

Activity of ϵ -Polylysine against *Escherichia coli* O157:H7, *Salmonella* Typhimurium and *Listeria monocytogenes* in Food Extracts

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ABSTRACT

The homopolymer of L-lysine, ϵ -polylysine, is used widely in foods in certain countries, and it has been shown to control growth of *Escherichia coli* O157:H7, *Salmonella* Typhimurium, and *Listeria monocytogenes* in a bacteriological broth. Limited information, however, is available on its antimicrobial activity in foods. This study investigated the antimicrobial activity of ϵ -polylysine against the aforementioned three pathogens in food systems. Antimicrobial activity was evaluated (two replications; three samples each) in extracts (10% wt/wt in distilled water) of fat-free and whole fat milk, beef, bologna, rice, vegetables (broccoli and cauliflower), and tryptic soy broth + 0.6% yeast extract (TSBYE). ϵ -Polylysine was tested at 0.005 and 0.02% (wt/vol) for *E. coli* O157:H7 and *L. monocytogenes*, and 0.02 and 0.04% (wt/vol) for *S. Typhimurium*. The substrates were inoculated (5 log CFU/ml) with five-strain (*E. coli* O157:H7, *S. Typhimurium*) or 10-strain (*L. monocytogenes*) mixtures of the pathogens. Survival/growth of inoculated bacteria was determined periodically during incubation at 12°C for 6 days by plating on tryptic soy agar + 0.6% yeast extract and appropriate selective media. In the absence of ϵ -polylysine, all pathogens grew ($P < 0.05$) to approximately 7-9 log CFU/ml in all substrates, except beef, within two days of incubation. The lowest ϵ -polylysine concentrations tested against *L. monocytogenes* (0.005%) and *S. Typhimurium* (0.02%) resulted in bactericidal effects in all food extracts, but not in the broth. ϵ -Polylysine at 0.005% was also bactericidal against *E. coli* O157:H7 in beef, bologna, rice and vegetable extracts, but not in milk or broth. In general, non-detectable levels (<0.0 log CFU/ml) of all three pathogens were obtained for all tested ϵ -polylysine concentrations in rice and vegetable extracts. Under the conditions of the study, ϵ -polylysine exerted greater antimicrobial activity in food extracts than in broth. Additional research is needed to explore its potential antimicrobial effects in real food systems.

OBJECTIVE

To determine and compare the effects of ϵ -polylysine against *Escherichia coli* O157:H7, *Salmonella* Typhimurium, and *Listeria monocytogenes* in food systems, represented by extracts of various foods, as compared to effects obtained in a culture broth medium.

INTRODUCTION

ϵ -Polylysine (ϵ -PL) is a naturally produced, cationic homopolymer of 25-30 L-lysine residues (Shih et al., 2006; Shima and Sakai, 1977). This compound was discovered, approximately 30 years ago in Japan, in culture filtrates of *Streptomyces abuluis* ssp. *lysinopolymers* strain 346, originally isolated from soil (Hiraki et al., 2003; Shima and Sakai, 1977). ϵ -PL is characterized as being edible, non toxic to humans, water-soluble, stable at high temperatures, and of low environmental impact due to its biodegradability (Hiraki et al., 2003; Shih et al., 2006; Yoshida and Nagasawa, 2003). It is also reported to have a wide antimicrobial spectrum, against both Gram-negative and Gram-positive bacteria, yeasts and molds (Geornaras and Sofos, 2005; Hiraki, 2000; Shima et al., 1984; Yoshida and Nagasawa, 2003). Due to these properties, ϵ -PL is now industrially produced in Japan, by aerobic fermentation, and is used as a food preservative in several staple Japanese foods (Hiraki et al., 2003). This compound is also approved for use in South Korea and the US (FDA, 2004; Shih et al., 2006). Considering its promising antimicrobial properties, there are only a limited number of studies published on this subject, all of which report on inhibitory concentrations of ϵ -PL against microorganisms in culture broth media (Geornaras and Sofos, 2005; Hiraki, 2000; Shima et al., 1984). Information on the antimicrobial activity of ϵ -PL in foods, however, is lacking.

