A STUDY OF COLIFORMS AND ENTEROCOCCI IN SOIL

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Robert Chauncey Cooper

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This is to certify that the

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A STUDY OF COLIFORMS AND ENTEROCOCCI IN SOIL

By

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INTRODUCTION

A perennial problem for investigators has been the sanitary significance of members of the coliform group of organisms when found in water and soils; and, in particular, the Aerobactor aerogenes types which are usually thought of as soil or grain organisms. It is thought that the presence of the enterococcus group might aid in determining the significance of the coliform organisms that are present. The purpose of this investigation is to determine the possible occurrence of the enterococci in various soils and to establish their relationships to the coliform organisms occurring in the same soil samples.

The presence of streptococci in sewage was originally reported by Laws and Andrews (1894) when they isolated them from hospital sewage in England. Winslow and Hunnewell (1902) gave the first report of sewage streptococci in the United States. They had isolated these organisms from the hands of school children in conjunction with Escherichia coli and found them to be similar to those found in Boston sewage. One of the first reports on the sanitary significance of these organisms was that of Houston (1898), who concluded that the streptococci in water are of sanitary significance and that they indicate more recent pollution than do the coliforms. Due to this report the organisms received the name "sewage streptococci of Houston". Savage and Read (1917) demonstrated that

no streptococci were present in polluted waters and are of undoubted value as evidence of excretal contamination. Hallmann (1928) found that E. coli tends to increase or multiply in swimming pools while the streptococci do not, demonstrating that the streptococci are better indicators of pollution in unsafe swimming pools. Winslow et al (1947) stated "although the significance of the streptococci as sewage organisms is not established with the definiteness which marks our knowledge of the coliform group, these bacteria have been isolated so frequently from polluted sources and so rarely from normal waters that it seems reasonable to regard their presence as indicators of pollution."

The intestinal streptococci are quite widespread in nature and usually in conjunction with the intestinal discharges of animals. Winslow and Palmer (1910) isolated the enterococci from the intestines of horses, cows, and man, making their differentiations by using acid production in sugar as a criterion for identification of the enterococcus. Orcutt (1926) isolated enterococci from the normal digestive tract of calves and demonstrated that these streptococci were not a homogeneous group. Steinhouse (1941) isolated Streptococcus fecalis from a number of insects including seven orders of the class Hexapoda. Sylvester and Benedict (1941) isolated 65 true enterococci and 15 related forms from the viscera of the fox and mink explaining their presence in the viscera as being due to migration from the intestine to the gut after death. Winter

and Sandholzer (1946) found enterococci in sewage, water, sea water, and the feces of man, domestic and wild animals. Ostrolenk and Hunter (1946) made a study of the distribution of enteric streptococci and found the organism present in the feces of humans, cats, mice, guinea pigs, dogs and other animals and insects. These workers found soil to be negative; however, they only tested two soil samples. Mallmann and Litsky (1951), in a study on the survival of various enteric organisms in soil, stated that there were no streptococci present in untreated soil.

It can thus be seen from the work done on the distribution of the enterococci that they have been isolated mainly from animal excreta. Of all the citations made in the literature, few have been made concerning the presence or absence of these organisms in the soil; in those cases where soil was included in the study no streptococci were found.

To be a good indicator of pollution an organism should neither be able to persist for great lengths of time outside of its normal habitat nor to multiply outside of its own surroundings. The enteric streptococci seem to fit this classification somewhat better than do the coliform organisms. Savage and Wood (1918) studied both coliforms and enterococci in tanks of water with small amounts of organic matter present, and found that the streptococci die out in about two weeks, while the coliform persisted longer and in some cases multiplied. They concluded that the presence of streptococci in water may

be a better indication of pollution than the coliform group. Mallmann (1940) demonstrated that in sewage there are instances when the coliform organisms will multiply while such is not the case with members of the enterococcus group. This again indicates that the streptococci would be better indicators of contamination. Mallmann and Litsky (1951), in a study on the survival of enteric organisms in various types of soil, inoculated lysimeters with material (soil) containing sewage in various forms. They found that the enterococci died out rapidly in soil while coliforms were found to persist for long periods of time. Young and Greenfield (1923) found that under certain natural conditions the coliform group will multiply and thereby fail to be an indicator of pollution. In sterile soil they found coliforms surviving for as long as 17 months; in unsterilized soil they found an increased number of coliforms. Skinner and Murray (1926) inoculated soil with coliforms and found that the organisms lasted longer than 122 days; while Kulp (1932) reported that A. aerogenes and E. coli lasted almost four years in the soil, demonstrating what he called "the protective influence of the soil on the coliform organisms."

