EFFECT OF pH ON LOW TEMPERATURE GROWTH OF *SALMONELLA*[1]

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(Received for publication July 22, 1971)

Abstract

The combined effects of pH and temperature on growth of three serotypes of salmonellae are reported. *Salmonella heidelberg* grew on the surface of agar over the pH range of 5.0 to 9.0. Minimum growth temperatures of 5.3, 5.2, and 5.3 C were obtained at pH values of 6.0, 7.0, and 8.0, respectively. In broth, S. *heidelberg* increased in number over the pH range of 6.0 to 8.0, with the lowest growth temperature of 6.0 C obtained at pH 7.0. *Salmonella typhimurium* increased in number in broth at pH values of 6.0 to 9.0. A time-temperature effect was shown by this organism after 16 days. *Salmonella derby* grew over the pH range of 6.0 to 8.0 in broth, with the lowest growth temperature of 9.0 C obtained at pH 6.0 and pH 7.0. The results indicate that food-poisoning *Salmonella* serotypes grow only over a narrow pH range at low temperatures.

Several reports have been published on the minimum growth temperatures of salmonellae in foods. Prescott and Geer *(8)* and Prescott and Tanner *(9)* reported that no growth of salmonellae occurred in foods held below 5 C. This was later confirmed by Angelotti et al. *(2)*, who also indicated that minimum growth temperature may vary with the foodstuff. Thus, there was no growth between 4.4 and 10 C in custard and ham salad, but growth did occur within 5 days in chicken a la king. Similarly, Matches and Liston *(6)* reported that salmonellae would not grow below 11 C in picked crabmeat but would grow at 8 C on fish fillets. These results suggest that properties of the food as a milieu for growth affect the minimum temperature at which salmonellae will grow. The pH of foods varies widely either as a result of natural composition or because of deliberate addition of acid or alkaline flavoring or preserving agents. Surprisingly little has been published on the influence of pH on salmonellae growth. In an early paper, Dernby *(5)* reported on the pH range which permitted 40 different organisms to grow, apparently at their optimum temperatures. *Salmonella typhosa* grew between pH 6.2 and 7.6; *Salmonella paratyphi* between 4.5 and 7.8; and *Salmonella schottmuelleri* between 4.5 and 8.0. Chung and Goepfert *(4)* studied the growth of salmonellae on medium adjusted with different acidulants and reported growth as low as pH 4.05. Ayres *(3)* reported that salmonellae were rapidly destroyed in lemon juice, pH 2.3, and lime juice, pH 2.5. He also reported that tomato juice, pH 4.3, was bactericidal to these organisms. Alford and Palumbo *(1)* reported growth of 23 strains of *Salmonella* at pH 5.8 and 6.5, but only one strain at 5.0, in broth containing 2% salt, incubated at 10 C.

Nothing seems to have been published concerning the minimum growth temperatures of salmonellae over the pH range occurring in foods. Yet the combined effects of low temperature (refrigerated storage) and lowered pH are commonly used to preserve foods. This paper reports results of a study on the ability of three common *Salmonella* serotypes to grow at low temperatures over the pH range 4.0 to 9.0.

Materials and Methods

Test organisms

Salmonella heidelberg, ATCC 8326; *Salmonella typhimurium*, ATCC 6994; and *Salmonella derby*, ATCC 6966, were used in these studies because they were among the serotypes most frequently isolated from human sources *(10)* and because data were available on their minimum growth temperatures.

Inoculum

The inoculum for the temperature-gradient incubator was 18 hr trypticase soy broth cultures which were spread on the agar surface by means of a sterile cotton swab.

The inoculum for the polythermostat was standardized by adding 18 hr trypticase soy broth cultures of the test organisms to 0.1% peptone water to obtain 55% transmittency at 660 nm in a Bausch and Lomb spectrophotometer, yielding 10^8 cells/ml. Appropriate serial decimal dilutions for inoculation were prepared in 0.1% peptone water, chilled in ice, and added to flasks of chilled sterile trypticase soy broth. The chilled suspension was added to 26 × 55 mm screw-cap tubes (12 ml/tube) and held chilled until placed in the polythermostat.

