

Growth Inhibition of *Listeria monocytogenes* by Sodium Polyphosphate as Affected by Polyvalent Metal Ions

ABSTRACT

Sodium polyphosphate (SPP, average chain length = 13) increased the lag time of *L. monocytogenes* Scott A (Lm) in brain-heart infusion broth (BHI). Polyvalent metal ions (1–10 mM) reversed inhibition of Lm growth by 0.5% SPP (nominal 3.6 mM). 10 mM Ca^{2+} or Mg^{2+} , 5 mM Fe^{3+} , 2 mM Mn^{2+} or 1 mM Zn^{2+} added to SPP-containing BHI, pH 6.0, at 19°C resulted in growth comparable to control cultures. Fe^{2+} partially restored growth; Ni^{2+} , Co^{2+} , Cu^{2+} or Al^{3+} were ineffective. SPP inhibited growth at 28°C in BHI, pH 5.0, and Lm grew upon addition of Ca^{2+} , Mg^{2+} or Mn^{2+} , but not Zn^{2+} or Fe^{3+} . Addition of 0.5% SPP to mineral-rich foods, such as pureed beef, green beans or sweet potatoes, did not delay growth.

INTRODUCTION

SODIUM POLYPHOSPHATES (SPP) are salts of polymers of phosphoric acid [$\text{Na}_{(n+2)} \text{P}_n \text{O}_{(3n+1)}$]. They are widely used food additives with several different functions: buffers, emulsifiers, dispersants, antioxidants, chelators or moisture binding agents (Ellinger, 1972; Molins, 1991; Steinhauer, 1983). Also, they may have antimicrobial properties (Ellinger, 1972; Molins, 1991; Tompkin, 1983; Wagner, 1986).

Listeria monocytogenes (Lm) is widely distributed in nature and has been isolated from many of foods. Outbreaks of listeriosis have sometimes resulted in fatalities or recalls of food products. We have examined the effects of sodium polyphosphates (SPP) on growth of *L. monocytogenes* in BHI media of pH 6.0 (Zaika and Kim, 1993). Short chain SPP had little effects on growth, but the higher polymers (Sodaphos, Hexaphos and Glass H, average $n = 6, 13$ and 21 , resp.) exhibited significant bacteriostatic effects which increased with increasing SPP concentrations. Later studies (Scullen and Zaika, 1994) determined effects and interactions of temperature (4, 12, 19°C), NaCl (0.5, 2.5, 4.5%) and initial pH (5.0, 5.5, 6.0, 7.0) on growth inhibition of *L. monocytogenes* by Hexaphos (0, 0.1, 0.3, 0.5, 1.0%). Growth inhibition by SPP increased with decreasing temperature, decreasing pH and increasing NaCl concentration. Growth inhibition induced by SPP was accompanied by changes in cellular morphology. These included cell elongation, thickening of the cell envelopes along the cylindrical region of the cell body and accumulation of cell wall material at the septa (Zaika et al., 1991). However, normal growth was observed when the SPP-containing media had been supplemented with MgSO_4 (Zaika and Kim, 1993). Several studies (Elliott et al., 1964; Jen and Shelef, 1986; Knabel et al., 1991; Lee et al., 1994; Post et al., 1963) have indicated that certain polyvalent metal ions, such as Mg^{2+} , Ca^{2+} and Fe^{3+} , can reverse the inhibitory effects of polyphosphates on microorganisms.

Our objective was to evaluate the effectiveness of polyvalent metal ions in reversing growth inhibition of *L. monocytogenes* by SPP in BHI media and to assess the ability of the bacterium to grow in foods containing added SPP.

MATERIALS & METHODS

Microorganism

Listeria monocytogenes Scott A was used. To prepare the inoculum, the organism was cultured for 18–24 h at 37°C in brain-heart infusion (Difco) broth (BHI) and the culture was diluted with sterile 0.1% peptone water (Difco).

Sodium polyphosphate (SPP)

Hexaphos™ (sodium polyphosphate, glassy, avg. chain length, $n = 13$, FMC Corp., Philadelphia, PA) was used. It had a P_2O_5 content of 66.5–68.0% by wt., and was infinitely soluble in water giving a 1% solution of pH 6.7–7.2. The SPP was added to sterile media as 10% aqueous filter-sterilized solution.

