

Direct Isolation of *Yersinia enterocolitica* and *Yersinia pseudotuberculosis* from Meat

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Received 20 March 1985/Accepted 17 June 1985

Studies were done to determine the usefulness of dilute alkali (KOH) treatment of meat samples for direct isolation of *Yersinia enterocolitica* and *Yersinia pseudotuberculosis*, without enrichment. Virulent *Y. enterocolitica* and *Y. pseudotuberculosis* in pork contaminated with 10^2 , 10^3 , and 10^4 cells per g survived the direct KOH treatment and were never recovered by using KOH postenrichment treatment. From 6 (4.8%) of 125 samples of retail ground pork, four biotype 4 serotype O3 and one biotype 3B serotype O3 strains of *Y. enterocolitica* and one *Y. pseudotuberculosis* serotype 4b strain were recovered by using direct KOH treatment without enrichment. As these isolations were attained without using enrichment cultural procedures, they represent an important time-saving alternative to simplify and speed isolation of *Yersinia* spp. from meat.

Many virulent *Yersinia enterocolitica* and *Yersinia pseudotuberculosis* strains have been detected in mammals, especially pigs (3, 11). *Y. enterocolitica* and *Y. enterocolitica*-like organisms have been isolated from foods such as beef, pork, lamb, and fowl, but *Y. pseudotuberculosis* strains have not been isolated from such sources (6). Many researchers have used some form of cold enrichment in a phosphate buffer solution to recover *Y. enterocolitica* from meat (6). Because of the long period required for cold enrichment, efforts have been made to devise selective enrichment techniques in which preparations could be incubated for shorter times and at higher temperatures (1, 9). However, Lee et al. (5) pointed out that high levels of indigenous microorganisms could become overgrown in modified selenite broth and mask the presence of *Y. enterocolitica* in food. Hence, a postenrichment medium or treatment is needed for *Yersinia* selection. Aulisio et al. (1) described a technique to separate *Yersinia* strains from background bacteria present in food enrichments, in which a KOH postenrichment treatment was used. However, previous investigations of yersiniae in food have been performed by using products isolated from enrichment samples. In the present study, I attempted to isolate directly yersiniae from artificially contaminated pork samples and from retail ground beef, pork, and chicken by using a direct KOH treatment, without enrichment.

A total of five strains of *Yersinia* were used. Two strains of *Y. enterocolitica* (a biotype 4 serotype O3 strain and a biotype 2 serotype O5,27 strain) were isolated from pig feces, and a *Y. enterocolitica* biotype 2 serotype O9 strain, was isolated from human feces in Shimane Prefecture, Japan. The remaining organisms, a *Y. pseudotuberculosis* serotype 5a strain and a *Y. enterocolitica* biotype 1 serotype O8 strain were kindly provided by M. Tsubokura, Department of Veterinary Microbiology, Faculty of Agriculture, Tottori University, Tottori, Japan. Cultures were grown in Trypticase soy broth (BBL Microbiology Systems) at 25°C for 48 h. Viable cell counts were made by plating suitable dilutions onto Trypticase soy agar and incubating the preparations at 25°C for 48 h. Cefsulodin-irgasan-novobiocin (OxOID) and MacConkey (Nissui Pharmaceutical Co. Ltd.) agar plates were used to recover the *Yersinia* isolates. Potassium hydroxide pellets (Wako Pure Chemical Indus-

tries Ltd.) were weighed and dissolved in 0.5% NaCl to give a 0.4 M (0.72%) solution (4).

