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A Research Note

Use of Temperature-Sensitive Gel for the Concentration of *Escherichia Coli* in Milk

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ABSTRACT

Temperature-sensitive gels (cross-linked, partially hydrolyzed polyacrylamide gels) with the property to absorb solutes less than 1 nm in diameter at 4°C and collapse at higher temperatures (19°C) were used to concentrate *Escherichia coli* from 2% low-fat milk. Milk samples seeded with bacteria were reduced eight-fold in volume with average recoveries of 40-66% at an optimal pH of 5.5. Repeated use of the gel did not affect its efficiency.

Infection and disease have been associated with the consumption of milk for many years. Raw unpasteurized milk, both certified and uncertified, has been found to be contaminated with pathogenic bacteria such as *Salmonella, Listeria, Yersinia, Campylobacter,* and *Escherichia (1,5,6,10,11).* Pathogenic strains of *Yersinia enterocolitica* have been detected in pasteurized milk *(8).* Although large numbers of contaminating bacteria can be easily detected by routine procedures, small numbers of bacteria may elude detection and cause infection in consumers. The sensitivity of the standard methods could be greatly enhanced if the contaminating bacteria are first concentrated in milk samples prior to their being tested.

Temperature-sensitive gels (cross-linked, partially hydrolyzed polyacrylamide gels) have been previously used to concentrate solutions as much as 20 fold, the gels being selective in their absorption of solutes less than 1 nm in diameter and exclusive to solutes of greater than 3 nm in diameter *(2).* The size-selectivity is a function of cross-linkage which in turn is a function of pH, temperature, solvent concentration, and electric field *(3,4,9).* Roepke *et al. (7)* have used these gels to concentrate avian influenza virus from infected allantoic fluids with a 12-fold decrease in volume and an average recovery of 84%. Their unique property to swell at 4°C and collapse at higher temperatures (19°C and above) makes these gels ideal for concentration of bacteria and viruses from solutions. The present study was undertaken to determine if temperature-sensitive gels can be used to concentrate bacteria from milk.

MATERIALS AND METHODS

Bacteria and bacterial assay

Overnight cultures of *Escherichia coli* (ATCC-11303) were propagated in trypticase soy broth, centrifuged, washed, and suspended in phosphate buffered saline (PBS). This bacterial suspension was used to spike milk samples. Bacteria were enumerated by inoculating 0.1 ml of serial ten-fold dilutions on duplicate plates of trypticase soy agar by the spread plate method. After incubating the plates at 37°C overnight, bacterial colonies were counted.

Gel Production

The gel was produced by the previously described method *(2,7).*

Concentration procedure

Samples of 2% low fat milk (30-50 ml volumes) were seeded with 1 ml of a 1:10 dilution of *E. coli* suspension. After thorough mixing, 1 ml was removed for bacterial quantification. The pH was adjusted to desired levels followed by the addition of 2-4 g of gel to the mixture. It was then stored at 4°C for various time intervals, or until a ten-fold reduction in volume occurred. Milk was separated from gel by sieving through a Buchner funnel, the gel was washed with 1-2 ml of PBS, and the eluate was collected along with the milk. After noting the volume, the eluate was assayed for bacteria. Samples of milk containing bacteria, but not gel, were kept at 4°C and were assayed at 60-min intervals for 4 h.

Reuse of gel

The expanded gel, following concentration, was autoclaved at 121°C for 20 min to collapse and simultaneously sterilize the gel. The collapsed gel was washed continuously under running hot water to remove any milk proteins that had adhered to it.

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TABLE 1. *The effect of pH on concentration and recovery of* Escherichia coli *from milk*[a].

pH	Volume (ml)		Concentration factor[b]	Percent recovery[c]
	Initial	Final		
4.5	30	3.4	9	7
5.5	50	6.5	8	66
6.5	50	8.0	6	57
7.5	50	23.0	2	42

[a]Results are an average of 2-5 experiments.

[b]Concentration factor = $\frac{\text{Initial volume}}{\text{Final volume}}$

[c]Percent recovery = $\frac{\text{Final volume} \times \text{Final count}}{\text{Initial volume} \times \text{Initial count}} \times 100.$

TABLE 2. *The effect of amount of gel on concentration*[a,b].

pH	Amount of gel (g)	Time (h)	Volume (ml)		Concentration factor
			Initial	Final	
5.5	2	3:45	50	26.5	2
5.5	3	5:00	50	6.5	8
5.5	4	3:15	50	6.6	8

[a]Results are an average of 2-5 experiments.

[b]Concentration factor = $\frac{\text{Initial volume}}{\text{Final volume}}$

TABLE 3. *The effect of pH and amount of gel on concentration and recovery of* Escherichia coli *from milk*[a].

Amount of gel (g)	pH	Time (h)	Volume (ml)		Concentration factor[b]	Percent recovery[c]
			Initial	Final		
3	5.5	5:00	50	6.5	8	66
	6.5	5:00	50	8.0	6	57
	7.5	5:00	50	23.0	2	42
4	5.5	3:00	50	6.0	7	40
	6.0	3:00	50	5.0	10	35
	6.5	3:00	50	11.5	4	32

[a]Results are an average based on 2-5 experiments.

