Yersinia enterocolitica

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Genes involved in Cold-Adaptation

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genen betrokken bij aanpassing aan lage temperatuur

(met een samenvatting in het Nederlands)

PROEFSCHRIFT

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The Road goes ever on and on
down from the door where it began.
Now far ahead the Road has gone
and I must follow, if I can.
Pursuing it with weary feet
untill it joins some larger way,
where many paths and errands meet.
And whither then? I cannot say...

(Bilbo and Frodo Balings, hobbits,
in: 'The Lord of the Rings',
J.R.R. Tolkien)

Opgedragen aan
mijn vader
(† 07-09-1992)

CONTENTS

PREFACE

Scope of the thesis

This thesis concerns the bacterium *Yersinia enterocolitica*, with special attention given to the molecular regulation of its ability to grow and multiplicate at temperatures around 0°C.

Y. enterocolitica is an important human entero-invasive pathogen with a global distribution [Bottone 1977, 1997; Ostroff 1995]. In contrast to other common bacterial enteropathogens, such as *Salmonella, Campylobacter* and *Shigella* species, which cease to grow below circa 8°C, *Y. enterocolitica* is able to grow near 0°C [Stern & Pierson 1979] and even at sub-zero temperatures [Bergann *et al.* 1995]. On account of this cold-tolerance, it has been frequently suggested that the ever increasing application of refrigeration in food storage plays a central role in the continuing expansion of *Y. enterocolitica* as a human pathogen [Christensen 1987; Mollaret 1995]. It could plausibly be argued that this cold-tolerant organism has taken advantage of the forthcoming of a new niche, mindful of Baas Becking's conclusion that "... *life is everywhere, but the environment determines its manifestation.*" [Baas Becking 1927]. Obviously, on the other hand, a causal connection is difficult to prove. Nevertheless, the fact that this hypothesis has never been scientifically documented makes it hard to rate the significance of bacterial cold-tolerance as an indirect threat to human health at its true value. Gaining insight into the suggested relationship requires a closer look at both the history of this bacterium and the milestones in the development of the 'Cold Chain' of food preservation.

Assuming that the ability of *Y. enterocolitica* to proliferate at 5°C or less, either in foods, water or other products, such as blood, truly constitutes a specific hazard for human health, the questions arise whether and how its growth at low temperature could possibly be suppressed. Answering this question, however, requires fundamental knowledge about the specific metabolic factors which enable this organism to multiply at refrigeration temperatures. Unfortunately, although the behaviour and physiology of *Y. enterocolitica* at low temperatures have been investigated extensively, only very few studies addressed the underlying mechanisms. In the present study an attempt is made to determine the vital link(s) in the metabolism at low temperatures, as a first step in elucidating how *Y. enterocolitica* copes with cold.

Outline of the thesis

Chapter 1 is devoted to *Y. enterocolitica* and describes its history and characteristics, as well as its importance as a human pathogen. In Chapter 2, the evolution of chilling into an indispensable factor of modern food preservation is delineated, including the rise of cold-loving ('psychrophilic') and cold-tolerant ('psychrotrophic') organisms in food spoilage and human disease. In Chapter 3, the present knowledge about the molecular aspects of bacterial adaptation to low temperature is reviewed. After these literature-based introductions to the bacterium under study, the development of the Cold Chain, and the phenomenon of microbial cold-adaptation, the Chapters 4 to 7 are based on experimental work with *Y. enterocolitica* and related species. In order to contribute to improved estimations of the prevalence and significance of *Y. enterocolitica*, a rapid and reliable detection method has been developed, which is described in Chapter 4. The central theme in the Chapters 5 and 6 is the identification of factors involved in cold-tolerance, as accomplished by mapping of specific phenotypic adaptations and by analysis of mutations that lead to disruption of the psychrotrophic phenotype. By the latter approach, it was deduced that expression of the *pnp*-gene, encoding polynucleotide phosphorylase (PNPase), is necessary for the ability of *Y. enterocolitica* to grow at low temperatures. Chapter 7 addresses the regulation of *pnp*-expression in *Y. enterocolitica* and other *Yersinia* species. Finally, an evaluation of the results described in the preceding chapters, and a discussion of the possible role of PNPase in the mechanisms that might enable growth at low temperature is the topic of Chapter 8.

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1

Yersinia enterocolitica

a versatile human enteric pathogen

ABSTRACT

Y. enterocolitica **is a Gram-negative bacterium which belongs to the family of the Enterobacteriaceae. Within this species, several bio-/serotypes are distinguished which are pathogenic to humans. Infection with virulent *Y. enterocolitica* mainly causes an acute gastroenteritis, called 'yersiniosis', which in many cases is due to consumption of contaminated foods. The pathogenesis of the bacterium is strongly associated with its enormous capacity to adapt to varying environmental conditions. This versatility enables the organism to multiply at temperatures near zero (e.g. in refrigerated foods), but also to switch over to a life at 37°C (e.g. in a warm-blooded host). This switch includes the expression of 'invasion factors' (to enable entrance of the host's tissues), and the production of serum resistance- and anti-phagocytosis factors (to restrain the host's immune response). In so doing, the bacterium can avoid the host's defense mechanisms, and may easily spread from the intestinal tract to other organs, which leads to a wide spectrum of serious post-infective extra-intestinal diseases and long-term sequela.**

Y. enterocolitica, **although first isolated in North America in the 1930s, emerged as an important enteric pathogen in much of the industrialized world in the 1970s and 1980s. There is a considerable geographic variation in the incidence of yersiniosis, ranging from < 0.0001% in low-incidence countries to ≥ 0.01% in high-incidence regions (<1 to ≥100 cases/year/ 1 million inhabitants).**

The gastrointestinal tract of swine is the main natural habitat of virulent strains. The improvement of slaughtering procedures (i.e. to prevent the contamination of carcasses), and the ameloriation of household hygiene (i.e. to repel the consumption of raw pork and the risk of cross-contaminations in the kitchen) have lead to a decline of yersiniosis, especially in the high-incidence countries, in the 1990s.