The literature on the significance of the coliform organisms in soil is quite confusing. The many workers draw divergent conclusions, particularly concerning the intermediate strains that lie somewhere between the true E. coli and the true A. aerogenes. Koser (1924 and 1926) found that there were

colon organisms resembling those of intestinal origin in relation to the methyl red-Vogues Proskauer test in apparently unpolluted soil. He felt, further, that the intestinal E. coli may be separated from the soil E. coli by using his citrate medium; a positive citrate being evidence of non-intestinal E. coli. In contrast, Vaughn and Levine (1942) gave evidence showing that citrate utilizing organisms are present in feces, soil and water. They felt the statement, "intermediate groups of coliforms are necessarily non-fecal in origin" to be unjustified. Parr (1941) found that some strains of coliforms are not stable on Simmons citrate. He felt that different strains exhibit varying grades of stability. Some citrate positive organisms will reproduce both citrate positive and negative colonies. Carpenter and Fulton (1937) reported that 49.8 percent of the fecal samples they examined contained citrate positive strains and 13.3 percent demonstrated intermediate groups. They, therefore, concluded that the citrate positive group may have sanitary significance. Gray (1932) found A. aerogenes universally present in human stools and predominating over E. coli in soil.

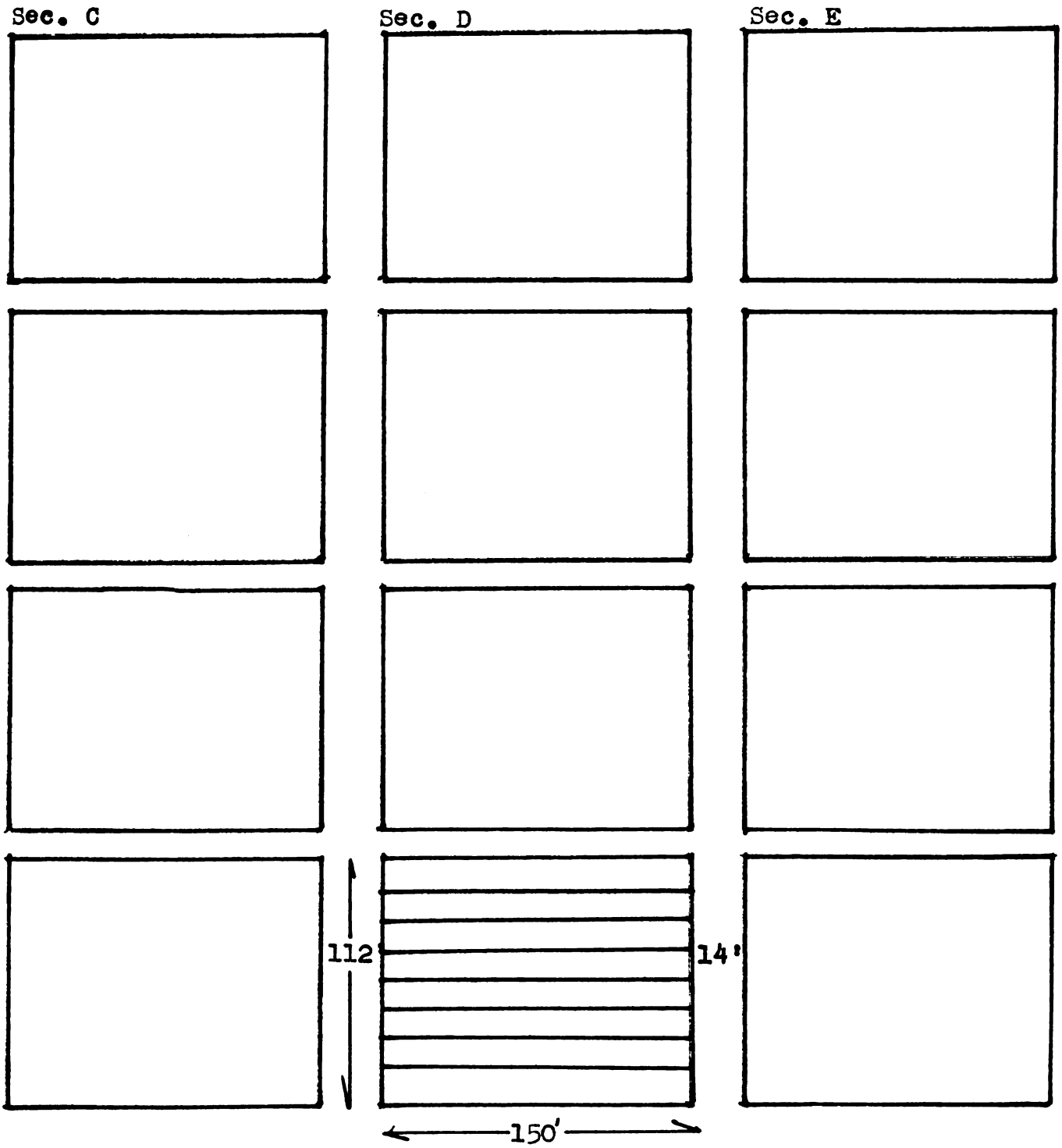
EXPERIMENTAL PROCEDURE

To enhance the value of the study it was deemed necessary to sample soils of known history in order that their sanitary conditions might be known. This would show the incidence of coliforms and enterococci, if any, in soil known to be of both good and poor sanitary quality.