Medium

The pH of media used in both the temperature-gradient incubator and the polythermostat was adjusted with HCl or NaOH prior to sterilization so that the desired pH of 4.0 to 9.0 was obtained after sterilization. Medium adjusted to a different pH was used in each of the channels of the temperature-gradient incubator and in the tubes in each of the channels of the polythermostat.

[1]Contribution No. 354, College of Fisheries, University of Washington, Seattle, Washington 98195. This investigation was supported by the U. S. Public Health Service Research Grant 5 RO1 UI 00248-02 from the Consumer Protection and Environmental Health Service, Food and Drug Administration.

The temperature-gradient incubator (7) consists of an aluminum block with six longitudinal channels (1 × 1 1/4 × 30 inches) machined into one surface. The channels are filled with agar, each adjusted to a different pH, and the surface inoculated with the test organism. The temperature-gradient incubator was converted into a polythermostat by placing screw-cap tubes into the channels and filling the channels around the tubes with 2% agar in water.

The incubator and polythermostat were cooled at one end by circulating refrigerant and warmed at the other end by circulating water. During operation a linear temperature-gradient was obtained along the length of the aluminum block. Temperature was monitored constantly by thermocouples placed at intervals along the gradient and recorded on a Brown 6-point recording potentiometer.

Enumeration and detection

The minimum growth temperature of *Salmonella* on the surface of agar in the temperature-gradient incubator was determined by visual observation of the point at which growth could no longer be seen, and calculation of the actual temperature on the basis of the gradient. *Salmonella* counts were determined by the drop-plate method. Duplicate or triplicate 0.1 or 0.01 ml volumes of the appropriate dilutions from each tube in the polythermostat were inoculated onto the surface of trypticase soy agar. The plates, with duplicate or triplicate samples, were incubated at room temperature (about 22-23 C) and the colonies counted after 16-24 hr with the aid of a dissecting microscope. The organisms grew rapidly at room temperature, with a generation time of approximately 1 hr. At higher incubation temperatures, growth was more rapid and colonies grew together, making counting difficult. Therefore, room temperature incubation was used and the small colonies, although easily seen, were more easily distinguished with the 12× dissecting microscope.

RESULTS

The growth of S. *heidelberg* on the surface of trypticase soy agar in the temperature-gradient incubator was determined after 7 days of incubation over a temperature range of −1 to 15.5 C. Experiments of longer than 7 days duration were not conducted because moisture evaporated from the warm end of the temperature-gradient incubator and condensed on the cold end. The average results of three experiments are shown in Fig 1. After 7 days of incubation at pH 4.0, no growth of *Salmonella* could be detected even at the warm end of the incubator. On agar adjusted to pH 5.0, S. *heidelberg* was able to grow, but the minimum growth temperature at this pH was only 9.5 C. As the pH of the medium was adjusted closer to neutrality, the minimum growth temperature began to drop. At pH values of 6.0, 7.0, and 8.0, the minimum growth temperatures were 5.3, 5.2, and 5.3 C, respectively. The minimum growth temperatures then increased to 6.4 C at pH 9.0.

When *Salmonella* was grown in broth in the polythermostat over a temperature range of 2 - 12 C, the incubation period was extended for as long as 28 days. At each sampling time the numbers of organisms were determined in each tube by the drop-plate method. An increase in numbers of cells was not considered significant and recorded as an increase unless the number of cells had increased by 0.5 log.