In addition to contracting yersinosis via the oral route, infection with the bacterium may also occur after transfusion with contaminated blood products, which leads in most cases to a life-threatening septicaemia. Transfusion-associated infections are not seldomly caused by blood products which had been obtained from asymptomatically infected donors, and which had been stored at 4°C for several weeks prior to the transfusion. Features like its psychrotrophic character, its ability to invade eukaryotic cells, its resistance to intracellular killing at low temperatures, and its capacity to benefit from iron enrichment due to aging erythrocytes, all contribute to proliferation of the bacterium under these conditions.

In conclusion, reduction of the contamination level of raw materials and subsequent chilling is not enough to restrain the cold-adapting pathogen *Y. enterocolitica*, neither in foods nor in blood products. Hence, additional methods are required to prevent the organism from unfolding its psychrotrophic character during storage at low temperature.

Yersiniosis: a foodborne disease

In september 1976, more than two hundred children at five schools in a restricted area of the USA fell ill with symptoms of abdominal pain, fever and diarrhoea, whereupon dozens of them were hospitalized and appendictomized upon suspicion of acute appendicitis. In the same year, two outbreaks of gastric infection with identical symptoms, involving ca. 150 schoolchildren, were reported in Canada. In 1981, over two hundred members of a summer camp population in the USA fell victim to the gastrointestinal symptoms described above, and the next year two outbreaks of the same kind affected more than a thousand people, spread over several states in the USA. Between 1972 and 1984, ten explosive outbreaks of a similar illness, each affecting several hundreds up to over a thousand children of rural primary schools and junior-highschools, occurred in Japan. Outbreaks of the same kind had previously been reported from nursery schools in Chezchoslovakia and, at a smaller scale, in families in Hungary and the USA, and in hospitals in Finland. All of these cases of human gastric infection had in common the fact that the causative agent identified was not one of the thus far commonly found enteropathogenic bacteria, but another species, called *Yersinia enterocolitica*. The disease was therefore called yersiniosis. An important resemblance in these outbreaks of yersiniosis was the presumed or proven implication of contaminated food as the source of infection.

1.1 HISTORY

1930s: First appearance

The organism presently known as *Yersinia enterocolitica* can be labelled as a 'recent' organism: its written history dates back only to 1934. In that year, a bacterium isolated from the facial ulcers of an American farm dweller was described that could not be identified as any

species known at that time, although its morphology and certain biochemical characteristics indicated "... *a similarity to the Pasteurella genus.*" [McIver & Pike 1934]. The isolate fell into oblivion for a couple of years, but attracted renewed interest in 1939, when at the New York State Department of Health three look-alike bacterial cultures were received that had been isolated from patients with life-threatening intestinal infections [Schleifstein & Coleman 1939]. The New York investigators thought these organisms, including a fifth identical culture from the NYSDH-collection (that had been isolated as early as 1923 from a chronic skin lesion in a carpet worker), particularly resembled *Pasteurella pseudotuberculosis*, a microbial species that had been known since the end of the last century to cause serious disease in animals. In the following years, annual reports of the New York State Health Department contained sporadic notes of the isolation of bacteria similar to those described in 1939 [Gilbert 1940]. Since these isolates mostly originated from children suffering from enteritis, the yet unclassified organism was temporarily called *Bacterium enterocoliticum* [Schleifstein & Coleman 1943].

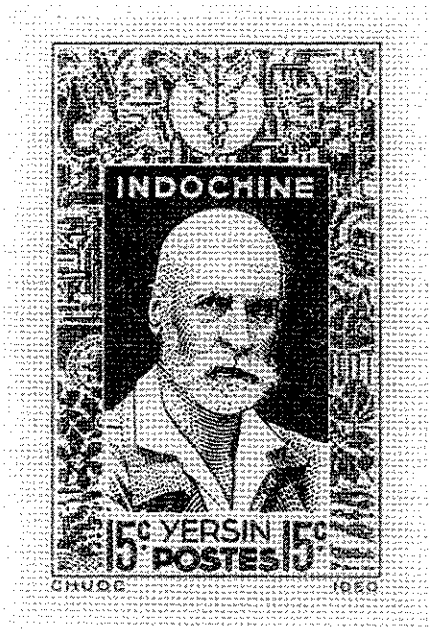
1940s - 1960s: Secluded existence

Little was heard about *B. enterocoliticum* for about twenty years, and from 1957 to 1968 complete silence surrounded the organism in the USA, when no cases of human infection due to it were reported. In the meantime, however, bacteria 'resembling *P. pseudotuberculosis*' were also recognized to be involved in human illness in Europe. The isolation of two such strains from patients who had died of septicemia was reported in Switzerland in 1949, whereupon the species was temporarily designated *P. pseudotuberculosis* ssp. *rodentium* [von Hässig *et al.* 1949].

