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DISTURBING FACTORS IN THE PRESUMPTIVE TEST FOR B. COLI

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The matter of the presumptive test for the presence of B. coli has been repeatedly brought to the attention of bacteriologists over a considerable period of time. Two reasons for this may be advanced: (1) the importance of detecting B. coli in infected waters, (2) the irregularities noticed in the presumptive test.

That the production of gas by B. coli, when growing in lactose broth, is considered only a presumptive test for that organism indicates that it does not fulfil all the requirements of a specific test. Chief among the defects is the production of gas when no B. coli can be demonstrated.

Frost (1) and scores of other workers have reported considerable variation in the presumptive test, as regards the demonstrability of B. coli. Numerous reasons for this irregular gas production have been advanced. Klein (2), Creel (3), and others have reported gas in lactose broth produced by anaerobic spore-forming bacilli. That this is a considerable source of the irregular gas formation is somewhat doubted by Meader and Bliss (4). E. M. Meyer (5), and others, have isolated aerobic spore-forming bacilli which give gas in lactose broth. Levine (6) confirmed this, and he advocated, as had Hall and Ellefson (7) previously, the use of gentian violet of various strengths to inhibit these organisms.

Sears and Putnam (8) have recently offered some explanations of gas production that seem applicable to this problem. They found that gas was produced by organisms in symbiosis, when none of the organisms concerned would produce gas when growing in pure culture, in the same medium. They concluded that the medium, inoculated with a symbiotic complex (two or more organisms), was first attacked by one of the organisms present, and that a second organism produced the gas by taking advantage of a stage in the decomposition due to the first. Most of their work was done with the usual cultures

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constituting a laboratory stock, chiefly pathogenic organisms. Their suggestion that this might have a bearing upon the failure of the presumptive test for B. coli seemed quite reasonable, and it was decided to investigate this phase of the question.

In considering the problem it was deemed essential to investigate from a dual standpoint. The first problem was to ascertain whether individual members of gas producing complexes were to be met with frequently in natural locations, from which they might easily gain access to a water supply. The second problem was to determine whether gas forming complexes occur in water samples giving a positive presumptive test, yet which are negative for B. coli.

SYMBIOTIC GAS PRODUCTION IN LACTOSE BROTH WITH ORGANISMS ISOLATED FROM SAMPLES SELECTED AT RANDOM

This phase of the investigation was concerned solely with the detection of organisms capable of becoming members of a gas producing complex, in water, soil, sewage, or such other locations from which they might readily enter a water supply, consequently no attention was given to the occurrence together, in the samples, of the different members of a complex.

Samples were obtained from a variety of sources, over a wide territory, including Oregon, Washington, Idaho, British Columbia, Alberta and Saskatchewan.

A portion of each sample obtained was inoculated into lactose broth, and another portion into nutrient broth for use as a stock culture. From the 48 hour culture on lactose, streaks were made on lactose litmus agar. From these plates, individual colonies were fished, and inoculated into nutrient broth. From these twenty-four hour cultures, the organisms isolated were transferred to lactose, and glucose, broths. Cultures giving gas on lactose were of course discarded. The remaining organisms, with their fermentation reactions as a basis, were divided into two groups, and various combinations of representatives of these two groups were inoculated into lactose broth, in large Smith fermentation tubes, and incubated at 37°C. At the end of forty-eight hours gas readings were recorded. As far as possible, equal amounts of the various cultures were used, the inoculation in each case being a loopful of the organism, from a 24 hour culture in nutrient broth. The inability of the organisms to produce gas from lactose, in pure culture, was established by special controls for each symbiotic complex.

In the tables given herewith, the number of any particular organism has to do solely with the numerical order in which it was

TABLE 1

CONSECUTIVE NUMBER OF CULTURE	SOURCE	GLUCOSE		LACTOSE	
		Acid	Gas	Acid	Gas
1	Swimming Pool	+	−	+	−
2	Sewage	+	+	−	−
3	Well	+	+	−	−
4	Sewage	+	−	+	−
5	Water	+	+	−	−
6	Well	+	+	−	−
7	Sewage	+	−	+	−
8	Sewage	+	−	+	−
9	Water	+	−	+	−
10	Water	+	+	−	−
11	Water	+	−	+	−
12	Water	+	+	−	−
13	Sewage	+	−	+	−
14	Well	+	−	+	−
15	Well	+	−	+	−
16	Well	+	−	+	−
25	Water	+	+	−	−
26	Sewage	+	+	−	−

