# THE EFFECT OF METABOLITES OF ESCHERICHIA COLI ON THE GROWTH OF COLI-AEROGENES BACTERIA

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For many years controversial reports have appeared in the literature to the effect that when bacteria grow in a culture medium more or less specific autoinhibitory agents are produced. Some investigators have maintained that inhibition in culture media is due primarily to a depletion of food supply.

Among investigators who believe that autoinhibitory substances are produced during bacterial growth are Garré (1887), De Giaxa (1889), Miquel (1889), Coplans (1910), Chesney (1916), McLeod and Govenlock (1921), Besredka (1923), Rideal (1923), Cornwall and Beer (1926), Grundel (1927), Weichardt (1927), Rogers (1928), Chaillot (1930), Fischer (1933), Powers (1934), Powers and Levine (1937), and Wheeler and Stuart (1937).

In contrast to these workers, Graham-Smith (1920), Barnes (1931), and Hershey and Bronfenbrenner (1937) ascribe the cessation of growth of bacteria to lack of available food material.

Layne-Claypon (1909) and Cleary, Beard, and Clifton (1935) thought inhibition to be caused by a combination of unavailability of food and the production of growth-inhibiting substances. As to the nature of the postulated growth-inhibiting products, Garré (1887), Miquel (1889), McLeod and Govenlock (1921), Rogers (1928), Powers (1934), and Powers and Levine (1937) believed such substances to be specific to a high degree. On the other hand, Weichardt (1927), Ninni and Molinari (1928), Fischer (1933), and Wheeler and Stuart believed that, while growth-inhibiting products are formed by bacteria, these products are nonspecific.

De Giaxa (1889), Miquel (1889), Eijkmann (1904), and Besredka (1923) considered the growth-inhibiting products of bacteria to be thermolabile, but Cornwall and Beer (1926), Roger (1928), and Wheeler and Stuart (1937) hold the view that such products are heat-stable. Marmorek (1902), Besredka (1923), and Wheeler and Stuart (1937) reported the growth-inhibiting products to be filterable, whereas Cornwall and Beer (1926), Grundel (1927), Rogers (1928), Powers (1934), and Powers and Levine (1937) were of the opinion that such substances were nonfilterable, i.e., the inhibiting effect is removed or markedly reduced by filtration.

The objective of the present study was to determine whether inhibition is due to autoinhibitory agents produced during bacterial metabolism.

## METHODS

In the present study the technique employed was, briefly, as follows: One liter of sterile 1.0 per cent Difco proteose peptone broth (buffered with 0.1 per cent

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$K_2HPO_4$) contained in a 2-liter Erlenmeyer flask was inoculated with a culture of a member of the coli-aerogenes group and incubated at 37 C for 2 or more days. At the end of the incubation period the contents of the flask were warmed to 43 C, and equal portions of this "staled"[2] broth and a 3.0 per cent agar gel (previously warmed to 45 C) were mixed thoroughly. The mixture of broth culture and agar gel, which constituted the so-called "staled-agar-substrate," was then poured into sterile petri dishes (15 to 20 ml per dish) and allowed to harden. The outer faces of the bottoms of the petri dishes were marked off in quadrants by means of a glass-marking pencil. Each quadrant was inoculated by streaking across it a loopful of a culture of a test organism, grown for 18 to 24 hours, at 37 C in proteose peptone (1 per cent) broth. The following were employed as controls:

(1) Each organism was streaked on nutrient agar to determine "normal" vigor of growth.

(2) On smearing a loopful of a broth culture on staled media the workers sometimes observed that a film resembling very faint growth developed. To eliminate any error in respect to this phenomenon, several autoclaved broth cultures were streaked on staled media as controls.

All plates were incubated at 37 C for 48 hours, and the relative vigor of growth was recorded as follows:

0 = no growth
0.5 = slight growth
1.0 = fair growth
2.0 = moderate growth
3.0 = good growth
4.0 = heavy growth

At times interpolations between the values listed above were made. For example, 2.5 represents growth that is apparently better than that expressed by 2.0 but less than that expressed by the value 3.0.