1960s: Coming out again

In the early 1960's, the bacterium was recognized as an animal pathogen, when *P. pseudotuberculosis*-like bacteria were reported to be causative agents in enzootics among various wild and captive animals, including chinchillas in Europe and North- and Central America, hare in Europe, and pigs in North Africa [Akkermans & Terpstra 1963; Daniëls & Goudzwaard 1963; Mollaret 1964; reviewed by Hurvell in 1981]. At approximately the same time, the organism, then referred to as *Germe X* or *P. pseudotuberculosis X* or *type B*, was again shown to be involved in human infections, but now in Sweden, France, and Belgium [Carlsson *et al.* 1964; Mollaret & Destombes 1964; Winblad *et al.* 1966; Vandepitte *et al.* 1973]. In addition, various animal hosts, such as deer and pigs, frequently appeared to be healthy carriers of the bacterium [Dickinson & Mocquot 1961; Wetzler & Hubbert 1968].

From then onwards, this intriguing organism became the focal point of intensive investigations. In 1963/64, the similarity was established between the American and European strains of both animal and human origin bearing the aforementioned epithets [Knapp & Thal 1963], and it was then proposed to define this organism as a new species in the genus *Yersinia*, which in 1944 had been split off from *Pasteurella* [Frederiksen 1964]. The name *Yersinia* for this new genus had



previously been chosen to honour the French bacteriologist Dr. Alexandre Jean Emile Yersin who, in 1894, first isolated the infamous plague bacillus, which is now known as *Yersinia pestis* [Butler 1983; Solomon 1995]. The suffix *enterocolitica* refers to the organism's most frequent habitat in cases of human disease: the intestine and the colon.

Alexandre Yersin (1863-1943), shown on a stamp, issued in 1944 in Indochine. Even today, Yersin is a legendary figure in this region, the present Vietnam. He is still greatly hounoured, not only for his work in beating the plague but also for his many other contributions to improve the welfare of the Vietnamese people, including the introduction of the rubber tree (*Hevea brasiliensis*) and the quinine tree (*Cinchona leidgeriana*) in this part of the world.

1970s - 1990s: Expansion and establishment

Whereas by 1965 less then 30 cases had been reported world-wide [Weir 1985], over 600 reports appeared on the association of *Y. enterocolitica* with human disease during the second half of the 1960s [Morris & Feeley 1976]. Simultaneously, a rapid expansion of the geographical distribution of the bacterium was seen: it was rediscovered in the USA in 1968 [Sonnenwirth 1968], and at approximately the same time reported from the Netherlands [Wulf *et al.* 1969], Canada [Albert & Lafleur 1971], South Africa [Rabson & Koornhof 1973] and Japan [Zen-Yoji & Maruyama 1972]. Within a decade, the collection of the International Reference Centre at the Pasteur Institute in Paris comprised more then 6,600 strains covering 35 countries on six continents [Mollaret *et al.* 1979]. In the 1970s and 1980s, yersiniosis evolved into a serious threat to human health, when it was implicated in recurrent outbreaks of foodborne disease in Japan [Zen-Yoji 1981] and North America [Shayegani & Parsons 1987], and gained endemic character in north western Europe and some Asian regions [WHO 1981; Markov *et al.* 1989; Dmitrovsky *et al.* 1998]. To some extent, the steady increase in reported isolates of *Y. enterocolitica* obviously reflects the growing interest of microbiologists in this species. However, worldwide surveillance data show an explosion in the number of reported non-outbreak isolates and cases of yersiniosis in the last two decades. Obviously, the increased investigative activity can only marginally account for it, and this notice inclined several authors to refer to *Y. enterocolitica* as a worldwide emerging enteric human pathogen [Cover & Aber 1989; McCarthy & Fenwick 1990; Lee *et al.* 1991; Ostroff 1995; Tauxe 1997].

1.2 CLASSIFICATION

1.2.1 Characteristics

Yersinia enterocolitica is a Gram-negative, non-sporeforming, facultatively anaerobic rod of 1.3-3.5 x 0.5-1.0 μm in size. Based on morphological and overall biochemical characteristics, this bacterium belongs to the family *Enterobacteriaceae* [Bercovier & Mollaret 1984]. The optimum growth temperature of *Y. enterocolitica* is about 28°C, but the organism is able to multiply at 40°C, as well as at temperatures around zero [García de Fernando *et al.* 1995; Greer *et al.* 1995; Bergann *et al.* 1995; Miller *et al.* 1997]. The ability to grow at refrigerator temperatures is a feature shared with all the other members of the genus, including *Y. pseudotuberculosis* and the ill famed *Y. pestis* [Bercovier & Mollaret 1984; Gray 1995]. *Y. enterocolitica* is motile by means of several flagellae when grown in cultures at 30°C or less, but non-motile when grown at 35-37°C (Figure 1.1). Many other phenotypic characteristics, such as lipopolysaccharide (LPS) composition and virulence determinants like enterotoxin production and synthesis of secreted proteins (the so-called Yops), are temperature-dependent [Straley & Perry 1995].