Polyvalent metal salt solutions and foods

Reagent grade crystalline salts (chlorides, sulfates or nitrates) were used. Aqueous solutions of the salts, 0.1 or 0.2M, were filter-sterilized and added to sterile media. Commercially available sterile foods that did not require further handling were used. These were baby foods: beef, sweet potatoes, butternut squash, green beans, beets and carrots.

Growth in liquid cultures

BHI was prepared to 90% dilution as follows: 37g BHI powder was dissolved in ≈ 800 mL distilled water and adjusted to the required pH with 2N HCl. The medium was diluted with water to 900 mL and 45-mL aliquots were sterilized by autoclaving. Sterile water, 10% Hexaphos and polyvalent metal salt solutions (5 mL total volume) were added to the sterile media. The final media contained 0 or 0.5% Hexaphos and 0, 1, 2, 5 or 10 mM metal ion. All flasks were inoculated with 0.5 mL of *L. monocytogenes* culture to an initial level of 10^3 CFU/mL and incubated on a rotary shaker (150 rpm) at 19 or 28°C.

At appropriate intervals microbial populations were determined by surface plating the cultures or dilutions in sterile 0.1% peptone water on Tryptose Agar (Difco) using a Spiral Plater (Spiral System Instruments, Inc., Bethesda, MD). The plates were incubated for 24 hr at 37°C and counted.

Growth in foods

Foods, 95g, were aseptically transferred to sterile 300-mL beakers, mixed with 5 mL sterile distilled water or 10% Hexaphos solution and inoculated with *L. monocytogenes* to 10^4 CFU/g. The beakers were capped with sterile aluminum foil and incubated at 19°C. For determination of microbial population, 1g of food was weighed into a test tube containing 9 mL sterile 0.1% peptone water and mixed thoroughly using a vortex. The mixture was poured into a filter stomacher bag (SFB-0410, Spiral Biotech., Bethesda, MD) to remove particulate matter and the filtrate plated on tryptose agar as described.

Determination of growth kinetics parameters

Bacterial growth curves were generated from microbial population data using the Gompertz equation (Gibson et al., 1987) in conjunction with ABACUS, a nonlinear regression program that employed a Gauss-Newton iteration procedure (Damert, 1994). The Gompertz parameter values (A, B, C, M) were used to calculate exponential growth rates (EGR) [$(\log_{10} \text{CFU/mL})/\text{h}$], generation times (GT) (h), lag phase durations (LPD) (h) and maximum population densities (MPD) ($\log_{10} \text{CFU/mL}$) as described by Gibson et al. (1987) and Buchanan et al. (1989).

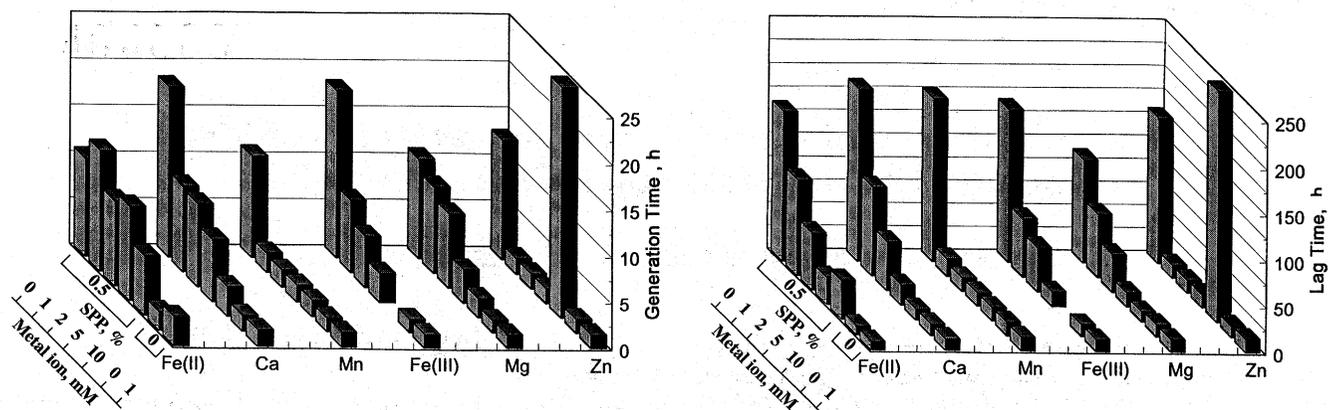


Fig. 1—Effect of SPP (0, 0.5% Hexaphos) and polyvalent metal ion concentration (0–10 mM) on growth of *Listeria monocytogenes* at 19°C in BHI, pH 6.0. (A) Generation time, h; (B) Lag time, h.