Soil samples were taken from experimental plots, records of which have been kept for a number of years. These histories include what has been placed in the soil by way of treatment and also included when the treatment was carried out. The soil plots are maintained by the Soils Department of Michigan State College. Their physical measurements are 112 feet wide and 150 feet deep. They are each divided into strips 14 feet wide making eight strips, each receiving a different treatment. There are 12 of these plots in the section used for soil samples, arranged three deep and four across (see map). The center section (D) was used in taking the samples because section (E) is on a road and Section (C) is adjacent to a different set of soil plots. This center section appeared to be the least susceptible of the three to foreign contamination.

The soils samples had been treated with sewage sludge, straw manure and peat; the material being placed on the soil and subsequently plowed under. Control plots, used as a

EXPERIMENTAL SOIL PLOTS



positive control when testing the effect of the above materials (in soil) on various crops, were also sampled. The control plots have had no treatment whatsoever. The "virgin soil" was taken from a heavily wooded area in the vicinity of the experimental plots. (The term "virgin soil" is used to indicate an area that has never been cultivated.)

To take the actual soil sample a simple instrument was required: one which would easily penetrate the soil and which would retain about ten grams of earth when removed. Ordinary cheese samplers - which are hollow cylinders with a sharp edge and perpendicular handle - were selected. In taking the sample the area to be tested was scraped clear of the first few centimeters of soil with a sterile tongue blade. The sterile cheese sampler was then driven into the soil to a depth of about four inches and the sample removed. The specimen was then placed in a sterile, wide-mouthed water sampling bottle with a ground glass stopper and transported to the laboratory for testing.

Random sampling was employed in taking the soil specimens, that is, there was no pre-determined distance between sampling points along the length of the test plot. The number of samples taken per plot was approximately ten; five in the spring and five in the fall.

In the laboratory, the sample was transferred to a sterile mortar and ground with a pestle to insure an even mixture of all depths represented. Two grams of the sample was weighed

out and placed in 100 ml. of sterile, distilled water. Using this procedure the coliform and streptococcus count of the 100 ml. will equal the total count present in the two grams of soil. The counts were made using most probable numbers based on three portions each of three dilutions. Dilutions of 1.0, 0.1 and 0.01 ml. for the coliforms, and 10.0, 1.0 and 0.1 ml. for the streptococci proved to be the most satisfactory.

The coliform tests were run according to standard methods procedures (1946) and were carried out through the completed test. Lauryl tryptose broth (Difco) was used for the presumptive test because, as was reported by Mallmann and Darby (1941), this medium will inhibit spore formers and shorten the lag phase of growth. Since soil is predominantly inhabited by spore forming organisms, many of which are capable of fermenting lactose with the production of gas, it was felt that the above medium would eliminate a large number of false positive presumptive tests. Confirmatory tests were carried out in brilliant green bile lactose broth. The completed test was performed using nutrient agar slants and lauryl sulfate tryptose broth. In running the indole, methyl red and citrate tests for the coliform identification, eosin methylene blue plates were streaked with inoculum from positive presumptive tubes and isolated colonies were picked and transferred to the various media required for the tests.

Methods for determining the presence of the enterococci have been significantly improved in recent years. Through

advances in technique, moreover, the enterococci have been shown to be much more numerous in polluted sources than was previously thought. Prescott and Baker (1904) gave a method for the detection of enteric streptococci which allowed the sample to incubate for four to five days in lactose broth; by the end of this time period the coliforms died out and the streptococci predominated. The resulting growth was then observed microscopically for coccus forms. This is the method upon which much of the work to date has been based.

In 1940 Mallmann developed a medium for the enterococci which included sodium azide as an inhibitor of gram negative organisms. This medium is selective for enterococci and gave better counts than any previous methods. Hajna and Perry (1943) independently devised a medium quite similar to Mallmann's, the only difference being that Hajna and Perry's medium requires an incubation temperature of 45 degrees centigrade. Mallmann and Seligmann (1950) made a study of various media for the detection of the enterococci. Their data indicated that azide dextrose broth is the best medium of the group tested which included standard lactose broth, sodium azide broth (Mallmann, 1940), and Hajna and Perry's S. F. Broth. This medium, dextrose azide, was chosen as the enrichment medium to be used in testing for the enterococci in soil. The azide dextrose broth could not be used as a direct presumptive test because the sedimentation of the soil in the sample confused the readings as to whether growth was present or absent. All

tubes of the aforementioned medium were assumed to be positive, and transfers were made from these into ethyl violet azide broth. Litsky, Mallmann and Fifield (1953) reported on the use of ethyl violet azide broth as a confirmatory medium for the enterococci. They found this medium to be highly selective and to give higher counts than any other medium yet used.