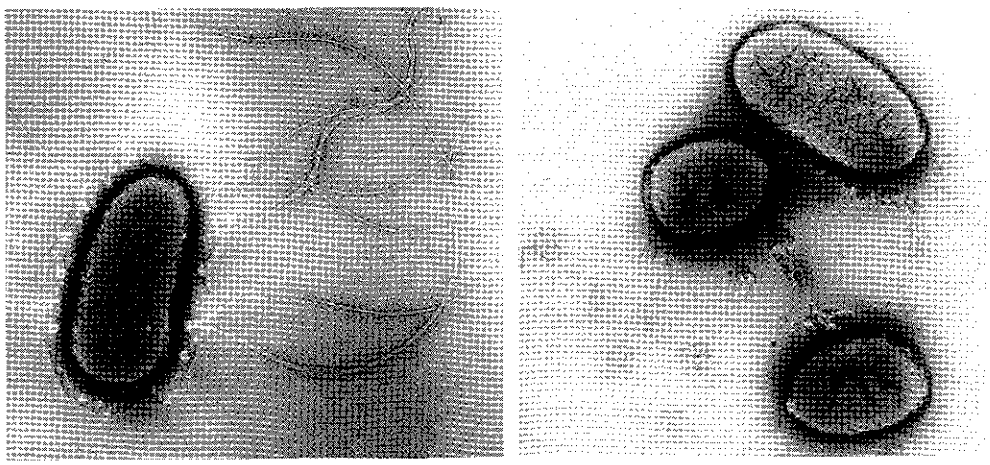


Figure 1.1 *Yersinia enterocolitica.* On the leftside: cells grown at 5°C; rightside: cells grown at 37°C

1.2.2 Related Species

Because the biochemical criteria initially proposed for the species *Y. enterocolitica* incorporated a rather heterogeneous group of bacteria of human and animal origin, several bio- and serotyping schemes have been developed to sub-group these isolates [Winblad 1967; Niléhn 1969; Wauters 1970; Knapp & Thal 1973]. Classification by these methods soon revealed certain

relationships between biotypes, ecological distribution and virulence [Alonso *et al.* 1976; Mollaret 1976; Mollaret *et al.* 1979]. Moreover, taxonomic studies applying DNA hybridization techniques elucidated differences in DNA-relatedness between typical *Y. enterocolitica* strains and those which were aberrant in phenotypic characteristics to such an extent that they had been referred to as '*Y. enterocolitica*-like' [Bercovier *et al.* 1980a; Brenner 1979; Brenner *et al.* 1976 1980a]. Hence, several groups of strains were reclassified as separate species and renamed *Y. intermedia* [Brenner *et al.* 1980b, *Y. kristensenii* [Bercovier *et al.* 1980], or *Y. frederiksenii* [Ursing *et al.* 1980]. The latter names were chosen in honour of the Danish microbiologists Kristensen and Frederiksen, who played important roles in the unraveling of the relationships of Yersinia-like organisms. In 1935, Dr. Martin Kristensen published a large study on so-called 'Paracolibacilli', originating from human faeces or urine [Kristensen *et al.* 1935], and in the 1960s one of these isolates was recognized as a Yersinia by Dr. Wilhelm Frederiksen. This strain was later chosen as the type strain for the newly defined species *Y. frederiksenii.*

When the relationships between the various strains became further unravelled, several other species were newly defined and - with reference to some other famous players in the continuing story of *Yersinia* - designated *Y. aldovae* [Bercovier *et al.* 1984], *Y. rohdei* [Aleksić *et al.* 1987], *Y. mollaretii* or *Y. bercovierii* [Wauters *et al.* 1988a]. Initially, these close relatives of *Y. enterocolitica* were very rarely associated with yersiniosis. However, their innocence is questionable, since several atypical cases of yersiniosis due to these strains have been described, more recently [Lewis & Chattopadhyay 1986; Cafferkey *et al.* 1993; Necrasova *et al.* 1998b].

1.2.3 Bio- and Serotypes

Despite the split off of the aforementioned related species, the bacteria currently classified as *Y. enterocolitica* by no means constitute a homogeneous group: yet several biotypes, based on their biochemical profiles, and a still increasing number of over 60 different serotypes, based on their somatic (O) and flagellar (H) antigens, can be distinguished [Wauters 1981; Wauters *et al.* 1991; Fenwick *et al.* 1996]. The situation is further complicated by the fact the H-antigens are species-specific, while the O-antigens are not [Aleksić 1995].

Although *Y. enterocolitica* was first recognized in relation to human illness, it soon appeared to be ubiquitous in nature: the bacterium has now been isolated from many vertebrate wild and domestic animals, from a variety of terrestrial and freshwater ecosystems, from drinking water and from raw and prepared food products [Mollaret *et al.* 1979]. As the number of isolates increased, a striking dichotomy was seen, with on the one side acknowledged pathogens and on the other side a range of so-called environmental strains. A fair correlation appeared to exist between biogroups, antigenic patterns and ecologic behaviour. Virulence was associated with only a dozen bio/-serotypes, whereas the vast majority of strains recovered from environmental sources were either non-typeable or serotypes which have never been implicated in human infections (Table 1.1, adapted from [Aleksić & Bockemühl 1990]).

Table 1.1 *Yersinia enterocolitica* bio- and serotypes which are regularly involved in human disease: ecological & geographical distribution and recognized transmission vehicles.

