AN AEROBIC SPORE-FORMING BACILLUS GIVING GAS IN LACTOSE BROTH ISOLATED IN ROUTINE WATER EXAMINATION

E. M. MEYER

Laboratory for Field Investigations of Stream Pollutions, United States Public Health Service, Cincinnati, Ohio

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In the course of routine water examinations at this station, the writer has, on several occasions, isolated aerobic lactose-fermenting organisms which have been demonstrated to be spore-forming. So far as can be ascertained by a fairly complete review of the published literature, there has been no such organism previously described.[1] In water work two large groups of bacteria generating gas from lactose are recognized. The first are the aerobic non-spore-forming bacilli of the coli-aerogenes group, and the second the anaerobic spore-forming bacilli of the sporogenes group. The significance of the presence in a water of members of either of these groups has been pretty well established. The organism to be described lies midway between these two groups, in that it is aerobic as well as spore-forming. Just what its sanitary significance is, remains to be established.

ISOLATION

It has been the routine procedure in this laboratory to isolate for study one culture of *B. coli* from each sample of water examined. This is effected by carefully fishing one isolated colony

[1] Since this article went to press, the writer has come across an article on systematic bacteriology of water by S. De M. Gage and E. B. Phelps in Am. Pub. Health Ass. Rep. 1902, **28**, 402, 412. In the table given in this article a few cultures are noted as being aerobic lactose fermenters. In the opinion of Mr. Gage it is very unlikely that the organism now being described could be among those described by him.

from the confirmatory Endo's medium plate, made from the lactose broth tube inoculated with the water sample. The colony is inoculated into lactose broth, to confirm gas production, and if this tube is positive the organism is again plated on Endo's medium to determine if it be pure. If this plate reveals the presence of but one form, a colony is fished to an agar slant. A smear from the culture on the slant, after forty-eight hours' growth at 37°C., is stained by Gram's method, and if only Gram-negative, non-spore-forming bacilli are seen, the culture is considered pure and made up of *B. coli*.

Following the above outlined procedure, eight cultures from one source[2] were found which gave gas in lactose broth, but which on the agar slant grew very differently from *B. coli*. They appeared on staining to be large Gram-negative, fusiform rods containing spores, together with large Gram-negative vegetative bacilli. For some time the writer was under the impression, gained through previous experience, that this spore-bearer was a contamination form and that the gas production would finally be found to be due to *B. coli*. Accordingly, attempts were made to purify the culture by plating on Endo's medium, agar and gelatine. The colonies on all of the media appeared to contain but one organism. When transferred to agar slants the growth was macroscopically and microscopically like the agar slant culture first alluded to. That the gas production was due to the spore-forming organism was definitely proved by the heat-resistance experiments next performed.

RESISTANCE TO HEAT

The organism will live and generate gas in lactose broth after being subjected to 95°C. for twenty minutes or to boiling water temperature for ten minutes, but is killed if subjected to the latter temperature for fifteen minutes. The writer has been checked in these results by another bacteriologist in this labora-

[2] Since the writing of this article the organism has been isolated three additional times—once from the tap water of Covington, Ky., and twice from raw tannery trade wastes. These three cultures resemble the eight previously isolated in all particulars.

tory, working independently. The tests were run as follows: tubes of standard lactose broth, with inverted vials, were placed in a water-bath, brought to the desired temperature and regulated to within 1°C. After the tubes had attained the temperature of the water-bath, 0.1 cc. of a light suspension of the organism taken from the surface of a seventy-two hour old agar slant was carefully added to the tube and the time noted. At suitable intervals, two tubes were withdrawn and put into cold water to cool. After seventy-two hours' incubation at 37°C. the tubes were examined and the growth and gas production recorded. Where there was no gas production the tubes were sterile. Agar slant cultures made from the broth tubes showing gas revealed the spore-former in pure culture.

MORPHOLOGY AND STAINING CHARACTERISTICS

In smear preparations made from forty-eight hour old agar slants three distinct forms, representing different stages of the bacillus, are seen. First there is the vegetative cell, a regular rod with rounded ends, Gram negative, 4.5 to 5.5 by 0.8 to 1 μ Next is the spore-bearer, spindle-shaped, Gram-negative, about 3 by 1.5 μ. With ordinary stains the spore is shown as a central, oval, unstained area, and with Müller's spore stain is readily demonstrated. The third form is the free spore, oval, regular, 2 by 1.5 μ, showing deep red with the spore stain. These occur most frequently and regularly in older cultures.

No capsules could be demonstrated by the use of the Welch capsule stain in smear preparations from milk cultures. When stained by Löffler's method, the organisms show numerous peritrichic flagella, some bacilli showing as many as 16 to 18. Notwithstanding this fact, motility has not been observed when using the hanging drop method.

APPEARANCE AND REACTIONS IN VARIOUS MEDIA

Agar slant. Growth quite distinctive. At 37°C., in twenty-four hours, thin transparent veil-like growth over entire surface except the very top. Growth lobate along upper edge. Micro-

scopically—in twenty-four hours mainly vegetative forms, in forty-eight hours spore-bearing forms and later only free spores.