[b]Concentration factor = $\frac{\text{Initial volume}}{\text{Final volume}}$

[c]Percent recovery = $\frac{\text{Final volume} \times \text{Final count}}{\text{Initial volume} \times \text{Initial count}} \times 100.$

The washed gel was transferred to a glass petri-dish, dried in a 150°C oven overnight and was used in subsequent experiments.

RESULTS

Absorption of milk by gel was difficult at pH 4.5 because of curdling of milk. This resulted in low efficiency of bacterial recovery because elution was also not optimum. At pH 5.5 the consistency of milk was ideal for absorption by gel and resulted in high bacterial recovery (66%) (Tables 1 and 3). In subsequent trials, it was found that the concentration factor was directly proportional to the amount of gel added. Larger amounts of gel also resulted in time savings while yielding good concentration (Table 2). A pH of 7.5 resulted in a significantly lower concentration factor in spite of the long refrigeration time of 5 hrs (Table 3). The bacterial counts in control milk samples, kept at 4°C, did not increase or decrease significantly over the 4-h refrigeration time.

DISCUSSION

The results of this study indicate that temperature-sensitive gels can be used to concentrate *E. coli* from milk. We were able to achieve an 8-fold reduction in volume with recoveries ranging from 40-66% at a pH of 5.5. The procedure is simple and requires minimal equipment.

Roepke *et al (7)* have successfully used this gel to concentrate avian influenza virus from infected allantoic fluids with > 80% recoveries and an eleven-fold reduction in volume. Our recoveries were not as high with milk probably due to the viscous nature of milk. Roepke *et al (7)* achieved greatest recoveries of influenza virus from allantoic fluid after complete absorption of fluid by the gel prior to elution. Due to the condensed state of milk after "complete" absorption, elution invariably required a larger volume of PBS, resulting in an inefficient concentration procedure. On the other hand, by not allowing complete absorption, recoveries were lower due to the loss of some concentrate that tends to adhere to the gel. Since we were working with small volumes of milk, elution was achieved by gravity alone. We suspect, however, that some sort of pressure may be required with larger volumes of milk, which may also reduce time for elution, and may make the whole process more efficient by dislodging the concentrated milk and bacteria from the gel to yield better recoveries.

The gel was found to be reusable except that it had to be washed several times with hot water which was achieved by placing the funnel with gel under running hot tap water while constantly stirring with a glass rod. This step was very important, as failure to wash prior to drying in the oven resulted in the gel being caked with milk proteins, reducing the efficiency of absorption considerably.

Although this study employed *E. coli* only, the procedure may also be suitable for the concentration of other pathogens from milk, e.g. *Listeria* and *Campylobacter*. The only limiting factor is that the solutes be greater than 3 nm in diameter *(2)*. In addition, the gel can perhaps be used for other fluids as well. Thus, we have been able to apply this procedure for the concentration of viruses from urine (Maheshkumar *et al*, manuscript in preparation).

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REFERENCES

1. Bryan, F. L. 1983. Epidemiology of milk-borne diseases. J. Food Prot. 46:637-649.
2. Cussler, E. L., M. R. Stokar, and J. E. Vaarberg. 1984. Gels as size-selective extraction solvents. Am. Inst. Chem. Eng. J. 30:578-582.
3. Gehrke, S. H. 1985. Gels as selective extraction solvents. Ph.D. Thesis, University of Minnesota, Minneapolis, MN.
4. Ilavsky, M., J. Hrouz, and K. Ulbrich. 1982. Phase transition in swollen gels, III. The temperature collapse and mechanical behavior of poly (N,N-diethylacrylamide) networks in water. Polymer Bull. 7:107-110.
5. Kornacki, J. L., and E. H. Marth. 1982. Foodborne illness caused by *Escherichia coli*: a review. J. Food Prot. 45:1051-1067.
6. Marth, E. H. 1969. Salmonellae and salmonellosis associated with milk and milk products. A review. J. Dairy Sci. 52:283-315.
7. Roepke, D. C., S. M. Goyal, C. J. Kelleher, D. A. Halvorson, A. J. Abraham, R. F. Freitas, and E. L. Cussler. 1987. Use of temperature-sensitive gel for concentration of influenza virus from infected allantoic fluids. J. Virol. Method. 15:25-31.
8. Swaminathan, B., M. C. Harmon, and I. J. Mehlman. 1982. A review: *Yersinia enterocolitica*. J. Appl. Bacteriol. 52:151-183.
9. Tanaka, T. 1981. Gels. Sci. Amer. 1:124-138.
10. Vidon, D. M., and C. L. Delmas. 1981. Incidence of *Yersinia enterocolitica* in raw milk in Eastern France. Appl. Environ. Microbiol. 41:355-359.
11. Vogt, R. L., A. A. Little, C. M. Patton, T. J. Barrett, and L. A. Orciari. 1984. Serotyping and serology studies of campylobacteriosis associated with consumption of raw milk. J. Clin. Microbiol. 20:998-1000.