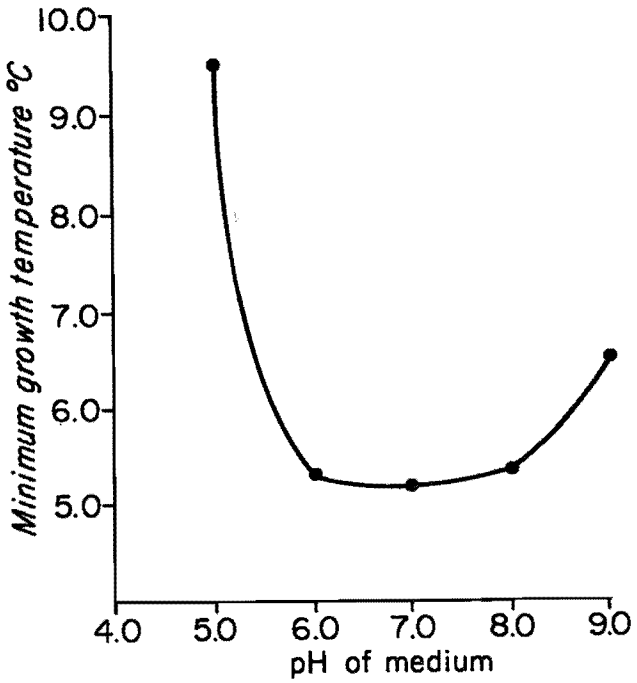

Figure 1. Minimum growth temperatures of *Salmonella heidelberg* after 7 days' incubation over the pH range of 4.0 to 9.0.

The minimum growth temperature of each organism for each pH value was determined at intervals for 28 days. The average results for two experiments are shown in Table 1. Increases in number of cells were obtained in all tubes incubated at temperatures higher than those given in Table 1. The actual minimum growth temperature may fall between the recorded temperature in the tube showing growth, and the temperature of the next colder tube which does not show an increase in numbers of cells.

When grown in broth at pH 4.0 and 5.0, the inoculum for the three serotypes decreased throughout the 28-day incubation period from an initial count of 10^4 to 10^6 cells/ml to fewer than 10 cells/ml.

Salmonella heidelberg showed greater pH effects in broth than on an agar surface. At pH 4 a decrease in cell numbers from 10^6 to <10/ml was obtained at all temperatures tested over the incubation period. An increase was not obtained at pH 5.0, but the number of cells remained almost constant throughout the incubation period. Increases in numbers of cells were obtained at pH 6.0, 7.0, and 8.0, and the minimum growth temperatures obtained at these pH values were 6.5, 5.9, and 7.7 C, respectively. At pH 9.0 the inoculum decreased from 16^6/ml to <10/ml in 6 days.

TABLE 1. INCUBATION TIME AND MINIMUM TEMPERATURE AT WHICH GROWTH OF SALMONELLA WAS DETECTED AT EACH pH VALUE.

pH	*S. heidelberg*		*S. typhimurium*		*S. derby*	
	Days incubation	Minimum temperature (°C)	Days incubation	Minimum temperature (°C)	Days incubation	Minimum temperature (°C)
5.0	No Growth		No Growth		No Growth	
6.0	6	6.5	2	12.0	5	10.0
	13	7.1	6	10.4	12	9.0
	20	7.1	16	7.2	19	9.0
	27	7.1			26	9.0
7.0	7	6.5	2	12.0	6	9.0
	14	5.9	6	9.6	13	9.0
	21	6.5	16	7.2	20	9.0
	28	6.5			27	9.0
8.0	7	7.7	2	11.2	6	9.5
	14	7.7	6	8.8	13	9.5
	21	7.7	16	6.4	20	9.5
	28	7.7	23	6.4	27	9.5
9.0	No Growth		16	8.0	No Growth	
			23	8.0		

At pH values of 6.0, 7.0, 8.0, and 9.0 an increase in the numbers of viable *S. typhimurium* cells was obtained. This increase showed a time-temperature effect with a longer lag at the lower temperature. The lowest minimum growth temperature, 6.4 C, was obtained at pH 8.0. This minimum increased to 7.2 C at pH 6.0 and 7.0 and to 8.0 C at pH 9.0.

The growth pattern obtained with *S. derby* was similar to that obtained with *S. heidelberg*. The numbers of viable cells decreased throughout the incubation periods at pH 4.0, 5.0, and 9.0. Increases in numbers of cells were obtained at pH 6.0, 7.0, and 8.0, and the minimum growth temperatures obtained at these pH values were 9.0, 9.0, and 9.5 C, respectively. The minimum growth temperature for *S. derby* over the pH range was higher than that obtained for either *S. typhimurium* or *S. heidelberg*.

DISCUSSION

The use of HCl and NaOH to adjust pH inevitably causes production of NaCl in the medium. However, the quantities used in these studies were insufficient to raise the NaCl content of the medium by even 0.5%. We have shown in a separate study (to be published in this Journal) that there is little effect on minimum temperature of growth of *Salmonella* of NaCl concentrations in the range 0.5 to 3%. NaCl may thus be ignored as a factor in the pH investigation.

