ANTAGONISM BY AEROBACTER STRAITS

E. STATEN WYNNE

Department of Bacteriology, University of Texas, Austin, Texas

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The literature on the antagonistic action of members of the genus *Aerobacter* is rather scanty. Winslow and Cohen (1918*a*, 1918*b*) reported that when a mixture of 46 per cent *Bacillus aerogenes* cells and 54 per cent *Bacillus coli* cells suspended in unsterilized tap water was stored 60 days, only 29 per cent of the remaining cells were *B. coli*. A slight degree of antagonism might conceivably have existed; however, Powers and Levine (1937) failed to demonstrate appreciable inhibition of *Escherichia* and "intermediate" strains on "staled" media made by adding agar to 10-day broth cultures of *Aerobacter aerogenes* and *Aerobacter cloacae*. Gundel (1927) claimed *Bacterium lactis-aerogenes* to be antagonistic to *Bacillus anthracis* but stated that the strains which he used could be distinguished from colon bacilli solely on the basis of capsule formation. Ivanovics (1931) reported only one out of six *B. aerogenes* strains moderately inhibitory toward *Eberthella typhosa* in plain broth, but all six were markedly inhibitory in sodium tetrathionate broth. Wynne and Williams (1945) confirmed Ivanovics' finding of antagonism of *A. aerogenes* toward *E. typhosa* but found a much higher degree of antagonism in plain broth than in sodium tetrathionate medium. Waksman (1945) has cited Fadeeva and Tchernobaiev's 1935 report of antagonism of *A. aerogenes* toward *Bacillus pestis*.

EXPERIMENTAL

All species used in this work were repurified by single colony isolation before the investigation was initiated. The 21 species tested against the *Aerobacter* strains were from the stock culture collection of the Department of Bacteriology at the University of Texas, where their characteristics had been checked at intervals over a considerable period of time. These included *Alkaligenes faecalis, Bacillus anthracis, Bacillus cereus, Bacillus mycoides, Bacillus mycoides* 24-V (*cereus*-like variant), *Bacillus polymyxa, Bacillus subtilis* Koch-Novy, *Eberthella typhosa, Escherichia coli, Neisseria catarrhalis, Proteus vulgaris, Pseudomonas fluorescens, Salmonella aertrycke, Salmonella enteritidis, Salmonella paratyphi, Salmonella schottmuelleri, Serratia marcescens, Shigella paradysenteriae* (Flexner), *Shigella paradysenteriae* (Hiss), *Shigella sonnei*, and *Staphylococcus aureus*.

The eight *Aerobacter* strains used were *Aerobacter cloacae, A. aerogenes* L, *A. aerogenes* 29, and five strains of *A. aerogenes* numbered 1 to 5. The first three strains were obtained from the same source as the test species. Except where otherwise specified, *A. aerogenes* 29 was used throughout the investigation. The remaining five *A. aerogenes* strains (1 to 5) were isolated from stool specimens. All seven *A. aerogenes* strains were gram-negative, nonmotile short rods producing acid throughout and gas in the butt of Russell double sugar, acid and gas from

lactose, sucrose, and glycerol, no liquefaction of gelatin, positive Voges-Proskauer tests, negative methyl red tests, and typical colonies on eosin methylene blue agar. The latter were pink, usually with dark reddish-purple centers, mucoid and capitate. The *A. cloacae* strain differed in being motile, not fermenting glycerol, and producing flatter colonies with a marked spreading tendency. It had apparently lost its power of liquefying gelatin, a not uncommon event according to Bergey *et al.* (1939).

All except four of the media employed were prepared from Difco dehydrated products. Eosin methylene blue agar with sucrose only was made according to the formula of the Difco *Manual* (1939), except that 10 grams per liter of sucrose were used instead of 5 grams each of sucrose and lactose. Eosin methylene blue agar with lactose only was prepared similarly. Buffered glucose broth for Voges-Proskauer and methyl red contained 5 grams per liter each of proteose peptone, K_2HPO_4, and glucose. Finally, glycerol broth was prepared from Difco nutrient broth to which was added 1 gram of Na_2HPO_4, 10 ml of Andrade's indicator, and 5 grams of glycerol per liter.

