NOTES

Initiation of Staphylococcal Growth in Processed Meat Environments

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The probability of staphylococcal growth initiation in laboratory-made cured meats is investigated and compared with growth initiation in broths.

Genigeorgis et al. (4) developed equations to predict the probability that one staphylococcal cell will initiate growth aerobically in Brain Heart Infusion (BHI) broths of various pH values and NaCl concentrations at 30 C. The studies reported here tested the reliability of the developed equations in predicting the probability of staphylococcal growth in a variety of laboratory-prepared processed meats.

Thirty-nine processed meats at different pH values and with various concentrations of NaCl and $NaNO_2$ were utilized during the course of four experiments. The meats were prepared by homogenizing defatted ground pork loin or beef sirloin in a meat grinder with desired amounts of NaCl and $NaNO_2$.

After 24 hr of refrigeration, the pH of each meat was adjusted to low and high levels by the addition of 1.75% (w/w) glucono-δ-lactone powder and NaOH (5 ml of 2% NaOH/100 g), respectively, and the meats were again homogenized. After this, they were cured for an additional 2 days and then pasteurized for 15 min (6 min to 121 C, 7 min at 121 C, and 2 min to room temperature), after which the meat juices were drained off.

Staphylococcus aureus cells of strain S-6 or 137, grown in 3%–3% PHP-NAK broth (1) for 4 hr in the shaker (37 C), were washed twice with saline and then resuspended in saline to desired concentrations. From each type of meat, multiple samples of 1, 5, or 10 g each were placed in sterile polycarbonate centrifuge tubes and inocu-

lated with different levels of staphylococcal cells. The numbers of cells per sample were: (experiment 1) 5×10^0, 5×10^2, 5×10^4, and 5×10^6; (experiment 2) 4×10^0, 4×10^1, 4×10^2, 4×10^3, and 4×10^6; (experiment 3) 3×10^0, 3×10^1, 3×10^2, 3×10^3, and 3×10^6; (experiment 4) 2×10^0, 2×10^1, 2×10^2, 2×10^3, and 2×10^6. Strain S-6 was used in experiments 1, 2, and 3 and strain 137 was used in experiment 4. The volume of the inoculum was 0.01 ml for the 1-g samples and 0.1 ml for the 5- and 10-g samples.

The tubes containing meats with the same brine concentration were placed in a can along with a solution of NaCl equal in concentration to that of the brine concentration of the meats. The cans were covered with plastic lids, and the cultures were then incubated aerobically for 7 days at 30 C. Samples of the various types of meats were then analyzed for pH, brine concentration, water, and nitrite according to procedures described previously (5).

After incubation, the meats were homogenized with 10 to 20 ml of sterile distilled water, and portions were plated on blood-agar (BHI base) for staphylococcal and total plate counts. The remainder of the homogenate was centrifuged, and the supernatant fluid was lyophilized after dialysis and treatment with chloroform (2). The lyophilized samples were rehydrated with 0.6 ml of phosphate-buffered 2.8% saline (pH 7.2, 0.02 M) and then tested by the single-gel diffusion (6) and microslide (3) techniques for the presence and amounts of enterotoxins B and C.

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TABLE 1. *Initiation of staphylococcal growth and production of enterotoxin B by Staphylococcus aureus strain S-6 in cured pork and beef meat environments*

Expt	Sample	HNO$_2$ (ppb)	NaCl^a (%)	pH	Cells/inoculation	Cells needed for growth^b (95% confidence limits)	Final staphylococcal counts/sample	Enterotoxin B^c
1, Pork (10-g samples)	1 L^d	234	1.04	5.00	5	1–1.3 × 10^4	8.6 × 10^9	−+++
	1 M	105	1.15	6.40	5	1–76	<10^{3e}	−+++
	1 M	105	1.15	6.40	500	1–76	4 × 10^{8e}	
	1 H	39	1.06	7.00	5	1–17	8 × 10^{8e}	++++
	4 L	278	3.25	5.15	5	5–4.7 × 10^4	<10^3	−+++
	4 L	278	3.25	5.15	500	5–4.7 × 10^4	6 × 10^{10}	
	4 M	95	3.79	6.39	5	1–501	2 × 10^9	−+++
	4 H	45	3.62	6.79	5	1–219	5 × 10^{8e}	−+++
	8 L	249	6.22	5.10	5	159–1.5 × 10^6	2 × 10^8	−−−−
	8 M	79	6.92	6.35	5	1–1.2 × 10^4	3 × 10^{8e}	−+++
	8 H	40	6.18	6.75	5	1–2,800	7 × 10^{8e}	++++
	10 L	313	10.00	5.06	5	1.2 × 10^4–1.2 × 10^7	1.5 × 10^4	−−−−
	10 L	313	10.00	5.06	500	1.2 × 10^4–1.2 × 10^7	2 × 10^{10}	
	10 M	93	9.01	6.27	5	14–1.3 × 10^5	10^8	−−−−
	10 H	39	9.20	6.75	5	6–6.3 × 10^4	5 × 10^8	−−−−
2, Beef (5-g samples)	4 L	6,206	3.72	4.33	4 × 10^6	2,700–2.6 × 10^7	<750	−−−−−
	4 M	906	3.87	6.02	4	1–1,500	3.8 × 10^7	−−−−−
	4 H	475	3.86	6.42	4	1–480	4 × 10^7	+++++
	8 L	19,440	6.79	4.26	4 × 10^6	1.75 × 10^5–1.6 × 10^9	<750	−−−−−
	8 M	1,050	7.56	5.96	4	9–8.3 × 10^4	<750	−−−−−
	8 M	1,050	7.56	5.96	40	9–8.3 × 10^4	4.3 × 10^8	
	8 H	513	6.52	6.43	4	1–6,600	2.6 × 10^6	−−−−−
	10 L	31,181	9.28	4.20	4 × 10^6	4.7 × 10^6–4.5 × 10^{10}	<750	−−−−−
	10 M	1,059	11.01	5.97	4	331–3.2 × 10^6	<750	−−−−−
	10 M	1,059	11.01	5.97	40	331–3.2 × 10^6	4.5 × 10^3	
	10 H	468	9.73	6.45	4	19–1.8 × 10^5	3.9 × 10^9	−−−−−

^{a} Per cent in brine.

