

Chapter 21: *Yersinia enterocolitica*

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Potential Food Safety Hazard (Weagant et al., 1998) [Top](#)

Yersinia enterocolitica and bacteria that resemble it are ubiquitous, being isolated frequently from soil, water, animals, and a variety of foods. They comprise a biochemically heterogeneous group that can grow at refrigeration temperatures (a strong argument for use of cold enrichment). Based on their biochemical heterogeneity and DNA relatedness, members of this group were separated into four species: *Y. enterocolitica*, *Y. intermedia*, *Y. frederiksenii*, and *Y. kristensenii* (Bercovier et al., 1980). Through additional revisions, the genus *Yersinia* has grown to include eleven species (Aleksic et al., 1987; Bercovier, 1980; Bercovier et al., 1984; Wauters et al., 1988), three of which are potentially pathogenic to humans: *Y. pestis*, *Y. pseudotuberculosis*, and *Y. enterocolitica*. Of these, *Y. enterocolitica* is most important as a cause of food-borne illness.

Y. enterocolitica strains and related species can be separated serologically into groups based on their heat-stable somatic antigens. Wauters (Wauters, 1981) described 54 serogroups for *Y. enterocolitica* and related species. Aleksic and Bockemuhl (1984) proposed simplifying this to 18 serogroups within the *Y. enterocolitica* species. Presently, pathogenic strains belonging to serogroups O:1, 2a, 3; O:2a,3; O:3; O:8; O:9; O:4,32; O:5,27; O:12,25; O:13a,13b; O:19; O:20; and O:21 have been identified. Therefore, pathogenic strains can belong to diverse serogroups. Serogroups that predominate in human illness are O:3, O:8, O:9, and O:5,27.

The association of human illness with consumption of *Y. enterocolitica*-contaminated food, animal wastes, and unchlorinated water is well documented (Aulisio et al., 1982; Aulisio, 1983). Refrigerated foods are potential vehicles because contamination is possible at the manufacturing site (Aulisio, 1982) or in the home (Aulisio, 1983). This organism may survive and grow during refrigerated storage.

A number of virulence tests have been proposed to distinguish potentially pathogenic *Y. enterocolitica*. Some strains of *Y. enterocolitica* and related species produce an in vitro heat-stable enterotoxin (ST) that can be detected by intragastric injection of cultural filtrates in suckling mice and is very similar to *Escherichia coli* ST (Boyce et al., 1979). However, *Yersinia* spp. produce ST only at temperatures below 30°C. Many environmental strains of *Yersinia* produce this protein, whereas some otherwise fully virulent strains of *Y. enterocolitica* do not. The role of ST in the disease process of *Yersinia* remains uncertain.

Yersinia spp. that cause human yersiniosis carry a plasmid (41-48 Mdal) (Gemski et al., 1980; Kay et al., 1982; Zink et al., 1980) that is associated with a number of traits related to virulence: autoagglutination in certain media at 35-37°C (Aulisio et al., 1983; Laird and Cavanaugh, 1980); inhibition of growth in calcium-deficient media (Gemski et al., 1980) and binding of crystal violet dye (Bhaduri et al., 1987) at 35-37°C; increased resistance to normal human sera (Pai and DeStephano, 1982); production of a series of outer membrane proteins at 35-37°C (Portnoy et al., 1981); ability to produce conjunctivitis in guinea pig or mouse (Sereny test) (Sereny, 1955; Zink et al., 1980); and lethality in adult and suckling mice by intraperitoneal (i.p.) injection of live organisms (Aulisio et al., 1983; Carter and Collins, 1974; Prpic et al., 1985; Robins-Brown and Prpic, 1985). The plasmid associated with virulence can be detected by gel electrophoresis or DNA colony ! hybridization (Hill et al., 1983). Recent evidence, however, indicates that presence of plasmid alone is not sufficient for the full expression of virulence in *Yersinia* (Heesemann et al., 1984; Portnoy and Martinez, 1985; Schiemann, 1989). The intensity of some plasmid-mediated virulence properties such as mouse lethality and conjunctivitis is variable, depending on the genes carried on the bacterial chromosome (Pai and DeStephano, 1982; Pierson and Falkow, 1990; Portnoy et al., 1981; Robins-Brown et al., 1989) and the serogroup, suggesting that chromosomal genes also contribute to *Yersinia* virulence.