Ground pork was obtained from a local grocery store and examined the same day. Tenfold serial dilutions of the bacterial suspensions were made in sterile 0.85% NaCl; 0.1 ml of each bacterial dilution was added to 10 g of pork, and 0.1 ml of a 0.85% NaCl solution was added to 10 g of uncontaminated control pork. The contaminated and control pork preparations were mixed with a blender for 2 min. Portions (one loopful) of the resulting homogenates were streaked onto cefsulodin-irgasan-novobiocin and MacConkey agar plates. Then, 40-ml portions of a 0.067 M phosphate buffer solution (pH 7.6) were added to the contaminated and control pork preparations, and these samples were mixed with a blender for 2 min.; 0.5-ml portions of the resulting homogenates and of 10-fold dilutions of homogenates with the 0.85% NaCl solution were mixed with 0.5-ml portions of 0.72% KOH in 0.5% NaCl for 30 s. Portions (0.1 ml) of these mixtures were spread onto cefsulodin-irgasan-novobiocin and MacConkey agar plates at each concentration. Then, 50 ml of phosphate buffer solution was added to contaminated and control pork homogenates, and the homogenates were incubated at either 4 or 25°C. The samples were examined for recoverable *Yersinia* cells on days 1, 2, 7, and 14. Portions (0.2 ml) of enrichments were mixed with 0.8-ml portions of 0.4% KOH in 0.5% NaCl for 30 s and streaked onto cefsulodin-irgasan-novobiocin and MacConkey agar plates. The cefsulodin-irgasan-novobiocin agar plates were incubated at 32°C for 24 h, and the MacConkey agar plates were incubated at 25°C for 48 h. The numbers of yersiniae in the samples were calculated (in colony-forming units). *Yersinia* were identified as described previously (2).

The results obtained after direct KOH treatment and KOH postenrichment treatment of artificially contaminated and control ground pork samples are shown in Table 1. A *Y. enterocolitica* serotype O5,27 strain was recovered from pork samples contaminated with 10^3 and 10^4 cells per g without KOH treatment. The *Y. enterocolitica* serotype O3 strain in pork contaminated with 10 cells per g and all strains in pork contaminated with 10^2 , 10^3 , and 10^4 cells per g survived the KOH treatment. However, no strain was recovered from the enrichments at 4 or 25°C by using KOH postenrichment treatment.

TABLE 1. Recovery of *Y. enterocolitica* and *Y. pseudotuberculosis* from pork: comparison of saline and direct alkali treatment of fresh samples and alkali postenrichment treatment of enriched media incubated at 4 and 25°C^a

Species or culture	Serotype (biotype)	Initial inoculum (no. of cells per g of meat) ^b	Presence of <i>Yersinia</i> ^c					
			NaCl treatment (zero time)	Zero time	25°C		4°C	
					Day 1	Day 2	Day 7	Day 14
<i>Y. enterocolitica</i>	O3 (4)	10	- ^d	+	-	-	-	-
		10 ²	-	+(10 ²) ^e	-	-	-	-
		10 ³	-	+(10 ³)	-	-	-	-
	O5,27 (2)	10 ⁴	-	+(10 ⁴)	-	-	-	-
		10	-	-	-	-	-	-
		10 ²	-	+(10 ²)	-	-	-	-
	O9 (2)	10 ³	+	+(10 ³)	-	-	-	-
		10 ⁴	+	+(10 ⁴)	-	-	-	-
		10	-	-	-	-	-	-
	O8 (1)	10 ²	-	+(10)	-	-	-	-
		10 ³	-	+(10 ²)	-	-	-	-
		10 ⁴	-	+(10 ⁴)	-	-	-	-
<i>Y. pseudotuberculosis</i>	5a	10	-	-	-	-	-	-
		10 ²	-	+(10 ²)	-	-	-	-
		10 ³	-	+(10 ³)	-	-	-	-
		10 ⁴	-	+(10 ⁴)	-	-	-	-
Uncontaminated control			-	-	-	-	-	

^a Pork was homogenized to a concentration of 10% in phosphate buffer solution (pH 7.6).

^b The bacterial inocula were grown for 48 h in Trypticase soy broth at 25°C.

^c The percentages of recovery were as follows: 10% for the NaCl treatment at zero time; 70% for the KOH treatment at zero time; and 0% for all other treatments.

^d -, Organisms absent; +, organisms present.

^e The numbers in parentheses are the numbers of *Yersinia* cells recovered (in log₁₀ colony-forming units per gram).