^{b} Based on formulas developed from the studies with BHI broth as culture medium (*see* reference 4).

^{c} Four or five signs refer to enterotoxin production (+) or no production (−) by the four or five inoculum levels used, starting with the lower cell level.

^{d} L, low pH; M, medium pH; H, high pH.

^{e} For all four samples, 10^7 to 10^9 sporeforming bacteria per sample were present.

The results of the experiments are presented in Tables 1 and 2. Only the minimum numbers of cells which initiated growth have been included.

The present findings indicate that the meat environments were more conducive to growth of staphylococci than were the BHI broths. Also, at the same brine concentration and pH, it took fewer cells to initiate growth in meats than in BHI broths. This was particularly evident in meats with high brine concentration and low pH. Staphylococcal growth was initiated in such meats even though the levels of inoculated cells were below the minimum limits predicted by the equations (4) as necessary for initiation of growth.

As expected (6, 7), elevated yields of enterotoxins B and C were obtained at high pH values coupled with low brine concentrations. All meats supporting enterotoxin production had good staphylococcal growth to over 4 × 10^7 cells per sample. Yet, 70 samples with 10^8 cells per sample had no enterotoxin B or C production. The highest yields of enterotoxins B and C were 70 and 20 µg per sample, respectively.

The results obtained in these studies indicate that the equations derived from data based on previous studies with BHI broth (4) cannot reliably be used to predict the probability of initiating staphylococcal growth in processed meats. Experiments statistically designed to permit development of predictive equations derived from results obtained by direct staphylococcal inoculation of meats are now in progress.

TABLE 2. *Initiation of staphylococcal growth and production of enterotoxins B and C by Staphylococcus aureus strains S-6 and 137 in processed beef environments*

Expt	Sample	NaCl[a]	pH	Cells/ inoculation	Cells needed for growth (95% confidence limits)[b]	Final staphylococcal counts/sample	Enterotoxin[c]
3, Beef (1-g samples)	4 L	3.56	4.7	3,000	$135-1.3 \times 10^6$	2,000	$- - - - -$
	4 L	3.56	4.7	3×10^6	$135-1.3 \times 10^6$	5×10^7	$- - - - -$
	4 M	3.75	5.80	3	$1-3,000$	10^8	$- + + + +$
	4 H	4.07	6.25	3	$1-910$	10^8	$- + + + +$
	8 L	7.72	4.7	3,000	$1.2 \times 10^4-1.2 \times 10^8$	10^8	$- - - - -$
	8 L	7.72	4.7	3×10^6	$1.2 \times 10^4-1.2 \times 10^8$	2×10^7	$- - - - -$
	8 M	7.78	5.85	3	$16-1.6 \times 10^5$	10^9	$- - - + +$
	8 H	7.75	6.30	3	$3-3.5 \times 10^4$	6×10^9	$- - - + +$
	14 L	13.61	4.67	3×10^6	$1.2 \times 10^7-1.2 \times 10^{11}$	3×10^4	$- - - - -$
	14 M	13.81	5.80	3×10^6	$1.5 \times 10^4-1.5 \times 10^8$	10^8	$- - - - -$
	14 H	14.03	6.30	3	$3 \times 10^3-3.10$	2×10^7	$- - - - -$
4, Beef (1-g samples)	4 L	3.2	4.72	2	$3.6 \times 10^4-3.3 \times 10^8$	50	$- - - - +$
	4 L	3.2	4.72	20	$3.6 \times 10^4-3.3 \times 10^8$	3×10^8	
	4 M	3.6	6.08	2	$1-7,900$	50	$- + + + +$
	4 M	3.6	6.08	20	$1-7,900$	10^9	
	4 H	3.45	6.50	2	$1-620$	10^8	$- - + + +$
	8 L	7.85	4.72	2	$3.2 \times 10^5-2.9 \times 10^9$	50	$- - - - -$
	8 L	7.85	4.72	20	$3.2 \times 10^5-2.9 \times 10^9$	5×10^6	
	8 M	7.10	6.15	2	$31-2.8 \times 10^5$	50	$- - - + +$
	8 M	7.10	6.15	20	$31-2.8 \times 10^5$	6×10^5	
	8 H	6.00	6.62	2	$1-2,700$	8×10^{9d}	$- - - + +$
	14 L	12.60	4.70	2,000	$4.7 \times 10^6-4.3 \times 10^{10}$	3,800	$- - - -$
	14 L	12.60	4.70	2×10^6	$4.7 \times 10^6-4.3 \times 10^{10}$	1×10^6	
	14 M	12.30	6.09	2	$130-1.2 \times 10^6$	5×10^6	$- - - - -$
	14 H	13.86	6.60	2	$100-9.1 \times 10^5$	2×10^6	$- - - - -$

[a] Per cent in brine.

[b] Based on formulas developed from the studies with BHI broth as culture medium (*see* reference 4).

[c] Five signs refer to enterotoxin production (+) or no production (−) by the five inoculum levels used, starting with the lower level. We tested for enterotoxin B in experiment 3 and enterotoxin C in experiment 4.

[d] For all five samples, 10^7 to 10^9 sporeforming bacteria per sample were present.

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