## RESULTS

*Preliminary observation.* Many series of experiments were made in the manner described above, but as results were fairly similar, only those for one series are presented in table 1.

It will be noted that strains which belong to the same genus as that used in producing a staled substrate were markedly inhibited by that substrate. This was especially true of strains of *Escherichia* which were completely inhibited on the two *Escherichia coli* "staled" substrates employed. Similarly, but to a lesser extent than observed with strains of *Escherichia*, strains of *"Citrobacter," Aerobacter aerogenes*, and *Aerobacter cloacae* were inhibited by their homologous substrates.

Since complete inhibition of *Escherichia* strains was obtained with substrates of *E. coli* (strains 100 and 115), it was felt that old broth cultures of these strains

[2] "Staled" agar consisted of equal parts 3.0 per cent Difco agar and a proteose peptone (1.0 per cent) broth culture grown at 37 C for 48 (or more) hours.

merited further study. *E. coli* (strain no. 100) was arbitrarily selected and observations made of the effect of time and temperature of incubation on the inhibiting properties of the "staled" media.

TABLE 1

*Growth of members of coli-aerogenes group on staled* media*

| TEST STRAINS | STALED SUBSTRATES | | | | | |
|---|---|---|---|---|---|---|
| | *E. coli* (no. 100) | *E. coli* (no. 115) | "*Citrobacter*" (no. 277) | *A. aerogenes* (no. 174) | *A. aerogenes* (no. 108 A) | *A. cloacae* (no. 41) |
| | Relative vigor of growth, 48 hr, 37 C | | | | | |
| *Escherichia* | | | | | | |
| 107 | 0 | 0 | 0 | 1 | 2 | 0 |
| 2 | 0 | 0 | 0.5 | 1 | 1 | 1 |
| 115 | 0 | 0 | 0.5 | 1.5 | 1.5 | 2 |
| 200 | 0 | 0 | 1 | 1.5 | 1.5 | 2 |
| 100 | 0 | 0 | 1 | 1 | 2 | 2 |
| 101 | 0 | 0 | 1 | 1 | 1 | 0 |
| 133 | 0 | 0 | 0.5 | 1 | 1 | 1 |
| "*Citrobacter*" | | | | | | |
| 79B | 1.5 | 1.5 | 1 | 1.5 | 2.5 | 2.0 |
| 202 | 1 | 1 | 0 | 1.0 | 2.0 | 1.5 |
| 277 | 1 | 1 | 0 | 1.5 | 1.5 | 2.5 |
| 128C | 1 | 1 | 0 | 1.5 | 1.5 | 1.0 |
| 139S | 1 | 1 | 1 | 1.5 | 2.0 | 1.5 |
| 144S | 2 | 1.5 | 1 | 1.5 | 2.5 | 2.0 |
| 147 | 1 | 1 | | 1.5 | 1.5 | 1.0 |
| *A. aerogenes* | | | | | | |
| 244 | 1.5 | 2.5 | 1.0 | 0.0 | 0.0 | 2.0 |
| 73 | 2.0 | 2.0 | 1.5 | 1.0 | 1.5 | 1.5 |
| 174 | 3.0 | 3.0 | 2.0 | 0.0 | 2.5 | 2.5 |
| A1 | 3.0 | 3.0 | 3.0 | 1.0 | 2.5 | 3.0 |
| A2 | 1.5 | 1.5 | 1.5 | 0.0 | 2.5 | 2.5 |
| 66A | 1.5 | 1.0 | 1.0 | 0.5 | 0.0 | 2.5 |
| 108A | 2.0 | 2.0 | 1.0 | 0.0 | 0.5 | 2.5 |
| 180D | 2.0 | 2.5 | 3.0 | 1.5 | 2.0 | 3.0 |
| *A. cloacae* | | | | | | |
| 301 | 1.5 | 1.5 | 1.0 | 0.5 | 1.0 | 1.0 |
| 251 | 1.5 | 1.0 | 0.5 | 1.0 | 1.0 | 1.0 |
| 252 | 1.5 | 1.0 | 0.5 | 1.0 | 1.0 | 0.5 |
| 41 | 2.0 | 1.5 | 0.5 | 0.5 | 1.0 | 0.5 |
| $10A_2$ | 1.5 | 1.0 | 0.5 | 0.5 | 0.5 | 0.5 |
| 211D | 1.5 | 1.0 | 0.5 | 0.5 | 0.5 | 0.5 |
| $214D_1$ | 1.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.5 |

* Ten-day (37 C) cultures employed for staling media.

