Minnesota Section.

University of Minnesota, January 20, 1932.

5973

Lactose Fermenting Anaerobes in Soil and Their Relation to Sanitary Water Analysis.

C. E. SKINNER AND A. H. BASKIN.

From the Department of Bacteriology and Immunology, University of Minnesota.

The suggestion has been made that the search for lactose fermenting anærobes be made routinely in place of, or in addition to, the lactose fermenting ærobes (*Bacterium coli*). The purpose of this work was to determine the advisability of such a procedure. If lactose fermenting anærobes are to serve as an index of fecal pollution in water, it must be shown that they are constantly present in feces and that they are not found in large numbers in soil or elsewhere in nature where they might get into water supplies. Furthermore, as stated by Levine[1], it ought to be shown that they are able to exist in water for a short time (but only for a short time) after the water has been freed from pathogens. It would seem requisite also that they be readily susceptible to detection without too much time, equipment, or effort.

There is no doubt that lactose fermenting anærobes, including *Clostridium welchii*, are normally present in human feces, but not all authors have found them in all samples studied, possibly because of the technical difficulties of the isolation of anaerobes.

As to their incidence in nature outside of the intestinal tract, opinions differ. Greer[2], pointing out the importance of lactose fermenting anærobes, ignores the possibility of their wide distribution in soil. Topley and Wilson[3] believe that the normal habitat of anaerobes is soil, and that their presence in sewage and feces is inci-

[1] Levine, M., *Iowa State Col. Eng. Exp. Sta. Bul.*, 62, 1921.

[2] Greer, F. E., *J. Infec. Dis.*, 1928, **42**, 501.

[3] Topley, W. W. C., and Wilson, G. S., Principles of Bacteriology and Immunology. Wm. Wood & Co. 1929.

dental. This opinion, while possibly extreme, is, in the light of the literature and the work to be reported, probably nearer the correct one than that of Greer, and others, namely, that *Cl. welchii* is to some extent an index of fecal pollution.

Experimental. It is obvious to anyone who has isolated anaerobes that it is impractical to use *Cl. welchii* as an index of fecal pollution if it is necessary to isolate the organism each time a sample of water is to be tested. While not particularly involved, the isolation of anaerobes takes far too much time and is too uncertain for routine water work. As a substitute for isolation there are a few more or less satisfactory criteria used to identify this organism and its close relatives. First, the stormy fermentation of milk may be used. The authors used the following method: Dilutions of soil were added to milk, and heated for 10 minutes at 75° to drive off excess oxygen and to kill the vegetative cells including *Bacterium ærogenes* and *B. coli.* Sterile paraffin wax was added, as in the Weinzirl[4] technique. Gassy fermentation results in lifting the paraffin plug and the stormy fermentation may be detected by observing the character of the clot. It should be stated that all gradations of stormy fermentation from the extreme to none at all were observed, together with gas formation, sufficient to loosen the paraffin plug, and an arbitrary line of demarcation had to be drawn. If only lactose fermentation is desired, lactose broth with paraffin wax plugs or lactose broth with gas traps is applicable. To determine the approximate numbers of spores in soil, the dilution method was used. Five 1 cc. replicate samples of decimal dilutions were used, and the numbers determined from the tables of Halvorson[5].

The method of Wilson and McBlair[6] may also be used. This is not specific for *Cl. welchii*, or even for anaerobes, but Wilson and McBlair, Adams,[7] and Greer[2] have suggested its use. For our work we used a modification of this method since large petri plates were not available. Inverted standard size petri plate bottoms were inserted in tops and sterilized. The dilutions were placed in the depression of the top, and the iron, sulphite, and previously boiled dextrose agar, poured and mixed with the dilution. The bottom was placed over it, the convex side of the bottom fitting into the concave side of the top, care being taken to eliminate all air bubbles. Sterile agar was placed around the circular border formed by the

[4] Weinzirl, J., *Am. J. Pub. Health*, 1921, **11**, 149.

[5] Halvorson, H. O., unpublished data.

[6] Wilson, W. J., and McBlair, E. M. McV., *J. Path. and Bact.*, 1924, **27**, 119.