STRAIN TYPE		DISTRIBUTION		TRANSMISSION	
Bio type	**Sero-type**	**Ecological spread (main hosts)**	**Geographical spread (main areas)**	**Source / Vehicle in outbreaks or trans-fusion-acquired cases of yersiniosis**	**References**
1B	O:4	environment	USA/Canada, India	well-water? humans	*142* *417*
	O:8	pigs, dogs, rodents	worldwide (before 1980 only in the USA)	milk (-products) pre-cooked meat pork processing-water surface-water humans, pets	*77, 369, 376* *369* *220* *11, 59* *241* *191*
	O:13a,b	monkeys, environment	USA Europe	milk? well-water	*396* *261*
	O:18	environment	USA	well-water? milk?	*142* *396*
	O:20	dogs, rats	USA	pets blood	*435* *404*
	O:21	environment	USA/Canada	surface-water? milk? humans?	*276*
2	O:9	pigs, dogs, cats, rodents	Europe, Japan, Australia	pork humans, pets blood citrus-fruit	*342* *33, 417* *12, 404* *273*
	O:5,27	pigs	worldwide	pork, milk blood	*240* *404*
3	O:5,27	pigs	Europe, Asia	citrus-fruit	*273*
	O:1,2,3	chinchilla, pigs,	worldwide	humans? blood	*198,349* *404*
4	O:3	pigs, dogs, cats, rodents	worldwide (not in the USA before 1980)	pork, milk? processing-water well-water pets blood	*45, 262,263,401* *26* *124* *33* *12, 288,253,402*

In addition to the specific ecological spread of the distinct bio/serotypes, a certain geographical distribution was initially also manifest. One group of strains, i.e. the biotype 1B strains, comprising the serotypes O:4, O:8, O:13a/b, O:18, O:20 and O:21, were mainly isolated in the USA [Wilson *et al* 1976; Eden *et al.* 1977; Martin *et al.* 1982; Black *et al.* 1978; Shayegani *et al.* 1983; Tacket *et al.* 1984, 1985] and these were therefore referred to as 'American strains'.
On the other hand, the strains that were the most common causes of yersiniosis in Europe and Japan, i.e. serotypes O:3 and O:9, were virtually unknown from America. Only one pathogenic serotype, i.e. O:5,27, seemed to have a global spread from the very beginning. Since the early 1980s, however, the distinction between 'American' and 'non-American' strains no longer applies as a result of worldwide serogroup shifts, involving an increase in the proportion of formerly rare serotypes and a concomitant decline of others [WHO 1981; Bottone 1983; Neogi *et al.* 1985; Hoogkamp-Korstanje *et al.* 1986; Bottone *et al.* 1987; Lee *et al.* 1990]; and [Chiesa *et al.* 1991; Ichinohe *et al.* 1991; Prentice *et al.* 1991; Kontiainen *et al.* 1994; Stolk-Engelaar & Hoogkamp-Korstanje 1996][1].
Furthermore, evidence has been growing in the last decades that classification by bio- or serotyping may not always predict pathogenicity: whereas formerly only biotypes 1B, 2, 3, 4 and 5 were thought to be indicative for virulence, several sub-groups of biotype 1A have by now also been shown to be involved in human disease [Noble *et al.* 1987; Bissett *et al.* 1990; Greenwood & Hooper 1990; Burnens *et al.* 1996], especially among young children [Glenn Morris *et al.* 1991] and immuno-compromised persons [Sulakvelidze *et al.* 1998].

[1] In the first decades of *Y. enterocolitica* research, the strains which were pathogenic for humans could be divided in 'American' and 'non-American' strains, based on their restricted geographical distribution. Since the onset of the 1980s, however, the formerly uneven spread of certain serotypes has gradually flattened out. Serotype O:3, for example, which was rarely isolated in America until 1983, has since then increasingly been recovered from sporadic cases of yersiniosis in the USA. In 1990, this serotype was for the first time reported to be involved in an outbreak, and nowadays O:3 predominates in North America, whereas most of the serotypes implicated in early outbreaks are rarely seen.The reverse movement was seen with the 'American' serotype O:8. Such strains were initially unknown outside North America, but around 1985, isolations from human patients started to be reported from Asia and Europe. In fact, the first recorded case of yersiniosis in Bangladesh, which occurred in 1984, was due to infection with an O:8 strain. Although serotype O:8 is still very rare in Belgium and Scandinavia, it now forms approximately 4% of the recorded *Y. enterocolitica* isolations in the Netherlands and the United Kingdom. In addition to the arrival of the formerly absent serotype O:8, the initially uneven distribution of serotype O:9 over Europe gradually flattened out. In the British Isles, for example, serovar O:9 was unknown until 1980, but today, over 50% of the pathogenic strains isolated from human faeces belong to this serotype. Inversely, the proportion O:9 in Finland changed from 41% in 1974 to 1% in 1994, and this serotype is still extremely rare in Denmark.