TABLE 2

Gas production by symbiotic complexes

(Selected at Random)

CONSECUTIVE NUMBER OF COMPLEX	CONSECUTIVE NUMBER OF ACID FORMER	CONSECUTIVE NUMBER OF GAS FORMER	PER CENT GAS FORMED
1	1	2	5
2	4	3	10
3	9	5	15
4	13	10	Trace
5	17	6	10
6	14	10	5
7	15	12	15
12	16	5	35
23	18	10	30
24	19	25	5
33	4, 9	3, 5	25
29	4	2 + 3 + 10 + 26 + 25	20
30	9	26 + 6 + 25	25
42	1 + 4 + 11 + 9 + 13	3	35

isolated. The specific identification of the cultures was not considered necessary to this phase of the investigation.

Table 1 gives the fermentation reactions of the different pure cultures in lactose, and glucose, broths. Table 2 gives the gas readings in lactose broth of the various combinations that were tested. A comparison of the two tables will show at once that a combination producing gas in lactose broth contained at least one organism capable of fermenting lactose with acid production, and at least one organism capable of fermenting glucose, with gas production. This confirms the findings of Sears and Putnam. For convenience, the two types of organisms are listed separately in table 2. Negative tests are not included in the table. It may be stated, however, that the absence of either of the above types of organisms assured the non-appearance of gas.

The gas percentages given were selected at random from a number of repeated tests in which the results often varied within fairly wide limits. Those given undoubtedly represent a fair approximation to the average experience. The causes of the variation were not determined. It will be readily noted that the gas percentages obtained in the tests are, in a majority of the cases, high enough to be interpreted as a positive presumptive test for B. coli, ten out of the fourteen recorded combinations giving 10 per cent or more gas.

SYMBIOTIC GAS PRODUCTION BY ORGANISMS ISOLATED FROM WATER SAMPLES GIVING A POSITIVE PRESUMPTIVE TEST, BUT NEGATIVE FOR B. COLI

This phase of the investigation was concerned with the detection of gas forming complexes in samples of water giving a positive presumptive test, yet which were negative for B. coli. Several such samples of water were obtained through the courtesy of the director of the Oregon State Health Laboratory.

A broth culture was made from each sample obtained, experience having shown that some of the organisms died if left in an acid medium, such as existed in the discarded presumptive test tube. From the twenty-four hour broth cultures, each sample was inoculated into a large Smith fermentation tube, containing lactose broth, and incubated at 37°C. At the end of forty-eight hours gas readings were recorded, and streaks were made (or pour plates where necessary) on lactose litmus agar. From these plates, forty-eight

hours later, individual colonies were fished and transferred to nutrient broth, to serve as stock cultures for further study. Particular attention was paid to their fermentation reactions on lactose, and glucose, serum waters and broths. All the organisms from one sample were next re-inoculated together, in large Smith fermentation tubes containing lactose broth, and incubated at 37°C. At the end of forty-eight hours the gas formed was recorded. These figures are given in the last column of table 3.

Of the fifty samples tested, all but two failed to show organisms giving gas on lactose. In both these cases the organism concerned

TABLE 3

Symbiotic gas production by complexes isolated from positive presumptive tests, negative for B. coli upon confirmation

PRESUMPTIVE TEST NUMBER	SOURCE	GAS PER CENT WHEN OBTAINED	GAS PER CENT UPON INOCULATION	NUMBER OF ORGANISMS ISOLATED	ACID FORMERS	GAS FORMERS	GAS PER CENT UPON RECOMBINATION
272	Creek	10	0	1	1	0	0
277	Spring	20	20	4	3	1	20
284	River	30	20	3	1	2	20
288	Well	Trace	Trace	1*	—	—	Trace
303	Well	20	10	3	2	1	20
312	Spring	10	10	1*	—	—	10
329	River	40	0	3	2	1	0
330	River	Trace	10	2	1	1	10
578	River	30	30	2	1	1	35
654	Pool	20	20	4	3	1	20
662	Spring	Trace	20	2	1	1	15
717	Stream	20	20	2	1	1	25

* Spore-forming lactose fermenter.

was a spore-former. It will be seen that symbiotic complexes were isolated from many of the remaining tests. It further appears that each gas producing complex contains at least one representative of each of the two types of organisms referred to above. Recombining the organisms isolated from a sample in many instances resulted in as much gas formation as in the original sample, and in some cases more.