Incubations were at 37 C, except for *S. marcescens*, which was incubated at room temperature for maximum pigment production. All nutrient agar plates were incubated about 24 hours, but the eosin methylene blue media were incubated 12 to 16 hours to lessen *Aerobacter* overgrowth. Russell double sugar and carbohydrate indicator media were read at 24 hours, nutrient gelatin at 48 hours, bismuth sulfite agar at 4 days, Voges-Proskauer tests at 24 to 48 hours, and methyl red tests at 4 days.

Quantitative differentiation of the colonies of the test species from those of *A. aerogenes* 29, a necessary part of the experimental method employed, in the majority of cases afforded no special difficulty. Because of the rather slow disappearance of *E. typhosa* and *S. paradysenteriae* (Flexner) from mixed cultures, doubtful colonies on eosin methylene blue agar were picked to Russell double sugar agar for confirmation. Such a procedure was rendered necessary by the short (12 to 16 hours) incubation time used to reduce *Aerobacter* overgrowth, since an occasional slowly developing colony of the latter led to some confusion.

E. coli colonies on eosin methylene blue agar were typically purplish black and easily distinguished from colonies of *A. aerogenes* 29, but at times slow development of the latter and its production of a flat variant type led to some difficulty. The formation of colored colonies on eosin methylene blue agar is a function of acid production, as shown by Wynne, Rode, and Hayward (1942). Since the *E. coli* strain did not ferment sucrose, eosin methylene blue agar containing sucrose only (instead of sucrose and lactose) was also used, with resulting clear-cut differentiation between the colorless *Escherichia* colonies and the usual *Aerobacter* type.

Considerable difficulty was experienced at first in distinguishing colonies of the *S. sonnei* strain used, because of its rapid fermentation of lactose and the production by the *Aerobacter* strain of the flat variant mentioned above on eosin methylene blue agar. At 48 hours' incubation, however, it was found that colonies of the former species possessed a reddish-orange tinge aiding materially in their identification, as confirmed by numerous tests of doubtful colonies in

Russell double sugar agar and phenol red sucrose broth. As the *Shigella* strain did not ferment sucrose, eosin methylene blue agar containing sucrose only was also employed, with the same well-defined differences in colonies as was obtained in the case of *E. coli*. *P. vulgaris* presented difficulties in colonial differentiation quite similar to those experienced with *S. sonnei*. Since the former fermented sucrose but not lactose, eosin methylene blue agar with lactose only was employed to facilitate colony identification.

With most of the species for which nutrient agar was used, differentiation of colonies of the test organisms from those of *A. aerogenes* 29 occasioned no particular difficulty. It was found that the use of transmitted as well as reflected light was of value, particularly with *B. subtilis* Koch-Novy and *P. fluorescens*. The former produced a smooth variant type distinguished from *Aerobacter* colonies mainly by its flatter appearance and greater opacity.

Unless well isolated, *S. aureus* colonies were considerably smaller than those of *Aerobacter*. Since pigment production was too slow to be of value, gram stains of questionable colonies were made. A similar procedure was used for *B. polymyxa*, which was invariably gram-positive.

N. catarrhalis colonies were rather difficult to distinguish from those of *A. aerogenes* 29, especially since the latter regularly produced a rather bluish variant type less opaque than the usual colony. Furthermore, more than one type of *Neisseria* colonies was produced, with varying opacity. It was found that holding the plates at about arm's length between the observer's eye and a ceiling source of light allowed ready differentiation, however. Painstaking tests of colonies in phenol red glucose broth were confirmatory.

The general method used for semiquantitative determination of antagonism was similar to that of Ivanovics (1931), except that pure culture controls were included and the observations continued for a much longer period. Fulton (1937) has emphasized the importance of pure culture controls and has also cited Topley and Fielden's 1922 finding that certain organisms which could not be isolated from a young mixed culture might later become the predominant type.

