily demonstrated. The decomposition of cellulose by anaerobic bacteria is readily noted by the presence of the characteristic yellow color of the reaction. The samples to be tested are collected and placed in separate bottles. About five grams of a sample is placed in tubes containing about 30 cc. of the liquid media and incubated at room temperature, and at 37°C. for ten days.

TABLE 1. Effect of temperature in relation to amount of growth

SOURCE	TEMPERATURE		SOURCE	TEMPERATURE	
	25-28°C.	37°C.		25-28°C.	37°C.
Rotten wood.....	+	++	Zebra feces.....	+	++
Muck soil.....	+	++	Rat feces.....	+	++
Horse feces.....	+	++	Llama feces.....	—	++
Hog feces.....	+	++	Buffalo feces.....	—	++
Goat feces.....	+	++	Woodchuck feces...	+	+
Sheep feces.....	+	++	Skunk feces.....	+	+
Rabbit feces.....	—	++	Mice feces.....	+	++
Deer feces.....	—	++	Termite feces.....	+	++
Elk feces.....	—	++	Termite stomach...	+	++
Elephant feces.....	—	+	Wood borer.....	—	+

— No growth, + slow growth, ++ rapid growth.

Table I shows that the best results were obtained from those organisms grown on this type of medium at 37°C. From this table it may be concluded that cellulose-fermenting organisms and especially the anaerobic spore formers are widely distributed in nature, and no doubt they play a very important role. A further study of the role of these organisms should reveal very interesting data in connection with the digestion of cellulose in the intestinal tract of the animal concerned.

Isolation. The attempts to effect an isolation of each culture was first made by preliminary enrichment of the culture. This method increased the cellulose-fermenting organisms, but did not entirely eliminate the contaminants. Some workers were able to eliminate these by repeated transfers and heating. This method was tried, but with little success. The dilution method was used, but the results were unsuccessful since all the tubes had the contaminants growing with the cellulose-fermenting organisms.

Attempts then were made to grow the culture on a solid medium. Such media as an agar starch solution proved useless, as also did beef extract and agar, and peptone and agar. Attempts then were made with a medium using cellulose as one of the constituents. The methods used by Winogradsky, Khouvine, and Cowles and Rettger were unsatisfactory for these organisms. A method was devised to use cellulose ground to a very fine state and mixed with agar and the basal salt solution. The cellulose was ground in the ball mill in a water solution. This type of ground cellulose is free from salts and acids, and is very cheap to make.

The method used for anaerobic conditions was to inoculate the cellulose agar and pour it in the plates and let it become solid, then cover the agar with sterile vaseline at 60° C. and let it harden. This method proved to be an easy and suitable anaerobic one.

In the early work on isolation from this cellulose agar, colonies appeared in 10 to 15 days on the vaseline plates. These colonies of cellulose-fermenting organisms could be easily detected on the medium by the appearance of a clear zone in the cellulose agar. These spots have been known as the "halo" or "enzymatic rings." When the colonies

are examined with the microscope the agar is found to be free of cellulose fibers.

According to theory these colonies could be easily picked and a pure culture obtained, but it was not found to be the case. In many of the cultures the colonies which were picked grew in a feeble manner and in a few transfers the new culture would die or fail to grow. After working with several kinds of media, it was found that there was a deficiency in one food in this medium which caused these organisms to fail to grow after picking and transferring them to a new culture.¹ In all the new cultures that were picked, contamination was always present, although there were never more than 2 or 3 types present. With the aid of the above method the cultures were enriched into a semi-pure state containing 2 or 3 different types of organisms.

Morphology. In these purified cultures there was more than one type of organism present. The vegetative cells in the liquid medium were of several types. The cells ranged from a short slender rod about 0.3 micron in diameter and from 6 to 8 microns in length, to types which were oval in shape, about 0.4 micron in diameter and 1 to 4 microns in length. The different types occurred singly and in chains of 2 to 6 each. Some were curved in shape. In all cases the organisms formed spores. The spores were formed either on the middle of the rod or at the end. They stained readily with all the common dyes. The gram reaction stain is uncertain, but as a rule the cells seem to be gram positive. The organisms in all the cultures seem to be motile in the hanging drop.

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¹ Author's unpublished thesis—"The Decomposition of Cellulose by Bacteria Isolated from Intestinal Tract of Termites," which is in the university library.