1.3 CLINICAL MANIFESTATION

1.3.1 Gastrointestinal Syndromes

The clinical spectrum of *Y. enterocolitica* infections varies with age and underlying conditions [Bottone 1997]. The most common presentation of an orally acquired infection is a diarrhoeal disease, associated with low grade fever and abdominal pain, lasting for a few days to several weeks. This type of - usually mild - gastroenteritis is particularly found in infants and young children and is normally self-limiting. The symptoms can even be so faint and short-lived that yersiniosis it not diagnosed, despite faecal carriage [Ossel 1990]. Sometimes, however, the clinical course of the infection is much more serious and destructive. Syndromes like extensive ulceration of the intestine and subsequent peritonitis or an acute abdomen, due to invagination of the infected section of the intestine into a neighbouring part ('intussuspection'), are not uncommon in young children, and several fatalities have been reported [Gutman *et al.* 1973; Martin *et al.* 1982; Staatz *et al.* 1998]. In older children and adults, clinical syndromes known as 'terminal ileitis' and 'mesenteric lymphadenitis' are more common, which refer to strong inflammatory reactions in the distal small intestine and to swelling of regional lymph nodes [Bottone 1977]. Unlike infections with other common foodborne pathogens, yersiniosis frequently manifests itself with symptoms that mimic appendicitis, leading to sometimes unnecessary appendix operations [Shorter *et al.* 1998]. The upper part of the gastrointestinal tract may also be affected, leading to symptoms of pharyngitis [Gutman *et al.* 1973; Tacket *et al.* 1984]. In elderly people, or persons whose normal host-defense mechanisms have been compromised, the bacterium may persist in intestinal tissue, causing chronic inflammatory bowel diseases [Kallinowski *et al.* 1998].

1.3.2 Complications and Sequela

Due to subsequent spread of the bacterium via the blood stream, other body parts may become infected and a generalized, extraintestinal infection ('septicemia') may occur. Although this complication is especially found among people with an iron-overload [Piroth *et al.*, 1997; Adamkiewicz *et al.* 1998] or suffering underlying diseases [Jensen *et al.* 1995], septicemia may also occur in otherwise healthy persons [Hosaka *et al.* 1997]. Its manifestation includes not only relatively harmless skin inflammations [Gauthier & So 1997], but also abscess formation in liver and spleen [Schiemann 1989], as well as life-threatening infections of brain ('meningitis'), lung ('pneumonia'), heart ('endocarditis') and blood vessels ('aneurysm') [Challa & Marx 1980; Giamarellou *et al.* 1995; Donald *et al.* 1996; Mercié *et al.* 1996], and [Bin-Sagheer *et al.* 1997; Bottone 1997; La Scola *et al.* 1997; Tame *et al.* 1998].

As a result of the host's immune response, *Y. enterocolitica* may also induce secondary, post-infectious auto-immune diseases such as cutaneous granuloma on the extremities ('erythema nodosum') [Niemie *et al.* 1976; Schiemann 1989], and acute and chronic arthritis [Petrus *et al.* 1997;

Heyden *et al.* 1997], especially in adolescents and older adults possessing the tissue type HLA-B27 [Ahvonen & Rossi 1970; Larsen 1980; Falcão *et al.* 1995]. There is also substantial evidence that subclinical persistent infections with *Y. enterocolitica* may induce auto-immune thyroid diseases [Wenzel *et al.* 1996].

1.4 PATHOGENESIS

1.4.1 Entrance, Colonization and Spread

Historically, *Y. enterocolitica* is primarily a gastro-intestinal tract pathogen, although it has also emerged as a significant cause of blood transfusion-associated bacteraemia in the last two decades (see section 1.6.5). The sequence of events following ingestion of virulent *Y.enterocolitica* cells can be summarized by five steps: (i) invasion of intestinal epithelial cells, (ii) penetration of the lamina propria, (iii) multiplication in underlying tissues, (iv) drainage to mesenteric lymph nodes, and (v) entrance into the bloodstream eventually leading to systemic infection [Cornelis *et al.* 1987; Bottone 1997] (Figure 1.2).

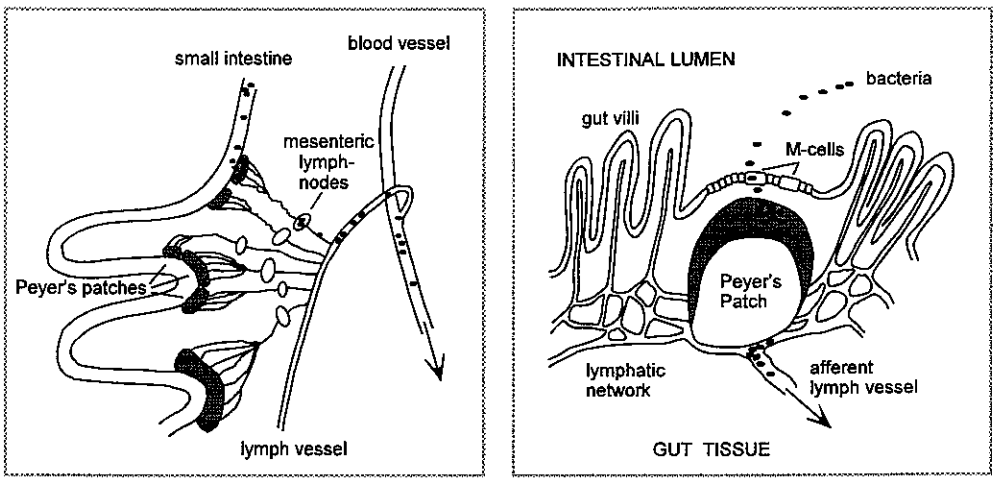


Figure 1.2 Entrance and spread of *Yersinia enterocolitica* in the human host

The first step, entry of the intestinal epithelium, is also seen in other entero-invasive bacteria, like *Shigella* and *Escherichia coli*, but while these enteropathogens usually invade the colonic epithelial layer, *Y. enterocolitica* preferentially localizes, like *Salmonella* species, to the distal