Virulent strains of *Yersinia* invade mammalian cells such as HeLa cells in tissue culture (Lee et al., 1977). However, strains that have lost other virulent properties retain HeLa invasiveness, because the invasive phenotype for mammalian cells is encoded by chromosomal loci. Two chromosomal genes of *Y. enterocolitica*, *inv* and *ail*, which encode the phenotype for mammalian cell invasion, have been identified (Miller and Falkow, 1988; Miller et al., 1989). Transfer of these genetic loci into *E. coli* confers the invasive phenotype to the *E. coli* host (Miller and Falkow, 1988). The *inv* gene allows high level *Yersinia* invasion of several tissue culture cell lines (Miller and Falkow, 1988). However, Southern blot analyses show that *inv* gene sequences are present on both tissue culture invasive and noninvasive isolates (Miller et al., 1989; Robins-Brown et al., 1989). Although this suggests that the *inv* gene in *Y. enterocolitica* may not be directly correlated with invasiveness, genetic evidence shows that *inv* genes are nonfunctional in the noninvasive isolates (Pierson and Falkow, 1990). The *ail* gene shows greater host specificity with regard to cell invasion and appears to be present only on pathogenic *Yersinia*. In disease-causing strains, all virulent *Y. enterocolitica* isolates were shown to be tissue culture-invasive and to carry the *ail* gene (Miller and Falkow, 1988; Portnoy et al., 1981). The *ail* locus, therefore, may be an essential chromosomal virulence factor in *Y. enterocolitica* (Miller et al., 1989; Robins-Brown et al., 1989).

Y. pseudotuberculosis is less ubiquitous than *Y. enterocolitica*, and although frequently associated with animals, has only rarely been isolated from soil, water, and foods (Fukushima et

al., 1989; Tsubokura et al., 1989). Among *Y. pseudotuberculosis* strains there is little or no variation in biochemical reactions, except with the sugars melibiose, raffinose, and salicin. Serologically (based on a heat-stable somatic antigen), the *Y. pseudotuberculosis* strains are classified into six groups, each serogroup containing pathogenic strains. Gemski et al. (1980) reported that serogroup III strains harbor a 42-Mdal plasmid as do serogroup II strains that are lethal to adult mice. The association of yersiniosis in humans with the presence of a 42-Mdal plasmid in *Y. pseudotuberculosis* has been established (Schiemann and Wauters, 1992).

Virulence genes present on the chromosome of *Y. pseudotuberculosis* have also been identified (Isberg et al., 1987; Isberg and Falkow, 1985). The *inv* gene of *Y. pseudotuberculosis* is homologous with that of *Y. enterocolitica*, and encodes for an invasion factor for mammalian cells. Transfer of *inv* gene into *E. coli* K-12 resulted in the expression of the invasive phenotype in *E. coli* (Isberg and Falkow, 1985). The *inv* gene is thermoregulated (Isberg et al., 1988; Isberg, 1989); it encodes for a 103 Kdal protein, invasins, which binds to specific receptors on mammalian cells and facilitates the entry of *Y. pseudotuberculosis* into tissue (Isberg and Leong, 1988). Tests for *Y. pseudotuberculosis* virulence are not as abundant as those for *Y. enterocolitica*; however, tissue cell-invasive and plasmid-carrying isolates of *Y. pseudotuberculosis* may be identified by DNA colony hybridization.

Control Measures

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Hazards from *Y. enterocolitica* can be prevented by: heating seafood sufficiently to kill the bacteria, holding chilled seafoods below 4.4°C (40°F) and preventing post-cooking cross-contamination (Ward et al., 1997).

FDA Guidelines

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FDA to assess situations on a case by case basis.

Growth

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[Limiting conditions for *Y. enterocolitica* growth.](#)

Heat Resistance

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Table 21-2. Heat resistance of *Y. enterocolitica*.

Temp.		D-Value	Medium	Reference
(°C)	(°F)	(min.)		
62.8	145	0.96	Milk	Lovett et al., 1982

Analytical Procedures

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Commercial Test Products

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Table 21-3. Commercial test products for *Yersinia* spp.

Test	Analytical Technique	Approx. Total Test Time	Supplier
Yersinia Test [A presumptive test for <i>Yersinia</i> spp.]	Uses prepared traditional media	5 days	Biomedix Contact: Claver Bundac 1105 #F North Golden Springs Dr. Diamond Bar, CA 91765 Phone: 800/674-8648 #4282; 909/396-0244 E-mail: cb4biomedx@aol.com

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