A standardized loopful from a well-shaken 24-hour nutrient broth culture of the test species in question was inoculated into a tube containing about 4 ml of nutrient broth along with a similar inoculum from a like culture of the *Aerobacter* strain (or a dilution therefrom). After agitation, a carefully standardized loopful (or more) of the mixture was distributed over the surface of a differential agar plate by means of a glass elbow rod. After appropriate incubation, which varied somewhat with the medium and the test organism (usually 14 to 24 hours), the initial control ratio of colonies of test species to *Aerobacter* colonies was determined. Even though Fulton (1937) failed to confirm the contentions of Etinger-Tulcynska (1932, 1934) and Neufeld and Kuhn (1934) that antagonistic action is dependent on the relative initial numbers of two given organisms, all tubes giving initial control ratios of $> 3:1$ or $< 1:3$ were discarded, and in the great majority of cases the limits established were 2:1 and 1:2. Ordinarily 100 to 400 colonies or more were counted in determining these ratios.

After incubation of the mixed cultures for the intervals listed in table 1, each tube was thoroughly agitated and a loopful transferred to about 4 ml of sterile

TABLE 1

Ratios of test colonies to those of Aerobacter aerogenes 29 and no. of colonies on pure test culture controls

TEST SPECIES	STREAKING MEDIUM	DILUTION OF AEROBACTER	INITIAL CONTROL	24 HR		48 HR		72 HR		96 HR		120 HR		14 DAYS	
				Ratio	P. C.	Ratio	P. C.	Ratio	P. C.	Ratio	P. C.	Ratio	P. C.	Ratio	P. C.
Alkaligenes faecalis	E.M.B.	Undiluted	1-2	1-2	—	1-1	—	2-1	—	6-1	—	5-1	—	1-1	—
Bacillus anthracis	N.A.	1-100	2-1	0	15	0	—	0	—	0	—	0	—	0	—
Bacillus cereus	N.A.	1-10	1-1	1-100	20	1-30	16	1-50	50	1-4	45	1-7	—	1-1	—
Bacillus mycoides	N.A.	1-100	1-2	0	1	1-120	1	1-120	1	1-18	1	1-35	1	1-9	23
Bacillus mycoides 24-V	N.A.	1-100	1-1	0	19	0	10	1-45	—	1-60	—	1-60	—	1-18	—
Bacillus polymyxa	N.A.	1-100	1-2	0	16	0	—	0	—	0	—	0	—	0	—
Bacillus subtilis Koch-Novy	N.A.	1-10	1-1	1-100	25	1-25	40	1-45	75	1-8	—	1-9	—	1-6	—
Eberthella typhosa	E.M.B.	Undiluted	1-2	1-8	400	1-14	600	1-100	—	1-150	—	1-300	—	0	—
Escherichia coli	E.M.B.S.O. + E.M.B.	Undiluted	1-1	1-2	—	1-2	—	1-2	—	1-2	—	1-3	—	1-2	—
Neisseria catarrhalis	N.A.	Undiluted	1-1	5-1	—	5-1	—	4-1	—	5-1	—	6-1	—	2-1	—
Proteus vulgaris	E.M.B.L.O. + E.M.B.	Undiluted	1-1	1-1	—	1-2	—	1-2	—	1-2	—	1-2	—	2-1	—
Pseudomonas fluorescens	N.A.	Undiluted	2-1	2-1	—	9-1	—	35-1	—	3-1	—	8-1	—	25-1	—
Salmonella aertrycke	E.M.B.	Undiluted	1-2	1-2	—	1-3	—	1-3	400	1-4	280	1-3	270	4-1	330
Salmonella enteritidis	E.M.B.	Undiluted	1-2	1-4	—	1-5	—	1-6	750	1-10	700	1-9	500	9-1	750
Salmonella paratyphi	E.M.B.	1-10	2-1	1-30	400	1-12	—	1-20	—	0	—	0	—	0	—
Salmonella schottmuelleri	E.M.B.	Undiluted	1-1	1-1	—	1-1	—	1-1	250	1-6	200	1-10	150	1-2	210
Serratia marcescens	N.A.	Undiluted	2-1	3-1	—	1-1	260	1-2	200	1-200*	460	0*	260	0*	260
Shigella paradysenteriae (Flexner)	E.M.B.	1-10	2-1	1-25	170	1-60	—	1-300	—	0	—	0	—	0	—
Shigella paradysenteriae (Hiss)	E.M.B.	1-10	1-1	0	130	0	—	0	—	0	—	0	—	0	—
Shigella sonnei	E.M.B.S.O. + E.M.B.	Undiluted	1-1	1-2	—	1-2	—	1-2	—	1-2	210	1-7	150	1-120	120
Staphylococcus aureus	N.A.	Undiluted	1-1	0	1,000	0	—	0	—	0	—	0	—	0	—

N.A. = nutrient agar.
E.M.B. = eosin methylene blue agar.
E.M.B.S.O. = eosin methylene blue agar with sucrose only (instead of lactose and sucrose).
E.M.B.L.O. = eosin methylene blue agar with lactose only (instead of lactose and sucrose).
— = not run.
* Indicates median rather than average ratio.

