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In Vitro Persistence of *Salmonella typhimurium* in a Dually Inoculated Medium. III. With *Aerobacter aerogenes*

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In two previous studies, the persistence of *Salmonella typhimurium in vitro* was not found to be significantly inhibited by varied numbers of *Proteus morganii* during a 19 day period (4) nor during a 38 day period by *Aerobacter cloacae* (5). The pathogen multiplied over 35 times during the first day of each study. This paper represents an extension of these studies to *Aerobacter aerogenes*.

Materials and Methods

Modified S-F broth (MSFB) in a series of flasks was inoculated with varied ratios of *S. typhimurium* and *A. aerogenes* / cc medium. Most of the materials and methods used throughout this study are presented in two previous papers (4 and 5) and will not be repeated in detail here. In addition, the pH of MSFB in flasks 28-48 was adjusted with m/15 NaH_2PO_4 and Na_2HPO_4 from a normal 6.9 to 5.8 prior to inoculating it. A Leeds and Northrup pH meter was used to determine the pH of MSFB in these flasks post-incubation and after autoclave sterilization. *Aerobacter aerogenes* was obtained from the Carolina Biological Supply Company, Burlington, North Carolina.

The identity of *A. aerogenes* was based on: (a) positive triple-sugar iron agar tests (an acid slant, an acid butt, and the production of gas exclusive of H_2S); (b) positive phenol red tartrate agar tests (no color change in medium); (c) negative urea broth tests (lack of the hydrolysis of urea as indicated by no color change in the broth); (d) negative macroscopic slide agglutination tests with polyvalent *Salmonella* antiserum. A serial dilution range of from 1:20 to $1:1024 \times 10^{10}$ was used in the enumeration of bacteria found in inoculated MSFB post-incubation.

Results

The results of this study are presented in Tables 1 and 2.

Salmonella typhimurium based on the differences in the numbers inoculated and the numbers detected in MSFB post-incubation, multiplied extensively in the presence of *A. aerogenes* during the first day of incubation. A high-level count of viable *S. typhimurium* persisted throughout a 44 day experimental period. The growth and persistence of this species was evident in MSFB irrespective of an initial pH of 6.9 or 5.8 and inoculum ratios of 1:0, 1:400, 1:800 (medium

pH = 5.8), 1:1, 1:16 x 10^5, 1:36 x 10^5 (medium pH = 6.9) / cc medium. No difference was reflected by the 10 serial dilutions employed in the growth cited and persistence of *S. typhimurium* in MSFB irrespective of the pH and pathogen-nonpathogen ratio employed. *Aerobacter aerogenes* coexisted at high level with the pathogen throughout the experimental period.

Discussion

A study of the multiplication of *S. typhimurium* in this work was not of prime interest. However, a difference of approximately 51.2 x 10^9 in the number of *typhimurium* inoculated and the numbers detected / cc medium irrespective of the pH and pathogen-nonpathogen inoculum ratios used and following a 24 hour incubation does indicate that a decided multiplication of this species occurred during the first day. This multiplication represents a minimum for the species since the number of viable cells in the highest serial dilution was sufficient to give higher counts as evidenced by growth on SS agar plates had the dilution range been extended. A generation time of 54.7 minutes for *typhimurium* as computed by $g = t/n$ is thought to be high. Thirty-five generations during the first day of incubation as determined by $n = \log p^1 - \log p^2 / \log 2$ is considered to be low. Certain closely related species (e.g., *Eschericheria coli*, g = 20 minutes) are known to show a greater number of generations and shorter generation periods than those calculated in this work for *typhimurium*.