[7] Adams, B. A., *Water and Water Eng.*, 1929, **31**, 412.

insertion of the inverted bottom in the top, thus making anærobiosis more complete. Tests with pure cultures of *Clostridium botulinum* and *Cl. welchii* have demonstrated that anaerobes grow well in such plates. All plates were incubated in moist chambers to prevent cracking of the agar and subsequent ærobic conditions near the cracks. The colonies of five or ten replicate plates were counted, only those being considered which showed the typical black colonies which the British authors found to be usually *Cl. welchii* and its relatives.

By means of these three methods, determinations of the "numbers" of *Cl. welchii* in soil were obtained. The soil samples were from forests, peat bogs, or unmanured fields. Most of them were virgin soils, that is, they had never been cultivated although they may have been logged off. Fecal pollution, if present, must have been at a minimum. Four of the samples had been air dried for five years in the laboratory. The samples plated on Wilson and McBlair's medium were not the same as those inoculated into milk. Although *Cl. welchii* has been found in soil by many investigators, it is believed that these are the only quantitative figures in the literature.

Seven separate strains of lactose fermenting anærobes were isolated from the soil. They fermented glucose, lactose, sucrose, and maltose, forming acid and copious gas. They caused a stormy fermentation of milk, and liquified gelatin. They were either *Cl. welchii* or closely related anærobes. Morphologically, they were similar to a strain of *Cl. welchii* obtained originally from Hall's laboratory.

The results show that if lactose fermentation with gas or a stormy fermentation of milk inoculated with a heated suspension or a blackening with typical large colonies on Wilson and McBlair's[6] medium be taken as a criterion for *Cl. welchii,* these organisms can scarcely be taken to indicate fecal pollution. It is hardly conceivable that organisms so numerous in soil would fail to get into water supplies. A sudden increase in their numbers may indicate a surface drainage due to rain or to agitation of the mud in streams, or to many other factors but not necessarily to sewage pollution. The spores are known to be resistant, and there is no reason to believe that they will not exist in water long after the water has been freed from pathogens by treatment or autopurification. For these reasons, it would seem that sufficient data have accumulated to indicate that lactose fermenting spore formers are so numerous in soil as to preclude their use for indicating fecal pollution. And if, as some maintain, these organisms in themselves are dangerous when taken

TABLE I.
Number of lactose fermenting spores in soil determined by stormy fermentation of milk and by gas formation, using dilution method.

Soil Samples	No. of tubes showing stormy fermentation. (5 inoculated with 1 cc. soil dilution)			Most probable No. per gm. of soil	No. of tubes showing gassy fermentation. (5 inoculated with 1 cc. soil dilution)			Most probable No. per gm. of soil
	1:10	1:100	1:1000		1:10	1:100	1:1000	
1	5	1	0	33	5	5	4	1620
2	5	4	1	171	5	5	2	542
3	5	2	0	49	5	4	1	171
4	5	5	4	1620	5	5	5	>10,000
5	5	5	3	918	5	5	5	>10,000
6	5	5	2	542	5	5	4	1620
7	1	0	0	2	4	0	0	13
8	5	5	5	>10,000	5	5	5	>10,000
9	5	4	2	221	5	5	5	>10,000
10	4	2	1	27	5	2	1	60
11	5	0	0	23	5	1	0	33
12	5	5	3	918	5	5	3	918
13	3	0	0	8	3	0	0	8
14	5	3	1	109	5	5	3	918
15	4	1	0	17	5	3	0	79
16	4	0	0	13	5	1	0	33
17	5	5	3	918	5	5	5	>10,000
18	5	5	1	349	5	5	5	>10,000
19	5	5	4	1620	5	5	5	>10,000
20	5	4	1	171	5	5	3	918

TABLE II.
Numbers of bacteria causing large black colonies on Wilson and McBlair's anaerobic medium.

Sample	No. per gm. of soil	Sample	No. per gm. of soil
1	532	10	25
2	483	11	160
3	860	12	42
4	1000	13	390
5	3700	14	5600
6	230	15	11
7	6	16	130
8	320	17	9
9	6210	18	4900

into the alimentary tract, such uncooked foods as carrots, tomatoes, radishes, lettuce, strawberries, cucumbers, and others grown in, on, or near the soil, should be viewed with suspicion. Likewise, most water supplies, if the above be true, are potential sources of *Cl. welchii* infection, since chlorination does not eliminate all the spores. However, evidence that these organisms from the soil are dangerous in food or water is far from conclusive.