

*A Research Note***Effect of Acids on Selenite Inhibition of
Salmonella typhimurium and *Salmonella dublin***

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ABSTRACT

The minimum pH for growth (MPG) in selenite-cystine enrichment medium was determined for a number of acids. The MPG for lactic, citric, hydrochloric, aspartic, malic, succinic, pyruvic, and tartaric acids was 5.80; acetic acid was considerably more inhibitory, giving a MPG of 6.30-6.40; the MPG's for fumaric and pyruvic acids were 6.00 and 5.70, respectively. The least inhibitory acid was α -ketoglutaric acid which gave a MPG lower than or equal to 5.1. Inoculum size had little or no effect on the MPG.

Selenite as a selective agent for salmonellae was first used by Guth (3) in a 1% concentration in agar media. Leifson (5) showed this concentration to be very toxic to many salmonellae and devised a liquid medium with 0.4% selenite (Leifson's selenite-F medium). North and Bartram (6) reported that incorporation of 0.01% cystine in the selenite-F medium considerably enhanced salmonella recovery. This was confirmed by Byrne et al. (2).

Salmonella survival experiments (unpublished) in Lebanon bologna gave anomalous results in our laboratory. The Lebanon bologna was inoculated before fermentation with either *Salmonella dublin* or *Salmonella typhimurium* and aliquots were cultured after fermentation. The original technique for the most probable number was to add three parts of selenite enrichment to one part of the fermented bologna, blend, and remove aliquots representing 100, 10, and 1 g. This blended material was then diluted 10- or 100-fold to give 0.1- and 0.01-g portions. The diluted portions sometimes gave positive salmonella results whereas the 100-, 10- and 1-g aliquots gave negative results. It was postulated that lactic acid inhibited the organisms in the selenite media. Evaluation of this theory prompted the investigation reported here.

MATERIALS AND METHODS

Cultures and media

Selenite Cystine Medium (Difco) in 5-ml amounts in 13 × 150 mm tubes was used in these studies with incubation at 37 C for 5 days. The salmonella cultures were laboratory strains of *S. typhimurium* and *S. dublin* and were maintained in Tryptic Soy Broth (TSB). Twenty-hour TSB cultures were used as inocula. The optical density was standardized against Tryptic Soy Agar plate counts and the broth diluted to give the requisite number of cells in 0.1 ml that was added to the selenite media. The salmonella count of the diluted TSB was determined by surface plating on Brilliant Green Agar (BGA).

Acids used

Solutions of 1.0 N lactic, acetic, aspartic, malic, succinic, tartaric, fumaric, α -ketoglutaric and pyruvic acids were used to adjust the pH values of the selenite media. The media were made up to 90% of the final concentrations (10/9 \times) adjusted to required pH values with the acids and then made to volume.

Determination of minimum pH for growth (MPG)

The minimum pH for growth (MPG) was defined as the lowest pH of tubes that showed visual evidence of growth (turbidity and/or selenite reduction) or that gave cultural evidence of cell proliferation when plated on BGA. The former was termed visual MPG; the latter was cultural MPG. All tubes not showing visual growth were plated on BGA. Adjustments of pH were made to the second decimal point.

RESULTS AND DISCUSSION

The effect of lactic, acetic, citric, and hydrochloric acids on the growth of *S. typhimurium* in selenite medium inoculated with 15, 150, or 1500 cells/ml indicated that inoculum size was not significant in causing growth inhibition. Acetic acid inhibited at pH 6.30 while the other acids inhibited at 5.80.

A previous study (4) indicated that *S. dublin* was less acid-resistant in TSB than was *S. typhimurium*. These organisms were compared for MPG in selenite medium, with acetic, citric, and hydrochloric acids used to adjust the pH (Table 1). Acetic acid inhibited both organisms at pH 6.30, both were inhibited at pH 5.70 with citric acid while hydrochloric acid inhibited *S. dublin* at pH 5.50 and *S. typhimurium* at pH 5.70. Thus, *S. dublin* was not more acid resistant in the selenite than was *S. typhimurium*.