Isolation of the streptococci, for identification, was done by the pour plate method. Transfers were made from positive ethyl violet azide tubes to brain heart infusion broth and allowed to incubate at 35° C for 24 hours. Serial dilutions of these broth cultures were carried out and pour plates, using brain heart infusion agar, were made. After 24 hours incubation isolated colonies were picked and placed in brain heart infusion broth again, and the above procedure was repeated. In this way two pour plate dilutions were made before a colony was considered pure. The ethyl violet is bacteriostatic against gram positive spore formers, and when enrichments are made from media containing this substance the gram positive rods will grow; one must, therefore, be careful when trying to establish a pure culture. Microscopic checks were made to insure that the isolated organism was not a gram positive rod.

In making identifications of the enterococci two distinct steps must be carried out; first, one must ascertain whether the isolated colony fits within the group referred to as the enterococci; second, it must be identified as a single species.

In this work Bergey's manual (1948) was used as the authority. The writer is aware that this method is not very accurate as has been shown by Sherman (1937), Sherman, Stark and Yawger (1938), and Sherman (1938), but some standard of reference was required for the sake of clarity.

To justify placing the isolated streptococcus into the enterococcus group it must grow at 45° C and in broth containing 6.5 percent sodium chloride. These are the most important requirements. Also included in the identification were reduction of litmus milk, growth in ethyl violet azide broth and a microscopic examination which indicated the presence or absence of gram positive streptococci. To determine to which of the four species included in the group enterococcus the organism belonged, the following schema was used (Bergey's manual):

I. Not Beta Hemolytic
   a) Gelatin not liquefied. S. fecalis
   b) Gelatin liquefied. S. liquefaciens

II. Beta Hemolytic
   a) Mannitol and Sorbitol fermented. S. zymogenes
   b) Mannitol and Sorbitol not fermented. S. durans

DISCUSSION

Tables I through VI show the results of the laboratory tests on the soil samples taken from the various experimental fields. In Table I both the logarithmic and the numerical averages of the number of enterococci and coliforms per gram of soil are given. The logarithmic average was determined by taking the sum of the logarithms of the number of organisms per gram of soil in each sample and dividing this by the total number of samples taken in that particular series. The antilog of this number is called the logarithmic average of the number of organisms present in the samples. It should be noted that the logarithm of one equals zero; therefore, a negative sample may be considered to have from zero to one organism per gram. The logarithmic average is the most representative, as it adjusts for wide variations in the number of organisms present; it gives a less misleading average when there are a number of negative or low count samples with one or two high count specimens which, when using the numerical average, will make a higher average per sample than actually is the case. In most cases the logarithmic average will be lower than the numerical except when the latter is less than one.

Fields treated with peat in 1950 and 1951 gave enterococcus counts of zero with the exception of one series (Table II) from a field treated in 1950 in which one sample gave a

TABLE I

THE NUMBER OF COLIFORMS AND ENTEROCOCCI IN VARIOUS TREATED AND UNTREATED SOILS

Soil Treatment Date	Date of Sampling	Logarithmic Average of M.P.N. Per Gram of Soil		Numerical Average of M.P.N. Per Gram of Soil	
		Coliform	Enterococci	Coliform	Enterococci
Peat-Fall 1950	6/2/53	4	1	19	0.4
Peat-Fall 1950	9/17/53	2	0	3	0
Peat-Fall 1951	5/19/53	5	0	15	0
Peat-Fall 1951	9/17/53	2	0	15	0
Sewage Sludge Fall-1951	5/19/53	39	0	46	0
Sewage Sludge Fall-1951	9/26/53	3	0	34	0
Sewage Sludge Spring-1953	5/27/53	5	0	20	0
Sewage Sludge Spring-1953	9/26/53	2	0	7	0
Straw Manure Fall-1951	6/22/53	11	6	54	97
Straw Manure Fall-1951	9/20/53	2	0	4	0
Straw Manure Spring-1953	6/23/53	51	26	159	105
Straw Manure Spring-1953	9/20/53	4	2	290	3
No Treatment*	7/7/53	13	3	197	53
"Virgin Soil"**	7/17/53	248	4	832	58
River Bank***	7/20/53	306	198	315	260

* These soils have had no treatment

** Soil has not been tilled

*** Soil from the bank of the Red Cedar River

count of two organisms per gram, or a logarithmic average for the field of one organism per gram. The number of coliform organisms remained relatively constant for both years represented, the number being quite low, ranging from two to five organisms per gram of soil. A field treated with peat was considered non-polluted, which consideration was supported by the very low coliform count and illustrated even more strikingly by an essentially negative count for the streptococci.