0.85 per cent NaCl solution. Following agitation, an amount sufficient to give approximately 200 to 300 colonies was streaked on appropriate agar plates, and the ratio of colonies of the test species to *Aerobacter* colonies was again determined.

It should be emphasized that the ratios in table 1 are rough approximations arrived at by taking the arithmetical averages of ratios of separate experiments, except for median ratios in three instances where extreme variation made average ratios give distorted values. For example, in 13 out of 16 experiments no *S. marcescens* colonies appeared on plates streaked from 14-day-old mixtures, while the remaining three trials gave ratios of 1:3, 1:5, and 1:40. The average ratio of 1:25 obviously does not represent the typical behavior, which is more accurately given by the median ratio of 0. No attempt was made to establish cases of very slight antagonism such as Fulton's finding that *S. schottmuelleri* produced only about two-thirds as many "cell hours" in the presence of *E. coli* as was theoretically expected from pure culture controls. Ratios showing no more than threefold decreases from the initial control ratios were regarded as of no significance, and larger decreases were not interpreted as indicative of antagonistic action unless repeated pure culture controls of the test species clearly indicated such to be the case.

The pure culture controls were subjected to exactly the same treatment as the mixtures, but were run only on organisms apparently inhibited and were examined only at the intervals deemed necessary to establish the presence or absence of antagonism. Their function is best explained by examples. Colonies of *S. paradysenteriae* (Hiss) never appeared on plates streaked from mixtures; i.e., all test ratios were 0. Since pure culture controls showed an average of 125 colonies at 24 hours' incubation, marked inhibition was clearly evident. On the other hand, *B. mycoides* gave ratios of 0 at 24 hours, and 1:120 at 48 hours and 72 hours. Assuming a total of roughly 250 colonies per plate, an average of only two colonies of *B. mycoides* appeared at the last two intervals. Nevertheless, antagonism was not present, since the corresponding pure culture controls averaged only one colony. Furthermore, the mixtures yielded a 1:9 ratio at 14 days, or about 25 *B. mycoides* colonies per plate, while the pure culture controls averaged 23.

It is important in this connection to note Fulton's observation that the total growth in "cell hours" of *S. schottmuelleri* and *E. coli* in mixed cultures was 53 per cent of their total combined growth in pure culture controls. It would follow, in the absence of antagonism, that a pure culture control of a given species with a growth rate comparable to *Aerobacter* strains should yield approximately twice as many test colonies as a mixture. It is doubtful, however, on theoretical grounds of space limitations and maximum cell concentrations, that such discrepancies hold for species such as *B. mycoides* with growth rates appreciably slower than those of the *Aerobacter* strains. Ample experimental confirmation has been obtained for both conclusions.

By comparison of the data for pure culture controls with those for mixtures given in table 1, the 21 test species may be divided into four groups on the basis of the antagonistic action exerted toward them by *A. aerogenes* 29, viz.:

Group 1. Organisms so markedly inhibited that their colonies never appeared on plates streaked from incubated mixed cultures, with pure culture controls indicating that an appreciable number of colonies should have appeared in the absence of antagonism. Included are *B. polymyxa*, *B. anthracis*, *S. paradysenteriae* (Hiss), and *S. aureus*.

Group 2. Species whose colonies appeared on plates streaked from young mixed cultures, but which were not detectable or were barely detectable after a variable incubation priod. Pure culture controls showed no corresponding disappearance of or marked decrease in colonies. Included are *E. typhosa*, *S. paratyphi*, *S. paradysenteriae* (Flexner), *S. sonnei*, and *S. marcescens*.