Agar stab at 37°C. Growth along entire line of inoculation into depths of agar. Growth somewhat echinulate. In meat infusion agar many gas bubbles, due to fermentation of muscle sugar (inosite).

Agar plate. Nutrient agar twenty-four hours at 37°C., colonies discrete, round, thin and small (1 mm. diameter), edges smooth.

Endo's plates at 37°C. Twenty-four hours, colonies pink with red center, irregular contour, 1 to 2 mm. diameter, little or no sheen. Colonies forty-eight hours, deep red, much sheen in colonies and surrounding medium. Latter point distinctive.

Gelatin plate at 20°C. Forty-eight hours, colonies small (0.5 to 1 mm. diameter), round, *incipient* liquefaction. Colonies seventy-two hours—liquefaction, round, edges regular, 2 to 3 mm. diameter.

Gelatin stab at 20°C. Forty-eight hours, beginning liquefaction; in seventy-two hours liquefaction infundibuliform, slight precipitate.

Carbohydrates. In standard extract broth to which has been added one percent of the following carbohydrates, acid and gas are formed: (1) Glucose, (2) laevulose, (3) raffinose, (4) maltose, (5) sucrose, (6) lactose, (7) inulin, (8) starch, (9) glycerol, (10) mannitol. No acid or gas and little growth in dulcite broth, which remained clear and limpid. In other broths gas usually appeared in twenty-four hours. Media uniformly clouded, slight stringy precipitate, no pellicle. Media forty-eight hours, slightly viscous.

Glucose neutral-red broth. Same reaction as that of *B. coli*, i.e., yellow fluorescence with gas formation.

Clark's glucose, peptone, phosphate, medium (0.5 per cent glucose, 0.5 per cent K_2HPO_4 and 0.5 per cent peptone broth), typical reaction of "Grain" type coli, in forty-eight hours at 37°C., i.e. reaction alkaline to methyl red, Voges-Proskauer test positive.

Limiting hydrogen-iron concentrations. Clark's glucose peptone-phosphate medium was used after being adjusted with NaOH or HCl to various hydrogen-ion concentrations. Growth between $P_H = 5.0$ to $P_H = 9.0$ inclusive. In tube $P_H = 9.0$ very scanty growth and only bubble of gas. In all other tubes much gas, and growth luxuriant.

Indol production in 1 per cent peptone, four days at 37°C. No indol detected when tested for by the nitrite and by Ehrlich's para-dimethyl-amido-benzaldehyde method.

Reduction of nitrates. In 0.1 per cent peptone + 0.02 per cent $NaNO_3$ solution, four days at 37°C., no reduction to nitrites.

Litmus milk at 37°C. In twenty-four hours acid, in forty-eight hours partially reduced, coagulated with extrusion of whey, beginning digestion of curd.

Lactose bile at 37°C. Ninety-six hours, no gas or growth.

Chromogenesis. None noted on any media used.

OCCURRENCE AND SIGNIFICANCE

Dr. J. S. Bolten, working in this laboratory, isolated what the writer believes to have been the same or a similar organism from a sample of sewage which had been taken from Mill Creek during the winter of 1916 and stored for forty-one days. Reference to his unpublished notes which are on file at this station, shows that the organism had morphological and cultural characteristics similar to those detailed above. Owing to rather variable and uncertain heat resistance experiments, which Dr. Bolten deemed inconclusive, he was unable to state definitely that the spore-bearer was the gas-former. However, Dr. Bolten used for seeding material in the heat resistance experiments, cultures in lactose broth, in which medium, sporulation is indefinite and delayed.

Regular examinations are made in this laboratory of samples of water from various sources. These include the Ohio River and its tributaries in the vicinity of Cincinnati, and the tap supplies of Cincinnati and two Kentucky cities on the Ohio River opposite this city. The organism herein described has

been isolated from but one of these sources—the water supply of Newport, Kentucky. Up to date it has been obtained from the samples collected on the following eight days: January 30, February 8, 10 and 26, March 31, and April 2, 11, and 20, 1917. Newport, Kentucky, uses Ohio River water after subjecting it to treatment and storage. Treatment consists of addition of small amounts of calcium hypochlorite, and of lime and iron, and the total storage is estimated at twenty days.

This organism during the times of its occurrence might cause considerable error in the determination of the colon index. During the months of January to April, 1917, inclusive, of 40 aerobic gas formers isolated from 91 samples of Newport tap water, 32 or 80 per cent were of the coli-aerogenes group while 8 or 20 per cent were this spore-former. Due to its rarity and limited occurrence, however, it could not constitute a source of appreciable error in routine water examinations in most localities. Over 17,000 samples have been examined at this station within the past three years, and this spore-former has been isolated but eight times and, as detailed above, from but one source. Other water workers have never reported its occurrence, and it is likely that in most waters it is exceedingly rare, if not entirely absent.

SUMMARY

An aerobic bacillus, giving gas from lactose, and demonstrated to be spore-forming, has been isolated eight times between January 30 and April 20, 1917, from the tap water of Newport, Kentucky. This organism is believed to be a species whose isolation has never before been described.

The writer wishes to acknowledge his indebtedness to Surgeon W. H. Frost, under whose direction this study has been made.