RESULTS & DISCUSSION

Growth in liquid cultures

The effects of SPP (0.5% Hexaphos) and polyvalent metal ions (Ca^{2+} , Mg^{2+} , Mn^{2+} , Zn^{2+} , Fe^{2+} and Fe^{3+}) were compared on the growth kinetics parameters of *L. monocytogenes* at 19°C in BHI medium, pH 6.0 (Fig. 1). In absence of added metal ions SPP exerted a considerable bacteriostatic effect. The median values for generation time and lag time were 11.9 and 148.4h, respectively, compared with corresponding values of 1.8 and 12.3h for the control media (Table 1). When the SPP-containing media were supplemented with metal ions, faster bacterial growth occurred with decreased generation times (Fig. 1a) and lag times (Fig. 1b). All metals reversed the growth inhibition by SPP even at the lowest concentrations tested, with Mn^{2+} and Zn^{2+} being most effective. Growth rate increased with increasing concentration of added metal in the SPP-containing media; however, the bacterium did not grow in the presence of 10 mM Zn^{2+} . Other metal ions (Cu^{2+} , Co^{2+} , Ni^{2+} and Al^{3+}) were tested in a similar manner in BHI, pH 6.0, at 19°C, but they were not effective in reversing growth inhibition by SPP (data not shown). Growth was comparable in control media and in media supplemented with 1 mM concentration of all metal ions tested in the absence of SPP, but higher concentrations of the metals, except Mg^{2+} , Ca^{2+} and Mn^{2+} , were inhibitory.

Growth of *L. monocytogenes* at 19°C was much slower in control media at pH 5.0 (generation time = 4.9h, lag time = 59.2h, Table 1) and did not occur during 400h incubation in SPP-containing media in the absence of added metals. However, growth occurred in SPP-containing media with the higher levels, 5 or 10 mM, of Mg^{2+} or Ca^{2+} (data not shown). Therefore, subsequent growth studies in media of pH 5.0 were carried out at 28°C (Fig. 2). In control media the generation time and the lag time were 1.2 and 16.8h, respectively, while in SPP-containing media growth did not occur during 200h incubation (Table 1). Inhibition by SPP was effectively reversed by addition of Mn^{2+} , Ca^{2+} or Mg^{2+} . In the absence of SPP, growth in media supplemented with 1 mM Mg^{2+} , Ca^{2+} , Mn^{2+} or Fe^{3+} was similar to that in control cultures; however, the bacterial population decreased in presence of 1 mM Zn^{2+} .

It is evident that the antimicrobial activity of SPP was associated with its ability to form complexes with metal ions. However, little information is available on the nature of such complexes. Because standard methods of studying complexes cannot be applied, the SPP-metal complexes have not been defined in terms of a chemical formula and a dissociation constant (Van Wazer and Campanella, 1950). The number of phosphorus pairs connected to each metal atom in the chelate structures may be determined by the relative concentration of metal and polyphosphate in solution, with the maximum number depending on the ratio of ionic radius of the metal to that of oxygen. When the number of attached phosphorus pairs is small, the complex would tend to

become insoluble and a precipitate may form. When more than one phosphorus pair is connected to any metal atom, they could come from the same or different polyphosphate chains. Studies with SPP, where $n = 5$, showed that alkali metals formed weak complexes and other metal ions formed strong complexes (Van Wazer and Campanella, 1950). Long chain SPP effectively bound Ca^{2+} and Mg^{2+} over a wide pH range (Irani and Callis, 1962), while binding of Fe^{3+} increased with decreasing pH (Irani and Morgenthaler, 1963). The nature of the complexes between metal ions and polyphosphates is further complicated. Commercial long chain polyphosphates are mixtures described by an average chain length, and interactions of metal ions and polyphosphates are possible with other components in complex media.

High levels of polyvalent metal ions may be toxic, but microorganisms require certain metal ions for normal growth and metabolism. Studies on the growth of *L. monocytogenes* in minimal media indicated that, of the polyvalent metal ions, the organism required only Mg^{2+} and Fe^{3+} (Premaratne et al., 1991).