Group 3. Organisms against which *A. aerogenes* 29 exerted a definite but temporary antagonistic action. With *B. cereus*, *B. mycoides* 24-V, and *B. subtilis* Koch-Novy this effect was manifested initially, but it did not become well defined in case of *S. enteritidis* and *S. schottmuelleri* until about 96 hours. Pure culture controls in each case demonstrated the reality of antagonism.

Group 4. Organisms not appreciably affected by the *Aerobacter* strain, insofar as could be determined by means of the method employed. Included are *A. faecalis*, *B. mycoides*, *E. coli*, *N. catarrhalis*, *P. vulgaris*, *P. fluorescens*, and *S. aertrycke*.

In table 2 are presented the results of testing seven strains of *A. aerogenes* and one strain of *A. cloacae* against *E. coli*, *S. aureus*, and *S. paratyphi*. The sources of the organisms have been given above. It is evident that some strain differences were exhibited, but the same general pattern of antagonism occurred with all *Aerobacter* organisms. None had appreciable effect against *E. coli*, but all exerted a marked action against *S. aureus*. After an initial inhibition, *S. paratyphi* effected a relative increase with all *Aerobacter* strains except *A. cloacae* to reach a maximum ratio at 48 to 96 hours' incubation, after which it disappeared from streaked plates.

The question naturally arises as to whether complete destruction occurred in cells of the species in group 1, toward which the antagonistic effect of *A. aerogenes* 29 was most pronounced. Fourteen-day-old mixed cultures of *B. anthracis* and *B. polymyxa* with the *Aerobacter* strain were heated at 60 C for 1 hour to kill vegetative cells; inoculation of 0.1 ml to nutrient broth followed. Growth occurred on incubation and each culture was identified by cellular morphology and colonial characteristics on nutrient agar as a pure culture of the test species. It may be concluded that either resistant spores were introduced in the inoculum for these two species, or else they were formed in the presence of *A. aerogenes* 29.

For nonsporulating organisms evidence was less conclusive. Smears of 14-day mixtures of *S. aureus* and the *Aerobacter* strain revealed no cells of the former, but in such old cultures staphylococci would very probably be gram-negative and quite difficult to distinguish from the coccoid *Aerobacter* cells. Furthermore, mere absence of cells of the antagonized species in smears cannot be interpreted as signifying their complete destruction.

Since no feasible experimental method suggested itself for testing this point with *S. paradysenteriae* (Hiss), the remaining organism in group 1, it was in-

vestigated with *E. typhosa*. By use of the bismuth sulfite medium of Wilson and Blair, quantitative estimations of this organism could be made fairly accurately in the presence of *A. aerogenes* 29, the large black *Eberthella* colonies with brownish halo being easily distinguished from the few smaller black colonies which the *Aerobacter* strain was able to produce on this selective medium. The accuracy of this differentiation was tested by the following procedure: 24-hour pure cultures of *E. typhosa* showed an average of 165,000,000 cells per ml by