Very few *in vitro* studies on microbial competition between enteric pathogens and nonpathogens have been conducted. Knuckles (4 and 5) observed no appreciable inhibitory effect on the persistence of *typhimurium* by competitive action due to *P. morganii* or *A. cloacae* in mixed cultures. The pathogen multiplied over 35 times in the presence of either of these species during the first 24 hours of incubation. The ability of *typhimurium* to multiply in the presence of *A. cloacae*, *A. aerogenes* and *P. morganii in vitro* and its ability to survive in bicontaminated *P. regina* (3) indicate that these nonpathogens do not significantly impair the multiplication of *typhimurium in vivo*, but when coupled with ecological factors within certain flies, may play a part in its destruction. These findings and those of a previous paper (5) do not agree with the observations by Bowling and Wynne (1) that several strains of *Aerobacter* produced complete inhibition of *Salmonella paratyphi* on staled agar. A probable inability of *S. paratyphi* to feed on the dehydrated agar, the presence of bacteriophage or some unknown factor may be a more feasible explanation for the inhibition observed by them rather than the presence of *Aerobacter*.

Aerobacter aerogenes demonstrated no significant inhibitory action on the survival of *typhimurium* as was evidenced by the persistence of a high-level count of at least 51.2 x 10^9 *S. typhimurium* / cc medium throughout a 44 day period in MSFB whose initial pH was 6.9 and for 31 days in a similar medium whose initial pH was 5.8. Jung and Shaffer (2) showed that *S. typhimurium* and *Salmonella montevido* could survive in human feces for at least 14 days and that the initial

concentration of these organisms appeared to have had no influence on the duration of their survival.

MSFB whose initial pH was 5.8 showed an immediate conversion toward a more alkaline pH when this medium was inoculated with only *S. typhimurium* and incubated, but the pH changed immediately to a more acidic one when inoculated with *A. aerogenes* and *S. typhimurium*, and incubated (Table 2). The conversion of the pH to a more acidic one in the latter case was followed by a pH change to a more alkaline one. This indicates that *S. typhimurium* can tolerate a pH of at least 4.8 and that apparently it is able through its metabolic products to alter a pH condition toward one which is more optimum for its persistence and growth. Such may be a reason why *aerogenes* showed no significant inhibitory action on the persistence of *typhimurium* in this study.

Table 1

In Vitro Persistence of *Salmonella typhimurium* in the Presence of *Aerobacter aerogenes*

Flask number	Number of pathogen inoculated / cc medium	Number of nonpathogen inoculated / cc medium	Incubation period (days)	Number of viable bacteria detected / cc medium by serial dilution *S. typhimurium*	/ *A. aerogenes*
1	750	7.50×10^2	1	512×10^8	512×10^8
2	750	7.50×10^2	3	512×10^8	512×10^8
3	750	7.50×10^2	6	512×10^8	512×10^8
4	750	7.50×10^2	8	512×10^8	512×10^8
5	750	7.50×10^2	10	512×10^8	512×10^8
6	750	7.50×10^2	13	512×10^8	512×10^8
7	750	7.50×10^2	38	512×10^8	512×10^8
8	750	7.50×10^2	41	512×10^8	512×10^8
9	750	7.50×10^2	44	512×10^8	512×10^8
10	750	1.20×10^9	1	512×10^8	512×10^8
11	750	1.20×10^9	3	512×10^8	512×10^8
12	750	1.20×10^9	6	512×10^8	512×10^8
13	750	1.20×10^9	8	512×10^8	512×10^8
14	750	1.20×10^9	10	512×10^8	512×10^8
15	750	1.20×10^9	13	512×10^8	512×10^8
16	750	1.20×10^9	38	512×10^8	512×10^8
17	750	1.20×10^9	41	512×10^8	512×10^8
18	750	1.20×10^9	44	512×10^8	512×10^8

19	25	9.00×10^7	1	512×10^8	512×10^8
20	25	9.00×10^7	3	512×10^8	512×10^8
21	25	9.00×10^7	6	512×10^8	512×10^8
22	25	9.00×10^7	8	512×10^8	512×10^8
23	25	9.00×10^7	10	512×10^8	512×10^8
24	25	9.00×10^7	13	512×10^8	512×10^8
25	25	9.00×10^7	38	512×10^8	512×10^8
26	25	9.00×10^7	41	512×10^8	512×10^8
27	25	9.00×10^7	44	512×10^8	512×10^8
*28	750	0	2	512×10^8	0
29	750	0	4	512×10^8	0
30	750	0	6	512×10^8	0
31	750	0	8	512×10^8	0
32	750	0	10	512×10^8	0
33	750	0	16	512×10^8	0
34	750	0	31	512×10^8	0
35	750	3.00×10^5	2	512×10^8	512×10^8
36	750	3.00×10^5	4	512×10^8	512×10^8
37	750	3.00×10^5	6	512×10^8	512×10^8
38	750	3.00×10^5	8	512×10^8	512×10^8
39	750	3.00×10^5	10	512×10^8	512×10^8
40	750	3.00×10^5	16	512×10^8	512×10^8
41	750	3.00×10^5	38	512×10^8	512×10^8