Growth in foods

To test for antilisterial potential of sodium polyphosphates added to foods we selected single-ingredient sterile foods without additives (baby foods). Growth curves and growth kinetics parameters (Table 2) were determined for *L. monocytogenes* inoculated into the six foods with or without added SPP (0.5% Hexaphos) and incubated at 19°C. In the absence of SPP growth occurred in all foods except beets, where the bacterial population remained essentially unchanged for extended times. The fastest growth occurred in beef, possibly due to a higher nutrient content or due to its higher pH (6.0) relative to vegetables (5.0–5.2) (Table 2). Rapid growth also occurred in sweet potatoes. Growth was somewhat slower in butternut squash, carrots and green beans. Growth of *L. monocytogenes* was essentially similar in the presence and in the absence of SPP (Table 2). Although additional verification is needed, it appears that in some foods, faster growth (shorter lag times) occurred in presence of the polyphosphate. This may be due to a slight increase in pH (0.1 to 0.3 pH units) for the foods, resulting from addition of 0.5% Hexaphos (Table 2). Note that changes in pH due to addition of polyphosphates to foods may affect the potential for growth or survival of microorganisms. Shelef et al. (1990) showed that a slight inhibitory effect on *Staphylococcus aureus* in cooked beef and custard was due to a decrease in pH upon addition of 1% sodium acid pyrophosphate (SAPP), while 0.5 or 1% of sodium polyphosphate ($p = 13$) had no effect. Previous work in our laboratory (Rajkowski et al., 1994) showed that addition of SPP (0.5 or 1.0% Hexaphos) to UHT-sterilized milk had no significant effect on the growth of *L. monocytogenes* at 12, 19, 28 or 37°C (Table 2). Concentrations in foods of metals important to microbial growth (Mg, Ca, Fe, Zn) have been re-

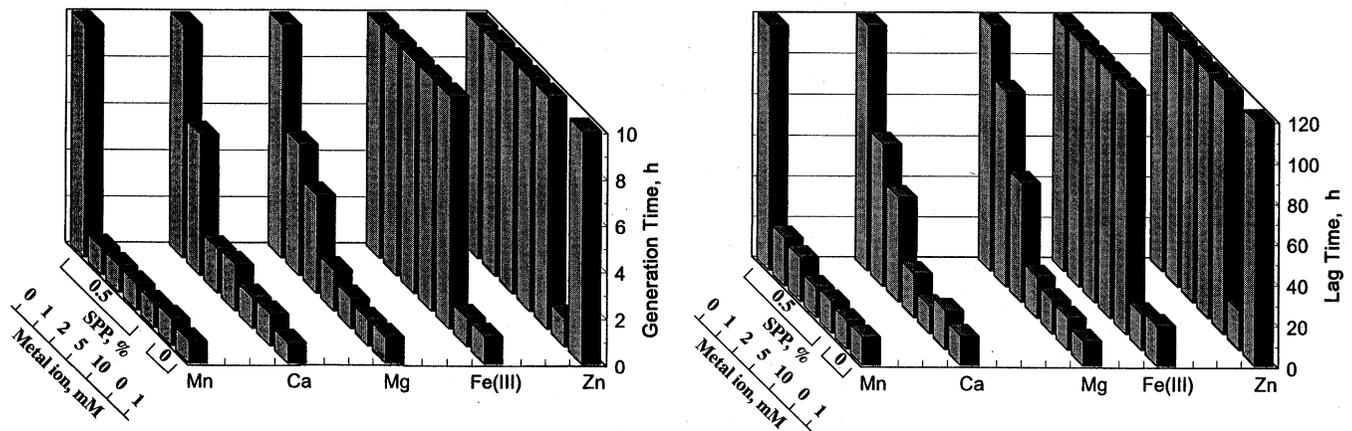


Fig. 2—Effect of SPP (0, 0.5% Hexaphos) and polyvalent metal ion concentration (0–10 mM) on growth of *Listeria monocytogenes* at 28°C in BHI, pH 5.0. (A) Generation time, h; (B) Lag time, h.