The minimum growth temperature-pH relationship for *S. heidelberg* on solid medium (Fig. 1) indicates the pattern of events for the three serotypes tested. In all instances the pH range permitting growth at minimum temperatures was pH 6 to pH 8. The actual minima observed under these conditions are in good agreement with values reported earlier *(7)*. A pH of 5 seemed to represent a break point in the ability of salmonellae to grow at temperatures in the refrigerated food range, although Chung and Goepfert *(4)* reported the growth of salmonellae as low as pH 4.05 at 25 and 32 C. Cells held at pH 4 died off rapidly at temperatures below 10 C. The organisms showed somewhat marginal growth at pH 9 when temperatures were below 10 C but the high pH seemed, not unexpectedly, to have less effect than pH values in the acid range.

Of the three serotypes tested, *S. heidelberg* showed the greatest tolerance of varied pH conditions. This organism has also shown the lowest minimum growth temperature and may in fact be somewhat more psychrotrophic than other *Salmonella* serotypes. It is probable that the difference in response of this organism when grown on the surface of solid media and in liquid media simply reflects a greater availability of oxygen on the solid medium. Chung and Goepfert *(4)* were able to get growth of *Salmonella senftenberg* at a lower pH with aeration than without. Obviously, physiologically important factors such as temperature, acidity, and oxygen availability interact in affecting the growth of microorganisms.

The combined effect of pH conditions and low temperature on the outgrowth time of salmonellae is of interest in relation to control of these organisms in foods. Where growth occurred at the extreme pH values of 5 and 9, it was greatly delayed. However, only *S. typhimurium* showed a marked time-temperature growth effect in the pH range permitting growth at below 10 C.

On the basis of these studies it appears that three of the more common food-poisoning serotypes of

Salmonella grow only over a narrow pH range between 6 and 8 at temperatures close to 5 C. The results reinforce earlier published conclusions that foods held at temperatures below 5 C will not support growth of *Salmonella*.

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(Continued from Page 48)

Peppler, G. H. Richardson, R. L. Saffle, F. M. Sawyer, C. F. Swift, B. A. Twigg, C. Vanderzant, H. B. Warren, H. Wistreich, and E. R. Wolford. One member of the Editorial Board, Dr. B. J. Liska, resigned during 1971 because he became Scientific Editor of the *Journal of Food Science*.

The following persons not on the Editorial Board have reviewed papers during the first one-half of 1971 and their help is gratefully acknowledged: C. H. Amundson, R. L. Bradley, Jr., A. L. Branen, C. L. Duncan, W. Gjomerac, J. M. Goepfert, R. V. Lechowich, D. B. Lund, N. F. Olson, T. Richardson, H. Sugiyama, and J. H. von Elbe.

E. H. MARTH
Editor
Journal of Milk and Food Technology

TABLE 1. SUMMARY OF CONTENTS OF *Journal of Milk and Food Technology*, 1967-1970

Item	Volume 30 (1967)	Volume 31 (1968)	Volume 32 (1969)	Volume 33 (1970)
1. Total pages, including covers	512	540	624	688
2. Research papers				
a. Number	30	32	47	66
b. Pages	137	142	205	280
c. Percent of total pages	26.7	26.3	32.9	40.7
3. General interest papers-technical				
a. Number	11	16	14	18
b. Pages	47	74	87	99
c. Percent of total pages	9.2	13.7	12.2	14.3
4. General interest papers-non-technical				
a. Number	23	14	26	20
b. Pages	72	65	91	64
c. Percent of total pages	14.1	12.0	14.6	9.3
5. Association affairs				
a. Pages	64	68	62	49
b. Percent of total pages	12.5	12.6	9.9	7.2
6. News and events				
a. Pages	51	42	36	23
b. Percent of total pages	9.9	7.8	5.8	3.4
Percent of pages-technical material	35.9	40.0	45.1	55.1
Percent of pages-non-technical material	36.5	32.4	30.3	20.0
Percent of pages-covers, standards, index, advertising, etc.	27.6	27.6	24.6	24.9[a]

[a]Includes 48 pages of E-3-A and 3-A Standards in the March and April issues.