GROWTH OF EBERTHELLA TYPHOSA AND AEROBACTER AEROGENES IN ASSOCIATION IN TETRATHIONATE BROTH

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The observation by Muller (1923) of the value of sodium tetrathionate broth as a selective enriching medium for certain members of the enteric group of bacteria has been confirmed by a number of workers. Kaufmann (1930), for example, reported 30 per cent more positive isolations from specimens enriched in this medium than were obtained by direct plating, whereas Schaeffer (1935) obtained four times as many. Similar results by others have led to extensive use of the medium in public health laboratory work.

Ivanovics (1931), however, has reported observations which cast some doubt on the value of the medium. In one series of experiments he noted marked inhibition of *Eberthella typhosa* by each of 6 strains of *Aerobacter aerogenes* after incubation in mixed culture in sodium tetrathionate broth for 24 hours. With 3 of these strains no *Eberthella* colonies appeared on plates streaked from the mixture. In another series of experiments with 4 strains of *Aerobacter* he was unable to recover *Eberthella* in any instance from 24-hour mixed cultures of the two organisms. Only one of the *Aerobacter* strains which he used showed antagonistic action against *Eberthella* in plain broth, and in this one instance the effect was very slight. Since *Aerobacter* is of common occurrence in stool specimens, the serious practical import of the work of Ivanovics is obvious.

In connection with some other work we recently have repeated the experiments of Ivanovics, using a strain of *Aerobacter* which, unlike those strains tested by him, did show moderate antagonism in plain broth toward *Eberthella*. Both the *Aerobacter* and the *Eberthella* strains which were used have been maintained in stock culture for a number of years and have been found repeatedly, both before and during the present work, to be culturally and biochemically typical. The purity of each was established by picking from isolated colonies on eosin methylene blue agar plates.

The tetrathionate broth was prepared from a proteose peptone oxgall base. All other media were prepared from Difco dehydrated products.

The experimental method followed very closely that used by Ivanovics, differing only in that the observations were extended over a longer period of incubation and that a pure culture control of *Eberthella* in both plain and in tetrathionate broth was included. A loopful of a 24-hour nutrient broth culture of each species was planted in tubes of plain and of sodium tetrathionate medium. After agitation, a standardized loopful from each tube was spread on a plate of eosin methylene blue agar with a glass elbow rod. After some preliminary work it became possible to obtain regularly an initial ratio of *Eberthella* to *Aero-*

bacter of 1:1 to 1:2 as determined by counting from 400 to 600 colonies on plates which had been incubated for from 12 to 16 hours. Ordinarily no difficulty was encountered in differentiating the two species on the basis of colony appearance. In case there was doubt as to the identity of a colony type a transfer was made to double sugar medium for confirmation.

After incubation of the tubes for the intervals shown in the tabulation below, each culture was thoroughly shaken and a dilution made in sterile 0.85 per cent sodium chloride solution for streaking on eosin methylene blue agar plates. The proportion of culture to salt solution was varied when necessary so as to obtain about 300 colonies per plate. Growth in the tetrathionate medium was not so heavy as in the plain medium, and, consequently, 4 to 5 loopfuls of the tetrathionate culture were required to obtain the same number of colonies as would appear from the dilution of a single loopful of plain broth culture in an equal amount of salt solution. From these, plate counts were made to determine the effect of growth in association upon the ratio of *Eberthella* to *Aerobacter*. An equal amount of the pure culture of *Eberthella* was treated the same way in order to give a crude quantitative determination of the luxuriance of growth in pure culture. By comparison of the pure culture data with the mixed culture data it is possible to evaluate in a rough way the degree of antagonism exerted by *Aerobacter* in each medium provided one fact is taken into consideration, namely, that the total amount of growth of each organism in mixed culture is considerably less—roughly about one half—than that of either in pure culture, so that the total number of cells per unit volume of medium after incubation is approximately the same for both the pure culture and for the mixture. This fact was noted by Fulton (1937) for *Salmonella schottmuelleri* and *Escherichia coli* grown in association, and it has been repeatedly confirmed in connection with the present work. In the absence of antagonism two species of comparable growth rates grown in association should show approximately equal numbers of cells per unit volume of medium, and the total of the two should approximate the total growth of one grown in pure culture. If one species is antagonistic to the other, then the ratio of the one to the other at intervals of time may be interpreted as an indication of the degree of antagonism.

The results shown in table 1 are fully typical of those obtained.

The ratios given in column II support the statement that the strain of *Aerobacter* showed antagonism against the strain of *Eberthella* in plain broth. An initial ratio of 1:2 changed to a ratio of 1:300 over a 5-day period, and after 14 days no *Eberthella* colonies could be detected. These ratios, it will be recalled, are based upon a usual count of approximately 300 colonies. In column III appears the crude comparative quantitative count of the pure culture of *Eberthella*. At 24 hours, on the basis of the pure culture count, an *Eberthella* count from the mixture of between 150 and 200 would be predicted in the absence of antagonism. The ratio of 1:8 means an *Eberthella* count of about 35. Similarly at 48 hours and at 14 days the results speak for an inhibition of *Eberthella* by *Aerobacter*.

With tetrathionate broth the ratio values given in column IV taken alone

would indicate that the *Aerobacter* had little or no effect on the *Eberthella* since no decided change in ratio developed. Columns V and VI, however, indicate that antagonism in this medium did occur, since otherwise the counts in column VI should be roughly one half of the corresponding counts in column V. A comparison of the ratio values in columns II and IV, however, shows that the antagonism was much greater in plain broth than in tetrathionate medium. No significance can be attached to the tetrathionate results at the 14-day interval since by this time *Eberthella* had died out in this medium.

Although tetrathionate broth will not support so luxuriant a growth of either organism as will plain broth, it is initially more favorable to *Eberthella* than to *Aerobacter*, or else it exerts an initial selective inhibitory action on *Aerobacter*.

TABLE 1

Growth of Eberthella in pure culture and in mixture with Aerobacter in plain and in sodium tetrathionate broth

I TIME IN HOURS	II RATIO E/A IN PLAIN BROTH	III TOTAL EBERTHELLA. PURE CULTURE IN PLAIN BROTH	IV RATIO E/A IN TETRATHIONATE	V TOTAL EBERTHELLA. PURE CULTURE IN TETRATHIONATE	VI TOTAL EBERTHELLA. MIXED CULTURE IN TETRATHIONATE
0	1–2	—	1–1	—	
24	1–8	400	1–3	500	50
48	1–14	600	1–2	700	120
72	1–100	—	1–3	900	100
96	1–150	—	1–2	650	150
120	1–300	—	1–3	350	110
*14 days	Pure A	150	Pure A	Sterile	Pure A

— not determined.

* Inoculum at 14 days from plain broth was 4 to 5 times as great as at other intervals; from tetrathionate 4 to 5 times less than at other intervals because of heavy late multiplication of *Aerobacter*.

Whatever may be the explanation, the results obtained are exactly the reverse of the results reported by Ivanovics.

In view of the fact that the ratio of contaminants to significant bacterial type, after a short incubation period, is the factor of greatest importance in the practical use of tetrathionate medium, the results obtained are in favor of the use of the medium for enrichment purposes.

SUMMARY

A strain of *Aerobacter aerogenes* which was moderately antagonistic for *Eberthella typhosa* was found to show less antagonism in tetrathionate medium than in plain broth.

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