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Effect of Volatile Fatty Acids on *Salmonella typhimurium*[1]

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It has been established for many years that volatile fatty acids have an inhibitory or bactericidal effect on a number of bacterial species. D. J. Hentges (3) noted that shigellae were inhibited when grown in association with *Klebsiella* sp. He identified the compounds responsible for this inhibition as formic acid and acetic acid, products of the metabolism of *Klebsiella*. In a separate publication, Hentges (4) demonstrated that the effect of the fatty acids was *p*H-dependent. Brownlie and Grau (1) reported that both *Escherichia coli* I and *Salmonella* were rapidly eliminated from the rumen and were rarely detectable in the feces of cattle fed a normal daily ration of hay. They attributed the demise of the enteric microorganisms in part to the high volatile fatty acid and low *p*H values in the rumen of these cattle.

[1] Published with the approval of the Director of the Wisconsin Experiment Station.

In order to define the conditions affecting the sensitivity of *S. typhimurium* to volatile fatty acids, the following experiments were performed. In all experiments the inoculum of *S. typhimurium* was prepared by diluting a 24-hr nutrient broth culture to provide an initial level of 5×10^3 to 5×10^4 cells per ml in the test solution. The test solutions consisted of a nutrient broth base to which the desired concentration of fatty acid was added. The *p*H was adjusted with HCl or KOH prior to autoclaving at 121 C for 15 min. Tubes containing the test solution were tempered to the appropriate temperature in a water bath prior to inoculation. Samples were withdrawn periodically and surviving salmonellae were enumerated by spread-plating on nutrient agar.

TABLE 1. *Effect of pH on the bactericidal activity of volatile fatty acids*

Acid	*p*H	No. of survivors per ml				
		Incubation time (hr)[a]				
		0	8	12	24	48
Formic	5.0	1.4×10^4	<10			
	5.5	1.1×10^4	1.1×10^3	3.0×10	<10	
	6.0	1.0×10^4	7.6×10^3	4.5×10^3	2.6×10^3	6.4×10^2
Acetic	5.0	1.5×10^4	<10			
	5.5	1.2×10^4	4.8×10^3	1.9×10^3	8.2×10^2	6.0×10
	6.0	1.1×10^4	8.6×10^3	6.3×10^3	4.4×10^2	3.9×10^2
Propionic	5.0	1.8×10^4	<10			
	5.5	1.8×10^4	4.8×10^3	1.5×10^3	1.2×10^2	<10
	6.0	1.3×10^4	6.3×10^3	4.0×10^3	3.8×10^3	5.9×10^2
Butyric	5.0	1.7×10^4	$<10^2$	<10		
	5.5	1.9×10^4	5.3×10^3	9.0×10^2	$<10^2$	1.0×10
	6.0	1.3×10^4	1.8×10^4	1.7×10^4	1.1×10^4	1.9×10^3
Control[b]	5.0	1.7×10^4		6.9×10^7	1.9×10^8	
	5.5	6.3×10^3		5.2×10^8	$>10^8$	

[a] Incubations at 37 C. Acid concentration was 0.5% in nutrient broth.
[b] Control: *p*H adjusted with HCl.

Table 1 shows the effect of *p*H on the bactericidal activity of formic, acetic, propionic, and butyric acids. The effectiveness of the acid diminished as the *p*H of the medium was increased.

These results are in keeping with the premise that the undissociated acid molecule is the bactericidal moiety (5). Although not of a pronounced nature, a general trend of decreasing bactericidal effect with increasing chain length of the fatty acid was noted.

The rate of death of the cells in the presence of volatile fatty acids at a given *p*H and temperature was concentration-dependent (Fig. 1). An increase in temperature increased the killing rate at fixed *p*H and concentration (Fig. 2). Lowering the temperature resulted in a longer lag period before the maximal death rate. A similar lag, although of longer duration, was noted at 7.5 C and 13 C, as reported previously (2). The lag period preceding the maximal death rate is unexplained, but characterized many of the survivor-time plots, particularly at lower concentrations of acid or higher *p*H values.

Various quantities of peptone, glucose, and sucrose were added to the test medium to ascertain whether proteins or sugars would protect the salmonellae. Each of the added substances was protective; the degree of protection increased with the concentration of the substance. Table 2 shows that glucose was the most protective substance when each was tested at the 20% level. It is possible that this reflects the larger number of glucose molecules present at the 20% level. The mechanism of this protection has not been elucidated.