0 —no growth. 2.0—moderate growth.
0.5—slight growth. 3.0—good growth.
1.0—fair growth. 4.0—vigorous growth.

*Effect of length of staling period on inhibiting properties of staled media.* A series of 2-liter Erlenmeyer flasks (containing 1 liter of 1.0 per cent proteose peptone) was inoculated with *E. coli* no. 100 and incubated at 37 C for 2 to 25 days. At the end of various incubation periods a flask was removed and staled agar plate tests were made as previously described. In these tests 198 strains were used, as follows: *Escherichia*, 50 strains; *A. aerogenes*, 49 strains; *A. cloacae*, 49 strains; and *"Citrobacter,"* 50 strains.

TABLE 2

*Effect of age of broth culture of E. coli no. 100, employed to prepare staled agar, on growth of members of coli-aerogenes group*

| TEST STRAINS | AGE OF CULTURE EMPLOYED FOR STALING | | | | |
|---|---|---|---|---|---|
| | 2 Days | 4 Days | 10 Days | 20 Days | 25 Days |
| | Cumulative vigor of growth, 48 hr, 37 C | | | | |
| *Escherichia* (50 strains) | 2.5 | 0 | 3 | 0 | 0 |
| *A. aerogenes* (49 strains) | 77.5 | 72 | 53.5 | 51.5 | 30.5 |
| *A. cloacae* (49 strains) | 46.0 | 46.0 | 28.0 | 25.5 | 18.0 |
| *"Citrobacter"* (50 strains) | 37.0 | 27.5 | 21.0 | 15.5 | 11.5 |
| | Per cent of strains completely inhibited | | | | |
| *Escherichia* (50 strains) | 90.0 | 100.0 | 94.0 | 100.0 | 100.0 |
| *A. aerogenes* (49 strains) | 0.0 | 4.1 | 4.1 | 6.2 | 2.0 |
| *A. cloacae* (49 strains) | 26.5 | 20.4 | 26.5 | 26.5 | 34.3 |
| *"Citrobacter"* (50 strains) | 20.0 | 30.0 | 36.0 | 56.0 | 68.0 |

The results are summarized in table 2, which gives the cumulative vigor of growth for each group of test organisms for a given medium. The cumulative vigor of growth for any group of organisms is a figure derived by adding the values for vigor of growth for each strain of that group. (These individual values were scored as in the preceding experiments.) For example, the data of table 2 indicate that the cumulative vigor of growth for 50 strains of *Escherichia* after 4 days' incubation of the substrate was 0. In other words, not one of the *Escherichia* strains showed visible growth upon that particular substrate. In table 2

are also given the percentages of strains completely inhibited on the various staled media.

It is evident from the results obtained that incubation of the culture substrates for as little as 48 hours was sufficient almost completely to prevent growth of *Escherichia* strains, and that the degree of inhibition of the strains of *A. aerogenes*,