Fields treated with sewage sludge gave much the same picture as (was obtained from) the peat-treated soils. The coliform count was somewhat higher with a logarithmic average of 39 organisms per gram in one series; the number, however, still would not be considered of sanitary significance. The enterococcus count was completely negative in all samples taken, again giving striking evidence of no pollution. It is of interest to note that the coliform count was higher in those plots which were treated in 1951 than those treated in 1953. This could possibly be due to the achievement of a natural balance in the former plots with a low rate of reproduction, while the latter has not yet reached such a stage.

The soil plots that had been treated with straw manure gave results that were somewhat different from the aforementioned two plots. In this case there were a number of samples which gave positive results for the enterococci as well as

TABLE II

THE NUMBER OF COLIFORMS AND ENTEROCOCCI IN SOILS TREATED WITH PEAT

Date of Application	Sample No.	Date of Sampling	Organisms per Gram of Soil	
			Coliforms	Streptococci
Fall 1950	1	6/2/53	0	0
	2	"	75	2
	3	"	0	0
	4	"	18	0
	5	"	0	0
	6	9/17/53	0	0
	7	"	0	0
	8	"	0	0
	9	"	15	0
	10	"	0	0
Fall 1951	1	5/19/53	0	0
	2	"	45	0
	3	"	0	0
	4	"	15	0
	5	9/17/53	0	0
	6	"	75	0
	7	"	0	0
	8	"	0	0
	9	"	0	0

TABLE III

THE NUMBER OF COLIFORMS AND ENTEROCOCCI
IN SOILS TREATED WITH SEWAGE SLUDGE

Date of Application	Sample No.	Date of Sampling	Organisms per Gram of Soil	
			Coliforms	Streptococci
Fall 1951	1	5/19/53	15	0
	2	"	80	0
	3	"	45	0
	4	"	45	0
	5	9/26/53	0	0
	6	"	135	0
	7	"	0	0
	8	"	0	0
Spring 1953	1	5/27/53	0	0
	2	"	55	0
	3	"	0	0
	4	"	45	0
	5	"	0	0
	6	9/26/53	0	0
	7	"	0	0
	8	"	0	0
	9	"	36	0
	10	"	0	0

TABLE IV

THE NUMBER OF COLIFORMS AND STREPTOCOCCI

IN SOILS TREATED WITH STRAW MANURE

Date of Application	Sample No.	Date of Sampling	Organisms per Gram of Soil	
			Coliforms	Streptococci
Fall 1951	1	6/22/53	0	0
	2	"	215	0
	3	"	36	15
	4	"	0	0
	5	"	18	460
	6	9/20/53	0	0
	7	"	0	0
	8	"	18	0
	9	"	0	0
	10	"	0	0
Spring 1953	1	6/23/53	0	12
	2	"	75	5
	3	"	460	460
	4	"	45	12
	5	"	215	37
	6	9/20/53	0	0
	7	"	0	0
	8	"	0	0
	9	"	1450	12
	10	"	0	0

TABLE V

THE NUMBER OF COLIFORMS AND ENTEROCOCCI FOUND IN EIGHT SAMPLES OF "VIRGIN SOIL"

Sample No.	Date of Sampling	Organisms per Gram of Soil	
		Coliforms	Streptococci
1	7/17/53	45	1
2	"	55	0
3	"	75	0
4	"	370	12
5	"	1100	22
6	"	195	0
7	"	4600	430
8	"	215	2

TABLE VI

THE NUMBER OF COLIFORMS AND ENTEROCOCCI FOUND IN CONTROL PLOTS (These soils have had no treatment)

Sample No.	Date of Sampling	Organisms per Gram of Soil	
		Coliforms	Streptococci
1	7/7/53	1450	460
2	"	15	8
3	"	18	0
4	"	45	12
5	"	45	0
6	"	0	0
7	"	0	0
8	"	0	0
9	"	18	0

the coliform organisms. Again the number of coliforms was low. The plot treated in the fall of 1951 gave an average (logarithmic) count of 11 coliform organisms per gram when sampled in the early summer while the plot treated in the spring of 1953 gave an average count of 51 per gram when sampled at the same time. This difference in count is to be expected, as one was treated two years previous to sampling while the other was treated in the same year. Both revealed, as did all the plots except those treated with peat, a decline in the number of coliforms during the summer. The enterococci counts were much lower than that of the coliforms in these plots, and fluctuated with the coliform count, a lowering in the number of coliforms being accompanied by a lowering in the number of enterococcus which dropped to zero in the case of the plot treated in 1951.