The acid forming organisms, in the series of tests examined, were for the most part gram-positive, non-motile, non-sporing organisms, growing readily on the usual laboratory media. The ma-

jority of these were chiefly streptococcus non-hemolyticus I and fecalis (Holman). Several were staphylococci; a few were streptococcus hemolyticus I (Holman).

The gas forming organisms were in every case found to be bacilli, most of which grew fairly well on the ordinary laboratory media.

TABLE 4

Characteristics of Organism

CONSECUTIVE NUMBER	MORPHOLOGY OF ORGANISM	GRAM STAIN	SPORES	MOTILITY	LIQUEFACTION GELATIN	INDOL PRODUCTION	GLUCOSE		LACTOSE		SACCHAROSE		MALTOSE		HEMOLYSIS OF BLOOD	MANNITE	SALICIN
							Acid	Gas	Acid	Gas	Acid	Gas	Acid	Gas			
1	Streptococcus	+	−	−	−	−	+	−	+	−	+	−	+	−	+	−	+
2	Bacillus, long, slender	−	−	+	−	−	+	+	−	−	−	−	+	+			
3	Bacillus	−	−	+	−	−	+	+	−	−	−	−	+	+			
4	Bacillus	−	−	−	−	−	+	−	+	−	−	−	+	−			
5	Bacillus	−	−	−	−	−	+	+	−	−	−	−	+	+			
6	Bacillus	−	−	+	−	−	+	+	−	−	−	−	+	+			
7	Bacillus-thick	−	−	+	+	−	+	−	+	−	+	−	+	−			
8	Streptococcus	+	−	−	−	−	+	−	+	−	+	−	+	−	−	+	−
9	Bacillus	−	−	+	−	−	+	−	+	−	+	−	+	−			
10	Bacillus	−	−	+	−	−	+	+	−	−	−	−	+	+			
11	Staphylococcus	+	−	−	+	−	+	−	+	−	+	−	+	−	−	−	−
12	Bacillus	−	−	+	−	+	+	+	−	−	+	+	+	+			
13	Staphylococcus	+	−	−	+	−	+	−	+	−	+	−	+	−	−	+	−
14	Bacillus	−	−	+	−	−	+	−	+	−	+	−	−	−			
15	Bacillus	−	−	+	+	−	+	−	+	−	+	−	+	−			
16	Staphylococcus	+	−	−	+	−	+	−	+	−	+	−	−	−			
17	Streptococcus	+	−	−	−	−	+	−	+	−	+	−	+	−	−	+	+
18	Bacillus	+	−	+	−	−	+	−	−	−	+	−	−	−			
19	Streptococcus	+	−	−	−	−	+	−	+	−	+	−	+	−	−	−	−
25	Bacillus	−	−	+	−	−	+	+	−	−	−	−	+	+			
26	Bacillus	−	−	+	−	−	+	+	−	−	−	−	+	+			
27	Bacillus	+	+	−	−	−	−	−	−	−	−	−	−	−			
28	Bacillus	+	−	−	−	−	+	−	+	−	−	−	+	−			

Several of these were gram-negative, non-sporing, motile organisms, which produced acid and gas on glucose, but failed to alter saccharose, or produce indol; these appear to be organisms related to the paratyphosus enteritidis-hog cholera group. A smaller number fermented sucrose, and produced indol, and appear to be members of the B. proteus group.

In addition to those named above, the bacillary forms encountered included some members of the subtilis, fecalis alcaligenes, and fluorescens types. Many of these were inert as far as the symbiotic complex is concerned. Six Gram-negative bacilli were found for every five Gram-positive bacilli; of these Gram-positive bacilli only about 10 per cent had any effect upon the gas producing properties of the complex.

Table 4 gives in detail the characteristics and cultural reactions of the organisms encountered.

From the small number of samples tested, it is obvious that no general conclusion can be drawn as to the percentage of occurrence of these gas producing complexes in water, and their importance as an explanation of the non-confirmation of B. coli in samples giving a positive presumptive test. The material available in a large water laboratory would be better suited, than that at our disposal, to an investigation of this question.

In the present investigation it has been demonstrated that organisms belonging to the types shown by Sears and Putnam to be capable of producing gas symbiotically from lactose broth occur widely distributed in nature and in locations from which they may readily enter a water supply. It is further shown in a definite small number of instances, that samples of water, negative for B. coli, but giving a positive presumptive test, actually contained gas producing symbiotic groups.

That the occurrence together of organisms belonging to the two groups mentioned does not necessarily lead to gas formation is evident. Symbiotic gas production in the absence of either of these groups, however, did not occur.

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