A total of 120 samples each of ground beef, pork, and chicken were obtained from 10 local grocery stores between April 1984 and March 1985, and 5 pork samples were obtained from one local grocery store in April 1985. All samples were refrigerated and assayed within 4 h after purchase. A 25-ml portion of phosphate buffer solution was added to 25 g of ground beef, pork, or chicken, and then the samples were mixed with a blender for 2 min. The resulting homogenates were subjected to the same procedure used for recovery of *Yersinia* from artificially contaminated pork. From the beef, chicken, and pork samples obtained between April 1984 and March 1985, 1,793 *Yersinia* strains were isolated from 68 beef samples (56.7%), 66 pork samples (55.0%), and 70 chicken samples (58.3%). Virulent *Y. enterocolitica* strains were isolated from five pork samples obtained in December 1984 (two pork samples), February 1985 (one pork sample), and April 1985 (two pork samples), and these strains belonged to biotype 4 serotype O3 (four strains) and biotype 3B serotype O3 (one strain). *Y. pseudotuberculosis* serotype 4b was isolated from one pork sample obtained in April 1985. Only one *Y. enterocolitica* biotype 4 serotype O3 strain was isolated on day 7 after the cold enrichment cultural procedure. Only six isolates of *Y. enterocolitica* serotype O3 and *Y. pseudotuberculosis* serotype 4b were positive for autoagglutination and calcium dependency. Of 630 environmental *Yersinia* isolates from beef, 418 belonged to *Y. enterocolitica* biotype 1, 158 belonged to *Yersinia intermedia*, 38 belonged to *Yersinia frederiksenii*, and 16 belonged to *Yersinia kristensenii*. Of 584 environmental *Yersinia* isolates from pork, 337 belonged to *Y. enterocolitica* biotype 1, 185 belonged to *Y. intermedia*, 39 belonged to *Y. frederiksenii*, and 23 belonged to *Y. kristensenii*.

Of 558 environmental *Yersinia* isolates from chickens, 262 belonged to *Y. enterocolitica*, 238 belonged to *Y. intermedia*, 25 belonged to *Y. frederiksenii*, and 33 belonged to *Y. kristensenii*. The most common *Y. enterocolitica* biotype 1 strain was a serotype O13,7 strain, the second most common strain was a serotype O5 strain, and the third most common strain was a serotype O6 strain; other strains belonged to serotypes O8,19, O9, O11, O12, O14, O16, O18, O21, O22, and O34, and some were untypable. The *Y. intermedia* strains belonged to serotypes O4, O5, O6, O8,19, O10, O11, O14, O16, O17, O18, O21, O22, and O34, and some were untypable. The *Y. frederiksenii* strains belonged to serotypes O3, O4, O5, O6, O13,7, O14, O17, O18, O21, O22, and O34, and some were untypable. The *Y. kristensenii* strains belonged to serotypes O11, O12, and O16, and some were untypable.

The *Yersinia* strains in the artificially contaminated pork samples showed comparatively high resistance to KOH, and all *Yersinia* strains were recovered from the pork samples contaminated with more than 10² cells per g after direct KOH treatment, without enrichment. Aulisio et al. (1) reported that a KOH postenrichment treatment increased the yield of yersiniae fourfold and the sensitivity 100-fold, shortened the incubation period, and appreciably decreased the growth of non-*Yersinia* isolates from various foods. In this study, however, virulent *Yersinia* isolates in pork samples contaminated with less than 10⁴ cells per g were never recovered by using KOH postenrichment treatment. Thus, direct KOH treatment of meat samples proved to be a valuable rapid method for direct isolation of *Yersinia* from meat contaminated with more than 10² cells per g.

In this investigation, *Y. enterocolitica* serotype O3 and *Y.*

pseudotuberculosis serotype 4b strains were directly isolated from retail ground pork. This isolation of *Y. pseudotuberculosis* from retail meat was probably the first such isolation in the world. This finding suggests that there is a close relationship between harboring of virulent *Y. enterocolitica* and *Y. pseudotuberculosis* in pigs and contamination with these virulent *Yersinia* species in pork and that pork may be an important source of *Y. enterocolitica* and *Y. pseudotuberculosis* infection.

Recently, serotypes O5, O6, O13,7, and O21 of *Y. enterocolitica* biotype 1 were isolated from patients with yersiniosis in the United States and Canada (7, 8, 10). In this investigation, although the most common strain of *Yersinia* was a *Y. enterocolitica* biotype 1 serotype O13,7 strain, the second most common strain was a *Y. enterocolitica* biotype 1 serotype O5 strain, and the third most common strain was a *Y. enterocolitica* biotype 1 serotype O6 strain, all of these strains were avirulent strains that were negative for autoagglutination and calcium dependency.

I thank M. Ohara (Kyushu University) for reading the manuscript.

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