The significance of the streptococci in low numbers in the straw manure-treated plots is difficult to evaluate. Their presence may be due to contamination by small animals or to the cow dung present in the manure. There is a large population of small animals on these fields, and many workers have shown that these animals harbor enterococcus organisms in their intestines. The larger number present (26 per gram) in the plot treated in 1953 is possibly due to both contamination from the straw manure itself and from small animals. The fact remains, however, that only one out of 37 samples taken from the peat and sewage sludge-treated fields was positive

for the enterococci. Animal contamination, therefore, may not be as significant as we believe. These plots, sewage-sludge and peat treated, are adjacent to those treated with straw manure and yet have a negative incidence of enterococci. Such evidence seems to indicate that the incidence of intestinal streptococci in straw manure is due to the presence of cow dung rather than animal contamination as all the plots would be frequented by the same animals. A very low incidence of enterococci was found in the control plots and in "virgin soil". In these cases the soil is supposedly unpolluted: the control plots having had no treatment and the "virgin soil" having been taken from a heavily wooded area in which the soil had not been tilled. The enterococci count was low and relatively stable in both soils, three to four organisms per gram. The best explanation for their presence seems to be contamination by small animals present in the area. The same problem, however, arises: some of the fields exposed to animal contamination have negative enterococci counts.

It is interesting to note that the enterococci count remained fairly constant in the "virgin" and untreated soil while the coliform count varied from 13 per gram in non-treated soil to 248 per gram in the "virgin soil".

To test soil from a source known to be polluted two samples were taken from the bank of the Red Cedar river, the water of which is known to be contaminated. Here the logarithmic

averages of the coliforms and streptococci were quite high in comparison to samples taken from the test plots, (see Table I) 306 per gram for the coliforms and 198 per gram for the enterococci, thus showing that in polluted samples there was an increase in the numbers of coliforms and a very marked increase in the streptococci present. The average (logarithmic) coliform count for virgin soil was 248 per gram, and in the river bank sample, 306 per gram. The small difference makes an evaluation between the two very difficult in terms of coliforms alone, but in conjunction with the enterococci content there was quite a large difference between the polluted and non-polluted samples.

An examination of Table VII shows the types of coliforms isolated from the soils tested. All of them were proved to be coliforms by the completed standard methods test. The majority, 72 percent, was of the Aerobacter aerogenes type, which would be expected in most soils. Twenty-two percent were aerobacter intermediates that produce acid in peptone broth (methyl red positive). Three percent were E. coli intermediates utilizing citrate. All of the coliform organisms isolated from these soils were able to utilize citrate. When streaked on eosin methylene blue agar none of these organisms gave a metallic sheen which is typical of E. coli. In comparison of these, two samples were taken from the banks of the Red Cedar river and run through the same process as used for all the samples. When streaked on eosin methylene blue

agar typical E. coli colonies developed and strains of E. coli positive for indole and methyl red and negative for citrate were obtained. This demonstrates that typical E. coli can be shown to be present by the methods used in handling these samples.

From the above we have not much choice other than to assume that E. coli was not present in any of the samples tested with the exception of those taken on the river bank. If we use E. coli alone as the standard of pollution, then none of the plots can be considered even slightly contaminated. This would assume that the intermediates are of no consequence. Many workers have stated, however, that the intermediates of the coliform group, including those that utilize citrate, do have some significance. If this is the case, then possibly the presence of members of the enterococcus group may aid in evaluating their significance. For example, the absence of E. coli in the fields treated with sewage sludge was a good indication that there was no contamination present. This was supported by the fact that the enterococcus count was negative. However, in the fields treated with straw manure no E. coli was isolated, and the coliform count was roughly the same as in those fields treated with sewage sludge, but in this case enterococci may indicate that there is a small amount of fecal contamination present and therefore indicates that the coliforms present in these particular samples may have been derived from a similar source of contamination. If this is true then

TABLE VII

DIFFERENTIAL REACTIONS AMONG COLIFORMS ISOLATED

No. of Isolations	Indole	Methyl Red	Citrate	Percent
23	-	-	+	72
7	-	+	+	22
1	+	-	+	3
1	+	+	+	3
Total 32				100

All organisms tested gave positive completed tests

None of these organisms produced a sheen on E.M.B.

the enterococci would be of great aid in evaluating the presence of coliform intermediates in regard to sanitation.

It should be noted that no soil plot showed any significant predominance of one intermediate over another; all of the fields gave similar results.

Table VIII gives the results obtained in identifying the types of enterococci present in the soil samples. The predominant type was Streptococcus liquefaciens with 72 percent of the isolates belonging to this group. Seventeen percent of the enterococci were S. zymogenes and 11 percent S. fecalis. No member of the S. durans species was isolated. The results of these identifications can not be relied upon completely because of the variations that may occur among these organisms in the various biochemical tests prescribed by Bergey's manual. As was stated previously, however, some standard reference was needed and at present this classification is the most acceptable.