Flask number	Number of pathogen inoculated / cc medium	Number of nonpathogen inoculated / cc medium	Incubation period (days)	Number of viable bacteria detected / cc medium by serial dilution *S. typhimurium* / *A. aerogenes*	
42	750	6.00×10^5	2	512×10^8	512×10^8
43	750	6.00×10^5	4	512×10^8	512×10^8
44	750	6.00×10^5	6	512×10^8	512×10^8
45	750	6.00×10^5	8	512×10^8	512×10^8
46	750	6.00×10^5	10	512×10^8	512×10^8
47	750	6.00×10^5	16	512×10^8	512×10^8
48	750	6.00×10^5	38	512×10^8	512×10^8

*The pH of the medium in flask 28-48 was adjusted to 5.8 prior to inoculating it.

Table 2

Changes In pH Values of Modified Selenite-F Broth Inoculated With *S. typhimurium* and *A. aerogenes*

Flask number	Pathogen/ nonpathogen ratio	Incubation period (days)	pH of medium
*28	1:0	2	5.8
29	1:0	4	6.0
30	1:0	6	6.0
31	1:0	8	6.0
32	1:0	10	6.1
33	1:0	16	6.4
34	1:0	31	6.4
35	1:400	2	4.8
36	1:400	4	4.8
37	1:400	6	4.8
38	1:400	8	5.4
39	1:400	10	5.4
40	1:400	16	5.5
41	1:400	38	6.2
42	1:800	2	4.8
43	1:800	4	5.1
44	1:800	6	5.1
45	1:800	8	5.3
46	1:800	10	5.5
47	1:800	16	5.9
48	1:800	38	6.1

*The initial pH of MSFB in each flask was 5.8.

Summary

Salmonella typhimurium underwent a 35 fold multiplication during the first day in MSFB inoculated with this species and *A. aerogenes* irrespective of varied ratios in their inoculum-numbers. Neither of the species appeared to have had a significant inhibitory effect on the growth of the other. The persistence and multiplication of *S. typhimurium* in mixed cultures with *A. aerogenes* appear to be reduced primarily by the availability of appropriate nutrients for the total bacterial population.

Salmonella typhimurium persisted at high-levels in MSFB (initial pH of 6.9) for 44 days and 38 days in MSFB (initial pH of 5.8) irrespective of the *typhimurium-aerogenes* inoculum-ratio employed.

Salmonella typhimurium is able to tolerate a pH of 4.8 but demonstrated an ability to alter this pH toward a more alkaline one.

Literature Cited

1. Bowling, R. E. and Wynne, E. S., 1957. Studies on the mechanism of antagonism by *Aerobacter* strains. J. Infect. Diseases, 88-84:277-281.

2. Jung, R. E. and Shaffer, M. F., 1952. Survival of ingested *Salmonella* in the cockroach *Periplaneta americana*. Am. J. Trop. Med. and Hyg., 1:990-998.

3. Knuckles, J. L., 1959. Studies on the role of *Phormia regina* (Meigen) as a vector of certain enteric bacteria. Doctoral dissertation, Univ. of Connecticut, Storrs, Connecticut.

4. Knuckles, J. L., 1967. *In vitro* persistence of *Salmonella typhimurium* in a dually inoculated medium. I. with *Proteus morganii*. Savannah State College Faculty Res. Bulletin, 21:177-184.

5. Knuckles, J. L., 1967. *In vitro* persistence of *Salmonella typhimurium* in a dually inoculated medium. II. with *Aerobacter cloacae*. Savannah State College Faculty Res. Bulletin, 21:185-191.