small intestine, the ileum. Secondly, *Y. enterocolitica* has, in contrast to other enteropathogens which usually remain and multiply locally in the epithelial cells, a strong propensity to penetrate the underlying lamina propria and to invade the gut associated lymphoid tissue, especially the organized lymphoid follicles known as Peyer's patches [Hanski *et al.* 1989]. Virulent strains of *Y. enterocolitica* can survive at this site, and multiply as extracellular microcolonies because they are able to resist phagocytosis by macrophages and polymorphonuclear leucocytes.
On the other hand, the organism can withstand intracellular killing by non-professional phagocytes. Hence, the bacterium can use leucocytes to translocate through endothelial monolayers [Rüssmann *et al.* 1996], thus allowing them to drain from the Peyer's patches into lymphatic vessels and to colonize the regional lymph nodes, the liver, and the spleen. Eventually, entrance into the bloodstream may lead to further spread of the infection, inducing various systemic diseases and immunologically mediated sequelae.
Based on their role in the various steps of pathogenesis, several types of virulence factors can be distinguished in *Y. enterocolitica.*

1.4.2 Virulence Factors

Invasion factors (Figure 1.3)
The fist steps of infection - adherence to and invasion of the epithelial layers of the host gut - require at least two chromosomal factors, called *ail* (for Adhesion Invasion Locus) and *inv* (for invasion) [Miller & Falkow 1988].
The *inv* gene is present in virulent as well as in non-virulent strains, whereas the *ail* gene is only found in pathogenic serotypes of *Y. enterocolitica* and in *Y. pestis* and *Y. pseudotuberculosis*, [Miller *et al.* 1989]. The *inv* product of *Y. enterocolitica*, invasin, is a ca. 90 kDa outer membrane protein that mediates cellular entry by binding to integrin receptors on the surface of certain epithelial cells, the so-called M-cells [Pierson 1994]. These cells, which cover the Peyer's patches, are specialized in delivering internalized particles to the underlying macrophages, and the bacterium thus exploits this host cell function to pass through the cellular barrier of the intestinal epithelium and invade the underlying tissue [Finlay & Cossart 1997]. The mode of action of the 17 kDa *ail* product, Ail, which is also an outer membrane protein, has not been elucidated so far.
In addition to the chromosomal factors, at least one extrachromosomally encoded factor directly contributes to invasion. This factor, formerly known as POMP1, P1 or YopA (for Yersinia Outer Protein A) [Cornelis *et al* 1987 1989] but now generally referred to as YadA (for Yersinia adhesin), is the product of the *yadA* (or *yopA*) gene, which is one of the genes present on pYV, the Yersinia virulence plasmid (see below). YadA consists of subunits of about 50 kDa and forms a fibrillar structure on the surface of the bacterium, which, among other functions, mediates clumping and adherence to intestinal mucin [Straley & Perry 1995].

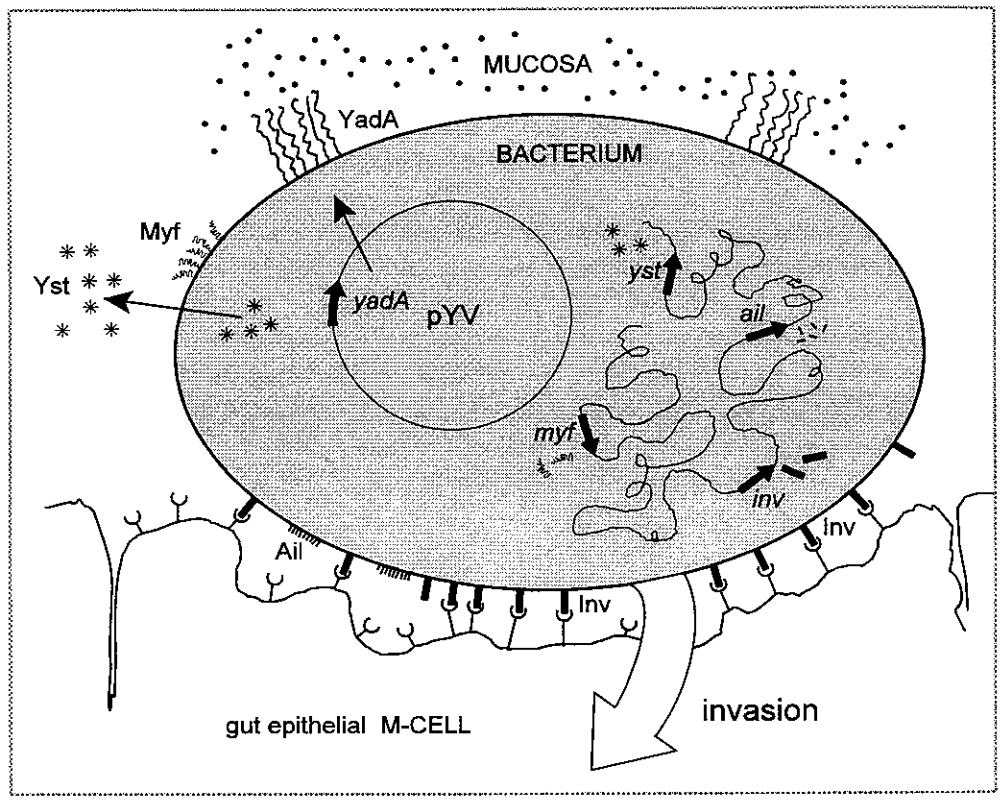


Figure 1.3 Yersinia enterocolitica in the intestine:
genes and gene-products which are involved in the invasion of the host epithelial tissues