In testing soil for coliforms a great number of false positive presumptives occurs due to the tremendous load of spore forming organisms that have the ability to ferment lactose with gas production. In view of such a condition it was necessary to perform completed coliform tests on all samples which gave positive presumptive results. Table IX shows graphically and tabularly the number of false positive presumptive and confirmatory tests. Eighty seven and six-tenths percent of all the samples taken gave positive presumptive tests, while

TABLE VIII

TYPES OF ENTEROCOCCI ISOLATED FROM SOIL

Species	Number	Percent
S. durans	0	0
S. fecalis	2	11
S. liquefaciens	13	72
S. zymogenes	3	17
Total	18	100

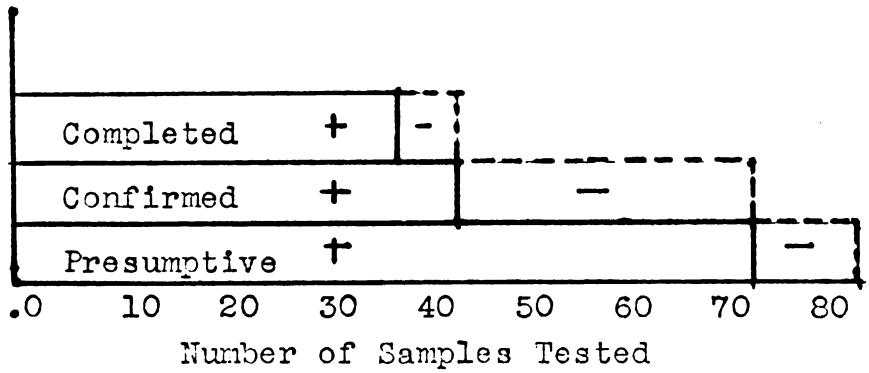
only 43 of these or 53.2 percent of all the samples gave positive confirmed tests. Of these 43 samples only 37 were shown to be coliform organisms by the completed test, that is, of all the samples tested only 45.6 percent were coliforms.

The analysis of the soils for enterococci using ethyl violet azide broth as the selective medium gave no false positive results. Positive tubes were checked microscopically and all contained gram positive cocci in chains. At no time were any other organisms observed to be present. All the isolations made for typing the organisms were taken from the ethyl violet azide medium and in every case enterococci were isolated. The number of false positives in the dextrose azide broth, which would be used as a presumptive medium if water or like samples were used, could not be determined because the amount of sedimented soil which was present made reading the tubes very difficult. It is most probable that the number of positive azide dextrose tubes that do not contain enterococci would be great, because this medium will not inhibit gram positive rods of which there are many in the soil. It is not known how many false negatives occurred with the two organisms as no tests were made for this purpose.

TABLE IX

RESULTS OF STANDARD METHODS

COLIFORM TESTS ON SOIL SAMPLES



Test	Number of Samples	Number of Positives	Percent of All Samples
Presumptive	81	71	87.6
Confirmed	71	43	53.2
Completed	43	37	45.6

SUMMARY

Coliform organisms were found to be present in all fields tested, and, in most instances, in low numbers. No typical E. coli was isolated from any of the samples. All the coliforms found were A. aerogenes or intermediates and all were able to utilize citrate. The sanitary significance of these coliforms is questionable as they were found in similar numbers in both polluted and unpolluted soils.

The enterococci were found to be present in some of the soils that would not be considered contaminated, namely "virgin soil" and untreated soil; however, they were present in very low and relatively constant numbers. They were markedly absent from sewage sludge and peat treated soils. In contrast, they were present in much higher numbers in polluted soil taken from the bank of the Red Cedar river. The enterococci present in the soils sampled were predominantly S. liquefaciens followed by S. zymogenes and S. fecalis. No S. durans was isolated.

The enterococci were always present in lower numbers than the coliform organisms but no definite ratio between them was observed. At no time was there a sample positive for the enterococci and negative for the coliforms, but the reverse of this happened quite often. There was an effect on both the coliform and enterococci content in the test soils during the summer months: both dropped in number during this period.

The presence of the enteric streptococci in a soil may aid in evaluating the significance of A. aerogenes and intermediate coliform organisms that are present.

BIBLIOGRAPHY

Breed, Robert S., et al. Bergey's Manual of Determinative Bacteriology. 6th ed. Abridged. New York: Biotech. Pub. 1948.

Carpenter, Philip L. and MacDonald Fulton. Escherichia-Aerobacter Intermediates from Human Feces. Am. Jour. Pub. Hlth., 27:822, 1937.

Gray, J. D. The Significance of B. Aerogenes in Water. Jour. Hyg., 32:132, 1932.

