

CHAPTER 10

Isolation and Identification of *Yersinia enterocolitica*

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Introduction:

Yersinia enterocolitica is a particularly versatile food-borne pathogen which can survive in many habitats both within and external to host animals. Pathogenic *Yersinia enterocolitica* organisms are significant causes of human disease in many parts of the world including India. The association of human illness with consumption of *Y. enterocolitica* from contaminated food, animal wastes, and unchlorinated water is well reported. This organism may survive and grow during refrigerated storage. Because contamination is possible at the manufacturing site or in the home, refrigerated foods are potential vehicles.

Yersinia is a genus in the family of Enterobacteriaceae, which are psychrotrophic, oxidase negative, catalase positive and non-lactose fermenting bacilli. Species of these groups are differentiated based on biochemical traits, such as urease test, motility test and sugar fermentation reactions. The molar percentage of G+C content within the genus *Yersinia* is consistent with that of Enterobacteriaceae and ranges from 46 to 50 and is consistent with that for Enterobacteriaceae species. *Yersinia* are gram negative, facultatively anaerobic, non-spore forming straight rods or coccobacilli. They are often more active biochemically at 25°C than 37°C; most of them are motile when grown below 30°C with peritrichous flagella.

The genus *Yersinia* comprises eleven species, with *Y. enterocolitica*, *Y. pseudotuberculosis*, *Y. pestis*, *Y. rohdei*, *Y. ruckeri*, *Y. frederiksenii*, *Y. intermedia*, *Y. kristensenii*, *Y. mollaretii*, *Y. bercovieri*, and *Y. aldovae*. Among these, *Y. enterocolitica*, *Y. pseudotuberculosis*, *Y. pestis*, and *Y. ruckeri* are known pathogens for humans and animals. *Y. enterocolitica* and *Y. pseudotuberculosis* are enteropathogenic organisms distributed worldwide that share common modes of transmission mainly through food and water. *Y. pestis* is a causative agent of the bubonic plague. *Y. ruckeri* is considered as causative agent of red enteric mouth disease in Salmonids. The most prevalent symptoms of *Yersinia* infections are abdominal pain and fever. However, other gastro-intestinal disorders such as diarrhea, nausea, headache, and vomiting may also be associated with the illness. The incubation period is about 24-36 hours which goes to a periods of up to 11 days have been reported.

Isolation of *Y. enterocolitica*:

The following procedure may be used for isolation *Yersinia* from food, water, and environmental samples -

1. Aseptically weigh 25 g sample into 225 ml PSBB. Homogenize 30 s and incubate at 10°C for 10 days (Don't freeze the samples).
2. If high levels of *Yersinia* are suspected in product, 0.1 ml may be spread plated on CIN agar before incubating broth. Also transfer 1 ml homogenate to 9 ml 0.5% KOH in 0.5% saline, mix for 2-3 seconds, and spread-plate 0.1 ml on MacConkey and CIN agars.
3. Incubate agar plates at 30°C for 1-2 days.
4. On day 10, remove enrichment broth from incubator and mix well. Transfer one loop-full of enrichment to 0.1 ml 0.5% KOH in 0.5% saline and mix for 2-3 s. Successively streak one loopful to MacConkey plate and one loopful to CIN plate.
5. Transfer additional 0.1 ml enrichment to 1 ml 0.5% saline and mix 5-10 s before streaking, as above. Incubate agar plates at 30°C for 1-2 days.

Isolation of *Yersinia*

6. Examine CIN plates after 1 day incubation. Select small (1-2 mm diameter) colonies having deep red center with sharp border surrounded by clear colorless zone with entire edge.



Fig. : *Y. enterocolitica* on YSA (CIN) agar

7. Inoculate each selected colony into LAIA (Lysine arginine iron agar) slant, Christensen's urea agar slant, and bile esculin agar plate or slant by stabbing with inoculation needle. Incubate 48 h at RT. Isolates giving alkaline slant and acid butt, no gas and no H₂S (KA–) reaction in LAIA, which are also urease-positive, are presumptive *Yersinia*. Discard cultures that produce H₂S and/or any gas in LAIA or are urease-negative.

Table 1. Biochemical Characteristics^(a) of *Yersinia* species

Reaction	<i>Y. enterocolitica</i>	<i>Y. pseudo-tuberculosis</i>	<i>Y. pestis</i>	<i>Y. intermedia</i>
Lysine	-	-	-	-
Arginine	-	-	-	-
Ornithine	+	+ ^(c)	-	+
Motility at RT (22-26°C)	+	+	+	+
35-37°C	-	-	-	-
Urea	+	+	+	+
Mannitol	+	+	+	+
Sorbitol	+	+	-	+
Cellobiose	+	+	-	+
Adonitol	-	-	-	-
Simmons citrate	+/-	-	-	
Voges-Proskauer	+	+/-(+)	-	+/-
Indole	+	+/-	-	+
Salicin	+	+/-	+/-	+
Esculin	+	+/-	+	+
				+

Table 2. Biotype scheme for *Y. enterocolitica*

Biochemical test	Reaction for biotypes						
	1A	1B	2	3	4	5	6
Lipase	+	+	-	-	-	-	-
Esculin/salicin (24 h)	+/-	-	-	-	-	-	-
Indole	+	+	(+)	-	-	-	-
Xylose	+	+	+	+	-	V	+
Trehalose	+	+	+	+	+	-	+
Pyrazinamidase	+	-	-	-	-	-	+
β -D-Glucosidase	+	-	-	-	-	-	-

Biochemical test	Reaction for biotypes						
	1A	1B	2	3	4	5	6
Voges-Proskauer	+	+	+	+/-	+	(+)	-

Composition:

Peptone Sorbitol Bile Broth

Na ₂ HPO ₄	8.23 g
NaH ₂ PO ₄ · H ₂ O	1.2 g
Bile salts No. 3	1.5 g
NaCl	5 g
Sorbitol	10 g
Peptone	5 g
Distilled water	1 liter

Autoclave 15 min at 121°C. Final pH, 7.6 ± 0.2.

Cefsulodin-Irgasan Novobiocin (CIN) Agar or Yersinia Selective Agar (YSA)

- **Basal medium**

Special peptone	20 g
Yeast extract	2 g
Mannitol	20 g
Pyruvic acid (Na salt)	2 g
NaCl	1 g
MgSO ₄ ·7H ₂ O (10 mg/ml)	1 ml
Agar	12 g
Distilled water	756 ml

- **Irgasan (Ciba-Geigy) solution and Novobiocin solution**

Irgasan 0.40% in 95% ethanol	1 ml
Novobiocin, [0.25 mg/ml]	10 ml
Cefsulodin[1.5 mg/ml]	10 ml

- May be stored at -20°C up to 4 weeks.