Anti-phagocytosis factors

After invasion of the intestinal mucosa, the bacterium has to defend itself to the non-specific immune response of the host, especially to phagocytosis by polymorphonuclear leucocytes The anti-phagocytosis strategy relies mainly on a dozen secreted proteins, called Yops (for Yersinia outer protein's), their individual cytosolic chaperones, called Syc proteins (for Specific Yop chaperone), a dedicated secretion apparatus which is made up of a twenty Ysc (for Yop secretion) proteins, and several regulatory proteins [Cornelis 1994; Boland *et al.* 1996].

Both the *yop*, *syc* and *ysc* genes, as well as the *vir* genes that regulate their expression, are found on a high molecular weight (70-75 kb) plasmid called pYV (for Yersinia Virulence) [Cornelis et al. 1987 1989; Bliska 1994; Iriarte & Cornelis 1998]. This plasmid is highly conserved among all virulent *Yersinia* species and serotypes, and pathogenicity is lost upon loss of pYV [Gemski

et al. 1980; Schiemann & Devenish 1982; Heesemann *et al.* 1983; Portnoy & Martinez 1985]. Cells which harbour pYV require Ca^{++} for growth at 37°C. In the absence of calcium ions, virulent Yersiniae restrict their growth at 37°C and synthesize, instead, large amounts of Yops. This phenomenon reflects a phase transition that allows the bacterium (i) to adapt to its environment - the infected host - and (ii) to proceed with the successive steps in infection.

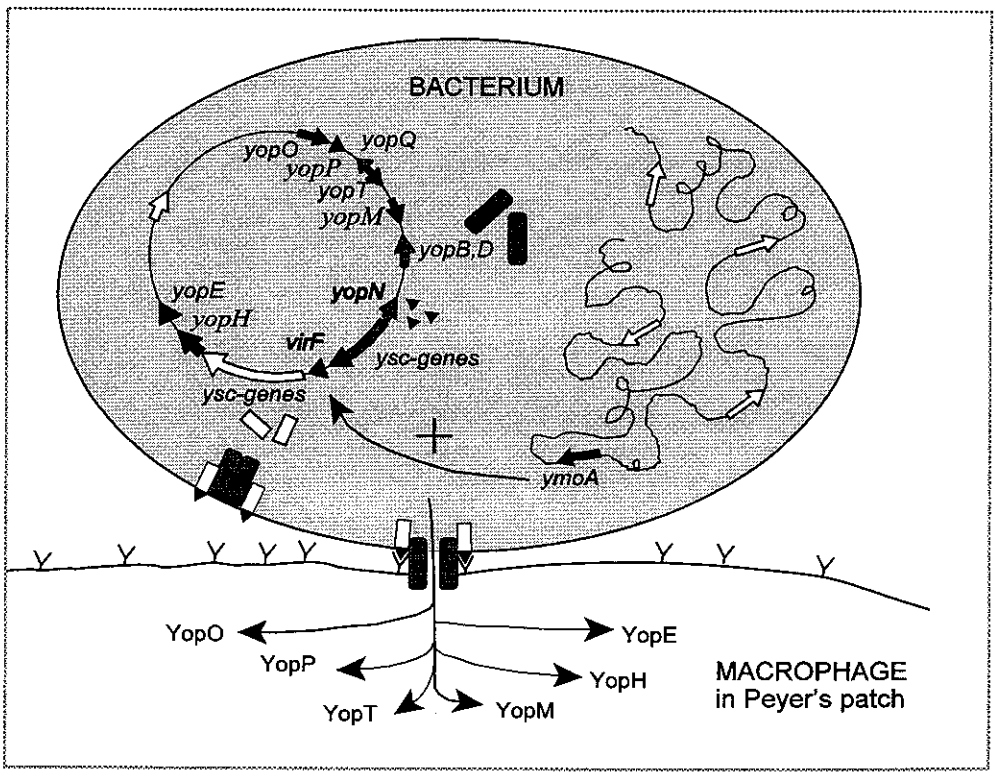


Figure 1.4 *Yersinia enterocolitica* in the Peyer's patch: genes and gene-products involved in the process of anti-phagocytosis.

The mode of action of several Yops has now largely been elucidated and a model has been proposed to explain the interaction between *Y. enterocolitica* and the target cell [Cornelis & Wolf-Watz 1997] (Figure 1.4). Upon contact of bacteria with the host cell surface, the membrane associated proteins YopB and D act, in co-operation with the membrane-bound Ysc secretion system, as a translocation apparatus to inject YopE, H, M, O, P and T into the phagocytic cells [Rosqvist *et al.* 1994; Persson *et al.* 1995; Boland *et al.* 1996; Mills *et al.* 1997; Iriarte & Cornelis 1998]. YopN