Although acetic acid gave a MPG of 6.30 with both

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organisms, there was some cell proliferation at pH 6.30 and 6.20 but not enough to show visible evidence of growth (Table 1). Hydrochloric acid, on the other hand, allowed no increase in cells at the visual MPG. Citric acid permitted an increase in cells with *S. dublin* at the visual MPG. Incubation for 5 days did not produce visible evidence of growth in tubes showing cell proliferation.

The effect on MPG of several organic acids is shown in Table 2. Acetic acid was the most inhibitory. The others were all similar except α -ketoglutaric acid which was not inhibitory at the lowest pH tested, 5.10.

Well-fermented Lebanon bologna had a pH of 5.20 when blended with three parts of selenite cystine

medium, a pH of 5.90 with five parts of the medium, and a pH of 6.50 with 10 parts.

The observation that the MPG of lactic acid in the selenite medium was 5.80 indicates that this is the source of the inhibition of salmonellae noted in Lebanon bologna experiments. Salmonellae are generally quite acid tolerant (4) with most strains growing well at pH 5.1-5.3; however, this report indicates that in the presence of selenite, the acids become much more inhibitory. One method of insuring against acid-inhibition would be to adjust the pH of the selenite medium after blending with the fermented product to 6.40 to 7.0. Such pH adjustment is recommended for all foods by the FDA in its compilation of microbiological methods (1).

TABLE 1. Inhibition of *S. dublin* and *S. typhimurium* in a selenite medium by acetic, citric, and hydrochloric acids.

pH	<i>S. dublin</i>						<i>S. typhimurium</i>					
	Acetic		Citric		HCl		Acetic		Citric		HCl	
	Visible ^a growth	Cell ^b conc	Visible growth	Cell conc	Visible growth	Cell conc	Visible growth	Cell conc	Visible growth	Cell conc	Visible growth	Cell conc
6.50	+		+		+		+		+		+	
6.30	+		+		+		+		+		+	
6.10	0	2400	+		+		0	1400	+		+	
5.90	0	2000	+		+		0	200	+		+	
5.70	0	0	+		+		0	0	+		+	
5.50	0	0	0	1200	+		0	0	0	0	0	0
5.30	0	0	0	0	0	0	0	0	0	0	0	0

Incubation was for 5 days at 37 C

^aTurbidity and/or selenite reduction.

^bDetermined by surface plating 0.01 ml on BGA. Only those tubes showing no visible evidence of growth were plated. 0 = no colonies. Original cell concentration was 160/ml for *S. dublin* and 240/ml for *S. typhimurium*.

TABLE 2. MPG^a of organic acids for *S. typhimurium*^b in selenite medium.

Acid	0.05 N ^c				0.1 N			
	Selenite		Tryptic soy		Selenite		Tryptic soy	
	Visual ^d	Cultural ^e	Visual	Cultural	Visual	Cultural	Visual	Cultural
Acetic	6.40	6.40	—	—	6.60	6.50	6.00	6.00
Aspartic	5.80	5.80	—	—	5.80	5.50	≤5.50	≤5.50
Malic	5.80	5.80	—	—	5.80	5.70	≤5.50	≤5.50
Succinic	5.80	5.80	—	—	5.80	5.80	≤5.50	≤5.50
Tartaric	5.80	5.70	—	—	5.80	5.80	≤5.50	≤5.50
Lactic	5.80	5.80	≤5.50	≤5.50	5.80	5.80	6.00	6.00
Fumaric	—	—	—	—	6.00	6.00	≤5.10	≤5.10
α -Ketoglutaric	—	—	—	—	≤5.10	≤5.10	≤5.10	≤5.10
Pyruvic	—	—	—	—	5.70	5.70	≤5.10	≤5.10
Citric	—	—	—	—	5.80	5.80	≤5.50	≤5.50

^apHs adjusted with HCl or NaOH in 10/9 × medium, then made to volume.

^bCell concentration was 12/ml.

^cFinal concentration of acid.

^dBased on turbidity and/or visual selenite reduction.

^eBased on presence of colonies in 0.01 ml medium streaked on Brilliant Green Agar.

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