TABLE 2

Relative antagonistic powers of Aerobacter strains

TEST SPECIES	AEROBACTER STRAIN	STREAKING MEDIUM	DILUTION OF AEROBACTER	RATIO OF TEST COLONIES TO AEROBACTER COLONIES						
				Initial control	24 hours	48 hours	72 hours	96 hours	120 hours	14 days
Staphylococcus aureus	*A. aerogenes* 29	Nutrient agar	Undiluted	1-1	0	0	0	0	0	0
	A. aerogenes L	Nutrient agar	Undiluted	1-1	0	—	—	—	—	—
	A. aerogenes, 1	Nutrient agar	Undiluted	1-1	1-70	1-400	0	—	—	—
	A. aerogenes, 2	Nutrient agar	Undiluted	1-1	1-200	0	—	—	—	—
	A. aerogenes, 3	Nutrient agar	Undiluted	1-1	0	—	—	—	—	—
	A. aerogenes, 4	Nutrient agar	Undiluted	1-1	1-200	0	—	—	—	—
	A. aerogenes, 5	Nutrient agar	Undiluted	1-1	1-100	0	—	—	—	—
	A. cloacae	Nutrient agar	Undiluted	1-1	0	—	—	—	—	—
Salmonella paratyphi	*A. aerogenes* 29	E.M.B.	1-10	2-1	1-30	1-12	1-20	0	0	0
	A. aerogenes L	E.M.B.	1-4	1-1	1-16	1-8	1-10	1-30	1-40	0
	A. aerogenes, 1	E.M.B.	1-10	3-1	1-13	1-20	1-20	1-25	1-60	0
	A. aerogenes, 2	E.M.B.	1-10	2-1	1-100	1-30	1-40	1-100	1-100	0
	A. aerogenes, 3	E.M.B.	1-10	3-1	1-150	1-25	1-12	1-10	1-20	0
	A. aerogenes, 4	E.M.B.	1-10	3-1	1-15	1-12	1-30	1-40	1-25	0
	A. aerogenes, 5	E.M.B.	1-4	1-1	1-25	1-20	1-13	1-45	1-40	0
	A. cloacae	E.M.B.	1-10	5-1	1-30	1-200	0	0	—	—
Escherichia coli	*A. aerogenes* 29	E.M.B.S.O.	Undiluted	1-1	1-2	1-2	1-2	1-2	1-3	1-2
	A. aerogenes L	E.M.B.	Undiluted	1-1	1-1	1-2	1-2	1-2	1-2	1-1
	A. aerogenes, 1	E.M.B.	Undiluted	1-1	1-1	1-1	1-2	1-1	1-1	1-1
	A. aerogenes, 2	E.M.B.	Undiluted	1-1	1-3	1-2	1-2	1-2	1-3	1-2
	A. aerogenes, 3	E.M.B.	Undiluted	1-1	1-1	1-3	1-2	1-1	1-1	1-1
	A. aerogenes, 4	E.M.B.S.O.	Undiluted	1-1	1-2	1-3	1-3	1-3	1-5	1-2
	A. aerogenes, 5	E.M.B.	Undiluted	1-1	1-1	1-2	1-3	1-3	1-5	1-2
	A. cloacae	E.M.B.S.O.	Undiluted	1-2	1-3	1-3	1-2	1-3	1-4	1-2

E.M.B. = eosin methylene blue agar.
E.M.B.S.O. = eosin methylene blue agar with sucrose only.
– = not run.

plate counts in bismuth sulfite medium, whereas mixtures with the *Aerobacter* strain yielded 40,000,000 or roughly one-fourth as many *Eberthella* cells. As shown in table 1, an average ratio of 1:8 exists between the two species in 24-hour mixtures, which should theoretically mean about 320,000,000 *Aerobacter* cells per ml—if the 40,000,000 per ml figure for *E. typhosa* is valid. Initial control ratios in table 1 reveal that *A. aerogenes* 29 produces approximately twice as many cells as *E. typhosa* in 24 hours, and 320,000,000 is roughly twice 165,000,000.

Fourteen-day old pure cultures of *E. typhosa* gave a count of 64,000,000 per ml in bismuth sulfite, or about 40 per cent of the 24-hour count. If the same

normal decrease in numbers be assumed for the mixed cultures, 40 per cent of 40,000,000 or about 15,000,000 *Eberthella* cells per ml, should have been present at 14 days in mixtures in the absence of antagonism. As 230,000 per ml were actually found, it may be assumed that roughly 64 out of 65 of the theoretical number were killed. Unfortunately, the antagonistic action toward *E. typhosa* was only moderate, so that these findings may not be interpreted to indicate that none of the nonsporulating bacteria affected by *Aerobacter* strains suffer complete destruction.

Filtrates of cultures of *A. aerogenes* 29 appeared completely devoid of antagonistic activity. Seventy-two-hour cultures were filtered through a Berkefeld W candle and the filtrate was added to triple strength nutrient broth in the ratio 2:1 so as to give a final concentration of nutrients at least equal to the usual broth. After incubation for sterility testing, inoculations were made of *B. anthracis*, *B. polymyxa*, *S. paradysenteriae* (Hiss), and *S. aureus*. In all cases growth was obtained which grossly equaled that in nutrient broth controls. Uninoculated controls were invariably sterile. Both 3- and 14-day-old cultures of the *Aerobacter* strain filtered through Seitz filters showed precisely the same results.