Hajna, A. A. and C. H. Perry. Comparative Study of Confirmatory Media for Bacteria of the Coliform Group and Fecal Streptococci. Am. Jour. Pub. Hlth., 33:550, 1943.

Houston, A. C. Bacterioscopic Examination of Drinking Water, with Particular Reference to the Relations of Streptococci and Staphlococci with Waters of this Class. Report of the Med. Officers to Local Board, 1898-9. (Supplement XXVIII. Ann. Rep. L. G. B.) as cited in Science, U. S., 15:827, 1902.

Koser, S. A. Differential Tests and Their Relation to Habitat of the Coli Aerogenes Group. Jour. Bact. 11:77, 1926.

Kulp, W. C. A Note Concerning the Effect of a Specific Environment on the Characteristics and Viability of Several Strains of A. Aerogenes and E. Coli. Jour. Bact. 24:317, 1932.

Laws, J. P. and F. W. Andrews. Report on the Result of Investigations of the Micro-organisms of Sewage. Report to London County Council, 1894. As cited in Science, U. S., 15:827, 1902.

Litsky, Warren, Walter L. Mallmann and C. W. Fifield. A New Medium for the Detection of Enterococci in Water. Am. Jour. Pub. Hlth., 43:873, 1953.

Mallmann, W. L. Streptococcus as an Indicator of Swimming Pool Pollution. Am. Jour. Pub. Hlth., 18:771, 1928.

Mallmann, W. L. A New Yardstick for Measuring Sewage Pollution. Sew. Works Jour., 12:875, 1940.

Mallmann, W. L. and C. W. Darby. Uses of a Lauryl Sulfate Broth for the Detection of Coliform Organisms. Am. Jour. Pub. Hlth. 31:127, 1941.

Mallmann, W. L. and W. Litsky. Survival of Selected Enteric Organisms in Various Types of Soil. Am. Jour. Pub. Hlth. 41: , 1951.

Mallmann, W. L. and E. B. Seligmann. A Comparative Study of Media for the Detection of Streptococci in Water and Sewage. Am. Jour. Pub. Hlth., 40: , 1950.

Orcutt, Marion L. A Study of Enterococci From the Digestive Tract of Calves. Jour. Bact., 11:129, 1926.

Ostrolenk, M. and A. C. Hunter. The Distribution of Enteric Streptococci. Jour. Bact. 51:735, 1946.

Parr, Leland W. Studies on a Series of Coliform "Citrate-Mutants" With Particular Reference to Their Stability. Jour. Bact. 41:29, 1941.

Prescott, S. C. and S. K. Baker. The Cultural Relations of Bacillus Coli and Houston's Sewage Streptococci and a Method for the Detection of These Organisms in Polluted Waters. Jour. Infectious Dis. 1:193, 1904.

Savage, W. G. and W. J. Read. The Significance of Streptococci in Water Supplies. Jour. Hyg. 15:334, 1917.

Savage, W. G. and D. R. Wood. The Vitality and Viability of Streptococci in Water. Jour. Hyg. 16:227, 1918.

Sherman, J. M. The Streptococci. Bact. Revs. 1:1-97, 1937.

Sherman, J. M. Enterococci and Related Streptococci. Jour. Bact. 35:81, 1938.

Sherman, J. M., Pauline Stark, and E. S. Yawger. The Enterococcus and Lactic Groups of the Streptococci. Jour. Bact. 33:25, 1937.

Skinner, C. E. and T. J. Murray. The Viability of B. Coli and B. Aerogenes in Soil. Jour. Infect. Dis. 38:37, 1926.

Steinhouse, Edward A. A study of Bacteria Associated with Thirty Species of Insects. Jour. Bact. 42:757, 1941.

Sylvester, J. C. and R. G. Benedict. Some Observations on the Physiological and Serological Character of the Enterococci. Jour. Bact. 42:138, 1941.

Vaughn, Reese H. and Max Levine. Differentiations of the Intermediate Coli-like Bacteria. Jour. Bact. 44:437, 1942.

Winslow, C. E. A. and M. P. Hunnewell. Streptococci Characteristic of Sewage and Sewage Polluted Waters. Science, U. S., 15:827, 1902.

Winslow, C. E. A. and G. T. Palmer. A Comparative Study of Intestinal Streptococci from the Horse, the Cow and Man. Jour. Infect. Dis. 7:1, 1910.

Winslow, C. E. A., S. C. Prescott and M. MacCrady. Water Bacteriology. 6th Ed. New York: Wiley and Son. 1947.

Winter, C. E. and L. A. Sandholzer. Isolation of Enterococci from Natural Sources. Jour. Bact. 51:588, 1946.

Young, C. C. and H. Greenfield. Observations on the Viability of the Bacterium Coli Group under Natural and Artificial Conditions. Am. Jour. Pub. Hlth. 13:270, 1923.

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