Table 1—Effect of 0.5% Hexaphos (SPP) on growth kinetics parameters^a of *Listeria monocytogenes* in BHI broth

	n ^b	EGR ^c (log CFU/mL)/h	GT ^c (h)	LPD ^c (h)	MPD ^c (log CFU/mL)
19°C, pH 6.0					
BHI	11	0.173 (0.041)	1.84 (0.51)	12.28 (1.80)	9.84 (0.30)
BHI + SPP	9	0.027 (0.010)	11.91 (4.05)	148.43 (32.78)	9.43 (0.40)
19°C, pH 5.0					
BHI	4	0.065 (0.019)	4.94 (1.47)	59.16 (20.00)	9.45 (0.06)
BHI + SPP	4	0.000		> 400	
28°C, pH 5.0					
BHI	6	0.254 (0.028)	1.19 (0.12)	16.80 (2.14)	9.53 (0.16)
BHI + SPP	6	0.000		> 200	

^a Numbers in parentheses are standard deviations.

^b n = number of replicate cultures.

^c See text for explanation to abbreviations.

Table 2—Effect of 0.5% Hexaphos (SPP) on growth kinetics parameters^a of *Listeria monocytogenes* in foods at 19°C

	pH	n ^b	EGR ^c (log CFU/g)/h	GT ^c (h)	LPD ^c (h)	MPD ^c (log CFU/g)
Beef	6.0	2	0.106 (0.002)	2.82 (0.06)	12.52 (3.37)	10.20 (0.03)
Beef + SPP	6.2	2	0.110 (0.013)	2.78 (0.32)	18.74 (2.18)	10.32 (0.03)
Beets	5.0	3	NG ^d			
Beets + SPP	5.3	3	NG			
B. squash	5.0	2	0.044 (0.001)	6.92 (0.23)	49.80 (3.64)	9.49 (0.01)
B. squash + SPP	5.3	2	0.055 (0.010)	5.60 (1.00)	37.60 (22.44)	9.37 (0.06)
Carrots	5.1	4	0.055 (0.019)	6.05 (2.30)	35.71 (21.45)	9.03 (0.12)
Carrots + SPP	5.2	3	0.048 (0.004)	6.24 (0.52)	36.79 (8.34)	9.01 (0.07)
G. beans	5.1	4	0.050 (0.017)	6.77 (3.19)	52.16 (20.13)	8.86 (0.20)
G. beans + SPP	5.2	4	0.049 (0.017)	6.70 (2.40)	45.47 (36.65)	8.91 (0.11)
S. potatoes	5.1	2	0.064 (0.006)	4.74 (0.40)	18.84 (2.96)	9.88 (0.09)
S. potatoes + SPP	5.4	2	0.068 (0.004)	4.42 (0.22)	10.78 (3.11)	9.82 (0.05)
Milk ^e	6.5		0.11 (0.01)	1.68 (0.06)	4.6 (0.02)	
Milk + SPP ^e			0.12 (0.01)	1.62 (0.01)	4.9 (0.43)	

^a Numbers in parentheses are standard deviations.

^b n = number of replicate cultures.

^c See text for explanation to abbreviations.

^d NG = no growth.

^e Data from Rajkowski et al. (1994).

ported (Wardlaw and Insel, 1993). All the foods we tested contained adequate levels of metals (more than 9 mM, predominantly Ca²⁺ and Mg²⁺) to overcome inhibition by 0.5% (nominal 3.6 mM) Hexaphos. In contrast, the total concentration of the four metals in BHI broth was low (0.43 mM) (Jen and Shelef, 1986). Antibacterial activities of polyphosphates were reported to be highest in low protein - low mineral media, such as nutrient broth or BHI (Zessin and Shelef, 1988). Published information on effects of polyphosphates on *L. monocytogenes* in foods is scarce. Flores et al. (1996) reported minimal or no effect on *L. monocytogenes* at 4°C in smoked sausage or boneless ham formulated to contain 0.5% of a phosphate blend (sodium polyphosphate, metaphosphate and orthophosphate).

CONCLUSIONS

THE GROWTH of *L. monocytogenes* in low mineral medium such as BHI broth was inhibited by long chain sodium polyphos-

phates (SPP). Addition of polyvalent metal ions, such as Mg²⁺ or Ca²⁺, to the SPP-containing medium reversed the growth inhibition. Addition of SPP had little effect on growth of *L. monocytogenes* in several mineral-rich foods.

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