MATERIALS AND METHODS

Inocula:

- 5-strain composite of *E. coli* O157:H7:
 - > ATCC 43889, ATCC 43895, ATCC 51657, ATCC 51658 and EO 139 (kindly provided by Dr. M.P. Doyle, University of Georgia, Griffin, GA)
- 5-strain composite of *S. Typhimurium*:
 - > ATCC 700408, ATCC 14028, UK1 (equine outbreak isolate; kindly provided by Dr. J.W. Foster, University of South Alabama, Mobile, AL) and two isolates of DT104 var Copenhagen (beef animal hide isolates)
- 10-strain composite of *L. monocytogenes*:
 - > NA-3 (serotype 4b, pork sausage isolate), NA-19 (serotype 3b, pork sausage isolate), 101M (serotype 4b, pork sausage isolate), 103M (serotype 1a, pork sausage isolate), 558 (serotype 1/2, pork meat isolate), PVM1, PVM2, PVM3 and PVM4 (serotype not known, pork variety meat isolates), and Scott A (serotype 4b, human isolate)

Procedure:

SUBSTRATES		CULTURE BROTH:
FOOD EXTRACTS (10% wt/wt in distilled water): <ul style="list-style-type: none"> • Fat-free milk • Whole fat milk • Fresh beef (inside rounds; 97% lean) • Bologna (formulated without antimicrobials; 25% fat) • Arborio rice (cooked) • Vegetables (50:50 ratio of broccoli and cauliflower) 		Tryptic soy broth supplemented with 0.6% yeast extract (TSBYE)
ϵ-PL CONCENTRATIONS (%)		
<i>E. coli</i> O157:H7:	<i>S. Typhimurium</i> :	<i>L. monocytogenes</i> :
0, 0.005, 0.02	0, 0.02, 0.04	0, 0.005, 0.02
INOCULATION LEVEL		
5 log CFU/ml		
STORAGE		
12°C, 6 days		

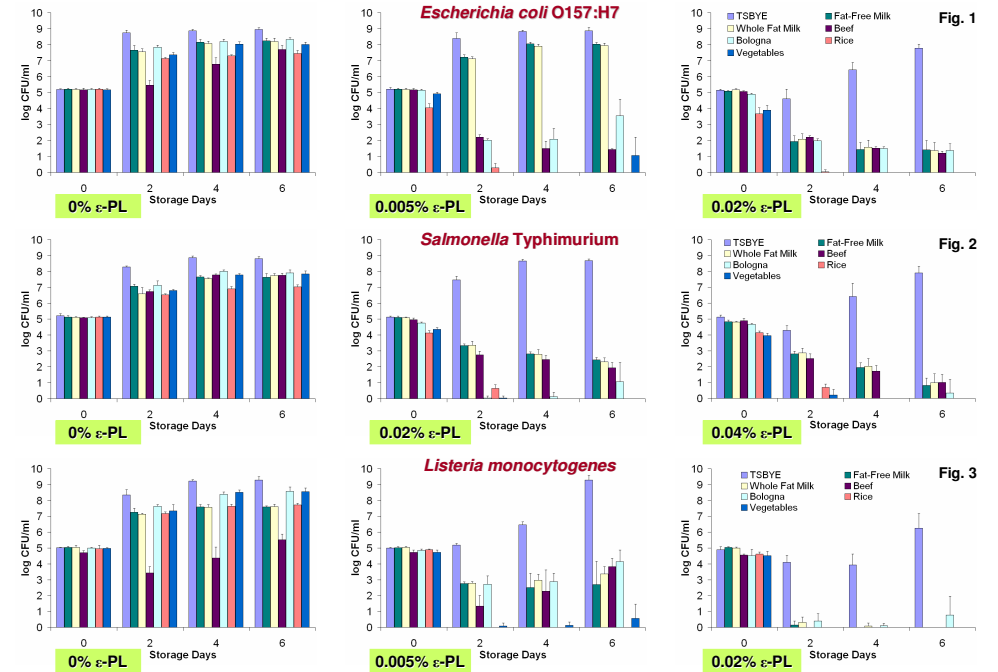
- Surviving populations enumerated (two replications; 3 samples each) immediately after inoculation (day-0), and on days 2, 4 and 6 of storage [tryptic soy agar supplemented with 0.6% yeast extract (TSAYE), and appropriate selective media for each pathogen].
- pH of substrates measured on each analysis day [Denver Instrument (Arvada, CO) pH meter and glass electrode].