TABLE 3

*Effect of temperature of incubation of culture used to prepare staled agar* on growth of coli-aerogenes group*

| TEST STRAINS | STALED SUBSTRATES | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | *E. coli* (no. 100) | | *E. coli* (no. 115) | | *A. aerogenes* $A_3$ | | *A. aerogenes* (no. 174) | |
| | INCUBATION TEMPERATURE | | | | | | | |
| | 37 C | 30 C | 37 C | 30 C | 37 C | 30 C | 37 C | 30 C |
| | Cumulative vigor of growth | | | | | | | |
| *Escherichia* (50 strains) | 0 | 0 | 0.5 | 0 | 44.0 | 43.0 | 45.5 | 36.5 |
| *A. aerogenes* (49 strains) | 77.0 | 72.0 | 80.0 | 67.5 | 6.0 | 14.0 | 12.5 | 8.0 |
| *A. cloacae* (49 strains) | 36.0 | 55.5 | 41.5 | 46.5 | 0.5 | 1.5 | 0.5 | 0.5 |
| "*Citrobacter*" (50 strains) | 35.5 | 45.0 | 40.5 | 44.5 | 41.0 | 43.5 | 33.5 | 46.5 |
| | Percentage of strains completely inhibited† | | | | | | | |
| *Escherichia* (50 strains) | 100 | 100 | 98 | 100 | 0 | 0 | 2 | 18 |
| *A. aerogenes* (49 strains) | 0 | 0 | 2 | 2 | 77 | 43 | 51 | 71 |
| *A. cloacae* (49 strains) | 27 | 14 | 18 | 20 | 98 | 94 | 98 | 98 |
| "*Citrobacter*" (50 strains) | 22 | 8 | 18 | 8 | 16 | 4 | 16 | 8 |

* Twelve-day-old cultures used.

† Percentage calculated to nearest whole number.

*A. cloacae*, and "*Citrobacter*" employed increased progressively with the age of the staled substrate. The effect of age of staling was particularly marked with the "*Citrobacter*" strains, which showed a progressive rise (from 20 per cent for the 2-day to 68 per cent for the 25-day-old staled medium) in the number of cultures completely inhibited as the age of the staling culture increased.

The *Escherichia* strains were completely inhibited by a 4-day staled medium, while the cumulative vigor of growth for the *Aerobacter* strains was 72 in contrast to the low value of 27.5 for the *"Citrobacter"* strains. This relationship (i.e., the *Escherichia* strains being completely inhibited, the *Aerobacter* strains showing the least degree of inhibition, with the *"Citrobacter"* strains falling in between these) held true for each of the 5 staled media observed. It therefore appears that the *"Citrobacter"* strains are not only intermediate on the basis of the V.P.-M.R.-citrate reactions, but they are also intermediate with respect to vigor of growth on *Escherichia* staled agar media.

*Effect of temperature of incubation of staled broth on inhibitory properties.* The effect of temperature of incubation on the inhibitory properties of staled agar was observed, the same technique and 198 test cultures being employed as in the preceding experiments. Duplicate staled agar substrates were prepared using two sets of the following organisms (one set incubated at 37 C and the other at 30 C for 12 days): *E. coli* no. 115, *E. coli* no. 100, *A. aerogenes* $A_2$, and *A. aerogenes* no. 174. Each test culture was streaked on each substrate, and the plates were incubated at 37 C for 48 hours, after which the vigor of growth was recorded.

The results are summarized in table 3, where values for the cumulative vigor of growth are recorded for each group of test strains upon each substrate, and also the percentages of strains completely inhibited. The results indicate that, though the individual strains showed some variations, there was no significant difference in the inhibitory properties of staled broth produced by cultures incubated at 30 C or 37 C for 12 days.

## SUMMARY AND CONCLUSIONS

Some observations were made on the production of growth-inhibitory substances by members of the coli-aerogenes group.[3]

Members of the coli-aerogenes group growing in 1.0 per cent proteose peptone broth buffered with 0.1 per cent $K_2HPO_4$ produced autoinhibitory agents. Those produced by *Escherichia coli* were especially effective as indicated by complete inhibition of 50 *Escherichia* strains on a staled agar made from a 48-hour (at 37 C) culture of *E. coli*.

The degree of inhibition increased with the time of incubation of the broth cultures used for preparing a staled medium.

There was no marked difference in the inhibitory agents produced by incubation of broth cultures at 37 C as compared with that at 30 C for a period of 12 days.

The inhibitory substances produced by members of the coli-aerogenes group in old broth cultures may serve as a basis for differentiation or for isolation of the various members of the coliform group from mixtures.

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