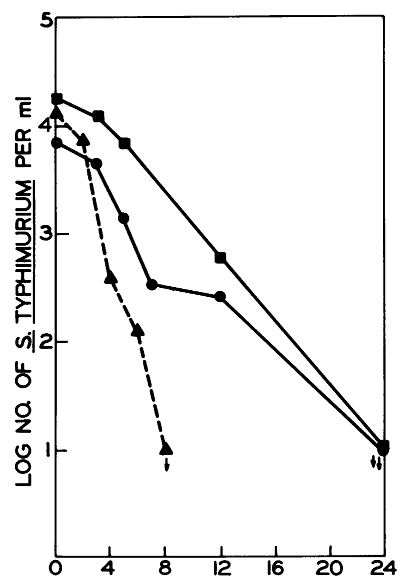

FIG. 1. *Effect of concentration of acetic acid on the rate of death of S. typhimurium in nutrient broth at pH 5 and 37 C. Symbols:* (■) *0.1% HAc,* (●) *0.2%,* (▲) *0.5%.*

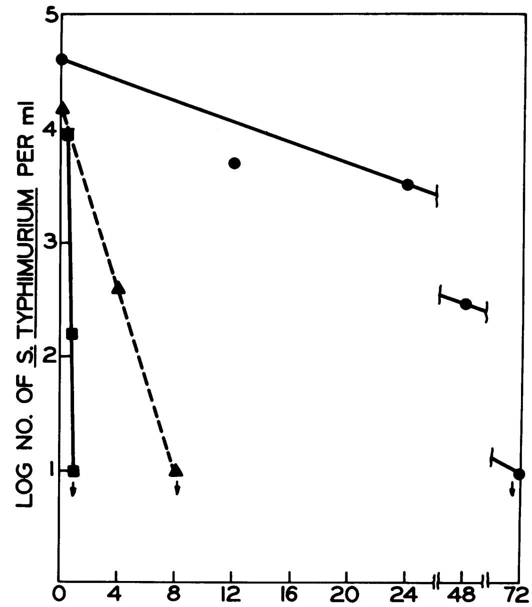

FIG. 2. *Survival of S. typhimurium in nutrient broth with 0.5% acetic acid at pH 5. Symbols:* (●) *25 C,* (▲) *37 C,* (■) *43 C.*

TABLE 2. *Effect of added glucose, sucrose, and peptone on the rate of death of S. typhimurium in 0.5% acetic acid at pH 5 and 37 C*

Incubation time (hr)	Number of survivors per ml			
	20% Glucose	20% Sucrose	20% Peptone	Control[a]
0	2.0×10^4	4.9×10^3	1.8×10^4	1.3×10^4
2	1.9×10^4	4.4×10^3	2.6×10^4	1.8×10^3
6	1.8×10^4	2.2×10^3	2.9×10^4	1.6×10^2
12	1.3×10^4	6.8×10^2	1.4×10^4	<10
24	3.5×10^3	<10	<10	
48	2.3×10^2			
72	<10			

[a] Control was nutrient broth plus 0.5% acetic acid at *p*H 5.0.

In summary, the sensitivity of *S. typhimurium* to volatile fatty acids depended not only on the *p*H of the medium but also on the chain length and concentration of the acid and the temperature and composition of the medium. Other data (Goepfert, *in preparation*) has indicated that available moisture plays a key role in the sensitivity of *Salmonella* to acetic acid.

LITERATURE CITED

1. Brownlie, L. E., and F. H. Grau. 1967. Effect of food intake on growth and survival of salmonellas and *Escherichia coli* in the bovine rumen. J. Gen. Microbiol. **46**:125–134.
2. Goepfert, J. M., N. F. Olson, and E. H. Marth. 1968. Behavior of *Salmonella typhimurium* during manufacture and curing of cheddar cheese. Appl. Microbiol. **16**:862–866.
3. Hentges, D. J. 1967. Inhibition of *Shigella flexneri* by the normal intestinal flora. I. Mechanisms of inhibition by *Klebsiella*. J. Bacteriol. **93**:1369–1373.
4. Hentges, D. J. 1967. Influence of *p*H on the inhibitory activity of formic and acetic acids for *Shigella*. J. Bacteriol. **93**:2029–2030.
5. Levine, A. S., and C. R. Fellers. 1940. Action of acetic acid on food spoilage microorganisms. J. Bacteriol. **39**:499–515.