Experiments on the occurrence of antagonism in solid media gave results entirely compatible with the failure to demonstrate a filterable antibiotic "substance." Nutrient agar plates heavily seeded with *B. anthracis*, *S. paradysenteriae* (Hiss), and *S. aureus* were massively streaked down the center with *A. aerogenes* 29. On incubation none of the organisms showed visible evidence of antagonism, though it could have occurred in a very narrow zone at the surface where the *Aerobacter* growth appeared without being detectable. That this was highly probably was shown by the ease of demonstrating antagonism when living *Aerobacter* cells were in close proximity to all cells of the test species. Thirty-hour and 18-day broth cultures of *A. aerogenes* 29 were solidified with an equal quantity of a cooled solution of double strength nutrient agar, and plates prepared in this manner were streaked heavily down the center with *B. anthracis*, *B. polymyxa*, *S. paradysenteriae* (Hiss), and *S. aureus*. At 4 days' incubation, the last three species showed no visible growth, but *B. anthracis* produced a zone of growth about half as wide as that on control nutrient agar plates. Repetition of this experiment with 18-day broth cultures of *A. aerogenes* 29 heated to 60 C and 80 C for 1 hour showed no real evidence of antagonism, though the growth of the two *Bacillus* species was slightly less than on nutrient agar controls.

The extreme variability of *S. marcescens* noted above, as well as the temporary inhibition of the organisms of group 3, might be explained if resistant forms could be shown to develop from these species. Strains of *B. cereus*, *B. subtilis* Koch-Novy, and *S. enteritidis* were obtained by picking colonies developing on plates streaked from mixtures with *A. aerogenes* 29 after the initial inhibition was past and delayed growth had occurred. One strain of *S. marcescens* was obtained from a colony appearing on a plate streaked from a 14-day mixed culture that had shown no *Serratia* colonies at 120 hours, and a second strain from colonies appearing on agar prepared by solidifying a 24-hour broth culture of *A. aerogenes*

29 with 3 per cent agar-agar and streaked with the test species. Comparison of these strains with the parent cultures showed no appreciable increase in resistance, however.

A. aerogenes 29 produces a bluish, translucent, rather flat variant colony which has not been observed to revert to the usual type. Since this variant appears in varying numbers in broth cultures, any lesser degree of antagonism exhibited by it might have a bearing on the delayed growth of species in group 3. Comparison of the antagonistic effects exerted by this stable variant and those of the parent culture against *B. cereus*, *S. enteritidis*, and *S. aureus* showed no appreciable differences. Against *B. mycoides* 24-V and *B. subtilis* Koch-Novy the variant exhibited a definitely greater effect, in fact.

DISCUSSION

Though the exact mechanism of the antagonistic action of *Aerobacter* strains is unknown, it is possible that it may involve some direct action of living cells. It is interesting in this connection to note the reports of Gundel and Kliewe (1932) and Isabolinski and Sobolewa (1934) that only living cells of *E. coli* were antagonistic to *B. anthracis*. Stickelbroch (1929) also claimed that the antagonistic effect of *E. coli* toward dysentery organisms was destroyed by heating to 60 C.

The findings here reported may have a bearing on the numerous observations by many workers of the disappearance of one species of bacteria in association with another in cases where a definite antibiotic substance could not be demonstrated. Neufeld and Kuhn (1934) have used the term "direct antagonism" to describe such phenomena.

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SUMMARY

A single strain of *Aerobacter aerogenes* 29 was tested against 21 common species of bacteria and found antagonistic in varying degrees toward 14 of them.

Comparison of 8 *Aerobacter* strains against 3 test species showed only minor differences in antagonistic effects.

Whether or not complete destruction of the cells of the most strongly affected nonsporulating species occurred is uncertain. The sporeforming species most strongly affected either produced resistant spores in the presence of *A. aerogenes* 29, or these were present in the inocula.

Filtrates of *Aerobacter* cultures did not exhibit antagonism.

Heating to 60 C for 1 hour destroyed the antagonistic effect of *A. aerogenes* 29.

Attempts to demonstrate resistant forms of species only temporarily affected were unsuccessful.

A common stable variant of *A. aerogenes* 29 was found not to vary appreciably from the parent culture in its antagonistic effects.

The mechanism of the antagonistic action of *Aerobacter* strains is unknown but may involve some direct action of living cells.

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