TABLE 1: Initial pH Values of Substrates

Substrate	ϵ -Polylysine Concentration (%)								
	<i>E. coli</i> O157:H7		<i>S. Typhimurium</i>		<i>L. monocytogenes</i>				
TSBYE	7.28	7.29	7.37	7.27	7.33	7.38	7.26	7.26	7.32
Fat-free milk	7.16	7.33	7.77	7.05	7.71	7.97	7.02	7.22	7.72
Whole fat milk	7.13	7.35	7.82	7.09	7.73	7.94	7.04	7.26	7.69
Beef	5.68	5.78	6.06	5.72	6.06	6.28	5.52	5.68	5.98
Bologna	6.28	6.40	6.63	6.19	6.52	6.75	6.23	6.34	6.58
Rice	6.95	7.93	8.25	6.79	8.20	8.37	6.84	7.90	8.37
Vegetables	6.43	6.92	7.46	6.53	7.42	7.68	6.43	6.98	7.50

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RESULTS

Survival/Growth of Pathogens

- > In general, all three pathogens reached 7-9 log CFU/ml within 2 days in control substrates (no ϵ -PL) (Fig. 1-3; presented data are from counts obtained on TSAYE).
- > Immediate bactericidal effects ($P < 0.05$) following exposure to ϵ -PL were obtained in the rice (all pathogens) and vegetable (*E. coli* O157:H7 and *S. Typhimurium*) extracts.
- > During storage, antimicrobial effects of ϵ -PL were more pronounced in the food extracts than in the broth medium.
- > The greatest antimicrobial activity for all three pathogens during storage was obtained in the rice and vegetable extracts.
- > In the other food extracts (fat-free milk, whole fat milk, beef, and bologna), both ϵ -PL concentrations tested generally resulted in lower ($P < 0.05$) pathogen levels at the end of storage compared to initial counts, with better bactericidal effects exerted by the higher of the two ϵ -PL concentrations.
- > Small increases (1.0 to 2.5 log CFU/ml from day-2 to day-6) in populations were obtained towards the end of storage in the bologna (all pathogens) and beef (*L. monocytogenes*) extracts containing the lower of the two ϵ -PL concentrations tested.
- > Comparison of counts obtained on TSAYE and selective media used to enumerate *E. coli* O157:H7 and *S. Typhimurium* indicated potential cell injury due to exposure to ϵ -PL (data not shown).

pH Values of Substrates

- > Addition of ϵ -PL did not change the pH of TSBYE; however, increasing ϵ -PL concentrations led to increases in the resultant pH of the food extracts (Table 1).
- > As expected, the pH of substrates during storage decreased in those samples where significant ($P < 0.05$) growth of pathogen populations was obtained (data not shown).

CONCLUSIONS

- > The antimicrobial activity of ϵ -PL in all six food extracts was better than that obtained in the laboratory broth culture medium.
- > Exposure of the pathogens to both tested levels of ϵ -PL in all food extracts generally led to lower ($P < 0.05$) populations by the end of storage compared to initial counts.
- > The antimicrobial activity of ϵ -PL was more pronounced in the food extracts that did not contain high protein levels (i.e., rice and vegetables).
- > Small increases in populations towards the end of storage in bologna extract and beef extract containing the low ϵ -PL concentration tested against each pathogen potentially indicated one or more components of these products may be responsible for loss of activity with extended storage; higher ϵ -PL levels, however, may be adequate for complete control of the high inoculum levels tested in these substrates.

REFERENCES

- FDA (Food and Drug Administration). 2004. Available from: <http://www.cfsan.fda.gov/~rdb/opa-g135.html>. Accessed 28 May 2007.
- Geornaras I, Sofos JN. 2005. J. Food Sci. 70:M404-8.
- Hiraki J. 2000. Fine Chem. 29:18-25.
- Hiraki J, Ichikawa T, Ninomiya S, Seki H, Uohama K, Seki H, Kimura S, Yanagimoto Y, Barnett JW Jr. 2003. Regul. Toxicol. Pharmacol. 37:328-40.
- Shih L, Shen MH, Van YT. 2006. Bioresour. Technol. 97:1148-59.
- Shima S, Sakai H. 1977. Agric. Biol. Chem. 41:1807-9.
- Shima S, Matsukawa H, Iwamoto T, Sakai H. 1984. J. Antibiot. 37:1449-55.
- Yoshida T, Nagasawa T. 2003. Appl. Microbiol. Biotechnol. 62:21-6.